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PROCEEDINGS

of
PAKISTAN CONGRESS OF ZOOLOGY

Volume 42 (2024)



FORTY SECOND PAKISTAN CONGRESS OF ZOOLOGY
April 23-25, 2024

held under the auspices of
THE ZOOLOGICAL SOCIETY OF PAKISTAN

at
DEPARTMENT OF ZOOLOGY
UNIVERSITY OF AZAD JAMMU & KASHMIR, MUZAFFARABAD

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*All research articles in this Proceedings were refereed
by experts in respective disciplines*



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**DEPARTMENT OF ZOOLOGY,
UNIVERSITY OF AZAD JAMMU & KASHMIR
King Abdullah Campus, Chatter Klass: Muzaffarabad**

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PROCEEDINGS OF THE CONGRESS

Editor

Prof. Dr. A.R. Shakoori

Composed & designed by Fakhar Mahmood Shahid

ACKNOWLEDGMENTS

Department of Zoology, University of Azad Jammu & Kashmir, Muzaffarabad hosted the 42nd Pakistan Congress of Zoology (International).

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PROGRAMME



42nd

**PAKISTAN CONGRESS OF ZOOLOGY
(INTERNATIONAL)**

April 23-25, 2024

Organized by:
Department of Zoology
University of Azad Jammu & Kashmir
King Abdullah Campus, Chatter Klass,
Muzaffarabad



42nd PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

UNIVERSITY OF AZAD JAMMU & KASHMIR, MUZAFFARABAD

April 23-25, 2024

DAY ONE

TUESDAY, APRIL 23, 2024

- 08:30 AM REGISTRATION
- 09:00 AM Inauguration: Recitation from the Holy Quran
- 09:05 AM Welcome address
- 09:10 AM Address by the Secretary General, Zoological Society of Pakistan
- 09:20 AM Address by the President, Zoological Society of Pakistan
- 09:30 AM Address by the Vice Chancellor, University of Azad Jammu & Kashmir
- 09:40 AM Distribution of Medals and Awards
- 10:10 AM Address by the Chief Guest
- 10:35 AM Vote of Thanks
- 10:40 AM Refreshment

11:30 AM, JOINT SESSION I: PLENARY LECTURES

CHAIRPERSON: Prof. Dr. Anwar-ul-Hassan Gilani

CO-CHAIRPERSON: Prof. Dr. Muhammad Wajid

1. **Prof. Dr. Telat YANIK**, Aquaculture Department, Faculty of Fisheries, Ataturk University, Turkey
Some General Aspects of Fish Farming
2. **Amael Borzee**, Laboratory of Animal Behaviour and Conservation, College of Life Sciences, Nanjing Forestry University, Nanjing, People's Republic of China
How to Integrate Multiple Fields in Amphibian Conservation Research?
3. **Qurra-tul-Ann Afza Gardner**, School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore
Insights into the Biological Significance of Insulin Variants

01:30 PM LUNCH AND PRAYER BREAK (ZUHAR)

02:15 PM - HALL-1		02:15 PM - HALL-2		02:15 PM - HALL-3	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 1 Chairperson: Prof. Dr. M. Naeem Khan Co-Chairperson: Dr. Soumble Zulfiqar		SECTION III ENTOMOLOGY SESSION 1 Chairperson: Prof. Dr. Riffat Sultana Co-Chairperson: Dr. Naheed Bano		SECTION V FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY SESSION 1 Chairperson: Prof. Dr. Naeem Tariq Narejo Co-Chairperson: Dr. Asma Shakir Khawaja	
1. S. Ali (GCU, Lahore)	CBGP-3	1. A.N. Memon (GUBDC, Dadu)	ENT-1	1. M.A. Brohi (SWD, Karachi)	FEWFM-1
2. M. Munir (UAJK, Muzaffarabad)	CBGP-13	2. A. Ansari (GHSSSKL, Matlari)	ENT-2	2. K. Saeed (UB, Buner)	FEWFM-2
3. S. Nazer (UAJK, Muzaffarabad)	CBGP-17	3. F. Shaikh (US, Jamshoro)	ENT-3	3. S. Jalbani (SBBUVAS, Sakrand)	FEWFM-4
4. S. Aziz (UAJK, Muzaffarabad)	CBGP-20	4. S. Kumar (CUVAS, Bahawalpur)	ENT-4	4. M. Khalid (US, Jamshoro)	FEWFM-5
5. F. Shafique (US, UK)	CBGP-25	5. A. Iqbal (CUVAS, Bahawalpur)	ENT-5	5. N. Naheed (MNSUA, Multan)	FEWFM-6
6. W. Ali (GCU, Lahore)	CBGP-29	6. M.A. Rameez (CUVAS, Bahawalpur)	ENT-6	6. A. Karim (SBKWU, Quetta)	FEWFM-7
7. I. Riaz (UAJK, Muzaffarabad)	CBGP-56	7. M. Waseem (CUVAS, Bahawalpur)	ENT-7	7. T. Mahmood (AAU, Rawalpindi)	FEWFM-8
8. K. Maqbool (UAJK, Muzaffarabad)	CBGP-57	8. M. Irfan (CUVAS, Bahawalpur)	ENT-8	8. A. Qureshi (UAJK, Muzaffarabad)	FEWFM-9
9. N. Tahir (UAJK, Muzaffarabad)	CBGP-58	9. M.Z. Abedin (CUVAS, Bahawalpur)	ENT-9	9. H. Khalid (UAJK, Muzaffarabad)	FEWFM-10
10. N. Kakar (UT, Kech)	CBGP-60	10. S. Iqbal (CUVAS, Bahawalpur)	ENT-10	10. R. Zareen (UAJK, Muzaffarabad)	FEWFM-11
11. A. Hussain (UK, Kotli)	CBGP-61	11. Amna (CUVAS, Bahawalpur)	ENT-11	11. T. Khaleeq (CUVAS, Bahawalpur)	FEWFM-12
12. Z. Bibi (GCU, Lahore)	CBGP-66	12. S. Batoool (CUVAS, Bahawalpur)	ENT-12	12. K. Ahmad (UB, Buner)	FEWFM-13
13. I. Fatima (GCU, Lahore)	CBGP-67	13. S. Soomro (US, Jamshoro)	ENT-13	13. N. Zahid (CUVAS, Bahawalpur)	FEWFM-14
14. A. Ali (GCU, Lahore)	CBGP-68	14. S.P. Memon (US, Jamshoro)	ENT-14	14. A. Khan (US, Swabi)	FEWFM-15
15. F. Hussain (SBBUVAS, Sakrand)	CBGP-100	15. A. Chandio (US, Jamshoro)	ENT-16	15. B. Baloch (SBBUVAS, Sakrand)	FEWFM-16
		16. A.A. Babar (US, Jamshoro)	ENT-17		
04:30 PM TEA BREAK AND PRAYER BREAK (ASAR)					
05:00 PM - HALL-1		05:00 PM - HALL-2		05:00 PM - HALL-3	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 2 Chairperson: Prof. Dr. Farah Rauf Shakoori Co-Chairperson: Dr. Asia Bibi		SECTION III ENTOMOLOGY SESSION 2 Chairperson: Prof. Dr. Unser Naeem Ullah Co-Chairperson: Dr. Santosh Kumar		SECTION V FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY SESSION 2 Chairperson: Prof. Dr. Basharat Ahmad Co-Chairperson: Dr. Roheela Yasmeen	
1. S. Khan (US, Swabi)	CBGP-1	1. A. Soomro (US, Jamshoro)	ENT-18	1. M.A. Shah (BUKC, Karachi)	FEWFM-17
2. M. Paras (US, Jamshoro)	CBGP-2	2. B. Mahar (US, Jamshoro)	ENT-19	2. M. Ali (US, Jamshoro)	FEWFM-18
3. G. S. Sharif (UP, Lahore)	CBGP-4	3. F.D. Soomro (US, Jamshoro)	ENT-20	3. N. Ahmad (BUKC, Karachi)	FEWFM-19
4. S. Rathore (FUUAAT, Karachi)	CBGP-5	4. F.A. Hussain (US, Jamshoro)	ENT-21	4. T. Afzal (UP, Rawalakot)	FEWFM-20
5. R. Sehar (CUVAS, Bahawalpur)	CBGP-6	5. M.J. Børdar (US, Jamshoro)	ENT-22	5. N. Afsar (UK, Karachi)	FEWFM-21
6. Bano (CUVAS, Bahawalpur)	CBGP-7	6. J. Das (US, Jamshoro)	ENT-23	6. M. Tabish (UK, Karachi)	FEWFM-22
7. S.M. Arshad (HU, Mansehra)	CBGP-8	7. S. Larailb (US, Jamshoro)	ENT-24	7. R. Nasir (UK, Karachi)	FEWFM-23
8. K. Fatima (CUVAS, Bahawalpur)	CBGP-9	8. M. Jamil (US, Jamshoro)	ENT-25	8. A. Hameed (UK, Karachi)	FEWFM-24
9. M. Sharif (IUB, Bahawalpur)	CBGP-10	9. M.A. Jakhrani (US, Jamshoro)	ENT-26	9. F.A. Mangi (UK, Karachi)	FEWFM-25
10. M. Chaudhary (IUB, Bahawalpur)	CBGP-11	10. N.G. Khattak (US, Jamshoro)	ENT-27	10. Q.B. Kazmi (UK, Karachi)	FEWFM-26
11. S. Andleeb (UAJK, Muzaffarabad)	CBGP-12	11. N. Soomro (US, Jamshoro)	ENT-28	11. F.S. Ghory (UK, Karachi)	FEWFM-27
12. A. Naseer (UAJK, Muzaffarabad)	CBGP-14	12. R. Shah (US, Jamshoro)	ENT-29	12. Habibullah (LUAWMS, Uthal)	FEWFM-28
13. R. Riaz (UAJK, Muzaffarabad)	CBGP-15	13. S.A. Talpur (US, Jamshoro)	ENT-30	13. N. Yaqoob (PINSTECH, Isl)	FEWFM-29
14. K. Tahir (UAJK, Muzaffarabad)	CBGP-16	14. M.S. Dayo (US, Jamshoro)	ENT-31	14. R. Kausar (UB, Quetta)	FEWFM-30
15. D.A. Bukhari (GCU, Lahore)	CBGP-18	15. H.A. Lalika (IUB, Bahawalpur)	ENT-33	15. T. Hamid (UK, Karachi)	FEWFM-31
16. A. Fatima (GCU, Lahore)	CBGP-19	16. A. Alladino (GCU, Hyderabad)	ENT-34		
17. S. Zahoor (CUVAS, Bahawalpur)	CBGP-21				
6:25 PM PRAYER BREAK (MAGHRB)					

06:40 PM - HALL-1		06:40 PM - HALL-2		06:40 PM - HALL-3	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 3 Chairperson: Prof. Dr. Abdul Rehman Co-Chairperson: Dr. Saba Irshad		SECTION III ENTOMOLOGY SESSION 3 Chairperson: Prof. Dr. Naheed Kaka Co-Chairperson: Dr. Asifa Hameed		SECTION V FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY SESSION 3 Chairperson: Dr. Noor-us-Saher Co-Chairperson: Dr. Tariq Mahmood	
1. T. Mazhar (CUVAS, Bahawalpur)	CBGP-22	1. S. Soomro (GCU, Hyderabad)	ENT-35	1. M. Mairaj (UK, Karachi)	FEWFM-33
2. M. Riaz (UoL, Lahore)	CBGP-23	2. M. Rizwan (BHFPRS, Rawalpindi)	ENT-36	2. S. Siddique (LUAWMS, Uthal)	FEWFM-34
3. A. Zarreen (ZMH, Rawalakot)	CBGP-24	3. R.Z. Khizar (MNSUA, Multan)	ENT-37	3. Habibullah (LUAWMS, Uthal)	FEWFM-35
4. R. Sultana (TA&MU, USA)	CBGP-26	4. S. Khaskheli (US, Jamshoro)	ENT-38	4. M. Rasheed (UK, Karachi)	FEWFM-36
5. Yusra (US, Swabi)	CBGP-27	5. U.A.A.Shaikh (UP, Rawalakot)	ENT-39	5. S. Majeed (BUK, Karachi)	FEWFM-37
6. M. Younus (CUVAS, Bahawalpur)	CBGP-28	6. S. Ali (UVAS, Lahore)	ENT-40	6. E.E. Valeem (LEMR, Karachi)	FEWFM-38
7. A.A. Abbasi (MUST, Mirpur)	CBGP-30	7. A. Hassan (UVAS, Lahore)	ENT-41	7. N.U. Saher (UK, Karachi)	FEWFM-39
8. M. Mahaz (AWKUM, Mardan)	CBGP-31	8. M.S. Abbas (MNSUA, Multan)	ENT-42	8. Z. Imdad (UK, Karachi)	FEWFM-40
9. S. Farooq (UP, Lahore)	CBGP-32	9. B. Ahmad (HU, Mansehra)	ENT-43	9. D. Shahwar (UK, Karachi)	FEWFM-41
10. A. Naveed (UP, Lahore)	CBGP-33	10. H.A.A. Khan (UP, Lahore)	ENT-44	10. A.H. Narejo (ZSP, Islamabad)	FEWFM-42
11. S. Gul (UP, Lahore)	CBGP-34	11. S.A. Channa (US, Jamshoro)	ENT-45	11. N.U. Saher (UK, Karachi)	FEWFM-43
12. K. Shaikh (GCU, Hyderabad)	CBGP-35	12. Z. Ahmed (FUUAST, Karachi)	ENT-46	12. A. Mukhtiar (UK, Karachi)	FEWFM-45
13. M. Alladino (GCU, Hyderabad)	CBGP-36	13. R. Mushtaq (FUUAST, Karachi)	ENT-47	13. S.I.H. Jafri (Karachi)	FEWFM-47
14. M. Buriro (GCU, Hyderabad)	CBGP-37	14. S.H.M. Bokhari (MNSUA, Multan)	ENT-48	14. S. Siyal (SBBUVAS, Sakrand)	FEWFM-48
15. S. Jawaid (GCU, Hyderabad)	CBGP-38	15. A.H. Soomro (SAU, Tandojam)	ENT-49		
16. M. Tayyab (GCU, Hyderabad)	CBGP-39	16. A.R. Bhatti (SAU, Tandojam)	ENT-50		
17. B. Aziz (PMASAAU, Rawalpindi)	CBGP-40				
7:00 PM EXECUTIVE COUNCIL MEETING					
8:30 PM DINNER					

DAY TWO**WEDNESDAY, APRIL 24, 2024****09:00 AM, JOINT SESSION II: PLENARY LECTURES****CHAIRPERSON: Prof. Dr. Mazhar Ayaz****CO-CHAIRPERSON: Prof. Dr. Ali Muhammad Yousafzai**

- Muhammad Khan**, Institute of Zoology, University of the Punjab, Quaid-e-Azam campus Lahore
Targeting Tumor Energy Metabolism With Plants-Derived Multi-Target Bioactive Molecules: A Promising Strategy for Cancer Therapy
- Syed Murtaza Hassan Andrabi**, Animal Reproduction and Genetics Program, Animal Sciences Institute, PARC-National Agricultural Research Centre; Animal Sciences Division, Pakistan Agricultural Research Council, Islamabad
An Update on Breeding Technologies in Water Buffalo (*Bubalus bubalis*)
- Muhammad Siddique Awan**, Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad.
Status of Biodiversity and Management Prescriptions for Ghamot National Park, District Neelum, Azad Jammu and Kashmir

10:30 AM - HALL-1

SECTION I
CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS,
PHYSIOLOGY, TOXICOLOGY, VIROLOGY
SESSION 4
Chairperson: Dr. Muhammad Afzal Ghauri
Co-Chairperson: Dr. Abdul Rauf Janjua

- N. Zulfiqar (AAU, Rawalpindi) CBGP-41
- R. Nisar (MUST, Mirpur) CBGP-42
- S. Khushal (MUST, Mirpur) CBGP-43
- M. Shadab (MUST, Mirpur) CBGP-44
- R. Shoukat (MUST, Mirpur) CBGP-45
- S. Farooq (MUST, Mirpur) CBGP-46
- T. Ahmed (MUST, Mirpur) CBGP-47
- A. Munawar (CUVAS, Bahawalpur) CBGP-48
- A.A. Latif (ICWU, Lahore) CBGP-49
- S.S. Hussain (SAU, Khairpur) CBGP-50
- F. Ishaq (UAJK, Muzaffarabad) CBGP-51
- S.N. Panhwar (SAU, China) CBGP-52
- M. Bibi (UAJK, Muzaffarabad) CBGP-53
- S. Sehar (UoL, Lahore) CBGP-54
- M. Ijaz (UVAS, Lahore) CBGP-55
- N. Muhammad (GCU, Lahore) CBGP-59
- S. Ameer (NIAB, Faisalabad) CBGP-62

10:30 AM - HALL-2

SECTION III
ENTOMOLOGY
SESSION 4
Chairperson: Dr. Zain Ul Abidin
Co-Chairperson: Dr. Nadir Ali Birmani

- A.A. Gil (SAU, Tandojam) ENT-51
- G.M. Lakho (SAU, Tandojam) ENT-52
- S.M. Jafery (SAU, Tandojam) ENT-53
- I. Khatri (SAU, Tandojam) ENT-54
- A.P. Soomro (SAU, Tandojam) ENT-55
- M.A. Gandahi (SAU, Tandojam) ENT-56
- H. Mannan (SAU, Tandojam) ENT-57
- A.A. Sarki (US, Jamshoro) ENT-58
- A.A. Khalique (US, Jamshoro) ENT-59
- A.M. Shaikh (GCU, Hyderabad) ENT-60
- N. Baloch (US, Jamshoro) ENT-61
- S.A. Larik (SALU, Khairpur Mirs) ENT-62
- M.A. Mahar (SALU, Khairpur Mirs) ENT-64
- S. Soomro (US, Jamshoro) ENT-65
- S. Suhriani (SALU, Khairpur Mirs) ENT-66

10:30 AM - HALL-3

SECTION V
FISHERIES, ECOLOGY, WILDLIFE,
FRESHWATER BIOLOGY, MARINE BIOLOGY
SESSION 4
Chairperson: Prof. Dr. M. Siddique Awan
Co-Chairperson: Dr. Muhammad Rais

- M. Zaman (UH, Haripur) CBGP-64
- V. Kumari (US, Jamshoro) ENT-15
- S. Sanam (CABI, Pak) ENT-32
- W.A. Panhwar (US, Jamshoro) ENT-63
- D.B. Mishori (GCU, Hyderabad) FEWFM-3
- N. Shoaib (UK, Karachi) FEWFM-32
- N. Shoaib (UK, Karachi) FEWFM-46
- S. Ali (SBBUVAS, Sakrand) FEWFM-66
- S.A. Minhas (UAJK, Muzaffarabad) FEWFM-78
- G. Rabbani (UP, Lahore) FEWFM-91
- K. Ahmed (UP, Lahore) FEWFM-92
- K. Sahar (HBP, Islamabad) FEWFM-93
- A. Marium (GSCWU, Bahawalpur) FEWFM-94
- M. Hanif (UP, Lahore) FEWFM-95
- M. Waqas (UE, Lahore) FEWFM-96
- Q.A. Shabbir (CISS-AJK) FEWFM-97
- S. Riaz (UK, Karachi) FEWFM-98

11:45 AM - TEA BREAK

12:00 NOON - HALL-1	12:00 NOON - HALL-2	12:00 NOON - HALL-3
SECTION 1 CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 5 Chairperson: Dr. Muhammad Khan Co-Chairperson: Dr. Beenish Shahid	SECTION III ENTOMOLOGY SESSION 5 Chairperson: Dr. Abdul Manan Shaikh Co-Chairperson: Dr. Nadir Ali Shah	SECTION V FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY SESSION 5 Chairperson: Prof. Dr. Zulfiqar Ali Co-Chairperson: Dr. Nasra Ashraf
1. M.U. Laghari (US, Jamshoro) CBGP-63 2. S. Younas (GCU, Lahore) CBGP-65 3. Shiza (GCU, Lahore) CBGP-69 4. A. Rehman (UP, Lahore) CBGP-70 5. K. Ashiq (CUVAS, Bahawalpur) CBGP-71 6. I. Zulfiqar (CUVAS, Bahawalpur) CBGP-72 7. S. Hussain (US, Swabi) CBGP-73 8. K. Malik (UP, Lahore) CBGP-74 9. F. Tabssum (UE, Lahore) CBGP-75 10. A. Fatima (UK, Karachi) CBGP-76 11. S. Naz (LCWU, Lahore) CBGP-77 12. D.A. Solangi (LUMHS, Jamshoro) CBGP-78 13. Z.H. Soomro (CH, Karachi) CBGP-79 14. S.G.Ali (QU, Islamabad) CBGP-80 15. D. Saood (AWKU, Mardan) CBGP-81 16. M. Ihsan (UAJK, Muzaffarabad) CBGP-82 17. S. Imtiaz (UAJK, Muzaffarabad) CBGP-83	1. S.R. Soomro (SALU, Khairpur Mirs) ENT-67 2. H. Mannan (SAU, Tandojam) ENT-68 3. F. Asif (UAJK, Muzaffarabad) ENT-69 4. H. Sajjad (SALU, Khairpur) ENT-70 5. M. Summer (GCU, Lahore) ENT-71 6. N. Ehsan (UA, Faisalabad) ENT-72 7. S.M. Usamma (GCU, Hyderabad) ENT-73 8. M.A. Sahito (GCU, Hyderabad) ENT-74 9. B. Mal (GCU, Hyderabad) ENT-75 10. V. Kumar (GCU, Hyderabad) ENT-76 11. A. Solangi (GCU, Hyderabad) ENT-77 12. A. Waheed (GCU, Hyderabad) ENT-78 13. A. Ayaz (GCU, Hyderabad) ENT-79 14. T.J. Ursani (US, Jamshoro) ENT-80 15. A.M. Sabir (RRI, Kala Shah Kaku) ENT-81	1. S. Batool (IUB, Bahawalpur) FEWFM-49 2. S. Khalid (UAJK, Muzaffarabad) FEWFM-51 3. A. Safdar (UP, Lahore) FEWFM-52 4. A.S. Khan (UP, Lahore) FEWFM-53 5. B. Sabahat (UP, Lahore) FEWFM-54 6. M.S. Hassan (UP, Lahore) FEWFM-56 7. R. Ahmad (LCWU, Lahore) FEWFM-57 8. M. Tanveer (UP, Lahore) FEWFM-58 9. A. Kakar (UB, Quetta) FEWFM-59 10. K. Mehmood (UK, Kotli) FEWFM-60 11. I. Ahmed (NARC, Islamabad) FEWFM-61 12. S. Ali (UAJK, Muzaffarabad) FEWFM-62 13. L.A. Khan (UAJK, Muzaffarabad) FEWFM-63 14. F. Bari (UC, Chitral) FEWFM-64
01:30 PM LUNCH BREAK (ZUHAR)		
02:00 PM - HALL-1	02:00 PM - HALL-2	02:00 PM - HALL-3
SECTION 1 CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 6 Chairperson: Dr. Muhammad Akhtar Ali Co-Chairperson: Dr. Ansar Ahmed Abbasi	SECTION II PESTS AND PEST CONTROL SESSION 1 Chairperson: Prof. Dr. Tahira Jabeen Ursani Co-Chairperson: Dr. Waheed Ali Panhwar	SECTION V FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY SESSION 6 Chairperson: Prof. Dr. Nuzhat Shafi Co-Chairperson: Dr. Riaz Aziz Minhas
1. R. Komal (UAJK, Muzaffarabad) CBGP-84 2. N. Fatima (GCU, Hyderabad) CBGP-85 3. A. Sadq (GCU, Hyderabad) CBGP-86 4. K. Ansari (GCU, Hyderabad) CBGP-87 5. Mahnoor (GCU, Hyderabad) CBGP-88 6. Hafsa (GCU, Hyderabad) CBGP-89 7. G. Fatima (GCU, Hyderabad) CBGP-90 8. Faiza (GCU, Hyderabad) CBGP-91 9. E. Aqeel (GCU, Hyderabad) CBGP-92 10. A. Kumar (GCU, Hyderabad) CBGP-93 11. N. Irfan (NIAB-C PIEAS, Faisalabad) CBGP-94 12. N. Suleman (KFUEIT, RYK) CBGP-95 13. M.U. Mazhar (NIAB, Faisalabad) CBGP-96 14. B. Shahid (UAJK, Muzaffarabad) CBGP-97 15. A. Ullah (US, Swabi) CBGP-98 16. M. Aftab (VU, Pak) CBGP-99 17. I. Uddin (SBBUVAS, Sakrand) CBGP-101	1. K.H. Dhilloo (SAU, Tandojam) PC-1 2. A. Lashari (SAU, Tandojam) PC-2 3. M.Z. Majeed (US, Sargodha) PC-3 4. Z.A. Abro (US, Jamshoro) PC-4 5. A. Amur (GCU, Hyderabad) PC-5 6. I. Khan (NIFA, Peshawar) PC-6 7. N. Bano (MNSUA, Multan) PC-7 8. A. Hamid (CUVAS, Bahawalpur) PC-8 9. S. Tariq (CUVAS, Bahawalpur) PC-9 10. F. Syed (CUVAS, Bahawalpur) PC-10 11. F. Anum (US, Jamshoro) PC-11 12. N. Bhanger (US, Jamshoro) PC-12 13. F. Abbasi (US, Jamshoro) PC-13 14. M. Ali (US, Jamshoro) PC-14 15. M.M. Jakhani (US, Jamshoro) PC-15 16. S.U. Mahmood (GAS, China) PC-16 17. M.S.Q. Zaman (MNSUA, Multan) PC-17	1. T. Iftikhar (WUAJK, Bagh) FEWFM-67 2. M. Siddique (LGU, Lahore) FEWFM-68 3. M. Tahir (UA, Faisalabad) FEWFM-69 4. W. Mustafa (UA, Faisalabad) FEWFM-70 5. A. Habib (UP, Lahore) FEWFM-71 6. S. Aslam (AAU, Rawalpindi) FEWFM-72 7. F. Jabeen (AAU, Rawalpindi) FEWFM-73 8. N. Ghafoor (UA, Faisalabad) FEWFM-74 9. B.A. Bughio (US, Jamshoro) FEWFM-79 10. W. Shaikh (UK, Karachi) FEWFM-90 11. A. Rehman (CISS, AJK) FEWFM-99 12. U. Sajjad (UP, Rawalakot) FEWFM-100 13. A. Naseer (UP, Lahore) FEWFM-101 14. M. Imran (UP, Lahore) FEWFM-102 15. M. Ain (UK, Karachi) FEWFM-103
04:30 PM TEA BREAK AND PRAYER BREAK (ASAR)		

05:00 PM - HALL-1		05:00 PM - HALL-2		05:00 PM - HALL-3	
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 7 Chairperson: Dr. Bushra Muneer Co-Chairperson: Dr. Zahid Latif		SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 8 Chairperson: Dr. Bibi Naazia Murtaza Co-Chairperson: Dr. Iram Liaqat		SECTION IV PARASITOLOGY SESSION 1 Chairperson: Prof. Dr. Mian Syed Khan Co-Chairperson: Dr. Suleman	
1. A. Beenash (WUAIK, Bagh)	CBGP-102	1. R. Tahir (UK, Karachi)	CBGP-119	1. A.S. Hullo (US, Jamshoro)	PAR-1
2. B. Younis (WUAIK, Bagh)	CBGP-103	2. S. Ghayyur (HU, Mansehra)	CBGP-120	2. B. Soomro (GCU, Hyderabad)	PAR-2
3. U. Farooq (IUB, Bahawalpur)	CBGP-104	3. W. Naz (HU, Mansehra)	CBGP-121	3. S.F.A. Shah (UVAS, Lahore)	PAR-3
4. M.H. Lashari (IUB, Bahawalpur)	CBGP-105	4. M. Nadeem (CUVAS, Bahawalpur)	CBGP-122	4. M. Munir (UVAS, Lahore)	PAR-4
5. T. Almas (UAIK, Muzaffarabad)	CBGP-106	5. T. Nauroze (UE, Lahore)	CBGP-123	5. Jemi (US, Jamshoro)	PAR-5
6. A. Manzoor (UA, Faisalabad)	CBGP-107	6. G. Maryum (CUVAS, Bahawalpur)	CBGP-124	6. S. Ullah (MNSUA, Multan)	PAR-6
7. M. Tariq (UA, Faisalabad)	CBGP-108	7. M. Ahmad (CUVAS, Bahawalpur)	CBGP-125	7. K.H. Burro (GDC, Daulatpur)	PAR-7
8. A. Tahir (UA, Faisalabad)	CBGP-109	8. M. Toufiq (CUVAS, Bahawalpur)	CBGP-126	8. M. Khan (US, Swabi)	PAR-8
9. R. Iqbal (BZU, Multan)	CBGP-110	9. A. Batool (CUVAS, Bahawalpur)	CBGP-127	9. T. Fatima (SALU, Khairpur)	PAR-9
10. S.A. Raja (CUI, Islamabad)	CBGP-111	10. M. Abrar (UoL, Lahore)	CBGP-128	10. S.S. Bukhari (WUAIK, Bagh)	PAR-10
11. F. Bashir (LCWU, Lahore)	CBGP-112	11. I. Ismail (UoL, Lahore)	CBGP-129	11. M. Farman (UK, Karachi)	PAR-11
12. S. Naz (LCWU, Lahore)	CBGP-113	12. S. Ashraf (UoL, Lahore)	CBGP-130	12. M. Hussain (NARC, Islamabad)	PAR-12
13. N. Ali (US, Swabi)	CBGP-114	13. S. Amjad (LGU, Lahore)	CBGP-131	13. T. Leghari (US, Jamshoro)	PAR-13
14. A. Waheed (US, Swabi)	CBGP-115	14. A. Fatima (GCU, Lahore)	CBGP-132	14. A. Khan (PARC, Karachi)	PAR-14
15. A. Fatima (VU, Pak)	CBGP-116	15. M. Hammad (GCU, Lahore)	CBGP-133	15. S.N. Das (US, Jamshoro)	PAR-15
16. S.A. Memon (GCU, Hyderabad)	CBGP-117	16. A. Akbar (UA, Faisalabad)	CBGP-134	16. D. Saood (AWKU, Mardan)	PAR-16
17. S. Soomro (GCU, Hyderabad)	CBGP-118	17. M.U. Ijaz (UA, Faisalabad)	CBGP-135		
08:00 PM GENERAL BODY MEETING					
08:30 PM DINNER/ CULTURE NIGHT					

DAY THREE**THURSDAY, APRIL 25, 2024****09:00 AM, JOINT SESSION III: PLENARY LECTURES****CHAIRPERSON: Prof. Dr. Muhammad Afzal****CO-CHAIRPERSON: Mr. Abdul Aziz Khan**

- Abdul Aziz Khan**, Pakistan Agricultural Research Council, Islamabad
Deterioration Impacts of Indian Crested Porcupine, *Hystrix Indica* on Forest Plantations of Pakistan
- Javed Iqbal Qazi**, Institute of Zoology, University of the Punjab, Lahore
Influences of Different Fruit Peels and Spices on the Efficacy of Antibiotics and Antidiabetic Drugs: In Vitro Approaches.

10:30 AM - HALL-1	10:30 AM - HALL-2	10:30 AM - HALL-3
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 9 Chairperson: Dr. Irfan Zia Qureshi Co-Chairperson: Dr. Dil Ara Abbas Bukhari	SECTION II PESTS AND PEST CONTROL SESSION 2 Chairperson: Dr. Inamullah Khan Co-Chairperson: Dr. Mansoor Ali Shah	SECTION V FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY SESSION 7: Chairperson: Dr. Zaib-un-Nisa Burhan Co-Chairperson: Dr. M. Younas Leghari
1. M.F. Hayat (UA, Faisalabad) CBGP-136 2. S.S. Zahara (UA, Faisalabad) CBGP-137 3. M. Khalil (UA, Faisalabad) CBGP-138 4. N. Ghafoor (UA, Faisalabad) CBGP-139 5. R. Azmat (UA, Faisalabad) CBGP-140 6. M.Z. Salar (UA, Faisalabad) CBGP-141 7. Z. Manzoor (UA, Faisalabad) CBGP-142 8. R. Eman (UA, Faisalabad) CBGP-143 9. G. Mustafa (UA, Faisalabad) CBGP-144 10. M.M. Jalal (UA, Faisalabad) CBGP-145 11. QA Sattar (UA, Faisalabad) CBGP-146 12. M. Jameel (UA, Faisalabad) CBGP-147 13. S.A.Zainab (SALU, Khairpur) CBGP-148 14. A. Rani (UAJK, Muzaffarabad) CBGP-149 15. D.S. Tahir (NIC, Rabwah) CBGP-150 16. Faiza (GCU, Lahore) CBGP-151	1. M. Ishtiaq (MNSUA, Multan) PC-18 2. M. Salman (NIFA, Peshawar) PC-19 3. N. Nazir (UP, Rawalakot) PC-20 4. H.A. Sajjad (MNSUA, Multan) PC-21 5. M. Ahmad (MNSUA, Multan) PC-22 6. M. Ashfaq (MNSUA, Multan) PC-23 7. M. Alizaz (MNSUA, Multan) PC-24 8. J.L. Khan (DPP, Karachi) PC-25 9. U. Sharif (MNSUA, Multan) PC-26 10. A. Khalid (MNSUA, Multan) PC-27 11. I. Saif (CUVAS, Bahawalpur) PC-28 12. I. Maryyam (MNSUA, Multan) PC-29 13. A. Hameed (MRI, Multan) PC-30 14. K. Azam (MNSUA, Multan) PC-31 15. H. Hira UA, Faisalabad) PC-32 16. M.A. Shah (US, Jamshoro) PC-33	1. F. Naz (UK, Karachi) FEWFM-44 2. Z. B. Mirza (IWMB, Islamabad) FEWFM-50 3. M. Tanveer (UP, Lahore) FEWFM-55 4. I. Larailb (UoL, Lahore) FEWFM-65 5. R.G. Mustafa (UAJK, Muzaffarabad) FEWFM-75 6. A. Hameed (UAJK, Muzaffarabad) FEWFM-76 7. A. Farooq (UAJK, Muzaffarabad) FEWFM-77 8. S. Ali (IWMB, Islamabad) FEWFM-80 9. M. Saeed (IWMB, Islamabad) FEWFM-81 10. Z. Ali (IWMB, Islamabad) FEWFM-82 11. S. Khanam (UK, Karachi) FEWFM-83 12. S. Halder (MNSUA, Multan) FEWFM-84 13. U. Jamil (UP, Lahore) FEWFM-85 14. U. Ahmad (UP, Lahore) FEWFM-86 15. A. Abid (PMASAAU, Rawalpindi) FEWFM-87 16. F. Ahmed (CFPC) FEWFM-88 17. H. Amin (NFU, China) FEWFM-89
11:45 AM - TEA BREAK		

12:00 NOON - HALL-1		12:00 NOON - HALL-2		
SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 10 Chairperson: Dr. Saiqa Andleeb Co-Chairperson: Dr. Shaukat Ali		SECTION I CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY SESSION 11 Chairperson: Prof. Dr. Javed Iqbal Qazi Co-Chairperson: Dr. Munawar Saleem Ahmad		
1. T. Naveed (GCU, Lahore)	CBGP-152	1. A. Shehzadi (UE, Lahore)	CBGP-168	
2. A. Naveen (UP, Lahore)	CBGP-153	2. S. Mushtaq (UP, Lahore)	CBGP-169	
3. F. Bakhtawar (UP, Lahore)	CBGP-154	3. M. Khalid (WUAIK, Bagh)	CBGP-170	
4. I. Babar (GCU, Hyderabad)	CBGP-155	4. Madeeha (US, Swat)	CBGP-171	
5. R. M. Tariq, (UK, Karachi)	CBGP-156	5. F. Channa (US, Jamshoro)	CBGP-172	
6. R. Shereen (QU, Islamabad)	CBGP-157	6. A.Z. Jamshoro (UVAS, Lahore)	CBGP-173	
7. A. Rafique (QSS-AJK, Muzaffarabad)	CBGP-158	7. S. Mumtaz (WUAIK, Bagh)	CBGP-174	
8. U.A. Awan (NUMS, Islamabad)	CBGP-159	8. A. Bibi (WU, Multan)	CBGP-175	
9. M. Awais (US, Sargodha)	CBGP-160	9. S. Shahnawaz (WU, Multan)	CBGP-176	
10. M.I. Khan (QU, Islamabad)	CBGP-161	10. D. Asghar (WU, Multan)	CBGP-177	
11. S. Khan (QU, Islamabad)	CBGP-162	11. Q. Ain (WU, Multan)	CBGP-178	
12. T. Ahmed (QU, Islamabad)	CBGP-163	12. S. Miraj (WU, Multan)	CBGP-179	
13. K. Zubaida (US, Jamshoro)	CBGP-164	13. S. Altaf (WU, Multan)	CBGP-180	
14. M.K. Ali (UP, Rawalakot)	CBGP-165	14. A. Rabbani (WU, Multan)	CBGP-181	
15. S. Khurshid (UP, Rawalakot)	CBGP-166	15. M. Fayyaz (GCU, Lahore)	CBGP-182	
16. S. Zulfiqar (UP, Lahore)	CBGP-167	16. G.M.A. Ghani (DG Khan)	CBGP-183	
02:00 PM LUNCH BREAK (ZUHAR)				

03:00 PM CONCLUDING CEREMONY	
03:00 PM	Recitation
03:05 PM	Congress Report by President, ZSP
03:15 PM	Award Ceremony
03:45 PM	Concluding Remarks by the Chief Guest
03:55 PM	Vote of Thanks
04:00 PM REFRESHMENT	

MEMBERS OF THE CONGRESS

BAHAWALPUR

Altaf, M. (Dr.)
 Batool, S.
 Chaudhary, M.
 Farooq, U. (Dr.)
 Iqbal, S.
 Irfan, M.
 Khalil, S. (Dr.)
 Khan, J.M.
 Kumar, S. (Dr.)
 Lashari, M.H. (Dr.)
 Munawar, A.
 Naz, S. (Dr.)
 Rameez, M.A.
 Sharif, M.
 Tariq, S.
 Waseem, M.
 Younus, M.
 Zain-Ul-Abedin, M.

BUNER, KPK

Ahmad, K.
 Alam, I.
 Ali, J.
 Khan, K.A.
 Rehman, H.
 Saeed, K.

CHARSADDA

Ullah, R. (Dr.)

CHENAB NAGER

Ashfaq, N.
 Jalal, A.
 Khabeer, A.
 Manan, A.
 Manan, R.
 Naeem, N.
 Naseem, A.
 Nayab, T.
 Samin, D. (Dr.)
 Shafiq, A.
 Shakeel, K.
 Siddiqa, K.
 Tahir, D.S. (Dr.)
 Tahir, S.S.

CHINA

Mahmood, S.U.

FAISALABAD

Akbar, A.
 Awan, M.H.
 Azmat, R.
 Bashir, R.
 Batool, T.
 Bibi, I.
 Ehsan, N. (Dr.)
 Ghafoor, N.
 Hayat, M.F.
 Hira, H.
 Ijaz, M.U. (Dr.)
 Khalil, M.
 Khan, S.M.D.
 Maqbool, F.
 Mustfa, W.
 Nadeem, S. (Dr.)
 Salar, M.Z.

HARIPUR, KPK

Zaman, M.

HYDERABAD

Memon, S.A.
 Shaikh, A.M. (Dr.)
 Soomro, B. (Dr.)

ISLAMABAD

Abbas, M.
 Ahmed, I. (Dr.)
 Ali, S.
 Ali, S.G.
 Ali, Z.
 Falak, A.
 Hussain, M. (Dr.)
 Khan, A.A.
 Mehtab, M.
 Mirza, Z.B. (Late) (Dr.)
 Murtaza, T.
 Qasim, M. (Dr.)
 Razzaq, A. (Dr.)
 Saeed, M. (Dr.)
 Sehar, U.
 Shereen, R.
 Yaqoob, N. (Dr.)

JAMSHORO

Abbasi, F.
 Al-Hussain, F.
 Ali, M.

Anum, F.
 Babar, A.A.
 Bhanger, N.
 Bibi, M.
 Bozdar, M.I.
 Bughio, B.A. (Dr.)
 Chandio, A.
 Das, J.
 Dayo, M.S.
 Jaffery, S.L.
 Jakhani, M.A.
 Jakhani, M.M.
 Jamil, M.
 Khattak, N.G.
 Kumari, V.
 Laghari, M.Y. (Dr.)
 Mahar, B.
 Memon, S.P.
 Paras, M.
 Sanam, S.
 Shah, M.A. (Dr.)
 Shah, R.
 Sheikh, M.
 Soomro, A.N.
 Soomro, F.D.
 Soomro, N.
 Soomro, S.
 Soomro, Z.H.
 Sultana, R. (Dr.)
 Talpur, S.A.

KARACHI

Ahmad, N. (Dr.)
 Ali, I. (Dr.)
 Burhan, Z.N. (Dr.)
 Farman, M.
 Fatima, A.
 Hassan, A.
 Jabeen, H. (Dr.)
 Jafri, S.I.H. (Dr.)
 Kazmi, M.A. (Dr.)
 Khan, A. (Dr.)
 Khwaja, S. (Dr.)
 Majeed, S. (Dr.)
 Mushaiyada Mairaj (Dr.)
 Narejo, N.T. (Dr.)
 Nisa, N. (Dr.)
 Qureshi, A.
 Rasheed, M. (Dr.)
 Riaz, S. (Dr.)

Saher, N. (Dr.)
 Shafique, S. (Dr.)
 Shah, S.M.A.
 Shaikh, W.
 Shoaib, N. (Dr.)
 Tahir, R. (Dr.)
 Umer, A.
 Urooj, S.

KHAIRPUR, SINDH

Fatima, T.
 Sajjad, H.

KOTLI, AZAD KASHMIR

Ahmed, M.
 Akrim, F. (Dr.)
 Akrim, F. (Dr.)
 Anwaar, A.
 Batool, K.
 Bibi, K.
 Bibi, S.
 Fatima, F.
 Fatima, I.
 Ghulam, A.
 Habib, U.
 Hammad-Bin-Shoukat
 Hussain, A.
 Hussain, A. (Dr.)
 Hussain, A. (Dr.)
 Ikhlaq, M.W.
 Ikhlaq, M.W.
 Iqbal, N.P.
 Irm, M. (Dr.)
 Israr, A.
 Jabbar, A.
 Kosar, M.
 Kousar, S.
 Laiqat, H.
 Mansoor, M.
 Maroof, F.
 Maroof, J.
 Matloob, D.K.
 Miran, F.
 Muneer, S.
 Muneer, T.
 Mushtaq, A.
 Nisar, S.
 Qasim, S.
 Qayyum, M.T.
 Qayyum, T.
 Rehman, Z.
 Rukhsar, M.Z.
 Sabir, I.

Sadiq, T.
 Saghir, G.
 Saghir, H.
 Shabbir, Z.
 Shoukat, H.B.
 Tareef, S.
 Yaqoob, F.
 Zafar, D.
 Zahid, I.

LAHORE

Adil, F.
 Ahmad, R.
 Ahmad, U.
 Ahmad, U.
 Ahmed, K.
 Ali, M.A. (Dr.)
 Ali, S.
 Ali, S. (Dr.)
 Ali, Z. (Dr.)
 Amin, H.
 Aslam, M.
 Bibi, Z.
 Bokhari, S.S. (Dr.)
 Chughtai, A.L.
 Durrani, A.Z. (Dr.)
 Fatima, S.N.
 Habib, A.
 Hamid, M.
 Hassan, A.
 Hassan, M.S.
 Ijaz, M. (Dr.)
 Irshad, S. (Dr.)
 Jamil, U.
 Javed, M.U.
 Khan, A.S.
 Khan, H.A.A. (Dr.)
 Khan, M. (Dr.)
 Khan, M. (Dr.)
 Malik, K.
 Manzoor, R.
 Manzoor, T.
 Muneer, B. (Dr.)
 Naveed, M.
 Qazi, J.I. (Dr.)
 Rabbani, G.
 Rafiq, M.
 Rahman, A.
 Sabahat, B.
 Safdar, A.
 Saqib, Z.A. (Dr.)
 Shabbir, S.
 Shakoori, A.R. (Dr.)

Shakoori, F.R. (Dr.)
 Sharif, G.S.
 Tanveer, M.
 Ullah, A.
 Yasmeen, R. (Dr.)
 Younas, S.
 Zulfiqar, S. (Dr.)

MALAKAND, KPK

Khan, W. (Dr.)

MANSEHRA

Arshad, S.M.
 Ghayyur, S. (Dr.)
 Naz, W.
 Ullah, Z.

MARDAN

Khan, H.A.
 Khan, M.
 Saood, D.

MULTAN

Aftab, R.
 Afzal, B.
 Ajmal, I.
 Ali, A.
 Altaf, S.
 Asghar, D.
 Ashraf, I.
 Bashir, M.
 Batool, H.
 Bibi, A.
 Bibi, A. (Dr.)
 Bibi, S.
 Bibi, Z.
 Eman, E.
 Fareed, I.
 Fatima, M.
 Ghaffar, U.
 Gulzar, A.
 Hameed, A. (Dr.)
 Hassan, N.
 Iqbal, R. (Dr.)
 Jabeen, S.
 Kausar, N. (Dr.)
 Khan, B.
 Latif, M.
 Liaqat, N.
 Malik, F.
 Maryam, A.
 Naheed, N.
 Naz, F.

Naz, M.
 Nazeer, T.
 Nimra
 Noor, M.
 Pervez, A.
 Qurat-ul-Ain
 Rabbani, A.
 Riaz, I.
 Saeed, A.
 Samad, Y.
 Shabbir, H.
 Shahnawaz, S. (Dr.)
 Shoukat, H.
 Talib, S.
 Um-e-Hani

MUZAFFARABAD, AJK

Ali, S.
 Ishfaq, F.
 Komal, R.
 Nazli, F.

NANKANA SAHIB

Afzal, M. (Dr.)

NAUSHARO FEROZE, SINDH

Channa. F. (Dr.)

OKARA

Wajid, M. (Dr.)

PATTOKI

Ali, S. (Dr.)
 Hassan, A.
 Munir, M.

PESHAWAR

Hassan, Z. (Dr.)
 Khan, G.Z. (Dr.)
 Salman, M.

QUETTA

Karim, A. (Dr.)

RAWALAKOT, AJK

Afzal, T.

RAWALPINDI

Ammar, M.

Ayesha, S.Q.
 Aziz, N.
 Bano, S.
 Batool, R.
 Bibi, N.
 Farid, Z.
 Fatima, A.
 Fatima, L.
 Gill, U.
 Hussain, S.A.
 Ibrahim, A.
 Javed, M.
 Kainat, N.
 Kashaf, M.
 Mahmood, T. (Dr.)
 Malik, L.A.
 Mushtaq, B.
 Muzafar, G.
 Naseem, N.
 Naz, G.
 Naz, S.
 Rais, M. (Dr.)
 Rais, M. (Dr.)
 Saher, S.
 Sardar, N.
 Shahid, V.
 Sheraz, A.
 Waqar, U.
 Yaseen, A.
 Zulfiqar, N.

SAKRAND, SINDH

Jalbani, S. (Dr.)
 Panhwer, S.N. (Dr.)

SARGODHA

Awais, M.
 Majeed, M.Z. (Dr.)

SWABI, KPK

Abdullah
 Ahmad, M.
 Ali, N.
 Ali, S.
 Ali, Z.
 Aziz, F.
 Hasan, B.
 Hussain, S.

Hussain, S.
 Khan, A.
 Khan, A.T.
 Khan, M.A.
 Khan, M.S. (Dr.)
 Maghaz, M.
 Mehran, M.
 Raziq, F.
 Rehman, A.
 Saeed, A.
 Shah, S.
 Suleman (Dr.)
 Tahir, F.
 Ullah, M.K.
 Umer, M.
 Usman, A.
 Usman, M.
 Waheed, A.
 Zaman, A.

SWAT

Riaz, M. (Dr.)
 Wahab, A. (Dr.)

TANDOJAM

Bhatti, A.R.
 Dhiloo, K.H. (Dr.)
 Ghandahi, M.A.
 Gil, A.A.
 Jafery, S.M.
 Khatri, I.
 Lakho, G.M. (Dr.)
 Lashari, A.
 Shaikh, H.M.
 Soomro, A.H.
 Soomro, A.P.

TURBAT, BALOCHISTAN

Kakar, N. (Dr.)

UTHAL, BALOCHISTAN

Bano, A. (Dr.)
 Habibullah
 Siddique, S. (Dr.)



Evaluation of the Health Risks Associated with the Seafood Quality of Pakistani Exports

Saima Majeed^{1*}, Tayyaba Asif², Aisha Qureshi³, Ayesha Umer³ and Naveed Ahmad³

¹Department of Maritime Sciences, Bahria University Karachi, Karachi 75260 Pakistan

²Department of Applied Sciences, Hamdard University, Karachi, Pakistan

³Aquatic Diagnostics and Research Centre, Bahria University Karachi, Karachi 75260 Pakistan

ABSTRACT

The quality of seafood is one of the significant concerns to food processors, buyers, and general wellbeing specialists. This investigation attempted to research the universality of microbial quality and antibiotic chloramphenicol residue in export-oriented frozen and fresh seafood to verify and control contamination for public health safety and international trade. A total of 32 frozen and fresh seafood samples were randomly collected for microbiological analysis including crabs, shrimps, and tin-packed tuna fishes, collected from 10 diverse processing plants situated at the Karachi fish harbor, Pakistan. The examination contains the assurance of total viable aerobic count (TVAC) by the standard plate count method estimated under 5×10^5 colony forming unit /g, *E. coli* count was found below 5 MPN/g, yeast and mold count were found under 1000 colony forming unit /g, all analyzed seafood test results were observed under the average permissible limit. Explicit fish pathogens such as *Salmonella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Listeria monocytogene*, and *Staphylococcus aureus* were likewise inspected yet discovered missing in all the samples under examination. All the frozen seafood samples were judged as protected food from a microbiological perspective and methods are satisfactory limit quantified by the international commission of microbiological specification for food.

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Authors' Contribution

SM wrote the manuscript. TA and AQ edited the manuscript and tested microbiology. AU tested biochemistry. NA helped in over improvement of the study and manuscript.

Key words

Seafood quality, Microbial analysis, Food assessment, Food standards, Food safety

INTRODUCTION

Fish and fishery items are one of the indispensable food segments from olden times, it is viewed as a great source of income for the coastline communities, international traders, and trade workers from various nations around the world (Salik *et al.*, 2015; Rashed *et al.*, 2013). Pakistan's fisheries face difficulties contribute discreetly to economic improvement and social advancement. However global demand for protein-rich fisheries products is rising, providing chances for ocean-facing countries such as Pakistan (Yousuf *et al.*, 2008). The trade of frozen fish, dry fish, salted and dehydrated fish is growing gradually from Pakistan to an extensive range of international markets including Japan, Vietnam, China, Taiwan, Hong Kong, Indonesia, Egypt, Saudi Arabia, UAE, Malaysia, Sri Lanka,

UK, Bahrain, Qatar, Singapore, Kuwait Thailand, South Korea, and Bangladesh, etc. Although there are 55 seafood processing plants in Pakistan. Therefore, preserving the quality of frozen fish and fisheries is important for its acceptance in global markets as well as for the well-being of consumers (Khan and Abbas, 2023).

Seafood is an essential component of a healthy and balanced diet because of its rich nutritional content. The World Health Organization emphasized its addition to diet as it helps to prevent different diseases. In Europe alone, more than 70 % of non-communicable health-related issues are associated with food that is consumed regularly. Consumption of seafood once or twice a week is recommended for maximum health benefits to human health (Liu and Ralston, 2021).

Pakistan is home to different ethnic and cultural societies along the people here consume different processed and non-processed foods. For the proper quality control checks different food safety and food quality standards are implemented in the industry to reduce food fraud and provide better food security. Pakistan Standards and Quality Control Authority (PSQCA) and Pakistan Council of Scientific and Industrial Research (PCSIR) are working in these domains and provide food consultation, testing, and certification services. These organizations are working under government and provincial offices are working to

* Corresponding author: saima.bukc@bahria.edu.pk
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regulate and enforce quality standards, ensuring safe food production and distribution, and correct labeling. These objectives could be achieved through inspections, testing, and penalties in case of non-conformity (Asgar *et al.*, 2023). Food security is a perplexing subject, where fish and fishery items are commonly viewed as hazardous commodities concerning their pathogen contents, regular pollutants, and other possible impurities and adulterants (De-Almeida *et al.*, 2019; Adebayo-Tayo *et al.*, 2012). Fish and shellfish consumption might cause sickness because of contamination or inebriation, some of these illnesses have been explicitly connected with microbes, which are impervious to antibiotics. Proper preservation and storage during the processing of seafood are also important, it has been assessed that more than 80 million cases annually of fish-borne infirmities on antibiotic resistance in the USA, and the expense of these illnesses are in billions of dollars each year (Gnanambal *et al.*, 2005). Foodborne pathogens, such as *Vibrio* spp. and *Salmonella* spp. is normally present in oceanic conditions and might cause contamination (Hernández-Cortez *et al.*, 2017; Osunla and Okoh, 2017). The third-highest number of shellfish-associated illnesses is caused by *Vibrio cholerae*, the infections result in septicemia and mild gastroenteritis while the occurrence of *Salmonella* contamination as a result of seafood consumption is still low (Novoslavskij *et al.*, 2016). Microbes may contaminate the fish from outside during off-hand management, packing, and cutting, the major external sources of bacterial loads are crushed ice and salt. A portion of the original microbial populace present on the surface of shrimps and crabs known as Specific Spoilage Organisms (SSO) causes the decay by producing metabolites that are liable for off-flavors and the organoleptic rejection of the item. Such conditions are resolved generally by the temperature and environmental situations during storage (Gram and Huss, 1996).

Seafood is highly liable to both microbiological and synthetic deterioration because of its high-water content, neutral pH, relatively huge amounts of free amino acids, and naturalistic occurrence of autolytic enzymes (Saloko *et al.*, 2014). The most serious seafood safety issues that result in contaminated products are related to microbial and particularly bacterial pathogens (Jeyasekaran *et al.*, 2006). But it can also be the result of cross-contamination due to the inappropriate handling and storage of fish (Bonnell, 2012; Huss, 1994). HACCP principles are being implemented, and various educational programs have been developed for food handlers and the consumer. Microbiological methods are utilized to gauge bacterial number, fish freshness, and hygiene, and to evaluate the conceivable presence of microbial organisms of public health concern (Cruz-Romero *et al.*, 2008).

The fisheries sector in Pakistan has assumed a considerable part in the reduction of destitution and achievement of food security in the last few years, the fare of fish and fishery products from Pakistan has grown remarkably to earn foreign exchange. As indicated by the centre for disease prevention and control (CDC), from 1973 to 2006, a variety of bacteria, viruses, and parasites were responsible for seafood contamination. The defilement might also be caused by the icing and water applied for washing (Fleming *et al.*, 2018). So, proper preservation of the accurate value of the products is observed as vital for attaining the desired achievement in the international market of seafood products. The most common seafood exported from Pakistan is brown prawn (*Metapenaeus monoseros*), flower prawn (*Penaeus semisulcatus*), pink shrimp (*Metapenaeus dobsoni*), tiger prawn (*Penaeus monodon*), white prawn (*Penaeus indicus*) and karikadi (*Parapenaeopsis styliifera*), mud crab (*Scylla serrata*), blue crab (*Portunus pelagius*), ribbon fish (*L. savala*), mackerel (*Rastrelliger kanagurta*), tuna (*Thunnus albacares*).

The seafood industry of Pakistan costs around \$1.2 billion among those exports worth \$370 million per annum. It is estimated that 0.8 million people and their families rely on this industry still the major threats faced by the sector include overfishing, poor quality control of catch, nets used for fishing, and less storage and preservation facilities in the trawler as well as processing plants. Overfishing impacts badly on the population of fish as random fishing reduces the population while on the other side, poor quality control and fewer storage facilities damage the catch and increase the waste (Ilyas *et al.*, 2022).

MATERIALS AND METHODS

Preparation of media and glassware

All the chemicals and glassware used in the current study are of analytical grade and were purchased from Sigma Aldrich, Daejung, Merck, and BDH. The glassware was washed and rinsed with distilled water and was further dried and sterilized in a hot air oven at 160°C for 1 h as per the protocol (Adibe and Eze, 2004). Commercial dehydrated culture media were purchased from Oxoid and BDH and prepared as per the instructions of the manufacturer and were further sterilized in an autoclave at 121°C for 15 min. The wire loop was sterilized before using each time on Bacti-cinerator sterilizer.

Study area and sample collection

In the current study, different seafood samples were collected from 10 different processing plants located at nearby locations in Karachi Fish Harbor, Karachi, Pakistan. All samples were packed in isolated polystyrene

boxes with ice and shifted to the laboratory within 1 h. All the samples were in a sterile container under aseptic conditions and kept on ice. These samples include blue crabs (*Portunus pelagicus*), mud crab (*Scylla serrata*), giant tiger prawn (*Penaeus monodon*), white prawn (*Penaeus indicus*), tongue sole fish (*Cynoglossus broadhursti*), cuttle fish (*Sepia officinalis*), ribbon fish (*Lepturacanthus savala*), mackerel (*Rastrelliger kanagurta*), tuna (*Thunnus albacares*). The collected samples were permitted to defrost at 4°C. The experiments were performed in triplicate for their microbiological flora analysis including total viable aerobic count (TVAC), total yeast and mold count (TYMC), and enumeration of *E. coli*. detection of *Salmonella* Spp., *Vibrio cholera*, *Vibrio parahaemolytica*, *Listeria monocytogenes*, and *Staphylococcus aureus*.

Sample processing

The sample was processed for microbiological testing by the method (Pomeranz, 2013). Ten gram raw and uncooked samples of crab meat and shrimp meat and 10 g cooked samples of fish were homogenized in 90 ml of phosphate buffered saline (1:10 dilution). These samples were further serially diluted in phosphate buffer saline up to 10⁻⁶.

The sterile sample was aseptically treated and minced properly followed by the transfer of 1 ml sample into 9 ml sterile saline and mixed properly. Serial dilution was prepared by transferring 1 ml sample from each tube to the next tube till 10⁻⁶.

Assessment of bacterial contents and enumeration of total aerobic microorganisms and yeast molds

The bacterial load of frozen food was assessed by the protocol of Sielaff *et al.* (2019). For the enumeration of total aerobic bacteria and total yeast and mold count, 1 ml of each sample was inoculated for total plate count agar (TPCA) and sabouraud dextrose agar (SDA) through pour plate technique. For total aerobic count, TPCA media plates were incubated at 37 °C for 2 days, and for the assessment of fungus, SDA media plates were incubated at 25°C for 5 days in the dark.

The calculation procedure for the total bacterial and fungal count was mentioned by (Rahimi *et al.*, 2019). The total colonies were estimated and shown in colony-forming units per gram (CFU g⁻¹).

$$\frac{\text{Total number of colonies} \times \text{total dilution factor}}{\text{Volume of cultured plated in ml}}$$

Isolation and identification of pathogenic strains

For the isolation of *Coliforms*, *Salmonella* spp., *Vibrio* spp., and *Staphylococcus* spp., samples were pre-enriched in a pre-enrichment broth of Butterfield's phosphate-

buffered water and lactose broth, respectively.

The 50 g sample was inoculated in 450 ml of buffer water and 25 g of sample in 225 ml of lactose broth and blended well, respectively. The samples were further incubated at 35 ± 2 °C for 6 to 8 h and 24 ± 2 h for *Vibrio* spp., *Salmonella* spp., *Staphylococcus* spp., and *Coliforms*, respectively. The 1.0 ml of sample for the isolation of *Salmonella* spp., was then further transferred into 10, 10 ml of pre-incubated RVS and T.T broth at 24 ± 2 h and 42 ± 2 °C, respectively. The 100 µl samples were streaked using a sterile glass spreader on pre-incubated plates of thiosulphate citrate bile salt sucrose (TCBS) agar, xylose lysine deoxycholate (XLD), mannitol salt agar (MSA), and MacConkey agar, EMB agar for *Vibrio* spp., *Salmonella* spp., *Staphylococcus* spp., and *Coliforms*, respectively. The plates were incubated at 37 °C for 24 to 48 h for *Escherichia coli* while coliform plates were incubated at 44.5°C for 24 h. While for the isolation of other pathogenic strains, plates were incubated at 37 °C overnight.

All isolates were purified through the four-plate streak method of streaking further purified cultures were sub-cultured and gram-staining was performed for confirmation. Identification of bacterial and fungal isolated strains was performed as described by El-Sayed *et al.* (2015), Ibrahim *et al.* (2014) and Eze *et al.* (2011).

Different biochemical tests were performed as recommended by bergey's manual of systematic bacteriology (Vos *et al.*, 2011) for identification of bacteria.

Detection of Chloramphenicol residues in sea food samples

A total of 20 g of tissue samples were isolated from each processing plant sample and then transferred to the University of Karachi for analysis of chloramphenicol through HPLC (Akter-Mou *et al.*, 2021). Ten grams of smashed seafood meat samples were mixed with equal volume of PBS buffer (pH = 6.88), shaken vigorously for 10 min and centrifuged for 5 min at 5500 rpm to collect the upper layer. Ethyl acetate (10 ml) was added, and centrifuged again. The extract was reduced with a nitrogen stream in a water bath at 45–50 °C. Two ml of methanol. 10 ml of 4% NaCl solution and 20 mL of hexane were added. The mixture was shaken for about 30 sec. The top layer of hexane was removed by aspiration and discarded. The aquens layer was analyzed by HPLC.

RESULTS AND DISCUSSION

Bacterial development in frozen fish is one of the primary sources of food deterioration or contamination of fish. Thus, the microbial investigation of the frozen fish sample including processing water and ice works as a marker of fish quality assurance (Jacquin *et al.*, 2020;

Zeitoun and Mehana, 2014; Fisher *et al.*, 2013). The main sign of spoilage as an off-flavor and unsavory taste of fish is a result of fungal contamination and may pose a public health risk and financial losses (Lund *et al.*, 2000). The fungal infection of fish could be credited to improper hygiene during the catching, handling, manufacturing, storage, transportation, and marketing of fish. These findings were view reported (El-Ahl and Rasha, 2010). Many species of fungi may cause pulmonary infections, urinary tract infections, arthritis, osteomyelitis, dermatitis, endocarditis, meningitis, and eye infections (Hernández-Cortez *et al.*, 2017). Fish caught in a polluted area may also raise the level of contamination (Ylitalo *et al.*, 2012)

The current examination revealed that the entirety of the fish, shrimps, and crabs alongside the materials satisfy the guideline levels proposed by ICMSF which showed that aseptic and legitimate sterile conditions were kept up appropriately all through all means, for example, catching, landing, transportation, processing, management, and preservation.

The TVAC maximum microbiological limit for seafood is 5×10^5 cfu/g (Boyd and Tucker, 2012). The studied samples TVAC within the limit of 1.8×10^4 cfu/g which was lower than the maximum satisfactory limit as shown as in Figure 1A. Thus, all the samples meet the acceptable limit of TVAC specified by ICMSF which confirms the good quality of the frozen seafood. It also indicates that processing plants maintain a good temperature maintenance system which protects the seafood from spoilage.

Total yeast and mold count (TYMC) has a limit of 1×10^3 . Yeast and mold were detected in the study at less than 1000 CFU/g, indicating that all seafood samples were within the permissible range for health and safety standards. This also suggests that the samples were taken from clean water and that food handlers and processors kept aseptic conditions throughout the processing (Fig. 1B).

E. coli in seafood is allowed in 1 MPN/g to 4 MPN/g. In the present study, *E. coli* loads of all the seafood samples were within the recommended limits the *E. coli* ranged from 1 MPN/g to 4 MPN/g showing that all the samples were received from pollution-free water which is probably a brackish water sample. The food processors and the controllers kept aseptic conditions and better temperature checks during overall processing as shown in Figure 1C.

In the 1930s the use of *E. coli* has been first reported as a sanitary indicator for fish samples (Gephart *et al.*, 2017). *E. coli* is normally related to seafood contamination in the tropics. The lower number of *E. coli* can be valuable for identifying the accuracy of safety procedures during processing and handling (Hassan *et al.*, 2007). There is a

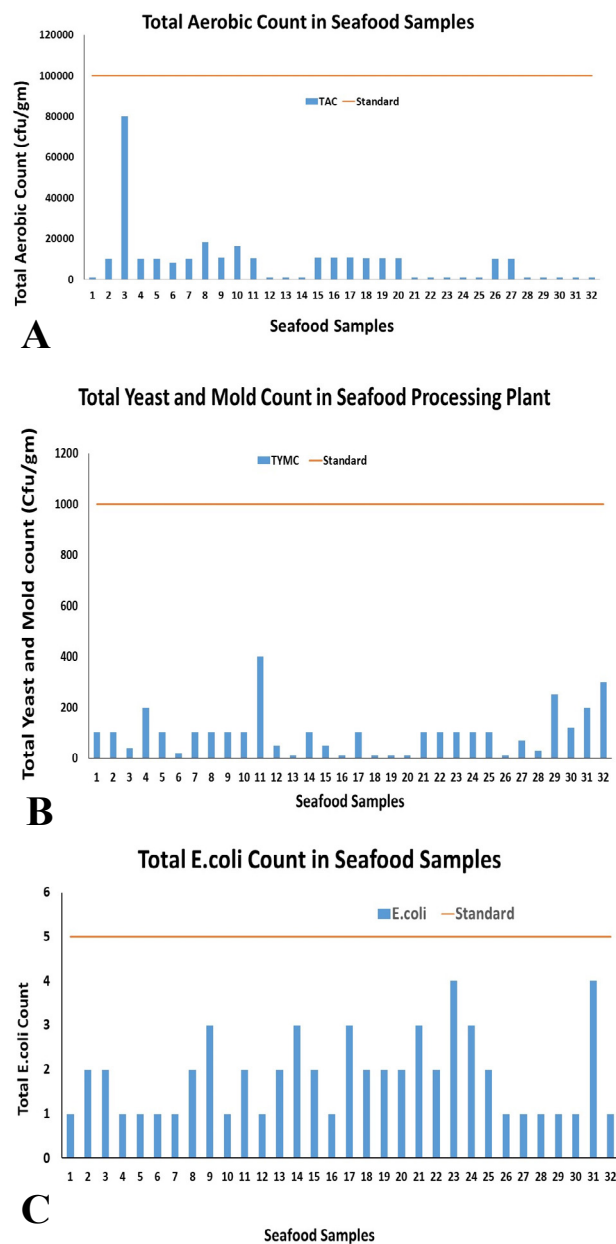


Fig. 1. Total aerobic count (cfu/gm) (A), total yeast and mold count (cfu/gm) (B) and *E. coli* count (MPN/g) (C) of seafood samples collected from processing industries of Karachi.

complete absence of other pathogenic species in seafood-based Products. Pathogenic load absence of all the seafood samples suggested and represented that all the samples were received from pollution-free water, and the hygienic condition of the seafood was maintained after capturing. It also indicated that the seafood processors and the controllers kept aseptic conditions throughout the processing which

ultimately resulted in healthy and free of contamination. Seafood microbial contamination risk associated with human disease in fresh seafood is not low. Storage and transportation in ice (0 °C) or freezing is the most effective prevalence of bacterial loads, cooking properly immediately before consumption is also an effective way of reducing hazards of fresh fish-borne disease. The most commonly recognized microbiological criteria for frozen raw fish are those set for aerobic plate counts (APC) at 25 °C, and *E. coli* proposed by the International Commission on Microbiological Specifications for Foods (ICMSF).

Regarding other pathogens none of the 32 seafood samples collected from Karachi fish harbor seafood processing industries had any of *Salmonella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Listeria monocytogene*, and *Staphylococcus aureus*. The microbiological quality of seafood in warm water areas is uncertain where fish are to be consumed raw and an increase of APC to levels in excess is usually indicative of unsatisfactory refrigeration, it may be necessary to test for pathogens like *Salmonella* and *Vibrio* spp. Proper processing, storage, and handling procedures of fish should be employed. Satisfactory dealing procedures before the consumption of fish are essential. Fish that physically indicate spoilage should not be retailed in the markets. In addition, manufacturing practices should be monitored by the trade authority to abate the risk of seafood products. An understanding of the etiologic agents and mechanisms of contamination that are agreeable to control is thus needed for the inhibition of seafood-associated infection outbreaks. There is a need to practice surveillance systems with pathogen-specific techniques to elude any future outbreaks.

Centers for disease prevention and control (CDC) revealed that the presence of profoundly pathogenic microorganisms i.e., *Salmonella* identified by the consumption of frozen raw tuna fish in 11 states of the USA and caused 18 outbreaks, 374 ailments, and 28 hospitalizations. The contamination was portrayed by diarrhea, fever, and intestinal cramps (Iwamoto *et al.*, 2010). Pathogenic *Vibrio* causes hazardous foodborne diseases and is spoken to as a significant microbial representative of sporadic and epidemic human contamination (Espineira *et al.*, 2010). Fish consumption isn't a hazard because of ocean pollution by anthropocentric exercises and ecological factors (Hassan *et al.*, 2018). The investigation of the microbial variety of fish is currently required as there is very little data available on the effect of water pollutants on fish life and its effect on human health since we need to handle new difficulties that will influence human well-being by consumption of contaminated fish, climate change and antimicrobial resistance. In this regard, few reports were going through with the emphasis

on water quality and its effect on the health, and mental wellbeing of consumers (Marshall *et al.*, 2019). The foundation of astute techniques toward fish security and quality affirmation inside the setting of such difficulties will add to sustainable development goals and one health.

However, in Pakistan, food-borne ailment from fresh or frozen fish consumption has not been reported and information on this issue is as yet deficient. The majority of the isolated genera are heat-labile and satisfactory cooking will inactivate it (Samarasekera *et al.*, 2017; US Food and Drug Administration, 2011). While inappropriate dealing with and cross-pollution or crude fish dietary patterns may represent a well-being peril, particularly to susceptible populaces such as the immunosuppressed i.e., youngsters and older individuals.

High-pressure processing (HPP) has much applicability in seafood processing plants as it eliminates most seafood pathogens, such as *Vibrio* and *Listeria* spp., and slows down the growth of microorganisms responsible for food spoilage. As the water used in the system is cold water as a pressure medium, the sensory and nutritional properties of the seafood remain constant resulting in the development of clean-label seafood products. The same technology also helped to recover the maximum amount of edible meat from oysters, lobsters, crabs, mussels, clams, and scallops. HPP of seafood helps to confine the organoleptic and functional properties of seafood for an extended period, especially during refrigeration. It also helps to maintain a balance between safety, quality, processing efficiency, and regulatory compliance (Roobab *et al.*, 2022).

In the present study chloramphenicol (CAP) residues of all the seafood samples were within the recommended limits of the European law ranging from 0 ppb to 0.1 ppb showing that all the samples were received from an antibiotics-free environment (Fig. 2).

The existence of chloramphenicol in food products is a violation of European law and many other countries, as it is related solely to the illegitimate practice of chloramphenicol in food production, very low residue levels pose a risk to consumer health, 0.3 ppb as a minimum required performance limit (MRPL) (Byrne, 2003).

This research revealed that the seafood under scrutiny from various processing plants in Karachi, Pakistan was of acceptable quality with low microbial load, and CAP residue might be because of a reduced amount of seawater pollution. So, can be concluded that the investigated frozen fishes, shrimps, and crabs were prepared with appropriately treated microorganism-free water and ice and kept up under great stockpiling conditions. Thus, these frozen fishes, shrimps, and crabs were qualified enough for trade additionally as human utilization according to a

bacteriological perspective.

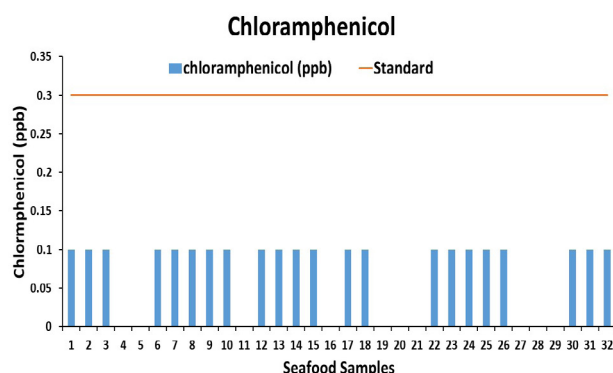


Fig. 2. Chloramphenicol detection in seafood samples collected from processing industries of Karachi.

CONCLUSION

In conclusion, the microbiological quality in terms of microbial growth and antibiotic chloramphenicol CAP residue in exported frozen seafood from Pakistan was within the recommended limit of ICMSF. Hence, the examined frozen fishes were capable enough for export as well as for human consumption from the microbial and CAP residue point of view. The presence of viruses, parasites, viable but non-culturable (VBNC) state of the pathogenic bacteria, and biochemical parameters such as histamine risk might be a problem with frozen fish products which is the limitations of this study. It is revealed from the present finding that the seafood exported from Pakistan is well managed (Capturing, handling, freezing, and packing) according to the standard. To observe more rigorous quality standards of the trading countries, these quality parameters must be taken into consideration.

DECLARATIONS

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Ethical statement

All applicable institutional, national and international guidelines for the care and use of animals were followed.

Statement of conflict of interest

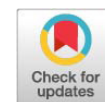
The authors have declared no conflict of interest.

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Optimizing Biofuel Yields from *Spirogyra* Microalgae: The Role of Extraction Techniques and Environmental Factors

Muhammad Waqas^{1*}, Razia Bashir¹, Haroon Ali², Arslan Shahzad¹ and Tehmina Saddique³

¹Department of Zoology, Division of Science and Technology, University of Education, Lahore, Pakistan

²Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Pakistan

³Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan

ABSTRACT

The increasing challenges of global warming, fossil fuel depletion and rising petroleum prices underscore the urgent need for renewable, eco-friendly, and sustainable biofuels. Fossil fuels, which currently meet over 80-85% of global energy demands, are rapidly depleting, with Pakistan heavily dependent on them for nearly 88% of its energy needs. This study aimed to evaluate the bioenergy potential of *Spirogyra* microalgae for biodiesel production, offering a sustainable solution to fossil fuel dependence and environmental degradation. Lipids from microalgae *Spirogyra* were extracted using n-Hexane as a solvent by both the hot plate magnetic stirrer method and simple solvent extraction method. The effects of habitat nature, n-Hexane to algal biomass ratio, algal biomass particle size, and contact time on the percentage yield of extracted lipids were studied and analyzed. The study showed that algae from agricultural runoff had the highest lipid extraction efficiency at 3.9%, while industrial effluent samples had the lowest at 3.56%. The hot plate magnetic stirrer method achieved 1.38% efficiency. An algae to n-Hexane ratio of 1:3 and smaller particle sizes yielded higher lipid extractions. The findings suggest that the Hot Plate Magnetic Stirrer method is more effective in lipid extraction from *Spirogyra* microalgae for biodiesel production. This underscores the importance of optimizing extraction methods for maximum lipid yield, contributing to the advancement of sustainable energy production. This study highlights the importance of nature-inspired solutions in addressing the complex challenges of energy security and environmental degradation. By harnessing the potential of *Spirogyra* microalgae for biodiesel production, it offers a pathway towards sustainable energy production and environmental conservation.

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Authors' Contribution

MW conducted the core research, formatted the manuscript, analysed and interpreted data, and presented the work. RB conceived the research idea, supervised the study, and revised the manuscript. HA collected samples and assisted in conducting experiments. AS contributed to sample collection. TS provided support in manuscript writing.

Key words

Biofuels, *Spirogyra* microalgae, Biodiesel production, Sustainable energy, Environmental conservation

INTRODUCTION

Global warming, running out of fossil fuels, and the rising prices of petroleum are the main issues our world is facing today (Baig *et al.*, 2018; Brennan and Owende, 2010). That is why there is a lot of focus on finding new ways to make renewable, eco-friendly and sustainable biofuels that would not harm the environment.

Fossil fuels are a key source in meeting global energy needs, over 80-85%, from heating our homes to powering our automobiles (Dale, 2023; Friedemann, 2021; Ritchie *et al.*, 2022; Keleş, 2011) especially Pakistan is heavily dependent on fossil fuels to meet nearly 88% of its energy requirements (Ali *et al.*, 2021). In 2020, the distribution of world energy use was approximately 83.1% from fossil fuels, 12.6% from renewable sources, and 4.3% from nuclear power (Masson *et al.*, 2021; Dale, 2023). It is true that the fossil fuels are still being formed, but at an entirely negligible rate compared to the rate at which we are consuming them. They will be run-off within 50 years if we continue our current usage due to their finite resources (Dale, 2023; Friedemann, 2021; Ritchie *et al.*, 2022; Mitchell and Robinson, 2021). Predicting the exact timeline for the complete depletion of fossil fuels is challenging, but it is widely acknowledged that these resources are finite and face a future of diminishing availability.

* Corresponding author: waqassiddique614@gmail.com, mtf2202199@ue.edu.pk
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The problems related to fossil fuels extend beyond depletion. The combustion of fossil fuels releases carbon dioxide, contributing significantly to global warming (UNEP, 2020) that leads to climate change (Keeling *et al.*, 1995; Houghton, 1997; Houghton *et al.*, 2001; Walther *et al.*, 2002; Mann and Jones, 2003; Karl and Trenberth, 2003; Rogelj *et al.*, 2018; Le Quéré *et al.*, 2020). This is now considered the primary threat to the future of humanity by the leading scientists (Masson *et al.*, 2021; IPCC, 2018; Ripple *et al.*, 2017, 2021). When we burn fossil fuels (coal, oil, and natural gas) to generate energy, gases are released into the atmosphere. This atmospheric pollution is leading to health problems, even fatalities (Ristinen and Kraushaas, 2006). We tried in our study to focus on the replacement of fossil fuels with renewable and sustainable energy sources because this is considered to be the most practical single pathway to climate stabilization when physical, financial, political, and environmental factors are all considered (Masson *et al.*, 2021; Dale, 2023; Heinberg and Friedly, 2016; Hickel and Kallis, 2019; IEA, 2021).

Bioenergy has received a significant attention in recent times due to the rapid growth of fuel prices, fast fossil fuel depletion, environmental deterioration caused by fossil fuels, and shifts in the global climate. It is considered a potent choice as a renewable energy source in order to reduce fossil fuel use and plays an important role in net-zero emission pathways (Seo *et al.*, 2024; Ali *et al.*, 2022). In the context of modern bioenergy, various products contribute to its diverse portfolio, including bioethanol, biodiesel, biomass energy, biogas energy, biohydrogen, biopower, biopropane, and biochar. However, among these, biodiesel and bioethanol emerge as the primary outputs (Rasool and Hamlatha, 2016). Notably, these two major bioenergy products serve as viable alternatives for traditional transportation fuels, hence are referred to as biofuels.

Within the context of bioenergy, microalgae emerge as a frontrunner due to their exceptional growth rates, high lipid content, and versatile biochemical composition (Chisti, 2007; Mata *et al.*, 2010). Algal biofuel has garnered increasing attention in recent years because algae boast higher production yields than terrestrial crops, making them a promising alternative to gasoline, jet fuel, and diesel in the transportation industry. In terms of growth and oil content, the growth rate of algae is approximately 20-30 times faster than conventional food crops and the oil content of algae is around 30-40% and this value can rise up to 85% for many species (Khan *et al.*, 2017; Ullah *et al.*, 2015). Consequently, the per hectare cultivation yield of microalgae can be boosted up to ten times higher than any oil crop. Algal biofuel has the capacity to store and accumulate substantial amounts of triacylglycerides

(TAGs), which serve as the primary feedstock for biodiesel production.

Microalgae have the innate ability to grow in all waters such as brackish, saline, waste waters, etc. (Tsai *et al.*, 2015) and can multiply their mass several times a day, yielding more oil than other plant crops (Demirbas, 2010). Due to the simple cellular structure of microalgae, they can proficiently harness solar energy. The cultivation of algae decreases the CO₂ concentration in the atmosphere by CO₂ biofixation and converting it to oxygen (Laamanen *et al.*, 2016). 183 tons of CO₂ can be captured when 100 tons of microalgal biomass is produced (Chisti, 2008; Razeghifard, 2013). Additionally, photoautotrophic microalgae can metabolize inorganic carbon dioxide through photosynthesis and absorb environmental pollutants like nitrates and phosphates (Gouveia, 2009; Veillette *et al.*, 2012). Moreover, after oil extraction the remnant algae can be used as fertilizers or as fish feed in fish and oyster farms (Ullah *et al.*, 2015).

Among the diverse array of microalgae species, *Spirogyra* stands out as a particularly promising candidate for biofuel production (biodiesel), owing to its swift growth in various aquatic environments and favorable lipid accumulation characteristics (Becker, 2007; Khan *et al.*, 2009; Dineshkumar *et al.*, 2019). *Spirogyra* microalgae possess inherent advantages over terrestrial biomass feedstocks, such as rapid biomass generation, minimal land and water requirements, and the ability to thrive in nutrient-rich wastewater streams (Hu *et al.*, 2020; Sharma *et al.*, 2021). It produces lipids, carbohydrate, and proteins that can be utilized for the production of biodiesel, or bioethanol (Khan *et al.*, 2009). Utilizing *spirogyra* microalgae for biodiesel production has the potential to significantly contribute to sustainable and environmentally friendly energy production. This approach could help alleviate the challenges associated with fossil fuel dependence and environmental degradation.

The purpose of the study was to evaluate the bioenergy potential of *Spirogyra* microalgae through lipid extraction using n-hexane as a solvent and to compare the efficacy of simple solvent extraction method and hot plate magnetic stirrer method. Effects of habitat, n-hexane to algal biomass ratio, algal biomass particle size and contact time on the percentage yield of oil extracted were studied.

MATERIALS AND METHODS

Chemicals

The laboratory experiment utilized analytical-grade chemicals including n-hexane, methanol, and ethanol without additional purification. The entire experimental procedure was carried out within the Zoology Laboratory

at the University of Education, Lahore, Pakistan.

Algae sample collection

The algae samples were collected from three different locations from different habitats to assess the relationship between lipid quantities with habitat nature. These samples were collected from University of Education, Lahore (open pond), Kasur city (rainfall water) and Gujranwala City (agricultural runoff) by Muhammad Waqas, Haroon Ali and Arslan Shahzad, respectively.

Algae identification and preparation for oil extraction

The collected samples were examined under NOVEX HOLLAND 86.025 Compound Microscope and identified as microalgae *Spirogyra*. The samples were spread under the sun for 4 days to dry them properly. The dried samples were grinded with the help of pestle and grinder and the fine powder was separated by passing it through different microsieves. The ground algae were dried in an incubator at 75 °C for 30 min. The algae powder was stored in sealed jars for later use.

Oil extraction from algae

In the simple solvent extraction method, n-hexane was mixed with algae powder in three different ratios (i.e., 1:1, 1:2 and 1:3) in a 250 ml separating funnel, thoroughly stirred for proper mixing, and left undisturbed for 24 h to allow for lipid extraction and settling of solid particles. The same procedure was repeated for all samples.

Similarly, in the hot plate magnetic stirrer method, n-hexane was mixed with algae powder in three different ratios (i.e., 1:1, 1:2 and 1:3) in a 250 ml round bottom flask, placed on a hot plate magnetic stirrer, and stirred for five h at a controlled temperature to enhance solvent penetration and mixing. The round bottom flask containing mixture was kept for 40 min after stirring for settlement of organic layers. After the extraction period, the round bottom flask was carefully opened to allow for phase separation, and the organic phase containing the dissolved algae oil was carefully transferred into pre-weighed 50 ml beakers using a glass pipette. To remove any remaining solid particles from the extracted oil, the organic phase was filtered using Whatman quantitative filter paper grade 40. The filtered algae oil was collected in pre-weighed beakers to determine the mass of the extracted oil accurately. Finally, to remove residual n-hexane from the extracted oil, the collected oil samples were subjected to evaporation in a water bath set at 30 °C, ensuring the evaporation of hexane while preserving the reliability of the extracted algae oil. All extraction procedures were performed in triplicates to ensure the reliability and reproducibility of the results, with statistical analysis conducted to compare the efficiency of the two extraction methods and validate the findings.

The lipid yield (wt. %) was then calculated by using the following equation (Arun *et al.*, 2017; Chen *et al.*, 2015).

$$\text{Extracted lipid efficiency} = \frac{\text{Mass of lipids extracted (grams)}}{\text{Total mass of algal biomass (grams)}} \times 100$$

RESULTS

Lipid extraction from algal samples by solvent extraction method

Table I described the results of lipids extraction by solvent extraction method from dried algal powder collected from different sources. The dried algal powder was mixed with n-hexane in different ratios. The results showed that the minimum lipids 0.78 g with extracted efficiency 2.6 were extracted by using algal to n-hexane in 1:1 ratio and maximum lipids 1.12 g with extracted efficiency 3.73 were extracted by using Algal to n-hexane in 1:3 ratio. The dried algal powder of sample from Kasur city was mixed with n-hexane in different ratios. The results showed that the minimum lipids 0.72 g were extracted with extracted efficiency 2.4 by using algal to n-hexane ratio 1:1 and the maximum lipids 1.07 g with extracted efficiency 3.56 were extracted with ratio 1:3. Likewise, the dried algal powder of samples from Gujranwala city were mixed with n-hexane in different ratios. The results showed that the minimum lipids 0.8 g with extracted efficiency 2.66 were extracted by using algal to n-hexane in 1:1 ratio and the maximum lipids 1.16 g with extracted efficiency 3.86 were extracted by using algal to n-hexane in 1:3 ratio.

Table I. Lipids extraction from samples collected from University of Education, Township, Kasur city and Gujranwala cities by solvent extraction method after 24 h of settling time.

Sam- ple	Algal biomass (g)	n-Hex- ane (g)	Algae to n-hexane ratio	Lipids extracted (g)	Extracted efficiency (%)
University of Education sample					
1	30	30	1	0.78	2.6
2	30	60	2	0.92	3.06
3	30	90	3	1.12	3.73
Kasur city sample					
1	30	30	1	0.72	2.4
2	30	60	2	0.89	2.96
3	30	90	3	1.07	3.56
Gujranwala sample					
1	30	30	1	0.8	2.66
2	30	60	2	0.97	3.23
3	30	90	3	1.16	3.86

Lipid extraction from algal samples by hot plate magnetic stirrer method

Table II explained the results of lipids extraction by solvent extraction method by using hot plate magnetic stirrer, from dried algal powder collected from University of Education, Lahore. The mixture was stirred for 5 h with help of hot plate magnetic stirrer and then let settled for 40 min to separate the layers. The dried algal powder was mixed with n-Hexane in different ratios. The results showed that the minimum lipids 0.82 g with extracted efficiency 2.73 were extracted by using algal to n-hexane in 1:1 ratio and maximum lipids 1.14 g with extracted efficiency 3.8 were extracted by using algal to n-hexane in 1:3 ratio.

Table II. Lipids extraction from samples collected from University of Education, Township, Kasur city and Gujranwala cities by using hot plate magnetic stirrer.

Sam-ple	Algal biomass (g)	n-hex-ane (g)	Algae to n-hexane ratio	Lipids extracted (g)	Extracted efficiency %
University of Education					
1	30	30	1	0.82	2.73
2	30	60	2	0.94	3.13
3	30	90	3	1.14	3.8
Kasur city sample					
1	30	30	1	0.74	2.46
2	30	60	2	0.92	3.06
3	30	90	3	1.15	3.83
Gujranwala sample					
1	30	30	1	0.82	2.73
2	30	60	2	0.98	3.26
3	30	90	3	1.17	3.9

Table II described the results of lipids extraction by solvent extraction method by using hot plate magnetic stirrer, from dried algal powder collected from Kasur city. The mixture was stirred for 5 h with the help of hot plate magnetic stirrer and then let settled for 40 min for settlement of the layers. The dried algal powder was mixed with n-Hexane in different ratios. The results showed that the minimum lipids 0.74 g with extracted efficiency 2.46 were extracted by using algal to n-hexane in 1:1 ratio and maximum lipids 1.15 g with extracted efficiency 3.83 were extracted by using algal to n-Hexane in 1:3 ratio. Table II shows the results of lipids extraction by solvent extraction method by using hot plate magnetic stirrer, from dried algal powder collected from Gujranwala city. The mixture was stirred for 5 h with help of hot plate magnetic stirrer

and then let settled for 40 min to separate the layers. The dried algal powder was mixed with n-hexane in different ratios. The results showed that the minimum lipids 0.82 g with extracted efficiency 2.73 were extracted by using algal to n-hexane in 1:1 ratio and maximum lipids 1.17 g with extracted efficiency 3.9 were extracted by using algal to n-hexane in 1:3 ratio.

DISCUSSION

The lipid extraction efficiency varied significantly among the different sources of algal samples, revealing interesting insights into the influence of environmental conditions on lipid contents in algae. Our study showed that algae collected from agricultural runoff had the highest lipid extraction efficiency at 3.9%. This finding aligns with literature suggesting that agricultural runoff, which often contains nutrients such as nitrogen and phosphorus from fertilizers, can enhance algal growth and lipid accumulation. Previous studies have documented increased lipid content in algae due to nutrient-rich environments provided by agricultural runoff (Li *et al.*, 2013; Tang *et al.*, 2014). The elevated levels of these nutrients can stimulate lipid biosynthesis pathways, thereby increasing lipid content in algal cells.

In contrast, samples from industrial effluents exhibited the lowest lipid extraction efficiency at 3.56%. This lower efficiency may be attributed to the presence of toxic substances in industrial effluents, which can inhibit algal growth and lipid production. Similar observations have been reported in other studies, where industrial effluents negatively impacted algal lipid content due to the presence of heavy metals and other contaminants (Ahmad *et al.*, 2017; Singh *et al.*, 2019). Heavy metals, such as cadmium and lead, can interfere with cellular metabolism and reduce the overall lipid content by disrupting enzyme activities essential for lipid biosynthesis (Rai *et al.*, 2020).

The hot plate magnetic stirrer method demonstrated a lipid extraction efficiency of 1.38%, outperforming other solvent extraction methods for *Spirogyra* microalgae. This method's efficiency can be attributed to its ability to maintain uniform temperature and agitation, facilitating better solvent penetration and lipid solubilization. This finding is supported by existing research, which highlights the importance of controlled heating and agitation in enhancing lipid extraction efficiency (Wang *et al.*, 2010; Lee *et al.*, 2012). Uniform temperature control prevents the degradation of thermolabile lipids, while consistent agitation ensures that solvent molecules interact effectively with algal cell walls, promoting efficient lipid extraction (Sathish *et al.*, 2013).

Our study identified an algal biomass to solvent

(n-hexane) ratio of 1:3 as the most effective for lipid extraction. This ratio likely provides an optimal balance between solvent availability and algal biomass concentration, ensuring maximum lipid dissolution without excessive solvent use. Literature supports the use of n-hexane due to its non-polar nature, which efficiently extracts lipids from algal cells (Folch *et al.*, 1957; Bligh and Dyer, 1959). Similar studies have shown that varying the solvent ratio can significantly impact lipid yield, with an optimal ratio often lying within the range of 1:2 to 1:4 (Zheng *et al.*, 2013; Yoo *et al.*, 2014). Excessive solvent use can lead to saturation and inefficiency, while insufficient solvent can result in incomplete lipid extraction (Johnson and Wen, 2009).

Our results indicate that smaller algal biomass particle sizes yield higher lipid extractions compared to larger particles. This outcome can be attributed to the increased surface area of smaller particles, which enhances solvent contact and lipid release. Previous research has similarly reported that reducing particle size improves lipid extraction efficiency by facilitating better solvent penetration and interaction with intracellular lipids (Park *et al.*, 2015; Kim *et al.*, 2016). The increased surface area allows for more effective disruption of cell walls and membranes, leading to greater lipid release into the solvent (Lee *et al.*, 2010). Furthermore, mechanical pretreatment methods, such as milling or homogenization, can significantly reduce particle size and improve overall lipid recovery (Günerken *et al.*, 2015).

Integrating our findings with the existing literature underscores the critical role of environmental factors, extraction methods, solvent ratios, and biomass particle size in optimizing lipid extraction from algae. The superior performance of samples from agricultural runoff suggests that nutrient enrichment plays a pivotal role in lipid biosynthesis. This insight is critical for selecting optimal cultivation conditions for algae intended for biodiesel production. Additionally, the effectiveness of the hot plate magnetic stirrer method emphasizes the need for precise control in extraction processes to maximize lipid yield and quality. Moreover, optimizing the biomass to solvent ratio and particle size can significantly enhance lipid yields, offering practical guidelines for improving biodiesel production from algal biomass.

These insights not only contribute to the body of knowledge on algal lipid extraction but also have practical implications for scaling up biodiesel production. Future research could focus on exploring other nutrient sources, such as wastewater from different industrial and domestic sources, and refining extraction techniques to further enhance lipid yields, ultimately supporting the development of sustainable biofuel technologies.

Investigating the synergistic effects of combining different solvents and mechanical pretreatment methods could also provide valuable insights into further optimizing the lipid extraction process.

CONCLUSION

This study demonstrates that environmental conditions, extraction methods, solvent ratios, and biomass particle sizes significantly impact lipid extraction efficiency from algae. Algae from agricultural runoff showed the highest lipid extraction efficiency at 3.9%, likely due to nutrient-rich conditions, whereas industrial effluents had the lowest at 3.56% due to the presence of toxic substances. The hot plate magnetic stirrer method proved most effective for *Spirogyra* microalgae with an efficiency of 1.38%, benefiting from uniform temperature and agitation. An algal biomass to n-hexane solvent ratio of 1:3 was optimal for lipid extraction, and smaller particle sizes further enhanced lipid yields by increasing surface area and solvent contact. These findings offer practical guidelines for optimizing biodiesel production from algae and highlight the need for precise control over cultivation and extraction conditions to maximize lipid yields, suggesting future research should explore diverse nutrient sources and refine extraction techniques to support sustainable biofuel technologies.

DECLARATIONS

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IRB approval

The Advance Study and Research Board at University of Education, Lahore approved the protocol of the present study.

Ethical statement

Permission from Ethical Committee, University of Education, Lahore, was taken for the research work.

Statement of conflict of interest

The authors have declared no conflict of interest.

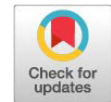
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Thyroid Dysfunction and Prevalence of Both Clinical and Subclinical Form of Hypothyroidism and Hyperthyroidism in District Swabi, Khyber Pakhtunkhwa, Pakistan

Madeeha¹, Mehreen Riaz^{2*} and Abdul Wahab³

¹Women University Swabi, Swabi, Pakistan

²University of Swat, Swat, Pakistan

³Doctor Khan Shaheed Govt. Degree College Kabal, Swat, Pakistan

ABSTRACT

Thyroid dysfunction is one of the most prevalent endocrine abnormalities, varies with age and gender. The study objective is to find thyroid dysfunction and the occurrence of clinical and subclinical hypothyroidism and hyperthyroidism in people of district Swabi, Khyber Pakhtunkhwa, Pakistan. Blood samples were taken from 104 people, and information was gathered via questionnaire. CLIA kit, Auto Bio Diagnostics LTD, China was used to detect TSH, T3, and T4 quantitatively by using Chemiluminescence Immunoassay (CLIA). Out of 104 individuals, 64 were females and 40 were males. A total of 24 people (23.04%) were affected by thyroid dysfunction, with 7 (17.5%) men and 17 (26.5%) women suffering from clinical and subclinical forms of hypothyroidism and hyperthyroidism, respectively. The occurrence of subclinical hypothyroidism was found to be the highest, with 10 cases (9.6%) recorded, followed by subclinical hyperthyroidism with 7 cases (6.7%), clinical hyperthyroidism with 4 cases (3.84%), and clinical hypothyroidism with 3 cases (2.9%). The study also revealed that the highest incidence of thyroid dysfunction occurred among individuals aged 31-40, with 6.73% affected, followed by those aged 41-50, with 5.8% affected. To conclude the women were more probable than men to have thyroid dysfunction in the area of District Swabi, and older people were more probable to have it than younger people. Iodine deficiency is the most typical cause, whereas in females, breastfeeding and pregnancy were the typical reasons.

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Authors' Contribution

M conducted the survey, laboratory work, and data analysis, utilized software and prepared the results. M wrote the manuscript according to the journal guidelines. MR proofread the manuscript for English language, reviewed the manuscript before submission.

Key words

Thyroid dysfunction, Hypothyroidism, Hyperthyroidism, TSH

INTRODUCTION

The thyroid gland in vertebrates functions as an endocrine gland and is located in the neck region of humans. It consists of two lobes linked by a thin tissue band known as the thyroid isthmus. The gland is situated below the Adam's apple and has a spherical structure called the thyroid follicle, which functions as the fundamental unit of the thyroid gland. The composition of this follicle consists of cells known as follicular cells or thyrocytes, and may sometimes contain Para follicular cells that surround a lumen filled with colloid (Hall and Guyton, 2011). In adult

humans, the thyroid gland weighs approximately 25 g, and it is typically larger in women than in men. During pregnancy, the gland tends to become wider. Each lobe of the gland measures approximately 5 cm in length, 3 cm in width, and 2 cm in thickness, while the isthmus is about 1.25 cm in both height and width (Gray and Standring, 2008; Bogart and Ort, 2007). The thyroid gland produces three hormones: triiodothyronine (T3), thyroxine (T4), and the peptide hormone calcitonin. Thyroid hormones control growth and development in children while also influencing protein synthesis and metabolic rate. Calcium homeostasis requires calcitonin. Thyroid-stimulating hormone (TSH), which is secreted by the anterior pituitary gland, stimulates the secretion of the two thyroid hormones. The hypothalamus is responsible for the production of thyrotropin-releasing hormone (TRH), which controls TSH (Hall and Guyton, 2011). The hypothalamus discharges TRH into the hypothalamic-hypophyseal portal system of the anterior pituitary gland. TRH activates the thyrotropin cells in the anterior pituitary to release TSH. The peptide hormone TRH is synthesized by the cell bodies located within the paraventricular nucleus

* Corresponding author: drmehreen1986@gmail.com
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(PVN) of the hypothalamus and these cell bodies extend their neurosecretory neurons into the hypophyseal portal circulation, where TRH can collect before reaching the anterior pituitary (Braun and Schweizer, 2018). According to (Bauer, 2013), thyroid disease refers to a medical illness that impairs the thyroid gland's ability to function. It can happen to anyone, regardless of age or gender, although its frequency fluctuates in various regions as well as in various age and sex groups (Lamfon, 2008).

Due to a lack of iodine doses, incidences of thyroid issues have been reported in more than 110 countries around the world, exposing 1.6 billion people at risk. Africa, Asia, and Latin America make up the majority of these, along with other emerging countries. About 20 million people who live in iodine-deficient areas in Pakistan, among whom 8 million suffer from some form of iodine deficiency and about 1 million have mental issues (Lotfi *et al.*, 1996). Under or over secretion of hormones are the key features of thyroid disorders, which are distinguished by distinct symptoms and signs in both conditions. Underbalanced thyroid function or thyroid gland hypertrophy might have an impact on these conditions (Alam *et al.*, 2002). The primary disorders of the thyroid gland are endocrine diseases, where certain endocrine glands directly react to metabolic glands while others respond to hormones produced by the pituitary gland (Kasper *et al.*, 2015). Among all chronic problems, thyroid abnormalities are one of the more common. Their symptoms differ greatly from region to region and are highly calculated by the level of iodine in the diet. For the definition of overt hypothyroidism and subclinical hypothyroidism, the specimen selection process used, the impact of age, sex, genetic inheritance, and lifestyle exposures, the numerous methods used to evaluate thyroid hormones, and the comparative insufficiency of epidemiology are just a few of the drawbacks of randomized trials of thyroid issues (Vanderpump, 2005). Iodine deficiency affects nearly one-third of the global population (Zimmermann *et al.*, 2008). Mostly people with thyroid abnormalities in iodine-rich countries suffer from autoimmune diseases that can extend between primary necrotic hypothyroidism to Hashimoto's thyroiditis to graves disorder thyrotoxicosis. The incidence and transmission of thyroid antibodies in many primarily Caucasian, communities were documented by inter investigations in Europe, the United States, and Japan (Vanderpump, 2005). Hollowell *et al.* (2002) reported that thyroid dysregulation rises the possibility of ischemic (decreased blood flow and oxygen), neurological problems, arthritis, dyslipidemia (imbalance of lipids), and metabolic disorders.

There are four main categories of thyroid disease (hypothyroidism, hyperthyroidism, structural

abnormalities and tumors). Symptoms of thyroid disease vary by type (Hall and Guyton, 2011). Hypothyroidism results from a reduced effect of thyroid hormone on tissues. It is more common in females, with a combined prevalence of 1% to 2% that increases with age (Canaris *et al.*, 2000). Hyperthyroidism occurs when the thyroid gland excessively produces thyroid hormones. The main states of hyperthyroidism are Graves' disease, toxic thyroid nodule, thyroid storm, toxic nodular stroma and hashitoxicosis. Approximately 1.2% of the population in the United States is affected by hyperthyroidism (Bahn *et al.*, 2011). The structural abnormality is an abnormal increase in size of the thyroid, including goiter, lingual thyroid, and thyroglossal duct cyst (Perros, 2007). Thyroid tumors, the clinical manifestation of thyroid cancer typically manifests as an expanding goiter or a single thyroid nodule. Despite the prevalence of thyroid nodules, thyroid cancer is uncommon (Perros, 2007).

MATERIALS AND METHODS

The study was conducted at Bacha Khan Medical Complex in Swabi Pakistan with a sample size of 104 individuals, randomly selected from the population under study. The data were collected through a survey questionnaire and by quantitatively estimating the levels of TSH, T3, and T4 hormones in the patients using a chemiluminescence immunoassay test. The study involved 40 male and 64 female subjects who underwent thyroid function tests to determine their thyroid status, which was classified as clinical, subclinical, or mixed hypothyroidism or hyperthyroidism. 5 cc of blood was taken from each subject, and the serum was used for further analysis to study the subjects' thyroid status.

Procedure adopted

The study involved blood samples being taken to the laboratory for analysis, where the levels of TSH, T3, and T4 hormones were measured using the chemiluminescence immunoassay (CLIA) method with AccuLite CLIA Micro Wells and TSH, T4, and T3 kits. The reference ranges for TSH, T3, and T4 were also established. Thyroid dysfunction was categorized into hyperthyroidism, subclinical hyperthyroidism, hypothyroidism, and subclinical hypothyroidism, each with its own specific hormone level changes. The TSH, T3, and T4 tests were conducted based on the principles of sandwich and competitive immunoassays, with each test having a total duration of about 18 min. The TSH test is used to measure the levels of TSH in a blood sample, while the T3 and T4 tests measure the levels of T3 and T4, respectively.

Data analysis

The analysis of the data was completed using two software prog: SPSS version 20 and Microsoft Excel version 2016. The appropriate statistical test used to analyze the results was the Chi-squared test. To determine the significance of the results, the p-value was calculated and considered significant if it was less than 0.05. The data obtained from the analysis were presented in various forms, including charts, tables, and statistical measures. The data was represented using different statistical measures such as percentages, frequencies, means, and standard deviations. These measures helped provide a better understanding of the data and insights into the results obtained from the analysis.

RESULTS

Table I shows the gender-wise distribution of clinical and subclinical forms of hypothyroidism and hyperthyroidism. Out of a total of 104 samples, 40 (38.5%) were males and 64 (61.5%) were females. The overall number of affected individuals was 24 (23.04%), with 7 (17.5%) being males and 17 (26.5%) being females. The ratio of clinical hypothyroidism was 3 (2.9%) in the total sample (Fig. 1), with 1 (2.5%) being male and 2 (3.1%) being female. The ratio of clinical hyperthyroidism was 4 (3.84%) in the total sample (Fig. 1), with 1 (2.5%) being male and 3 (4.7%) being female. On the other hand, the ratio of the subclinical form of both hypothyroidism and

hyperthyroidism was higher. The ratio of subclinical hypothyroidism was 10 (9.6%) in the total sample, with 3 (7.5%) being male and 7 (10.9%) being female. The ratio of subclinical hyperthyroidism was 7 (6.7%) in the total sample (Fig. 1), with 2 (5%) being male and 5 (7.8%) being female. The p-value was found to be 0.87, which is not statistically significant.

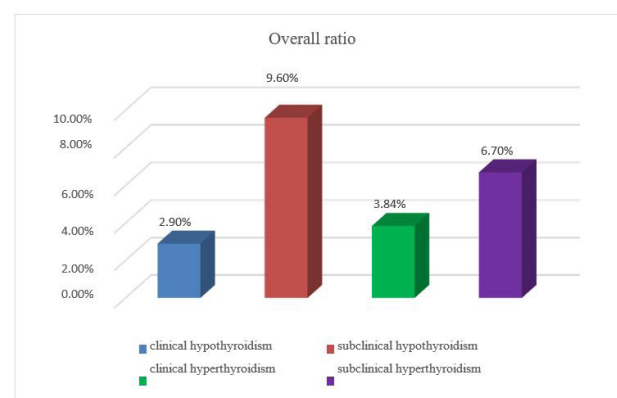


Fig. 1. Overall ratio.

Table II demonstrates the age-wise proportion of both clinical and subclinical forms of hypothyroidism and hyperthyroidism. The highest ratio of thyroid dysfunction was found in the age group 31-40 (6.73%), followed by the age groups 41-50 (5.8%). The occurrence of clinical

Table I. Distributions of thyroid dysfunctions in males and females.

Prevalence of thyroid dysfunction	Male =40	Female =64	Total =104	P- value
Clinical hypothyroidism	1 (2.5%)	2 (3.12%)	3 (2.9%)	0.87
Subclinical hypothyroidism	3 (7.5%)	7 (10.93%)	10 (9.6%)	
Clinical hyperthyroidism	1 (2.5%)	3 (4.7%)	4 (3.84%)	
Subclinical hyperthyroidism	2 (5%)	5 (7.8%)	7 (6.7%)	
Total	7 (17.5%)	17 (26.5%)	24 (23.04%)	

Table II. Distribution of thyroid dysfunctions in different age groups.

Age	Clinical hypothyroidism	Subclinical hypothyroidism	Clinical hyperthyroidism	Subclinical hyperthyroidism	Total
21-30	0 (0%)	1 (0.97%)	1 (0.97%)	2 (1.9%)	4 (3.84%)
31-40	1 (0.97%)	4 (3.8%)	1 (0.97%)	1 (0.97%)	7 (6.73%)
41-50	1 (0.97%)	3 (2.9%)	0 (0%)	2 (1.92%)	6 (5.8%)
51-60	0 (0%)	0 (0%)	1 (0.97%)	1 (0.97%)	2 (1.92%)
>-60	1 (0.97%)	2 (1.92%)	1 (0.97%)	1 (0.97%)	5 (4.80%)
P. value	0.93				

hypothyroidism was the same in the 31-50 and >60 age groups (0.97%), and it was not found in the 21-30 and 50-60 age groups. On the other hand, the occurrence of subclinical hypothyroidism was high in the 31-40 age group (3.8%), followed by the 41-50 age group (2.9%), and it was not found in the 51-60 age group. The prevalence of clinical hyperthyroidism was the same in all age groups (0.97%), except in the 41-50 age group where no cases were found. Conversely, the prevalence of subclinical hyperthyroidism was high in the 21-30 and 41-50 age groups (1.92%), followed by other age groups. The p-value was 0.93, which is statistically non-significant.

Table III. Mean, standard deviation, minimum and maximum values of thyroid hormones.

Thyroid hormones	Mean	SD	Minimum	Maximum
T3 (ng/ml)	1.05	0.41	0.04	3.27
T4 (µg/dl)	8.60	4.93	1.20	50.65
TSH (µIU/ml)	5.52	16.64	0.001	>100.00

Table III shows the mean, SD (standard deviation), maximum, and minimum values of the thyroid hormones T3, T4, and TSH. The mean of the T3 was 1.05, the SD was 0.41, the maximum value was 3.27, and the minimum value was 0.04. The mean of the T4 was 8.6, the SD was 4.93, the maximum value was 50.65, and the minimum value was 1.2. The mean of the TSH was 5.52, the SD was 16.64, the maximum value was greater than 100, and the minimum value was 0.001.

Table IV shows the standard deviation and mean of thyroid dysfunction levels by comparing thyroid hormone levels. The SD and mean of T3, T4, and TSH in normal individuals were 1.02 ± 0.25 , 8.06 ± 1.62 , and 1.66 ± 1.24 . In clinical hypothyroidism, the mean and SD of T3, T4, and TSH were 0.50 ± 0.52 , 5.18 ± 3.85 , and 35.13 ± 56.22 , respectively, while in subclinical hypothyroidism, they were 10.64 ± 1.39 , 5.52 ± 1.64 , and 33.82 ± 33.20 , respectively. Similarly, in hyperthyroidism, the mean and SD were 2.11 ± 0.96 , 25.03 ± 17.39 , and 0.23 ± 0.44 for T3, T4, and TSH, respectively, while in subclinical hyperthyroidism, they were 1.29 ± 0.23 , 10.65 ± 3.10 , and 0.72 ± 0.23 , respectively.

Table IV. Representation of dysfunction levels by comparing thyroid hormones levels.

Thyroid hormones	Normal (Mean±SD)	Hypothyroidism (Mean±SD)	Subclinical hypothyroidism (Mean±SD)	Hyperthyroidism (Mean±SD)	Subclinical hyperthyroidism (Mean±SD)
T3 (ng/ml)	1.02 ± 0.25	0.50 ± 0.52	10.64 ± 1.39	2.11 ± 0.96	1.29 ± 0.23
T4 (µg/dl)	8.06 ± 1.62	5.18 ± 3.85	5.52 ± 1.64	25.03 ± 17.39	10.65 ± 3.10
TSH (µIU/ml)	1.66 ± 1.24	35.13 ± 56.22	33.82 ± 33.20	0.23 ± 0.44	0.72

DISCUSSION

This study at Bacha Khan Medical Complex, Swabi Pakistan, aimed to determine the occurrence of clinical and subclinical hypothyroidism and hyperthyroidism. It involved 104 patients of different ages and genders, randomly selected from the outpatient department. Blood samples were taken and analyzed for TSH, T3, and T4 levels to diagnose thyroid dysfunction. Hypothyroidism is diagnosed with high TSH and low T3, T4 levels, while hyperthyroidism is identified with low TSH and high T3, T4 levels. The study aimed to identify risk factors for thyroid dysfunction in the local population. It was found that the overall prevalence of thyroid dysfunction in the population was 23.04%, with 2.9% having clinical hypothyroidism and 9.6% having subclinical hypothyroidism. Clinical hyperthyroidism was found in 3.84% of the population, while subclinical hyperthyroidism was found in 6.7%. The results indicate a significant number of undiagnosed cases and emphasize the importance of routine screening for early detection and treatment of thyroid dysfunction.

In previous studies conducted in Pakistan, Akhter *et al.* (2001) found a higher prevalence of clinical hypothyroidism (4.1%) and hyperthyroidism (5.1%), while subclinical hypothyroidism (5.4%) and hyperthyroidism (5.8%) were lower compared to the present study. Similarly, Alam *et al.* (2019) reported lower rates of clinical and subclinical hypothyroidism and hyperthyroidism in the District Mardan compared to the District Swabi. These studies also attributed thyroid dysfunction to iodine deficiency. In a study conducted in Libya by Nouh *et al.* (2008), the prevalence of clinical and subclinical hyperthyroidism was 0.84%, while clinical and subclinical hypothyroidism was 1.12% and 6.18%, respectively. These rates were lower than those found in the present study. In a study by Alanazi *et al.* (2018), involving 160 participants, 22.5% had thyroid disease. Among the cases, 30.5% had hyperthyroidism and 69.5% had hypothyroidism. The overall occurrence of thyroid dysfunction (22.5%) was similar to the present study (23.04%). According to Velayutham *et al.* (2015), the prevalence of hypothyroidism and hyperthyroidism was 7.3% and 0.3%, respectively. Strieder *et al.* (2003) found that 3.6% had hypothyroidism and 1.9%

had hyperthyroidism due to the presence of thyroid peroxidase autoantibodies. These results align with the current study, where hypothyroidism (2.9%) was higher than hyperthyroidism (3.84%). In contrast, [Moussa *et al.* \(2016\)](#) reported higher rates of hypothyroidism (56%) and hyperthyroidism (32.6%) in the Hail region of Saudi Arabia. According to [Vanderpump \(2019\)](#), hyperthyroidism occurs in women at a rate of 0.5-2%, while hypothyroidism occurs at a rate of 1-2%. Women are affected by these conditions ten times more frequently than men, which can be attributed to factors such as increasing age and iodine deficiency. In a study conducted by [Alam *et al.* \(2019\)](#) in the Mardan region, the occurrence of thyroid disorders was higher in females (22.06%) compared to males (17.18%). The rates of clinical and subclinical hyperthyroidism were 5.26% and 5.66% in females, respectively, and 3.12% and 4.16% in males. The rates of clinical and subclinical hypothyroidism were 5.06% and 6.07% in females, respectively, and 4.68% and 5.20% in males. Menstrual abnormalities, lactation, and pregnancy were identified as common causes of thyroid problems in females. A comparative study revealed slightly higher prevalence rate of both clinical and subclinical hypothyroidism in the Swabi region than in Mardan.

The current study, like others, found a higher prevalence of subclinical hypothyroidism compared to other forms of thyroid dysfunction. The overall prevalence of thyroid dysfunction was 23.04%, with subclinical hypothyroidism being the most common (9.6%). Different countries, such as Libya and Saudi Arabia, have also reported varying prevalence rates of thyroid dysfunction, with some showing a higher occurrence of hypothyroidism than hyperthyroidism. These variations may be attributed to factors like population differences, geographic location, methodologies, environmental factors, and various risk factors such as iodine deficiency and the presence of thyroid peroxidase (TPO) autoantibodies.

In terms of gender distribution, the study included a total of 24 affected individuals, with 7 (17.5%) being males and 17 (26.5%) being females. Clinical hypothyroidism accounted for 3 (2.99%) cases, with 1 (2.5%) being male and 2 (3.12%) being female. Clinical hyperthyroidism accounted for 4 (3.84%) cases, with 1 (2.5%) being male and 3 (4.7%) being female. On the other hand, the subclinical forms of both hypothyroidism and hyperthyroidism were more prevalent. Subclinical hypothyroidism accounted for 10 (9.6%) cases, with 3 (7.5%) being male and 7 (10.93%) being female. Subclinical hyperthyroidism accounted for 7 (6.7%) cases, with 2 (5%) being male and 5 (7.8%) being female. The calculated p-value of 0.87 indicates non-significance.

While examining thyroid dysfunction across different

age group, the study found that the highest prevalence was observed in the 31-40 age group (6.73%), followed by the 41-50 age group (5.8%). The occurrence of clinical hypothyroidism was the same in the 31-40 and >60 age group (0.97%), while it was not found in the 21-30 and 51-60 age groups. Subclinical hypothyroidism was more common in the 31-40 age group (3.8%), followed by the 41-50 age group (2.9%), and it was not found in the 51-60 age group. Regarding clinical hyperthyroidism, it had a consistent occurrence across all age groups, except for the 41-50 age group where no cases were found. The incidence of clinical hyperthyroidism seemed relatively consistent across different age groups, except for this age range. Subclinical hyperthyroidism was most prevalent in the 21-30 and 41-50 age group (1.92%), and the statistical analysis showed a non-significant p-value of 0.93.

The current study, along with previous research, highlights that thyroid dysfunctions, particularly subclinical hypothyroidism and subclinical hyperthyroidism, are more common in women compared to men. Several factors contribute to this gender difference, including lactation, pregnancy, menstrual abnormalities, and iodine deficiency. Autoimmune conditions, which are more prevalent in women, can also contribute to higher rates of thyroid diseases. The interaction between thyroid hormones and the hormonal changes during the menstrual cycle is believed to play a role in the higher incidence among women. Additionally, menopause and its fluctuating hormone levels can increase the likelihood of thyroid diseases. Some women may mistake the symptoms of menopause for thyroid issues, leading to delayed treatment-seeking.

CONCLUSION

In conclusion, this study in Swabi, Pakistan found that thyroid dysfunction is common, with a higher prevalence in females. Among the 104 samples, 61.5% were females, and the overall prevalence was 23.04%. Clinical hypothyroidism was 2.99%, slightly higher in females (3.12%), while clinical hyperthyroidism was 3.84%, with a higher prevalence in females (4.7%). Subclinical forms had higher ratios: subclinical hypothyroidism at 9.6%, with 10.93% in females and 7.5% in males, and subclinical hyperthyroidism at 6.7%, with 7.8% in females and 5% in males. The study also showed thyroid dysfunction across age group, with the highest prevalence in the 31-40 age group (6.73%) and the 41-50 age group (5.8%). Clinical hypothyroidism affected the 31-50 and > 60 age group (0.97%), not the 21-30 and 50-60 age group. Subclinical hypothyroidism was highest in the 31-40 age groups (3.8%) and the 41-50 age group (2.9%), while it was not present

in the 51-60 age group. Clinical hyperthyroidism had the same prevalence in all age group (0.97%), except for no cases in the 41-50 age group. Subclinical hyperthyroidism was highly prevalent in the 21-30 and 41-50 age group (1.92%), as well as other age group.

DECLARATIONS

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IRB approval

The research proposal was reviewed and approved by the Internal Research Project Committee of Women University Swabi.

Ethical statement

All the ethical issues was addressed according to the international standards.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Development and Validation of Rapid Multiplex PCR Assay for Identification of DNA Origins of Pork, Donkey and Cow Species in Real Food Samples

Muhammad Safdar^{1*}, Muhammad Younus², Faiz-ul Hassan¹ and Yasmeen Junejo³

¹Department of Breeding and Genetics, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan

²Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan

³Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan

ABSTRACT

Food composition and authenticity assessment is an important concern to protect the consumers from illegal or unwanted substitution; for economic, religious and health concerning reasons. Cow, pork and donkey meats are being used as a substitute ingredient for red meat. Muslim and Jewish populations avoid consumption of pork and donkey meats, even in min quantities, due to their religious faiths. In this background, there is a need of a rapid, economic and highly sensitive multiplex PCR instead of uniplex PCR for identification of DNA origins of pork, donkey and cow species in real food samples. For this purpose, a total of 150 samples including blood samples, tissue samples, laboratory prepared food samples and commercial foodstuffs including chicken karahi, beef biryani, chicken samosa, chicken pakora, haleem, nihari etc. were collected from Bahawalpur, Multan, and Rahim Yar Khan. DNA was extracted from all the targeted samples. We developed the first multiplex PCR assay to identify pork, donkey, and cow species origins in food products within a single tube. The assay utilizes specific primers [pork (*Sus scrofa*), donkey (*Equus asinus*), and cow (*Bos taurus*)] that amplify fragments of the mitochondrial COX1 (donkey; 184 bp), 12S rRNA (cow; 271 bp), and ATPase subunit 6 and 8 genes (pork; 459 bp), respectively, as well as universal 18S rRNA primers that amplify a 99 bp fragment. The detection limit of the assay was 0.005% for each species. In conclusion, this PCR assay is a simple, rapid, sensitive, precise, and economical method to identify donkey, pork, and cow species in commercial food samples.

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Authors' Contribution

MS conceived the idea and designed the experiments. MY and YJ performed the experiments. MS and FH analysed the data. MS, MY and YJ wrote the manuscript. FH and MS reviewed the manuscript.

Key words

Multiplex PCR, Universal primers, Species-specific primers, Sensitivity, Specificity, Commercial foodstuffs

INTRODUCTION

Food was a prerequisite for the first living organism to breathe billions of years ago, as biology has unequivocally demonstrated. Evolution, development and growth could not have occurred in the absence of nourishment. For survival, growth, and reproduction, all living things on Earth plants and animals alike need food (Brüssow and Brüßow, 2007). As the world gets busier,

more and more individuals are having to work longer hours at their jobs to keep up with the growing amount of work (Williams, 2023). Lack of time to cook their own meals is forcing an increasing number of people to settle for whatever they can obtain from a nearby restaurant or grocery store (Jurado-Gonzalez *et al.*, 2024). Thus, the demands on the restaurant business and its prospects, along with those for prepared foods like hot dogs, pizza, and sandwiches, are growing quickly (Ali *et al.*, 2012). Due to dietary changes and growing knowledge of the health advantages of meat, there is a growing global desire for premium animal protein, which includes meat and products produced from it. Since the sale of food has been a commercial activity, there have always been issues surrounding food authenticity, particularly those pertaining to fraudulent adulteration and misrepresentation (Safdar *et al.*, 2024). Due to the critical importance of consumer health, there is a greater focus on closely monitoring food intake and verifying its authenticity in order to prevent unwanted and illegal substitutes that are

* Corresponding author: msafdar@cuvas.edu.pk
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motivated by economic, religious, or health-related factors (Plowman and Close, 1988).

Pakistan currently lacks suitable circumstances for meat exports because there is no enforcement legislation and no readily available tests for precisely identifying the species of meat (Butt and Ahmed, 2020). Prohibited meat species or their hybridization with halal meat, as well as low-nutritional halal food species for financial benefit, have long been a sensitive topic in the press and public (Safdar *et al.*, 2023). All things considered; the nation's anti-adulteration laws present a dismal image. It is imperative that we resist this difficult situation. This means that in order to differentiate between haram species like some pig and donkey species and halal meat species like cattle scientific procedures are required (Safdar and Junejo, 2016). Similarly, mutton and beef are not interchangeable (Safdar and Ozaslan, 2022). Scientists have developed a number of techniques in recent decades to detect and identify the species origin, especially in industrial meat products, which are important for hygienic, religious, and financial reasons (Hossain *et al.*, 2021). To establish beyond a doubt that food fraud has occurred, food ingredients must be located and confirmed. It can be quite difficult to discern between replacement materials and primary components because, from a biochemical standpoint, they are often comparable. It is essential to determine the species of meat products in order to protect consumers from unknown influences and to detect adulteration or fraudulent replacement (Meyer *et al.*, 1995). Multiplex PCR has steadily gained popularity over simplex PCR due to numerous benefits, which include faster reaction times, reduced costs, and improved detection efficiency in just one reaction hole. Regular meat fraud has spread throughout the world, perhaps endangering food safety, violating market regulations and potentially endangering public health (Li *et al.*, 2020). In recent years, methods for determining the species of meat have undergone continual evolution (Chung and Hellberg, 2020). Polymerase chain reaction, or PCR, and DNA-based techniques together give more accurate ways for differentiating meat species since DNA molecules are found in every cell and have a high degree of stability (Mansouri *et al.*, 2020). Real-time PCR and conventional multiplex techniques are both regarded as trustworthy procedures with excellent sensitivity and specificity for identifying meat species (Liu *et al.*, 2021).

In Pakistan, there is an urgent need for PCR assays that offer simple, sensitive, specific, and cost-effective methods for DNA-based commercial analysis and the surveillance of foodstuffs. Therefore, we aimed to address this need by developing a multiplex PCR assay as a potential tool for the rapid, specific, sensitive, and cost-effective detection of small fragments of mitochondrial

DNA from pork, donkey, and cow species in foodstuffs. This assay utilizes both species-specific and universal primers, marking the first time such a method has been employed for this purpose.

MATERIALS AND METHODS

Samples preparation and collection

To optimize the simplex and multiplex PCR assays of the targeted species, the raw meats and grains of each targeted species such as cow, pork and donkey etc. were collected from Bahawalpur and 150 market food samples were collected from a different area of Bahawalpur, Multan and Rahim Yar Khan. Raw and processed meat samples were cut into small pieces and immediately stored at -20°C until use. We directly transported them to the Molecular Genetics Research Laboratory (CUVAS) and stored at -20°C until the extraction of the DNA in order to prevent the enzymatic degradation of DNA.

Validation of laboratory prepared samples

To validate the market food samples such as cow, pork and donkey etc. meats of each species were mixed in different %age and cooked in the laboratory (Table I). In order to evaluate the sensitivity test, a compound DNAs of all species (cow, sheep, horse, pork and donkey etc.) was mixed and cooked in different %age up to 0.005% (Table I). Finally, DNA was extracted from these samples and stored at -20°C until use.

Table I. Preparation of samples for validation and sensitivity.

Horse (NC) (%)	Donkey (%)	Pig (%)	Cow (%)	Sheep (NC) (%)
Samples for validation				
20	70	60	30	80
50	0.05	10	0	50
70	20	0	50	30
100	0	40	55	0
Samples for sensitivity				
24	24	24	24	4
12	12	12	12	52
6	6	6	6	76
3	3	3	3	88
1	1	1	1	96
1.5	1.5	1.5	1.5	94
0.75	0.75	0.75	0.75	97
0.05	0.05	0.05	0.05	99.8
0.005	0.005	0.005	0.005	99.98
0	0	0	0	100

Table II. List of targeted species primers that were used in this study.

Primers	Species	Genes	Positions	Oligonucleotides primers 5' → 3'	Amplicons (bp)
Pork	<i>Sus scrofa</i>	<i>COX1</i>	chrM:7096+7554	F: CTACTATCCCCTGCCAGTT R: GAATAGGAAGATGAAGCC	459
Donkey	<i>Equus assinus</i>	<i>12S rRNA</i>	PP049798.1	F: GAACAAGAAGCTCAACCCAAACGG R: CTTTCATATGTTTGGATCATGGTTTGTG	184
Cow	<i>Bos taurus</i>	<i>ATPase sub-unit 6and8</i>	chrM:8107+8377	F: GCCATATACTCTCCTTGGTGACA R: GTAGGCTTGGGAATAGTACGA	271
Eukaryotes	-	<i>18S rRNA</i>	CP143370.1	F: AGGATCCATTGGAGGGCAAGT R: TCCAACCTACGAGCTTTTAACTGCA	99

DNA extraction

DNA was extracted from 50-100 mg of laboratory prepared and market collected food samples using instructions and protocol of Genomic DNA Purification Tissue and Blood Kit (Thermo-Scientific) and DNeasy® Kit (Germany) as per instructions of protocol given in the kit. DNA concentration (quantification) in a solution was measured using a Gel Doc XR+ with imaging Lab instrument. It produced high-quality photographs with exceptional sensitivity and resolution.

Oligonucleotide's primers

Specific gene loci, such as the COX1 gene for pork, the 16S rRNA for donkeys, and the ATPase subunits 6 and 8 for cows, were amplified using oligonucleotide primers. Next, we used the BLAST and NCBI (National Center for Biotechnology Information) tools to verify the specificity of the DNAs in the DNA databank (Table II). All the primers were synthesized by Synbio Technologies, Russia.

Simplex qualitative PCR

A final amount of 20 µl, comprising 1 µl of each primer, 3 µl of DNA template, and WizPure™ PCR 2X Master mix, was used for PCR amplification. Amplification was carried out in a Thermocycler Techne under the following cycling conditions: 35 cycles were programmed following an initial heat denaturation step of 5 min at 95 °C: 30 sec intervals at 95°C, 59–61°C, 72°C for one min, and a 5-min extension at 72°C were all used.

Multiplex qualitative PCR

Using every primer set previously chosen for the simplex PCR, an a single-step multiplex PCR was employed for simultaneous identification of every species. The WizPure™ PCR 2X Master mix, 0.4µl of each primer, and 1µl of DNA template were added to a total volume of 20 µl for PCR amplification. The following cycling parameters were used for the amplification process

in a Thermocycler Techne: 35 cycles were scheduled for 95 °C for 30 s, 59–61 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min, after an initial heat denaturation stage at 95 °C for 5 min.

RESULTS

Optimization of simplex and multiplex PCR

Figure 1 shows the multiplex PCR using the same primers using DNA extracted from cooked meats of cow, pork, donkey, etc., after several repetitions.

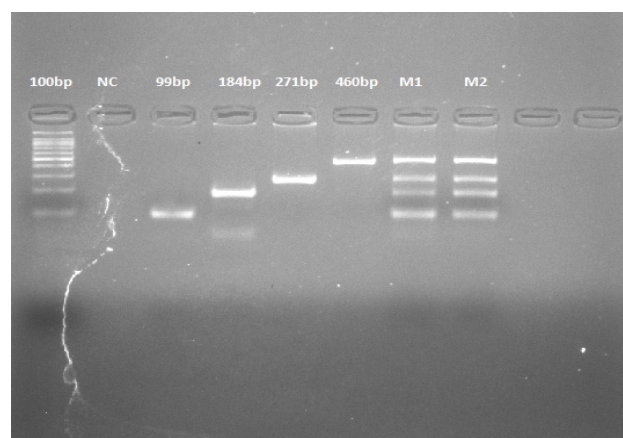


Fig. 1. 100bp: Ladder, NC: Negative Control (Everything without primer of cow), Cow (Positive Control), Universal primer (99bp), Donkey (184bp), Cow (271bp), Pork (459bp). M1; Multiplex PCR 1, M2; Multiplex PCR 2 (Repeat).

Figure 2 shows the sensitivity of the multiplex PCR, targeting the same species of the DNAs, was evaluated down to a minimum concentration of 0.005%.

To validate the PCR assay for market food samples, including meats such as cow, pork, and donkey, the mixtures of these species' meats were prepared in different

percentages and cooked in the laboratory. DNA was then extracted from these samples and validated the multiplex PCR as shown in Figure 3.

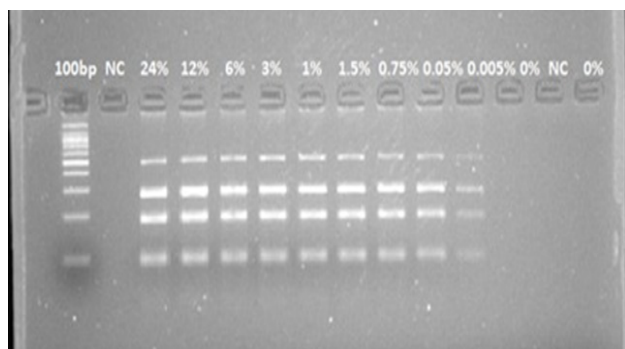


Fig. 2. Evaluation of multiplex PCR assay sensitivity for universal, cow, donkey and pork in cooked labortaty prepared samples; M: Marker 100-bp. NC: Negative control (reagents with primers without DNAs); Eu: Eukaryotic DNA positive control. (1) 24%, (2) 12%, (3) 6%, (4) 3%, (5) 1.5%, (6) 0.75%, (7) 0.05% (8) 0.005% (9) 0%.

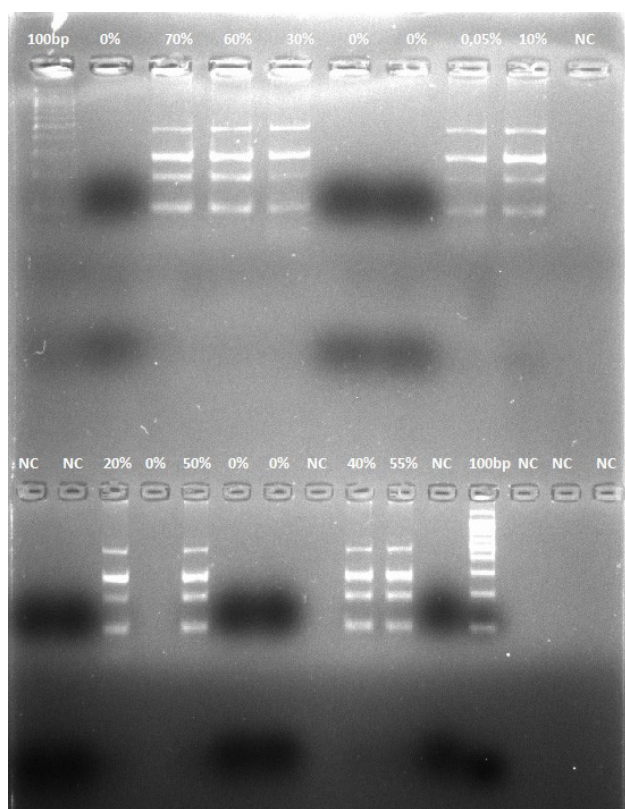


Fig. 3. Evaluation of multiplex PCR assay validity was performed on cow, donkey and pork in cooked labortaty prepared samples. M, Marker 100-bp; NC, Negative control (reagents with primers without DNAs).

Multiplex PCR assay

We have applied our developed assay on 150 commercial food samples. We had not found any contamination with pork and donkey in 150 collected samples, while we had found 15% adulteration that may be intentionally or un-intentionally by producers (Fig. 4).

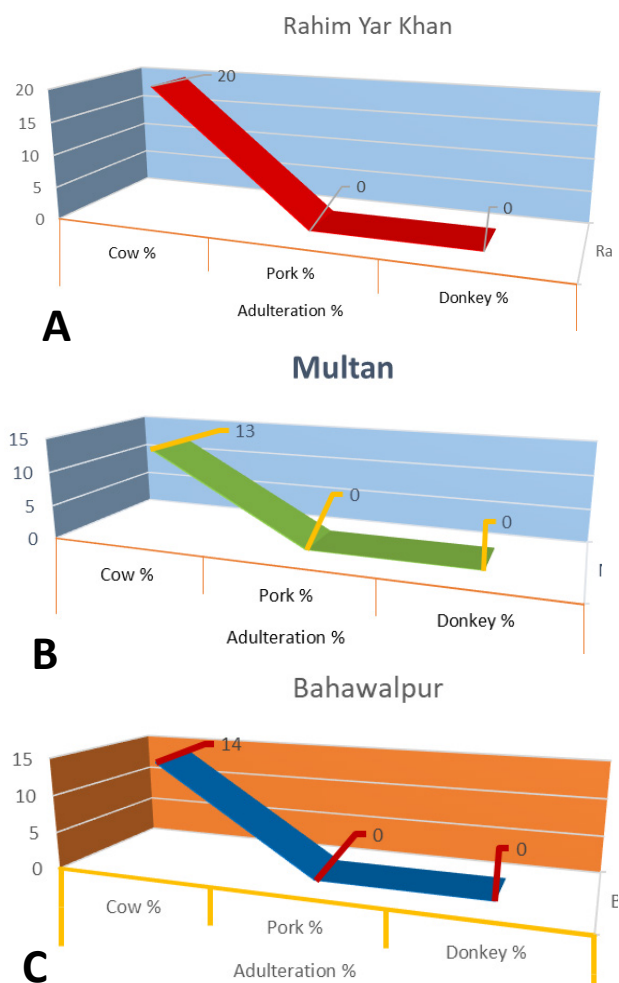


Fig. 4. Adulteration in commercial food samples at RYK district (A), Multan district (B) and Bahawalpur district (C).

DISCUSSION

This study highlights the critical need for quick, accurate, sensitive, and affordable identification methods to evaluate cocktail food items and confirm the legitimacy of labels, especially when it comes to identifying donkey, pork and beef species. To identify these animal species in food products with species-specific PCR assays have been published in previous studies (Palle-Reisch *et al.*, 2014).

This PCR assays, however, have a number of advantages over previous methods. In a single step, the multiplex PCR assay that identify donkey, pig and beef in mixed food products without the need for specialized knowledge, unusual tools, or extra chemicals. Simple agarose gel analysis is being used to detect species-specific DNA, which makes it affordable first screening method for large-scale applications. The detection limit of our assay is 0.005%, demonstrating its high sensitivity and reliability as compared to others. Some researchers have reported higher detection limits for meat species identification in food products, with a minimum detection limit of 0.5%-0.1% (Ha JeongChul *et al.*, 2006; Rahmati *et al.*, 2016). With a limit of detection of 0.01% DNA, Safdar and Junejo (2015) presented a multiplex PCR to concurrently identify the bovine, ovine, caprine, and fish species in feedstuffs. Therefore, our method is notably more attractive due to its superior sensitivity. This method's unique characteristics also pave the way for detecting and distinguishing other closely related species, such as turkey, quail, pigeon, goat, buffalo, deer and donkey etc. in food as well as feed products.

The development of real-time PCR has significantly advanced the identification and quantification of meat species in foodstuffs (Pegels *et al.*, 2014). However, real-time PCR assays are notably more expensive compared to conventional PCR methods that utilize species-specific primers and probes (Safdar and Abasiyanik, 2013). Additionally, the requirement for specialized equipment and trained personnel restricts the implementation of real-time PCR in many feed control laboratories, particularly those with limited resources. In contrast, multiplex PCR assays using species-specific primers offer a cost-effective alternative that can be performed with minimal resources while still providing accurate verification of commercial foodstuffs. The high sensitivity, specificity, and reproducibility of multiplex PCR make it a feasible and ideal method for the rapid analysis of food samples, ensuring the detection of potential fraud or risks associated with zoonotic diseases (Zia *et al.*, 2020).

The limitations of multiplex PCR include potential issues with primer compatibility, which can lead to non-specific amplification or reduced sensitivity. Additionally, optimizing reaction conditions for multiple targets simultaneously can be challenging, and there is a risk of preferential amplification of certain targets over others, affecting overall assay accuracy.

CONCLUSION

The findings of this study demonstrate that the multiplex PCR assay developed can effectively detect the

presence of meat species, specifically cow, donkey, and pork, in foodstuffs. The assay is capable of simultaneous, rapid, and routine detection of both animal and plant species in food products, with a detection limit as low as 0.005%. Validation across various matrices confirmed the assay's applicability in practical identification of these species in both food and feed products. We anticipate that this assay will be adopted by food regulatory bodies for screening and monitoring the presence of cow, donkey, and pork species in foodstuffs.

DECLARATIONS

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Ethical statement

No animal was harmed or disturbed during this research.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Comparative Evaluation of RF and Anti-CCP Tests for Rheumatoid Arthritis Diagnosis: Insights from a Pakistani Population

Muhammad Waqas*, Razia Bashir and Muhammad Arshad

Department of Zoology, Division of Science and Technology, University of Education, Lahore, Punjab, Pakistan.

ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease that principally affects joints. Various diagnostic techniques are in use to detect RA based on the presence of auto-antibodies in patients' blood like rheumatoid factor and anti-cyclic citrullinated peptides. We aimed to detect RF and anti-CCP antibodies for diagnostic evaluation of RA. RF was done by the latex agglutination method while anti-CCP test was performed on Abbot AxSYM system using micro-particle enzyme immunoassay method. The analysis showed that RF test is more prevalent than anti-CCP to determine whether an individual has RA or not. In the present study RA is also found to be associated with age of the patients. To conclude RA is an age-related disorder that can be detected earlier by the presence of RF autoantibodies as compared to anti-CCP. These findings can be helpful for the clinical diagnosis of RA.

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Authors' Contribution

MW conducted the research and drafted the manuscript. RB contributed to manuscript writing and revisions. MA supervised the research work and provided guidance.

Key words

Rheumatoid arthritis, RF, Anti-CCP

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that primarily targets joints leading to their inflammation and destruction. Progressive damage to joints results in disability (Firestein, 2003). However, its impact extends beyond joints, affecting various tissues and organs such as ligaments, tendons, muscles, cartilage, and bone (Firestein, 2017; Turesson *et al.*, 2003). This complex ailment exerts its influence globally, afflicting approximately 1% of the world's population, with a prevalence of 0.14% to 0.55% among Pakistanis (Ghouri *et al.*, 2023). It demonstrates a gender bias, affecting females three times more frequently than males. Although the onset of RA can occur at any stage but mostly it affects people between 40 to 60 years of age (Alamanos and Drosos, 2005; Alamanos *et al.*, 2006). Moreover, the influence of this autoimmune disorder is heightened in smokers, with a

prevalence up to three times higher, especially among those who test positive for rheumatoid factor (Bang *et al.*, 2010).

RA is a mysterious ailment with origins still poorly understood. Genes, hormones, infections, and other autoimmune disorders are considered significant contributors (Firestein, 2017). While biological agents like bacteria, viruses and fungi are suspected culprits, we haven't proven it yet. Environmental factors such as tobacco use, exposure to silica minerals, and existing chronic conditions are prone to RA development (Klareskog *et al.*, 2006). The complex nature of RA involves inflammation driven by cytokines, specifically pro-inflammatory ones like leukotriene B4 (LTB4), tumor necrosis factor-alpha (TNF-alpha), interleukin-6, and interleukin-1b. These increased cytokine levels may lead to the deterioration of joints' synovium, synovial fluid, and cartilage, as revealed by serological studies (Brod, 2000). Genetic factors also play a substantial role, contributing to 50-60% of RA development (MacGregor *et al.*, 2000).

Patients with RA have a great variety of auto-antibodies in their serum. Detection of RA is based on these serological markers. The most commonly occurring auto-antibodies include rheumatoid factor (RF), cyclic citrullinated peptides (CCP), anti-perinuclear factor (APF), anti-keratin antibodies (AKA) and anti-filaggrin antibodies (AFA) (Nienhuis and Mandema, 1964; Young *et al.*, 1979; Hoet *et al.*, 1991; Schellekens *et al.*, 1998, 2000). RF is a classical antibody in RA diagnosis (Jaskowski *et al.*, 2010).

* Corresponding author: waqassiddique614@gmail.com, mtf2202199@ue.edu.pk
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According to American College of Rheumatology criteria for Rheumatoid Arthritis (RA), RF is the only reliable diagnostic antibody with high sensitivity (Avouac *et al.*, 2006). However, false negative results were also reported in many autoimmune diseases like chronic hepatitis, systemic lupus erythematosus (SLE) and Sjogren's syndrome (Dörner *et al.*, 2004; Sauerland *et al.*, 2005).

The Waaler-Rose hemagglutination assay is the prevailing method to analyze RF during the last 50 years (Plotz and Singer, 1956a, b). Many additional methods have also been developed, including different latex particle agglutination assays and isotype-specific enzyme immunoassays using various sources of IgG antibodies. The biological role of RF is not clearly understood. Therefore, various studies using different detection methods have contradictory results regarding sensitivity and specificity of RF test (Bas *et al.*, 2002).

Cyclic citrullinated peptides (CCP) are particular type of proteins. During inflammation arginine residues in proteins such as vimentin can be enzymatically converted into citrullinated peptides, in a process called citrullination. If their shapes are significantly changed, then these proteins may be taken as antigens by the immune system, provoking an immune response. The reactivity of RA sera depends on the citrulline residues present, since the same peptides in which the citrulline was replaced by any other amino acid were not antigenic (Raptopoulou *et al.*, 2007). These auto-antibodies can be detected even prior to development of clinical symptoms of joint destruction and inflammation. Recent studies have documented that anti-CCP antibodies showed good specificity in early detection of RA than any other class of antibodies (Rantapää-Dahlqvist *et al.*, 2003; Vallbracht *et al.*, 2004). Several assays for detecting anti-citrullinated peptide antibodies (ACPAs) were developed employing mutated citrullinated vimentin (MCV-assay), flaggrin-derived peptides (CCP-assay) and viral citrullinated peptides (Coenen *et al.*, 2007; Nishimura *et al.*, 2007).

When patients are positive for CCP as well as for RF, it is very likely that they may develop a severe form of the disease. When patients are negative for either CCP or RF, then the clinical symptoms are more important in determining the disease outcome. When patients are negative for both the CCP and RF, it is less likely that they may have RA (Feldmann *et al.*, 1996). Present study aims to clarify the comparative role of these auto-antibodies in diagnosis of RA in a local Pakistani population.

MATERIALS AND METHODS

Blood samples were collected from suspected RA patients from different Laboratories in Lahore for

diagnosis of RA from February 2022 to February 2023. Total number of samples collected was 65 out of which 29 were males of 12-74 years of age and 36 were females of 15-70 years of age. These samples were tested for RF and Anti-CCP.

For qualitative determination of RA in serum the latex agglutination method described by Plotz and Singer (1956a, b) was used. For the determination of Anti-CCP, diagnostic kit of ABBOT AxSYM was used which determined the IgG class of autoantibodies specific to cyclic citrullinated peptides in human serum and plasma (Schellekens *et al.*, 1998, 2000). The data was analyzed by using Chi-square test.

RESULTS

Table I describes overall prevalence of RF and anti-CCP in RA. Out of 65 samples for suspected RA patients, 41 were found positive for RA on the basis of RF, which constituted about 63% on the other hand 29 of this sampled population were positive for RA on the basis of anti-CCP with a prevalence of 44.6%. All anti-CCP positive patients were those that are RF-RA positive as well. Statistically significant difference was observed between two groups for the detection of RA (p -value <0.05).

Table I. Overall prevalence of RF and Anti-CCP in RA.

Total	RF +ve (%)	RF -ve (%)	Anti-CCP +ve (%)	Anti-CCP -ve (%)
65	41 (63%)	24 (37%)	29 (44.6%)	36 (55.4)
p -value		0.03		
Chi-square value		4.45		
Df		1		

Table II. Gender wise prevalence of RF and anti-CCP in RA.

RF +ve		Anti-CCP+ve	
Male	Female	Male	Female
19	22	12	17
p -value 0.68			
Chi-square value 0.17			
Df 1			

Table II describes the gender wise prevalence of RF and anti-CCP in RA. Out of 29 males, 19 were positive for RF, which constituted 65.5%, while 22 females were positive for RF out of 36 which constituted 61.1%. The individuals positive for anti-CCP were 29, out of them 12 were male (41.3%) while 17 were females with a prevalence of 58.7%. No significant difference was

observed between males and females with reference to positivity for anti-CCP and RF (p -value >0.05).

Table III. Age wise prevalence of RF and anti-CCP in RA.

RF +ve		Anti-CCP +ve	
≤ 32 years	> 32 years	≤ 32 years	> 32 years
9	32	1	28
p -value 0.02			
Chi-square value 4.749			
Df 1			

Table III describes the age wise prevalence of RF and anti-CCP in RA. Among sampled population, 23 were in the age of 32 years or below while 42 were above 32 years of age. In the age group of 32 years or below, 9 were positive for RF with a prevalence of 39.1%. In the age group of above 32 years, 32 were positive for RF with a prevalence of 76.1%. In the age group of 32 years and below 3 were positive for anti-CCP (13%). On the other hand in the group above 32 years of age, 28 were positive for anti-CCP (61.9%). A significant difference (p -value < 0.05) was observed in two age groups for the prevalence of RF and anti-CCP in RA patients.

DISCUSSION

RA is disease of an unknown origin. Infections, genes, hormones and certain environmental factors such as smoking and exposure to heavy metals are considered to be associated with disease onset. It usually begins with a minor joint pain, stiffness and fatigue (Klareskog *et al.*, 2006; Linn-Rasker *et al.*, 2006). Being systemic illness, it also affects other organs, including skin, heart, blood vessels, lungs and liver etc. (Scott *et al.*, 2010).

In the present study, suspected RA patients were analyzed for RF and anti-CCP auto-antibodies. The prevalence of RF was found to be higher (63%) in patients, than that of anti-CCP. Among RF +ve RA cases it was observed that 70% were positive for anti-CCP. It reflected that RF is more sensitive than anti-CCP in RA patients. Similar findings are reported by Jaskowski *et al.* (2010). They observed that RF has a prevalence of 80% while anti-CCP has a prevalence of 65% among suspected RA patients. There were more anti-CCP +ve specimens among RF +ve subset of RA. The prevalence of anti-CCP positivity was 76% in RF +ve patients of RA. Scherbaum *et al.* (1984) observed the increased levels of RF while studying an association between RA and autoimmune thyroiditis (Scherbaum *et al.*, 1984).

Results of the present study for greater sensitivity of RA are supported by van Venrooij *et al.* (2002). They described that RF test has good sensitivity for RA but its specificity is low for the disease as it can also be detected in the serum of patients with other rheumatic or inflammatory diseases and even in the healthy population (van Venrooij *et al.*, 2002). However, during the present study RF sensitivity was studied in RA exclusively. Other autoimmune diseases were not considered that may have some confounding effects on presence of RF.

Contradictory results are also reported by various studies for comparative efficacy of RF and anti-CCP tests. van Gaalen *et al.* (2005) observed that although CCP-assays provide a sensitivity comparable with that of RF, however analysis of the correlation of anti-CCP antibody titer with RA disease activity yielded conflicting results (van Gaalen *et al.*, 2005). Gottenberg *et al.* (2005) observed in a comparative analysis of RF with anti-CCP that the proportion of rheumatoid factor (75.6%) and the proportion of anti-CCP positivity (75.9%) were significantly high (Gottenberg *et al.*, 2005).

Oliver and Silman (2006) reported that females are three times more affected by RA than males. RA is more common among females than males, which may be due to gender related hormonal differences (Oliver and Silman, 2006; Gibofsky, 2012). Alamanos and Drosos (2005) reported that females are three times more affected by RA than males (Alamanos and Drosos, 2005). During the present study gender wise prevalence of RA was also studied. More females were found positive for RF and anti-CCP than males. However, the difference was not significant statistically ($p > 0.05$) which might be due to short sample size in our study.

RA is considered an age-related disorder. The age wise prevalence of RA was also studied in RF +ve and anti-CCP +ve cases. In the age group of 32 years and below the prevalence of the disease was very low; on the other hand prevalence was significantly high in the group of above 32 years of age. These findings are in accordance with the findings of Alamanos and Drosos (2005) who reported that onset of the disease occurs more frequently between 40 to 60 years of age. Results of the present study are also similar to the findings of Scott *et al.* (2013) who reported the peak age of RA onset is above 40 years of life. This high prevalence may be related to age related hormonal changes, nutritional factors, long term exposure to heavy metals and oxidative stress as associated risk factors with the onset of RA in late stages of life.

CONCLUSION

From results of the present study, it is concluded that

RF is more efficient, sensitive and cost-effective strategy than anti-CCP for diagnosis of RA. Moreover, the onset of disease is also found to be associated with the age of the patients as well. However small sample size is a limitation of this study. Future studies involving large population fraction may be helpful in revealing greater details.

DECLARATIONS

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Ethical statement and IRB approval

All procedures were in agreement with the declaration of Helsinki. The Advance Study and Research Board at University of Education, Lahore approved the protocol of the present study. Permission from Ethical Committee, University of Education, Lahore, was also taken for the research work.

Consent for publication

All authors consented for the publication.

Availability of data and materials

Data and materials will be made available as required.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Statement of conflict of interest

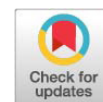
The authors have declared no conflict of interest.

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Contamination of White and Brown Rice with Aflatoxin and Evaluation of Strategies for their Decontamination

Roheela Yasmeen^{1*}, Sadia Amjad¹ and Naseem Zahra²

¹Lahore Garrison University, Pakistan

²Pakistan Council of Scientific and Industrial Research, Pakistan

ABSTRACT

Rice is a primary food crop for over 50% of the world's population, with approximately 500 million tons of milled rice produced globally each year. Significant food wastage occurs due to poor storage conditions, with an estimated economic loss of US\$1.6 million in the rice industry attributed to aflatoxins (AFs), which also pose serious health risks. Various research efforts are underway to improve storage methods and reduce AF contamination. In this study, white and brown rice samples were analyzed for aflatoxin contamination and treated for detoxification. A total of 50 samples, 30 white rice and 20 brown rice were collected from different areas in and around Lahore, Pakistan. Aflatoxins were quantified using Thin Layer Chromatography. Various detoxification methods, including physical, chemical, and natural approaches, were applied to contaminated rice. Contamination was found in 36% of white rice samples, with 23% exceeding European Union permissible limits. In brown rice, 40% of samples were contaminated, with 20% exceeding permissible levels. The highest aflatoxin concentration ($16.45 \pm 0.06 \mu\text{g/kg}$) was found in brown rice. Detoxification results showed a maximum reduction of 40.09% through cooking, 62.31% with 10% citric acid, and 82.24% with 10% clove oil for physical, chemical, and natural methods, respectively. Among these, essential oils proved to be the most effective for detoxification. The study concludes that aflatoxins are a major cause of rice spoilage, and the use of natural storage methods, such as essential oils, offers a promising solution for reducing contamination.

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Authors' Contribution

RY, SA: Investigation. NZ: Supervision.

Key words

Aflatoxins, Thin layer chromatography, Contamination, Detoxification, Essential oils

INTRODUCTION

Rice (*Oryza sativa* L.) is a major staple food all over the world (Trucksess *et al.*, 2011; Choi *et al.*, 2015). It is consumed by around 75% of the global population and 60% of South Asians. In grain crops, rice (*Oryza sativa* Linn.) are second large crop after wheat (Lutfullah and Hussain, 2012). Rice production in Pakistan is approximately 4.9 tons/hectare which makes it world's 11th biggest producer and it contributes 1.3% in gross domestic production of country (Eslami *et al.*, 2015; Suleman *et al.*, 2018; Arshad *et al.*, 2019; Khan *et al.*, 2019). A lot of food waste occurred due to storage incapability as approximately US\$ 1.6 million economical loss is only related to rice industry due to AFs. AFs are a group of nearly

20 structurally similar mycotoxins found in a wide range of foods, such as cereals, spices, nuts, figs, and dried fruit. Though, the highest levels are produced in the food crops that cultivated in the tropical regions around the globe. Because of the worldwide trade of important crops, AFs are at high concern not just for producing countries as well as for consuming ones (Prandini *et al.*, 2009). AFs (AFTs) production is caused by the fungi *Aspergillus flavus*, *Aspergillus nomius*, *Aspergillus parasiticus* and *Aspergillus pseudotamarii* (Hassan *et al.*, 2017; Iqbal *et al.*, 2019; Nazir *et al.*, 2019). Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), and Aflatoxin G2 (AFG2) are the four primary naturally occurring AFs labelled depending on their color variations in Ultraviolet (UV) light and their retention factor (Rf) on Thin Layer chromatography (TLC). AFG2 < AFB2 < AFG1 < AFB1 is the sequence of poisonous of above mentioned AFs from acute to chronic (Tahir *et al.*, 2018).

Rice is mostly grown in subtropical climates that are known for being hot and moist. Rice is usually dried after harvesting, however when stored improperly, it can become an ideal substrate for the mould production (Reiter *et al.*, 2010; Lai *et al.*, 2015). Improper storage conditions lead to the fungal development and other harmful substances

* Corresponding author: raheelasattar44@gmail.com
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resulting in the loss of 15% of produced rice each year as per FAO (Dors *et al.*, 2009).

AFs pose a potent threat to human health, either through direct intake of food or through the transmitted AFs and their byproducts in milk and meat (Nordkvist *et al.*, 2009; Reiter *et al.*, 2010; Naseer *et al.*, 2014). AF B1 is the most hazardous of the AFs, having being linked to hepatocellular carcinoma (HCC) along with growth retardation, immunological alteration, and malnutrition (IARC, 2012). Considering AF toxicity, a lot of states have established AF tolerance levels in foods. The maximal tolerated levels for the AFs in white rice are 4 µg/kg for all AFs (AFG1 + AFG2 + AFB1 + AFB2) and 2 µg/kg for AF B1 and in brown rice these are recorded as 10 µg/kg for all AFs and 5 µg/kg for AF B1 as set by the European Unions (EU, 2010).

For the study of AFs, different techniques such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and ELISA can be employed (Matabaro *et al.*, 2017). However, Thin layer chromatography is one of the best technique which has potential to detect levels of toxins and can produce good and reliable results (Namir *et al.*, 2019). AFs have been found in rice and rice products reported from various countries such as Iran (Mazaheri, 2009), Pakistan (Majeed *et al.*, 2013; Iqbal *et al.*, 2014), China (Zhu *et al.*, 2013), and Scotland (Ruadrew *et al.*, 2013). The escalating number of studies on AFs prevalence in foodstuff and feed necessitates the development of detoxification processes that not only lowers the AFs concentration to “secure” levels under established limits but also meet the following criteria: simple to utilize, affordable and free of the risk of producing toxic substances or affecting the nutrient profile of the treated item (Méndez-Albores *et al.*, 2004).

This study was aimed to determine the AF concentration in white and brown rice, to compare the incidence levels in both types of rice and was compared to European Union maximum limits. The study also purposed to see appropriate detoxification methods such as physical, chemical and natural for reduction of AF contamination in white and brown rice.

MATERIALS AND METHODS

Collection of samples

A total of 50 samples of white (30) and brown (20) rice were collected from various shops of five areas in and around Lahore that included Gulberg, Iqbal town, Walton, Shahdara and Kasur during August to October 2020. Ten samples were collected from each area; 6 of white rice and 4 of brown rice. One kg of rice sample was taken from a large sized jute sacks from 2-3 different places of jute

bag diagonally. The collected rice samples were then passed through sample divider and obtained 200g of rice from each sample and mixed thoroughly and grinded to fine powder in a grinding mil for experimental analysis (Trucksess, 2005). All the samples were properly labelled as Gulberg (G1-G10), Iqbal town (I1-I10), Walton (W1-W10), Shahdara (S1-S10), and Kasur (K1-K10) and quantified for AFs by TLC.

Extraction of aflatoxin from rice samples

The extraction procedure was performed by taking approximately 50 g of powdered rice samples in a 500 ml conical flask. Then 150 ml chloroform and 25 ml distilled H₂O was added the flask. The flasks containing rice samples were put on shaking on wrist-action shaker for 30 mins and samples were filtered in beakers by filter paper. Approximately 50 ml of filtrate was placed on heavy duty hot plate for evaporation. Dilutions for spotting were obtained in micro-liter. 25 µL spot of the sample solution was put on a TLC plate with micro syringe. 5 or 10 µL standard spots of AFs (B1, B2, G1, and G2) were also placed on the same plate as internal standards. Then the TLC plate was subjected to two TLC tanks containing mobile phases. The TLC plate was developed with anhydrous ether in first TLC tank up to the mark. After the plate was completely developed it was taken out from tank and dried. Then plate was redeveloped in the same direction in the second TLC tank with acetone and chloroform concentration of 1:9. TLC plate was then removed and left for drying (Nisa *et al.*, 2016; Summia *et al.*, 2021).

Identification and quantification of AFs in rice samples

For the identification and quantification of AFs in the test solution spots, the plate was scanned using ultraviolet light scanner. Comparison was made between fluorescing concentrations of specimen spots with standard spots of AFs. In case of fluorescing spot of sample lied between two standard spots, the average value of two standard spots was taken into consideration. Another very important step in the AF analysis was the fluorescing sample spots. This was carried out by spraying evenly the TLC plate with aqueous sulphuric acid (50/50 v/v). After the spraying TLC plate was allowed to dry and then viewed under UV light (365 nm).

Decontamination of contaminated samples

Detoxification of AFs were carried out by physical methods using washing and cooking (Hwang and Lee, 2006; Park and Kim, 2006), by chemical using hydrochloric acid (0.3% HCl) and citric acid (10%) (Zahra *et al.*, 2013) and natural methods using essential oils such as 10% clove oil and cinnamon oil (Anzlovar *et al.*, 2017).

RESULTS

In this study, 50 rice samples, including 30 white rice and 20 brown rice samples, were analyzed for aflatoxins (AFs). Among the 30 white rice samples, 11 (36%) were found to be contaminated, with 7 samples (23%) within the permissible range and 4 samples (13%) exceeding the permissible range (Table I).

Of the 20 brown rice samples, 8 (40%) were contaminated, with 4 samples (20%) within the permissible range and 4 samples (20%) exceeding it. A larger proportion of brown rice samples (40%) were found to be contaminated compared to white rice samples (36%) (Table II).

Detoxification of highly contaminated samples was performed using methods such as washing, cooking, acids, and oils. Physical methods were the least effective, reducing AFs by 24.31% to 40.09%. Chemical treatments achieved a reduction of 53.73% to 62.31%. However, the most effective results were observed with natural methods using plant oils, where clove and cinnamon oils reduced AFs by 73.88% to 82.24% (Table II).

DISCUSSION

Rice is a primarily essential food crop in several states. Rice is mainly cultivated in these countries during the rainy season. During this season, sun drying of rice, as is done by majority of the farmers, sometimes may not be enough to keep the moisture level of grains low enough to avoid fungal growth. As a result, rice grains having moisture level greater than the required threshold (>14%) may enter the storage

system. Change in color of the grain or hull, quality loss, loss in vitality, and toxin contamination are all detrimental outcomes of such fungal invasion (Reddy *et al.*, 2009).

Table I. Aflatoxins contamination of white rice and brown rice samples collected from different sites of Lahore permissible range according to EU (2010) is 4 µg/kg for white rice and 10 µg/kg for brown rice.

S. No.		Sample ID	Mean ± SD
White rice samples			
1	Gulberg	G3	1.89 ±0.02
2	Iqbal Town	I4	1.74 ±0.01
3	Walton	W2	2.42 ± 0.03
4		W3	1.85 ± 0.07
5		W5	7.44 ± 0.2
6	Shahdara	S1	8.73 ± 0.02
7		S4	2.41 ± 0.03
8		S6	1.67 ± 0.04
9	Kasur	K3	4.60 ± 0.04
10		K5	1.54 ± 0.03
11		K6	5.45 ±0.1
Brown rice samples			
1	Gulberg	G1	13.21 ± 0.03
2		G3	6.83 ± 0.1
3	Walton	W2	4.14 ±0.02
4		W4	3.92 ± 0.05
5	Kasur	K4	1.35 ± 0.02
6	Iqbal Town	I2	16.45 ± 0.06
7	Shahdara	S1	15.72 ± 0.01
		S4	11.06 ± 0.04

Table II. Detoxification of contaminated white rice samples S1 and brown rice sample I2 by different physical, chemical, and natural methods.

Sample	Initial AF contaminations (µg/kg)	Methods	Type	AF concentration after decontamination process	Reduction in contamination (%)
Shahdara SI (white rice)					
	8.73 ± 0.02	Physical	Washing with water	6.45 ± 0.03	26.11
			Cooking	5.23 ± 0.1	40.09
			0.3% HCl	3.97 ± 0.07	54.52
		Chemical	10% Citric acid	3.29 ± 0.12	62.31
		Natural	10% Clove oil	1.55 ± 0.2	82.24
			10% Cinnamon oil	2.28 ± 0.1	73.88
Iqbal Town I2 (Brown Rice)					
	16.45 ± 0.06	Physical	Washing with water	12.45 ± 0.02	24.31
			Cooking	10.03 ± 0.05	39.02
		Chemical	0.3% HCl	7.61 ± 0.3	53.73
			10% Citric acid	6.58 ± 0.1	60
		Natural	10% Clove oil	2.97 ± 0.04	81.94
			10% Cinnamon oil	4.84 ± 0.15	70.57

38% of all samples were found tainted with further differentiation of 36% from white rice and 40% from the brown rice in the present study. A research study that was carried out in Pakistan revealed the prevalence of higher AFs contamination of 70% in the rice samples and the mean contaminated levels were 4.9 µg/kg that surpassed the secure level established by European Union 4 µg/kg. AF B1, AF B2, AF G1, and AF G2 were present in levels up to 18, 10.4, 8.4, and 12 µg/kg, accordingly (Ali *et al.*, 2011).

Various varieties of rice, comprising parboiled, white, brown, paddy, and broken, were documented to have AFs (Iqbal *et al.*, 2012) and results of white and brown rice samples were comparable to our existing findings. Paddy rice were majorly infected (64%), while brown rice were least effected (33%) followed by white rice (42%). The study also claimed dietary rice as a main source of AFs in human beings. Asghar *et al.* (2014) documented AFB1 and AFB2 prevalence in 95.4% and 7.6% in Pakistani brown rice, respectively that as very high as compared to our findings for the brown rice that was 40%. Whereas AFG1 and AFG2 were not found in any sample and that was very similar to current study. Overall AFs occurred between 1.07 and 27.27 µg/kg.

Different physical, chemical and biological methods were also adopted to reduce AF levels in rice. Park *et al.* (2005) concluded that the reduction of AFB1 in rice after washing with water ranged from 20 to 24%, with an average of 22% which support our results.

In different studies conducted in Korea, cooking positively decreased total AFs 34% and 31-36% in originally contaminated refined rice (Park *et al.*, 2005; Park and Kim, 2006). Both the studies were in line with our work. The impact of steaming at 160 °C by ordinary cooker on AF deposits in the cooked rice had been documented previously by others (Park *et al.*, 2005).

Zahra *et al.* (2013) documented 55% decrease of AFB1 in rice by 0.3% HCl which is in agreement with the results of our experiment. As HCl is a strong acid so its mild concentration is used in experiment because high concentration can change the organoleptic properties of food.

Citric acid (C₆H₈O₇) was utilized for the conservation measures. It has particular, distinctive, corrosive and tart flavors, incorporated into different kinds of beverages and edibles. Lemon juice comprises of 5 % citric acid. Reaction with the diluted citric acid degraded AF B1 in rice. In present study tainted samples were immersed in lemon juice for decontamination. The outcomes of that experiment revealed that citric acid was 63.59% to 90% effectual, against AFB1 in the various types screened (Safara *et al.*, 2010; Karlovsky *et al.*, 2016).

Few essential oils and compounds in plant extracts are taken up as generally recognized as safe (GRAS). Different amounts (0, 2, 4, 6, 8, and 10% (w/v)) of *Syzygium aromaticum* pure diluted extracts were screened for their potential resistive impact on *Aspergillus* growth and AF formation in 50 g of rice. The outcomes revealed that *Syzygium aromaticum* at 10% (w/v) and restricted the *Aspergillus* growth and also lowered the AF formation rate in rice (Thanaboripat *et al.*, 1997). Reddy *et al.* (2009) noticed that mold growth and AF development were totally restricted after employing of diluted extracts from clove on healthy kernels of a rice cultivator for five days. Eugenol (C₁₀H₁₂O₂) is a phenolic substance obtained chiefly from buds and leaves of *Syzygium aromaticum* and from *Cinnamomum zeylanicum*, comprising the most important and active compound of clove oil (85 to 95%) along with methyleugenol and iso-eugenol (Souza *et al.*, 2005). The most effectual compounds of cinnamon essential oil because of their biological activities were suggested to be cinnamaldehyde and eugenol (Siddiqua *et al.*, 2015).

Physical and chemical decontamination strategies may have certain restrictions like loss of minerals, ineffectiveness and variations in the organoleptic properties of food. But, the application of natural methods (plant oils) is a secure way of detoxification of AF contamination in rice samples. This study provided updated knowledge regarding AFs contamination in different areas and results provided a good contribution to food authorities and they can form and imply regulations to control this.

CONCLUSION

It was concluded that the contamination percentages of aflatoxins (AFs) differed between brown and white rice samples, with higher concentrations detected in brown rice. Rice samples collected from various locations exhibited varying levels of AF contamination, with the highest levels found in samples from Walton and Shahdara, likely due to improper storage conditions. While physical and chemical detoxification methods were moderately effective, natural methods proved more effective for long-term storage. This study provides updated insights into AF contamination across different rice types and regions, contributing valuable information for food authorities regarding the implementation of safety regulations.

DECLARATIONS

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Statement of conflict of interest

The authors have declared no conflict of interest.

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Unraveling Renal Complexities in Thalassemia Major: A Comprehensive Nephrological Inquiry in Central Punjab, Pakistan

Muhammad Waqas^{1*}, Razia Bashir¹, Khadija Munir¹ and Muhammad Arshad²

¹Department of Zoology, Division of Science and Technology, University of Education, Township, Lahore, Punjab, Pakistan.

²Department of Zoology, Government College University, Lahore, Pakistan

ABSTRACT

This research investigates the intricate nexus between thalassemia major and renal dysfunction in Central Punjab, Pakistan. A comprehensive analysis was conducted on a diverse cohort of beta-thalassemic patients to explore the prevalence and factors influencing renal profiles. The study encompassed demographic factors, family history, and caste systems, shedding light on their associations with beta-thalassemia. Furthermore, gender and age-specific assessments were performed, focusing on serum creatinine and urea levels to provide a nuanced understanding of renal function. The findings revealed a higher prevalence of beta-thalassemia in the 11-20 years age group, emphasizing the critical role of adolescence and early adulthood in the manifestation of this genetic disorder. Family history emerged as a significant factor, with patients having positive family histories showing a higher prevalence. Caste-based analysis highlighted variations in prevalence, with Arains and Rajpoot exhibiting the highest rates. Renal profile assessments, including serum creatinine and urea levels, indicated stable renal function across genders and age groups. No significant variations were observed, reaffirming the stability of renal parameters in beta-thalassemic patients. The study contributes valuable insights for targeted healthcare strategies, genetic counseling, and awareness programs in managing thalassemia major in the Central Punjab population.

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Authors' Contribution

MW contributed as a co-researcher, provided technical assistance and formatted the research article. RB participated in the revision process and contributed to manuscript preparation. KM conducted the research work. MA supervised and guided the overall research.

Key words

Thalassemia major, Renal dysfunction, Serum creatinine, Serum urea

INTRODUCTION

Thalassemia, a complex and heterogeneous group of disorders, is recognized as an autosomal recessive blood disorder with a global prevalence across all races (Badeli *et al.*, 2019). This genetic and inheritable disease is characterized by the defective production of hemoglobin, the crucial protein responsible for oxygen transport (Bakr *et al.*, 2014). First identified in the early 1920s by Dr. Denton Cooley, thalassemia major presented a distinctive clinical profile in seven children of Italian or Greek origin (Borgna-Pignatti, 2010). The term thalassemia, originally coined by Sarrai (2012), Mary *et al.* (2017), finds its roots in the Greek words thalassa (sea)

and haima (blood), underlining the condition's impact on blood composition (Galanello and Origa, 2010).

Beta thalassemia major, the focal point of this inquiry, involves three pivotal pathophysiological factors: chronic anemia hemolysis, ineffective erythropoiesis, and secondary iron overload (Bekhit *et al.*, 2017). Associated symptoms encompass a spectrum of clinical manifestations, including failure to thrive, progressively pale color, feeding problems, diarrhea, recurrent fever episodes, delayed growth, jaundice, dark urine, poor muscle development, chronic hypoxia, hepatosplenomegaly, and the formation of masses due to extramedullary hematopoiesis (Raza *et al.*, 2016). Skeletal alterations arising from bone marrow expansion contribute to long bone deformities in the legs, while distinctive craniofacial changes involve skull protuberance, prominent malar eminence, nasal bridge depression, and a tendency for exposed upper teeth (Sultan *et al.*, 2016).

In addition to the hematological challenges, thalassemia major poses a significant risk of renal complications. Renal failure, indicative of the kidneys' inability to perform excretory functions, results in the retention of nitrogenous waste products in the blood. The renal profile, a comprehensive measure of kidney functions, encompasses key markers such as creatinine,

* Corresponding author: waqassiddique614@gmail.com, mtf2202199@ue.edu.pk
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urea, uric acid, and glomerular filtration rate (GFR). Chronic kidney disease, defined by kidney damage or a GFR less than 60 mL/min per 1.73 m² for three months or more, is a formidable concern for individuals with thalassemia major (Demosthenous *et al.*, 2019). Notably, renal issues rank as the fourth most common cause of morbidity, following endocrine, cardiovascular, and hepatic concerns, underscoring the multifaceted nature of thalassemia major and its impact on overall health (Demosthenous *et al.*, 2019).

Creatinine levels in serum serve as a widely utilized test for assessing renal function. Creatinine, a breakdown product of creatine phosphate in muscle, is typically produced at a constant rate by the body, dependent on muscle mass (Economou *et al.*, 2010). While freely filtered by the glomeruli, under normal conditions, Creatinine is not re-absorbed by the tubules to any appreciable extent. Elevated blood creatinine levels are indicative of marked nephron damage, making it less suitable for detecting early-stage kidney disease. The apparent differences in serum creatinine levels may be attributed to factors like age and weight, with lower values potentially reflecting the influence of younger age and smaller body weight, as serum Creatinine is affected by muscle mass (Economou *et al.*, 2010).

Urinary N-actly-D-beta glycosidase (NAG) excretion emerges as a valuable tool for early detection of proximal tubular epithelial cell malfunction in beta thalassemia major patients, offering a reliable marker for tubular cell status (Bekhit *et al.*, 2017). Identifying high-risk patients prone to renal damage is crucial for implementing timely interventions to mitigate the progression of renal injury and reduce the incidence of renal impairment. Recognizing the significance of urinary NAG excretion in monitoring tubular cell status aids in tailoring specific measures for individual patients (Bekhit *et al.*, 2017).

Chronic anemia, iron overload, and iron chelation therapy are implicated in the etiology of these abnormalities. Anemia induces alterations in renal hemodynamics, disrupting renal plasma flow and glomerular filtration rate (GFR) in beta thalassemia patients (Bekhit *et al.*, 2017). Despite an initial increase in renal plasma flow due to glomerular hyperfiltration and renal hyperperfusion resulting from anemia, prolonged anemia leads to a significant decrease in GFR over time. Additionally, non-progressive increases in serum creatinine levels have been noted following exposure to certain iron chelators. Case reports, such as a 62-year-old man experiencing renal function effects with Deferasirox, underscore the importance of monitoring and managing iron chelation therapy to prevent adverse renal outcomes (Brosnahan *et al.*, 2008; Musallam and Taher, 2012).

Post-marketing surveillance has revealed several cases of acute kidney injury associated with the oral chelator Deferasirox, reinforcing the need for careful consideration and monitoring in thalassemia patients undergoing iron chelation therapy (Musallam and Taher, 2012).

In conclusion, this research endeavors to shed light on the intricate nexus between thalassemia major and renal dysfunction in Central Punjab, Pakistan. By comprehensively characterizing the prevalence and genetic aspects of thalassemia major in this unique demographic, the study aims to unravel the specific nature of renal complications associated with the disorder. Additionally, the research seeks to identify early predictors of renal dysfunction, exploring the potential utility of markers such as urinary N-actly-D-beta glycosidase (NAG) excretion. Through a meticulous examination of factors influencing renal function, including chronic anemia, iron overload, and iron chelation therapy, the study aspires to provide region-specific insights that inform both clinical management and public health strategies. Ultimately, this research contributes to the global understanding of thalassemia major and offers tailored insights for optimizing healthcare practices in the Central Punjab, Pakistan population.

MATERIALS AND METHODS

The experimental study was conducted at the Department of Zoology, University of Education Lower Mall Campus Lahore, Pakistan. Blood samples were collected between August 2019 and June 2020 from individuals diagnosed with thalassemia major at Fatimid Foundation Johar Town Lahore, Pakistan. A total of 185 blood samples were obtained from patients whose thalassemia status was confirmed through genetic and hemoglobin analyses.

Study subjects

The study included 185 patients diagnosed with thalassemia major based on comprehensive genetic and hemoglobin analyses.

Sample collection

Blood samples were collected via vein puncture, with meticulous attention to proper site sterilization using antiseptic measures to prevent contamination. Sterile, disposable, 23-gauge Shifad syringes (5ml) were used for blood sampling, with 3ml of blood collected from each patient. The collected samples were deposited into serum separating tubes (SST or yellow top vials) containing anticoagulant dipotassium ethylenediamine tetra acetic acid (K2-EDTA). Subsequently, samples were centrifuged (Cryofuge 16) for 2 min at 3500 rpm, and the serum

samples were stored at 2-8 °C until further analysis.

Laboratory analysis

Quantitative determination of creatinine in serum utilized the CREJ2 diagnostic kit (lot no. 04810716) with a measuring range of 0.17-24.9 mg/dL. Urea levels in serum were quantitatively determined using the diagnostic kit (Cat. No. 04460715) with measuring ranges of 3.0-240 mg/dL urea. For uric acid quantification in serum, the diagnostic kit Cat. No. 03183807 with a measuring range of 0.2-25.0 mg/dL was employed. These analyses were conducted using the Cobas c 111 Roche automatic analyzer. The selection of these specific diagnostic kits and analyzer ensured accurate and standardized measurements for the assessment of renal function parameters in thalassemia major patients.

Statistical analysis

The data collected for the study underwent meticulous coding and entry using the statistical package for social science (SPSS) software. The dataset was comprehensively summarized utilizing measures such as mean and standard deviation to provide a robust overview of the key parameters. Group comparisons were performed employing the Kruskal-Wallis test, which is particularly suitable for non-parametric data. The threshold for statistical significance was set at a P value of ≤ 0.05 , ensuring a rigorous evaluation of the study outcomes.

RESULTS

Gender wise prevalence of β -thalassemia

The gender-wise distribution of beta-thalassemia patients, as illustrated in Figure 1, revealed intriguing patterns. Categorized into male and female groups, the prevalence of beta-thalassemia was notably higher among males, accounting for 54.54%, compared to females, where the distribution stood at 45.45%. This significant gender-based disparity in the prevalence of beta-thalassemia underscores the need for further exploration into potential biological or societal factors influencing the observed variations. Incorporating the Kruskal-Wallis test into the statistical analysis ensured robust assessments of group differences, providing a more comprehensive understanding of the data. The utilization of SPSS software for data management and analysis adds a layer of reliability to the study's findings, enhancing the credibility of the observed trends.

Age wise prevalence of β -thalassemia

Examining the age-wise prevalence of beta-thalassemia (Fig. 2) across four distinct age groups (1-10 years, 11-20 years, 21-30 years, and 31-40 years)

revealed noteworthy insights. The prevalence of beta-thalassemia in the 11-20 years age group was strikingly higher at 45.45%, highlighting a significant concentration during adolescence and early adulthood. In contrast, the 31-40 years age group exhibited the lowest prevalence at 1.2%, indicating a potential decrease in beta-thalassemia cases with advancing age. While in the age groups of 1-10 and 21-30 the prevalence of beta-thalassemia was noted 30.30% and 23.03%, respectively. The prevalence order across different age groups was observed as 11-20 > 1-10 > 21-30 > 31-40, emphasizing the pivotal role of screening and intervention strategies, particularly during the adolescent years. These findings provide valuable data for targeted healthcare initiatives and underscore the need for age-specific awareness programs in addressing beta-thalassemia prevalence.

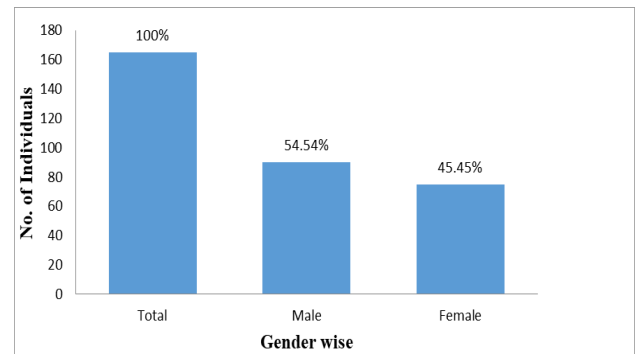


Fig. 1. Gender wise prevalence of β thalassemia.

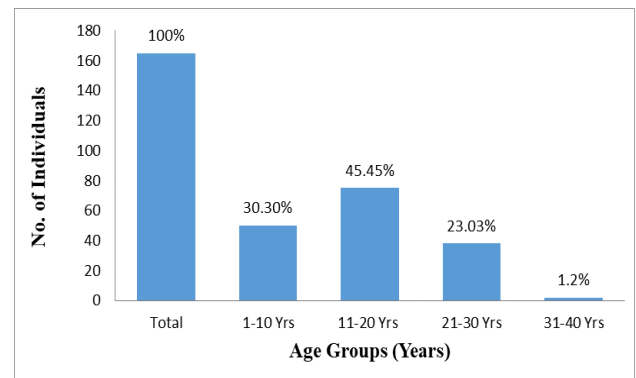


Fig. 2. Age wise prevalence of β thalassemia.

Family history among β -thalassemic patients

The analysis of family history among beta-thalassemic patients revealed two distinct categories: Positive and negative family histories (Fig. 3). The data demonstrated that 57.58% of beta-thalassemia cases had a positive family history, while 42.42% had a negative

family history. This observation underscores a higher prevalence of beta-thalassemia among individuals with a positive family history compared to those without. The significant influence of familial factors on the occurrence of beta-thalassemia highlights the importance of genetic screening and counseling within affected families for effective preventive measures.

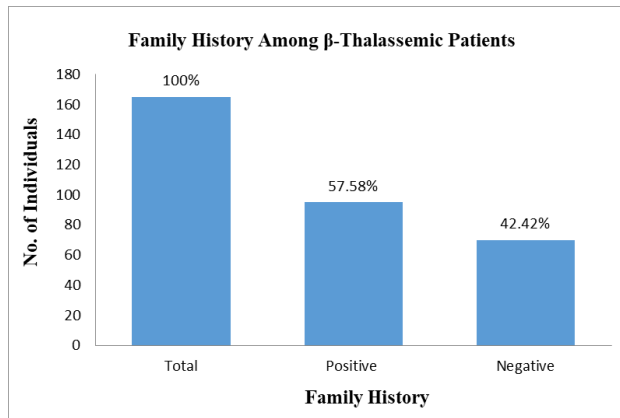


Fig. 3. Family history among β thalassemic patients.

Prevalence of β -thalassemia in different cast systems

Figure 4 shows the assessment of beta-thalassemia prevalence among various caste systems in Pakistan. The Arains and Rajpoots shared the highest prevalence at 19.39%, surpassing other castes. Following closely were Sheikh, Jutt, and Khokhar, with prevalence rates of 12.12%, 9.09%, and 4.48%, respectively. Notably, thalassemia frequency in other castes collectively formed 35.15%, with prevalence less than 5%, providing a distinct subgroup for further analysis. This caste-based distribution underscores the significance of considering diverse sociodemographic factors in understanding beta-thalassemia prevalence, advocating for targeted awareness campaigns and genetic screening initiatives within high-risk caste groups.

Gender wise serum creatinine and urea level (mg/dl) in β thalassemic patients

Table I provides insights into the gender-wise levels of serum creatinine (mg/dl) in beta-thalassemic patients, categorizing them into male (n=88) and female (n=72) groups. The measurement of serum creatinine was integral to understanding renal function as part of the renal profile assessment. The reference value for normal serum creatinine is 0.4 to 1.2 mg/dl. Upon comparing serum levels between genders, no statistically significant difference was observed ($p=0.09$). The mean value of serum creatinine in males was 0.539 ± 0.213 , while in females, it was 0.483 ± 0.201 . The results indicated that

serum creatinine values fell within the reference range, suggesting no variation in serum creatinine levels among beta-thalassemic patients. This observation provides reassurance regarding the stability of renal function within the studied population.

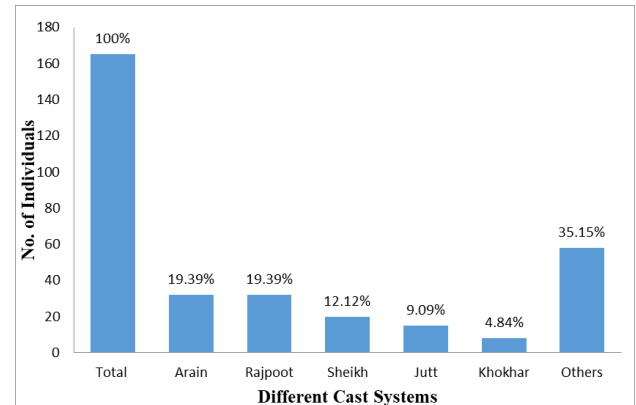


Fig. 4. Prevalence of beta thalassemia in different Cast system.

Table I. Gender wise comparison of serum creatinine level (mg/dl) and urea level (mg/dl) in beta thalassemic patients.

Gender	No.	Mean± SD	p-value
Creatinine level			
Males	88	0.539±0.213	0.09
Females	72	0.483±0.201	
Serum urea level (mg/dl)			
Males	79	20.19±11.33	0.06
Females	63	16.9±9.48	

Table I shows the gender-wise levels of serum urea (mg/dl) in beta-thalassemic patients, encompassing a study population of 142 individuals, of which 79 were males and 63 were females. Serum urea (mg/dl) was measured comprehensively in all beta-thalassemic patients to assess their renal function as part of the renal profile. The reference value for serum urea level (mg/dl) is 10-50 (mg/dl). The mean value of serum urea in males was 20.19 ± 11.33 , and in females, it was 16.9 ± 16.9 . Upon comparing serum urea levels (mg/dl) between genders, no statistically significant difference was observed ($p=0.06$). The results indicated that values of serum urea level (mg/dl) showed no variations, consistently falling within the reference range. This finding suggests stability in renal function across genders among beta-thalassemic patients.

Age wise serum creatinine and urea level (mg/dl) in Beta thalassemic patients

Table II delved into the age-wise levels of serum creatinine (mg/dl) in beta-thalassemic patients, categorizing them into three distinct age groups: 1-10 years (n=46), 11-20 years (n=71), and 21-30 years (n=37). The mean serum creatinine level (mg/dl) in the 1-10 years age group was 76.37, 74.22 in the 11-20 years age group, and 85.20 in the 21-30 years age group. Serum creatinine (mg/dl) measurement served as a crucial aspect of the renal profile assessment. Upon comparing serum creatinine levels in these age groups, no statistically significant difference was observed ($p=0.45$). The results indicated that values of serum creatinine level (mg/dl) exhibited no variations, as they consistently fell within the reference range. This finding suggests stability in renal function across different age groups among beta-thalassemic patients.

Table II. Age wise comparison of serum creatinine level (mg/dl) and serum urea level (mg/dl) in beta thalassemic patients.

Age groups	No.	Mean	P-value
Creatinine level (mg/dl)			
1-10 years	46	76.37	
11-20 years	71	74.22	
21-30 years	37	85.20	
Urea level (mg/dl)			
1-10 years	38	74.36	0.549
11-20 years	68	66.23	
21-30 years	33	72.76	

Table II provided insights into the age-wise levels of serum urea (mg/dl) in beta-thalassemic patients studied in Central Punjab of Pakistan, categorized into three distinct age groups: 1-10 years (n=38), 11-20 years (n=68), and 21-30 years (n=33). The mean level of serum urea (mg/dl) in the 1st age group was 74.36, 66.23 in the 2nd age group, and 72.76 in the 3rd age group. Serum urea (mg/dl) measurement served as a crucial aspect of the renal profile assessment. Upon comparing serum urea levels (mg/dl) in these age groups, no statistically significant difference was observed ($p=0.06$). The results indicated that values of serum urea level (mg/dl) exhibited no variations, consistently falling within the reference range. This finding suggests stability in renal function across different age groups among beta-thalassemic patients in Central Punjab, Pakistan.

CONCLUSION

Thalassemia, an autosomal recessive blood disorder with a global prevalence across all races, is characterized by the defective production of hemoglobin. The heightened prevalence of thalassemia major observed in the 11-20 years age group underscores the critical period of adolescence and early adulthood in the manifestation of thalassemia major. Positive family histories emerged as significant indicators, emphasizing the role of genetic factors in the prevalence of the disorder. Caste-based analysis further delineated variations, with specific castes exhibiting higher rates. The meticulous examination of renal profiles, encompassing serum creatinine and urea levels, demonstrated consistent stability across genders and age groups. These findings have crucial implications for healthcare strategies, genetic counseling, and targeted awareness programs tailored to the unique characteristics of the Central Punjab, Pakistan population.

DECLARATIONS

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Ethical statement and IRB approval

Ethical approval of this study was obtained from the University's Ethical Committee. All procedures adhered to the principles outlined in the Declaration of Helsinki.

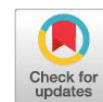
Statement of conflict of interest

The authors have declared no conflict of interest.

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Bioaccumulation of Heavy Metals in Freshwater and Marine Fish Species of Pakistan

Hina Jabeen*, Usman Irshad, Kainat Azhar, Alisha Fatima, Asma Zaheer Abbasi, Tayyaba Sadia and Jaweria Aqeel

Department of Biological Sciences, Virtual University of Pakistan, Lahore

ABSTRACT

Fish have a higher likelihood of surviving under hypo toxic conditions when exposed to heavy metals, but they can accumulate in humans' brain, liver, kidney, lungs, and muscles after consumption, posing a significant health risk. The fish meat quality is the biggest concern in the food chain of the present time. Both water and fish species are contaminated with heavy metals due to anthropogenic activities i.e., domestic, sewage, industrial and agricultural runoff. Heavy metals concentration in the seven fish species was analyzed from different riverine and marine sites of Pakistan. Freshwater fish were collected from Rawal Dam (Islamabad), Mini Dam at Fateh Jang (Attock) and Sulemanki Headworks at River Satluj, Head Sulemanki at River Satluj, River Jhelum, River Chenab and marine fish species were collected from Karachi coastal region. The concentrations of cadmium, chromium, nickel, lead and iron were analyzed in the fish muscles by Atomic Absorption Spectrophotometer. Among metals, the highest concentrations of Pb ($3.391 \pm 0.002 \mu\text{g/g}$) followed by Cr ($0.311 \pm 0.001 \mu\text{g/g}$) were estimated in the freshwater fish species *L. rohita* collected from Rawal Dam and *W. attu* from River Chenab, respectively. The maximum concentrations of Cr ($4.589 \pm 1.008 \mu\text{g/g}$) and Fe ($41.176 \pm 8.123 \mu\text{g/g}$) was observed in marine fish species *S. cavalla* and *Pampus* sp., while Cd showed the intermediate concentration throughout in all fish species. Results showed that the concentration of Pb and Fe exceeded the limit of WHO and FAO which is the potential threat specifically the marine species due to their high consumption rate.

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Authors' Contribution

HJ supervised the study. UI and AF detected metal concentration in the lab. AZA and JA collected fish samples and did statistical analysis. UI and KA wrote the paper.

Key words

Ecotoxicology, Bioaccumulation, Metal contamination, Fish muscles, Anthropogenic activities

INTRODUCTION

Fish play a substantial role within aquatic ecosystems and are recognized as an exceptional protein source for human dietary needs. Globally, fish contribute approximately a quarter of the animal-derived protein that people consume. With the global population on the rise and a growing demand for sustenance, the necessity for fish and related products is escalating (Bahnasawy *et al.*, 2009). As emphasized by the American Heart Association, consuming fish at least two times per week is a vital element in averting coronary heart diseases, ensuring an adequate intake of omega-3 fatty acids (Martin and Genner, 2009).

The accumulation of heavy metals in fish hinges on both the absorption rate of these metals by fish and the metabolic activity of the species. However, in aquatic

ecosystems, fish tend to occupy higher positions within the food chain, consequently resulting in elevated heavy metal concentrations within their bodies. The degree of this accumulation relies on the balance between the rate of intake and excretion within the fish's physiological system (Karadede *et al.*, 2004). Multiple research endeavors have unveiled a range of biotic and abiotic factors contributing to the accumulation of inorganic contaminants in fish tissues and organs. These factors encompass fish species diversity, the trophic level occupied by fish in the ecosystem, feeding patterns, fish age and size, species-specific variances in susceptibility to different metals, the concentration of heavy metals within water bodies, sediment concentrations in the environment, the composition of fish diets, water quality influenced by physical and chemical aspects, the intrinsic nature and chemical properties of heavy metals, and the availability of these metals for uptake (Griboff *et al.*, 2018; Moiseenko and Gashkina, 2020). Given their inherent toxicity, a heightened focus has been placed on the evaluation of heavy metal content in various food sources, particularly marine fish consumed as part of meals. Poultry chicken, owing to elevated arsenic levels in their diet, can harbor heavy metals within their meat (Nachman *et al.*, 2012). Inversely, heavy metals accumulation in fish may absorb into their systems through the aquatic environment, including water, sediments, and feeding intake (Pal and

* Corresponding author: hina.jabeen@vu.edu.pk
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Maiti, 2018; Kumar *et al.*, 2020). Considering current resources, a consistent effort is required to understand how pollutants or metal contaminants invade through food chains in the complex consumption web. Abiotic factors like soil and water are being studied less frequently than live organisms due to the increased awareness of the importance of life (Tashla *et al.*, 2018; Fatima *et al.*, 2020). Furthermore, according to Abdel-Mohsien and Mahmoud (2015), cadmium (Cd) adversely affects kidneys and exerts a lasting toxic influence on reproductive functions, as well as renal and hepatic systems.

Due to their elevated toxicity, enduring presence, and propensity to accumulate, heavy metals rank among the most perilous pollutants within aquatic environments, capturing worldwide attention (Ali *et al.*, 2016, 2018; Karunanidhi *et al.*, 2017; Lao *et al.*, 2019). Their capacity to inflict harm extends to aquatic ecosystems, affecting fish, sediments, and water (Agusa *et al.*, 2007; Hajebe *et al.*, 2009; Bhuyan and Bakar, 2017). These heavy metals accumulate within marine ecosystems, imparting detrimental impacts on both the environment and human well-being (Batvari *et al.*, 2015; Islam *et al.*, 2018). As indicated by several investigations (Terra *et al.*, 2008; Yi *et al.*, 2011; Islam *et al.*, 2016; 2017), the contamination of aquatic settings with these heavy metals raises potential concerns for human health, given that these contaminants enter the human food chain through the consumption of fish and other aquatic products. A prominent global challenge concerning the health risks associated with fish consumption pertains to the contamination of seafood. The nutritional and therapeutic benefits of fish consumption have driven a global surge in its intake in recent years. Fish are notably rich in protein content (accounting for 60% of daily protein requirements), essential minerals, and vitamins (Medeiros *et al.*, 2012; de Anda *et al.*, 2019; Hossein *et al.*, 2021). However, it has been revealed that the consumption of fish tainted with harmful metals has been linked to the emergence of various severe disorders. For instance, chromium (Cr) contamination in fish has been associated with kidney lesions, anuria, nephritis, and extensive lesions (Proshad *et al.*, 2018; Rahman *et al.*, 2012). Cadmium toxicity has been linked to compromised fertility, kidney dysfunction, tumors, hypertension, and hepatic malfunction (Al-Busaidi *et al.*, 2011; Rahman *et al.*, 2012), while lead poisoning is known to cause liver damage and renal failure (Lee and Hieu, 2011). It has been noted that fish gills and skin can be contributing factors to the accumulation of heavy metals in fish (Al-Busaidi *et al.*, 2011). Fish have become significantly contaminated by toxic metals within aquatic environments, primarily through consuming water and organisms like zooplankton and phytoplankton as direct sources of food. As inhabitants

of various trophic levels, spanning all age groups and sizes, and serving as a crucial food source for human populations (Burger, 2002; Kuklina *et al.*, 2014; Karunanidhi *et al.*, 2017; Svobodová *et al.*, 2017), fish are commonly employed as bio-indicators of hazardous metal pollution. The enduring, toxic, and accumulative properties of these metals can give rise to ecological challenges and detrimentally impact aquatic ecosystems (Rahman *et al.*, 2010; Islam *et al.*, 2017). Since fish inhabit and feed within aquatic habitats, they cannot evade the adverse effects of pollutants, rendering them particularly sensitive and susceptible to contamination (Saha and Zaman, 2013). The potential danger of heavy metal ingestion hinges on factors like the absorbed dosage, the mode of exposure, and the duration of exposure, whether it's acute or chronic, as explored in the study by Islam *et al.* (2016). Fish, due to their heightened vulnerability to diverse toxic substances, offer valuable insights as test subjects, aiding in the evaluation of ecosystem health.

Untreated industrial and domestic effluent negatively impacts ecosystems and people's health, affecting fishing, boating, and tourism. To prevent marine extinction and biodiversity loss, frequent monitoring is crucial. Preventing marine pollution along the Karachi Coast is essential for safety, health, and socioeconomic viability (Jilani, 2015). In River Satluj, anthropogenic activities such as dumping of domestic effluents, untreated industrial and sewage water have significantly contributed to the decline of aquatic fish species (Sharma and Singh, 2015). In Rawal Lake, pollutants enter the reservoir from a variety of sources, including garbage from nearby human settlements, car washes, and poultry farms (Malik and Maurya, 2014). Low-slung water quality effect directly affects the aquatic community (Qadir *et al.*, 2008) and humans due to their consumption of aquatic organisms specifically fishes which have pathological changes in their tissue (Ahmed *et al.*, 2014).

The aim of this study was to investigate the accumulation of heavy metals from muscle tissues of relevant marine and freshwater fish species across various regions of Pakistan.

MATERIALS AND METHODS

Sampling sites and collection

Seven fish species were collected from six different sites *Labeo rohita* from Mini Dam Fateh Jang (Attock), River Satluj, and Rawal Dam (Islamabad), *Cyprinus carpio* from River Satluj, *Wallago attu* from River Chenab, *Hypophthalmichthys molitrix* and *Xenentodon cancila* from River Jhelum, and *Scomberomorus cavalla* and *Pampus* sp. from Karachi coastal area in the post monsoon

season from September to November 2023. These samples were packed in thermo-cool boxes with ice packs and transported to the laboratory for further analysis.

Concentration of heavy metal analysis

Dissection of the fish sample was done on the same day in the laboratory with the help of stainless steel and plastic-coated tweezers pretreated with the acetone and rinsed with D₃ water before taking samples from the fish. Fish muscles of 3g were taken in the Pyrex vessel which further pretreated with 8 ml of 65% HNO₃ and 1.5 ml of 30% H₂O₂ to digest the sample. Samples with chemical were kept in the water bath at 80°C for 24 h to get fully digested sample. After heating the samples were cooled down at room temperature and diluted with 7 ml of D₃ water and obtained filtrate was analyzed through atomic absorption spectrophotometer from the given method by [Howell \(1984\)](#).

Statistical analysis

The metal absorption in fish muscles was measured in µg/g (per wet weight). One way ANOVA was used to estimate the difference between metal concentrations by using SPSS version 2.0.

RESULTS AND DISCUSSION

Heavy metals are the major concern in the developed and developing countries because of their toxic nature, persistence and bioaccumulation which cannot be degraded especially in the aquatic environment

([Gerhardt and Fieldes, 1999](#); [Adeyeye et al., 2016](#)). The concentration of these metals differs in different body parts of animals. Fish is widely consumed as food as a rich source of protein which is mostly captured from the riverine and marine waters which are polluted by heavy metals through different point sources. So, it is the most important topic to study heavy metals in the tissues of consumable fishes for formulating future strategies. With these objectives in mind present study was conducted to access the concentration of heavy metals like chromium, cadmium, nickel, lead and iron in seven main fish species which are commonly used to consume in Pakistan. A close assessment of the statistics suggested some significant trends which are discussed here.

The heavy metal concentration in muscles of fish species is given in [Table I](#). All metal accumulation patterns were significantly different ($p < 0.001$) between different localities except for lead ([Table I](#)). There was not a consistent increase observed in metals across all the freshwater fish species except the fishes collected from marine waters (*Scomberomus cavalla* and *Pampus sp.*) for the concentration of chromium and iron, respectively ([Table I](#)).

The mean concentration of cadmium was showing the highest in the muscles of *Labeo rohita* collected from Fateh Jang (0.015 ± 0.001 µg/g) followed by *Cyprinus carpio* (0.014 ± 0.002 µg/g) and lowest in *Xenotodon cancila* (0.009 ± 0.001 µg/g). Chromium concentration was observed highest in marine water fishes

Table I. Mean concentrations of heavy metals (µg/g) in fish samples collected from different sites of Pakistan.

Species	Locality	Number of samples	Metal concentration \pm SD (µg/g)				
			Cadmium (Cd)	Chromium (Cr)	Nickel (Ni)	Lead (Pb)	Iron (Fe)
<i>Labeo rohita</i>	Rawal Dam	4	0.011 ± 0.001	0.016 ± 0.003	0.017 ± 0.002	3.391 ± 0.002	-
	Fateh Jang	3	0.015 ± 0.001	0.005 ± 0.001	0.084 ± 0.003	0.029 ± 0.001	1.085 ± 0.007
	River Satluj	10	0.013 ± 0.002	0.007 ± 0.001	0.089 ± 0.003	0.056 ± 0.002	1.239 ± 0.143
<i>Cyprinus carpio</i>	River Satluj	10	0.014 ± 0.002	0.005 ± 0.001	0.026 ± 0.004	0.044 ± 0.005	1.822 ± 0.086
<i>Hypophthalmichthys molitrix</i>	River Jhelum	3	0.008 ± 0.001	0.007 ± 0.001	0.097 ± 0.001	0.042 ± 0.001	1.267 ± 0.002
<i>Xenotodon cancila</i>	River Jhelum	3	0.009 ± 0.001	0.008 ± 0.002	0.059 ± 0.004	0.017 ± 0.003	1.151 ± 0.008
<i>Wallago attu</i>	River Chenab	3	-	0.311 ± 0.001	0.133 ± 0.003	0.176 ± 0.003	2.786 ± 0.013
<i>Scomberomus cavalla</i>	Karachi coast	5	-	1.723 ± 0.260	0.409 ± 0.127	0.230 ± 0.071	41.176 ± 8.123
<i>Pampus sp.</i>	Karachi coast	5	-	4.589 ± 1.008	0.051 ± 0.006	0.185 ± 0.013	4.723 ± 1.339
One-way ANOVA							
df			11	20	20	20	20
F			15.667	767.522	219.095	0.912	139.439
P			<0.001	<0.001	<0.001	<0.05	<0.001

Pampus sp. ($4.589 \pm 1.008 \mu\text{g/g}$) and *Scomberomus cavalla* ($1.723 \pm 0.260 \mu\text{g/g}$), and lowest similar concentrations observed in the *L. rohita* collected from Fateh Jang and *C. carpio* ($0.005 \pm 0.001 \mu\text{g/g}$), respectively. The concentration of nickel was observed highest in the marine fishes *S. cavalla* ($0.409 \pm 0.127 \mu\text{g/g}$) and freshwater fish species *Wallago attu* ($0.133 \pm 0.003 \mu\text{g/g}$) while lowest concentration was observed in *L. rohita* collected from Rawal dam ($0.017 \pm 0.002 \mu\text{g/g}$). Similar pattern was observed for iron concentration which was highest in marine fishes *S. cavalla* ($41.176 \pm 8.123 \mu\text{g/g}$), *Pampus* sp. ($4.723 \pm 1.339 \mu\text{g/g}$) and in freshwater species *W. attu* ($2.786 \pm 0.013 \mu\text{g/g}$) while lowest in *L. rohita* collected from Fateh Jang ($1.085 \pm 0.007 \mu\text{g/g}$). Lead mean concentration was ranged from $0.017 \pm 0.003 \mu\text{g/g}$ in *X. cancila* to $3.391 \pm 0.002 \mu\text{g/g}$ in *L. rohita* collected from the Rawal Dam (Table I). The statistical estimations presented in Table I shows that there is a significant greater accumulation of lead in muscle tissues ($p < 0.05$).

Amongst the studied fish species, cadmium showed intermediate muscle concentrations in all, displaying a similar pattern for chromium. Conversely, *Labeo rohita* exhibited a higher chromium accumulation in Rawal Dam compared to other locations. Nickel distribution diverse, with intermediate levels in muscle of *L. rohita* showed slightly low concentration except *Scomberomus cavalla* which showed maximum concentration level. Chromium exhibits a high bonding for sediments due to its low water solubility and sturdy binding to particulate matter. These results in considerably elevated concentrations in sediments compared to the overlying water column. As a result, direct bioaccumulation of chromium by fish from the water phase is limited. Instead, chronic contact primarily occurs through the food chain via eating of impure benthic organisms and their associated detritus. This biomagnifications effect has been documented by Adeniyi *et al.* (2008) and Gupta *et al.* (2009) observed higher chromium levels in fish tissues when compared to water, often surpassed only by lead.

Metal uptake by fish also depends upon the zone of the water body occupied by fish. Kilgour (1991) has indicated that animals which have proximity with sediment show relatively high accumulation of metals while cadmium contamination exhibits negligible presence in fish tissues. This examination aligns with earlier studies by Has-Schön *et al.* (2008) and Öztürk *et al.* (2009) consistently identified cadmium as the metal with the lowest accumulation. Nickel deviated from this pattern, displaying a unique distribution. While its concentration remained lesser than lead and chromium in water and fish, it pointed high in sediments. This suggests low water retention, active sediment sequestration, and minimal uptake by benthic organisms

from both water and sediment. These findings concur with the notion that, although nickel can accumulate in aquatic life, it does not experience biomagnifications through the food chain. Furthermore, nickel mainly exhibits partial mobility, except under acidic conditions, as noted by Mahmood (2003).

Inter-site difference revealed a noticeable spatial variation in nickel (Ni) tissue concentrations in different fish species. Notably, at Head Sulemanki Headworks of River Satluj, Ni levels were constantly at upper point across all studied tissues, while at Rawal Dam exhibited the lowest mean values in *L. rohita*. Further, within Rawal Dam and Head Sulemanki, *L. rohita* depicted higher Pb concentrations compared to other species. This observation hinted potential species-specific factors influencing Pb bioaccumulation patterns, even within a seemingly less contaminated environment. Notably, the less Pb level in water at both sites implies limited direct uptake from this medium in *C. carpio*. This shows the food web may play a more significant role in Pb bioaccumulation for these fishes. Toxicological effects of industrial effluents deposited in River Chenab on *Wallago attu* in Muzaffargarh region, contrast with this one. The observations demonstrate that a larger concentration of tendency of bioaccumulation of metals in *W. attu* due to the fact that fish's physiology is vulnerable to a particular kind of metal (Yousafzai *et al.*, 2010). In this study, Fe was the maximum in the tissues of analyzed marine species (*Scomberomus cavalla* and *Pampus* sp.) at Karachi coast, followed by Cr, Ni and Pb. Similar data patterns were found by other researchers (Tepe *et al.*, 2008; Türkmen *et al.*, 2009). The calculated averages and standard deviations provided valuable insights into the dispersion and variability of heavy metals in *S. cavalla*.

CONCLUSION

The findings underscored the spatial and species-specific variations in heavy metal bioaccumulation, highlighting the need for continued monitoring and assessment of riverine and marine ecosystems. The concentration was largely detected in marine fish species than freshwater species. Overall, the metal concentration was estimated from high to low in the following order:

$$\text{Fe} > \text{Pd} > \text{Ni} > \text{Cr} > \text{Cd}$$

Additionally, the calculated averages and standard deviations offered crucial information for understanding the potential environmental impact and guiding future conservation efforts in the freshwater reservoirs and coastal regions of Pakistan.

DECLARATIONS

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Statement of conflict of interest

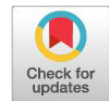
The authors have declared no conflict of interest.

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Seasonal Prevalence and Impact of Environmental Variables in Emergence of *Aedes* Mosquito in Multan, Pakistan

Faheem Ali* and Amjad Farooq

Institute of Zoology, Bahauddin Zakariya University, Multan, Punjab, Pakistan.

ABSTRACT

Mosquito borne infection is considered a most important public health problem all over the world including the dengue virus. The dominant occurrence of *Aedes* mosquitoes under the influence of environmental factors (temperature, relative humidity, and rainfall) of Multan city was assessed during 2014-2016. In this study, *Aedes* larvae were collected by using the entomological dipping method and identified in the laboratory. The results showed mean (\pm SD) temperature, rainfall, and relative humidity during 2014-2016 ranged 12.88-35.43°C, -0.33-87.43mm, and 36.17-72.17%, respectively. Likewise, maximum *Aedes* larvae were found during the month of November, with 348.67 *Aedes* larvae from indoor while 71.67 from outdoor. The emergence of *Aedes* larvae was found after rainy season while decline trend was observed at non-rainy, extreme temperatures and relative humidity conditions. This study could be used in further studies in order to evaluate *Aedes* mosquito emergence in relation with different environmental variables, and to establish integrated vector control strategies in the future.

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Authors' Contribution

AF conceived and designed the study. FA performed research, provided reagents and materials; and drafted the manuscript. Both authors revised and approved the manuscript.

Key words

Aedes, Temperature, Rain fall, Relative humidity

INTRODUCTION

Dengue fever is a well-known viral infection, influenced by many environmental factors (Mahmud *et al.*, 2019) that infect human and threatened 975 million peoples around the world specifically in tropically and sub-tropically areas of South-Eastern Asia, America and the Pacific region (Haider and Iqbal, 2016). Although, dengue virus belonged to family flaviridae which has 4 strains or serotypes that are serologically similar but different by genetically (Zanotto *et al.*, 1996). Additionally, *Aedes aegypti* and *Aedes albopictus* are the mosquito vectors that are responsible for the transmission of the dengue virus in human causing symptoms such as high grade fever, painful muscles and joints, vomiting, nausea, skin rashes, severe headache, and pain behind the eyes (Manzoor *et al.*, 2015; Habib *et al.*, 2016). *Aedes* mosquito is primarily responsible for the spread of dengue in all regions of the world (Manzoor *et al.*, 2015) while later is usually thought to be a weak with minor epidemics (Kim-Lien *et al.*, 2015).

The *Aedes* population has a major effect on the human populations as its life cycle is closely related with the human activities (Madzlan *et al.*, 2016) and can adopt different habitats such as *aegypti* occupied urban residences however *albopictus* invade rural, sub-urban and urban environmental conditions (Kim-Lien *et al.*, 2015). *Aedes aegypti* established itself in the urban areas and lesser extent to semi-urban area but is not established in the rural area. On the other side, *Aedes albopictus* population had established itself in rural areas rather than urban areas. Moreover, for breeding, *Aedes aegypti* preferred indoor and *Aedes albopictus* preferred both indoor and outdoor (Herath *et al.*, 2022).

Aedes is not only adopt the local domestic environments but can also adopt demographic growth and migrate through human (Carneiro *et al.*, 2017). In tropical and sub-tropical lands of Asia, this viral infection is mostly spread by *Aedes aegypti* and *Aedes albopictus* and existed in the surroundings of human. On the other hand, *Aedes albopictus* is originate in sub-tropical zones than *Aedes aegypti* due to its tolerance to low temperature. Additionally, extreme invasion of dengue infestation is related with the adult vectors under the ideal setting of humidity and temperature in rainy seasons (Carneiro *et al.*, 2017).

Moreover, *Aedes* mosquito required clean watery environment during initial three developmental stages i.e., egg, larvae and pupae both in natural and artificial conditions (Madzlan *et al.*, 2016), depends upon water temperature and its environmental conditions (Gubler, 1997). Furthermore,

* Corresponding author: khanmultani2@gmail.com
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mosquitos completed their three developmental stages (egg to adult mosquito) in 7-10 days. The female *Aedes* mosquito lays its eggs in potential water containers (CDC, 2019) where eggs are fastened with the walls of container and could survive up to 8 months. Eggs hatched within a few days to months-long duration when enclosed with water till emergence of larvae in water container and later converted into pupae in next 5 days. In next 2–3 days pupae develop into mature mosquito (CDC, 2019).

After the onset of the monsoon, dengue virus infestations occurred as a burning health issue due to higher arrival of *Aedes* mosquito in Pakistan (Manzoor *et al.*, 2015). Due to greater suitable vector environment and habitats, dengue virus has occupied in many regions in Pakistan in last decade. This disease victimized mainly the adult population of human in Pakistan as well as all over the world (Naqvi *et al.*, 2015). Present study was planned to evaluate the impact of different environmental variables such as rainfall, relative humidity, and temperature in the emergence of *Aedes* mosquito responsible for the infestation of dengue fever in district Multan, Punjab; Pakistan. Moreover, this study will be also helpful in future to control the dengue virus epidemics by earlier prediction by evaluating the impact of environmental variables that favored vector emergence in any region of the world.

Aim of the study was to evaluate the seasonal prevalence of *Aedes* mosquito and impact of environmental variables upon *Aedes* mosquito emergence in Multan, Pakistan during the year 2014-2016.

MATERIALS AND METHODS

Growth conditions

Previously it is demonstrated that the *Aedes* can adapt different habitats with human migration (Kim-Lien *et al.*, 2015; Carneiro *et al.*, 2017) as adapted different habitats of Multan. Multan is located at southern area of Punjab; Pakistan. Its climate is arid that usually experienced hot summer with higher temperature and cold winters with lowest temperature.

To determine the effect of arid climate of Multan in the emergence of *Aedes*, the vector larvae were collected from the natural environment of inside (Indoor) and outside (Outdoor) of the houses. During *Aedes* larvae sampling, temperature was recorded in Celsius degrees by using ordinary thermometer while relative humidity was measured in percentage by using wet and dry bulb thermometers and rainfall was measured in millimeters by using ordinary rain gauge.

Sample collection

Larvae were collected at different stages of its

lifecycle from different natural water containers and stagnant water ponds via dipping method (Robert *et al.*, 2002) by utilizing collection tools i.e., sampling scoop, transparent glass jar, magnifying glass, torch and strainer.

The wild urban populations of *Aedes* larvae were sampled randomly during the day times from two premises. In 1st premises (indoor sites), aqueous habitats of inside of houses which were considered for larvae sampling i.e., water containers used by human in washing and drinking, animal's drinking pots, room water coolers, main whole covers, used/spare tires, air condition water collection containers, leaking water taps, flower jars, house stagnant water, household rubbishes, roof top junks, refrigerator trays, garbage, and construction rubbish. Whereas, in 2nd premises (outdoor sites) aqueous habitats of outside of houses which were considered i.e., park shrubberies, tyres, street and road side trash, flower pots, leaked water taps, air coolers, open manholes/drains, water vessels used for puncture in the shops, sanitation wastes in buildings, park and tree holes, construction wastes, bird pots, air conditioner outlets, water tanks, fountains and stagnant water at roadside.

The *Aedes* larvae were collected during the year 2014-2016 with different month's interval at different temperature, average rainfall, and relative humidity (Table I). The collected *Aedes* larvae were taken to the laboratory and were identified by using standard taxonomic keys as prescribed previously (Darsie and Pradhan, 1990; Tyagi *et al.*, 2015) with little modifications.

Table I. Mean values of metrological parameters and total *Aedes* larvae found in Multan Pakistan during the year 2014-2016.

Months	Climate			<i>Aedes</i> larvae (Vector)		
	Average temperature (°C)	Average rain fall (mm)	Average relative humidity (%)	Indoor	Out-door	Total
January	12.88	1.63	72.17	8.33	0.00	8.33
February	16.55	10.60	64.67	7.33	1.33	8.67
March	21.10	47.17	63.17	41.67	10.67	52.33
April	27.98	14.17	44.00	83.00	12.67	95.67
May	33.35	27.00	36.17	59.67	9.33	69.00
June	35.43	16.83	42.83	12.33	2.00	14.33
July	33.62	87.43	60.50	52.00	6.67	58.67
August	32.62	60.80	62.67	134.67	32.00	166.67
September	31.47	5.27	59.67	188.00	37.00	225.00
October	27.68	11.23	56.00	257.67	53.00	310.67
November	20.62	0.07	61.00	348.67	71.67	420.33
December	15.32	-0.33	69.83	125.33	14.33	139.67

Statistical analysis

To analyze the emergence of *Aedes* larvae, variance analysis was performed. Temperature, average rainfall, and relative humidity were recorded daily in three repeats and average value was calculated for a month although error bars represent the average \pm SD of recorded values.

Correlation among total *Aedes* larvae and numerous metrological parameters were performed by using regression analysis in MS Excel. Asterisks over the values indicate a significance level among mean values of metrological parameters and total *Aedes* larvae was evaluated via regression analysis (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

RESULTS

Multan climate is hot and arid that favored *Aedes* mosquito breeding and its emergence in September, October and November. Consequently, the rainfall, relative humidity, and temperature were recorded in 2014-2016 (Table I). The maximum temperatures 35.43°C were recorded in June, while minimum values were recorded as 12.88°C in January during 2014 to 2016. Recorded data showed maximum rainfall 87.43 mm in July while maximum relative humidity was recorded (72.77%) in January during three consecutive years (2014-2016). Moreover, the mean prevalence of *Aedes* larvae were found highest in the month of November (348.67 from indoor and 71.67 from outdoor) while the lowest in January (8.33 from indoor and 0.00 from outdoor), showing that *Aedes* had the preference for indoor breeding, as described in Table I. Collectively, the maximum *Aedes* larvae (603 and 766) were collected in November, 2015 and October, 2016 when temperature, rainfall and relative humidity were found favorable for its breeding and development. Prevalence of *Aedes* (Dengue Vector) larvae in District Multan during years 2014-2016 is represented in Table II, indicating no significant difference between the prevalence of indoor and outdoor *Aedes* larvae. Table III shows the prevalence of *Aedes* (Dengue vector) larvae in District

Multan during years 2014-2016.

Table II. Prevalence of *Aedes* (Dengue Vector) larvae in District Multan during years 2014-2016.

Year	<i>Aedes</i> (Indoor)	<i>Aedes</i> (Outdoor)
2014	0.0000 A	0.2500 A
2015	0.5833 A	0.1667 A
2016	0.0833 A	0.2500 A

Means bearing different letters are significantly different from each other.

Table III. Prevalence of *Aedes* (Dengue Vector) larvae in District Multan during years 2014-2016.

Month	<i>Aedes</i> (Indoor)	<i>Aedes</i> (Outdoor)
January	0.0000 A	0.0000 A
February	0.0000 A	0.0000 A
March	0.0000 A	0.0000 A
April	1.6667 A	0.0000 A
May	0.3333 A	0.0000 A
June	0.0000 A	0.0000 A
July	0.6667 A	0.0000 A
August	0.0000 A	0.3333 A
September	0.0000 A	1.3333 A
October	0.0000 A	0.3333 A
November	0.0000 A	0.6667 A
December	0.0000 A	0.0000 A

Means bearing different letters are significantly different from each other.

Regression analysis (r value) was performed to determine the co-efficiency among different metrological parameters and *Aedes* larvae in Multan (Table IV). Findings of regression analysis provided that total larvae showed significant ($P < 0.001$) correlation with indoor and outdoor larvae, but insignificant ($P > 0.05$) correlation with relative humidity, temperature and rain fall. However, temperature significantly affected the relative humidity ($P < 0.001$) and rainfall ($P < 0.05$).

Table IV. Relationship among different metrological parameters and *Aedes* larvae found in Multan Pakistan during 2014-2016.

Parameters	Indoor larvae	Outdoor larvae	Total larvae	Relative humidity	Temperature	Rain fall
Indoor larvae	1					
Outdoor larvae	0.916***	1				
Total larvae	0.998***	0.940***	1			
Relative humidity	0.014 ^{NS}	0.017 ^{NS}	0.015 ^{NS}	1		
Temperature	0.063 ^{NS}	0.074 ^{NS}	0.065 ^{NS}	0.690***	1	
Rain fall	0.200 ^{NS}	0.149 ^{NS}	0.194 ^{NS}	0.064 ^{NS}	0.369*	1

*** $P < 0.001$, * $P < 0.05$, ^{NS} $P > 0.05$.

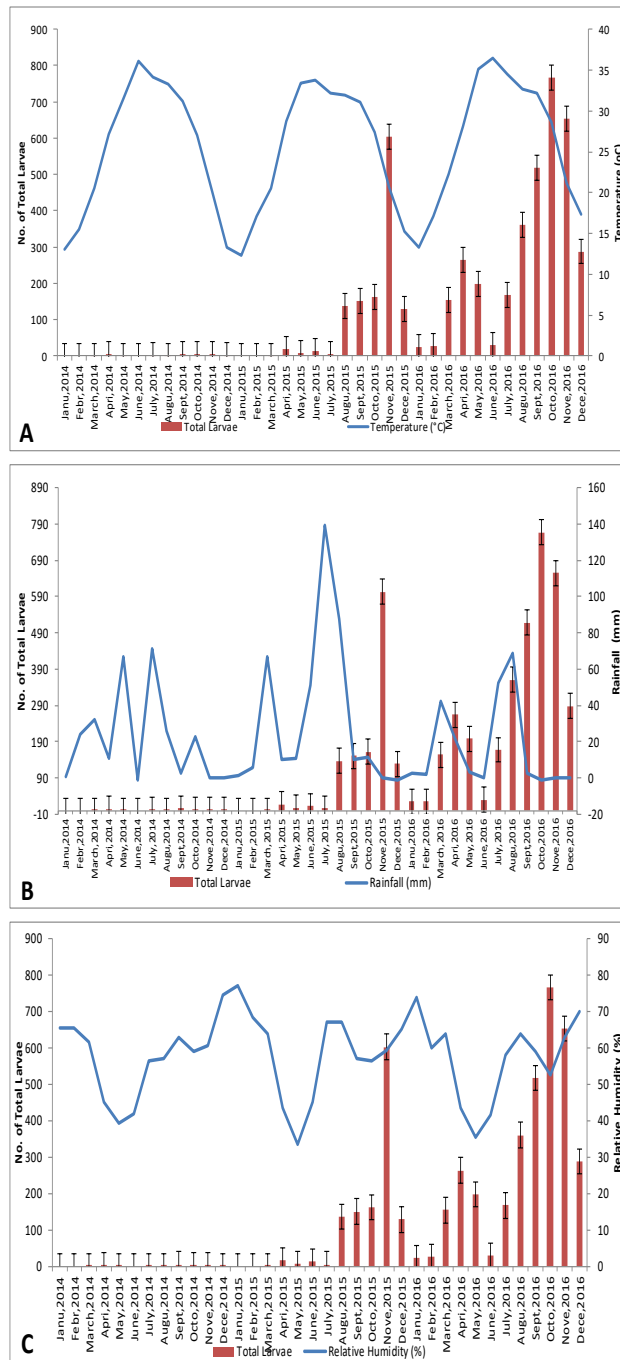


Fig. 1. Effect of temperature (A), rainfall (B) and relative humidity (C) on the total number of *Aedes* larvae found during the year 2014-2016 from District Multan.

Additionally, the dependent of temperature on the emergence of total number of *Aedes* larvae during the studied years (2014-2016) is shown in Figure 1A. Extremely high and low temperature suppressed the

breeding and growth of the *Aedes* larvae. At extremely low temperature (0-18°C) from January to February and high temperature (32-36°C) in May to July, no or less *Aedes* larvae were observed. However, during September to November when temperature ranged from 20-30°C, maximum larvae were found.

Furthermore, the role of rainfall in the emergence of *Aedes* larvae was also examined by measuring average rainfall and collecting total number of larvae (outdoor and indoor) after different month's interval in 2014 and 2016. Rainfall established water holdings and provide environment for female *Aedes* mosquito to lay its ova and then developed into larvae. Higher rainfall in July, 2015 and August 2016 gave ultimately larvae emergence during August to November with maximum larvae population in October, 2015 and November, 2016 (Fig. 1B). Hence, the results showed positive relationship between rainfall and larvae incidences.

Relationship between number of *Aedes* larvae and relative humidity was also plotted as Figure 1C, which showed peak of *Aedes* larvae incidences at 50 to 65%. Relative humidity in the atmosphere due to higher rainfall in July, 2015 and August 2016 gave favorable environment for maximum emergence of larvae during August to November 2015 and October 2016. Collectively, these results showed the positive roles of moderate temperature, rainfall and relative humidity in the emergence of larvae suggested and confirmed the important roles of these environmental factors in the hatching and breeding of *Aedes* larvae which in turn caused dengue epidemic in Multan in late 2015.

DISCUSSION

Dengue is usually considered to be an urban disease and its infestations are increasing because it is caused by the species of the *Aedes* mosquito (*Aedes aegypti* and *Aedes albopictus*). The incidence of dengue vectors to new geographical areas, its climate, concentration of population, household water and trash storage to recycle the waste materials can serve as risk factors for dengue virus infections (Madzlan *et al.*, 2016).

In this study, *Aedes* larvae were found higher from inside of the houses than outside of the houses of Multan during 2014-2016. These findings are in general agreement with that of reported by Herath *et al.* (2022), who have documented that *Aedes* was found moderately in the urban area and to a lesser extent in semiurban but not in the rural area and *Aedes* mostly preferred indoor breeding.

The higher number of *Aedes* larvae were collected in November, 2015 and October, 2016. The same findings were reflected by Sarwar and Rasool (2022) that revealed

the highest breeding of *Aedes albopictus* from Faisalabad district during 2019-2020 in the months of September-November and previously by Asrar *et al.* (2017) whom found *Aedes* prevalence in Multan city, during February-March and later in July, September and maximum in November.

Although temperature has great impact on mosquitoes growth because mosquitoes are poikilothermic organisms on which external environment directly influence their body temperature (Hawley, 1988) but the effect of meteorological variables on vector populations could be different amongst geographical locations with diverse climate conditions (Tuladhar *et al.*, 2019). In the current study, decline in *Aedes* population during extreme temperatures of cold weather (January to March and December) and hot weather (May to July) was observed in Multan. The peak population of the *Aedes* larvae during 2014-2016 was collected from Multan when temperature was calculated between 20-32 °C during September to November, and was in agreement with the results reported previously by Delatte *et al.* (2009) who found optimum immature stages development at 29 °C and Mohammed and Chadee (2011) whom proposed significant mosquito's eggs hatching success at 24-35 °C.

As it is reported previously that in the South-Eastern Asia, female vector preferred to lay its eggs in domestic containers and precipitations plays vital role in its distribution and abundance (Aziz *et al.*, 2012) indicated that water is one of the important factors involved in the growth and breeding of larvae. In this study, the highest rainfall recorded in July, 2015 (138.9 mm) then in August, 2016 (69 mm), increased the emergence of *Aedes* larvae population from August to November during 2015 and 2016 suggested that the rainfall has positive correlation with the breeding of larvae. These results are in accordance with the earlier findings that higher rainfall plays vital role to enhance the *Aedes* population (Vanzie, 2008; Aziz *et al.*, 2012). Additionally, the maximum population of *Aedes* larvae at 50-65% relative humidity in September to November during 2015 and 2016 demonstrated that relative humidity in the atmosphere due to higher rainfall had positive association with the emergence of larvae. The same findings were reported by Costa *et al.* (2010) that the gradual reductions in egg fertility at 60% relative humidity with increase in temperature, although such effect was not found at 80% relative humidity at 25 °C and 30 °C which also confirmed and authenticated this study findings.

CONCLUSION

The environmental variables played major role in seasonal emergence of *Aedes* mosquito. Favorable

environmental variables (watery environment or rain fall, temperature between 20-33 °C and suitable relative humidity between 50-65%) enhanced the growth of the *Aedes* population in Multan during this study especially in November 2015. Designed study and its findings will help in forecasting dengue epidemic especially in dengue endemic regions and will be helpful to control the spread of *Aedes* populations and dengue fever infestations under the impact of environmental variables by earlier adopting integrated vector management strategies.

DECLARATIONS

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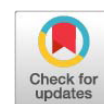
Statement of conflict of interest

All authors declared that there is no conflict of interest.

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Molecular Analysis of the Two-Component CusS/CusR Regulon for Copper Resistance: Cloning, Phylogenetics, and Structural Modeling

Soumble Zulfiqar* and Abdul Rauf Shakoori

School of Biological Sciences, University of the Punjab, Quaid-I-Azam Campus, Lahore 54590, Pakistan

ABSTRACT

Copper resistance in bacteria is mediated by regulatory systems that sense and respond to metal stress. In this study, the CusS and CusR from a copper-resistant *Klebsiella pneumoniae* strain were explored, which form a two-component regulatory system crucial for copper detoxification. The genes were successfully amplified and cloned into a vector. Their sequences were obtained through commercial services. The corresponding protein sequences were deduced and a comprehensive analysis was conducted, including multiple sequence alignments and phylogenetic tree construction. The findings reveal that CusS and CusR are conserved across a broad range of Proteobacteria, suggesting a widespread role in metal resistance. The proteins were further characterized through structural modeling to predict their three-dimensional conformations. The analysis identified key domains involved in the regulatory mechanisms of copper resistance. The study highlights the evolutionary conservation of this two-component system and provides insights into its functional dynamics across diverse bacterial species. These results enhance our understanding of metal resistance mechanisms and could inform future research on bacterial adaptation to environmental stresses.

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Authors' Contribution

SZ performed wet and dry lab work and wrote the manuscript. ARS supervised the whole work.

Key words

Klebsiella pneumoniae, CusS/CusR regulon, Copper resistance, Phylogenetics, Structural modeling

INTRODUCTION

Copper is an essential micronutrient required in several biological processes, but it becomes toxic when present in excessive amounts. Elevated copper levels, exacerbated by rapid industrialization, have led to widespread contamination of food chains, resulting in significant health issues for both humans and wildlife. Effective management of copper contamination is crucial for filtering it out of industrial waste to prevent its entry into food chains. While traditional chemical and physical methods for cleaning industrial waste can be expensive and environmentally damaging, the advent of biotechnology has introduced microbes with simple genomes and

structures as invaluable tools that offer a more sustainable and cost-effective solution. By leveraging microbes that can tolerate and detoxify high concentrations of copper, environmental contamination can be mitigated. Understanding the mechanisms bacteria use to handle toxic metals is fundamental for optimizing these biotechnological strategies and enhancing our ability to address metal pollution more effectively.

Since the early 1970s extensive research has been carried out in the field of metal resistance systems. Metal resistance systems may have developed shortly after prokaryotic life started and are present in nearly all bacterial types (Ji and Silver, 1995). Selected genes arose to deal with toxicity of metals at the same time as mechanisms for sugar and carbon sources (Bhagirath *et al.*, 2019; Ji and Silver, 1995). Resistance can be transferred among organisms through conjugation or transduction (Harnett and Gyles, 1984). Investigations of copper-resistant bacteria have revealed several mechanisms. However, recent molecular analyses suggest surprising similarities between copper resistance genes from diverse bacterial genera that were described as having different resistance mechanisms. The number of evolutionary paths that have led to copper resistance in bacteria may therefore be more limited than it would seem from the number of

* Corresponding author: soumbles.sbs@pu.edu.pk, soumbles@gmail.com
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proposed mechanisms. The finding of chromosomal genes with similarity to plasmid-borne copper resistance genes in pseudomonads and the identification of chromosomal genes which function in copper uptake and management in *Escherichia coli* have provided insight into copper resistance genetic determinants evolution in several bacterial species (Cooksey, 1993).

The emerging similarities between copper resistance systems from different bacterial taxa and from distinct geographical locations could be explained by plasmid transfer between bacteria, probably assisted by long-distance transport of resistant bacterial strains by human activities. However, considering that chromosomal copper transport and management systems could be common in different bacterial taxa, then independent evolutionary modifications of related ancestral transport genes could also have led to related copper resistance gene systems in different taxa. If parallel evolution of resistance from related but somewhat diverged ancestral genes occurred, then it would not be surprising to find some differences in specific functional and regulatory mechanisms, as is observed for the related copper resistance systems in *Pseudomonas syringae* from the United States and in *E. coli* from Australia. The characterization of chromosomal genes involved in copper transport and management may help to clarify their evolutionary relationship to the plasmid-borne resistance systems (Cooksey, 1993). Suggested copper resistance mechanisms in bacteria include reduced uptake by permeability barrier, active efflux of the metal, intracellular compartmentalization/sequestration, extracellular sequestration, enzymatic detoxification of a metal to a less toxic form, and reduction in metal sensitivity of cellular targets (Bhagirath *et al.*, 2019; Bruins *et al.*, 2000).

In contrast to Gram-positive bacteria, a Gram-negative bacterium not only needs to safeguard the cytoplasm but also the vital periplasmic compartments from metal-induced damage. A family of related transport systems found in Gram-negative bacteria is that of the proton-driven CBA-transport systems. These are involved in export of metal ions, xenobiotics, and drugs (Tseng *et al.*, 1999; Poole, 2001) and work under both anaerobic and aerobic conditions (Munson *et al.*, 2000).

The copper translocating Cus system is encoded on the chromosome as a determinant comprising of two operons transcribed in opposite directions (Li *et al.*, 2023). One operon encodes the regulators CusRS that form a two-component regulatory system. CusS is a histidine kinase located at the cytoplasmic membrane probably sensing copper ions in the periplasm. CusR acts as a response regulator. The CusRS, a sensor/regulator pair, activates the adjacent but divergently transcribed genes *cusCFBA*

(Bhagirath *et al.*, 2019; Munson *et al.*, 2000; Oshima *et al.*, 2002). CusA, CusB and CusC interact to form an active channel spanning the periplasm and connecting the cytoplasm to the outer membrane. Thus, CusCBA plays a role in efflux of excess cellular copper. A unique feature of the Cus system is the presence of a small periplasmic metallochaperone protein, CusF transporting copper to the CusCBA efflux complex and thus facilitating copper detoxification of the periplasm.

The present study aimed to explore the structural features of the CusRS two-component system in a copper-resistant bacterial isolate, utilizing computational and bioinformatics techniques. Study of its distribution and evolutionary relationship in bacterial taxa is likely to provide valuable information to the field of bacterial copper resistance.

MATERIALS AND METHODS

Isolation of genomic DNA

A Cu⁺⁺ resistant bacterial isolate *Klebsiella pneumoniae* KW (Acc. No. AB642256) reported by Zulfiqar and Shakoori (2012) was grown in LB medium supplemented with 1mM Cu⁺⁺. Genomic DNA of the strain was isolated as described by Rodriguez and Tait (1983) and subjected to RNase treatment. The quantity and quality of DNA was observed by taking spectrum between 200_{nm} and 280_{nm} in spectrophotometer (Bio Spec-1604 Shimadzu) as well as running on 0.8% agarose gel.

Amplification and cloning of *cusRS*

The regulatory operon comprising of *cusR* and *cusS* were amplified from the isolated genomic DNA. The primers used in amplification reaction included forward primer *cusRS-F1* and reverse primer *cusRS-R1* (Table I). These primers were designed from the sequence of *Klebsiella pneumoniae* subsp. *pneumoniae* MGH (ACC # CP000647) (McClelland *et al.*, 2006) using online available Primer3 version 0.4.0 program (Rozen and Skaletsky, 2000).

The PCR reaction mixture (50 µl) contained 250 µM dNTPs, 1.5 mM MgCl₂, 1x Taq Polymerase buffer, 2 µM each primer, 5 units Taq polymerase and 100-200 ng genomic DNA. The PCR cycle consisted of initial denaturation of 5 min at 94 °C followed by 30 cycles each of denaturation at 94 °C for 1 min, annealing at 58 °C for 90 sec and extension at 72 °C for 2 min with final extension at 72 °C for 10 min.

The PCR product was resolved on 0.8 % agarose gel using DNA ladder mix (Fermentas Cat # SM0331) as reference. The band, observed at right position, was excised from the gel through Qiagen gel extraction kit (Cat

28704) and ligated in pTZ57R/T vector using Fermentas InsT/A clone PCR Product Cloning Kit (Cat # K1214) according to manufacturers' instructions. *E. coli* DH5 α cells made competent by ice cold CaCl₂ method were transformed with the recombinant plasmid pTZ57/cusRS (Sambrook and Russell, 2001). Positive transformants, selected on the basis of blue white assortment, were grown in the presence of ampicillin and the recombinant plasmid was purified through method reported by Birnboim and Doly (1979).

Table I. Primers used for amplification and sequencing of *cusR* and *cusS* of *Klebsiella pneumoniae* KW.

Primer ID	Primer sequence (5' ? 3')	Position
cus RS-F1	GGCGTGACGGGAAAATGACAAAAC	5193500 – 5193524 *
cus RS-R1	TTATCTCCGGCCTGGTGCAAACTTC	5195815 – 5195791 *
RS-F2	GTAAAACCATTCGCCTTTGC	408-427
RS-F3	CATGATCCATTCGGTGAAGG	876-895
RS-F4	CATCGTGCTGTTGTGGTCT	1374-1393
RS-R2	CACTTCATCGGCCAGATCC	1800-1782
RS-R3	ATGGATAAGGCCATCAGCAG	1292-1273
RS-R4	GGTGGCGAGGCTAATAAAGA	846-827

* The positions of primers are according to *K. pneumoniae* subsp. *pneumoniae* MGH (ACC # CP000647). For rest of the primers, the position indicates number of nucleotides away from 5' end of the amplicon

The presence of insert was confirmed through restriction analysis. The recombinant plasmid was subjected to single digestion with either of *Pst*I and *Xba*I and double digestion with both of these endonucleases in the presence of 1x Y tango buffer. The digested products along with DNA marker were resolved on 0.8 % agarose gel.

For sequencing, the recombinant plasmid was isolated and purified through QIAprep® Spin Miniprep Kit (Cat # 12125). Sequencing was first performed using M13 F and M13 R primers. To get sequence of internal region, primers were designed from already sequenced regions (Table I). Sequencing was carried out on ABI PRISM 310 Automated DNA sequencer (Applied Biosystems) through a core facility of sequencing available in School of Biological Sciences, University of the Punjab.

Sequence alignment of *CusS* and *CusR* proteins and analysis

The nucleotide sequences of the two regulatory genes *cusR* and *cusS* were used to deduce respective protein

sequences. These sequences were subjected to protein BLAST analysis available online on NCBI site (<http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>) to calculate the identity of the *cus* determinants. The predicted protein sequences were also analysed for conserved domains through NCBI's Conserved Domain Database (CDD) (Marchler-Bauer *et al.*, 2009).

The multiple alignments of the deduced protein sequences were performed through online clustal W program available on EMBL-EBI site (<http://www.ebi.ac.uk/Tools/clustalw2/>) along with homologous proteins of some other strains of *Klebsiella pneumoniae*, *Klebsiella variicola*, *Klebsiella oxytoca*, other members of family Enterobacteriaceae (*Escherichia coli*, *Citrobacter koseri*, *Cronobacter sakazakii*, *Edwardsiella ictaluri*, *Salmonella enterica*, *Serratia marcescens* and *Shigella sonnei*) and organisms representing at inter-order (*Shewanella putrefaciens*, *Pseudomonas aeruginosa* and *Acinetobacter junii*) and at inter-class level (*Caulobacter segnis*, *Ralstonia pickettii*, *Herminiimonas arsenicoxydans* and *Thiobacillus denitrificans*).

Phylogenetic relationship among these sequences was inferred through neighbor-joining method (Saitou and Nei, 1987) using MEGA 4 software (Tamura *et al.*, 2007). The evolutionary distances were computed using the maximum composite likelihood method (Tamura *et al.*, 2004). Tree topology was evaluated by the bootstrap re-sampling method of Felsenstein (1985) based on 1000 replications.

Determination of membrane topology of regulatory proteins

Membrane topology of CusR and CusS proteins was predicted through hydropathy profile of each protein through online available software SOSUI (Mitaku *et al.*, 2002).

Determination of three-dimensional structure of regulatory proteins

Homology modeling for determining the tertiary structure of protein was carried out by using SWISS model server (Arnold *et al.*, 2006). Template structure was identified on the basis of maximum identity. This template structure along with the deduced protein sequence was submitted for automated modeling. The model obtained was viewed by deep view- Swiss-PdbViewer version 4.0 (Guex and Peitsch, 1997). Literature was surveyed for determining if any of the proteins, under study, functionally exists as oligo/multimeric form. Tertiary structures of these oligo/multimers were modeled using Symmdoc server (Schneidman-Duhovny *et al.*, 2005).

RESULTS

Characteristics of *cusRS*

Figure 1 shows an intact band of the isolated genomic DNA appeared on gel indicating its integrity (Fig. 1a). $OD_{260/280}$ was found to be 1.89 representing high quality of the isolated DNA.

A single band of *cusRS* amplicon (2.316 kb) was observed in the agarose gel with no nonspecific amplification (Fig. 1b). In no-template control reaction (NTC), no band was observed. The recombinant plasmid purified appeared in the form of three bands representing nicked, coiled and supercoiled forms of the molecule (Fig. 1c). Presence of a single ~ 5.1 Kb band in result of single restriction and two bands approximately at 2.8 and 2.4 Kb in double digestion confirmed the successful cloning of *cusRS* in the cloning vector (Fig. 1d).

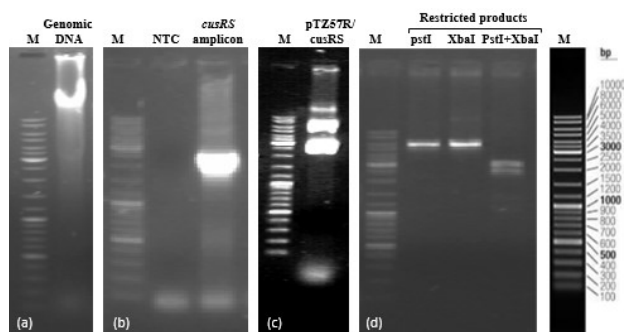


Fig. 1. Clonig of *cusRS*. (a) Genomic DNA of *K. pneumoniae* KW (b) *cusRS* amplicon (2.316 kb) (c) recombinant plasmid pTZ57R/*cusRS* (d) Restriction products of single and double digestion of the recombinant plasmid. Lanes M represent DNA marker (Fermentas cat # SM0331).

The DNA sequences of *cusS* and *cusR* obtained were submitted to DNA Data Bank of Japan (DDBJ) with the accession numbers AB640883 and AB641120, respectively. NCBI BLAST analysis revealed an 11 bp overlapping region (5' ATGCCCGCTAA) in the coding sequence of the two genes where ATG acts as start codon for *cusS* and TAA serves as stop codon for *cusR*.

In *K. pneumoniae* KW, the predicted CusS and CusR proteins were 477 and 227 amino acids in length, respectively. Blast analysis revealed that CusS and CusR proteins are widely and exclusively present in proteobacteria. Multiple alignment of each deduced protein sequence along with homologous protein sequences in other bacterial systems revealed its scope of similarity.

Phylogenetic analysis of regulatory proteins

Phylogenetic analysis of each deduced protein

sequence revealed its relationship at various levels, ranging from intra species up to inter class level.

CusS

Phylogenetic relationship of CusS was revealed through construction of neighbor joining tree (Fig. 2). CusS protein sequence of *K. pneumoniae* KW showed 96–98 % similarity with homologous proteins in the same genus. Among other members of family enterobacteriaceae, *Citrobacter* was the most homologous (69 %) followed by *Escherichia* (65 %) and *Shigella* (64 %). *Salmonella*, *Cronobacter* and *Serratia* were next, all showing 57 % homology. *Edwardsiella* was observed to be the least homologous (53 %) within the family Enterobacteriaceae. CusS in *Shewanella* was found slightly more homologous (56%) than *Edwardsiella*. Pseudomonadales (*Pseudomonas* and *Acinetobacter*) of same gamma class and members of beta proteobacteria (*Ralstonia*, *Herminiimonas* and *Thiobacillus*) possessed similarity between 32–40 %. No remarkable homologue of CusS was found in *Caulobacter*, a member of alpha proteobacteria.

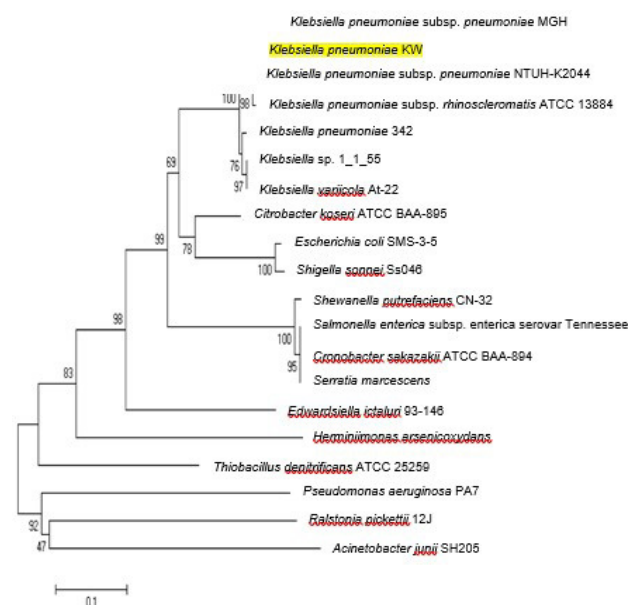


Fig. 2. Neighbor joining trees showing relationship of CusS protein of *K. pneumoniae* KW among various bacterial strains. Bootstrap values expressed as a percentage of 1000 replications are given at branching points. In each tree, bar represents substitutions per residue position.

CusR

Phylogenetic analysis of CusR of *K. pneumoniae* KW revealed that this protein was more homologous upto wider level as compared to other regulatory protein, CusS. Figure 3 depicts the phylogenetic status of this protein

among bacterial strains under consideration. CusR was found 98–99 % homologous in all *Klebsiella* spp., 93 % in each of *Escherichia* and *Shigella*, 92 % in *Citrobacter*, 87 % in each of *Salmonella*, *Cronobacter* and *Serratia* and 84 % in *Edwardsiella*. Thus, the same trend was observed as for CusS. *Shewanella* harboured 85 % homologue of CusR of *K. pneumoniae* KW. Members of beta proteobacteria (*Ralstonia*, *Herminiimonas* and *Thiobacillus*) were found more closely related (65–69 %) as compared to pseudomonadales (*Pseudomonas* and *Acinetobacter*) of the alpha proteobacteria (62 %). Gamma proteobacteria (*Caulobacter*) were observed as the least homologous (44 %).

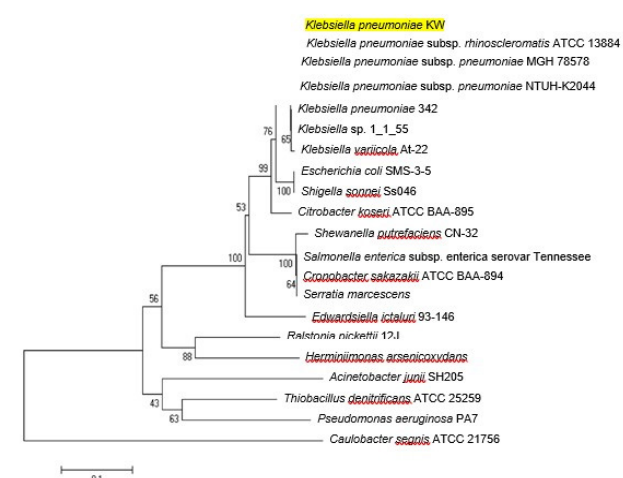


Fig. 3. Neighbor joining trees showing relationship of CusR protein of *K. pneumoniae* KW among various bacterial strains. Bootstrap values expressed as a percentage of 1000 replications are given at branching points. In each tree, bar represents substitutions per residue position.

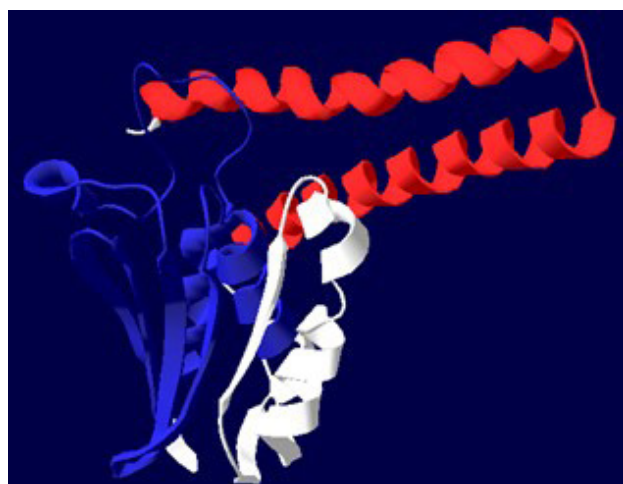


Fig. 4. Ribbon diagram of CusS. His KA domain is shown in red colour and HATPase_C domain is blue.

Structural analysis of CusS

Structural analysis of CusS and CusR included determination of three-dimensional structures on basis of homology modeling and topological analysis.

CusS, a sensor protein comprised 477 residues. Three-dimensional structure of CusS (Fig. 4) comprised of residues 255 to 477, as the template was available for only this part of protein. No appropriate template with HAMP domain along with N-terminal side was found in the database.

Conserved Data Domain (CDD) showed that this protein constituted three domains; HAMP, His KA and HATPase_C domains (Fig. 5). The structural and sequence analysis of this protein revealed many features of the three domains.

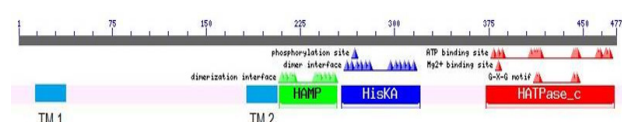


Fig. 5. Structural organization of CusS. TM 1 and TM 2 are trans-membrane regions followed by HAMP, His KA and HATPase-C domains.

HAMP domain (Histidine kinase, Adenylyl cyclase, Methyl-accepting protein, and Phosphatase) was formed of 206–254 residues. This domain contained two alpha helices connected by an extended linker. A dimerization interface was found in this domain involved in the formation of a parallel four-helix bundle. Figure 6 shows its multiple alignment with related domains found in CDD.

His KA domain (Histidine Kinase A) was composed of 256–320 residues. Dimerization and phosphoacceptor sites were observed in this domain. A histidine residue (position 269) involved in trans-autophosphorylation was found conserved in phosphoacceptor site. Phosphoacceptor site and dimerization interface are shown in the Figure 6 which harbors multiple alignment of related protein sequences available in CDD.

HATPase_c domain (Histidine kinase-like ATPase) playing its role in ATP binding, comprised residues 373 – 475. Two specific GXG motifs were found conserved in this domain.

Topological analysis of CusS

Topological analysis of CusS revealed that this was a membrane protein, comprising two trans-membrane regions TM1 (TRLTFFISLATVIAFFAFTWIMI) and TM2 (AELISAASIISLLIIAIVLFVVY) each of which was 23 residues in length. Figure 7 reveals that both of these two regions were present towards N-terminal side of the three domains. TM1 was present near the N-terminal end spanning residues 11–33 while TM2 (182–204) was

Structural analysis of CusR

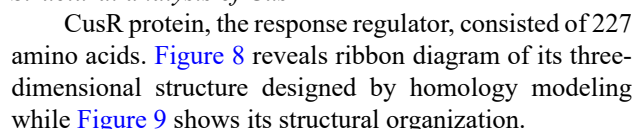


Diagram illustrating the structure of a protein, showing two distinct domains: the **Signal receiver domain** (colored red) and the **Effector domain** (colored blue). The domains are connected by a flexible linker.

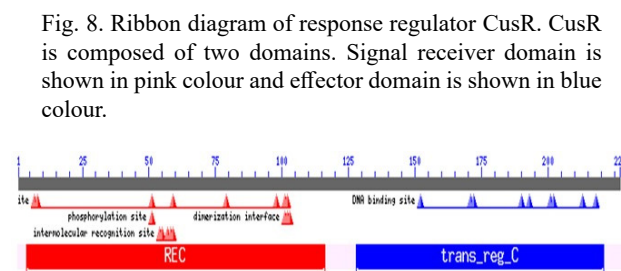


Fig. 9. Structural organization of CusR. This protein is composed of Signal receiver domain (REC) followed by effector domain (trans-reg-C).

Trans-reg-C (effector domain) was 94 amino acids in length spanning from residues 128 to 221. There were seven β strands and three α helices. Sequence analysis through CDD (Fig. 10) revealed that the effector domain contained DNA and RNA polymerase binding sites

required for its interaction with a specific site (cusR box) present in the promoter region of *cus* determinants and the RNA polymerase leading to enhanced transcription of four structural genes *i.e.*, *cusC*, *cusF*, *cusB*, *cusA*.

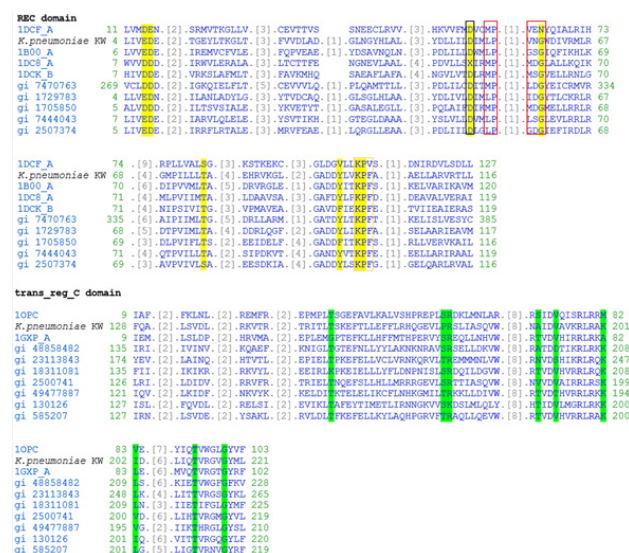


Fig. 10. Multiple alignment trans_reg_C domain in CusR of *K. pneumoniae* KW along with related protein sequences found in CDD. In REC domain, residues contributing active site are highlighted with yellow colour while phosphorylation site, dimerization interface and intermolecular recognition site are boxed with black, orange and red boundaries, respectively. In trans_reg_C domain, residues involved in DNA binding are highlighted with green colour.

In topological analysis of CusR no membrane associated region was observed and CusR was found as a soluble protein.

DISCUSSION

Copper is an essential transition metal. Owing to its redox property, it plays very important role in many biological processes. The same metal exerts lethal effects when present at higher concentration. Organisms have evolved some homeostatic and resistance mechanisms to cope this problem. These mechanisms include extra and intra cellular sequestration, compartmentalization, reduction of metal into less toxic form and active and passive efflux. Many bacteria have been reported that harbor one or more of these resistance mechanisms (Li *et al.*, 2014).

In this study, a bacterial strain *K. pneumoniae* KW was used. This strain exhibits high resistance to copper with minimum inhibitory concentration of 6.0 mM. It

uptakes a significant amount of Cu^{++} (~ 24 $\mu\text{g}/\text{mg}$ dry cell weight within 5 h in the presence of 3 mM Cu^{++} in the medium) and effluxes almost all the accumulated copper within 24 h (Zulfiqar and Shakoory, 2012).

The gram-negative bacteria deal with high amounts of copper mainly through export of excess copper. Passive efflux of copper is reported in *E. coli* and other bacteria through *cus* determinants (Li *et al.*, 2023). *cus* determinants include six genes grouped in form of two operons; *cusRS* and *cusCFBA*. These operons are present divergently and are regulated in opposite directions by a same promoter (Frank *et al.*, 2001, 2003). *cusRS* is regulatory in nature and is formed of two genes including *cusS* and *cusR* (Munson *et al.*, 2000). CusS and CusR constitute two components regulatory system in which CusS senses copper and CusR which is a response regulator binds DNA and enhances transcription of *cus* determinants (Fu *et al.*, 2020). *cusCFBA* constitutes four structural genes including *cusC*, *cusF*, *cusB* and *cusA* (Bhagirath *et al.*, 2019).

Considering the efflux ability of *K. pneumoniae* KW, an attempt was made to explore its *cusRS* system. For this, *cusS* and *cusR* genes were amplified from genomic DNA of the strain and cloned in a cloning vector. The nucleotide sequences were used to deduce the respective protein sequences that were later analyzed through multiple alignments with homologous proteins of protobacteria, construction of phylogenetic trees, three-dimensional modeling, and topological analysis.

Multiple alignments and phylogenetic analysis of deduced protein sequences of both *Cus* determinants through construction of neighbor joining trees revealed many features. It was found that *Cus* system exists widely in proteobacteria. In case of each *Cus* determinant, alteromonadales were found to be more homologous to enterobacteriales as compared to pseudomonadales. Surprisingly, alteromonadales were homologous even more than *Edwardsiella*, a member of family enterobacteriaceae. At interclass level, beta proteobacteria were found to be more homologous to gamma proteobacteria as compared to alpha proteobacteria in terms of *Cus* determinants homology. Moreover, it was found that CusS was highly homologous even at wider range, while homology of CusR was considerably low.

Analysis of deduced protein sequence of CusS through CDD and three-dimensional model revealed that this protein contains three domains; HAMP domain, His KA domain and HATPase_c domain. All these domains are present towards C terminal portion of the protein. HAMP domain contains two alpha helices connected by an extended linker (Aravind and Ponting, 1999; Swain and Falke, 2007). The dimerization interface present in this domain contains polypeptide binding site through which

two HAMP domains form a parallel four-helix bundle (Dunin-Horkawicz and Lupas, 2010). His KA domain contains dimerization and phosphoacceptor sites. Histidine Kinase A homo dimers are formed through parallel association of two domains creating 4-helix bundles. Then trans-autophosphorylation occurs at a conserved Histidine residue in phosphoacceptor sites by the catalytic domain of the histidine kinase (West and Stock, 2001). This phosphoryl group is subsequently transferred to the Asp acceptor residue of a response regulator protein (Stock *et al.*, 2000). HATPase_c domain plays its role in ATP binding. Two specific GXG motives are found conserved in this domain (Dutta and Inouye, 2000).

CDD and three-dimensional model of CusR revealed that this response regulator was composed of two domains; REC domain and trans-reg-C domain (Pao and Saier, 1995). REC domain is also known as signal receiver domain. It contains active site, phosphorylation site, intermolecular recognition site and dimerization interface. Upon receiving signal from CusS, this domain is transphosphorylated at a conserved aspartate residue present in phosphorylation site. Active site possessing an acidic pocket coordinates Mg^{++} required for event. Phosphorylation leads to a conformational change creating an exposed hydrophobic surface. This event triggers the formation of homodimers through the dimerization interface. CusR in homodimeric form binds to DNA and RNA polymerase through trans_reg_C domain also known as effector domain (Martinez-Hackert and Stock, 2001; Kenney, 2002). The effector domain is activated upon phosphorylation of REC domain which induces the cellular response. The effector domain contains DNA and RNA polymerase binding sites through which it interacts with a specific site (cusR box) present in the promoter region of *cus* determinants and the RNA polymerase. The binding of response regulator to RNA polymerase and DNA enhances the promoter activity in both directions leading to increased levels of transcription of four structural genes *i.e.*, *cusC*, *cusF*, *cusB* and *cusA* in one direction and two regulatory genes *i.e.*, *cusR* and *cusS* in the other direction.

Our findings emphasize the importance of the CusRS system in the broader context of bacterial copper resistance, highlighting its evolutionary significance across diverse bacterial taxa. These results may be helpful for future studies to explore the potential of targeting these systems in efforts to control toxic metals-based pollution.

CONCLUSION

Findings of this study not only deepen the understanding of the structural and functional aspects of the CusRS system in *Klebsiella pneumoniae* but also

underscore its critical role in bacterial copper resistance. These insights contribute to the broader field of environmental microbiology and suggest new avenues for exploring targeted strategies to mitigate copper toxicity in both natural and industrial settings.

DECLARATIONS

Statement of conflict of interest

There is no competing interest.

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Addition of Some Marine Ostracoda and Diplostraca from Pakistan (Northern Arabian Sea) and Restudy of Ctenopod *Penilia avirostris* and Cyprinid *Cypridina dentata*

Quddusi B. Kazmi* and M.A. Kazmi

Department of Zoology, University of Karachi, Karachi

ABSTRACT

This study was carried out in order to add in the existing fauna of the marine Ostracoda and Diplostraca of Pakistan. The paper summarizes published and unpublished species of planktonic and phthal Ostracoda and Diplostraca recorded from the Pakistan coast (northern Arabian Sea). We report hereby two halocyprid ostracods *Conchoecia* sp. and *Halocypria* sp. of the family Halocyprididae Dana, and podocypid ostracod species *Parasterope* sp. of the family Cyndroleberididae Müller; another species- *Ancohenia robusta* (Brady, 1890) of a myodocypid family Sarsiellidae and *Rutiderma* sp. of family Rutidermatidae Brady and Norman were also collected; an instar of an indeterminate myodocypid ostracod is added here. Additional descriptions of cyprinid *Cypridina dentata* (Müller, 1906), and a diplostracan *Penilia avirostris* Dana, 1849 of the order Ctenopoda in the family Sididae Baird are given here. Sample of *Penilia avirostris* is worth emphasizing as it is among the samples collected in the Northwestern Indian Ocean within the framework of the Office of Naval Research project (ONR: under Grant N00014-86-86-G-0230 1991), through partnerships with Pakistan academia and government, ONR coordinates and sponsors scientific research and technology development for the U.S. Navy and Marine Corps. All the species are described and illustrated.

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Authors' Contribution

QBK designed methodology of the study, wrote and edited the manuscript. MAK finalized the manuscript for publication.

Key words

Ostracoda, Diplostraca, Coastal, Oceanic species, Planktonic, Phthal, Taxonomy, Morphology

INTRODUCTION

This study was carried out in 1995 in order to determine Cladoceran now Diplostracan and Ostracodan fauna of the Pakistan coast, the studies were conducted in the areas very close to coast and sampling depth of the stations area changed 5 to 10m. The Ostracoda is a large class of relatively poorly known pancrustaceans. An important distinguishing feature Ostracods share with other arthropods is the bilateral symmetry of their body form. The paired body parts are enclosed in a dorsally hinged carapace. They are tiny crustaceans which inhabit virtually all aquatic environments and damp terrestrial, in shallow marine, deltaic, estuarine, lagoonal, lacustrine, fluvial and swamp environments. In tropical marine waters ostracods

also appear capable of attacking live animals, observed during night diving attacking fishes in swarms (Stephen and Brusca, 1985) or actual parasites on other marine animals (Monod, 1923; Harding, 1966; Felder, 1982; Cohen, 1983; Kretzler, 1984). The class Ostracoda is divided into 3 rescent subclasses (1) Myodocopa (2) Platycopida and (3) Podocopa; first two have representatives in marine environment (Pennak, 2001). Of the above mentioned three subclasses-two i.e., Podocopa and Mydocopa- occur in Pakistani marine waters. The samples collected from Karachi during 1995 form the basis of this study. Among the meiobenthic crustaceans, ostracods remain poorly studied that's why extant meiobenthic ostracods from this area are poorly documented and many living marine remain undescribed today. The Diplostraca is by far the most diverse and speciose superorder within the class Branchiopoda, most of which have flattened, leaf-like appendages. The superorder forms a monophyletic group of 7 orders, about 24 families, and more than 11,000 species. Many more species are undescribed. The diplostracans are important member of pelagic ecosystem and they serve as good source of food for fish and fish larvae during the warm periods. The diplostracan are represented by 8 species in marine environments (Belk, 2007), widely distributed in neritic and oceanic waters

* Corresponding author: qbkazmi@yahoo.com
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(Egloff *et al.*, 1997), sometimes, they produce “blooms” in marine environments. The marine cladocerans are adapted to neritic environments rather than offshore deep-sea environments (Egloff *et al.*, 1997; Onbé, 1999). Some species, however, are often observed in the open ocean over several kilometers from the coast (Della-Croce and Venugopal, 1972; Gieskes, 1971). The diplostracan are commonly used as live feed as a substitute for *Artemia* in aquaculture practices. They are major food source of many organisms like fish and other crustaceans and play an important part in the food chain, trophic levels and energy transfer in the ecosystem (Palanichamy, 1996). While going through literature we found little was known before 1990 about the deep pelagic ostracodan and diplostracan fauna of the Arabian Sea, was mainly known by collection made by the early oceanographic voyages of the German Tiefsee (1898) and the Danish Dana (1920, 1930), through a short paper on the planktonic ostracods was published by Cannon (1940) during the John Murray Expedition (1933–1934). Further data on the ostracods was published by George (1969) in a preliminary analysis of the plankton samples collected during the International Indian Ocean Expedition (IIOE, 1959–1965). Leveau (1967) also reported on a collection of halocyprids from the Arabian Sea, there have been a few follow-up studies of the halocyprids (James, 1975, unpublished thesis; George and Nair, 1980; Nair and Madhupratap, 1984; Smith and Madhupratap, 2005; Drapun and Smith, 2011) on the taxonomy and distribution of halocyprid ostracods from the Arabian Sea, based upon samples collected in the Northwestern Indian Ocean under the Netherlands Indian Ocean Program (NIOP, 1992–1993), the U.S. Joint Global Ocean Flux Study (US JGOFS, 1994–1996) and the U.S. Global Ocean Ecosystem Dynamics program (US GLOBEC, 1995). Mathew *et al.* (1996) studied planktonic distribution of Ostracoda collected from Arabian Sea; Purushothaman (2015) worked on the diversity of planktonic ostracods in the mixed layer of northeastern Arabian Sea.

Following marine planktonic and benthic diplostracan, ostracodan and ctenopodan species were reported from Pakistan (the source is given in parenthesis) *Podon* sp. planktonic (Khan, 1976), *Cylindroleberis mariae* (Baird, 1850) plankton (Khan, 1976 as *Asterope mariae* Baird), *Cypridina sinuosa* (Mueller, 1906), planktonic (Khan, 1976 as *Pyrocypris sinuosa* Muller), *Cypridina dentata* (Mueller, 1906), planktonic (Khan, 1976 as *Cypridina megalops*), *Gigantocypris mullerii* Skogsberg, 1920, planktonic, bathypelagic (Cannon, 1940), *Conchoecia* sp. 0.6 mm benthic (Shahab, 2002, unpublished project), *Euconchoecia aculeata* (Scott, 1894), planktonic (IndoOBIS), *Euconchoecia* sp.

planktonic (Khan, 1976), *Proceroecia procera* (Müller, 1894), planktonic, neritic (IndoOBIS), *Conchoecetta giesbrechti* (Müller, 1906) pelagic (IndoOBIS), *Orthoconchoecia atlantica* (Lubbock, 1856) pelagic (IndoOBIS), *Metaconchoecia rotundata* (Müller, 1890) pelagic (IndoOBIS), *Halocypris inflata* Dana, 1849 planktonic (IndoOBIS), *Paraconchoecia decipiens* (Müller, 1906) planktonic (IndoOBIS), *Mikroconchoecia curta* (Lubbock, 1860), planktonic (IndoOBIS), *Agelaiocypris pellucida* Mostafawi, 2003, littoral, 10–95m., subfossil (Mostafawi, 2003), *Argilloecia* sp. 5 benthic (Maddock, 1969), *Neonesidea phlegeri* (Mckenzie and Swain, 1967) on rocks with dense vegetation at low tide (CAS: INVERT 120542.0 Steiner, 1973), *Condonocera* sp., pelagic (Khan, 1976), *Philomedes* (*Philomedes*) *lilljeborgi* (Sars, 1928), planktonic (Khan, 1976 as *Philomedas lilljeborgi*), *Penilia avirostris* Dana, 1849 planktonic (Khan 1977; Nayeem, 1991), *Ancohenia hawaiiensis* Kornicker, 1976 benthic (Shahab, 2002, unpublished MSc project); Kazmi and Khatoon (2016), *Ancohenia robusta* (Brady, 1890) algal associated (Shazia, 2002; Kazmi and Khatoon, 2016), *Cylindroleberis bacescui* Kornicker and Caraion (1974), length, 2.5–4.0 mm, height, 0.55–1.7 mm. Bluish purple. algal associated in rocky area (Kazmi and Khatoon, 2016), *Cyprinotus inaequalis* Bronstein, 1928 brackish (Hartmann, 1964), *Neonesidea phlegeri* (Mckenzie and Swain, 1967) on rocks with dense vegetation (Maddocks, 1969). Khan *et al.* (2012) and Qureshi and Sultana (2016) merely mentioned of group ostracod without any further details. Recently Abbasi *et al.* (2018) reported, two genera of diplostracans – *Evadne* and *Penilia* from Karachi coast; Kazmi and Khatoon (2016) and Valeem *et al.* (2023) reported phytal marine ostracods namely *Cylindroleberis bacescui* and *Ancohenia robusta*, along with other symbiotic crustaceans. In spite of notable abundance of marine diplostracans, detailed information on the marine diplostracans in Pakistani waters is still lacking

MATERIALS AND METHODS

Onshore and offshore sampling was done. Offshore sampling was done in the Northwestern Indian Ocean within the framework of the Office of Naval Research project (ONR under Grant N00014-86-86-G-0230 1991), through partnerships with Pakistan academia and government, ONR coordinates and sponsors scientific research and technology development for the U.S. Navy and Marine Corps. The senior author had access to a large numbers of samples collected in the 1991. In shore algal samples were collected from June 1990 to May 1991; monthly algal collections were obtained from Manora and Buleji at low tide. The crustaceans were decanted.

Plankton net was used for collecting samples of plankton in standing bodies of waters in the tidal zone. The specimens were dissected and mounted on microscope slides. The line drawings were prepared with the aid of a drawing tube attachment. Abbreviations used in the text are cl and cb for carapace length and carapace breadth, respectively.

RESULTS

We report hereby brief descriptions of ostracodan halocyprids *Halocyprida* Dana and *Conchoecetta acuminata* Claus, 1890 in family Halocyprididae Dana; a podocopid *Xestoleberis* sp. and mydocopid ostracod *Parasterope* sp. of the family Cyllindroleberididae; *Rutiderma* sp. of family Rutidermatidae; *Ancohenia robusta* of family Sarsiellidae and an additional description of *Cypridina dentata* of Cyprinididae and *Penilia avirostris* - a Diplostracan of the order Ctenopoda in family Sididae. All the presently reported species are briefly described and illustrated.

KEY TO SUBCLASSES OF CLASS OSTRACODA

Latreille 1894 Ostracoda (after Anne et al., 2007).

Carapace bivalve elongate oblong to almost circular; with or without rostrum and incisur; valves usually not overlapping; 2nd antenna with large muscular protopod; long exopod with nine segments (rarely less) with long setae (often used for swimming); smaller (often much smaller) endopod with one to three segments, usually conspicuously dimorphic in adults; large lamellar furcae posterior to anus; with or without lateral eyes and Bellonci organ Subclass Mydocopa
-Carapace ovoid, inflated-sub triangular, oblong elongate, or compressed; no rostrum or incisur; valves overlap around free margin; 2nd antenna geniculate, pediform; with very small exopod with no more than two segments; much larger propulsive endopod with up to four segments; variable furca anterior to anus; no lateral eyes or Bellonci organ. Subclass Podocopa

Mydocopa Subclass Mueller, 1894 Order Halocyprida Dana, 1853

The Halocyprida is one of the two orders within the subclass Mydocopa. From the Arabian Sea forty-one species belonging to its family Halocyprididae have been described by [Drapun and Smith \(2012\)](#). In the present collection only 2 genera - *Conchoecetta* and *Halocyprida* are found.

Family Halocyprididae Dana, 1853

Conchoecetta acuminata Claus, 1890 (Fig. 2)

Material

Male, cl 0.89 mm.cb0.5mm.

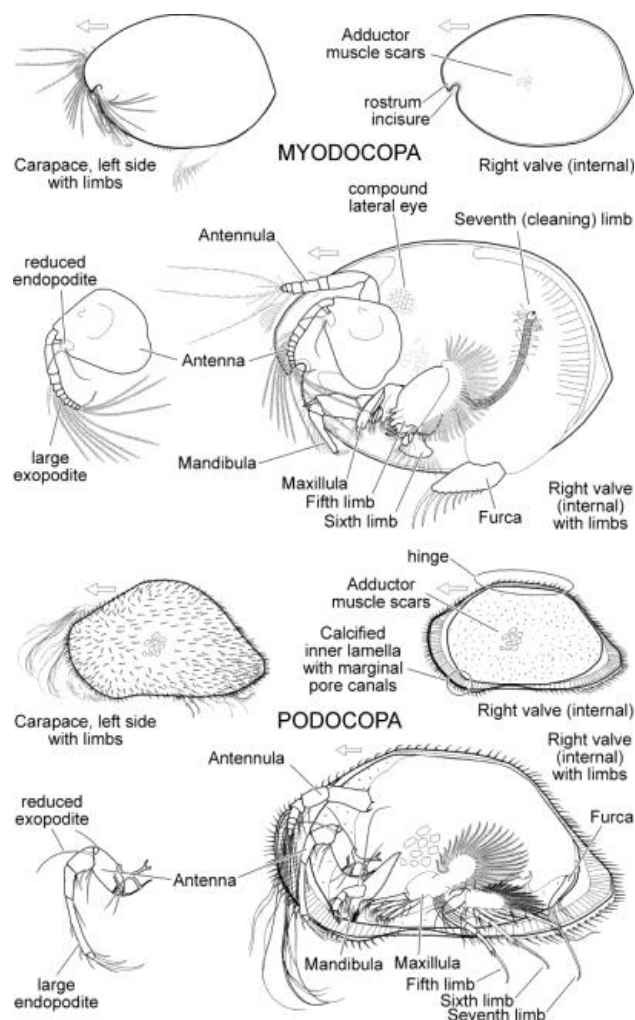


Fig. 1. Schematic diagrams of subclasses of Ostracoda (after, Rodriguez-Lazaro and Muñoz, 2011).

Description

Both the rostrum and the posterior dorsal corner of valve of the carapace are extended. The rostrum ([Fig. 2A](#)) is elongated and pointed. The front and back shoulder vaults are slightly raised. The antennule ([Fig. 2B](#)) is clearly segmented; at the distal end there are three small spines that are directed distally; just beyond the end of the armature the seta is hinged and is then flanged with very fine spinules along the edge of the flange. The protopod of antenna ([Fig. 2C](#)). is three times the length of the first exopod segment, and more than twice the combined

lengths of the remaining exopod segments that carry the swimming setae. There is a small terminal seta at the distal end of the first exopod segment that curves around the articulation with the second segment. The furca (Fig. 2D) has the typical structure for the genus with eight pairs of bare spine setae.

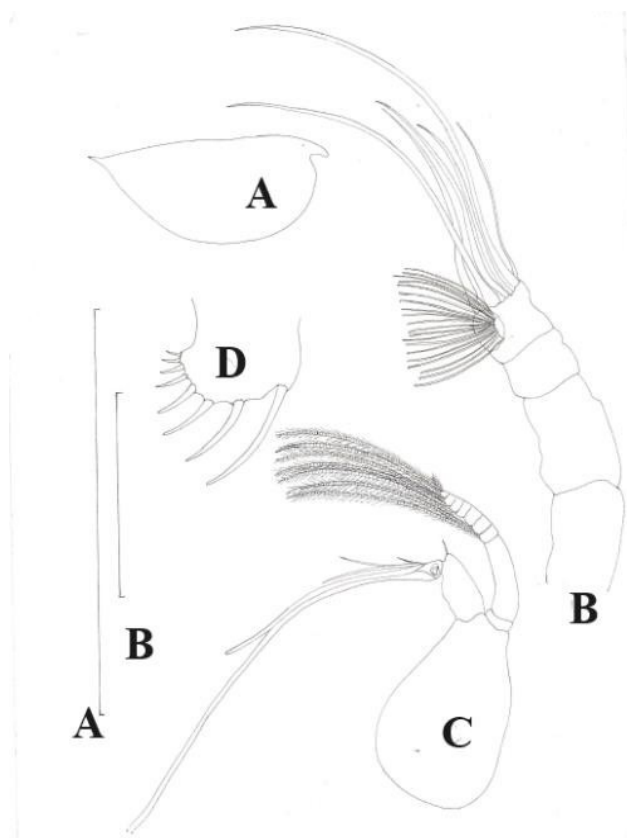


Fig. 2. *Conchoecetta acuminata*. A, carapace; B, antennule; C, antenna; D, furca.

***Halocypria globosa* Claus, 1874**
(Fig. 3)

Material

Female cl. 0.89 mm; cb. 0.5 mm

Description

The species lacks an obvious eye. Both the antennules are sexually dimorphic. In males the segments of the antennule are always clearly separated by sutures, but in the females segments are fused. In males three of the terminal setae are long, thick-walled and slender; the first exopod segment is slim composed of two segments;

the terminal segment carries five setae (Fig. 3B). In males there is a third segment, which is developed into a hook appendage. The antenna (Fig. 3C) has a bare protopod; on the endopod processus mamillaris is bluntly pointed. The mandible is with well-developed endopod (Fig. 3D), the basal segment is developed, the exopod is reduced to small pegs on the basale that carry a long plumose seta. The maxilla is as shown in Figure 3E. The fifth limb is as shown in Figure 3F and the sixth limb in Figure 3G. In males the limb is strongly developed and its three terminal setae are filamentous and subequal. In females the terminal setae are unequal. The furca (Fig. 3H) consists of two flanges which are armed with 7 pairs of hook setae, the longest first pair is off-set from the others, which progressively diminish in size posteriorly.

Remarks

Earlier identified as genus *Conchoecia* Dana.

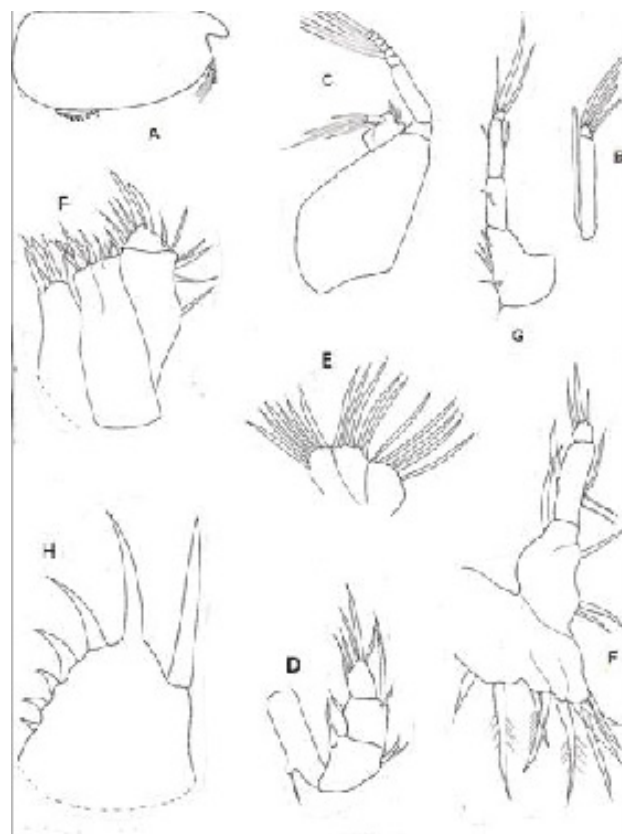


Fig. 3. A, *Halocypria globosa* carapace; B, antennule; C, antenna; D, mandibular endopod; E, endites of maxilla; F, fifth limb; G, sixth limb; H, furca.

Family Cylindroleberididae Müller, 1906

The family Cyndroleberididae is one of the largest myodocopid ostracod families with remarkable morphological diversity. The family has a worldwide marine distribution and can be found from shallow waters to depths of more than 4570 m (Kornicker, 1975). The Cyndroleberididae accounts for 225 species classified in 33 genera and the following four subfamilies: Cyndroleberidinae, Cyclasteropinae, Asteropteroinae, and Macroasteropteroinae. The Cyndroleberididae is represented in the present collection by the genus *Parasterope* Kornicker, 1975 which is the most speciose of all cyndroleberid genera of the subfamily Cyndroleberidinae with 47 species and subspecies currently listed on the World Ostracod Database. Poulsen (1965) described this genus as a new genus, but since no type species was designated, the name became only valid in 1975, when Kornicker designated a type. The genus has a global distribution.. Its species can be found in shallow waters, such as sandy mud flats near mangrove area, to abyssal depths of 4303 m.

***Parasterope* sp.**
(Fig. 4)

Material

Female, male, cl.1.5 mm. subtidal, planktonic.

Description

The male carapace (Fig. 4A) is more elongate than that of female (Fig. 4B) and with more open incisur;. Tha antennule (Fig. 4C) has a sensory seta is with robust stem and many filaments; first segment is bare second and fourth segments are without dorsal marginal spines; the margin is evenly but very weakly arched at middle; the ventral margin is straight; dorsal margin of third joint has 6 bristles; the fifth joint has sensory bristle with 2 long terminal filaments and. has 0+5 sensory bristles The antennal (Fig. 4D) endopod is prehensile and 3-jointed the exopod has 9 joints. The mandible coxale without bristle at basis of endite; a long, slender laterally placed bristle (Fig. 4E). The sixth limb (Fig. 4F) is hatchet shaped, its posteroventral margin with 29 setae with spines. The terminal part of the seventh limb is bulbous.

Remarks

Till now *Parasterope* is a genus with 49 species described so far, which makes it the most diverse representative of the subfamily Cyndroleberidinae, as well as the entire family Cyndroleberididae The cyndroleberid fauna of Pakistan is hardly known. Despite its global distribution no species of *Parasterope* have been reported from Pakistan. The present report of the genus is

the first one from Pakistan.

Order Myodocopida Sars, 1866
Family Cypridinidae Baird, 1850
***Cypridina dentata* (Müller, 1906) (Fig. 5) basionym**
***Pyrocypis dentata* Müller, 1906**

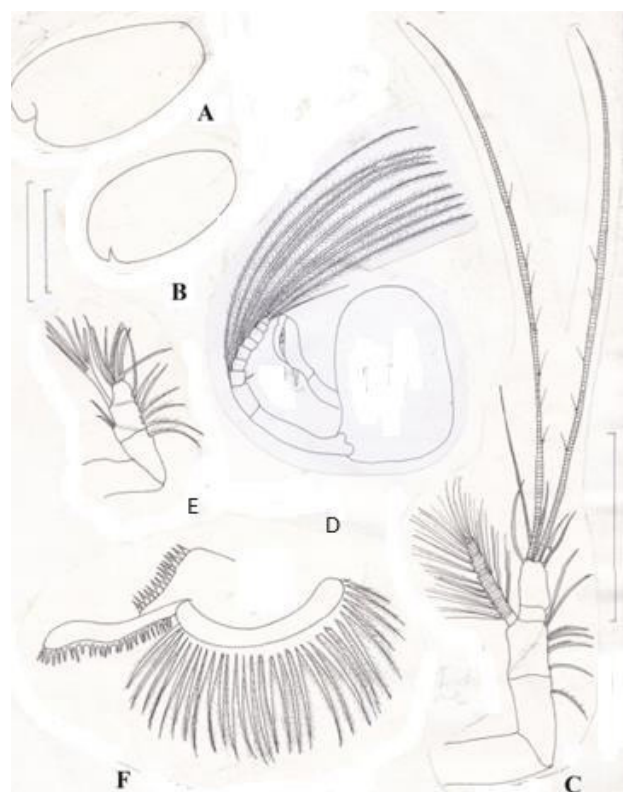


Fig. 4. *Parasterope* sp. A, B, carapaces; C, antennule; D, antenna; E, mandible; F, sixth limb.

Material

Female cl.1mm.; cb.o.7 mm

Description

Dorsal corner of rostrum is angular in lateral view (Fig. 5A), and with concave anterior margin and pointed ventral corner; caudal process is elongate with rounded tip. Outer surface of valves has 3 or 4 slender bristles along anteroventral margin near edge ventral to incisur. The rostral in fold with provided with 3 bristles forming row paralleling anterior edge of rostrum. The 1st segment of antennule (Fig. 5C) is bare. 2nd segment has no spines; 3rd segment is armed with 2 bare bristles. 4th segment is provided with 1 bare bristle. The sensory bristle of 5th segment has 2 short proximal filaments followed by 1 longer slender filament, 5 long stout filaments, 2 short

slender filaments. The 6th segment possesses a short bare medial bristle near dorsal margin. The 7th segment has a bare bristle, slightly longer and stouter than bristle of 6th segment; another bristle about half length of sensory bristle of 5th segment, with 4 short proximal filaments; one bristle about twice length of sensory bristle of 5th segment, with 8 short slender filaments, some with spines. Antennal protopod is with small distal medial bristle with few minute marginal spines (Fig. 5D); the endopod is jointed with 4 bristles. 1st segment of exopod is with few dorsal spines along concave margin; the bristle of 2nd segment reaches to about 6th segment; bristles of 3-8 segments are armed with natatory hairs but no spines; 9th segment with 3 bristles. The mandible (Fig. 5E) coxale endite is well developed, spinous, with 2 stout terminal spines, 1 on each side of small triangular process; small bristle near base of endite; basal: ventral margin with 2 small bristles; dorsal margin with 3 bristles, bare, 2 terminal; medial surface with few spines near dorsal margin; lateral surface with few proximal hairs near ventral margin. Exopod about same length as dorsal margin of 1st endopodial segment, hirsute, with terminal hirsute triangular pad and 2 sub terminal bristles. 1st endopodial segment is provided with 4 ventral bristles. 2nd endopodial segment ventral margin has few proximal spines, 2 single distal bristles, and paired terminal bristles unringed; 4 long spinous bristles, 1 shorter spinous bristle proximal to long bristles, and 1 short bristle medial to 2nd long bristle. 3rd endopodial segment with 3 equi-length claws and 4 bristles (modified from various sources).



Fig. 5. *Cypridina dentata*. A, entire; B, eggs; C, antennule; D, antenna; E, mandible; F, right furca; G, left furca; H, maxilla; I, endites of maxilla; J, fifth limb; K, seventh limb; L, seventh limb.

Instar of a myodocopid (Fig. 6)

Material

Station 132

Description

A bivalved carapace (Fig. 6A) encloses the entire soft body. one dorsodistal bristle on antennule (Fig. 6C). The seventh limb (Fig. 6B) is elongate bears bristles.

Remarks

In this order the life cycle consists of four to seven larval stages, followed by the adult. All stages are free living larval types. Instars (including nauplius): Newly hatched myodocopid ostracods have a similar morphology to that of the adults, except for their smaller size, fewer appendages, and smaller number of bristles. The free-living first larval stage, sometimes called the first instar and considered to be a nauplius, has a bivalved carapace that, when shut, encloses the entire soft body. Most useful characters are the number of bristles on the following limbs: the 4th segment of the 1st antenna, the ventral side of the 2nd segment of the endopod of the mandible, and the 6th limb; and the number and shape of bristles on the 7th limb.

The total number of larval stages in the suborder Myodocopina is four to six: The Rutidermatidae and Sarsiellidae have four larval stages; the Philomedidae has four or five; the Cypridinidae has five or six; and the Cylindroleberididae has four to six. The present larva cannot be attributed to any stage and to which family it belongs. Since in the third stage, the limb develops into the adult form and is usually furnished with many bristles as is in the present stage it is suspected that this is third stage (Atlas of Crustacean Larvae, 2014).

Family Sarsiellidae Brady and Norman, 1896

The family Sarsiellidae is divided into 2 subfamilies and 15 genera. The present genus *Ancohenia* belongs to the subfamily Sarsiellinae Brady and Norman, 1896. The genus *Ancohenia* currently has 3 taxonomic siblings. *Ancohenia hawaiiensis* Kornicker, 1976. *Ancohenia arostrata* (Kornicker, 1958) and *Ancohenia robusta* (Brady, 1890), the latter is found in study area.

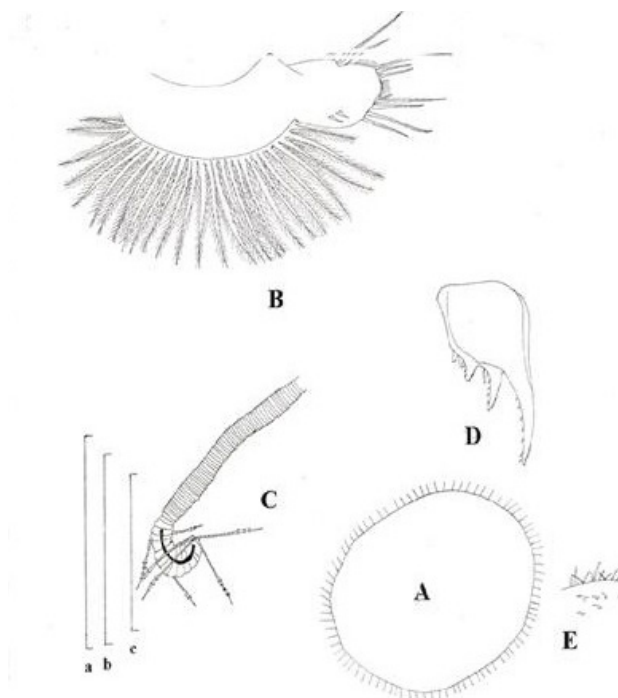


Fig. 6. Instar of a myodocopid. A, carapace; B, epipodal appendage of a limb; C, endopod of antenna, sensory tubule; D, claw; E, surface detail of carapace.

***Ancohenia robusta* (Brady, 1890)**
(Fig. 7)

Material

A large series of both sexes, cl. 1.5 mm colour golden yellow, phytal. Synonymies: *Pleoschisma robusta* Brady, 1890, *Sarstella robusta* Brady, 1897



Fig. 7. *Ancohenia robusta*. Entire.

***Eusarsiella robusta* Poulsen, 1965; Kornicker, 1981, 2002 new combination**

Material

A large series of both sexes.

Description

The carapace is with distinct but shallow sinus (Fig. 7) and truncate posterior; its dorsal margin is arched but fairly linear in posterior half, ventral margin is evenly convex. 1st segment of antennule is without any bristle. 2nd segment is with 1 distal dorsal bristle. 3rd and 4th segment are fused; third segment is short with single dorsal bristle; long 4th segment is with 2 bristles the 5th segment is small. 6th segment is long, with 1 shortmedial bristle. 7th segment has its a-bristle small; b-bristle about same length as a-bristle but with broad proximal part; c-bristle long, with 2 small proximal filaments. The 8th segment: d- and e-bristles are bare, with blunt tips; its d-bristle is shorter and narrower than e-bristle; f-bristle and g-bristle is long, clawlike proximally but bristle-like with rings distally, each bristle with short proximal filament. Antennal protopod bare. Endopod 3-jointed: 1st segment is short, with 2 proximal anterior bristles; 2nd segment is elongate with 2 long proximal bristles; 3rd segment is elongate, with broad rounded tip with transverse ridges and 2 minute bristles. Exopod 1st segment is elongate, remaining joints are short, decreasing in size distally, 9th segment is minute, with 2 bristles, 1 short, 1 fairly long with natatory hairs; 2-8 segments are provided with long natatory bristles, some with small slender ventral spines in addition to hairs. Basale: ventral margin fragmented but with 1 small proximal bristle (Modified from various sources)

Family Rutidermatidae Brady and Norman, 1896

Sparsely represented on much of the Indo-West Pacific.

***Rutiderma* sp.**
(Fig. 8).

Description

Figure 8A shows the surface of carapace is covered by circular dark dots. The incisur is deep and the dorsal margin is rather straight. 1st segment of first antenna is bare; 2nd segment is also bare; 3rd segment is fused with 4th, with 2 bristles (1 ventral, 1 dorsal). The 4th segment is elongate bearing long and short bristles (Fig. 8B).

Subclass Podocopa Mueller, 1894

The Podocopa is the only order that contains both marine and freshwater ostracods. Within Podocopa are two suborders: Metacopina and Podocopina. As will be described below a podocopin species of family Xestoleberididae Sars, 1928 in genus *Xestoleberis* Sars, 1866.

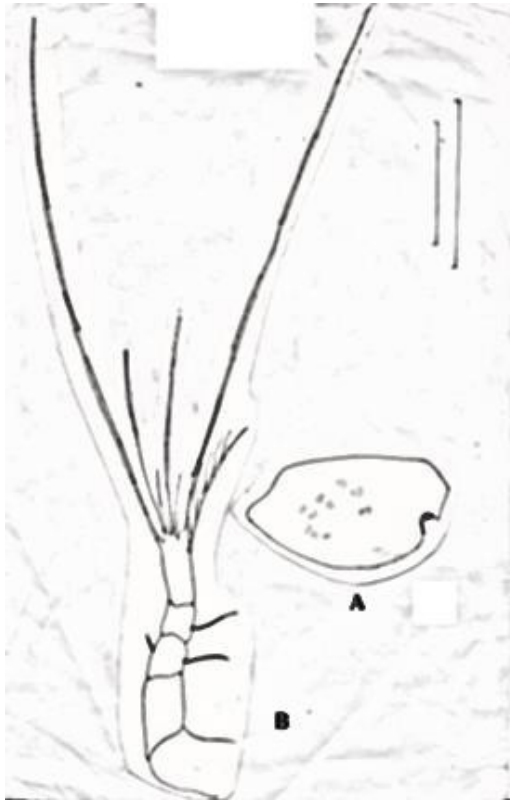


Fig. 8. *Rutiderma* sp. A, carapace; B, Antennule

Family Xestoleberididae Sars, 1928

Xestoleberis sp.

(Fig. 9).

Description

Carapace (Fig. 9A) is ovate, sub-rectangular and elongate. Ventral surface is almost straight. Maximum height is at mid-length. Maximum width is in posterior third. Dorsal margin is convex. Surface is typically smooth, margin is spiny (Fig. 9B). Eye-spot is almost inconspicuous. Antennule (Fig. 9C) consists of four articulated segments, terminal segment of the antenna with two claws; first segment with very long stout three-joined exopod; second segment trapezoidal, 1 hirsute

seta on posterior distal end; third segment shorter than first, 2 hirsute robust setae at basal anterior and posterior corners. Antenna (Fig. 9D) consisting of four segments, first segment having a very long stout two-joined exopod at anterior-distal end. Second segment trapezoidal with 1 hirsute seta on posterior distal end. Third segment not elongated, bearing 1 hirsute seta protruding from posterior edge, and 1 hirsute robust seta at apical posterior corner. Fourth segment the longest with setae on both anterior and posterior edges and 1 reduced seta on distal end and 2 stout setae on distal end. Mandible (Fig. 9E) with four segments, coxa stout. Basis carrying exopod with no setae, second segment of exopod bearing 1 apical seta at posterior distal corner, third segment of exopod with 2 simple setae and 7 setae at distal end. Vibratory plate of the mandible strong, carrying no setae.

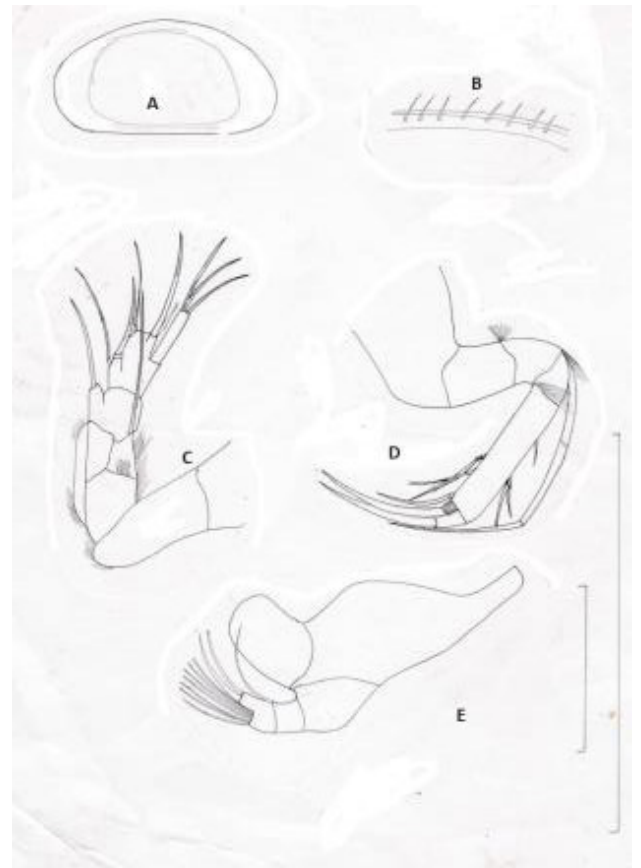


Fig. 9. *Xestoleberis* sp. A, carapace; B, carapace margin antennule; D, antenna; E, mandible.

Remarks

In order to assess the species more material is required. *Xestoleberis* Sars, 1866 is a large genus having nearly 200 species. It is one of the most common intertidal

ostracode genera in and around in certain areas of the world. The species at hand is near *Xestoleberis cornelii* Caraión, 1963 but not exactly so.

Class Branchiopoda Latreille, 1817

Order Ctenopoda G.O. Sars, 1865

Family Sididae Baird, 1850

***Penilia avirostris* Dana, 1849**

(Fig. 10A-D)

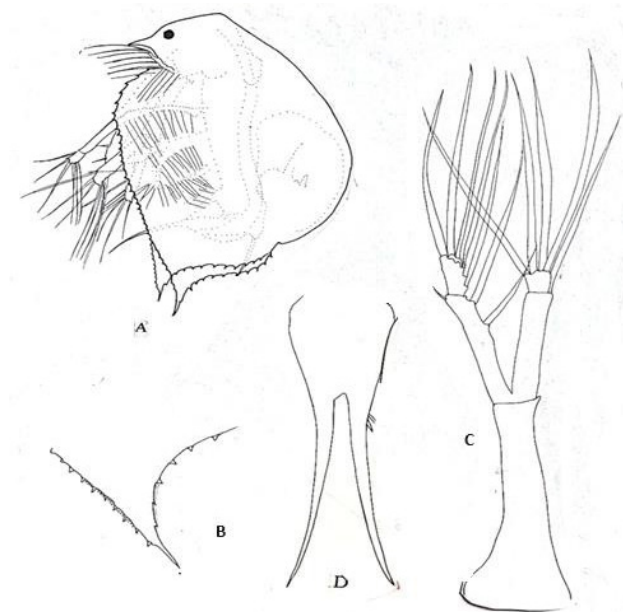


Fig. 10. *Penilia avirostris* 1.5 mm. A, entire lateral view; B, ventral shell rim; C, antenna; D, abdomen.

Synonyms: *Penilia orientalis* Dana, 1852, *Penilia pacifica* Kraemer, 1895, *Penilia schmackeri* Richard, 1895

Material

Station 25 A Female: 0.7-1.4 mm; Male: 0.6-1.3 mm.

Description

Body is transparent. The carapace is bivalve, opening ventrally, egg-shaped in lateral view; brood pouch with a large, marked spine in females (Fig. 10A), in males carapace is narrowing and strongly pointed in lateral view but without marked spine. The ventral edges of the carapace are lined with fine spines (Fig. 10B). The head is distinct, with a pointed rostrum. The abdomen bends sharply, ventrally, at the end of the carapace, and ends in two long spines (Fig. 10D). A small eye and six pairs of similar structure and feathery appendages are present. The antennule is clearly separated from the lower side of head, weakly developed in the female. The antenna (Fig.

10C) is biramous, and is as long as the carapace. Legs are short. 1st pair of legs is not elongated. Males are usually rare. The male is distinguished by its lack of a rostrum; its long antennules, which are nearly as long as the carapace; curved hook-like setae on the first pair of thoracic legs. Last segment of endopod of first pair of legs with small hook (Modified from Egloff *et al.*, 1997).

Remarks

The species is widely distributed in coastal regions throughout the world, but its true native range is still unknown. It was collected on sides of the Atlantic, the Mediterranean and Black Seas, the Indian Ocean, and the western Pacific (Ramner, 1933). It is probably the most abundant and most widely distributed of the Pakistani marine Ostracoda, also in estuaries and back waters.

DECLARATIONS

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Statement of conflict of interest

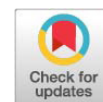
The authors have declared no conflict of interest.

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Population Frequencies and Proportional Efficacies of Acute and Anticoagulant Rodenticides Formulated Baits and Fumigant Against Rodent Pests of Wheat Crop

Muhammad Sarwar^{1*}, Areej Javaria², Muhammad Zain-ul-Abideen³,
Muhammad Farhan Sarwar⁴ and Muhammad Haroon Sarwar⁵,

¹Pakistan Atomic Energy Commission, National Institute for Biotechnology & Genetic Engineering (NIBGE), Faisalabad, Pakistan.

²ABWA Medical College, Faisalabad, Pakistan.

³Indus Medical College, Tando Muhammad Khan, Pakistan.

⁴University of Dundee, Dundee, Scotland, UK.

⁵Mayo Hospital, King Edward Medical University, Lahore, Pakistan.

ABSTRACT

The use of rodenticides is one of the most common approaches to control rodents in agricultural, urban and rural environments, while successfulness of a poison depends upon choice of suitable chemical, its formulation, and method and timing of applications. An investigation was carried out to detect the population frequency of rodent (Rodentia: Muridae) pests in wheat at various growth stages of crop and control operation against them. For evaluating the effectiveness of chemicals in pests control operation, two acute rodenticides (aldicarb, zinc phosphide), an anticoagulant rodenticide (racumin), and a fumigant (phostoxin), were applied at three growth stages (dough, maturation and pre-harvesting) of crop. A spot-baiting technique was adopted to apply poison baits to the rodent's burrows according to randomize complete block design of field. In all replications, the efficacy of rodenticides was assessed by recording the damaged wheat tillers and comparing the numbers of active burrows in the treated and uncontrolled fields fortnightly throughout the trial. During the crop growing season, amongst the trapped and identified pests, lesser bandicoot rat *Bandicota bengalensis* Gray was the primary species in wheat fields followed by Indian gerbil *Tatera indica* Hardwicke, house mouse *Mus musculus* L., soft-furred field rat *Millardia melitana* Gray and short tailed mole rat *Nesokia indica* Gray and Hardwicke. These different pests began to raid and damage the wheat crop right from sowing, depredation increased during booting stage and peaked up to the time of harvesting. Such damage was likely influenced by a number of factors, such as relative abundance of different growth stages of crop and their relative nutritional contents, palatability, tastes, and availability of other foods in the locality. The products tested demonstrated satisfactory efficacy in controlling of rodents in protected wheat plants compared to unprotected fields. During dough stage, the maximum percent of cut stems of wheat were 6.95% in the control field which was significantly ($P < 0.05$) greater from fields receiving rodenticide treatments (1.06% for aldicarb, 0.82% for zinc phosphide, 0.67% for phostoxin and 0.40% for racumin). Whereas, perusal of results on overall average numbers of active burrows revealed the maximum numeral were in control fields (6.80) and it displayed statistically significant results from aldicarb (1.33), zinc phosphide (1.03), phostoxin (0.90) and racumin (0.63) products. Biological efficacy indicates that the use of poison baits containing rodenticides, and fumigant could provide an economical and effective method of controlling the rodents in wheat fields.

INTRODUCTION

A diverse group of vertebrate pests possessing a backbone inflict economic damage to agricultural crops

* Corresponding author: drmsarwar64@gmail.com
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(Sarwar, 2018, 2019, 2023). Rodents (rats and mice) are very destructive pests and become a nuisance when they come in adjacent vicinity to humans, crops and storages. As well as spreading diseases to both humans and household pets, rodents can also cause destruction of goods and infect food sources in certain localities. These are one of the most important vertebrate pests that directly relate to the production, storage and utilization of field crops by man and livestock at the global level. The tropical climate enhances breeding and flare-up of rodents, and recurrently they continue to build extraordinary population levels in agricultural habitat (Sarwar, 2015a). At the worldwide level, rodents have been identified as the most successful

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Authors' Contribution

MS performed research work in field and laboratory, compiled the data of research work, and writeup of the manuscript for publication. AJ, MZ, MFS and MHS helped in lab works and field operations.

Key words

Rodenticides, Rat, Mouse, Muridae, Wheat, Pest control, Rodent baits, Aldicarb, Racumin, Zinc Phosphide, Phostoxin

mammalian agricultural pests. The effect of rodent damage causes huge amount of crop losses and food shortages in some parts of the world (Fayenuwo *et al.*, 2007). Among the rodents, most of the harmful and notorious recognized as pests are members of the family Muridae. As a result, a large number of crops during pre- and post-harvesting periods is being lost every year (Meerburg and Kijlstra, 2008).

Among farmers practicing wheat growing, there is considerable fear that rodents can become a serious problem from the seedling to crop maturity owing to increased vegetation cover. Rodents damaged 6.32-8.15% wheat tillers and the maximum damage has been recorded when the crop is at maturity stage. Lesser bandicoot rats *Bandicota bengalensis* Gray hoarded on an average about 0.486 kg ear-heads per burrow system (Sheikher and Jain, 1991). During the wheat growing season locally, various rodents species are involved in damaging to crop, but variations in their predominance have been detected. Of the trapped and identified pests, the lesser bandicoot rat *B. bengalensis* is the mainly crucial species in the wheat fields approached by Indian gerbil *Tatera indica* Hardwicke, house mouse *Mus musculus* L., soft-furred field rat *Rattus meltdada* Gray and short tailed mole rat *Nesokia indica* Gray and Hardwicke. These different pests begin to damage the wheat crop right from sowing, depredation increases during the booting stage and peaks up to the time of harvesting. Rodents directly damage to wheat grain and carry infection to the storage by posing a food safety risk. Rodents urinate on grain posing a food contamination threat and contaminated grain is unacceptable for consumption. The increase in rodent populations and economic damage they cause in a specific wheat field is influenced by several conditions. The species of rodent concerned, its reproductive condition, phase of its population cycle, type of edge surrounding the wheat field, history of the field, weather situations and all other factors around the field upset the potential for rodent damage (Sarwar, 2015b).

Rodenticides are commonly used for rodents control in residential, business and agricultural settings. Their effectiveness depends upon the selection of an appropriate compound, its formulation, and the method and timing of applications. Such lethal approaches, particularly the use of rodenticides provide an immediate solution to the problem and are considered the most practical, economical and effective method of combatting rodents (Buckle, 1994). Rodenticides are commonly used for rodent control, removal and to help get rid of in home, business site or field. A variety of lethal and non-lethal practices, comprising elimination, habitat adaptation, and trapping are existing that could successfully control these pests. However, investigations on rodent pests in the tropical farm

fields are of utmost importance as the crops losses due to rodents are reported high (Balakrishnan, 2004). Because, at the worldwide level, rodents have been identified as the most important mammals, the aim of present investigation is therefore to study proportional field efficacy of some acute, anticoagulant rodenticides formulated baits and fumigant against rats and mice pests of wheat crop.

MATERIALS AND METHODS

Study area

Study area of current research was comprised of about 50 acres of wheat fields in the vicinity of village 26/E.B., Arifwala in Sahiwal District (now included in District Pakpattan) in the Punjab province of Pakistan. It is located to the southwest of Pakpattan at latitude 30° 17' 38 N, longitude 73° 4' 25 E and altitude 252 m. The climate of Arifwala is extreme, reaching up to 50 °C (122 °F) in summer and down to 0 °C (32 °F) in winter, and the soil of the suburbs is very fertile. The experimental area was divided into 5 replications including check (untreated) each having an area of 10 acres arranged in randomized complete block design. Study program for controlling of the rodents problem in wheat fields was started after crop growing and continued till harvesting. The fields around study area were of heterogeneous type vegetation-ally in which wheat, fodder and some other crops together with a small tract of uncultivated sandy area were present. There were buffer areas between rodenticide treated fields that could minimize movement of rodents (immigration, emigration) among untreated plots.

Rodent's population in wheat fields

Before carrying out any control operation, all the replications were studied for rodent's damage, during seedling, tillering, booting, dough, maturation and pre-harvesting growth stages of wheat. To study the rodent's damage pattern to growing wheat, the data were recorded fortnightly from the wheat fields. To identify the pest specimens and their population dynamics, 10 acres of randomly selected wheat fields of the experimental area were trapped from the seedling to maturity periods. On each field of the selected wheat at each growth stage, four snap traps (16 × 10 cm metallic trap) supplied daily with food baits consisted of bread pasted with butter oil were operated per plot (2 acres) for three consecutive nights per fortnight.

Rodent control program

For managing of rodent pests, four different rodenticides representing three different groups were applied as control agents in wheat field. (1) Acute (single dose, fast acting) poisons, in which baits were prepared

by mixing zinc phosphide and aldicarb with suitable cereals and other materials. (2) Chronic (multiple doses, anticoagulant, death occurs one to two weeks after ingestion of the lethal dose) poison, in which baits were prepared by mixing of racumin (coumatetralyl) along with suitable cereals and other materials. (3) Fumigant, fumigation of live burrows with tablets of aluminum phosphide (i.e., phostoxin 3.0 g tablet). For evaluating the effectiveness of chemicals in pest control operation, the rodenticides were applied at three growth stages of wheat crop i.e., dough (75-90 days of sowing), maturation (105-120 days of sowing) and pre-harvesting (135-150 days of sowing). The following ready-to-use rodenticide baits were used on the experimental site of wheat during experimentation.

Acute rodenticides

To prepare the poison baits of acute rodenticides the basic grain used were wheat and maize, wherein only the healthy grain of both cereals were used during bait preparation. The ingredients of various rodenticides used in reducing rodent populations and damage in wheat crop for 2% zinc phosphide, aldicarb and racumin are given in the ensuing section.

1. zinc phosphide, 100 g; wheat flour, 2.5 kg; cracked wheat, 2.5 kg; butter oil, 100 g
2. aldicarb 10 G, 100 g; wheat flour, 2.5 kg; cracked maize, 2.5 kg; full cream dry milk, 100 g
3. racumin, 150 g; broken rice, 5 kg; vegetable oil, 100 g

For the preparation of baits of acute poisons, the flour and grains were mixed well with zinc phosphide/aldicarb separately. Then butter oil or full cream dry milk was added and mixed well accordingly. After that water was added and mixed well until a stiff barter formed. The baits so prepared were stored in a cool dry place until used.

Chronic rodenticide

To prepare racumin baits, the grains were coated with vegetable oil and then mixed while stirring, slowly racumin was added and blended till the grains coated evenly. The baits so prepared were used in the loose grain form.

Fumigant

The fumigation was done by applying one tablet of phostoxin per burrow. Immediately after that the treated burrows were plugged with grasses or leaves and then sealed with soil. They were rechecked next morning and burrows found open were again treated as mentioned above.

Application of poison baits

Pre-baiting was undertaken with the baits prepared according to above cited procedure, but without use of

poisons. Pre-baiting was undertaken on the spots where the rodents were likely to visit, in order to encourage feeding on the baits before the poison baits were applied. The poison baits were placed on the same spots where pre-baiting was done. Baits were applied by placing them on polythene paper near the active burrows. The extent of pre-baits consumed was considered as a rough indication about the amount of poisonous baits required. At spots where all the baits had been consumed, twice the amount previously used was placed. On the wheat fields, baiting was started about 9 weeks after sowing, when wheat crop was at its booting stage, during the middle of January. Likewise, second and third baitings were done during the middle of February and middle of March, respectively. Within each replication (10 acres each), each block of 5 treatments (control + 4 rodenticides) replicated 5 times were treated with zinc phosphide, aldicarb, racumin, phostoxin and untreated check according to randomization (2 acres each). The first application of rodenticides was confined to bunds and irrigation embankments. About 4 to 6 bait pieces weighing approximately 10 to 12 gm (zinc phosphide and aldicarb) for each baiting point were placed along each field edge and at every intersection of bunds. Second and third baitings were made inside the fields by spot baiting as well as along the bunds. This was done by walking 3-4 m from the intersection of bunds and making transect line inside the field. All active burrows were treated along this transect line and in each field three or four such transects were made and lively burrows treated accordingly.

Racumin baits were applied in loose grain form, weighing about 25 gm, for each baiting point. These were placed along with bunds and embankments at about 15-20 m intervals and also inside the fields near the active burrows along transect lines made in the same fashion as in the case of zinc phosphide and aldicarb baits placement. Cares were taken if the rain was anticipated, the baiting application avoided shortly for 2 to 3 days. The baiting was also avoided 2 to 3 days before or after irrigating the fields. The baits were placed at left or right side of active burrows and not in front of the open hole as these were likely to be buried by fresh dirt pushed out of the burrow mouth by the rodents. Normally, baits were placed throughout the infested area at points where there were signs of rodent's activity, such as fresh pest's feeding places, droppings and runways, and presence of tillers inside burrows.

Rodenticides evaluation

The results of all the treatments of four rodenticides used as rodents control agents in the wheat fields were recorded fortnightly. In all replications, the efficacy of the rodenticides was assessed by recording the damaged wheat

tillers and through comparing the number of active burrows in the treated and control fields. To count the damaged and undamaged tillers, 1×1 m quadrat delineated by iron frames was used as the sampling unit. Within each field (2 acres) the nearest corner in the first field was chosen and observation taken, then a diagonal line followed in the second field to the opposite corner of first field, observation drawn near this corner, and third sampling was drawn in the centre of both fields along predetermined diagonal (3 quadrates) line to count both damaged and undamaged tillers. Then total stems, cut stems and average percent damage of three samples were calculated. As the damage data were to be recorded fortnightly, therefore, after each interval only the fresh damaged tillers recorded. The criteria used to distinguish fresh rat's damage from old damage were: (1) Tillers with fresh rat damage were green, without or with decay at an early stage. (2) Tillers with old rat damages were turned brown or dry, with the decay at an advanced stage.

To distinguish potential rodents and other pests damaged tillers from each other, cut portion of wheat plant was observed. Though rats, mice and parrots (Psittaciformes: Psittacidae) cut wheat stem at the top near the base of ear, but rodents damage was scattered throughout the field and not clumped along the edges as the parrot injury happened. Furthermore, the teeth marks on damaged portion were noted when cut by rodents and they either left the half eaten ears or carry to the burrows, while parrots transfer it away.

The experimental site was also surveyed to record burrows distribution of the rats and mice, and for this purpose, study spots for all the burrows in the fields were observed. It was also noted whether a given burrow was located at the margin of the field, in field embankments separating two fields, on the embankment of water courses, or inside the fields. The observations on active burrows were recorded by walking along the straight lines 4 m apart from each other both within treated and control fields. Efforts were also made to know whether a given burrow system was active or abandoned. To meet these requirements, the following procedure was followed: (1) All the burrows which evidenced sign of recent digging activities of rodents such as soil at the opening were considered as active burrows having rodents inside. (2) Burrow systems which carried no fresh dirt in front of the burrow mouth, presence of web and dry leaves at burrow entrance and absence of foot prints, were considered signs of inactive burrows. (3) Filled in the mouth of all the rats or mice holes found, then again re-examined next day and all reopened holes indicated that a rodent was present and considered as active burrows otherwise inactive.

Safety precautions

The main expected hazards either directly or indirectly concerned with rodents and their control operation were the possibility of occurring the rodent-borne diseases and the risk of accidental poisoning to non-target species or man. By observing the important safety precautions, no any hazards were observed under the field conditions. Rodenticides, like all other pesticides, were handled responsibly and used in accordance with the label's instructions.

Data analysis

Data counts collected during sampling periods were defined as the percent damaged wheat tillers, the number of active burrows and number of rodents within treated and control sites. Pre- and post-treatment data were used to compute the efficacy of rodent baits containing rodenticides. All data were analyzed using SPSS (statistical product and service solutions) statistics version 15.0 for Windows (IBM, China). Differences between pre- and post-treatments, and within the treated and control sites, at fortnightly sampling periods were assessed using a LSD post-hoc test after the ANOVA, at $P=0.05$.

RESULTS

During the crop growing season, several rodents species were involved in damaging to wheat, but variations in their predominance were detected. The effectiveness of poison products displayed the highest variations in their efficacies, but the baits significantly reduced the prevalence of rodents in infested crop.

Rodent's population frequency

Of the trapped and identified rodent pests, the lesser bandicoot rat (*Bandicota bengalensis*), the Indian gerbil (*Tatera indica*), the house mouse (*Mus musculus*), soft-furred field rat (*Millardia melitada*) and short-tailed mole rat (*Nesokia indica*) constituted the numbers viz., 32, 17, 14, 7 and 1, respectively, from seedling to crop maturity (Standard error 0.553; critical value 1.2339; DF 14). This comparison on captures of the different species of rodents was at different growth periods of crop in wheat farmland during seedling, tillering, booting, dough, maturation and pre-harvesting stages. Bandicoot rat was the primary rodent pest of wheat; it invaded the wheat fields in appreciable numbers when the crop was in dough growth stage. In the subsequent stages of growth of the crop, this outnumbered the other species. The bandicoot rats began to damage the wheat crop right from sowing, their destruction increased during the flowering stage and peaked at the time of harvesting. Wheat fields also harbored the gerbils in good numbers during all growth stages, but the pest attained

maximum abundance during the dough stage, after which its numbers were continually decreased as the bandicoot rats became increasingly predominant. Corresponding to gerbil, the house mouse infested the wheat crop in almost all the stages of growth and fairly in good numbers too. This was observed that only during the pre-harvesting season its number dwindled considerably. The field rats infested the wheat fields in rather lower numbers than bandicoot, gerbil and house mouse in all growth stages of the crop. Their numbers remained relatively stable and did not fluctuate like those of all the other three pests species. This species was noted in the wheat fields maximum, when the crop was in booting and dough stages. However, they were scarce during the ripening and pre-harvesting stages. A single specimen of short-tailed mole rat was trapped in the investigational zone and it observed just earlier to harvest at crop maturation (Fig. 1). Generally, the incidence of rodents damage to the wheat-growing area was patchy.

The frequency of occurrence of stem density varied from 117-359/m² for all the wheat fields sampled from seedling to harvest. In general, it was observed that as the stem density per unit area increased, rats damage increased concomitantly. However, the damage distribution among fields having dense population of wheat stem per m² was very severe than surrounding fields. Virtually no damage was recorded for fields with less than 117 stems/m², but as the stem density approached 359 stems/m², pest's damage increased up to greater extent.

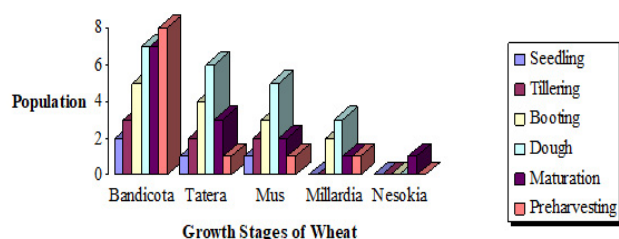


Fig. 1. A comparison of densities of different rodents species recorded at different growth stages of crop in wheat farmland.

During this study, wheat plants were found to possess a large potential for compensatory recovery from rat and mouse damage that occurred during the early growth stages. Beyond the vegetative or tillering stage, however, this potential disappeared rapidly. At the tillering stage, the secondary tillers were as productive as the original intact tillers and growth compensation offset the early damage. In the booting stage some plants compensation was observed, but panicle size was condensed because the grain had less time to mature fully. At the dough stage

there was no measurable plant compensation and panicles did not form before harvest. There was no opportunity for tillering's development when damage was caused at the mature stage, so losses at this time may be found to be very high.

Rodenticides evaluation

Table I shows the percent cut stems recorded before treatments during various growth stages viz., seedling, tillering and booting. It is clear from the results that seedling stage with 0.83% and tillering stage having 1.11% cut stems differed statistically from booting stage with 1.48% cut stems, while seedling (0.83%) and tillering stage (1.11%) reflected non-significant results. A perusal of the results on the percent cut stems recorded before treatments during various fortnights depicted that the crop damage started from first fortnight (15 days after crop sowing) and increased through second fortnight (30 days after sowing), which differed non-significantly. However, damage increased continuously up to the third to fourth fortnights (45-60 days after sowing), and cut stems differed significantly from first and second fortnights.

Table I. Pre-treatment data on comparative effectiveness of rodenticides in reducing rodents damage and burrows at different growth stages of wheat crop within the treated and control sites.

Wheat growth stage	Days after crop sowing	Percent cut stems of wheat	Number of active burrows
Seedling	15	0.83 b	1.40 b
Tillering	30	1.11 b	2.20 b
Booting	45-60	1.48 a	4.20 a
Standard error		0.129	0.400
LSD value		0.298	0.922

Means within columns followed by the same letters are not significantly different ($P = 0.05$).

The data recorded on the number of active burrows before treatment during various fortnights are also given in Table II. The results of statistical analysis depicted that the number of active burrows enhanced abruptly from seedling (1.40), tillering (2.20) and booting (4.20) stages, therefore, seedling and tillering growth stages differed significantly from booting stage. Perusals of these results indicate that the number of active burrows during third and fourth fortnights differed significantly, while in case of first and second fortnights these showed non-significant differences with each other. Though the numbers of active burrows increased tenuously, yet the abrupt activity of rodents had been recorded during third and fourth fortnights.

Table II. Comparative effectiveness of rodenticides in reducing rodents damage and numbers of active burrows in wheat fields.

Treatments	Growth stages of wheat crop					
	Dough (75-90 days of sowing)		Maturation (105-120 days of sowing)		Pre-harvesting (135-150 days of sowing)	
	Percent damage	Active burrows	Percent damage	Active burrows	Percent damage	Active burrows
	First baiting program		Second baiting program		Third baiting program	
Zinc phosphide	0.65 b	0.90 bc	0.97 b	1.00 bc	0.86 c	1.20 b
Aldicarb	0.84 b	1.30 b	1.06 b	1.50 b	1.28 b	1.20 b
Racumin	0.28 c	0.60 c	0.42 c	0.60 c	0.52 d	0.70 b
Phostoxin	0.57 bc	0.90 bc	0.79 bc	0.80 bc	0.66 cd	1.00 b
Control	4.78 a	5.80 a	6.75 a	6.80 a	9.34 a	7.80 a
St. error	0.152	0.325	0.237	0.354	0.125	0.431
LSD. value	0.308	0.657	0.480	0.716	0.254	0.872

Means within columns followed by the same letters are not significantly different ($P = 0.05$).

The data reflecting the comparison of test rodenticides on the average percent cut stems after each treatment at dough stage of wheat (75-90 days of sowing), indicated that maximum percent cut stems of wheat were recorded in control fields (6.75%) and it differed significantly from all other treatments. Whereas, the treatments of racumin (0.42%), phostoxin (0.79%), zinc phosphide (0.97%) and aldicarb (1.06%), provided non-significant results with one another. The results of statistical analysis given on the average percent cut stems recorded during maturation (105-120 days of sowing) growth stage depicted that all the treatments viz., zinc phosphide (0.65%), aldicarb (0.84%), racumin (0.28%) and phostoxin (0.57%) differed significantly from control fields (4.78%). All the treatments, zinc phosphide, aldicarb, racumin and phostoxin gave non-significant results with each other. But, the mean damage in racumin treatment evidenced lower level with that of phostoxin, zinc phosphide and aldicarb. The percent cut stems recorded after pre-harvesting (135-150 days of sowing) period and results of statistical analysis given depicted the racumin (0.52%), phostoxin (0.66%), zinc phosphide (0.86%) and aldicarb (1.28%) losses resulted after second fortnight of application. A perusal of these results revealed that all the treated areas differed significantly in injury from control fields (9.34%) damage.

The results obtained from statistical analysis regarding number of active burrows recorded during various growth stages viz., dough, maturation and pre-harvesting are given per plot in Table II. The perusal of the data in the relevant Table II revealed that number of active burrows during dough (75-90 days of sowing), were maximum in the control fields (5.80) and it differed significantly from all the rest of treatments, racumin (0.60

phostoxin (0.90), zinc phosphide (0.90) and adicarb (1.30) and showed statistically related results. The number of burrows recorded after maturation (105-120 days of sowing) of wheat of each treatment area indicated that the total active burrows were negligible in the treated areas, while maximum in untreated (6.80) fields. However, the data revealed a significant difference among control and all other treatments. As far as the treated area is concerned, the active burrows were maximum in aldicarb (1.50), followed by zinc phosphide (1.00), phostoxin (0.80) and racumin (0.60), which did not show any significant difference among themselves. The average numbers of active burrows recorded at pre-harvesting stage (135-150 days of sowing) presented in this table revealed that number of active burrows in the control area (7.80) were the maximum as compared to treated areas and showed significant results among each other. The aldicarb (1.20) and zinc phosphide (1.20) indicated good control of rats and mice, and the number of active burrows in these treatments differed non-significantly from that of racumin (0.70) and phostoxin (1.00).

The overall average percent cut stems recorded during dough, maturation and pre-harvesting stages after all the treatments are depicted in Figure 2. The maximum percent cut stems of wheat were in control fields (6.95%) and it differed significantly from all other treatments. Whereas, results of aldicarb (1.06%), racumin (0.40), zinc phosphide (0.82%) and phostoxin (0.67%) did not differ statistically (standard error 0.795; critical value 1.834; DF8). The overall average number of active burrows recorded at various growth stages after all treatments are also given in Figure 2, the perusal of results revealed that the number of active burrows were maximum in control fields (6.80) and

it differed significantly from all other treatments. However, zinc phosphide (1.03), aldicarb (1.33), racumin (0.63) and phostoxin (0.90) showed statistically non-significant results for each other (standard error 0.358; critical value 0.827; DF 8).

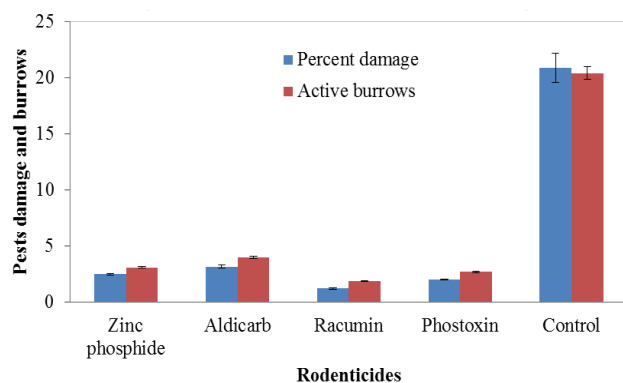


Fig. 2. Comparative effectiveness of rodenticides in reducing rodents populations and damage in wheat fields (Error bars= \pm standard error of Mean).

DISCUSSION

Present study observations made in the wheat fields reported the damage inflicted on the crop by the guild of 5 rodents species belonging to family Muridae namely *B. bengalensis*, *T. indica*, *M. musculus*, *M. meltada* and *N. indica* with *B. bengalensis* being the most prevalent species from sowing to harvesting. In fact, the surveys in the same locality revealed that the sugarcane crop was found infested with six species of rodents including five as mentioned above plus a squirrel *Funambulus pennanti* L. (Sarwar *et al.*, 2011). Furthermore, results revealed that guild of four rodent species viz., the lesser bandicoot rat (*B. bengalensis*), the soft-furred field rat (*M. meltada* Gray), the short-tailed mole rat (*N. indica*), and the house mouse (*M. musculus*) was observed damaging in the rice fields (Sarwar, 2015c). Such information on the presence of rodents in disturbed and primary habitats agrees with other earlier observations (Cabello *et al.*, 2006), confirming the wide ecological tolerance (niche breadth) of these pest species. However, abundance variations in the different biotopes suggest the importance of several environmental parameters, such as food, cover and predators of pests. These different responses of mice and rats, and also variations in their densities, highlight the importance of monitoring of targeted pests species during control operations (Bennett *et al.*, 2002). These findings also emphasize the importance of monitoring the densities of mouse populations, particularly prior to sowing, to

determine if management is warranted (Brown *et al.*, 2007). The variations in the predominance of species in different regions also relate to the cropping patterns of a locality as *B. bengalensis* predominant in fields of paddy-wheat rotation, *R. meltada*, *T. indica* and *Mus* spp., in cotton and groundnut-wheat rotations, and *B. bengalensis*, *R. meltada* and *T. indica* in millet and maize-wheat rotations (Parshad, 1991). The rodenticide treatments conducted in farmer's fields revealed that to protect the sugarcane crop from rodent's damage during the months of December-January, the rodenticide treatment may be applied either by double-baiting with 2% zinc phosphide followed by 0.005% bromadiolone after 15 days at 1 kg/ha each, or by single-baiting with 0.005% bromadiolone at 2 kg/ha. Likewise, the impact of rodenticide treatments in cane fields was also evident in the adjoining wheat crop fields where the incidence of rodent damage was less (0.97-3.24%) than in the fields surrounding untreated cane fields (3.53-6.22%) (Singla and Parshad, 2010). Hereafter, the rodent populations, which generally ceased to breed during the winter season, were forced to move from one crop to another following their harvesting at different times of the year. Consequently, the pest populations dispersed and then concentrated in certain fields where they could be easily destroyed using an appropriate rodenticide concentration in the vegetable, fodder and sugarcane fields (Durr-i-Shahwar *et al.*, 1999).

Our results indicate that the average numbers of cut stems and active burrows recorded during dough, maturation and pre-harvesting stages showed non-significant differences, yet all the treatments differed significantly from the control designating that losses caused by rodents and measures taken for their control during various growth stages had non-significant differences i.e. crop stages have no effect on rodent's damage, though the data of treatments differed significantly from control. Henceforth, when growers are engaged in rodents control in the wheat fields, a jointly concentration on their destroying through poison baiting in other minor and major crops of the area is utter most worthy before these pests start migrating to the wheat fields. If necessary, during October or November the poison baiting in these crops might be reiterated, earlier to the rodents begin to infest the wheat fields. Through this tactic, the rat and mouse individuals persisting in the October and November control actions can have not an opportunity to build up their populations for the reason that throughout the winter months these pests turn into reproductively quiescent, or else significantly slow down the practice of reproduction. When the food and shelter circumstances in the wheat cropland are momentarily refining above a massive range, then the probability of migration of rodents resulting from

lack of diet and housing can be very little or the chances of immigration from outside the protected area may be very negligible. In this context, pest management program in wheat may be operated on the fundamental concepts of the economic injury level and the action threshold value. However, in integrated cropping systems, pesticides may be used to control pests only when a risk of damage is established, and pesticide usage can be reduced as much as possible by using other cropping practices aiming at reducing the occurrence of pests e.g., resistant cultivars, minimum tillage and lower nitrogen fertilizer rates (Loyce *et al.*, 2012).

The present investigations assessed the efficacy of poison bait formulations in the wheat land using acute and anticoagulant rodenticides, and fumigant. During the crop growing season, the products tested demonstrated satisfactory efficacy in controlling of rodents in wheat crops, while product racumin displayed the highest efficacy. Rat activity was significantly reduced in poisoned compared with non-poisoned areas after bait stations were stocked. At present, most commonly used rodenticides are available in various formulations for control of rats and mice, thus facilitating as one component of an integrated pest management program. Most commercial poison baits are registered for rats and mice, and their level of susceptibility to the toxicants as well as the attractiveness and palatability of the bait formulation varies between the pests species and even between individuals of the same species. No rodent bait ingredient is universally highly acceptable, so, to achieve good control of rodents using rodenticides, selection of the appropriate toxicant and formulation (grain or attractant) as well as bait placement, are important considerations (Jokic *et al.*, 2010; Sakthivel and Neelananarayanan, 2014).

During this study, the data examination based on the average numbers of cut stems and active holes, and rodenticide efficacy measured in three stages of wheat crop displayed the products tested demonstrated satisfactory efficacy in controlling of rodents. This is due to the fact that anticoagulants are more toxic because these bind more tightly to the enzyme that makes blood clotting agents, can also interfere with other steps in Vitamin K recycling, are not easily excreted from the body, and they can be stored in the liver (Watt *et al.*, 2005; Mchomvu *et al.*, 2013). This information on the biological efficacy of chemicals for controlling of rats and mice in wheat-based fields is in compliance with the previous findings (Durr-i-Shahwar *et al.*, 1999; Pervez *et al.*, 2005; Singla and Parshad, 2010), wherein the researchers for controlling of rodents depredations in the croplands, used common rodenticides and the products tested demonstrated satisfactory efficacy for pests control. Thus, regular control and sustainable

operations are essential if rodents pest populations are to be kept within tolerable limits (Mulungu *et al.*, 2006). However, as the pesticides manufacturing is fairly vigorous, therefore the participation and contributions of several stakeholders in making rodenticides can be overemphasized.

During most of the session, the variations in the densities of rodents recorded in different trapping periods can be associated with the fluctuations in population size in the pests wherein there were more rodents in the wheat than in other networks harmoniously. Correspondingly, it is emphasized that rodent control programs could be more effective if applied during the pre-breeding season, which also coincides with the reproductive phase of the vegetation communities around (Workneh *et al.*, 2004). Further, as rodents get shelter in areas of natural vegetation around farmlands throughout the seasons, it is guided to follow up clean farming practices by clearing shrubs and rock piles in areas around farmlands, as an environmental means of rodent's control. Moreover, as the relative abundance of rats and mice varied significantly between pre- and post-harvest sessions, Jacob and Brown (2000) have emphasized that harvesting not only results in food scarcity for pests, but also exposes rodents to predators, leading to increased predation risk. Naturally, they move away from such areas to have more suitable areas, thus resulting in decreased population density in farmlands after harvest.

Conversely, there are numerous significantly undesirable aspects to the usage of rodent poisons. For the reason of the overuse of rodenticides, some rats and mice populations have developed resistance to the toxicants. Furthermore, the practice of rodenticides can be a dangerous exercise for the environment, thus, aim of integrated pest management (IPM) is to decrease two special things, the pest population and the quantity of pesticides desirable to achieve that objective. With the exception of emergency circumstances, entirely other existing control approaches must be used before using of a pesticide. Prior to use of any rodenticide in an IPM package, sanitation, maintenance and other less toxic control methods must be operated. Some of the most effective methods of prevention using sanitation and maintenance are by limiting rats and mice access to their nesting sites, and food and water supplies. Detection and monitoring are important in controlling of mouse and rat problems by observing nesting areas, food sources, water sources, access points, signs of rodent activity, and removal of individuals by trapping. For monitoring, rodent traps fall into three main categories including snap traps, live traps and glue boards. When mice and rats persist and other management techniques are insufficient, then it warrants control of pests problem

via chemical pesticides to handle emergency situations. For future rodents control operation, keep record of all chemicals applied in IPM logbook including name of the applicator, date of application, formulation used and the brand name of the chemical used (Sarwar, 2015d, 2016).

CONCLUSION

The key objective of this study is to advance an operational strategy for employment of rodent's management program by the use of rodenticides in wheat ecosystem and its implementation in diverse pest locations. The outcomes signpost the usage of poison baits containing rodenticides, and fumigant to deliver an economical and effective method of controlling the rodents in wheat fields. The picture of overall average percent cut stems and average numbers of active burrows recorded during dough to harvesting stages reflected that the maximum damage of wheat was in uncontrolled field and it differed significantly from other treatments i.e., aldicarb, zinc phosphide, phostoxin and racumin. On the other hand, the development of an operative controlling approach to reduce damage due to rodents necessitates an understanding of the exploitation and collaborations among the diverse habitat elements of the ecosystem. After the wheat is harvested from fields, the field populations of rodents may become markedly less abundant or evidence little change. When most of the wheat fields are harvested, the mouse can use the sheaves of the harvested plants for food and shelter. Further, the bandicoot rat, undoubtedly in consequence of its subterranean mode of life and grain storing habits, can be able to continue infestation of the harvested fields. Undoubtedly, such type of behaviors of rat and mouse enable the pests to continue their numbers in the harvested wheat fields. Soon after harvesting, flooding of the fields for the duration of irrigation and succeeding ploughing can have devastating effects on the bandicoot and mouse populations. This sort of rodent's disturbance can arise to wheat fields much earlier to prepare soil for the sowing of rice crop that is helpful to control pests without using of rodenticides. Feasibly, this can be done in the agroecosystem through restriction of the length of the harvesting and sowing periods of the gramineous crops. The fundamental hint in it, is to withdraw shelter and food for the rodents to make them more defenseless to predation and the mortality components. The future of rodents control permits the effectiveness and precision with the practice of using strategies, software and other progressive technology to help growers for no longer depends on products that can threaten the health of people and wildlife.

DECLARATIONS

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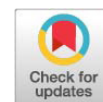
Statement of conflict of interest

The authors have declared no conflict of interest.

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Evaluation of a Novel Technique to Detect the Presence and Attraction of Wild Boar (*Sus scrofa*) towards Baiting Points

Irfan Ahmed¹, Uswah Sehar¹, Zakia Panhwar¹ and Muhammad Mushtaq²

¹Vertebrate Pest Management Programme, Institute of Plant and Environmental Protection, National Agricultural Research Centre, Islamabad, Pakistan

²Department of Zoology, Wildlife and Fisheries, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan

ABSTRACT

The main aim of this study was to evaluate the effectiveness of waste motor oil as a tool for detecting the presence of wild boar in forested environment and attracting them towards poison baiting sites/points. The study was conducted at two different locations, Muzafferabad, AJK and Attock, Punjab, Pakistan. The waste motor oil was applied on ground surface at 14 sites at both locations, each one measuring 2x2 square meters near established bait stations where wild boars had been regularly observed. The results indicated that wild boar activity of digging of soil surface, rolling over oily surface and fresh foot prints were found more toward treated sites compared to non-treated sites which indicated that wild boars have been attracted towards waste motor oil sites. These results suggest that scent based lures/bait such as waste motor oil is effective for detecting the presence of wild boars in forested environments and crop lands where quick assessment of population is required.

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Authors' Contribution

IA, US, ZP: Conceived and designed the study. IA, US, ZP, MM: Performed field work and data collection. US, ZP: Analyzed data. IA, US: Wrote the original draft, review and edited final version of manuscript. IA: Supervision.

Key words

Attractant, Lure, Feral swine, Waste motor oil, Scent rubbing

INTRODUCTION

Wild pigs are native of Eurasia but now they occur all over the world having 14 to 23 subspecies which include both endemic and invasive species. Wildlife management actions are always performed for managing wild pigs, most specifically in the areas where there is a risk of damage to human health and agricultural crops (Mayer and Brisbin, 2009). Beside damage to human health and agricultural crops wild boar also cause infection to livestock and wildlife in Spain, because it is a host for Foot and Mouth disease and bovine tuberculosis (Gortázar *et al.*, 2008). Feral pigs are generalists so they survive in greater range of habitat and their omnivorous nature allows them to eat different kind of food sources. Mostly their diet includes seeds, forbs, soft and hard mast such as roots, shoots, tubers, and grasses. The diet of feral

pigs also changes seasonally i.e., they consume nuts like a corn and hickory which are two important food items (Mungall *et al.*, 2001). The diet of wild pigs also includes invertebrates such as earthworms, centipedes, beetles, leeches, grasshoppers, eggs of ground nesting birds (Hellgren, 1993).

Pakistan being Islamic country, it is not hunted on large scale as an economic resource for consumption as it is forbidden to consume by Muslims. Also, there is very less number of predators to predate on it which is not able to pull down the existing population in the areas of high infestations. Environmental groups, agricultural producers and public health officials are worried by the wide distribution and rapid increase in the number of wild boars.

Both wild and domesticated pigs possess keen sensory abilities, encompassing evolutionary advantages and disadvantages. Wild boars rely on their nose tickling sense of smell to locate food, detect potential threats, and engage in communication with other members of their species. Most commonly, wild pigs are attracted towards a food commodity like grains of corn or maize as baiting material. The frequency of wild boar visitation to baiting locations/stations can fluctuate due to factors such as the presence of natural food sources, human interference, and the acceptance of new food sources, devices, and activities (Lavelle *et al.*, 2017). Birch wood tar has been used as an

* Corresponding author: rajairfanahmed@gmail.com
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attractant to confirm the presence of wild boar in an area. It was observed that these animals consistently left a higher number of activity signs, such as rubbing, tusk marks, and rooting, in the vicinity of trees treated with this substance compared to control trees treated with water (Massei *et al.*, 2021).

Reliable methods for detecting the presence of wild pigs are required, especially in regions where the species is sparsely distributed. These methods are crucial for monitoring natural range expansion, identifying the potential introduction of wild boars into new areas, whether intentional or accidental, and assessing the success of eradication efforts. These methods mainly involve the use of baited traps, deployment of carcasses or bait to attract those animals which are potential survivor, and on those pigs equipped with tracking devices to locate remaining groups of pigs (Cruz *et al.*, 2005; Parkes *et al.*, 2010).

The objective of this study was to evaluate the effectiveness of a potential attractant such as waste motor oil, to detect the presence and to attract the wild boar towards baiting points in forested environments and crop lands.

MATERIALS AND METHODS

Study area

This study was carried out at two different locations i.e., village Basnaraha (Union Council Chatter Klass), District Muzaffarabad, Azad Jammu and Kashmir (AJ & K) and Union Council Mirza, District Attock, Punjab Pakistan. The district Muzaffarabad is situated at coordinates N 34.359688 and E 73.471054, with elevations ranging from 737 meters to 1103 meters above sea level, covering a total area of 1642 square kilometres. The district receives an average annual rainfall of 1457 mm, with an average annual temperature of 20.2°C. Light snowfall in winter is a common occurrence in the mountains adjacent to Chatter class. The study area features a variety of habitats, including rivers, thickly vegetated areas and green fields. Major agricultural crops like wheat, maize, rice, and vegetables are grown in this area (GOAJK, 2013). Muzaffarabad's topography is characterized by mountainous and hilly terrain, featuring subtropical scrub and pine forests.

The Attock district is situated on the northern border of Punjab, positioned at 33.768051 North Latitude and 72.360703 East Longitude. It is located at an elevation of 384 meters above sea level. This region is categorized within agro-ecological zone-V, Barani (PARC, 1980). Climatically, the area adjacent to the mountains is relatively humid, while the southern part is semi-arid and experiences hot conditions. The average monthly rainfall

typically amounts to around 200 mm during the summer months and ranges from 36 to 50 mm in the winter. The major crops cultivated in the area include maize, groundnuts sorghum, wheat, millet, legumes and mustard (Rashid and Rasul, 2011).

Experimental design

The research trials were conducted in Attock district during the month of March 2023, in wheat crop at maturity stage and Muzaffarabad district during the month of May, 2023 in forest plantations. A cheap lubricant (waste motor oil) was evaluated as an attractant for attracting wild boar towards baiting points. The study aimed to determine whether these animals visited treated sites with the attractant more frequently compared to control sites. It was chosen for two reasons (i) it was the direct observation of local farmers in the field that this lubricant act as an attractant for wild boar and (ii) wild boars are used to roll over the waste motor oil on the soil surface to kill its ticks and mites on its thick body hairs (in majority cases reported by local farmers).

In both research trials, waste motor oil was applied on ground surface measuring 2x2 square meters near established bait stations (baiting points). Each bait station was at least 800-1000 meters apart. Bait stations were selected randomly where wild boars had been regularly observed in an area. Overall 14 baiting points were established in each location (i.e. seven control sites and seven treated sites). Culled poultry heads were applied as a new bait delivery system at each bait station of both treated and control sites (Khan *et al.*, 2011). Counted number of poultry heads and 50 ml motor oil was applied late in the evening before sunset and data was recorded next day early in the morning. Left over poultry heads were picked up to avoid consumption by non-target animals. Poultry heads were placed approximately 1 meter near the baiting points where motor oil was applied to see the consumption preference trend, compared to control sites. Consumption data was recorded for consecutive five days at both locations. Foot prints of wild boar, digging of soil surface and rolling over oily surface by wild boar were recorded from all bait stations where motor oil was applied.

Statistical analysis

Analysis of variance (Statistix 8.3) software was used to compare the consumption of bait towards treated and control sites with wild boar activity signs.

RESULTS

At both locations, wild boar activity of digging of soil surface, rolling over oily surface and fresh foot prints were

found more towards treated sites compared to control sites (Fig. 1). Moreover, no activity of any non-target species was observed near treated bait stations which suggest that waste motor oil did not act as an attractant for non-target species.

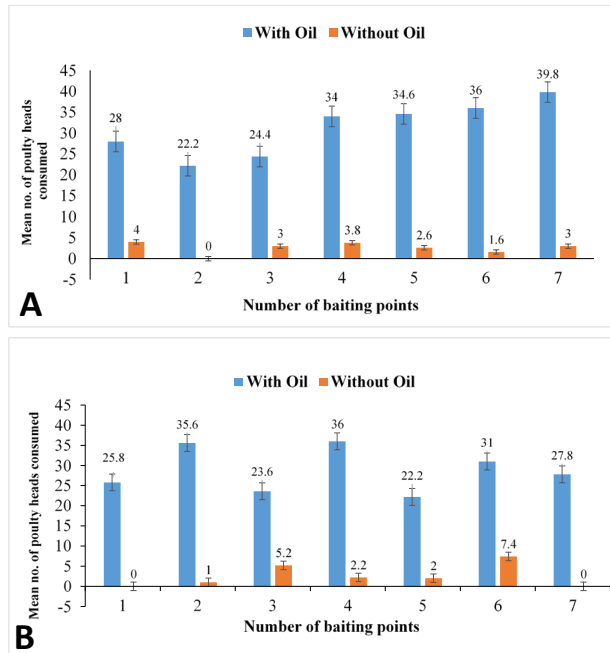


Fig. 1. Comparison in consumption of poultry heads from all bait stations in Muzaffarabad, AJ & K (A) and Attock (B).

Results showed that 93.19% of bait was consumed from those bait stations where waste motor oil was applied while only 8.73% bait was consumed where this lubricant was not applied. The application of waste motor oil to bait stations had significantly increased wild boar visitation and bait uptake in Muzaffarabad ($F = 257.74$, $df = 1$, $P < 0.001$) and Attock district ($F = 424.53$, $df = 1$, $P < 0.001$). In Muzaffarabad, a total of 1175 poultry heads were placed at 7 bait stations by using waste motor oil and out of this 1095 were consumed. While, 1030 poultry heads were placed where waste motor oil was not applied and out of this 90 poultry heads were consumed (Fig. 1A).

In Attock, a total of 1075 poultry heads were placed at 7 bait stations by using waste motor oil and out of this 1010 were consumed. While, 875 poultry heads were placed where waste motor oil was not applied and out of these 89 poultry head were consumed. Results showed that 93.95% of bait was consumed from those bait stations where waste motor oil was applied while only 10.17% bait was consumed from points where this lubricant was not applied (Fig. 1B).

Overall, high bait consumption and attraction of wild boar was recorded in Muzaffarabad followed by Attock. In Muzaffarabad, mean consumption of poultry heads towards treated and control site was 31.286 and 2.571 recorded respectively, and in Attock, similar pattern of consumption of poultry heads towards treated and control site was recorded, i.e., 28.857 and 2.542, respectively (Fig. 2).

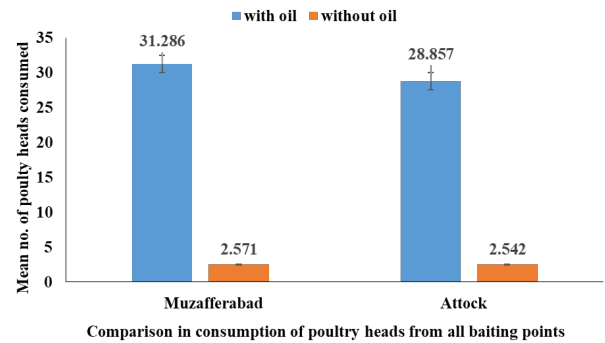


Fig. 2. Comparison in consumption of poultry heads from all bait stations at Muzaffarabad and Attock.

DISCUSSION

This study confirmed that wild boars exhibited stronger attraction towards treated sites where waste motor oil was applied compared to non-treated sites. The higher number of wild boar activity signs was observed from the areas where waste motor oil was applied which suggests that it can be used as an attractant to confirm the presence or absence of wild boar in an area. Waste motor oil was applied in the areas which were used as a regular pathway by wild boar. This lubricant might have attracted wild boars from neighbouring areas too. Wild boars emigrate from their core habitats and enlarge their range (Spencer and Hampton, 2005; Wilson, 2013).

Scent-rubbing is a poorly understood behaviour in which mammals rub their body with a bizarre array of substances, any of which have strong odours such as waste motor oil in the present study. Scent rubbing has attracted a variety of functional interpretations but no consensus has emerged so far. Explanations include comfort behaviour and increasing social attractiveness, i.e., status advertisement (Gosling and McKay, 1990). Further studies are required to explain scent marking behaviour of mammals using camera traps.

Limited efforts have been made so far to identify the presence of wild boars in a particular area. Some attractants like food additives and some lures have been used to test the effectiveness of these attractants (Lavelle *et al.*, 2017; Sandoval *et al.*, 2019). Sometimes food attractants are not effective source of attractants because of availability of

natural food resources which may limit the attraction of wild boars towards such attractants (Lavelle *et al.*, 2017).

When attracting targeted animals to traps then species specificity is important so that trapping of non-targeted species can be minimized. The specificity of the attractant is also important for attracting wild boars to bait that contains substances like toxins, vaccines, or contraceptives. This issue can only be solved by using boar operated system/bait delivery system (BOS) specifically designed to prevent bait consumption by non-target species (Massei *et al.*, 2010; Campbell *et al.*, 2011; Ferretti *et al.*, 2014).

Scent based lures are effective for several weeks and do not require replacement very often as compared to food attractants (Sandoval *et al.*, 2019). The use of waste motor oil in current study offered several advantages over other attractants which were previously employed: (1) little cost, as it is cheap and easily available from local motor workshops and 3.5 litres were used on the surface of 7 baiting points/sites (2) effectiveness, there is limited staff effort for initial application and monitoring the effect of attractant during subsequent visits; (3) no effect on non-target species; (4) it is an effective attractant for wild boar in all seasons even when natural food is available; (5) anticoagulant baits were successfully used against wild boar (Khan *et al.*, 2017). As anticoagulant baiting takes 17 days, this method will facilitate the regular visiting of wild boar towards the baiting points just from the start of control operation.

CONCLUSION

It can be concluded that waste motor oil is a potential attractant which can be used to attract wild boars towards baiting points and detect their presence in the areas which are newly colonized by these animals or the areas where density of wild boar is low. Further experiments must be performed to detect the presence of wild pigs in newly colonized sites, or the areas which have relatively low-density species. Moreover, further studies can be performed to access whether waste motor oil could enhance the effectiveness of trapping or attracting these species towards areas where poison baits are delivered in special devices like wooden boxes (environmentally safe bait dispensers) and other bait delivery systems like furrow baiting in different field crops.

DECLARATIONS

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IRB approval

The authors would like to confirm that the study protocol for this study was reviewed by the independent ethics committee.

Ethical statement

The authors comply with all the laws and regulations that apply to science and profession during the period of studies.

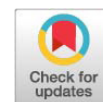
Statement of conflict of interest

The authors have declared no conflict of interest.

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Epidemiological Study of Warble Fly in Pakistan

Munib Hussain¹, Abdul Razzaq², Mohammad Qasim^{1*}, Muhammad Jamil³,
Abid Hussain⁴ and Najeel Ullah⁵

¹Animal Science Institute, Animal Health Program, National Agriculture Research Center, Islamabad

²Animal Sciences Division, Pakistan Agricultural Research Council Islamabad

³PARC Arid Zone Research Center, Dera Ismail Khan

⁴Balochistan Agriculture Research and Development Center, Quetta

⁵Livestock and Dairy Development Department, Punjab

ABSTRACT

Hypodermosis is an important endoparasitic infection of cattle and goats prevalent in hilly and semi-hilly areas of Pakistan and considered as an important disease of economic significance affecting ruminants especially cattle (*Hypoderma bovis* and *Hypoderma lineatum*) and goats (*Przhevalskiana silenus*). The major economic loss incurred by warble fly infection to livestock and leather industry is due to perforation of hide and skin. Therefore, the present study aims to investigate field epidemiology of warble fly in four federating units of Pakistan. As per farmers perceptions the warble fly prevalence was 1-66% in goats this year while in cattle it was 0-33% as compared to last year (20-80%). The warble fly larval prevalence was recorded physically (66%) in goats at Gilgit during autumn, followed by Ziarat (1-8%), Fort Munro (0-14%), D.I. Khan (0-5%), while in cattle it was 33% followed by Ziarat (0%), Fort Munro (2-0%), Gilgit (0-33%), D.I. Khan (1-13%).

Article Information

The article was presented in 42nd Pakistan Congress of Zoology (International) held on 23-25th April 2024, organized by University of Azad Jammu & Kashmir, Muzaffarabad, Pakistan.

Authors' Contribution

MH and AR designed the study. MQ and MJ executed the study, MH, AR and MQ wrote the manuscript. AR analysed the data. AH and NH critically reviewed the manuscript.

Key words

Warble fly, Myiasis, Hypoderma, lumpy skin disease

INTRODUCTION

Livestock is a crucial component of Pakistan's economy, particularly in rural areas where the majority of the population relies on agriculture. The livestock sector in Pakistan annually produces 36.3 million skins and 7.5 million hides, which are significant, export items contributing to 12 percent of the total exports. Leather and leather products are among Pakistan's largest sources of foreign exchange (Ahmad and Ali, 2023). Several factors contribute to low production in the livestock sector, one of which is ectoparasitic infestations. Myiasis caused by *Hypoderma* species larvae is a notable economic threat to both wild and domesticated ruminants. Hosts of this parasite include buffalo, cattle, deer, reindeer, sheep, and goats (Liaquat *et al.*, 2021). Warble fly infestation, affecting cattle

(*Hypoderma bovis* and *Hypoderma lineatum*) and goats (*Przhevalskiana silenus*), is significant in hilly, semi-hilly, and sandy areas of Pakistan. This infestation results in substantial economic losses due to hide and skin perforation, degraded meat quality, and reduced milk production (Arshad *et al.*, 2014). Infested skins can see price reductions of up to 70%, depending on the number of perforations caused by warble fly larvae (Oryan *et al.*, 2009). Hypodermosis is a significant endoparasitic infection prevalent in the hilly and semi-hilly areas of Pakistan, mainly affecting cattle and goats. Warble fly infestation (WFI), caused by larvae of *Przhevalskiana silenus*, results in economic losses by reducing milk yield and the quality of meat and hides. This disease is characterized by subcutaneous warbles on the dorso lumbar region of affected animals, with all three larval stages (L₁, L₂, and L₃) causing infestations over 7-9 months without internal migration, unlike other Hypoderminae members (Otranto *et al.*, 1999; Ahmad and Ali, 2013).

The prevalence of warble flies can vary depending on several factors, such as geography, host population density, and environmental conditions. In Europe and North America, the prevalence of warble flies is relatively high, and they are considered a significant problem for livestock farmers. In some regions, 100 % infestation rate was recorded in cattle and goats populations. While in

* Corresponding author: drqasim46@gmail.com
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Pakistan prevalence of Warble fly infestation was found to be 3.2% overall, with goats and cattle having higher rates of 18.4 %. Chemotherapy treatments have been effective against the adult fly and first larval stage in many European and North American countries (Liaquat *et al.*, 2021).

Keeping in view the importance of disease this study was conducted for field epidemiology of warble fly infestation in high risk areas of Pakistan.

MATERIALS AND METHODS

A team of scientists from the Animal Health Program and Animal Sciences Division, PARC, visited eight villages in four federating units (Punjab, Balochistan, Khyber Pakhtunkhwa, and Gilgit) of Pakistan for the physical examination of animals on an already developed questionnaire during spring (March-May 2023) and again during autumn (September-November 2023). In these visits, 225 livestock farmers holding 1685 goats and 653 cattle were also clinically examined for warble fly infestation (Table I).

Table I. Field epidemiological survey of warble fly in various areas of Pakistan.

Province/District	Season	Clinical observation of animals		Number of farmers
		Goats	Cattles	
Balochistan (Ziarat)	Spring	485	37	20
	Autumn	215	12	9
DG Khan (Fort Munro)	Spring	337	108	43
	Autumn	58	12	13
Gilgit (Gorikot/Astore)	Spring	240	149	57
	Autumn	50	12	16
Khyber Pakhtunkhwa (D.I. Khan)	Spring	188	237	43
	Autumn	112	86	24
	Total	1685	653	225

RESULTS

Prevalence of warble fly larvae (Przhevalskiana silenus) in goats

The warble fly larval *Przhevalskiana silenus* prevalence was higher (up to 66%) during autumn as compared to spring season (0-0.4%) in goats. Area wise higher prevalence was recorded in Gilgit followed by Fort Munro, Ziarat and D.I. Khan (Table II). As per farmer's perceptions, the warble fly prevalence was higher in last few years (20–80%) as compared to the current year (2023) this might be due to heavy usage of spray and ivermectin

for the control of lumpy skin disease.

Table II. Prevalence of warble fly larvae (*Przhevalskiana silenus*) in goats during two seasons at various areas of Pakistan.

Province/District	Season	No. of goats examined	No. of goats infected	Prevalence (%)
Balochistan (Ziarat)	Spring	485	2	0.41
	Autumn	215	18	8.37
Punjab (Fort Munro)	Spring	337	0	0
	Autumn	58	8	13.79
Gilgit (Gorikot/Astore)	Spring	240	0	0
	Autumn	50	33	66
KPK (D.I. Khan)	Spring	188	0	0
	Autumn	112	5	4.4
	Total/overall	1685	49	2.9

Prevalence of warble fly larvae (Hypoderma lineatum) in cattle

The warble fly larval (*Hypoderma lineatum*) prevalence was higher (up to 33%) during autumn as compared to spring season (0-1.85%) in cattle (Table III). Area wise higher prevalence was recorded in Gilgit followed by D.I. Khan, Fortmunro. The cattle in Ziarat area were found free of warble fly larvae.

Table III. Field epidemiological survey of warble fly in various areas of Pakistan during spring season in cattles.

Province/District	Season	No. of cattle examined	No. of cattle infected	Prevalence (%)
Balochistan (Ziarat)	Spring	37	0	0
	Autumn	12	0	0
DG Khan (Fort Munro)	Spring	108	2	1.85
	Autumn	12	0	0
Gilgit (Gorikot/Astore)	Spring	149	0	0
	Autumn	12	4	33.33
KPK (D.I. Khan)	Spring	237	2	0.84
	Autumn	86	11	12.79
	Total/overall	653	23	2.52

DISCUSSION

Warble fly infestation is a major economic concern for cattle and goats in Pakistan, particularly in hilly

and semi-hilly areas. This study aimed to document field epidemiology of warble fly in goats and cattle across four federating units (Punjab, Gilgit, Khyber Pakhtunkhwa, and Balochistan) of Pakistan. Warble fly larvae (*Przhevalskiana silenus*) prevalence in goats ranged from 1-8% in Balochistan (Ziarat), 14% in Punjab (Fort Munro), 5% in Khyber Pakhtunkhwa (DI Khan), and 66% in Gilgit-Baltistan. Compared to previous years, prevalence in Gilgit-Baltistan was lower (32% vs. up to 80%) as reported by (Khan *et al.*, 2012). For cattle, *Hypoderma lineatum* prevalence during the two seasons was 0% in Balochistan, 2% in Punjab (Fort Munro), 1-13% in Khyber Pakhtunkhwa (DI Khan), and 33.33% in Gilgit-Baltistan. Lower prevalence in Punjab, Balochistan, and Khyber Pakhtunkhwa among the districts might be due to management system, grazing patterns, pastures and extensive use of acaricidal spray and ivermectin for lumpy skin disease control. Similar findings were reported by (Ahmad and Ali, 2012; Arshad *et al.* 2014). Our results are in agreement with (Khan *et al.*, 2012) shows higher prevalence comparatively less warble fly prevalence's decreased due to heavy use of Acaricides spray and ivermectin in the last two years.

CONCLUSION

Field epidemiology of warble fly infestation revealed a significant decrease in prevalence, dropping to 32% from previous rates as high as 80%. This change in trend is attributed to the extensive use of acaricidal sprays and ivermectin for controlling lumpy skin disease, which also significantly reduced warble fly infestation. This information will aid in developing a long-term control strategy for warble fly.

DECLARATIONS

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IRB approval

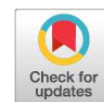
The study was approved from Departmental Ethical Committee of NARC.

Statement of conflict of interest

The authors have declared no conflict of interest.

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Diversity of Invertebrate Fossils in Pakistan

Syed Iftikhar Hussain Jafri

Flat No. C-5, Commercial-1, Gulshan e Shamim, Karachi

ABSTRACT

Occurrence of various invertebrate fossils groups is quite variable. Largest group was that of Gastropods (22%), Bivalves were (17%), Echinoderms were (15%), Corals (13%), Cephalopods (Nautiloids, Belminites, Ammonites) were (13%), Brachiopods (10%), Crustaceans and Foraminiferons were (5%) each. Fossils studied presently belong to 8 classes, 52 orders and 88 families. 136 genera are reported here from various provinces of Pakistan. 35% of these are from Sindh; 27% from KPK (Khyber Pakhtunkhwa); 21% from Balochistan and 17% from Punjab.

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Key words

Diversity, Fossils, Invertebrates, Pakistan

INTRODUCTION

Few isolated publications are available on invertebrate fossil fauna of Pakistan. Costello (1860) published the 1st report on rock types in the mountain ranges of Bannu Area in KPK. He recognized some Brachiobods and Molluscs. Some Tertiary crabs were reported from Sindh and Kutch, India (Stoliczka, 1871). Another early report on invertebrate fossil of Dera Bugti Area of Balochistan was published by Blandford (1883).

Siddiqui (1971) recorded some Ostracods from Pakistan. In a detailed study of Bannu and DI Khan Area (KPK), Grant (1968) discussed the occurrence of brachiobods, ammonites, bivalves, gastrobods and crinoids of triassic, jurassic and cretaceous age. Sillurain nummilites were reported from Drazinda formation in KPK (Ozyan et al., 2019). Nizami (2019) described the faunal assemblage of Jurassic age from Chichali gorge, salt range in Punjab. Fossils included foraminiferans, gastrobods, bivalves, bryozons, brachiobods, echinoids, sponges and corals. Paleozoic and cenozoic bivalves were recorded from Dhok Pathan formation (District Chakwal),

Punjab by Farooqui et al. (2018). Fiest et al. (2001), reported a new species of Trilobite *Newcolmonia chitralensis* from Chitral, KPK.

Siddiqui et al. (2009) recorded 4 species of Ammonites from Raskoh area of Balochistan. Similarly, 3 genera of Tertiary Nautiloids were reported from Katch area, Western India (Halder, 2012). Iqbal (1972) studied Gastropods fauna from Jhriak-Lakhra-Bara Nai (Sindh), Salt range, Punjab and Samana Suke range in KPK.

The area where collision of two tectonic plates (Indian and Eurasian) occurred, lies in Nagar state in Gilgit Baltistan. Pakistan geologically overlaps Indian and Eurasian tectonic plates. Sindh and Punjab provinces are located in north-western corner of Indian plate. Balochistan and KPK are within the Eurasian plate which is a part of Iranian plateau. The Northern Areas and Azad Kashmir lie mainly in central Asia along the edge of Indian plate. Pakistan belongs to eastern part of Gondwanaland while western part constitutes rest of the Indo-Pak plate (Wong et al., 2012). In Paleozoic age a type of platform developed which was known as Indian shield. In the north it forms the Hiamalayan foredeep and Penensular region. Early Eocene is exposed in Sakesar limestone of Nummal gorge in central salt range of Punjab. 9 genera of Foraminiferans were identified from this area (Ghazi et al., 2010).

Malkani (2011) discussed the general geology of Balochistan province. The rocks consists of Igneous, Sedimentary and Metamorphic deposits. Sulaiman and Kirthar Basins consist of Triassic to recent strata. Punjab province includes Tertiary and Mesozoic rocks. These are sedimentary in nature while Quarterny age rocks are also present. That saline series of salt range is located

* Corresponding author: syediftikharhusainjafri@gmail.com
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in northern Punjab and KPK. These deposits have been estimated to be of Pre-Cambrian age (Fig. 2). This area contains abundant micro-fossils in salt marle. Drazinda Formation located near Drazinda town (District D.I. Khan) in KPK province, which is in fact part of Sulaiman range, forms a lobe in this area (Powell, 1979; Kazmi and Rana, 1982). The lobe is widest at this point. The Sulaiman fold belt continues into Baluchistan and Sindh provinces up to Makran and Karachi coast.



Fig. 1. Map of Pakistan showing fossil collection sites.

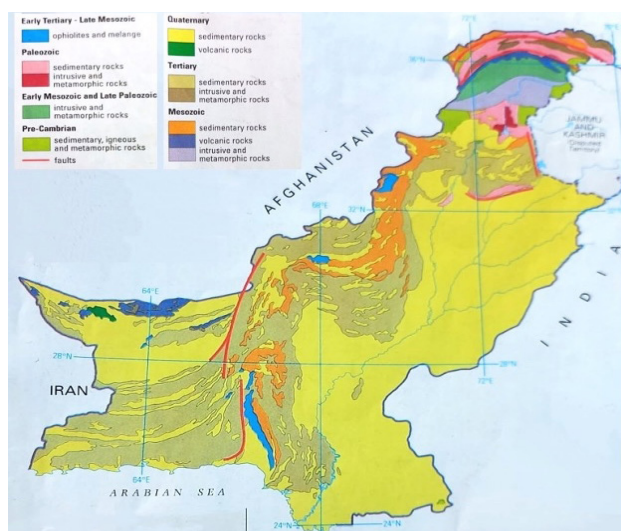


Fig. 2. Geological map of Pakistan showing various types of rocks.

Indian subcontinent collided with Eurasian plate about 55-60 million years ago during Paleocene age at closure of Tethys Sea. Since then, this subcontinent has witnessed three distinct cycles of flooding and receding of sea level (Gingerich *et al* 1979). Paleogeographic information on Cretaceous-Paleocene sequence of rocks in this area was reported earlier (Davies, 1939; Pinfold, 1937). Pleistocene sedimentary rocks are also found in the foot hills of Sulaiman range (Kazmi and Rana, 1982; Hamayun, 1990). Blanford (1879) coined the term 'Kirthar Formation' to describe the range in Balochistan and Sindh. Later, the stratigraphy of lower Indus basin. Drazinda shale of Kirthar range consists of gray clay stone and marle beds at lower level and are highly fossiliferous. The upper level consists of limestone deposit which contains abundant Foraminifera, Bivalves and Echinoids (Hemphill and Kidwai, 1973) reported some Shark teeth, Nummulites as well as some Mammalian remains of Oligocene age in the foot hills of Dera Bugti area of Balochistan.

A small group of low hills extend from Khairpur to Rohri in north-south direction in the province of Sindh. These hills are located on the right bank of river Indus and in fact are the eastern most limit of Kirthar range foot hills remnants. These hills mainly consist of stratified limestone which arises in fluvial plan of river Indus (Fig. 2). These are part of Brahui formation which extends up to Hyderabad, Thatta and Karachi districts (Blandford, 1879; Hunting Survey Corporation, 1960). In Indus basin area of Sindh, two flooding cycles in sea level have been recognized (Wakefield and Montil, 2002). These rocks are considered to be deposits of Eocene and Oligocene age and are highly fossiliferous. Soil profile studies at various points in these hills indicated presence of Calcite, gypsum, quartz granules and red clay deposits (Fig. 3). Limestone deposits have incursion of flint nodules on the surface as well (Biagi and Crumachi, 1988). Palynological (Fredrickson, 1994) and sedimentation studies indicate a shallow marine paleoenvironment in Indus plain area of Sindh (Mohan, 1982; Usmani, 1983; Usmani *et al.* 2008; Wakefield and Monteil, 2002) during tertiary period.

MATERIALS AND METHODS

The fossils specimens were collected from various places in Pakistan. Drazinda range in KPK yielded a considerable number consisting of many groups of invertebrates. Similarly, fossils are also collected from Khairpur Hills, Hyderabad and Jamshoro Area of Sindh Province. Some fossils are obtained from Dera Bugti and Khuzdar in Balochistan (Fig. 1). Due to paucity of published information on taxonomy of fossils groups, the

present specimen has been identified up to generic level.



Fig. 3. Various collection sites of fossils.

SYSTEMATIC PALEONTOLOGY

Phylum Protozoa

Class Rhizaria

Order Foraminifera

Family Nummulitidae

1. Genus *Nummulites*

Age: Eocene, about 40 million years old.

Locality: Drazinda formation, KPK and Dera Bugti, Balochistan.

2. Genus *Discocyclina*

Age: Miocene, about 20 million years old.

Locality: Sindh.

Order Fusulinida

Family Fusulinidae

3. Genus *Astrorhizona*

4. Genus *Fusilia*

Age: Permian, about 240 million years old.

Locality: Karachi, Sindh and Khuzdar, Balochistan.

Family Heterohelicidae

5. Genus *Heterohelise*

6. Genus *Lepidocyclina*

7. Genus *Metapolymerphina*

Age: Eocene, about 40 million years old

Locality: Khuzdar, Balochistan.

Order Sprinilida

Family Sprilininidae

8. Genus *Spirilina*

Age: Pliocene, 1.5 million years old.

Locality: Karachi, Sindh (Fig. 4).

Phylum Cnidaria

Class Anthozoa

Order Scleractinia

Family Merulinidae

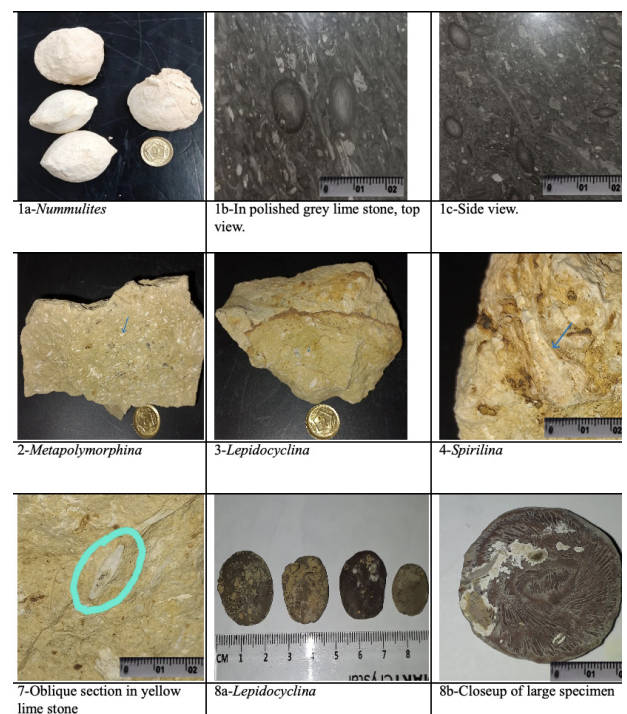


Fig. 4. Foraminiferan fossils.

9. Genus *Fevites*

10. Genus *Echinopora*

Family Fevidae

11. Genus *Fevia*

Age: Miocene, about 20 million years old.

Locality: Karachi, Sindh.

12. Genus *Astrocerium*

Age: Silurian, about 425 million years old.

Locality: Chinji Formation, Balochistan.

13. Genus *Paleofevosites*

Age: Silurian, about 425 million years old.

Locality: Wargal Formation, Balochistan.

14. Genus *Protaria*
Age: Silurian, 420 million years old.
Locality: Salt range, Punjab
15. Genus *Cladocora*
16. Genus *Cyphastria*
Age: Eocene, about 50 million years old.
Locality: Drazinda, KPK.
17. Genus *Leptastrea*
Age: Pleistocene, about 1.5 million years old.
Locality: Karachi, Sindh.

Class Hexacoralia**Order Rugosa****Family Lithostrotionidae**

18. Genus *Siphenodendron*
Age: Permian, about 280 million years old.
Locality: Khairpur, Sindh and Drazinda formation, KPK.

Family Disphyllidae

19. Genus *Hexagonaria*
Age: Silurian, 420 million years old.
Locality: Salt range, Punjab.
20. *Lithostrotion*
Age: Permian, 280 million years old.
Locality: Drazinda, KPK.

Order Tabulata**Family Syringophyllidae**

21. Genus *Calapoecia*
Age: Ordovician, about 460 million years old.
Locality: Kalat, Balochistan.
22. Genus *Acropora*
Age: Eocene, about 40 million years old.
Locality: Drazinda formation, KPK.

Family Lophophyllidae

23. Genus *Lophophyllidium*

Family Zephrentidae

24. Genus *Zaphrentis*
Age : Permian, about 250 million years old.
Locality: Drazinda formation, KPK and Sindh.
25. Genus *Heliophyllum*
Age: Permian, about 250 million years old.
Locality: Jamshoro Area, Sindh.

Family Paleocyclidae

26. Genus *Paleocyclus*
27. *Septasteria*
Age: Devonian, about 400 million years old.
Locality: Drazinda formation, KPK.

BRANCHED CORAL**Phylum Cnidaria****Class Tabulata****Order Feositida****Family Feositidae**

28. Genus *Coenites*
Age: Devonian, about 400 million years old.

Locality: Sindh.

29. Genus *Fevosites*
Age: Eocene, 40 million years old.
Locality: Kalat, Balochistan.

Family Heliolitidae

30. Genus *Proheleolites*
Age: Eocene, 40 million years old.
Locality: Kalat, Balochistan (Fig. 5).

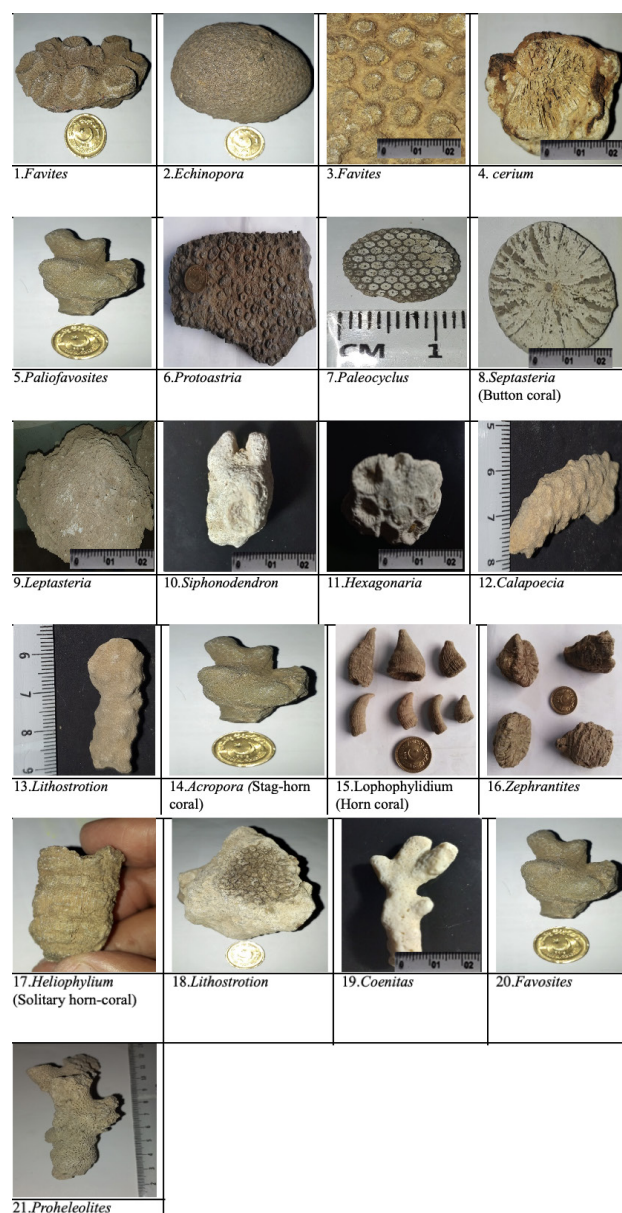
Phylum Echinodermata**Class Echinoida****Family Echinoidae**

Fig. 5. Fossil Corals.

31. Genus *Micraster*

Age: Eocene, about 40 million years old.

Locality: Drazinda formation, KPK.

Khuzdar, Balochistan.

32. Genus *Clypeas*

Age (a): Eocene, about 40 million years old.

Locality: Drazinda formation, KPK.

Age (b): Miocene, about 20 million years old.

Locality: Hyderabad, Sindh.

Age (c): Eocene, about 40 million years old.

Locality: Barkhan, Baluchistan.

Class Echinoidea**Order Clypeastroida****Family Clypridae**33. Genus *Heteraster*

Age: Cretaceous, about 70 million years old.

Locality: Gaj Formation, Sindh.

34. Genus *Clypeaster*

Age: Oligocene, about 30 million years old.

Locality: Khuzdar, Balochistan.

Class Echinoidea**Order Echinoida****Family Echinoidae**35. Genus *Psephechinus*36. Genus *Stomoechinus*37. Genus *Schizoaster*

Age: Eocene, about 40 million years old.

Locality: Barkhan, Balochistan.

Order Holasteroida**Family Holoasteridae**38. Genus *Echinocorys***Order Cidaroida****Family Cidaroidae**39. Genus *Cidaris*

Age: Pliocene, about 1.5 million years old.

Locality: Malir, Karachi, Sindh.

40. Genus *Tretocidaris*

Age: Cretaceous, about 70 million years old.

Locality: Gaj Formation, Sindh.

Order Echinoida**Family Echinolampidae**41. Genus *Echinolampus***Family Echinometritidae**42. Genus *Echinometra*

Age: Miocene, about 15 million years old.

Locality: Kirthar Formation, Sindh.

Order Spatangoida**Family Spatangidae**43. Genus *Spatangus* (Heart urchin cross section)

Age: Oligocene, about 30 Million years old.

Locality: Khuzdar, Balochistan.

CRINOIDS:**Class Crinoidia****Order Flexibilia**44. Genus *Onychocrinus*

Age: Eocene, about 50 million years old.

Locality: Drazinda formation, KPK.

Order Comatulida**Family Antedonidae**45. Genus *Anteodon* (Feather star, Theca)

Age: Eocene, about 20 million years old.

Locality: Hyderabad, Sindh.

CRINOID CALYX:**Class Crinoidia****Order Encrinida****Family Encrinidea**46. Genus *Encrinus*

Age: Triassic, about 240 million years old.

Locality: Salt Range, Punjab (Fig. 6).

CRUSTACEANS:**Crabs and Lobster:****Arthropoda****Class Crustacea****Order Decapoda****Family Carpiloidae**47. Genus *Proxicarpilius*

Age: Miocene, about 20 million years old.

Locality: Khairpur hills, Sindh.

Family Carpiloidae48. Genus *Paleocarpilius*

Age: Miocene, about 20 million years old.

Locality: Khairpur, Sindh.

Family Cancridae49. Genus *Lobocarcinus*

Age: Eocene, about 40 million years old.

Locality: Jamshoro, Sindh.

Order Decapoda**Family Thalassinidae**50. Genus *Thalassina* (Mud Lobster)

Age: Eocene, about 50 million years old.

Locality: Drazinda formation, KPK.

Order Balanomorphia**Family Balanidae**51. Genus *Notobalanus* (Acorn Barnacle)

Age: Eocene, about 40 million years old.

Locality: Sindh.

Order Cirripedia**Family Archeobalanidae**52. Genus *Semibalanus*

Age: Cretaceous, about 70 million years old.

Locality: Malir, Karachi, Sindh.

SEED SHRIMP:**Order Ostracoda****Family Mydocopidae**

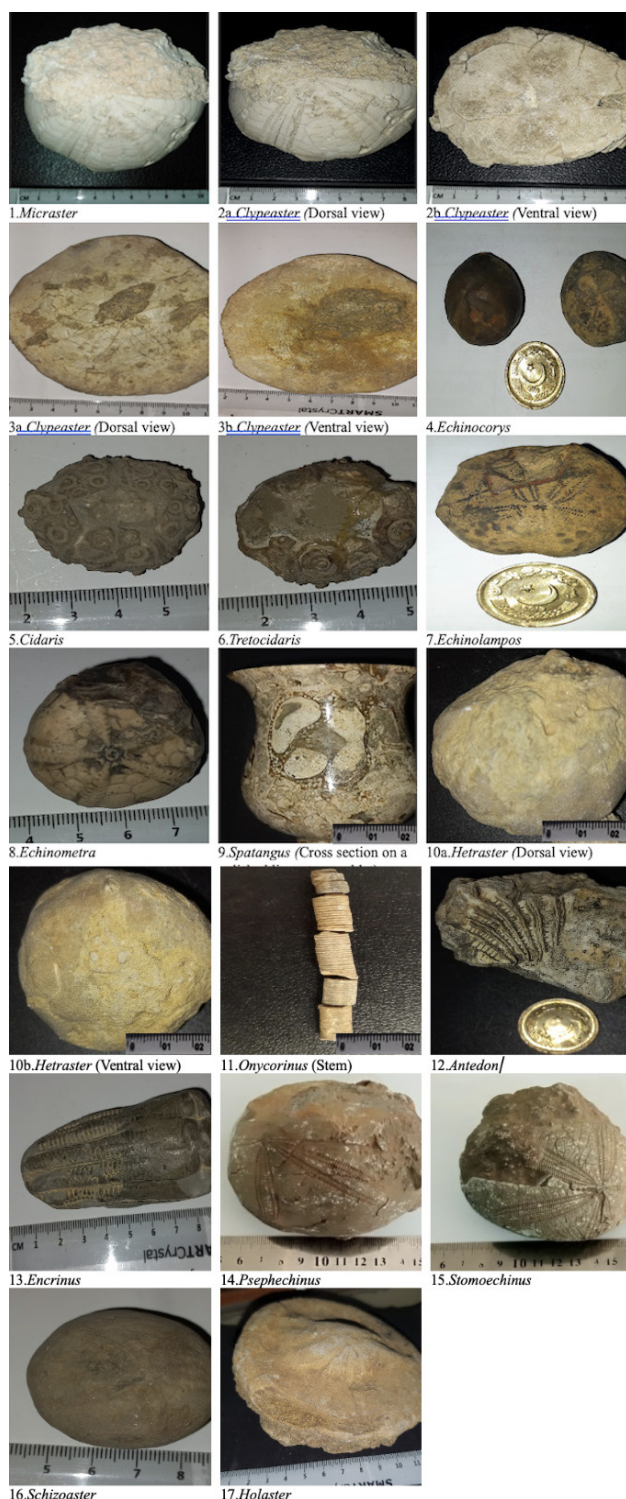


Fig. 6. Echinoderm fossils.

53. Genus *Cycloliberis*

Age: Oligocene, about 30 million years old.

Locality: Khuzdar, Balochistan (Fig. 7).

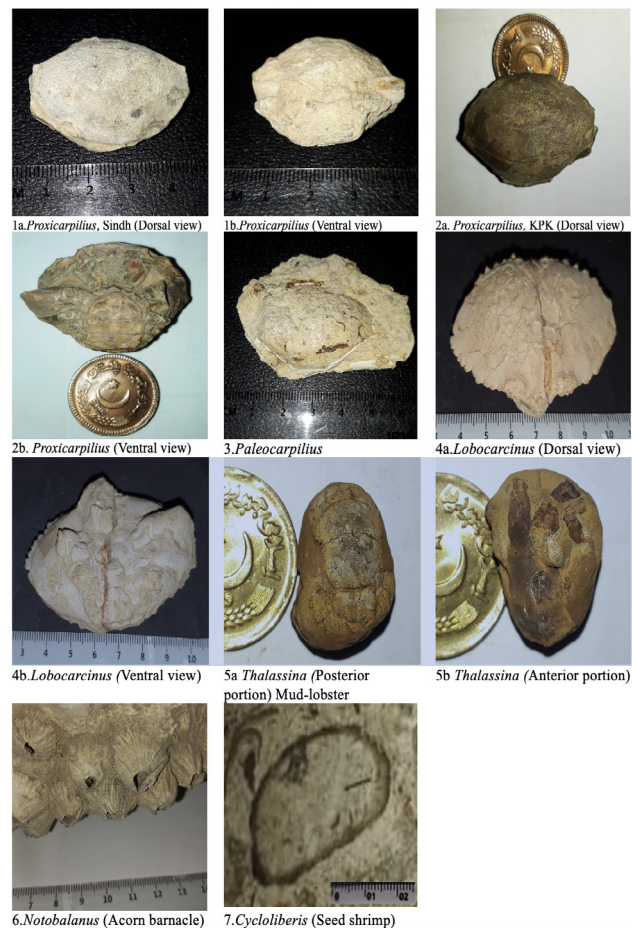
Phylum Brachiopoda**Class Rhynchonellata****Order Terebratulida****Family Terebratulidae**

Fig. 7. Crustaceans fossils.

54. Genus *Terebratulida*

Age: Triassic, about 280 million years old.

Locality: Drazinda formation, KPK.

Order Spiriferida**Family Spiriferidae**55. Genus *Spiriferina*

Age: Triassic, about 220 million years old.

Locality: Gadani, Balochistan.

Order Spiriferidina**Family Spinocrytidae**56. Genus *Spinocrytia*

Age: Devonian, about 390 million years old.

Locality: Salt Range, Punjab.

Order Orthida

Family Plarostomyidae

57. Genus *Plarostomys*

Age: Ordovician, about 400 million years old.

Locality: Salt Range, Punjab.

Order Spiriferida

Family Crytospiriferidae

58. Genus *Crytospirifer*

Age: Permian, about 250 million years old.

Locality: Salt Range, Punjab.

Class Strophomenata

Order Productida

Family Linoproductidae

59. Genus *Linoproductus*

Age: Permian, about 250 million years old.

Locality: Salt Range, Punjab.

Family Productidae

60. Genus *Antiquatinia*

Age: Carboniferous, about 300 million years old.

Locality: Salt Range, Punjab.

61. Genus *Penicularis*

Age: Carboniferous, about 300 million years old.

Locality: Salt Range, Punjab.

Order Trebratulida

Family Cancellothyridae

62. Genus *Ortholina*

Age: Cretaceous, about 100 million years old

Locality: Salt Range, Punjab.

Order Orthida

Family Platystrophiae

63. Genus *Vinlandostrophia*

Age: Ordovician, about 480 million years old.

Locality: Salt Range, Punjab.

Order Spiriferida

Family Neospiriferidae

64. Genus *Neospirifer*

Age: Cretaceous, about 100 million years old

Locality: Salt Range, Punjab (Fig. 8).

Phylum Mollusca

Class Cephalopoda

Order Nautiloidea

Family Nautiloidae

65. Genus *Dertoidovarrtius*

Age: Cretaceous, about 65 million years old.

Locality: Hub Range, Balochistan.

Order Nautilida

Family Solanochelidae

66. Genus *Acanthonautilus*

Age: Carboniferous, about 320 million years old.

Locality: Khuzdar, Balochistan.

Family Permoceratidae

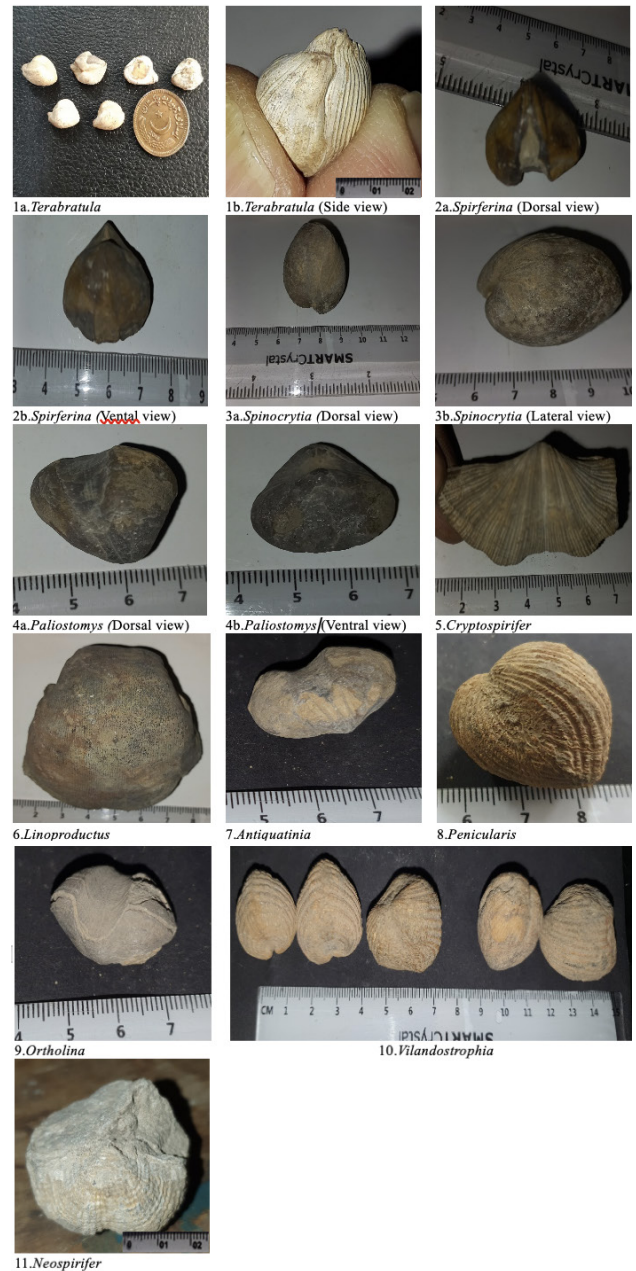


Fig. 8. Brachiopods fossils.

67. Genus *Permoceras*

Age: Permian, about 260 million years old.

Locality: Khuzdar, Balochistan.

Family Trigonoceratidae

68. Genus *Domatoceras*

Age: Triassic, about 230 million years old

Order Pseudorthocerida

Family Spiroceratidae

69. Genus *Spiroceras* (Cross section)

Age: Silurian, about 420 million years old.
 Locality: Khuzdar, Balochistan.

Order Tarphycerida

Family Uranocletoidea

70. Genus *Bionautilus*

Age: Silurian, about 420 million years old
 Locality: Salt range, Punjab

Order Nautiloidea

Family Nautiloidae

71. Genus *Eutrophoceras*

Age: Eocene, about 50 million years old.
 Locality (a): Drazinda formation, KPK.
 Age: Miocene, about 20 million years old.
 Locality (b): Jamshoro, Sindh.

BELEMENITES:

Order Coleoidea

Family Coleoidea

71 a. Genus *Phragmoteuthis* (Bellminite Guard)

Age: Cretaceous, about 70 million years old.
 Locality: Drazinda, KPK.

71b. Genus *Acroteuthis*

Age: Miocene, about 15 million years old.
 Locality: Sindh.

71c. Genus *Dimetobalus*

72. Genus *Belimentela*

Age: Cretaceous, about 60 million years old.
 Locality: Sindh.
 Ammonites:

Order Ammonoidea

Family Ammonoidae

73. Genus *Mammites*

74. Genus *Demotoceras*

Age: Cretaceous, about 65 million years old.
 Locality: Salt range, Punjab.

75. Genus *Collina*

Age: Jurassic, about 190 million years old.
 Locality: Sindh.

Order Ceratitida

Family Ceratitidae

76. Genus *Paradonticeras*

77. Genus *Ceretites*

78. Genus *Analcites*

79. Genus *Rohlites*

Age: Triassic, about 230 million years old.
 Locality: Chaghi, Balochistan.

Order Goniatitida

Family Agathiceratidae

80. Genus *Agathiceras*

Age: Permian, about 260 million years old.
 Locality: Balochistan.

Order Discorida

Family Phragmoceratidae

81. *Phylloceras*

Age: Silurian, about 420 million years old.
 Locality: Sindh.

Order Ammonitida

Family Phylloceratidae

82. Genus *Phragmoceras*

Age: Triassic, about 200 million years old.
 Locality: KPK.

Order Ceratitida

Family Ceratitidae

83. Genus *Egnoceras*

84. Genus *Protoegnoceras*

Age: Cretaceous, about 100 million years old.
 Locality: KPK.

Order Riocereda

Family Bactroceratidae

85. Genus *Bactroceras*

Age: Ordovician, about 450 million years old.

Order Ascocerida

Family Ascoceritidae

86. Genus *Ascoceras*

87. Genus *Plactoceras*

Age: Silurian, about 420 million years old.
 Locality: Khuzdar, Balochistan.

BIVALVES:

Class Bivalvia

Order Carditida

Family Carditidae

88. Genus *Venericardia*

Age: Pliocene, about 1 million years old.
 Locality: Karachi, Sindh.

Order Megalotontida

Family Megalontidae

89. Genus *Neomegalodon* (Heart clam)

Age: Jurassic, about 150 million years old.
 Locality: Drazinda formation, KPK.

Order Unionida

Family Unionidae

90. Genus *Parreysea*

Age: Eocene, about 50 million years old.
 Locality: Drazinda formation, KPK.

91. Genus *Lemillidens*

Age: Eocene, about 50 million years old.
 Locality: Drazinda formation, KPK.

Order Arcida

Family Arcidae

92. Genus *Anadara* (Ark clam)

Age: Eocene, about 50 million years old.
 Locality: Drazinda formation, KPK.

Order Myida

Family Anomiidae

93. Genus *Placonopsis*
Age: Eocene, about 50 million years old.
Locality: Drazinda formation, KPK.
Order Pholadoidea
Family Pholadidae
94. Genus *Pholas* (Rock borer)
Age: Eocene, about 50 million years old.
Locality: Drazinda formation, KPK.
Order Myidea
Family Corbulidae
95. Genus *Corbula* (Beaked clam)
Age: Eocene, about 50 million years old.
Locality: Drazinda formation, KPK.
Family Corbiculidae
96. Genus *Carbicula*
Age: Miocene, about 20 million years old.
Locality: Hyderabad, Sindh.
Order Limida
Family Limidae
97. Genus *Plagiostoma*
Age: Eocene, about 50 million years old.
Locality: Drazinda formation, KPK.
Order Veneroida
Family Veneridae
98. Genus *Circe*
Family Mactridae
99. Genus *Lutraria*
100. Genus *Lirophora*
Age: Eocene, about 50 million years old.
Locality: Drazinda formation, KPK.
Order Arcida
Family Cuculleidae
101. Genus *Cucullaea* (Deer Heart clam)
Age: Miocene, about 20 million years old.
Locality: Hyderabad, Sindh.
Order Ostereida
Family Ostereidae
102. Genus *Ostera*
Age (a): Miocene, about 20 million years old.
Locality: Khairpur, Sindh.
Age (b): Pleistocene, about 1.5 million years old.
Locality: Karachi, Sindh.
Order Ostereida
Family Ostereidae
103. Genus *Crossostrea*
Age: Pliocene, about 2 million years old.
Locality: Malir, Karachi, Sindh.
104. Genus *Astarte*
Age: Miocene, about 20 million years old.
Locality: Khairpur, Sindh.
105. Genus *Laevstaria*
Age: Eocene, about 40 million years old.
Locality: Drazinda, KPK.
Family Gryphaeidae
106. Genus *Gryphaea*
(Devil's toe-nail oyster)
Age (a): Eocene, about 50 million years old.
Locality: Drazinda formation, KPK.
Age (b): Miocene, about 20 million years old.
Locality: Sindh.
Order Arcida
Family Noeitedae
107. Genus *Noetia*
Age: Miocene, about 20 million years old.
Locality: Khairpur, Sindh.
Family Arcidae
108. Genus *Striarca*
Age: Eocene, about 40 million years old.
Locality: Drazinda, KPK.
Order Neotrigonida
Family Tregonidae
109. Genus *Neotrigonia*
Age: Eocene, about 40 million years old.
Locality: Punjab.
Order Myida
Family Myidae
110. Genus *Mya*
Age: Oligocene, about 20 million years old.
Locality: Sonmiani, Balochistan.
Order Pectinidea
Family Pectinidae
111. Genus *Argopecten*
Age: Miocene, about 20 million years old.
Locality: Hyderabad, Sindh.
112. Genus *Sinopecten*
Age: Eocene, about 50 million years old.
Locality: Rawalpindi, Punjab (Fig. 10).
Class Gastropoda
Order Neogastropoda
Family Busyconidae
113. Genus *Fulguropsis* (Crown Couch)
114. Genus *Spinifulgar* (Whelk)
Age: Pliocene, about 1 million years old.
Locality: Karachi Coast, Sindh.
Family Strombidae
115. Genus *Strombus*
Order Neogastropoda
Family Conidae
116. Genus *Conus*
117. Genus *Cylindrella*
Age: Pliocene, about 1.5 million years old.
Locality: Malir, Sindh.
Order Littorinimorpha
Family Scaphidae



Fig. 9. Molluscan fossils: Cephalopoda



Fig. 10. Bivalves

118. Genus *Terrabellum*
 Age(a): Miocene, about 20 million years old.
 Locality: Khairpur hills, Sindh.
 Age (b): Eocene, about 40 million years old.
 Locality: Drazinda Formation, KPK.
 Age (c): Eocene, about 50 million years old.
 Locality: Salt Range, Punjab.
Family Cypraeidae
119. Genus *Proadusta*
 Age: Pliocene, about 1 million years old.
 Locality: Karachi Coast, Sindh.
120. Genus *Trona*
 Age: Eocene, about 50 million years old.
 Locality: Rawalpindi, Punjab
Family Olividae
121. Genus *Olivauicularia*
 Age: (a) Eocene, about 50 million years old.
 Locality: Drazinda KPK.
 Age: Pliocene, about 1 million years old.
 Locality: Karachi Coast, Sindh.
Order Archeogastropoda
Family Macluritidae
122. Genus *Maclurites*
 Age: Cretaceous, about 66 million years old.
 Locality: Balochistan and Sindh.
Order Orthogastropoda
Family Hormotomidae
123. Genus *Hormotoma*
124. Genus *Orthonema*
125. Genus *Cyclonema*
Order Lepitellida
Family Fissurellidae
126. Genus *Medurafissurella*
 Age: Eocene, about 35 million years old.
 Locality: Khuzdar, Balochistan.
Order Neritimorpha
Family Paleotrochidae
127. Genus *Turbonopsis*
 Age: Devonian, about 370 million years old.
 Locality: Hyderabad, Sindh.
Order Trochida
Family Trochidae
128. Genus *Trochus*
 Age: Devonian, about 370 million years old.
 Locality: Khuzdar, Balochistan.
Order Cenogastropoda
Family Globocornidae
129. Genus *Angaria*
 Age: Miocene, about 10 million years old.
 Locality: Sindh.
130. Genus *Globocornus*
 Age: Eocene, about 55 million years old
 Locality: Salt Range, Punjab
 Family Megalomastidae
131. Genus *Tomocyclus*
 Age: Eocene, about 55 million years old
 Locality: Salt Range, Punjab
Family Nassariidae
132. Genus *Nassarius*
 Age: Eocene, about 55 million years old
 Locality: Salt Range, Punjab
Family Sibulitidae
133. Genus *Subulitus*
 Age: Triassic, about 200 million years old.
 Locality: Salt Range, Punjab.
Family Horiclanidae
134. Genus *Carinapex*
 Age: Pliocene, about 1.5 million years old.
 Locality: Malir, Karachi, Sindh.
Family Naticidae
135. Genus *Amacurellina* (Moon Snail)
 Age: Eocene, about 55 million years old.
 Locality: Drazinda Formation, KPK.
Family Turritellidae
136. Genus *Turritella*
Family Ampullinidae
137. Genus *Globularia*
Family Ovulidae
138. Genus *Morgula*
Family Volutidae
139. Genus *Scaphella*
Family Triviidae
140. Genus *Trivellona*
Family Hetrohelicidae
141. Genus *Hetrohelix* (in polished lime stone goblet)
 Age: Oligocene, about 30 million years old.
 Locality: Khuzdar Balochistan.
Family Cypridae
142. Genus *Umbilia*
 Age (a): Eocene, about 55 million years old.
 Locality: Drazinda, KPK.
 Age (b): Pliocene, about 1.5 million years old.
 Locality: Sindh.
Order Neogastropoda
Family Buccinidae
143. Genus *Neptuna*
 Age: Eocene, about 55 million years old.
 Locality: Drazinda, KPK.
Family Helicidae
144. Genus *Halicionella*
 Age: Carboniferous, about 300 million years old.
 Locality: Khuzdar, Balochistan.
Order Cenogastropoda
Family Strombidae

145. Genus *Volutispina*

Age: Pliocene, about 3 million years old.

Locality: Karachi Sindh.

Order Patellogastropoda

Family Patellidae

146. Genus *Prostcutum* (in lime stone slab)

Age: Cretaceous, about 100 million years old.

Locality: Punjab (Fig. 11).

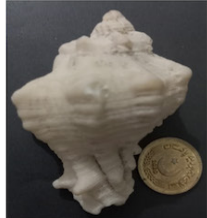
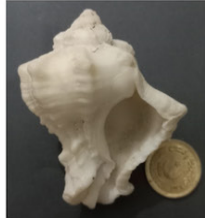
1a. *Fulgoropsis* (Dorsal view), Crown conch.1b. *Fulgoropsis* (Ventral view)2a. *Spinifulgar* (Dorsal view), Whelk.2b. *Spinifulgar* (Ventral view)3a. *Strombus* (Dorsal view)3b. *Strombus* (Ventral view)4a. *Conus* (Lateral view)4b. *Conus* (Dorsal view)5a. *Cylinder* (Dorsal view)5b. *Cylinder* (Ventral view)6a. *Terebellum* (Dorsal view)6b. *Terebellum* (Ventral view)7a. *Produsta* (Dorsal view)7b. *Produsta* (Ventral view)7c. *Prodiusta* (Lateral view)8a. *Oliva ancillaria* (Lateral view)9a. *Maclurites*, in lime stone.10. *Hormotoma*11a. *Orthonema* (Dorsal view)11b. *Orthonema* (Ventral view)12. *Cyclonema* (in polished lime stone goblet)13. *Medurafissurella* (in polish lime stone).14a. *Turbonopsis* (Dorsal view)14b. *Turbonopsis* (Ventral view)15. *Trochus*16a. *Globocornis* (Dorsal view)16b. *Globocornis* (Ventral view)17. *Angaria*18a. *Tomocyclus* (Lateral view)18b. *Tomocyclus* (Dorsal view)19. *Nssarius* (in lime stone)20. *Subulites*21a. *Carinapex* (Dorsal view)21b. *Carinapex* (Ventral view)22a. *Globularia* (Dorsal view)22b. *Globularia* (Ventral view)



Fig. 11. Gastropods.

DISCUSSION

Causes for rich invertebrate fossil fauna are probably due to impact of two global events, Cretaceous-Eocene–Paleogene crises and Paleocene–Eocene Thermal optimum in relation to the collision of Indian and Eurasian tectonic plates (Jablonski, 2008). Iqbal (1980) opines that faunal composition is significantly different from that of KPK and Balochistan. Observations of present study do not show this trend. Among Foraminiferans genus *Nummulites* was common in Eocene rocks of KPK and Balochistan, whereas genus *Disocyclus* was found in Miocene deposits of Sindh only. Genus *Astrorhiza* and *Fusulina* were found in deposits of Permian age in Sindh and Balochistan only. Genus *Spirulina* was found only from Sindh in Pleistocene rocks. Three genera of family Heterohalicidae are reported only from Khuzdar Balochistan in Eocene age rocks. Ozycan *et al.* (2019) has reported *Nummulites* of Silurian age from Drazinda formation, KPK and Salt range, Punjab. Siddiqui *et al.* (2022) studied the Jhimpir area, Lucky formation of Southern Indus basin. Three Eocene age genera of foraminiferans, *Globogerina*, *Morsovella* and *Acrinina* were recorded.

Ten species of fossil corals from Jiواني area of Balochistan are identified. In present study genus *Favites*, *Echinopora* and *Favia* have been recorded from Miocene rocks of Karachi, Sindh. Two Silurian age genera *Astrocerium* and *Paleofavites* have been identified from Balochistan. Among colonial Rugose corals, genus *Siphonodendrone* has been reported from Permian rocks of Dranzinda KPK and Khairpur Hills of Sindh. Similarly, Horn corals of genus *Lophophylidium*, *Zaphrentes* and *Heleophyallam* are recorded from Permian rocks of Sindh and KPK. Branched corals of genus *Favosites* and *Coenites* have been identified from Eocene rocks of Sindh only. Ghobadipour and Popove

(2022), recently studied the Ordovician corals from Iran, Afghanistan and Pakistan. *Cruzina rugosa* was recorded from KPK.

Among Echinoderms, Genus *Micraster* and *Clypeas* of Eocene age have been identified from KPK and Balochistan. Similarly, genus *Cidaris* (Pleistocene age) and *Tretocidaris* (Cretaceous age) have been recorded from Sindh. Three species of Ordovician echinoids were reported from Donghan Region of Alberz mountain range, Iran (Lefebvre *et al.*, 2005).

Crustaceans were recorded in very small number in present study. Crabs of genus *Proxicarpilius* and *Lobocarcinus* have been identified from Eocene age rocks of Sindh. Balanus of genus *Semibalanus* of Cretaceous age has also been recorded from Sindh. A small mud – lobster of genus *Thalassina* has also been identified from Eocene deposits of KPK. Hyzny *et al.* (2016) have described two new species of Ghost shrimp (f. Axiididea), *Newcallichernus khadroensis* and *N. lakharaensis* from Eocene deposits of Sindh (Fig. 12).

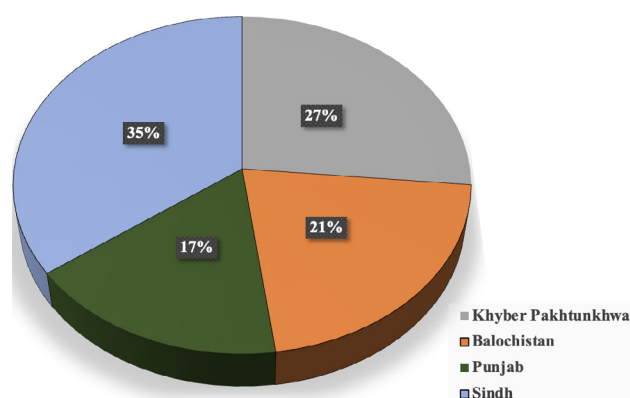


Fig. 12. Occurrence of fossils in various provinces of Pakistan.

Brachiopods flourished during Paleozoic era and became extinct by the end of Permian age. Only one genus is extant at present. In present study ten species of Brachiopods have been identified. Genus *Cryptospirifer*; *Antiquantia* and *Penicularis* belong to Carboniferous age. *Ortholina*; *Spiriferina* and *Trebratula* are from Triassic age, while *Plarostomys* and *Vinlandostrophia* belong to Ordovician age. According to Angiolini *et al.* (1999), late Carboniferous brachiopod fauna from Northern Karakoram area of Pakistan shows affinities with the fauna of contiguous pre-gondwanan rocks of Afghanistan and Lahasa block and are also related to those of Tethys-Himalayas.

Among Cephalopods, 8 genera of *Nautiloids* and *Belmenites* have been recorded while 14 genera of *Ammonites* have been identified in present study.

Cephalopods appeared during Cambrian age and became dominant during the Ordovician age. Ammonites and belemnites became extinct by the end of Cretaceous age. Few representatives of subclass Nautiloidea and Coleoidea are still found today in ocean of the world. Majority of Cephalopod fossils have earlier been found from salt range, Punjab (Spath, 1939) and Balochistan (Fatmi *et al.* 1999; Siddiqui *et al.*, 2009). In present study some Cephalopod fossils have been recorded from KPK and Sindh province as well. Earlier Fatmi *et al.* have recorded Jurassic age Cephalopod from Kishor- Marwat range of D.I. Khan KPK. Helder (2012) identified 3 new species of Cenozoic Nautiloids from Kutch area of W. India. Three Ordovician age Orthoconic cephalopods have been recorded from Albarz mountain range, Iran (Evans *et al.*, 2013).

In present study 23 genera of bivalves have been identified. Bivalves appeared in middle of Cambrian age. These flourished in Mesozoic and Cenozoic era. They are found in abundance in modern day seas (Tichart, 1966). Majority of fossils identified in present study belong to Eocene age. Only one genus, *Neomegelodon* (heart-clam) belongs to Jurassic age which has been recorded from Drazinda formation, KPK. Genus *Venecardia* belonging to Pliocene age has been recorded from Karachi, Sindh. Most of bivalves identified from Drazinda formation, KPK, belong to Eocene age. A scallop genus *Argopectin* belonging to the Miocene age has been recorded from Hyderabad, Sindh.

Gastropods from the largest group of fossils (30 genera) in present study (Fig. 13). Gastropods are a highly successful group of molluscs. Among 721 families of known gastropods, 245 are extinct today. Gastropods appeared in Paleozoic era (Cambrian age) and continue up to present age. Brohi *et al.* (2009) while discussing the economic significance of Tertiary rocks in Thana Bola Khan area of Sindh also recorded various invertebrate fossil groups from different formations. Lucky formation yielded large

Foraminiferans; Gastropods; bivalves and Echinoids. Tiyon formation contained the fossils of all the above groups. Similarly, Gaj formation; Nari formation and Manchar formation revealed the fossils of above-mentioned groups. In another study of fossil Gastropods (f. Volutidae) were studied from Lakhra formation of Sindh. Ten new species of Eocene age were identified. Harzausurs *et al.* (2017) recorded 19 species of Gastropods from Makran area of S.E. Iran. These included 3 new species.

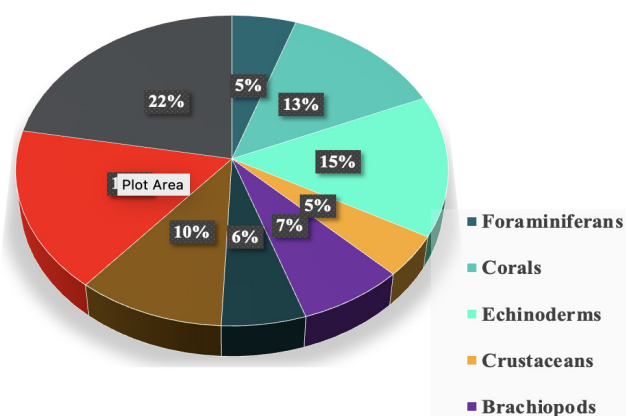


Fig. 13. Occurrence of various groups of fossil invertebrates.

DECLARATIONS

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Statement of conflict of interest

The authors have declared no conflict of interest.

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CITATIONS

Prof. Dr. Muzaffer Ahmad Lifetime Achievement Award 2024

**Prof. Dr. Javed Iqbal Qazi,
Former Dean,
Faculty of Life Sciences/
Institute of Zoology,
University of the Punjab, Lahore.**



Prof. Dr. Javed Iqbal Qazi retired as Dean, Faculty of Life Sciences / Director Institute of Zoology, University of the Punjab after 40 years of meritorious service on 05.04.2023. During his illustrious academic journey he supervised research work of 30 Ph.D. and more than 140 MS/M.Sc. students. He completed 16 Research Projects and Organized 15 International Scientific Conferences. His research had focused scientific exploitation of indigenous resources for economic uplift of the country.

Prof. Qazi has published more than 215 research articles with 2200 citation (Google Scholar). He has also authored 20 books/chapter in books published by national as well as international publishers. He has also written several books/articles in Urdu for public awareness on topics of core scientific disciplines as well as on social and religious issues.

Based upon his academic performances, Prof. Qazi was awarded Best University Teacher Award by Higher Education Commission in 2016, Research Productivity Awards by Pakistan Council for Science and Technology in 2012 and Zoologist of the year Award by Zoological Society of Pakistan in 2012.

**Prof. Dr. Muzaffer Ahmad
Lifetime Achievement Award
2024**

**Prof. Dr. Muhammad Siddique Awan,
Department of Zoology,
University of Azad Jammu and Kashmir,
Muzaffarabad.**



Professor Dr. Muhammad Siddique Awan, is a renowned academician and researcher, with a distinguished personality in the field of Zoology with specialization in Wildlife Ecology and Conservation Biology. Holding a robust academic background with M.Sc., M.Phil, and PhD degrees in Zoology, he served the University of Azad Jammu and Kashmir in various academic and administrative positions since 1990, where he dedicated his 33 years to teaching and research at the Department of Zoology. During his illustrious career, Professor Siddique Awan has made significant contributions to the field of wildlife research, with 75 research publications with impact factor exceeding 30 over 1400 citations h-index of 21, an i10-index surpassing 46. He is pioneer of the in research on wildlife of the state of Azad Jammu and Kashmir. He made significant contributions of discovery of Red Jungle Fowl and rediscovery of Cheer pheasant to his credit. He has mentored 2 Ph.D. and 80 M.Sc/M.Phil scholars. Recognizing his invaluable contributions, Prof. Awan has received seven consecutive Research Productivity Certificate Awards from 2013 to 2019.

He holds Life Fellowship of Zoological Society of Pakistan, and Fellowship of Biological Society of Pakistan. He has been member of National Curriculum Committee of HEC in Zoology. He has actively participated in over 100 conferences, symposia, and seminars. Prof. Siddique is Member of the Wildlife Board, Government of Azad Jammu and Kashmir, and HEC's National Core Group in Life Sciences (NCGLS).

**Prof. Dr. Muzaffer Ahmad
Lifetime Achievement Award
2024**

**Prof. Dr. Muhammad Naeem Khan,
Professor Emeritus,
Vice Chancellor,
University of Baltistan, Skardu.**



Prof. Emeritus Dr. Muhammad Naeem Khan is Vice Chancellor, University of Baltistan, Skardu. He obtained M.Sc. degree in Zoology from University of the Punjab in 1983 and Ph.D. degree in Zoology from University of Guelph, Ontario in 1996. He joined Department of Fisheries, Government of Punjab as Assistant Director Fisheries in 1986, and became Director of Fisheries in 1996. In 2003 he moved to University of Veterinary and Animal Sciences as Dean, Faculty of Fisheries & Wildlife. In 2004 he joined University of the Punjab as Professor of Zoology and later became Dean, Faculty of Science in 2015, the position he occupied till he became Vice Chancellor of University of Baltistan.

Dr. Khan has published 101 articles in prestigious international journals with citations 2357 and h-index 23. He has also written three books namely (1) Freshwater, Fish and the Future published by FAO, Michigan State University & American Fisheries Society, USA and (2) Building an expert judgment-based Model of Mangrove Fisheries, published by American Fisheries Society, USA (2015). (3) Aquaculture Handbook-Fish Farming and Nutrition in Pakistan- Feeding Pakistan published by US Dept. of Agriculture.

Prof. Dr. Muzaffer Ahmad
Lifetime Achievement Award
2024

Prof. Dr. Nasreen Memon,
Ex-Chairperson,
Department of Zoology,
University of Sindh, Jamshoro.



Meritorious Professor, Dr. Nasreen Memon, Ex-chairperson, Department of Zoology, University of Sindh, obtained her Ph.D. degree from University of Karachi in 2003, and post doctoral research experience from National University of Singapore in 2005 and Univesity of Nottingham, United Kingdom in 2010. She has put in move than 30 years of meritorious service in promotion of Zoological Sciences. She has been active member of National Curriculum Revision Committee of Higher Eudcation Commission and focal person of Higher Education Commission, Islamabad, Pakistan 2014-2016 under National Research Program for Universities (NRPU).

Prof. Memon has received, Best Female Teacher Award – 2014, for her Meritorious Services in teaching and research by Federal Ministry of Education, Pakistan. Zoologist of the Year Award - 2016 by Zoological Society of Pakistan and Gold Medal of Eminent Person (Life Achievement) by University of Sindh in 2019.

She won three research projects which were funded by Higher Education Commission, Islamabad. She established an Entomology Research Lab in 2004 and an Insectary in 2007 at Department of Zoology, University of Sindh, Jamshoro. She Organized 36th Pakistan Congress of Zoology (International) in collaboration with Zoological Society of Pakistan, in the Department of Zoology, University of Sindh, Jamshoro in February 2016.

Prof. Memon has produced 14 Ph.D. scholars and 37 M. Phil/MS research scholars. She has published 91 research articles with Impact Factor 30.5. Prof. Memon served as Vice president (South), of Zoological Society of Pakistan from 2013-2016, Prof. Memon is Fellow of Zoological Society of Pakistan since 2004, and Member of Entomological Society of America (ESA), Member of Royal Entomological Society, London, UK. Honorary member of Editorial Board of Pakistan Journal of Zoology Since 2006. Honorary member of Editorial Board of an International Journal Bulletin of Pure and Applied Sciences. (INDIA) since 2004.

Zoologist of the Year Award 2024

**Prof. Dr. Riffat Sultana,
Department of Zoology,
University of Sindh, Jamshoro.**




Dr. Riffat Sultana is a Professor at the Department of Zoology, University of Sindh, Jamshoro. She obtained her PhD degree in Zoology with a specialization in Entomology from the University of Sindh, Jamshoro, in 2008. She conducted her two postdoctoral research at the Department of Entomology, A&M University, USA, and the Tea Research Institute of CAAS, Hangzhou, China. Her expertise encompasses research areas such as Biodiversity, Insect Systematics, Chemical & Biological Control, and Food Preferences of Insects in general, with a particular focus on Orthoptera. Dr. Sultana has 199 published research articles to her credit, both in national and international journals of repute, accumulated Web of Science IF of >100, Google Scholar Citation indices: 919, h-index: 15, i10-index: 34, while on ResearchGate, her citation score is 1169, h-index: 15, and citation count: 727, with 56,653 reads. She has also authored 02 books and 02 chapters at national and international levels and 45 popular scientific articles in several daily newspapers and magazines about pests and pest control for common people. She contributed 19 entirely new entries to science. Up to now, she has produced 23 PhDs and 61 M.Phils. She has been awarded 06 research projects as Principal Investigator, including 01 international project by the National Geographic Society and 05 national projects sponsored by the Higher Education Commission Islamabad (HEC) and 01 by the Pakistan Science Foundation Islamabad (PSF).


Recently, she signed an LOA with FAO for trials of Biopesticides in Pakistan. She is the Founder Editor of the University of Sindh Journal of Animal Sciences (HEC Recognized), launched in 2019.

Dr. Riffat Sultana has achieved several prestigious awards, including the Prof. Dr. Mirza Azhar Beg Gold Medal in 2023, the Prof. Dr. Anjum Suhail Memorial Gold Medal in 2022, the Prof. Dr. I. Ahmed Gold Medal in 2018, and the Chancellor Gold Medal in 2018.

*Other applicants of this award were Prof. Dr. Najma Shaheen, Dr. Muhammad Sarwar, Dr. Muhammad Rais and Dr. Ihsan ul Haq



These accolades recognize her exceptional research contributions in the field of Entomology & Biodiversity. Additionally, she received a Competitive Research Grant award from the Governor of Sindh in 2017, an Incentive Award certificate of appreciation for 2011-2012, and the Pepsi Merit Scholarship award for an outstanding academic record from Sind Beverage (PTV). Due to her expertise and remarkable contributions, she was nominated as the iBOL Focal Person for Sindh Province, Pakistan, by iBOL (International Barcode of Life), University of Guelph, Guelph, Ontario, Canada. Additionally, the Ministry of Food Security, Islamabad, entrusted her as the Sindh Provincial focal person for the management of locust infestation in Sindh, Pakistan. Beside this, she developed research collaborations with many prestigious universities in the USA, Canada, Turkey, China, Malaysia, Sri Lanka, India, and Bangladesh.





Prof. Dr. A.R. Shakoori Gold Medal 2024


Dr. Azhar Rasul,
Assistant Professor,
Department of Zoology,
Govt. College University, Faisalabad.




Dr. Azhar Rasul is an Assistant Professor in the Department of Zoology at GCUF. He got his MSc and M.Phil degree in Zoology from Bahauddin Zakariya University, Multan and Ph.D. in Biological Sciences (Cell Biology) from Northeast Normal University, China. He secured a PhD fellowship jointly awarded by Ministry of Education (MOE), Pakistan and China Scholarship Council (CSC), China and completed his. He later received China postdoctoral fellowship in 2012, Japanese Society for Promotion of Science (JSPS) Postdoctoral fellowship in 2013, and subsequently Tokyo Biochemical Research Foundation (TBRF) fellowship in 2015. During his research career, he has made significant contributions in discovery of Topoisomerase II (Topo-II) and Tubulin I inhibitor as anti-cancer agents. He also identified novel inhibitors of 6PGD and Malic Enzyme 2 (ME-2), as non-cytotoxic anti-metastasis agents.

Dr. Rasul has published over 181 peer-reviewed articles with cumulative impact factor over 500 and with over 5863 citations. He has authored three books and he has published thirty book chapters. Additionally, he is the editor for two books. He has presented several keynotes and invited talks at National and International level. He has attended more than 20 International conferences in Japan, China, South Korea, Dubai, Sri Lanka, Qatar, Turkey, Thailand, Malaysia and France. Under his supervision, 24 MPhil students and 12 Ph.D students completed their research work.


*Other applicants of this award were. Prof. Dr. Umar Farooq, Dr. Yasir Waheed, Dr. Aziz-Ul-Rahman, Prof. Dr. Riffat Mehboob, Dr. Samia Afzal, Dr. Fakhar-ud-Din, Dr. Zia-ur-Rehman Mashwani, Dr. Muhammad Khan, Dr. Haroon Ahmed, Dr. Ali Hussain, Dr. Siddra Ijaz, Dr. Sana Javaid Awan, Dr. Muhammad Shahid, Dr. Shaukat Ali, Dr. Noshin Ilyas, Prof. Dr. Muhammad Ijaz, Dr. Shahzad Ali, Prof. Dr. Saima Sadaf, Dr. Q.A.A. Gardner and Dr. Aasia Karim





He was awarded with Young Scientist Program (YSP) Fellowship, 2018 by IUBMB and Mufti Mehmood Award” on the behalf of “Education and Science Committee” on December 9, 2018. He got RPA award from GCUF in 2023.

He has won 15 national and international research grants (11 as PI and 4 as Co-PI) from various funding organizations including PHEC, IFS, HEC-Pak Turk Mobility Grant, ISSESCO RG, HEC-NRPU RG(s), The Nagai Foundation Tokyo RG(s), PSF and COMSTECH-TWAS RG with total funding amount of 50 million PKRs. His lab is actively engaged in interdisciplinary research on novel tumor biomarkers, cancer-related health disparities, identification of non-toxic anti- cancer compounds for various hallmarks of cancer (cancer stem cells, cancer cell metabolism, and tumor hypoxia) from natural sources and development of highly efficient green extraction methodologies for preparation of active constituent enriched-bioactive extracts libraries.





Prof. Imtiaz Ahmad Gold Medal 2024


**Dr. Hafiz Azhar Ali Khan,
Associate Professor,
Institute of Zoology,
University of the Punjab, Lahore.**



Dr. Hafiz Azhar Ali Khan as an Associate Professor at the Institute of Zoology University of the Punjab. He obtained his B.Sc. (Hons.) degree in Entomology from Bahauddin Zakariya University, Multan, M.Sc. (Hons.)/M.Phil. Entomology from University of Agriculture, Faisalabad, and Ph.D. in Entomology from Bahauddin Zakariya University, Multan. He was conferred Gold Medal for securing overall first class first position in B. Sc. (Hons.) by the BZU, and ICI Pakistan Life-Sciences Gold Medal for the Best University graduate in 2008.

Dr. Khan's area of research work focuses on management of pests and insecticide resistance. He has published 120 research articles/monographs/book chapters in peer-reviewed journals of national and international repute with a cumulative impact factor 230, h-index 29, and more than 3000 citations. He received Best Research Paper award of Higher Education Commission, Pakistan, for the years 2013/2014 and 2017, Research Productivity Awards of Pakistan Council for Science and Technology for the years 2013 to 2017. Dr. Khan is working on insect pest management. Dr. Khan is also serving as an Associate Editor for the international journal "Discover Applied Sciences (formerly SN Applied Sciences)" published by the Springer-Nature, Switzerland.

*Other applicants of this award were Dr. Azhar Abbas Khan, Dr. Santosh Kumar and Dr. Muhammad Zeeshan Majeed



Dr. Abdul Aleem Chaudhary
Gold Medal
2024

Dr. Muhammad Altaf,
Institute of Forest Sciences,
The Islamia University of Bahawalpur.



Dr. Muhammad Altaf obtained an MSc (Zoology) from the University of the Punjab, Pakistan in 2008; an M.Phil. and a Ph.D (Wildlife and Ecology) from the University of Veterinary and Animal Sciences, Pakistan in 2010 and 2016 respectively. His Ph.D thesis work revolved around the human-wildlife interaction and ethnomedicinal uses of wildlife. Dr. M. Altaf started his professional career initially as an Assistant Professor of Zoology at Women's University of Bagh, Azad Jammu and Kashmir in 2016, later moved to at the Institute of Forest Sciences, The Islamia University of Bahawalpur, Pakistan in 2020. Dr. M. Altaf has published 63 indexed research articles with a 66.8 impact factor and has received more than 2000 citations. He has also published 6 books (3 national and 3 international) and 6 chapters (international), and has supervised the thesis work of 43 MS/M.Phil and 41 BS (Hons)/M.Sc. theses.

*Other applicants of this award were. Dr. Faraz Akrim and Dr. Ume Habiba

Prof. Dr. Mirza Azhar Beg
Gold Medal
2024

Dr. Rehana Iqbal,
Bahauddin Zakariya University,
Multan.



Dr. Rehana Iqbal obtained her PhD degree in Biological Sciences with specialization in Fish Nutrition from Bahauddin Zakariya University, Multan. She has supervised 70 M.Phil and 5 Ph.D. students and published 50 research papers in refereed scientific journals with 80 Impact factor and more than about 1000 citations. Dr. Rehana Iqbal has authored 4 International Books chapters presented papers in many national and international conferences. Dr. Rehana attended more than 50 seminars and workshops on the different zoological aspects and won scholarship for Training course on “Tilapia Hatchery Techniques and grow out of farming” 1-12 June 2015 at Asian Institute of Technology (AIT) Bangkok, Thailand. She is life fellow of Zoological Society of Pakistan, Pakistan Fisheries society and Applied Zoological Society of Pakistan. She has been awarded with Dr. Farhat Jabeen Gold Medal in Toxicology, by The Applied Zoological Society of Pakistan in 2019.

*Other applicants of this award were Dr. Mahroze Fatima and Dr. Shaista

**Z.B. Mirza Biodiversity (Faunal)
Gold Medal
2024**

**Dr. Syed Ahmed Zia,
Senior Scientific Officer,
National Insect Museum (NARC),
Islamabad.**



Dr. Syed Ahmed Zia is Senior Scientific Officer, National Insect Museum, NARC Islamabad. He obtained Ph.D. degree in 2010 in the field of Entomology with specialization in Biosystematics. Dr. Syed Ahmed Zia has worked on Biodiversity of Class Insecta including crop pests species, storage pests species, pollinators species, water pollution indicator species, medical importance species, environmental cleaners species, medicinal value species and even invasive insect pest species (insects & plant) of Pakistan. He has surveyed and documented faunal works from all the four provinces, Gilgit Baltistan and AJ&K including difficult border areas with India, Afghanistan and China. He has documented 100 new to country and two new to science species to the Biodiversity of Pakistan. Dr. Zia is serving as Adjunct Faculty Member at PIASA institute (at NARC) affiliated with Agriculture University Peshawar and QAU Islamabad since 2009.

Dr. Zia has Supervised and Co-Supervised > 50 M.Sc. and Ph.D. Students and have run National as well as International funded projects as PI & Co-PI. His biodiversity and faunal work have focused on conservation of red listed species with international collaboration. Acknowledging his services in the field of Odonata diversity, he has been awarded a Certificate of Honor from IDF, Germany. Dr. Zia is first Member of International Association of Neuroptology (IAN), USA and sole Pakistani member of Worldwide Dragonfly Association (WDA), Germany. He is also the only Representative Member for IUCN Species Survival Commission [Dragonfly Specialist Group (DSG)] from Pakistan.

*Other applicants of this award were Dr. Riaz Aziz Minhas and Dr. Suleman

Prof. Dr. Nasima M. Tirmizi
Memorial Gold Medal
2024

Dr. Zaib-un-Nisa Burhan,
Assistant Professor,
Centre of Excellence in Marine Biology,
University of Karachi, Karachi.




Dr. Zaib-un-Nisa Burhan, earned her M.Sc. in Zoology and M. Phil and Ph. D in Marine Biology from University of Karachi. She is serving at Centre of Excellence in Marine Biology (CEMB), University of Karachi (UoK) since the past 15 years. Before joining the service, Dr. Burhan worked as Research Associate in Office of Naval Research Funded Project, Pakistan Science Foundation and Honorary Research Associate in Higher Education Commission at CEMB, UoK.


During the past several years, she has been involved in collaborative research work in the field of Marine Microbial Ecology, Biodiversity and Conservation. She has been trained as marine microbial ecologist at *Scottish* Association for Marine Science, Scotland, UK under DIFID and HE Link Program. Dr. Burhan has taken part in many other national and overseas funded training courses on instrumental techniques, bioassays, biodiversity assessment and Integrated Coastal Management under British Council/ DelpHE Higher Education Link. Recently, she has been certified as Master trainer on Integrated Coastal Management from the Asian Institute of Technology, Bangkok, Thailand.

In addition, she was awarded Productive Scientist of Pakistan in 2016 & 2017 by Ministry of Science and Technology, Pakistan. She has contributed significantly in various Webinars, Conferences, Seminars and Training workshops within the country and abroad. She has participated as resource person in several training courses for undergrad and graduate students at CEMB, Institute of Environmental Sciences, UoK and Institute of Marine Sciences and Fisheries, Chittagong University, Bangladesh.

*Other applicants of this award were Mr. Muhammad Moazzam Khan. Dr. Farhana Shafiq Ghory, Dr. Amjad Ali, Dr. Nafisa Shoaib and Dr. Munawwer Rasheed



Dr. Burhan is HEC approved supervisor and supervising many student's research at M. Phil/Ph. D level. At present she is also serving as Peer Reviewer for several international impact factor journals. Dr. Burhan has authored 47 research publications and contributed 02 chapters in books and 04 articles in Proceedings and Newsletters at national and international level. Besides, she has taken part in organizing conferences, seminars and training workshops since the year 2000.



**RECIPIENTS OF
GOLD MEDALS AWARDED BY
THE ZOOLOGICAL SOCIETY OF PAKISTAN**

1. Muzaffer Ahmad Gold Medal 2024

The 29th Muzaffer Ahmad Gold Medal 2024 was received by Ms. Amina student of University of the Punjab, Lahore for standing first in the recent BS. Zoology examination.



Ms. Amina

2. Afsar Mian Gold Medal 2024

The 14th Afsar Mian Gold Medal 2024 was given to Ms. Tayyaba Mubeen a student of the Arid Agriculture University, Rawalpindi for standing first in the recent M.Sc. Zoology examination.



Ms. Tayyaba Mubeen

3. Muhammad Afzal Hussain Qadri Memorial Gold Medal 2024

The 25th Muhammad Afzal Hussain Qadri Memorial Gold Medal 2024 was awarded to Ms. Maria Zulfiqar student of Karachi University for standing first in the recent M.Sc. Zoology examination.



Ms. Maria Zulfiqar

4. Prof. Dr. S.N.H. Naqvi Gold Medal 2024

The 18th Prof. Dr. S.N.H. Naqvi Gold Medal 2024 was given to Dr. M. Naeem Khan for obtaining Ph.D. degree in Zoology with specialization in the field of Toxicology from University of Karachi.



Dr. M. Naeem Khan

5. M.A.H. Qadri Memorial Gold Medal 2024

The 22nd M.A.H. Qadri Memorial Gold Medal 2024 was given to Dr. Gul-e-Lala for obtaining Ph.D. degree in Zoology with specialization in the field of Parasitology from University of Karachi.



Dr. Gul-e-Lala

6. Mujib Memorial Gold Medal 2024

The 29th Mujib Memorial Gold Medal 2024 was given to Ms. Maria Zulfiqar a student of Karachi University for standing first in the recent M.Sc. Zoology examination with specialization in Parasitology.



Ms. Maria Zulfiqar

7. Prof. Dr. Muhammad Ali Gold Medal 2024

The 6th Prof. Dr. Muhammad Ali Gold Medal 2024 was awarded to Mr. Mussadaq Hussain a student of Government College University, Faisalabad for standing first in the recent BS (Hons) Zoology examination.



Mr. Mussadaq Hussain

8. Ahmed Mohiuddin Memorial Gold Medal 2024

The 23rd Ahmed Mohiuddin Memorial Gold Medal 2024 was awarded to Ms. Tooba Gul a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination.



Ms. Tooba Gul

9. Prof. Dr. S.S. Akbar Memorial Gold Medal 2024

The 9th Prof. Dr. S.S. Akbar Memorial Gold Medal 2024 was awarded to Mr. Raja Ram a student of University of Sindh, Jamshoro for standing first in the recent M.Sc. Zoology examination with specialization in Entomology.



Mr. Raja Ram

10. Prof. Dr. Muhammad Saeed Wagon Gold Medal 2024

The 4th Prof. Dr. Muhammad Saeed Wagon Gold Medal 2024 was awarded to Miss Dua a student of University of Sindh, Jamshoro for standing first in the recent BS Zoology examination.



Miss Dua

**SOME GLIMPSES OF
ACADEMIC SESSIONS
AND THE CONGRESS PARTICIPANTS**



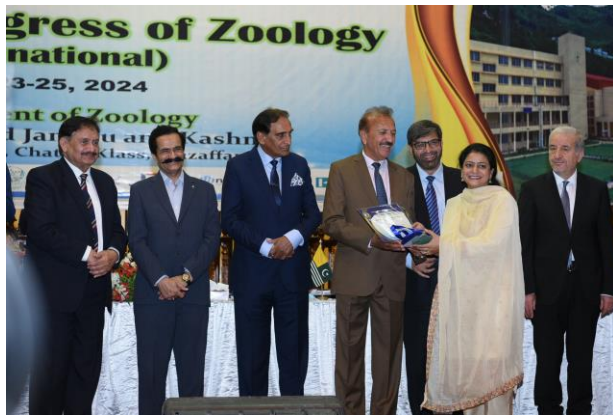














Abstracts of Plenary Lectures

PLENARY LECTURE-1**SOME GENERAL ASPECTS OF FISH FARMING****Prof. Dr. Telat YANIK***Aquaculture Department, Faculty of Fisheries, Ataturk University, 25240-Erzurum, Turkey.*Corresponding Author: tyanik@atauni.edu.tr

In aquaculture, it should be known at what stage fish require a certain amount of feed and how often to feed them. According to the availability of feeds or the type of food given to the fish, the system of aquacultural production can be defined as extensive (no feed is given), semi-intensive, and intensive. Cultured or wild fish eggs and milt can be fertilized and hatched up to a 97% success rate. However, if feeding fry and larvae are not provided successfully, all of the fish may be lost. That means sometimes, in fish culture, success depends on feeding. Fish feeding can be affected by many factors. These factors, classified as either outer or inner stress-bearing factors, include disturbances to fish, any change in water or feed quality, etc., and the physiological state of fish (such as reproduction). Therefore, certain facts, such as fish behavior, ecology (relationships between the living environment and the organism), nutrient requirements, energy metabolism, feed type, and size, should be known. On the other hand, the effective utilization of feed in a fish body can be indirectly called “metabolism.” Metabolism starts with the intake of feed particles into the body to provide the energy for normal life processes. It compensates for losses such as natural wear or excretion and produces enough energy for the growth of an organism. It ends with the discharge of nitrogenous wastes from the intestines in the form of urine and feces. The metabolism rate changes depending on the state of the fish, i.e., basal metabolism during rest, routine metabolism in normal swimming and looking for feed, and active metabolism in moving. In order to get the maximum growth rate, fish should be in a very comfortable medium.

Therefore, it is necessary to maintain good environmental conditions and prepare the best feed mixture to get the maximum weight. In general, the more feed is accepted by the fish and utilized effectively, the more the fish grow. In this presentation, some general aspects related to fish culture will be discussed based on the published scientific data.

PLENARY LECTURE-2**HOW TO INTEGRATE MULTIPLE FIELDS IN AMPHIBIAN CONSERVATION RESEARCH?****Amael Borzee***Laboratory of Animal Behaviour and Conservation, College of Life Sciences,
Nanjing Forestry University, Nanjing, People's Republic of China.**Amphibian Specialist Group, Species Survival Commission, IUCN*

Corresponding Author: amaelborzee@gmail.com

The surge in research focused on conservation, along with its novelty factor, has made it an appealing subject of investigation from multiple perspectives. This growing interest has fostered an interdisciplinary approach that has yielded significant innovations, ultimately bolstering efforts aimed at conserving the Earth's biodiversity. However, a critical challenge that confronts us is the need to effectively synthesize and organize research findings in a manner that can exert tangible influence on conservation initiatives. Of particular concern is the decline of amphibian populations, with over 40% of species worldwide now listed as threatened. Analysing the proportion of threatened amphibians within each biome, it becomes evident that East Asia stands out as a hotspot for both biodiversity and species at high risk of extinction. This pronounced trend can be attributed, in large part, to the transformation of wetland habitats into agricultural wetlands primarily driven by rice cultivation. The resulting modifications to the landscape have deleteriously impacted species diversity and abundance in these altered ecosystems. The approach presented here integrates science-based conservation strategies, thereby facilitating a comprehensive understanding of the threats faced by amphibians in East Asia predominantly attributed to landscape transformations driven by agriculture, climate change, and population displacement. This approach relies on a multi-faceted process that encompasses key domains of conservation, including the formulation of mitigation plans, policy recommendations, conservation applications, and monitoring protocols. In doing so, this approach strives to provide robust support for the conservation of East Asian biodiversity while concurrently fostering a sustainable coexistence between humans and other species inhabiting our planet.

PLENARY LECTURE-3**INSIGHTS INTO THE BIOLOGICAL SIGNIFICANCE OF INSULIN VARIANTS****Qurra-tul-Ann Afza Gardner***School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore*

Corresponding Author: qagardner.sbs@pu.edu.pk

In the early 1920s, Banting and Best, along with Macleod and Collip, made a remarkable history in the field of medicine and a protein of the 20th century, the first hormone, 'insulin' was identified. The discovery of insulin from Islets of Langerhans became the 'Islets of hope' for diabetics. It was the first protein, the primary structure of which was completely determined, crystallized, and chemically synthesized. The main challenge was to correctly align the six -SH groups to produce two interchain one intrachain disulphide bridges present in native insulin. The key pointer to the latter challenge was provided by the discovery of preproinsulin and proinsulin, the single-chain precursors of insulin by Steiner. Preproinsulin is the initial product of the insulin gene expression with signal peptide, which is removed by signal peptidase to give proinsulin consisting of three domains: the B and A chains linked via C-peptide. In the Golgi apparatus, proinsulin undergoes the removal of the C-peptide domain creating the two chains (A and B) present in insulin-core, followed by the trimming of the extra amino acids to give native insulin. When glucose level rises, insulin is released into the blood stream that helps to regulate blood sugar levels. Understanding the synthesis and processing of insulin has led to the recombinant production of insulin analogues with tailored properties, such as faster onset, shorter duration of action for rapid-acting analogs, whereas long-acting analogs provide more stable and prolonged action. The present talk describes the properties of various insulin variants, prepared in SBS, Lahore. Briefly, this involves, designing DNA constructs encoding insulin derivatives, their heterologous expression in *Escherichia coli*, processing of the expressed proteins through a multistep protocol involving trypsin and carboxypeptidase B to insulin derivatives, which are as active as commercially available recombinant human insulin. The future of insulin variants is promising, that could revolutionize diabetes management and continues to be a life-saving treatment for millions of people with diabetes globally.

PLENARY LECTURE-4**TARGETING TUMOR ENERGY METABOLISM WITH PLANTS-DERIVED MULTI-TARGET BIOACTIVE MOLECULES: A PROMISING STRATEGY FOR CANCER THERAPY****Muhammad Khan***Institute of Zoology, University of the Punjab, Quaid-e-Azam campus Lahore*

Corresponding Author: mkhan.zool@pu.edu.pk

The journey to develop cancer chemotherapeutics started at the beginning of 20th century. From 1950 to 2000, a large number of cancer chemotherapeutics had been developed and made available in cancer clinic to treat multiple human malignancies. However, these drugs mainly work by killing fast-proliferating cells and thus found equally toxic to normal growing cells. Around 1990, researchers and pharmaceutical companies started to develop targeted therapies and till date we have a significant list of targeted therapies which selectively bind and set the cancer cell on the road to ruin by blocking a particular signaling pathway, thereby sparing the normal cells. However, development of secondary drug resistance has appeared the major challenge and reduced the efficacy of targeted drugs in cancer clinic. Targeting altered tumor energy metabolism has emerged as a novel and pragmatic approach as it distinguishes cancer cells from normal cells and receive limelight as target for cancer therapy. It is well established now that cancer cells undergo aerobic glycolysis even in the presence of adequate amount of oxygen, a phenomenon known as “Warburg Effect” which is insufficient energy yielding process. To compensate energy loss, cancer cells overexpress several glycolytic enzymes including GLUT1, HK2, PK-M2 and LDH for uptake of glucose and enhanced glycolysis. This unique metabolic pattern makes cancer cells more sensitive to drugs that disrupt energy metabolism. In fact, a large number of glycolytic inhibitors have been developed but none of them have passed FDA approval for use in cancer clinic. Recent research has shown that cancer cells undergo metabolic reprogramming and shift to other energy metabolism when one is inhibited.

Thus, intervening both energy supply system simultaneously will be a feasible strategy to eradicate cancer cells effectively. Here, we have identified and characterized a natural bioactive molecule “Cryptotanshinone” which has potential to inhibit glycolysis and oxidative phosphorylation simultaneously, using ovarian cancer model. The anticancer mechanism and cellular targets of Cryptotanshinone has been investigated in detail using in silico, in vitro and in vivo models.

PLENARY LECTURE-5**AN UPDATE ON BREEDING TECHNOLOGIES IN WATER BUFFALO
(BUBALUS BUBALIS)****Syed Murtaza Hassan Andrabi*^{1,2}, Muhammad Hammad Fayyaz¹, Muhammad Shafiq Haider¹***¹Animal Reproduction and Genetics Program, Animal Sciences Institute,
PARC-National Agricultural Research Centre**²Animal Sciences Division, Pakistan Agricultural Research Council, Islamabad*
Correspondence Author: andrabi123@yahoo.com

This abstract provides an update on the advancements in breeding technologies being utilized to improve the reproductive efficiency in water buffalo (*Bubalus bubalis*). The buffalo plays a crucial role towards the dairy and meat sectors of Asian, especially the South-Asian countries. Breeders are increasingly drawn to buffalo farming due to its substantial socioeconomic impact and its pivotal role in advancing the livestock sector in the developing nations. Buffalo reproduction faces considerable challenges and has lower reproductive efficiency as compared to temperate cattle. Factors contributing to this include difficult to detect estrus overtly, silent estrus, seasonality, delayed puberty, inherently fewer primordial follicles. In this regard, researchers are working to optimize modern breeding technologies in buffalo. These technologies include estrus synchronization (ES), timed artificial insemination (TAI), transvaginal ultrasound-guided ovum pick-up (OPU), gamete/embryo preservation, in-vitro embryo production and transfer (IVEP&T) and sperm sex sorting. Regarding buffalo sperm cryopreservation, researchers have significantly improved the post-thawing quality through chemically defined extenders, optimized cooling, freezing and thawing rates. Likewise, researchers have standardized ES protocols and TAI. These synchronization protocols are meant to control both luteal and follicular functions and permit timed AI with higher pregnancy rates in low and peak breeding seasons. In recent past, there was scarcity in the outcomes of OPU/IVEP&T techniques in buffalo. Considering the limited success, researchers have optimized these techniques by standardizing superovulation protocols, media recipes, oocyte/embryo handling, incubation periods and using high quality spermatozoa. In the buffalo breeding sector, sex pre-selection also holds significant economic importance. To accomplish this, the sorting of sperm bearing X and Y chromosome shows a large potential. Flow-cytometry is the technique to sort X and Y chromosome-bearing sperm (based on DNA content), achieving an accuracy rate of more than 90%. Moreover, scientists have optimized cost effective and doable sperm sexing techniques, including modified swim-up and magnetic nanoparticles with 70% and 90% accuracy, respectively based on Real-Time PCR outcomes. In conclusion, the ongoing developments in breeding technologies for water buffalo are promising and aimed at overcoming the reproductive challenges.

PLENARY LECTURE-6**STATUS OF BIODIVERSITY AND MANAGEMENT PRESCRIPTIONS FOR GHAMOT NATIONAL PARK, DISTRICT NEELUM, AZAD JAMMU AND KASHMIR**

Muhammad Siddique*, Muhammad Jahangeer and Riaz Aziz Minhas
Department of Zoology, University of Azad Jammu and Kashmir Muzaffarabad,
Azad Jammu and Kashmir

Corresponding Author: siddique.awan@ajku.edu.pk

The present research aims to explore the socio-economic baseline, extent of biodiversity, management implications, and factors and issues that pose threats to the natural resources in the Ghamot National Park (GNP) during 2018-21. The study area extended over 27,271 ha, located a sub-valley of district Neelum and falls in the inner Himalayas, its altitude ranging from 2439–4949 m above sea level. The study area dispersed in 8 allied villages with a total population of 6,131 that comprised of 734 households having an average household size of 8.13 individuals. Rapid assessment prioritization of protected area management (RAPPAM) method, Participatory Rural Appraisals survey (PRAS), and a structured self-administrated questionnaires (n=980) were used to investigate study parameters. Floral and faunal attributes were studied using quadrat, point count and line transects methods. A total of 425 quadrates along 76 lines transects were laid that accounted for a total of 489 plant species belonging to 77 families representing trees (n=9; 1.84%), shrubs (n=32, 6.54%), and herbs (n= 448, 91.61%). Plant's families with maximum number of species were Asteraceae (n=46, 9%) followed by Poaceae (n=32, 7%), and Ranunculaceae (n=29, 6%), collectively contributed 37% of the local flora of study area. A total of 18 plant communities were studied from all localities of the study area. Phytosociological investigation revealed that the dominance plant species (based on average importance value index) were *Juniperus communis* (IVI=4.99), *Abies pindrow* (IVI=4.40), and *Salix flabellaris* (IVI=3.70). Faunal diversity was recorded using line transect (n=490) and point count (n=240) confirmed the presence of 201 species belonging to 74 families. Major animal groups were recorded as fish (n=5), amphibians (n=4), reptiles (n=11), butterflies (n=16), small mammals (n=14), large mammals (n=14) and birds (n=127).

Despite protected status of the study area, several issues and threats to its resources were recorded including deforestation, grazing and fodder collection (high intensity), fuel wood collection (19849.62 tons during 2020-21), timber collection (n=346 mature tree, during 2020-21), illegal extraction of medicinal plant, human-wildlife conflict (n=597 livestock losses during 2018-2021), illegal hunting and poaching of wildlife species (n= 114 reported during 2018-21), lack of community involvement in conservation, public

education and awareness, lack of management (insufficient staff), infrastructure, encroachment, and unregulated tourism. Major management prescriptions for addressing such threats include increasing community engagement in conservation approaches, providing livelihood opportunities, enhancing monitoring and management, reducing anthropogenic disturbance and pressure, zonation of the study area, enhancing institutional staff and financial sustainability of the wildlife and fisheries department of AJ&K. It is concluded that allied communities mostly relying on natural resources and are a deep level of dependence on the forest resources. Unemployment, insufficient basic necessities for life and remoteness of area are major causes of their dependence on natural resources. Present study offers an understanding of floral and faunal diversity in Western Himalayan ecosystems, particular to species composition, community structure, diversity, ecosystem services and sustainability. This provides valuable baseline information for managers of the park to make effective conservation decisions and for researchers wishing to conduct related ecological studies. In addition to tightening law enforcement activities to reduce the current human and livestock encroachment into the park, studies on the population structure and spatiotemporal habitat use, and the impacts of human-induced actions on the diversity of the park are needed to assist management plan formulation.

PLENARY LECTURE-7**DETERIORATION IMPACTS OF INDIAN CRESTED PORCUPINE,
HYSTRIX INDICA ON FOREST PLANTATIONS OF PAKISTAN****Abdul Aziz Khan***Pakistan Agricultural Research Council, Islamabad*

Corresponding Author: pestwatch360@yahoo.com

The Indian crested porcupine, *Hystrix indica*, is a generalist forager that exploits a wide variety of plant material. In the 'Forest Management Plans of Pakistan', it has been identified as a serious pest of silvicultural importance. Complete or partial debarkation of tree trunk by porcupine reduces radial growth and promotes stunted growth and is exposed to fungal diseases and insect's attack. Various studies conducted in Pakistan have indicated that the most important porcupine damage is inflicted in the man-made irrigated forest plantations in the Punjab and the coniferous forests in AJ&K and the Northern areas of Pakistan. As early as 1967, porcupine damage was estimated in Changa Manga plantation which revealed that about 15% of the trees in the plantation was damaged resulting into loss of increment amounting to 3853 cubic meter of wood. In another study, eight irrigated forest plantations located in Central and Southern regions of the Punjab, were sampled to assess the tree and nursery stock damage. The incidence of damage to *Morus alba*, *Dalbergia sissoo* and *Eucalyptus camaldulensis* averaged 9.36, 10.82 and 8.0%, respectively. On an average plant nurseries of *D. sissoo*, *M. alba* and *Bombax ceiba* received 9.0, 14.97 and 27.05% damage, respectively. In Chichawatni plantation, *Melia azedarach* has disappeared due to porcupine damage. Damage to *Azadirachta indica* in the rangelands of Sindh and 5-28% damage to wild pistachio (*Pistacia Khinjuk*) has been reported in Baluchistan. The results of these studies and damage information gathered from the scattered reports of Forest Departments suggest that the most susceptible tree species to porcupine damage in irrigated plantations of Punjab is *M. azedarach* followed by *M. alba* and *D. sissoo*. Severe stocking damage of 60% to *Pinus roxburghii* and 42% to *Robinia pseudoacacia* has been recorded in different reforestation areas of Tarbela Watershed Management Project. Throughout Pakistan, so far, thirty-four species of trees have been reported being damaged by *H. indica*. Economic losses due to porcupine damage in irrigated plantations of Punjab and natural forests in KPK have been estimated of about US\$ 60-70 per hectare. The accuracy of these economic losses could be much improved by more exact studies of the damage and its relation to the actual and potential value of timber extracted at different stages in the forest management cycle.

PLENARY LECTURE-8**INFLUENCES OF DIFFERENT FRUIT PEELS AND SPICES ON THE EFFICACY OF ANTIBIOTICS AND ANTIDIABETIC DRUGS: IN VITRO APPROACHES****Javed Iqbal Qazi***Institute of Zoology, University of the Punjab, Lahore*

Corresponding Author: qazi.zool@pu.edu.pk

Humans feedbacks of medicinal drugs must not be ignored. Rather such information be employed to dig out the possible scientific phenomena for further research and development. To answer that how our foods or their different ingredient hereof affect efficiencies antibiotics, a direct approach has been visualized in this study. Likewise, effects of antidiabetic drugs and some spices on the process of starch digestion have elucidated by establishing simulated gastrointestinal conditions. The experimental designs reported here are useful for further investigations to regulate more effective applications of antibiotics and antidiabetic medicines.

Abstracts

SECTION - I

CELL BIOLOGY, MOLECULAR BIOLOGY, GENETICS, PHYSIOLOGY, TOXICOLOGY, VIROLOGY

- 1. HERBAL MEDICINE, BIOCHEMISTRY, AND
BIOTECHNOLOGY**
- 2. CELL AND MOLECULAR BIOLOGY, CELL BIOLOGY,
GENETICS**
- 3. HUMAN AND ANIMAL DISEASES**
- 4. MICROBIOLOGY**
- 5. PHYSIOLOGY**
- 6. TOXICOLOGY**
- 7. VIROLOGY**
- 8. CANCER BIOLOGY**

1. HERBAL MEDICINE, BIOCHEMISTRY, BIOTECHNOLOGY AND BIOINFORMATICS

EVALUATION OF *SOLANUM NIGRUM* EXTRACTS FOR ANTIDIABETIC ACTIVITY AGAINST ALLOXAN INDUCED DIABETIC MICE

Sharafat Khan¹, Imran Khan², Dua Saleem² and Munawar Saleem Ahmad^{1*}

¹Department of Zoology, University of Swabi

²Department of Pharmacy, University of Swabi

*Corresponding Author: saleemsbs@gmail.com, saleemsbs@uoswabi.edu.pk

Diabetes mellitus (DM) is a universal endocrine disorder. Hypoglycemic from natural and synthetic sources are available to care for diabetes. The aim of this study was to induce experimental diabetes mellitus using alloxan monohydrate in common adult Swiss albino mice and to confirm the antidiabetic activity of changes in body weight, food and water consumption, urine volume and glucose level between common and diabetic mice to investigate. *Solanum nigrum* has been shown to be helpful in treating diabetes. The result of methanol and aqueous extract from *Solanum nigrum* leaves was examined under common conditions of glucose loading and alloxan monohydrate-induced diabetic mice. The polyherbal composition shows significant hypoglycemic activity.

ESTIMATION OF BIOCHEMICAL AND PHYTOCHEMICAL COMPOUNDS IN SOME SELECTED NATIVE PLANTS OF DISTRICT UMERKOT

Muzna Paras and Naseem Aslam Channa

Department of Biochemistry, University of Sindh

*Corresponding Author: muznaparas86@gmail.com

Plants have been used for medicinal purposes long before prehistoric period. The native plants of Sindh Thar region have not been studied properly for chemical analysis rather than a few physical, and some studies in India and Baluchistan. The Umerkot district of Sindh owns the wide variety of very important medicinal plants. Four medicinal plants included *Tecomellaundulta* (**rohida**), *Cenchrus Setigerus*(**sogobhurt**), *Artiplex* (**saltbush**), *Cymbopogonjuwarcusa* (**khavi**)were collected from PARC ARID Zone Research Centre Umerkot Sindh, and were identified by their scientific officer (Botanist).All medicinal plants were washed, dried, grinded, and extracted with ethanol. All plant extracts were quantitatively analyzed for phytochemicals and biochemical components included phenolics, tannins, alkaloids, flavonoids, carbohydrates and proteins using double beam spectrophotometer at Institute of Biochemistry, university of Sindh, Jamshoro. We found significantly increased concentration of phytochemicals & biochemical compounds in ethanol and mixed extracts. Significantly increased concentration of tannins and flavonoids were found in stem and leaves of *Cymbopogonjuwarcusa* and *Tecomellaundulta*, followed by flower, seeds and roots. We investigated that in vitro mixed extract and ethanol displayed with maximum phytochemicals & biochemical compounds, whereas, roots showed least concentration among all. Highest concentration of tannins was attained in stem of *Cymbopogonjuwarcusa* and leaves of *Tecomellaundulta*. Highest concentration of carbohydrates was found in *Artiplex*, followed by *Cenchrus setigerus*. Phenolic compounds were found significantly increased concentration in *Artiplex*. Proteins were found significantly increased in leaves and stem of *Tecomellaundulta*. We found highest

concentration of Calcium in leaves of *Cymbopogon J.* and iron. In conclusion, highest concentration of phytochemicals is found in ethanol extract of stem and leaves in *Tecomella undulata*, *Artiplex* and *Cymbopogon juwarcusa*

INVESTIGATING THE WOUND HEALING PROPERTIES OF *PSIDIUM GUAJAVA* AND *OCIMUM SANCTUM* CONJUGATED SILVER NANOPARTICLES IN DIABETIC MICE

Shaukat Ali*, Aqsa Bibi, Muhammad Summer and Hafiz Muhammad Tahir

*Applied Entomology and Medical Toxicology Laboratory, Department of Zoology,
Government College University, Lahore*

*Corresponding Author: dr.shaukatali@gcu.edu.pk

Natural products have been used for centuries in treating various ailments, including wound healing. In the current study, alloxan monohydrate was used to induce diabetes in Swiss albino mice. Subsequently, excision wounds were created using a 6 mm biopsy puncture. Diabetic wounds were treated with 5% *Psidium guajava* (PG) and 7% *Ocimum sanctum* (OS) leaf extract individually and in combinations (5% PG + 7% OS) along with their nanoparticles individually (PGNP and OSNP) and combined form (PGNP+OSNP). Parameters like wound contraction, healing duration, and histological analysis were evaluated. Serum levels of MMPs (MMP2, MMP7, MMP9), pro-inflammatory cytokines (TNF- α , IL-6, IL-8), and TIMPs were analyzed. PGNP+OSNP displayed the most favorable outcome, achieving complete wound healing in 12 days with 100.0 \pm 0.0% contraction. %. In contrast, wounds in the positive control (polyfax) and diabetic control (saline) groups showed contractions of 96.3 \pm 1.5% and 95.8 \pm 1.4%, respectively, by the 16th and 18th day. Histological analysis highlighted increased keratinocytes, fibroblasts, collagen fiber growth, blood vessels, and reduced inflammation in the PGNP+OSNP group. The combination also normalized disrupted blood serum biomarkers. The combination (PGNP+OSNP) significantly reduced serum levels of matrix metalloproteinases, including MMP2 (284.4 \pm 5.1pg/ml), MMP7 (268.0 \pm 3.4pg/ml), and MMP9 (180.8 \pm 7.1pg/ml), compared to the Diabetic Control group (MMP2=591.0 \pm 11.9pg/ml, MMP7=508.8 \pm 6.9pg/ml, MMP9=415.6 \pm 5.1pg/ml) (P<0.001). The serum level of pro-inflammatory cytokines i.e., TNF- α (20.0 \pm 1.1pg/ml), IL-6 (14.4 \pm 0.7pg/ml), and IL-8 (26.2 \pm 1.0pg/ml) in the same group were significantly lower than those in the Diabetic Control group (TNF- α =55.0 \pm 3.0pg/ml, IL-6=39.8 \pm 1.6pg/ml, IL-8=70.8 \pm 2.8pg/ml) (P<0.001). The serum level of TIMPs (209.6 \pm 8.4pg/ml) in this combination group considerably increased compared to the diabetic control (74.2 \pm 5.0pg/ml) (P<0.001). In conclusion, these nanoparticles synergistically enhance healing potential, offering a promising therapeutic approach for chronic wound healing in non-diabetic and diabetic individuals.

AVIAN HEALTH INVESTIGATIONS THROUGH HISTOLOGICAL AND HEMATOLOGICAL PROGRESSIONS IN HUMAN MODIFIED HABITATS

Gaitee Sahar Sharif¹, Zulfiqar Ali^{1*}, Rida Ahmad^{1,2}, Usman Ahmad³ and Komel Ahmed¹

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

A study conducted in the Punjab region of Pakistan aimed to assess the health status of wild birds and the impact of environmental pollutants on their well-being. The focus was on ten bird species from three

different study sites: farmlands in Kasur, Shahdara, and traffic-congested areas in Lahore. The research, conducted between May and September 2019, utilized various parameters to determine bird health, including behavior, body condition scores, physical parameters, and differential leukocyte count. Heavy metal concentrations, specifically Zinc, Nickel, Chromium, Cobalt, Manganese, and Copper, were analyzed in feathers, lungs, liver, muscle, brain, and kidney tissues. The study also performed histopathology on lung and liver tissues using Hematoxylin and Eosin. The findings revealed concerning trends, with a significant proportion of birds being underweight—65% in Kasur, 80% in Shahdara, and 70% in Lahore. Chromium concentration, the highest among the heavy metals studied, showed varying levels across the three locations, with Shahdara exhibiting the highest levels. Granivorous birds, particularly Little Brown Doves from Kasur, displayed elevated chromium concentrations in the lungs. Differential leukocyte analysis highlighted variations among bird species, with Kites and Egrets having higher heterophil percentages, while Red Vented Bulbuls exhibited the highest lymphocyte percentages. The Kites also had the highest H/L ratio, indicative of stress. Crows, Kites, Pigeons, and Common Mynas demonstrated greater resilience to stress conditions compared to House Sparrows, Little Brown Doves, Bank Mynas, and Red Vented Bulbuls. Despite outward appearances of health, histopathological analysis of lung and liver tissues revealed anomalies, with birds from Shahdara exhibiting the poorest health, followed by Lahore and Kasur. The study emphasized the need for ongoing monitoring due to the potential long-term effects of environmental pollutants on bird populations in the region.

HEMATOLOGICAL CHANGES AND EFFECTS OF TYPHOID FEVER ON CBC IN THE AREA OF NORTH KARACHI TOWN

**Saboor Rathore¹, Ambreen Akram¹, Rubina Mushtaq¹, Sobia Khwaja¹,
Aasia Karim² and Shagufta Riaz³**

¹Department of Zoology, Federal Urdu University of Arts Science and Technology, Karachi

²Department of Zoology, Sardar Bahadur Khan Women University, Quetta

³Department of Zoology, University of Karachi, Karachi

*Corresponding Author: ambadn@hotmail.com

Salmonella typhi is the cause of enteric fever and typhoid disease. Because of the lack of drinkable water and low standards of cleanliness, it is mostly a disease seen in poorer countries. The infection's most noticeable symptom is a temperature that progressively increases to a high plateau. Typhoid fever is becoming more common, and this trend is linked to a number of hematological characteristics. This study was carried out to determine the changes in various hematological parameters in typhoid individuals. Samples were obtained from volunteer subjects visiting Sindh govt. hospital north Karachi town. 104 typhoid positive samples were collected from subjects and comparison were made between experimental and control group. Widal test was carried out as a confirmatory test for typhoid fever and evaluation of the hematological parameters were performed. The hematological parameters considered includes hemoglobin (HB), hematocrit, (MCV), MCH, MCHC, RBC, Platelet count, WBC, Neutrophils, Lymphocytes, Eosinophils and Monocyte. The study includes their correlation with each other and effect of each parameter in both male and female typhoid fever patients. The result showed significant changes in number of different parameters such as decrease in Hemoglobin (HB), increase in number of Mean cell volume (MCV), increased number of lymphocytes, Eosinophil were normal but Monocyte were observed less than normal values. Furthermore, the study also includes findings of positive and negative correlation between these parameters showing their effects on each other as well. Typhoid fever has significant effect on some hematological parameters studied. These changes could be useful in the diagnosis of typhoid fever.

SUB-LETHAL EFFECTS OF LEAD ON CATALASE, PEROXIDASE, SUPEROXIDE DISMUTASE AND LIVER HISTOLOGY OF TILAPIA

Rabbiya Sehar^{1*}, Huma Naz¹, Tanveer Ahmed², Syed Qaswar Ali Shah¹, Sajid Abdullah³, Muhammad Umar Ijaz¹, Muhammad Usman⁴, and Murtaza Manzoor¹

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

³Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

⁴Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: rabbiyayousaf1@gmail.com, dr.humanaz98@gmail.com

The study was conducted to assess the sub-lethal effects of lead (Pb) on antioxidant enzymes viz. catalase (CAT), peroxidase (POx), and superoxide dismutase (SOD) and liver histology of tilapia (*Oreochromis niloticus*) for 15 days trial. The 1/3rd LC₅₀ of Pb was (25.891mg/L) used to exposed fish for 15 days. The analysis of results expressed that CAT, POx, and SOD activities were elevated in Pb-exposed fish. Histology of liver revealed changes in Pb-treated *O. niloticus* in terms of histo-morphological parameters viz. hepatocytes diameter, width of sinusoids and diameter of central vein. Hepatocytes diameter of Pb treated *O. niloticus* was significantly less and sinusoids width of Pb treated *O. niloticus* was significantly greater as compared to control. The central veins of Pb treated *O. niloticus* were congested than control *O. niloticus*. Some other liver histological disorders like hemorrhage, eccentrically situated nuclei, vacuolation, irregular shaped hepatocytes (loss of their polygonal shape), enucleated and necrosis were also seen in liver of Pb treated *O. niloticus* when compared with control.

ASSESSMENT OF OXIDATIVE STRESS AND HISTOLOGICAL PARAMETERS OF CR+CD EXPOSED LABEO ROHITA

Raisa Bano^{1*}, Huma Naz¹, Tanveer Ahmed², Sajid Abdullah³, Muhammad Usman⁴, Adnan Ahmed Qazi¹, Muhammad Adeel Hassan⁵, Iqra Zulfiqar¹ and Murtaza Manzoor¹

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

³Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

⁴Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

⁵Department of Parasitology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: raisabano13@gmail.com, dr.humanaz98@gmail.com

The study was planned to evaluate the effects of chromium (Cr) and cadmium (Cd) mixture on antioxidant enzymes and histology of *Labeo rohita*. Fish was exposed to 1/3rd of 96-hr LC₅₀ (6.22 mg/L) of Cr+Cd mixture for 20 days. After trial, fish was dissected and organs viz. gills, brain, heart, liver, kidney and muscles were isolated to check the antioxidant enzymes viz. catalase, peroxidase and superoxide dismutase. The activities of antioxidant enzymes viz. CAT, POx and SOD were significantly increased (p<0.05) in all organs of the fish treated with Cr+Cd mixture. Results showed that the fish exposed to Cr+Cd mixture revealed significant histological alterations in muscles and liver. In muscular tissues of treated fish, atrophy and degeneration of the muscle fibers, as well as a reduction in the diameter of the muscle fiber and muscle fascicle, were observed. In liver tissues many of the histological alterations were observed such as; diameter of hepatocytes in Cr+Cd mixture treated fish groups was significantly less than control fish group. Moreover,

width of sinusoids in Cr+Cd treated fish was significantly higher than control. Some other histological alterations were also observed in liver histology such as irregular shaped hepatocytes (loss of their polygonal shape), enucleated, eccentrically situated nuclei, vacuolation and necrosis.

ASSESSMENT OF HEMATO-BIOCHEMICAL AND GENOTOXIC EFFECTS INDUCED BY PANADOL EXTRA IN COMMON CARP (*CYPRINUS CARPIO*)

Sardar Maria Arshad*, Muhammad Fiaz Khan and Shehzad Ghayyur

Department of Zoology, Hazara University, Mansehra

Corresponding Author: mariaarshad549@gmail.com

The increasing presence of pharmaceutical residues in aquatic ecosystems poses a significant threat to non-target species, necessitating comprehensive investigations into potential sublethal impacts. This research focused on assessing the hemato-biochemical and genotoxic effects induced by Panadol Extra, a widely used analgesic, on freshwater fish *Cyprinus carpio*. The study employed a 96-hour exposure experiment with four different concentrations of Panadol Extra (0.5mg/L, 1mg/L, 1.5mg/L, and 2mg/L) alongside a control group. Hematological analysis revealed a significant ($p < 0.05$) decline in white blood cell (WBC) and platelet levels at 1.5mg/L and 2mg/L concentrations, while red blood cell (RBC) count decreased at all treated concentrations. Hemoglobin levels significantly ($p < 0.05$) declined at higher doses (1mg/L, 1.5mg/L, and 2mg/L). Hematological indices such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) did not show significant differences, indicating selective impacts on certain parameters. Serum biochemical analysis demonstrated significant ($p < 0.05$) reduction in total protein at higher concentrations (1.5mg/L and 2mg/L), while triglycerides declined at all treated concentrations. Glucose, urea, iron, and sodium levels increased significantly ($p < 0.05$) while calcium and potassium levels decreased at 1mg/L, 1.5mg/L, and 2mg/L. Genotoxicity assessment through the micronucleus test revealed a significant ($p < 0.05$) micronucleus index at higher concentrations (1.5mg/L and 2mg/L), which shows that this drug has a high degree of toxicity. This study highlights the potential adverse effects of Panadol Extra on *C. carpio* emphasizing the importance of monitoring pharmaceutical residues in aquatic environments and understanding their impacts on non-target species. The findings contribute to the broader understanding of the ecological consequences of pharmaceutical pollution in freshwater ecosystems.

BEHAVIORAL AND HEMATOLOGICAL STUDY OF THREE VARIETIES OF COTURNIX COTURNIX JAPONICA SELECTED FOR HIGH BODY WEIGHT AND EGG

Khushbu Fatima* Irfan Baboo, Anwar Khan and Kiran Ashiq

Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

Corresponding Author: khushiif4@gmail.com

This study was designed to assess several behavioral characteristics of Japanese quail, reared in the cages at normal environmental conditions. The behavioral inspections for 180 quails were tossed when the quails were 16-week-old and continued for 2 months. Birds were divided into 15 experimental units having 4 birds (3 female and 1 male). Feeding (F), drinking (D), activation (A), sitting / lying (S/L), standing (S), feather pecking (FP) and wing flapping (WF) were noticed twice a day in alternate days of a week, in the morning 9 to 12pm and in evening 5 to 8pm. The behavioral traits were observed once in every 5min during a 3h observation period by time-sampling method. The weight of the birds was checked before and after the experiment. The results showed that Japanese quails displayed high feeding, drinking, activation and wing

flapping behaviors, whereas; sitting / lying, standing and pecking behaviors befell at rather low levels. Hematological parameters (hemoglobin, WBC, RBC, HCT, MCV, MCH, MCHC, platelets, neutrophils, lymphocytes, heterophils, H/L) were evaluated at the time of slaughtering. Regarding behavioral traits, the maximum feeding was found in body weight line. Drinking, wing flapping and activation were found highly significant in egg number line with (P0.05). However, in hematological parameters hemoglobin, WBC, HCT / PCV were statistically least significant with (P0.05). Diurnal behaviors did not show any differences. According to the results, birds were comfortable with the intensive system.

DIAGNOSTIC EFFICACY OF HAND-HELD DIGITAL REFRACTOMETER FOR DETERMINING TOTAL SERUM PROTEIN IN INDIGENOUS SHEEP OF PAKISTAN

**Madiha Sharif^{1*}, Mushtaq Hussain Lashari¹, Umer Farooq²,
Musadiq Idris² and Muhammad Abrar Afzal²**

¹*Department of Zoology, The Islamia University of Bahawalpur*

²*Department of Physiology, The Islamia University of Bahawalpur*

*Corresponding Author: monasharif009@gmail.com

The study was designed to ascertain the diagnostic efficacy of hand-held digital refractometer in determining total protein. The Sipli sheep (n=141) were grouped as per gender (females=99, males=29) and age (G1=up till 1 year, G2=from 1 to 2 years, G3=above 2 years). The results regarding the overall mean (\pm SE) values and RIs for the TPs attained through serum chemistry analyzer (TP1) and hand-held digital refractometer (TP2) were non-significantly ($P \leq 0.05$) different (59.2 ± 1.6 g/L and 59.8 ± 0.5 g/L, respectively). However, the RIs were quite different between the two TPs being 45.1-95.7g/L and 57.0-67.0g/L for TP1 and TP2, respectively. Similar results were seen for gender-wise and group-wise results. On the contrary, the results regarding correlation coefficient and logilinear regression showed a negative correlation between the two TPs ($r = -0.0244$) with an adjusted r-square of 0.059 (5.9% probability). Furthermore, the results for Cronbach alpha and intraclass correlation coefficient between TP1 and TP2 showed that the values for single measure and average values were lower between TP1 and TP2 being -0.135 and -0.313. Bland and Altman test between TP1 and TP2 also showed a weak level of agreement between the two methods of detecting TP. A proportional bias on the distribution of data around the mean difference line was noticed between TP1 and TP2 (Mean= 0.5; 95% CI= 39.8 to -40.9) with a standard deviation of biasness being 20.58. In a nutshell, the hand-held digital refractometer cannot be used as an on-farm POCT device for determining serum TP in sheep. However, certain other models of refractometers with higher sensitivity and specificity may be utilized in future studies to establish these conclusions for other species of livestock.

FIRST REPORT ON CLINICAL FEASIBILITY OF DRIED BLOOD SPOT TECHNIQUE FOR HEMOGLOBIN ESTIMATION IN CHOLISTANI CATTLE

**Maryam Chaudhary^{1*}, Umer Farooq², Musadiq Idris², Mushtaq Hussain Lashari¹,
Saira Qasim¹, Muhammad Abrar Afzal², Musarrat Abbas Khan³ and Asad Ali²**

¹*Department of Zoology, The Islamia University of Bahawalpur*

²*Department of Physiology, The Islamia University of Bahawalpur*

³*Department of Animal Breeding and Genetics, The Islamia University of Bahawalpur*

*Corresponding Author: mrymch222@gmail.com

The present study is the first of its kind being reported for Hb estimation in Cholistani cattle (n=63) blood through dried blood spot (DBS) technique using filter paper. Three methods of Hb estimation were

implied viz. through veterinary hematology analyzer (HbA), and two indirect cyanmethemoglobin methods (HbIC and HbICX) using measured and unmeasured blood drops on the filter paper, respectively. Results for overall as well as for group-wise data (gender and sex-based) revealed that HbA and HbIC were non-significantly ($P \geq 0.05$) different from each other, being different from HbICX ($P \leq 0.05$). The HbICX gave overestimated values of Hb as compared to HbA and HbIC. The sensitivity, specificity, positive predictive value, and negative predictive value for HbIC were higher being 86.1%, 88.3%, 88.0%, and 86.0%, respectively as compared to the lower values of 45.0%, 12.0%, 12.0% and 45.0%, for HbICX. A better level of agreement was noticed between HbA and HbIC through Bland Altman test with no proportional bias on the distribution of data around the mean difference line (Mean = -0.16, 95% CI = 0.34 to -0.67). Similarly, for Cronbach alpha and intraclass correlation coefficient between HbA and HbIC, both the values for single measure and average values were higher between HbA and HbIC being 0.703 and 0.825 as compared to the values of 0.200 and 0.333 between HbA and HbICX. It is concluded that the indirect cyanmethemoglobin method for Hb estimation is reliable and accurate for cattle blood, if a measured quantity of blood drop is taken on a filter paper. We recommend this DBS technique for Hb estimation in cattle blood for resource-poor settings and for livestock herds being reared distantly from the laboratories. For future, it is recommended that DBS technique with various other modifications and for other hematochemical biomarkers may be validated for livestock blood.

VERMITECHNOLOGIES (VERMICOMPOSTING AND VERMIREMEDIATION): PROMISING AND SUSTAINABLE TECHNOLOGIES TO IMPROVE SOIL HEALTH

Saiqa Andleeb*

*Biotechnology and Vermi-technology Laboratory, Department of Zoology,
University of Azad Jammu and Kashmir, King Abdullah Campus, Muzaffarabad*

*Corresponding Author: drsaiqa@ajku.edu.pk; drsaiqa@gmail.com

Earthworms are probably the most important component of soil biodiversity. They are highly beneficial to agriculture and are soil-dwelling invertebrates. Sufficient knowledge is lacking on the indigenous fauna of worms concerning vermicomposting and vermiremediation in Azad Jammu and Kashmir, Pakistan. So, the aim of current research is the production and utilization of earthworm species for the management of solid waste and the remediation of heavy metals from the contaminated soil to improve soil health. In the current study, *Eisenia fetida* were used for vermicomposting and vermiremediation processes. Naturally contaminated soil samples were collected from various localities of Muzaffarabad, Azad Kashmir, Pakistan and heavy metals (lead, cadmium, chromium) were detected through atomic absorption spectrometry. Vermiremediation results revealed that heavy metals in natural soil were not lethal and the mortality rate was 0 % throughout the experimentation. The maximum and minimum fecundity was recorded in sample H and D ($93.33 \pm 0.04\%$ and $76.19 \pm 0.68\%$). The maximum growth rate (15.89 ± 0.11 g) and body weight ($99.87 \pm 0.07\%$) were recorded in garden soil. The lowest body weight was recorded in sample D and the lowest growth weight (3.95 ± 0.06 g) was recorded in sample E ($25.41 \pm 0.02\%$). Atomic absorption spectrometry (AAS) results revealed that *E. fetida* significantly ($P < 0.000$) and efficiently remediate the heavy metals from the naturally contaminated soil. The order of heavy metals accumulation by *E. fetida* was recorded as $\text{Cr} > \text{Cd} > \text{Pb}$ and the linear trendlines for bioaccumulation of heavy metals via *E. fetida* are recorded as Cd ($R^2 = 3\text{E-}06$, $P < 0.001$), Cr ($R^2 = 0.0282$, $P < 0.001$), and Pb ($R^2 = 0.0004$, $P < 0.001$). Vermicompost and gut of *E. fetida* rich in beneficial bacteria which involved in the vermiremediation and vermicomposting. Vermibacteria i.e. *Bacillus thuringiensis*, *Bacillus aryabhatai*, *Staphylococcus hominis*, *Bacillus toyonensis*, *Bacillus cabrialesii*, *Bacillus tequilensis*, *Bacillus mojavensis*, *Bacillus amyloliquefaciens*, *Bacillus toyonensis*, *Bacillus anthracis*, and

Bacillus paranthracis associated with gut of *E. fetida* not only involved in the remediation of heavy metals but also used as plant growth promoting bacteria. These bacteria produce all plant growth promoting traits such as indole acetic acid (IAA), amylase, protease, lipase, hydrogen cyanide, ammonia, and siderophore production, and also act as a phosphate solubilizers. Therefore, the current findings showed that vermitechnologies i.e. vermiremediation, vermicomposting, and vermiculture could be used for the improvement of soil health.

BIODEGRADATION OF POLYVINYL CHLORIDE USING VERMIBACTERIA UNDER VARIOUS PHYSICOCHEMICAL PARAMETERS

**Muqaddas Munir¹, Saiqa Andleeb^{1*}, Muhammad Ishtiaq Ali², Kaleem Imdad³ and
Muhammad Siraj ul Haq⁴**

¹Microbial Biotechnology lab, Department of Zoology, University of Azad Jammu and Kashmir,
King Abdullah Campus, Chattar Kalas, Muzaffarabad

²Department of Microbiology, Quaid-i-Azam University, Islamabad

³Bioscience Department, COMSATS University, Park Road, Chak Shahzad, Islamabad

⁴Department of Chemistry, University of Science & Technology, Bannu

*Corresponding Author: drsaiqa@gmail.com; drsaiqa@ajku.edu.pk

Plastic is hazardous to the environment. The problem of plastic's impact to the environment could be resolved by allowing it to degrade. Biodegradation of plastic wastes by using microorganisms is an environmental-friendly method. In the current study, vermibacterial strains i.e *Bacillus mycoides*, *Bacillus megaterium*, *Staphylococcus hominis*, *Bacillus licheniformis*, *Bacillus mojavensis*, *Bacillus toyonensis*, *Bacillus anthracis*, *Bacillus paranthracis*, were selected to evaluate the degrading impact on polyvinyl chloride (PVC) and tested under various physicochemical conditions such as pH (7, 5, 9), temperature (37 and 50 degree), carbon sources (Glucose and sucrose), and nitrogen sources (yeast extract and peptone). Liquid culture technique was used to investigate the chloride production and solid media experiment was employed for biodegradation of polyvinylchloride films. After primary screening via liquid culture technique at pH (7, 5, 9), temperature (37 and 50 degree) without carbon and nitrogen sources, the strains which showed credible degradation were selected for further studies. Different parameters including pH, temperature, carbon and nitrogen sources were evaluated. *Bacillus mojavensis* showed maximum chloride production 0.79 ± 0.779 at pH 5 and 37 degree in the absence of carbon and nitrogen sources and maximum chloride production 2.231 ± 0.019 at pH 7 and 50 degree in the presence of sucrose and peptone after 5 days of incubation while *Bacillus anthracis* showed 1.923 ± 0.02 chloride production at pH 9 and 50 degree in the presence of sucrose and peptone. Scanning electron microscopy was used to check the morphological changes of the PVC-film after vermibacteria treatments and results revealed that vermibacteria attached with PVC films and validated the changes in surface topography. Similarly, Fourier-transform infrared spectroscopy revealed the changes in functional group intensity on both vermibacteria treated PVC film and control. It was concluded that, vermibacteria could be used as potential candidates for plastic biodegradation.

ORGANIC MANURE EFFICACY ON VERMIREMEDIATION AND PHYTOREMEDIATION OF HEAVY METALS

**Anum Naseer¹, Saiqa Andleeb^{1*}, Abdul Basit¹, Shaukat Ali², Nazish Mazhar Ali²,
Iram Liaqat², Aisha Nazir³**

¹*Biotechnology and Vermi-technology Laboratory, Department of Zoology,
University of Azad Jammu and Kashmir, King Abdullah Campus, Muzaffarabad*

²*Department of Zoology, GC University, Lahore*

³*Institute of Botany, University of the Punjab, Quaid-e-Azam campus, Lahore*

*Corresponding Author: drsaiqa@ajku.edu.pk; drsaiqa@gmail.com

Heavy metals contamination raised significant concerns throughout the world. The aim of current research was to remediate heavy metals i.e. cadmium, lead, and chromium from artificial contaminated soil via vermiremediation and phytoremediation, and to evaluate the impact of organic manure (cow dung and buffalo dung) on both remediation techniques. *Eisenia fetida*, *Pheretima lignicola* and *Spinacia oleracea* were used. Results revealed that *E. fetida* tolerated heavy metals concentration (Pb; 280 mg), Cd; 150 mg, and Cr; 860 mg) compared to *P. lignicola*. The growth and reproduction of *Eisenia fetida* was efficient in the cow dung manure compared to buffalo dung. Similarly, seed germination and growth of *Spinacia oleracea* was better in cow dung media compared to buffalo dung. Bioaccumulation factor showed that that *E. fetida* showed higher accumulation of heavy metals in their tissues when vermi + phytoremediation was jointly applied (9.50 ppm of Pb, 24.166 of Cd, and 6.695 of Cr). Fourier-transform infrared spectroscopy and comet assay revealed that heavy metals had no genotoxic effect on the *Eisenia fetida* and *Spinacia oleracea*. It was concluded that both *Eisenia fetida* and *Spinacia oleracea* are appropriate for heavy metals remediation in cow dung manure.

SCREENING AND CHARACTERIZATION OF CATTLE DUNG ASSOCIATED BACTERIA POSSESSING PLANT GROWTH PROMOTING TRAITS

Rimsha Riaz, Iqra Riaz, Saiqa Andleeb* and Tasleem Akhtar

¹*Microbial Bioremediation and Vermi-technology laboratory, Department of Zoology,
University of Azad Jammu & Kashmir, King Abdullah Campus, Chattar Kalass, Muzaffarabad*

²*Department of Zoology, Government College University, Faisalabad*

⁴*Department of Zoology, Hazara University, Khyber Pakhtunkhwa*

*Corresponding Author: drsaiqa@ajku.edu.pk; drsaiqa@gmail.com

The current study was aimed to isolate and characterize the cattle dung associated bacteria which could be used as microbial biofertilizers. The objectives of the current study were 1. to isolate, identify, and characterize cow manure associated bacteria using microscopic, cultural media, and biochemical tests, 2. to determine the pathogenicity of cow manure associated bacteria, 3. to screen the enzymatic analysis of cow manure associated bacteria, and 4. to screen the antibiotic resistivity among cow manure associated bacteria through antibiogram analysis. Results revealed that twenty-four bacteria were isolated from the cattle manure using nutrient agar, MacConkey agar, mannitol salt agar, and Tryptone soya agar. Staining techniques result revealed that 3C, 6C, 9C, 13C, 16C, 17C, 18C, 19C, 20C, 22C, and 23C are gram-positive bacteria and 1C, 13C, 19C, 22C, and 24C are non-endospore forming bacteria. Biochemical characterization results indicated that all cattle dung associated bacteria do not produce ureases, oxidases, and proteases. Hemolytic results revealed that all bacteria are pathogenic in nature, all bacteria produce lipases, and Methyl red test indicates that 4C, 7C, 11C, 12C, 13C, 14C, 17C, 18C, 19C, 20C, 22C, and 23C produced fermented products. All bacteria involved in ammonia production, generate hydrogen cyanide, and almost all of bacterium isolates produced Indole acetic acid (IAA).

Antibiogram analysis results showed the antibiotic resistance towards Azithromycin (ATM), Piperacillin-Tazobactam (TZP), Ampicillin (AMC), Cefixime (CXM), and Amoxicillin (AML). Norfloxacin (NOR) showed the maximum inhibition of all tested bacteria except 19c, 20c, and 21c. Resistogram analysis revealed that lead is toxic for all cattle dung associated bacteria. It was observed that lead showed the maximum inhibition (21 ± 0.0 mm to 35 ± 0.0 mm) of all tested bacteria. On the other hand, cadmium showed the maximum inhibition (20 ± 0.0 mm to 30 ± 0.0 mm) of 3c, 5c, 12c, 17c, 18c, 19c, 20c, 21c, and 22c, respectively. All tested bacteria are chromium resistant except 18C. It was concluded that cattle manure associated bacteria possessed plant growth promoting traits and resistant to various antibiotics, so they could be used as potential microbial biofertilizers to enhance crop production.

ANTAGONISTIC EFFECT OF SPIROGYRA ASSOCIATED BACTERIA AGAINST WATER-BORNE BACTERIAL PATHOGENS

Kanwal Tahir¹, Saiqa Andleeb^{1*} and Kaleem Imdad²

¹Microbial Biotechnology Lab, Department of Zoology, University of Azad Jammu and Kashmir, King Abdullah Campus, Chattar Kalas, Muzaffarabad

²Bioscience Department, COMSATS University, Park Road, Chak Shahzad, Islamabad

*Corresponding Author: drsaiqa@gmail.com; drsaiqa@ajku.edu.pk

Spirogyra are a rich source of surface associated bacteria and are potential to possess antimicrobial molecules. In the present study, 12 bacterial isolates (SAB1-SAB12) were isolated from Spirogyra, were identified via various techniques such as cultural media, staining techniques, and biochemical tests. It was observed that spirogyra associated with *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumonia*. Antagonistic activity of spirogyra associated bacteria were determined against water borne (WBB) and nosocomial bacterial pathogens via agar well diffusion method. Results revealed that the maximum bactericidal effect of SAB1 was recorded against WBB5, WBB8, and WBB10 (15.0 ± 0.0 mm, 20.0 ± 0.0 mm, 18.0 ± 0.0 mm). On the other hand, SAB1 showed maximum activity against *Klebsiella pneumonia* (15.0 ± 0.0 mm), *Methicillin Resistant Staphylococcus aureus* (13.0 ± 0.0 mm), *Staphylococcus aureus* (20.0 ± 0.0 mm), *Serratia marcescens* (23.0 ± 0.0 mm), *Escherichia coli* (15.0 ± 0.0 mm), and *Pseudomonas aeruginosa* (9.0 ± 0.0 mm). The results revealed that effective bactericidal effect against nosocomial pathogens may be due to the presence of antimicrobial compounds such as enzymes. Similarly, disc diffusion method was used for antibiogram analysis and results revealed that mostly spirogyra associated bacteria and waterborne bacteria are not antibiotic resistant. It was concluded that spirogyra associated bacteria could be potent against waterborne bacteria but could be used as potential antibacterial agent against nosocomial bacterial pathogens.

ANTIBACTERIAL POTENTIAL OF BIOGENIC SYNTHESIZED SILVER NANOPARTICLES USING AJUGA BRACTEOSA ALONG WITH ANTIBIOTICS USING VARIOUS BIOLOGICAL ASSAYS

Sadia Nazer¹, Saiqa Andleeb^{1*}, Shaukat Ali² and Abida Raza³

¹Microbial Biotechnology Lab, Department of Zoology, University of Azad Jammu and Kashmir, King Abdullah Campus, Chattar Kalas, Muzaffarabad

²Department of Zoology, Government College University, Lahore

³University Institute of Biochemistry and Biotechnology, PMAS-Arid Agriculture University Rawalpindi

*Corresponding Author: drsaiqa@ajku.edu.pk; drsaiqa@gmail.com

The aim of present study was to synthesize, characterize, and evaluate the *in vitro* antibacterial efficacy of green synthesized silver nanoparticles (ABAgNPs) and *A. bracteosa* extract (ABaqu). The

silver nanoparticles synthesis (ABAgNPs) was carried out *via* maceration and boiling methods. The characterization was done using UV-Viz spectroscopy, scanning electron microscopy, and Fourier-transform infrared spectroscopy. After synthesis, both qualitative and quantitative phytochemical screening of ABAgNPs and ABaqu were carried out. Antioxidant activity of ABAgNPs and ABaqu was measured through both ABTS^{•+} decolorization and DPPH scavenging assays. Minimum inhibitory concentrations of ABaqu extracts and silver nanoparticles ABAgNPs (0.02 g/ml, 0.04 g/ml, 0.06 g/ml, 0.08 g/ml, and 0.10 g/ml) were tested against bacterial pathogens 14 bacterial pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* (clinical samples associated), *Lactobacillus delbrueki* and *Lactobacillus curvatus* (yogurt associated), *Staphylococcus warneri* (red meat associated), *Enterobacter amnigenus*, *Salmonella typhimurium*, and *Shigella flexneri* (spoiled fish samples) *via* broth dilution method *via* agar well diffusion assay. The maximum antibacterial activity of synthesized silver nanoparticles ABAgNPs and ABaqu were observed at 0.10 mg/ml. Time kill assay, biofilm inhibition effect (crystal violet assay), cell proliferation inhibition effect (MTT assay), antibiogram analysis and synergistic effect, TLC bio-autography, and TLC spot screening was done against seven bacterial pathogens such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Lactobacillus curvatus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* which were effectively inhibited by ABAgNPs and ABaqu at 0.10 mg/ml concentration. ABAgNPs showed maximum inhibition of *Klebsiella pneumoniae* (15.0 ± 0.0 mm), *Escherichia coli* (15.0 ± 0.0 mm), *Streptococcus pyogenes* (17.0 ± 0.0 mm), *Staphylococcus aureus* (15.0 ± 0.0 mm), and *Pseudomonas aeruginosa* (2) (17.0 ± 0.0 mm) at 0.1 mg/ml concentration. Similarly, ABaqu showed maximum inhibition of *Pseudomonas aeruginosa* (2) (15.0 ± 0.0 mm) at 0.1 mg/ml. The time-kill activity showed that Gram-positive bacteria and Gram-negative bacteria were killed after 4 h and 1 h of incubation. A strong synergistic effect of chloramphenicol and kanamycin in combination with ABaqu and ABAgNPs was recorded. Biofilm inhibition assay, cell proliferation inhibition assay, TLC bio-autography, and TLC spot screening persist the results of agar well diffusion and broth dilution assays. This research demonstrated that ABAgNPs and ABaqu extract could be considered as a therapeutic drug against pathogen associated diseases.

MOLECULAR STUDIES ON SYNERGISM OF HETERODIMERIC B-GALACTOSIDASE FROM LOCALLY ISOLATED *LACTOBACILLUS ACIDOPHILUS* MR-24

Dilara Abbas Bukhari and Arif Ullah

Department of Zoology, Government College University, Lahore

*Corresponding Author: arifullah@gcu.edu.pk

The objective of the current study was to isolate and characterize bacteria producing β -galactosidase from dairy products in Lahore, Pakistan. Among the 50 isolates, the identification of *Lactobacillus* was carried out using biochemical tests, focusing on their X-gal hydrolysis ability. The fifteen colonies with blue color on X-gal plates were selected to check the highest activity. *L. acidophilus* MR-24 exhibited the most significant β -galactosidase activity (905.15 U/l) identified on 16S rRNA gene analysis. The enzyme functioned optimally at 37°C and pH 7 within 48 hrs. By using a Sephadex-G-75 column for purification, enzyme activity increased tenfold. The β -galactosidase genes lacLM (2.8kb), lacL (1887 bp), and lacM (951 bp) were isolated from *L. acidophilus* MR-24. After PCR and agarose electrophoresis, these genes were inserted into the pTZ57R cloning vector using TA cloning. Cloning authenticity was confirmed via restriction digestion and colony PCR. Sequencing revealed that the β -galactosidase gene is composed of two overlapping sections, lacL and lacM. LacL encoded a larger subunit (628 amino acids, 70 kDa), while lacM encoded a smaller subunit (316 amino acids, 35 kDa). Homology with β -galactosidases from other

lactobacilli was evident. Coexpressing lacL and lacM in *E. coli* generated active protein, with a 30-fold activity increase after purification through ion-exchange column chromatography using DEAE cellulose, compared to crude native β -galactosidase. Characterization of the purified enzymes demonstrated stability at pH 7, with limited activity beyond pH 3.0 and pH 9.0. Optimal temperatures were 60°C and 45°C for native and expressed enzymes, respectively, retaining 40% activity at 90°C. The effect of inhibitors and activators was also determined. The β -galactosidase's 3D structure exhibited three distinct domains (Domain N, Domain A, and Domain C). The expressed enzyme from *L. acidophilus* MR-24 holds potential as a probiotic source in dairy products, offering benefits to those with lactose intolerance.

SILVER-CHITOSAN BASED BIODEGRADABLE COMPOSITE AS ANTIBACTERIAL FOOD PACKAGING

Alishba Fatima and Iram Liaqat*

Microbiology Lab, Department of Zoology, Government College University, Lahore

*Corresponding Author: dr.iramliqat@gcu.edu.pk, alishbabar@12gmail.com

Plastic pollution is increasing day by day, there is a trend to develop new technologies for eco-friendly, cost effective and biodegradability plastic based packaging. Chitosan is a biopolymer with excellent biocompatibility, while silver nanoparticles (AgNPs) are known for their antibacterial activity. In present study, silver-chitosan composites were synthesised by chemical reduction using AgNO₃. The structural and mechanical properties of the composite were examined by fourier transform infrared spectroscopy (FTIR), UV-Visible spectroscopy (UV-vis). In addition, the formed composite have a broad absorption peak at near 1657cm⁻¹ and indicate the binding of Ag nanoparticles to N-H bond of chitosan observed by FTIR, while UV-vis maximum absorption peak was observed at 413nm. The potential impacts of silver-chitosan (Ag-CH) composite on food safety and control will be evaluated by determining the antimicrobial activity of the synthesized silver-chitosan (Ag-CH) composite against different bacteria isolated from food spoilage. The findings of current study will highlight the potential of Ag-CH composite considered as biodegradable and biocompatible food packaging material and an alternate option for petroleum-based plastics.

ENCAPSULATION OF LETROZOLE WITH IN MODIFIED CHITOSAN-POLY (VINYL ALCOHOL)/IRRADIATED GO BASED HYDROGEL FOR DRUG DELIVERY

Summaya Aziz and Nuzhat Shafi*

Department of Zoology, University of Azad Jammu and Kashmir, King Abdullah Campus, Muzaffarabad

*Corresponding author: nuzhatshafi@gmail.com; nuzhatshafi@ajku.edu.pk

In this study, a complete, novel hydrogel containing irradiated Graphene Oxide (GO) linked with blend of chitosan poly (vinyl alcohol) (PVA) were synthesized. GO was imposed to gamma irradiation in order to induce defects and enhance oxidation. The effect of irradiated GO (iGO) at different absorbed dose on the physio-chemical properties of CHS/PVA blend was studied and characterized by FT-IR, XRD, TGA and SEM. SEM results have confirmed the uniform distributions of graphene oxide in chitosan/PVA hydrogel. Swelling response of the hydrogel was investigated at different pH values and decreased with increase in absorbed dose. All hydrogels showed low swelling in acidic and basic pH media, whereas maximum swelling was exhibited at neutral pH, while the thermal stability increased with the increasing of the irradiation dose. Letrozole was studied as a model drug and its release profile was studied in

phosphate-buffered saline (PBS) at a pH of 7.4. The results showed hydrogel release drug in a sustained way up to 100% in 3.5 hours. This study shows iGO reinforced CS/PVA with high bioactive properties are potential candidates for injectable controlled drug delivery and other applications in biomedical fields.

EFFECT OF PROBIOTIC SUPPLEMENTED DIET ON GROWTH, ANTIOXIDANT ENZYMES AND GASTRO-SOMATIC INDEX OF *OREOCHROMIS NILOTICUS*

Sidra Zahoor^{1*}, Huma Naz¹, Tanveer Ahmed², Tariq Mahmood¹, Kamal Niaz³, Syed Qaswar Ali Shah¹, Sajid Abdullah⁴, Muhammad Umer Ijaz¹, Tahreem Asad⁵ and Murtaza Manzoor¹

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

³Department of Pharmacology and Toxicology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

⁴Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

⁵Department of Poultry Sciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: sidrazahoor372@gmail.com, dr.humanaz98@gmail.com

The aim of the study was to investigate the effects of probiotics on the growth of *Oreochromis niloticus*, as well as on the gastro somatic index, antioxidant enzymes (catalase, peroxidase, and superoxide dismutase). All the growth parameters was measured during 8 weeks of experimental period; daily weight gain (DWG), daily length gain (DLG), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF) in probiotic fed group were greater as compared to the control group. Liver, gills, kidneys, brain and muscles were extracted to examine the antioxidant enzymes activities. Antioxidant enzymes activities in fish fed probiotic was significantly greater than control fish. In comparison of control fish, fish feeding on probiotic shown more significant result on gastro somatic index (GaSI). Fish fed with probiotic also show significant effects on gut histology in term of increased in villus height (VH), crypt depth (CD), muscularis externa (ME), villus width (VW) and muscularis mucosa (MM) at 2 % concentration.

EFFECT OF CHROMIUM ON *LABEO ROHITA* GILLS AND CATALASE ACTIVITY

Tuba Mazhar^{1*}, Huma Naz¹, Tanveer Ahmed², Syed Qaswar Ali Shah¹, Sajid Abdullah³, Khalid Abbas³, Muhammad Usman⁴, Tehreem Asad⁵ and Murtaza Manzoor¹

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

³Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

⁴Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

⁵Department of Poultry Sciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: tubamazhar108@gmail.com, dr.humanaz98@gmail.com

Aquatic pollution caused by heavy metals poses a growing threat to the health of living organisms, particularly fish. The study was planned to evaluate the effects of chromium (Cr) on histology and catalase activity of *Labeo rohita*. Fish were exposed to Cr for 15 days. After trial, fish were dissected and its organs (gills, brain, heart, and muscles) were isolated to check the catalase (CAT) activity. The gills histology of Cr treated *L. rohita* revealed severe damages including necrosis, epithelium lifting,

hyperplasia, fusion in secondary lamellae and oedema as compared to control group. The Cr treated *L. rohita* showed higher CAT activity in the heart, intestine, brain and muscles as compared to control fish. In the conclusion, that chromium is a toxic heavy metal discharge via effluents into aquatic environment caused severe alterations in histology and catalase activity in *L. rohita*. The parameters can be used as a biomarker to monitor the aquatic pollution. To eliminate and avoid the aquatic life loss there is a need to minimize the heavy metals pollution.

MEDICINAL USE OF ANIMALS AND THEIR PRODUCTS IN SARGODHA, PAKISTAN

Mehwish Riaz and Sana Ashraf*

Department of Zoology, The University of Lahore, Lahore

Corresponding Author: sanaashrafdr@gmail.com

Current study was conducted to know the medicinal importance of animals and their products by the local people. Data were collected from 100 informants of rural and urban areas. Total 82 species of animals were recorded from Sargodha. Animal groups used in medicines were Annelids, mollusks, arthropods, fishes, amphibians, reptiles, birds and mammals. Commonly used animal groups were mammals and birds. Different animal's parts were used like fat, skin, meat, bone, milk and blood. The mostly used animal part was meat. Animals derived products like honey and wax were also used in medicines. Common diseases treated were sexual enhancement, paralysis, epilepsy, lockjaw, anemia, weakness, skin issues, gastric issues and respiratory problems.

2. CELL AND MOLECULAR BIOLOGY, CELL BIOLOGY, GENETICS

GENETIC IDENTIFICATION OF BAGRID CATFISH SPECIES THROUGH DNA BARCODING FROM PAKISTAN

Atiya Zarreen*

Zarin Medical Hall, Near CMH, Rawalakot, Azad Kashmir

*Corresponding Author: atiyazarinkhan@gmail.com

Bagridae is scaleless family, identified by four pairs of barbels, serrated pectoral fin spines, adipose fin and a fairly developed anal fin but there are some limitations in morphological identification. The objective of this study was to identify six bagrid catfish species, collected from Chashma barrage (*M.seenghala* and *R.rita*) and Chashma lake (*M.bleekeri*, *M.vittatus*, *M.cavasius*, *M.tengara*) constructed on the left bank of Indus River, using COI gene as a DNA barcoding marker. DNA barcoding provides automatable, precise and quick species identification by means of tiny and uniform gene regions considered as internal species tags. Partial sequences of COI gene of fish species were amplified, PCR products were sequenced and analyzed by bioinformatic software. The level of similarity of fish species was 97-100% with the respective sequence in GenBank database and 98-100% in BOLD database. The overall base composition of bagrid catfish species was 30.51% of T, 26.64% of C, 25.17% of A and 17.69% of G, A+T contents 55.7% and G+C contents 44.33%. The Ts/Tv bias (R) was 2.76. Mean inter-specific sequence divergence for bagrid species was (0.25 ± 0.02) higher as compared to intra-specific sequence divergence (0.11 ± 0.02) that is in accordance with DNA barcoding principle. It is concluded that besides fish identification, nucleotide divergence among species could be calculated through DNA barcoding.

APPLICATION OF CRISPR-CAS12A TECHNOLOGY IN ZEBRAFISH TO SIMULATE β -GLOBIN GENE (HBB) MUTATIONS

Farheen Shafique^{1,2*}, Shaukat Ali^{2,3}, Edward Blackburn¹ and Mahreen-ul-Hassan^{1,4}

¹School of Bioscience, University of Sheffield, UK

²Department of Zoology, University of Azad Jammu and Kashmir Muzaffarabad

³Department of Zoology, Government College University Lahore

⁴Department of Microbiology, Shaheed Benazir Bhutto Women University Peshawar

*Corresponding Author: farheen.shafique@ajku.edu.pk

β -thalassemia, a genetic blood disorder resulting from mutations in the β -globin gene (HBB), poses significant therapeutic challenges. CRISPR-Cas12 technology offers a promising avenue for addressing such genetic disorders. This study aimed to evaluate the effectiveness of CRISPR-Cas12a gene editing technology in introducing precise genetic modifications into the zebrafish *hbbe1.1* gene, simulating thalassemic mutations. Thalassemic mutations were replicated in zebrafish to create a suitable model for

gene editing experiments. We targeted the embryonic hemoglobin gene *hbbe1.1* at larval stages using CRISPR-Cas12a technology, along with a phosphorothioated "rescue template" (ssDNA L33P), to introduce specific base edits into the zebrafish DNA sequence. These experiments successfully induced a single amino acid alteration (codon 33, CTG leucine to CCA proline ATC) at the protein level. Through this genetic editing method, we generated a novel zebrafish strain with specific amino acid alterations resembling pathogenic mutations seen in HBB for β -thalassemia. Notably, we observed the prevalence of MMEJ (Microhomology-Mediated End Joining)-mediated knock-in events, suggesting their role in germline inheritance during zebrafish gene editing. The findings have broader implications for gene editing therapies in human genetic diseases, including thalassemia. Our results highlight the significance of MMEJ-mediated knock-in events over HR (Homology-Directed Repair) during zebrafish gene editing. CRISPR-Cas12a technology holds promise for zebrafish models and potential therapeutic applications in human genetic disorders. Further research in this direction could enhance gene editing strategies for treating thalassemia and related conditions.

PHYLOGENY OF ACRIDOIDEA (ORTHOPTERA: CAELIFERA) OF PAKISTAN BASED ON MITOCHONDRIAL CYTOCHROME C OXIDASE I

Riffat Sultana^{1,2} and Hojun Song¹

¹Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX 77843-2475 USA

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: riffat.sultana@usindh.edu.pk

Acridids are known for their versatility in nature. Some emerge as major pests, especially during periodic local and large-scale outbreaks, causing significant economic damage. Others serve as sensitive indicators of ecosystem health; for instance, they have proven valuable for monitoring steppe succession or assessing the status of montane wetlands. However, outdated data hinders the accuracy of morphological identification in Acrididae. The lack of reference sequences for many taxa in GenBank further limits the effectiveness of molecular identification. No consistent analysis has yet been done on the phylogeny of Acrididae in Pakistan. Consequently, establishing a reliable morphological database with associated DNA barcoding is imperative. We sampled a total of 164 taxa, including 6 outgroups and 158 ingroup taxa, to explore the phylogenetic diversity within Acrididae using mitochondrial cytochrome *c* oxidase subunit I sequences (COI, total 2970 bp). The study was designed to present the first large-scale DNA barcoding effort for Pakistani Acrididae to test the monophyly of the family and different subfamilies, along with understanding the evolutionary relationships among taxa. Phylogenetic trees were reconstructed using Maximum Likelihood (ML) and Maximum Parsimony (MP) methods with Pamphagidae and Pyrgomorphidae as outgroups. In Pakistan, there are currently 14 recognized subfamilies within Acrididae. Among these, only four subfamilies (Acridinae, Cyrtacanthacridinae, Gomphocerinae, and Oedipodinae) exhibit a cosmopolitan distribution, while the others have more restricted ranges. The most species-rich subfamily reported was Oedipodinae, with 56 species. The highest abundances of species occurred during June to October, while the lowest was recorded during January to March and December. This database can be expanded in future investigations to include specific fauna, ecology, and biodiversity information. By combining morphological and molecular approaches, we aim to enhance the accuracy and reliability of Acridid taxonomy and phylogeny. The grant received from HEC Islamabad (No. 3-1/PDFP/HEC/2022(B-3)/2399/03) is highly acknowledged.

TOXICITY ANALYSIS OF LOCAL ISOLATES OF *BACILLUS THURINGIENSIS* AND CRY1F PROTEIN AGAINST SELECTED LEPIDOPTERA PESTS

Yusra¹, Javed Khan², Mukhtar Alam³ and Munawar Saleem Ahmad¹

¹Department of Zoology, University of Swabi, Swabi

²NARC, Islamabad

³Department of Agriculture, University of Swabi, Swabi

*Corresponding Author: saleemsbs@gmail.com, saleemsbs@uoswabi.edu.pk

It has been claimed that *Bacillus thuringiensis* has the potential to control a variety of insect pests, particularly Lepidoptera. Products made from *B. thuringiensis* have been used in agrochemical industries for years and have been shown to be harmless. For field applications, a number of *B. thuringiensis* pesticides have been described. *B. thuringiensis* is used to produce a number of crystal proteins. These crystal proteins are harmful to numerous insects or actions including Lepidoptera, Diptera, Coleoptera, etc. and have unusual insecticidal action. There have been several reported *B. thuringiensis* strains. Each *B. thuringiensis* strain yields a distinct crystal protein. Transgenic plants encode crystal proteins from the bacterium *B. thuringiensis*. *B. thuringiensis* toxins have been studied. These *Bt* poisons are extremely hazardous to pest's insects. It has been documented that *Bt* toxins are harmful to the Lepidoptera pest. These proteins are effective against a variety of insect pests. These insect pests are detrimental to the growth and ultimate crop output of *Bt* crops. Isolates of *B. thuringiensis* are highly resistant to Lepidoptera pests. Therefore, *B. thuringiensis* pesticides are effective in controlling Lepidoptera pests. *B. thuringiensis* strains' lethal concentrations (LC50 values) have been calculated. Proteins from the *B. thuringiensis* are historically significant and have a wide variety of agrochemical and other applications. They could be applied to environmental preservation, the delivery of drugs or genes, and the identification of fatal human diseases like cancer, among other things. In addition to being a popular food, vegetables are a vital source of nutrients. The goal of the current investigation was to evaluate the bio larvicidal effects of endotoxins produced by *B. thuringiensis* from the CRY1F gene against widely encountered lepidopteran pests (*Spodoptera litura* and *Helicoverpa armigera*). Insect-proof netting was used to contain the vegetable plants in a small field. Similar to this, *B. thuringiensis* with active CRY1F was cultivated in L.B media under controlled laboratory conditions for getting the most colonies possible for preparation of spore/crystal mixtures with different Concentrations (100 µg /ml1000 µg /ml). The larvae of some pests were also raised in a laboratory under control. Following tests, the L.C50 (96-hours) of the CRY1F protein-crystal mixture against lepidoptera pests was determined to be 158.37 µg/ml for *S. litura* and 170.73 µg/ml for *H. armigera*. The endotoxin mixes of CRY1F exhibit considerable potency, causing 100% death in *S. litura* with 500 µg/ml and *H. armigera* with 600 µg/ml, according to the results of the analysis of comparative potencies. The overall findings showed that the *Bt* Local isolates and CRY1F protein endotoxins provide effective and long-lasting results to prevent financially detrimental lowering agriculture output losses through the management of lepidopteran pests. According to our study's findings, the bio larvicidal action of *Bt* local isolates and CRY1F endotoxins against various lepidopteron pests indicate promising results for pest control in pest management.

A RAPID PCR ASSAY FOR IDENTIFICATION OF HARAM IN HALAL FOOD PRODUCTS

Muhammad Younus¹ and Muhammad Safdar²

¹Department of Zoology, Cholistan of Veterinary and Animal Sciences, Bahawalpur

²Department of Breeding and Genetics, Cholistan of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: msafdar@cuvas.edu.pk , younus31302@gmail.com

The mixing of haram (forbidden) meat with halal (permissible) meat is a significant concern in Islamic dietary practices. In Islamic law, certain types of meat are considered haram, such as horse and donkey etc. The admixture of haram meat into halal meat products can occur either unintentionally, due to cross-contamination in processing facilities, or intentionally, due to fraudulent practices. A new rapid simplex PCR technique has been established to identify the presence of three haram animal species in cow meat products. This method utilizes unique primers for horse (*Equus caballus*), donkey (*Equus asinus*), and cow (*Bos taurus*). These primers amplify specific DNA fragments of varying lengths (85 bp for horse, 184 bp for donkey and 271 bp for cow) from the 12S rRNA gene. In testing with reference food samples, the assay demonstrated a detection threshold as low as 0.05% for each of these species. Overall, the collected data confirms that this PCR assay is an efficient, quick, sensitive, specific, and cost-effective method for detecting horse, donkey, and cow DNA in food products. The authors express their gratitude to the Pakistan Science Foundation, Islamabad, for their financial support of this project under Project No. PSF/NSLP/P-CUVAS (933). Additionally, we would like to thank the Department of Breeding and Genetics, CUVAS, Bahawalpur for providing logistical support.

EVALUATION OF *BERGENIA CILIATA* CONJUGATED SILVER NANOPARTICLES FOR TREATING DIABETIC FOOT ULCERS IN MICE

Warda Ali, Shaukat Ali* and Muhammad Summer

Applied Entomology and Medical Toxicology Laboratory, Department of Zoology,

Government College University, Lahore

*Corresponding Author: dr.shaukatali@gcu.edu.pk

Diabetes, a serious metabolic disorder holds many complications specifically impaired healing of chronic wounds like diabetic foot ulcer. In current research, Swiss albino mice were injected with alloxan monohydrate for diabetes induction. Wounds were formed in rectangular shape at dorsal surface of mice foot. These diabetic wounds were treated with *Bergenia ciliata* extract in two different combinations (5% and 10%) and with their conjugated silver nanoparticles (5% and 10%). The healing effect of these biomaterials was analyzed by determination of healing time, percent wound contraction and histological analysis. Serum level of various blood biomarkers i.e., TNF- α , IL-6, IL-8, MMP2, MMP7, MMP9 and TIMPs was also evaluated. The excellent healing was observed in group treated with 10% BCAGNPs. This group was healed in 12 days with wound contraction up to 93 \pm 0.9 %. While the diabetic control group and positive control group (polyfax) were healed in 20 days (67.23 \pm 1.2 %) and 18 days (84.25 \pm 2.40 %) respectively. The histological analysis also showed best healing in same group as this group was observed having proper formation of epidermis, dermis, subcutaneous layer, large number of sebaceous glands and hair follicles, collagen fibers, fibroblasts, enhanced angiogenesis and reduced ulceration. The BCAGNPs 10% extract also alleviated the serum level of TNF- α (19.4 \pm 1.5 pg/ml), IL-6 (13.8 \pm 0.6pg/ml), IL-8 (24.8 \pm 1.2pg/ml) with respect to diabetic control group i.e., TNF- α (55.0 \pm 3.0 pg/ml), IL-6 (39.8 \pm 1.6 pg/ml), IL-8 (70.8 \pm 2.8 pg/ml) (P<0.001). Serum level of MMP2 (268.8 \pm 3.9pg/ml), MMP7 (266.0 \pm 4.4 pg/ml), MMP9 (180.8 \pm 7.1 pg/ml) (P<0.001) was significantly reduced in 10% BCAGNPs group as

compare to diabetic control (MMP2=591.0±11.9 pg/ml, MMP7=508.8±6.9pg/ml, MMP9=415.6±5.1pg/ml)(P<0.001).The serum level of TIMPs(200.8±5.5 pg/ml) was seen elevated in BCaGNPs 10% group in contrast of diabetic control (74.2±5.0 pg/ml) (P<0.001).In conclusion, BCaGNPs enhanced the healing potential and can be used as promising treatment for chronic wounds like DFU in normal as well as in diabetic patients.

WHOLE EXOME SEQUENCING IDENTIFIES CANDIDATE GENE OF NEUROLOGICAL DISORDERS IN KASHMIRI FAMILIES

**Ansar Ahmed Abbasi¹, Nadine Krämer,² Na Li³, Tauqeer Ahmed¹, Rameez Nisar¹,
Lena-Luise Becker², Xiang Fang³, Hao Hu³ and Angela M. Kaindal²**

¹Department of Zoology Mirpur University of science and technology (MUST) Mirpur AJ&K

²Institute of Cell Biology and Neurobiology and Center for Chronically Sick Children (SPZ), Charité –
Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

³Laboratory of Medical Systems Biology, Guangzhou Women and Children's Medical Center,
Guangzhou Medical University, Guangzhou, 510623, China

*Corresponding Author: ansar.zoology@must.edu.pk

Intellectual disability affects 1–3% of the general population and the environmental or hereditary etiology is identified in less than half of patients. Genetic studies have reported mutations in approximately 1000 different genes that may cause intellectual disability. We assessed patients from multiple consanguineous families from Kashmir region, exhibiting non-syndromal intellectual disability and postnatal microcephaly with whole exome sequencing (WES) followed by Sanger sequencing and cosegregation analysis. WES analysis identified novel candidate genes, novel mutations in known genes linked with neurodevelopmental disorders and previously reported mutations in known ID genes. Thus, our study expands the phenotypic spectrum of the genes linked with neurodevelopmental disorders and hence contribute towards better understanding of mechanisms involved in brain development and function.

PHYLOGENETIC AND COMPARATIVE ANALYSIS OF THE NUCLEAR RIBOSOMAL DNA AND NEARLY COMPLETE MITOCHONDRIAL GENOME OF *TANAISIA INNOPINA* COLLECTED FROM *COTURNIX JAPONICA* IN DISTRICT SWABI

**Malghalara Mahaz¹, Suleman^{2*}, Nasreen¹, Mian Sayed Khan², Daim Saood¹,
Hammad Ahmad Khan¹ and Nehaz Muhammad³**

¹Department of Zoology, Abdul Wali Khan University Mardan, Mardan

²Department of Zoology, University of Swabi, Anbar, Swabi

³Hebei Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology,
Hebei Collaborative Innovation, Center for Eco-Environment, College of Life Sciences, Hebei Normal
University, Shijiazhuang, Hebei Province, P. R. China

*Correspondence: zaheer0332@gmail.com

The superfamily Microphalloidea Ward, 1901, which is one of the most derived taxa of the digenean, includes the family Eucotylidae. Three genera, *Tamerlania*, *Tanaisia*, and *Paratanaisia*,

make up the subfamily Tanaisiinae. For a very small number of eucotylids, mtDNA and nuclear rDNA data are available. *Tanaisia innopina* samples were collected during a study on avian trematodes, and its mitochondrial genomes and 28S rDNA were sequenced. The newly sequenced 28S rDNA was aligned with the sequences of other eucotylid species to determine how closely related our specimen is to other members of the Eucotylidae family, phylogenetically. Based on 28S rDNA sequences, our eucotylid grouped with *Tanaisia* sp. with 100% nodal support. Similarly, phylogenetic analyses based on nucleotide sequences of PCGs was also carried out. The nearly completed mt genome of *T. innopina* measured 13870 bp and contained 36 genes, including 20 transfer RNA genes, two ribosomal RNA genes (rrnL and rrnS), and 11 complete PCGs and 1 partial PCG (cox3). The analyses of the average sequence identity, nucleotide diversity and Kimura-2-parameters of genetic distances indicated that Atp6, Nad5, Nad4L, and Nad6 are highly variable genes in the mitogenomes of eucotylids, suggesting that these genes are better molecular markers for the specific identification and intra-specific diversity. The phylogenetic analysis based on mtDNA sequences of 11 PCGs indicated that the Eucotylidae does not have a tight relationship with the other two families included in suborder Xiphidiata. This indicated the non-monophyletic character of the xiphidiatan trematodes. The availability of mitogenome sequences of *T. innopina* offers a rich source of molecular markers for identification, epidemiology, diagnosis and phylogeny of the Eucotylidae and other trematodes.

SPATIOTEMPORAL VARIATION OF TRACE ELEMENTS IN *MACROBRACHIUM* SP. FROM RAVI AND CHENAB RIVERS, PAKISTAN

Samia Farooq¹, Munib Ahmed Shafique² and Abdul Qadir¹

¹College of Earth and Environmental Sciences, University of the Punjab, Lahore

²Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad

*Corresponding Author: aqadir.cees@pu.edu.pk

The present study aimed to highlight the spatiotemporal variation of trace elements (As, Cu, Cr, Mn, Ni, Pb, Sr, Ti, and Zn) in *Macrobrachium* sp. from Ravi and Chenab rivers, Pakistan, and their potential hazardous risks. For this purpose, *Macrobrachium* sp samples were collected during the monsoon and fall seasons from the River Ravi and Chenab. These samples were acid-digested and analyzed using Inductively coupled plasma mass spectrometry (ICP-MS). The highest mean concentrations in the monsoon season in these metals were; As ($0.07 \pm 0.09 \mu\text{g/g}$), Mn ($0.12 \pm 0.09 \mu\text{g/g}$), at Qadirabad Barrage, Cu ($0.195 \pm 0.064 \mu\text{g/g}$), Cr ($0.076 \pm 0.153 \mu\text{g/g}$) and Zn ($0.995 \pm 0.377 \mu\text{g/g}$) both at Khanki Barrage, Ni ($0.017 \pm 0.005 \mu\text{g/g}$) and Ti ($0.142 \pm 0.129 \mu\text{g/g}$) both at Chiniot, Pb ($0.1 \pm 0.14 \mu\text{g/g}$) at, and Sr ($0.558 \pm 0.370 \mu\text{g/g}$) at Trimmu Barrage. The highest mean concentrations of the metals in the fall season were recorded as As ($0.025 \pm 0.035 \mu\text{g/g}$) and Zn ($1.977 \pm 2.472 \mu\text{g/g}$) at Chiniot, Cu ($0.107 \pm 0.097 \mu\text{g/g}$), Ni ($0.033 \pm 0.006 \mu\text{g/g}$), and Pb ($0.063 \pm 0.083 \mu\text{g/g}$) all three at Balloki Barrage, Mn ($0.093 \pm 0.006 \mu\text{g/g}$) at Khanki Barrage, and Ti ($0.142 \pm 0.129 \mu\text{g/g}$) at Qadirabad Barrage. Out of the selected metals, the concentration of Zinc was high in both seasons. The overall trend for the specimen entrapped in the monsoon season was recorded as; Zn > Sr > Cu > Pb > Mn > As > Ti > Cr > Ni, whereas, for the fall season, the trend of metal accumulation followed as; Zn > Sr > Ti > Cu > Mn > Pb > Ni > As > Cr. All metals were observed as non-significant except for Cu (monsoon samples), Mn, and Ni (fall samples). A comprehensive investigation of metal accumulation patterns and their impacts on freshwater prawns and river ecosystem.

THE NOTCH CHRONICLES IN OSTEOGENIC ALCHEMY: DECRYPTING THE NOTCH MEDIATED OSTEOGENIC POTENTIAL OF *CISSUS QUADRANGULARIS* ON PRE-OSTEOBLASTS

Ahad Naveed*, Qindeel Fatima and Abdul R. Shakoori*

School of Biological Sciences, University of the Punjab, Lahore

*Corresponding Author: arshakoori.sbs@pu.edu.pk, ahad.naveed@live.co.uk

Bone is an excellent 'servo' system that continuously integrates signal and responses to sustain its functions. Disruption to this system causes low bone density (LBD), leading to osteopenia and osteoporosis, which significantly burdens global health. Hence, investigating bone remodeling for innovative perspectives and interventions is imperative. Both ayurvedic and scientific literature recognize *Cissus quadrangularis* (CQ) as a prospective solution for osteo-pathologies. Yet deciphering its impact on signaling pathways has yielded inconclusive findings, clouding Notch signaling role in osteogenesis. Current study analyzes Notch signaling in osteogenesis and evaluates CQ and resveratrol for their Notch-mediated osteogenic effects. Utilizing omics tools, we analyzed the expression profiles of Notch- and osteoblast-related genes during osteoblast differentiation stages. Our results from microarray dataset GSE30393 revealed 113 genes associated with bone formation, Extra cellular matrix (ECM) synthesis, bone homeostasis, and Notch signaling. During lineage commitment, all Notch genes are temporarily downregulated, except for *Jag1*, *Dlk2*, and *Hes1*. *Notch1* shows significant expression during proliferation and maturity, with a notable transient decrease at the onset of differentiation compared to *Notch2*, *Notch3*, and *Notch4*. Through *in vitro* experimentations, osteogenic potential of CQ ethanolic extract was assessed in MC3T3-E1 cells, with peaked mitogenic activity at 10µg/ml along 0.02µg/ml of resveratrol, 3.0µM g-secretase inhibitor LY411575. 10µg/ml CQ-E showed mineralization but 0.02µg/ml resveratrol exhibited superior biomineralization action. However, composite treatment of 10µg/ml CQ-E and 3.0µM LY411575 augmented CQ-E induced mineralized nodule formation by demoting Notch signaling. Notch receptor, ligand, and downstream gene expression during osteoblast differentiation were quantified, with resveratrol used as a positive control and LY411575 as a Notch inhibitor to fine-tune outcomes. At gate of osteoblast lineage transition the expression of all Notch receptors decreased. However, *Hes1* was observed to be regulated independent of *Notch1* and 2, possibly via oscillatory mechanism increasing the efficiency of *Runx2*. Jagged1 reported to promotes calvarial osteoblast mineralization which justifies enhanced *Jag1* expression in all treatment. The Hey gene family exhibited abrupt high transcript level on 7th day of treatment showing induction via the Notch2/Hey/Runx2 axis. In conclusion, adequate Notch signaling is crucial for bone homeostasis and preventing osteoporosis. Further studies are needed to understand ligand/receptor interactions and molecular pathways in osteogenesis. However current study, integrating computational analyses with molecular insights, reveals that transient downregulation of Notch expression at the transition phase is crucial for osteoblast differentiation. It also suggests the potential influence of bioactive compounds like resveratrol in Notch-mediated osteogenesis.

MOLECULAR DOCKING-AIDED IDENTIFICATION OF NATURAL POLYPHENOLS AS POTENTIAL STAT3 INHIBITORS

Sameena Gul, Muhammad Faisal Maqbool and Muhammad Khan*

Institute of Zoology, University of the Punjab, Lahore

*Corresponding Author: mkhan.zool@pu.edu.pk

Natural polyphenols are secondary metabolites mainly distributed in the plant kingdom with pronounced biological, and chemoprevention potential. Many epidemiological studies have shown their

promising role in the reduction of certain cancer cases. Cancer is characterized by various well-established signaling pathways in which STAT3 (signal transducer and activator of transducer and activator of transcription 3) is one of the major players and plays a role in cancer cell survival, progression, and chemo-resistance development. Inhibiting STAT3 protein by polyphenolic compounds can be a potential approach against cancer. For this, we performed high throughput screening of 125 polyphenolic compounds by molecular docking via AutoDock Vina Tools. Molecular docking results revealed that the binding energy of only 27 compounds with STAT3 was greater than S3I-201, a commercial STAT3 inhibitor (-7.6 Kcal/mol). Out of all these compounds, 6-Hydroxyluteolin 7-O-rhamnoside (HOR) showed the highest binding energy with STAT3 (-9.1 Kcal/mol). Discovery Studio Visualizer showed that HOR formed three hydrogen bonds with GLN416, CYS418, and VAL374 along with other hydrophobic interactions with the DNA binding domain of STAT3. So, inhibition of STAT3 transcriptional activity by blocking its DNA binding domain can inhibit cancer cell survival and chemoresistance development in cancer. Further, in-vitro and in-vivo studies are required to validate these findings.

PREVALENCE AND DISPARITY OF HAIR LOSS (*ALOPECIA*) IN MALE AND FEMALE GENDER AT DISTRICT HYDERABAD, SINDH, PAKISTAN

Kiran Shaikh^{1*} and Bakhtawar Soomro^{*}

Department of Zoology, Government College University Hyderabad

*Corresponding Author: kiranshaikh058@gmail.com, bakhtawarsoomro145@gmail.com

Alopecia, the medical term for hair loss, exhibits varying patterns across genders. Alopecia is a widespread concern affecting individuals globally, with notable distinctions between males and females in terms of prevalence. This article seeks to provide a comprehensive analysis of the commonality of *Alopecia* in males compared to females, examining factors such as genetic predisposition, hormonal influences, and societal perceptions. This article delves into the distinctive prevalence of alopecia in males compared to females. Through an exploration of existing literature and statistical analysis, this study aims to shed light on the factors contributing to the gender-based differences in alopecia manifestation.

PHENOTYPIC AND GENETICAL VARIATION OF EYE COLOUR OF (HOMO SAPIENS) HUMANBEINGS AMONG DIFFERENT AGES AT GC, UNIVERSITY HYDERABAD, SINDH

Misbah Alladino, Bakhtawar Soomro*, Shakeel Ahmed Memon and Saheefa Soomro

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

Eye color is the specific hue that is present in a person's eyes. It can range from deep shades of brown to vibrant blues, captivating greens, subtle grays, and enchanting hazels. The color is determined by the amount and distribution of melanin in the iris, which is the colored part of the eye. Each eye color has its own distinct charm and can vary greatly from person to person. It's one of the many unique features that make each individual special. Everyone has different eye colors; the reason has to do with genetics and the unique combination of genes that each person inherits from their parents. The two main pigments involved are melanin and lipochrome. The genes that control the production and distribution of these pigments can vary from person to person, resulting in different eye colors. So, it's kind of like a genetic lottery that determines the beautiful diversity of eye colors we see in the world. This research aims to determine the eye color of (Homo sapiens) Human beings among the different ages at District Hyderabad, Sindh,

Pakistan. Present studies conducted on the survey basis under the umbrella of Government College, University Hyderabad. Around 100 samples were collected which reflects variation in colors viz. black, brown, blue, grey, green and hazels. Therefore, it is concluded that genetics play its role in transmission of phenotypic characters from parent to offspring.

REFLECTION OF PHENOTYPIC HEIGHT AT VARYING AGE AMONG THE STUDENTS OF GOVERNMENT COLLEGE, UNIVERSITY, DISTRICT HYDERABAD, SINDH, PAKISTAN

Muqadas Buriro* and Bakhtawar Soomro*

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: muqadasburiro06@gmail.com, bakhtawarsoomro145@gmail.com

During the present studies, the tall and dwarf characters of males ♂ and females ♀ were collected. Around 100 samples, were collected under the umbrella of Government College, University Hyderabad. Human height is a quantitative trait. During the present studies, characteristics of male and female gender measured in quantitative level, which are controlled by the multiple factors. About 60 to 80 percent of the difference in height between individuals is determined by genetic factors, whereas 20 to 40 percent can be attribute to environment effects, mainly nutrition. Current studies reflects that their height is different due to different age. Majority of samples collected with the height 5 feet. During the present studies samples of height including tall and dwarf were collected from the Government College University, Hyderabad, all parameters measured in feet or inches. This abstract explores role of height in genetics and how genes work and transmit their characters from parents to offspring.

THE RARITY OF (O) NEGATIVE BLOOD GROUP AMONG THE INDIVIDUALS OF GOVERNMENT COLLEGE, UNIVERSITY, HYDERABAD SINDH, PAKISTAN

Sadaf Jawaid* and Bakhtawar Soomro*

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: sadafjawaid18@gmail.com, bakhtawarsoomro145@gmail.com

Present studies reflects the outcomes derived from a blood group camp conducted within a university setting, aimed at determining the blood groups of 200 participants. The participants comprised 133 female and 66 male individuals. Among this cohort, a remarkable discovery emerged—solely one male participant exhibited the O negative blood group. The methodology encompassed a systematic blood group testing process, administered freely to attendees. Statistical analysis revealed an intriguing gender distribution within the identified O negative blood group, with its occurrence limited to a single male among the tested participants. These findings shed light on the rarity of the O negative blood group within the university community, particularly in the context of gender disparity. The results accentuate the importance of further research to comprehend the underlying factors contributing to such distinctive blood group distributions among university attendees. This study serves as a starting point for broader investigations into the prevalence and implications of O negative blood groups among specific demographics, urging a deeper exploration into the genetic, social, and medical dimensions associated with this exceptional blood type.

THE RELATIONSHIP BETWEEN GENETICS AND HUMAN PSYCHOLOGY ENCOMPASSES PERSONALITY, BEHAVIOR, MENTAL HEALTH, AND INTELLECTUAL ABILITIES

Muhammad Tayyab* and Bakhtawar Soomro*

Department of Zoology Government College University, Hyderabad

*Corresponding Author: mohammedtayyabchanna@gmail.com, bakhtawarsoomro145@gmail.com

Genetics is the study of transferring of physical and psychological characteristics from parents to offspring whereas Psychology is the study of behavior of an individual. There is debate and contrasting ideas among different Psychologists and Genetists regarding relationship of Genetics to psychology. The debate is whether these both, psychology and Genetists are influenced by each other or not, and if they, then how much? Present studies reflect that genetics plays crucial role to shape the psychological characters of human being. The literature studies from all over the world does not show the exact ratio of psychological factors being influenced by Genetics, different researches show different ratio. During the current studies, certain family as well as twin studies, adopted the methodology of different psychological, mental health and intelligence questionnaires, that have strengthened our research and from those studies and researches it has been cleared and convinced that psychological traits of personality, behavior, mental health and intellectual abilities are being influenced by Genetics. Keeping these traits in mind, twin studies, gene environment interaction and also family studies, have supported so much to make conclusion about relationship of Genetics to psychology. Various experiments at international and national repute and also current research have put a hypothesis, from those experiments and results that, psychological factors are surely being influenced by Genetics. According to twin studies the factors of homosexuality, openness, extroversion or introversion are largely influenced by hereditary information contained in DNA of chromosomes. Besides all, certain disorders such as hemophilia, color blindness all are linked to the heredity. Therefore, it is concluded that the human mental disorders, behavior and personality, these all factors are influenced by Genetically factors, and these traits are inherited from parents to offspring.

ASSOCIATION OF A SINGLE NUCLEOTIDE POLYMORPHISM (rs1136410) IN *PARP1* GENE WITH LOW SPERM COUNT IN A COHORT OF PAKISTANI INFERTILE MEN

**Bushra Aziz, Muhammad Irfan, Naila Zulfikar¹, Nagheen Javed,
Hina Ejaz, Shaheena Bibi and Kainat Abbasi**

Department of Zoology, Wildlife & Fisheries, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi

*Corresponding Author: zarreeneman000@gamil.com

Infertility is the failure to attain pregnancy after 12 months of frequent unprotected sexual contact with the same partner. Male factors contribute about 50 % to infertility. Single nucleotide polymorphisms (SNPs) found in genes of DNA repair produce damage in sperm DNA that may contribute to male infertility. The PARP1 gene is important for DNA repair thus, it is important for spermatogenesis. PARP1 gene is involved in DNA repair and chromatin remodeling steps (replacement of histone to protamine). Polymorphisms in the catalytic domain of PARP1 alter their function of sperm DNA repair, as a result, apoptosis occurs and a large number of sperms die. It was hypothesized that SNP (rs1136410) of the PARP1 gene may be associated with a low number of sperms in men. A case-control study was performed in Islamabad and Rawalpindi. A questionnaire was filled and blood samples were collected from infertile and fertile men. DNA extraction was done by the double salting out method. The Sanger sequencing method was used for genotyping. Results

were analyzed by logistic regression. Genotypic and allelic frequencies were calculated for both groups. The correlation results suggest that high BMI causes a decline in sperm motility. Infertile men exhibited comorbid conditions including typhoid, varicocele, allergies, and sexual dysfunction. Among these comorbid groups, infertility was more prevalent in men aged 21-40 years. The distribution of genotypic frequencies of TT, TC, and CC were 10(40.00%), 11(44.00%), and 4(16.00%) in patients and 4(40.00%), 4(40.00%), and 2(20.00%) in controls. The frequency of combined genotypes TC + CC in patients was 15(60.00%), whereas 6(60.00%) in control groups. The allelic frequency of C and T were 19(38.00%) and 31(62.00%) in patients and 8(40.00%) and 12(60.00%) in controls. Evaluation of genotypic and allelic frequencies of the SNP (rs1136410) in the PARP1 gene has revealed that there is no significant association with low sperm count.

ASSOCIATION OF *TNP2* GENE WITH HUMAN MALE INFERTILITY IN LOCAL PAKISTANI POPULATION

**Naila Zulfiqar*, Muhammad Irfan, Bushra Aziz, Shaheena Bibi, Hina Ejaz,
Naghen Javed and Kainat Abbasi**

*Department of Zoology, Wildlife and Fisheries, Pir Mehr Ali Shah
Arid Agriculture University, Rawalpindi*

*Corresponding Author: nailazulfiqar55@gmail.com

Infertility is the failure of a couple to achieve and conceive a pregnancy after one year of regular and unprotected sexual intercourse. Male infertility contributes to about 50% of cases. Single nucleotide polymorphism (SNP) found in the genes of spermiogenesis may contribute to male infertility. Spermiogenesis is the maturation of round sperm into active motile sperm. During this process, DNA becomes highly condensed which makes sperm actively motile. In DNA condensation nuclear histone proteins are replaced by nuclear transition protein (TNP2). Further, protamine replaces transition proteins, making DNA highly condensed and actively motile. It helps to condense the chromatin and protect it during the remodeling process, ensuring the efficient and proper packaging of the paternal DNA into the sperm head. DNA condensation protects DNA from mutagenesis and enhances sperm motility. Single nucleotide polymorphism (G1272C) of TNP2 alters the formation of transition protein, affecting the normal spermiogenesis process. It was hypothesized that the SNP of the TNP2 gene may be associated with an abnormal number of sperm in men. A case-control study was performed in Rawalpindi and Islamabad. A questionnaire was filled and blood samples were collected from both fertile and infertile men. The correlation result showed that high BMI was significantly associated with reduced sperm motility. Infertile men exhibited comorbid conditions, including premature ejaculation, erectile dysfunction, typhoid, varicocele, and allergies. Among these comorbid groups, infertility was more prevalent in men aged 21-40 years and those belonging to the Punjabi and Pathan ethnic groups. DNA extraction was done by the salting out method. Sanger sequencing was used for genotyping. The result was analyzed statistically by logistic regression. The SNP G1272C of the TNP2 gene was analyzed in 25 infertile and 10 fertile men. Both genotypic and allelic frequencies were calculated. The calculated frequency for the GG genotype was 52% in the cases and 50% in the controls and CC was 12% in the cases and 20% in the controls. The allelic frequency was not significantly different between the case and control group ($P < 0.05$). The odds ratio showed no association of G1272C with male infertility in the local Pakistani population.

MOLECULAR ANALYSIS OF *TRMT1* GENE INVOLVED IN AUTOSOMAL RECESSIVE INTELLECTUAL DISABILITY AND MICROCEPHALY

Rameez Nisar¹, Nadine Kraemer², Sundas Farooq¹, Angela M. Kaindal² and Ansar Ahmed Abbasi¹

¹*Department of Zoology Mirpur University of science and technology (MUST) Mirpur (10250), AJ&K*

²*Institute of Cell Biology and Neurobiology and Center for Chronically Sick Children (SPZ), Charité – Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany*

*Corresponding Author: ansar.zoology@must.edu.pk

The *TRMT1* gene (MIM#611669) encodes the protein tRNA methyltransferase 1 that transfers a methyl-group onto a single guanine residue present in most tRNAs and thereby modifies them post-translationally. The exact function of *TRMT1* is unknown, but such methyltransferases have been suggested to influence parameters such as RNA stability, translation, trafficking, localization, enzymatic activity or patterns of interactions with other molecules. Homozygous mutations in the *TRMT1* gene have been reported recently in a large screen for genes associated with autosomal recessive intellectual disability. So far only two families with a biallelic *TRMT1* mutation have been reported; however, no detailed clinical or radiological data was available. Here, we report four further individuals who carry homozygous mutations in the *TRMT1* gene and thereby expand the *TRMT1*-linked phenotype. We investigated affected and non-affected individuals of the two families through whole exome sequencing (WES) approach and identified a novel 1 bp insertion at the donor-site between exon 12 and 13 (c.1506+1G>T, in family 2 and an already reported homozygous deletion in the *TRMT1* gene (c.657_688del32, p.Q219Hfs* in family 1). Both reported mutations affect highly conserved amino acids within the functional domain of TRMT1.

RECURRENT MUTATIONS IN KNOWN OCA GENES ARE ASSOCIATED WITH HEREDITARY OCA IN KASHMIRI FAMILIES

Saiqa Khushal¹, Ansar Ahmed Abbasi¹*Ammama Ghaffar² and Zubair M Ahmed²

¹*Department of Zoology Mirpur University of science and technology (MUST) Mirpur (10250), AJ&K*

²*Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21202, USA*

*Corresponding Author: ansar.zoology@must.edu.pk

Oculocutaneous albinism (OCA), is known as a genetic disorder which occurs due to some error in biosynthesis of a pigment called melanin responsible for producing colour in body and it also gives diverse phenotypic appearances. Pathogenic variants in eight genes have been reported to be involved in OCA. In current study Kashmiri families segregating autosomal recessive Oculocutaneous albinism were recruited from different localities of Mirpur/Bhimber Azad Jammu and Kashmir. Tetra primer analysis was carried out in all the families. Most of the families showed mutations in *TYR* Gene. All the variants identified through whole exome sequencing will be confirmed by sanger sequencing. Altogether, our study highlights the significance of exome sequencing for the complete genetic diagnosis of inbred families and provides the ramifications of potential genetic interaction and digenic inheritance of variants in the *TYR* Gene.

AUTOSOMAL RECESSIVE NON-SYNDROMIC HEARING LOSS GENES IN PAKISTAN DURING THE PREVIOUS THREE DECADES

**Madiha Shadab^{1*}, Ansar Ahmed Abbasi¹, Ahsan Ejaz^{2,3}, Afif Ben-Mahmoud⁴,
Vijay Gupta⁴, Hyung-Goo Kim^{4,5} and Barbara Vona^{6,7*}**

¹*Department of Zoology, Mirpur University of Science and Technology, Mirpur*

²*Department of Physics, University of Kotli Azad Jammu and Kashmir, 11100, Kotli*

³*School of Nuclear Science and Technology, Lanzhou University, Lanzhou, 730000, PR China*

⁴*Neurological Disorders Research Center, Qatar Biomedical Research Institute,
Hamad Bin Khalifa University, Doha, Qatar*

⁵*College of Health & Life Sciences, Hamad Bin Khalifa University (HBKU), Doha, Qatar*

⁶*Institute of Human Genetics, University Medical Center Göttingen, 37073 Göttingen, Germany*

⁷*Institute for Auditory Neuroscience and Inner Ear Lab,*

University Medical Center Göttingen, 37075 Göttingen, Germany

*Corresponding author: madiha.shadab@hotmail.com, ansar.zoology@must.edu.pk

Hearing loss is a clinically and genetically heterogeneous disorder, with over 124 genes and 170 loci associated with its pathogenesis. The spectrum and frequency of causal variants vary across different genetic ancestries and are more prevalent in populations that practice consanguineous marriages. Pakistan has a rich history of autosomal recessive gene discovery related to non-syndromic hearing loss. Since the first linkage analysis with a Pakistani family that led to the mapping of the DFNB1 locus on chromosome 13, 51 genes associated with this disorder have been identified in this population. Among these, 13 of the most prevalent genes, namely *CDH23*, *CIB2*, *CLDN14*, *GJB2*, *HGF*, *MARVELD2*, *MYO7A*, *MYO15A*, *MSRB3*, *OTOF*, *SLC26A4*, *TMC1*, and *TMPRSS3* account for more than half of all cases of profound hearing loss, while the prevalence of other genes is less than 2% individually. In this review, we discuss the most common autosomal recessive non-syndromic hearing loss genes in Pakistani individuals, as well as the genetic mapping and sequencing approaches used to discover them. Furthermore, we identified enriched gene ontology terms and common pathways involved in these 51 autosomal recessive non-syndromic hearing loss genes to gain a better understanding of the underlying mechanisms. Establishing a molecular understanding of the disorder may aid in reducing its future prevalence by enabling timely diagnostics and genetic counselling, leading to more effective clinical management and treatments of hearing loss.

GENETIC CAUSES OF OCULOCUTANEOUS ALBINISM IN KASHMIRI FAMILIES

Rabia Shoukot¹, Saiqa Khushal¹, Ansar Ahmed Abbasi^{*1}, Ammama Ghaffar² and Zubair M Ahmed²

¹*Department of Zoology, Mirpur University of Science and Technology, Mirpur, Azad Jammu and Kashmir*

²*Department of Biochemistry and Molecular Biology, University of Maryland,
School of Medicine, Baltimore, MD 21202, USA*

*Corresponding Author: ansar.zoology@must.edu.pk

Melanin pigment helps protect our body from broad wavelength solar radiation and skin cancer. Among other pigmentation disorders in humans, albinism is reported to manifest in both syndromic and non-syndromic forms as well as with varying inheritance patterns. Oculocutaneous albinism (OCA), an autosomal recessive non syndromic form of albinism, presents as partial to complete loss of melanin in the skin, hair, and iris. OCA has been known to be caused by pathogenic variants in seven different genes, so far, according to all the currently published population studies. However, the detection rate of alleles

causing OCA varies from 50% to 90%. One of the significant challenges of uncovering the pathological variant underlying disease etiology is inter- and intrafamilial locus heterogeneity. This problem is especially pertinent in highly inbred populations. As examples of such familial locus heterogeneity, we present nine consanguineous Pakistani families with segregating OCA due to variants in one or two different known albinism-associated genes. All of the identified variants are predicted to be pathogenic, which was corroborated by several in silico algorithms and association with diverse clinical phenotypes. We report an individual affected with OCA carries heterozygous, likely pathogenic variants in TYR and OCA2, raising the question of a possible digenic inheritance. Altogether, our study highlights the significance of exome sequencing for the complete genetic diagnosis of inbred families and provides the ramifications of potential genetic interaction and digenic inheritance of variants in the TYR and OCA2 genes.

GENE PANEL SEQUENCING IDENTIFIES PREVIOUSLY REPORTED MUTATIONS IN KNOWN GENES INVOLVED IN HEREDITARY MICROCEPHALY IN CONSANGUINEOUS FAMILIES

Sundas Farooq¹, Ansar Ahmed Abbasi¹ and Muhammad Sajjid Hussain²

¹*Department of Zoology Mirpur University of science and technology (MUST) Mirpur (10250), AJ&K*

²*Cologne Center for Genomics (CCG), Faculty of Medicine, University Hospital Cologne, University of Cologne, 50931 Cologne, Germany*

*Corresponding Author: ansar.zoology@must.edu.pk

Autosomal recessive microcephaly is a heterogeneous genetic disorder characterized by reduced head circumferences and sloping forehead. Affected individuals also show mild to moderate intellectual disability. To date 27 genes have been identified which are involved in autosomal recessive microcephaly. In Pakistani Population *ASPM* and *WDR62* are most frequently involved genes and mutations in these two genes are responsible for MCPH. In present study 10 families were subjected to gene panel sequencing for *ASPM* mutation. Gene panel sequencing showed one missense c.8987G>A; p.Arg2996Gln, one nonsense mutation c.9286C>T; p.Arg3096*, one deletion c.8200_8201delAA; p.Asn2734Leufs*16 in 3 families. Remaining seven families are negative for *ASPM* mutation and will be subjected to Whole exome sequencing. Furthermore two families were screened with STS markers followed by Sanger sequencing which showed mutation in *WDR62* and *CENPJ* respectively. This study broadens the spectrum of mutations involved in hereditary microcephaly in Pakistani Population.

MUTATION IN *RAPGEF1* IS RESPONSIBLE FOR INTELLECTUAL DISABILITY IN CONSANGUINEOUS FAMILY

Tauqeer Ahmed, Rameez Nisar and Ansar Ahmed Abbasi*

Department of Zoology Mirpur University of Science and Technology (MUST) Mirpur, AJ&K

*Corresponding Author: ansar.zoology@must.edu.pk

Intellectual disability is a common neurological disorder in consanguineous populations. Intellectual disability is affecting approximately 1% of global population. It is an unresolved problem and majority of patients remains untreated due to lack of awareness and some other reasons. Because of extreme heterogeneity, genetic cause of disease is still unknown. Here, we report for the first time a homozygous mutation of *RAPGEF1* in a consanguineous family with two siblings affected by intellectual disability.

RAPGEF1 is a guanine nucleotide exchange factor responsible for transmitting extracellular signals to the Ras family of GTPase located at the inside of membrane.

**EPIDEMIOLOGY, MOLECULAR CHARACTERIZATION, GENETIC IDENTIFICATION AND
PHYLOGENETIC ANALYSIS OF ANAPLASMA SPECIES FROM TICK-BORNE
ANAPLASMOSIS IN GOATS OF SOUTH PUNJAB, PAKISTAN**

Adeel Munawar^{1*}, Syed Qaswar Ali Shah¹ and Abdullah Saghir Ahmad²

¹*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

²*Department of Parasitology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

*Corresponding Author: adeelmunawarofficial@gmail.com

Anaplasmosis is a tick-borne disease affecting ruminants and is caused by obligate intra-erythrocytic rickettsial microbes of the genus *Anaplasma*. The clinical manifestations of this disease in animals include fever, inappetence, decreased milk yield, anemia, depression, icterus, brownish urine, pale mucous membrane, labored breathing, and constipation. This research highlights the economic impact of anaplasmosis on agriculture, particularly in goat farming, which constitutes a significant portion of the economy in many regions. The study is set in South Punjab, Pakistan, where the climate is conducive to tick infestation, leading to a variety of anaplasmosis cases in goats. To address the limitations of traditional diagnostic methods, the study proposes using advanced techniques like Polymerase Chain Reaction (PCR) and Sequencing for accurate detection, molecular identification, genetic characterization, and phylogenetic analysis of *Anaplasma*. The research involves collecting blood and tick samples from goats in six cities during September and October 2023. The extracted DNA undergoes PCR, and the resulting products are sequenced for further analysis. The obtained sequences are then checked using BLAST through the National Center for Biotechnology Information (NCBI). Multiple alignments and phylogenetic analyses are conducted using MEGA software through the Neighbour Joining Method. In conclusion, the study aims to enhance the understanding and detection of anaplasmosis in goats in South Punjab, employing advanced molecular techniques for a comprehensive analysis.

3. HUMAN AND ANIMAL DISEASES

SEROPREVALENCE OF TOXOMPLASMA GONDII AND ASSOCIATED RISK FACTORS AMONG WOMEN VISITING DIFFERENT HOSPITALS OF GUJRANWALA, PAKISTAN

Asma Abdul Latif, Fatima Aslam, Hira Jabeen, Tooba Arooj and Shafaq Fatima

Department of Zoology, Lahore College for Women University, Lahore

*Corresponding Author: asma5latif@gmail.com

Toxoplasmosis is an infection spread throughout the world caused by parasite *Toxoplasma gondii*. Parasite infects at least one-third of the world's human population, making it one of the most effective diseases. The present study was conducted to find out the seroprevalence of Toxoplasmosis among women in different hospitals of Gujranwala. Total 360 blood samples were collected. 150 samples from pregnant women while 210 samples from non-pregnant were collected for comparison. Serum from blood samples were separated by centrifugation (3000 rpm). With the help of a questionnaire, information regarding the pregnancy, marital status, age, occupation, weight, socioeconomic status, education level, and contact with cats were collected. *Toxoplasma gondii* antibody index was determined by using the ELISA technique. The results showed that seroprevalence (%) of *Toxoplasma gondii* among women was 30%. The seroprevalence of *Toxoplasma gondii* is more in pregnant-women (35.3%) than non-pregnant women (26.1%). Statistical analysis after performing chi-square showed a significant ($p < 0.05$) association between pregnant and non-pregnant women. The infection can also spread from the mother to the fetus during pregnancy, maybe resulting in congenital defects. Risk factors include consuming raw or undercooked meat, coming into contact with infected cat feces, Ingesting water or food contaminated with the parasite's oocysts, handling soil or contaminated vegetables, unawareness, poor hygiene practices, and eating unwashed fruits and vegetables. It was concluded that Toxoplasmosis can be avoided by avoiding direct contact with cat litter or soil that may be contaminated with cat feces.

BURDEN OF MALARIA AT PIR ABDUL QADIR SHAH JEELANI INSTITUTES OF MEDICAL SCIENCES, GAMBAT, PAKISTAN

Sahiba Noor Hussain, Tahira Junejo and Khadim Hussain Memon*

Department of Zoology Shah Abdul Latif University, Khairpur

*Corresponding Author: khadim.memon@salu.edu.pk

Malaria is one of the major health problems caused by a vector-borne eukaryotic protist of the genus Plasmodium. Plasmodium is a parasite which is transmitted naturally by the bite of female mosquito anopheles called malarial vector. The cross-sectional study was aimed to determine the prevalence of malaria at Gambat Pakistan. Primary and secondary data was collected from August to September 2023 and from January 2022 to September 2023 respectively from Pir Abdul Qadir Shah Jeelani institutes of medical Sciences Gambat, Pakistan. Age, sex, and month wise data was collected. The identification of malarial parasite was carried out using both rapid diagnostic test and microscopic examination. In primary data a total of 200 malarial suspected patients were enrolled while in secondary data the records of 1,170 malarial patients were collected. Among the total cases (80%) were found positive for malarial parasite and (20%) were found negative. The malarial infection was found more common in males (52%) as

compare to females (28%) in study population. Among the positive cases (36.5%) were found *P. vivax* (3.5%) *P. falciparum*. In females (89.2%) *P. vivax* and (10.7%) were *P. falciparum*. In males (92.3%) *P. vivax* and (7.6%) were found *P. falciparum*. The month wise distribution of malaria in the present study indicated that malarial cases were high in the month of August (44%) followed by September (36%). While the patients were divided into the four age groups of 1-10 years, 11-20 years, 21-30 years and 31 and above years, on the basis of age groups higher number of cases (85.1%) was observed in age between 1-10 years with an average age of 5.3 ± 2.01 years. The minimum prevalence of malaria was observed (26.08%) in 31 and above years with an average age 34.91 ± 2.6 years. In secondary data maximum prevalence of malaria for females observed (45.45%) in November 2022, whereas in males' maximum positive cases were (46.87) in month of May 2022. While minimum prevalence for females observed (3.1%) in March 2022. Similarly in males' minimum cases were in month of April 2022 with (1%) positive cases, meantime in month of March 2023 maximum cases found in females with (19.04%) while (29%) were positive male in month of August 2023. whereas minimum percentage of female were found in May 2023 with total cases of (7.5%), while minimum positive male cases were in July 2023 with (10.34%). In conclusion, males were infected more as compared to females and high number of cases reported in age between 1-10 years. Maximum prevalence found in November 2022 for females and May 2022 for males, while minimum prevalence for females observed in March 2022 and April 2022 for male. Whereas in month of March 2023 maximum positive cases found in females and August 2023 for males meantime minimum cases for females in month of May 2023 and July 2023 for males. *P. vivax* is the dominant as compare *P. falciparum* in Gambat due to occurrence of host in that area.

EPIDEMIOLOGY AND ECONOMIC IMPACT OF LUMPY SKIN DISEASE IN CATTLE OF AZAD JAMMU & KASHMIR

Faiza Ishfaq¹, Beenish Shahid^{1*}, Muhammad Ijaz Khan² and Zeeshan Anjum²

¹*Department of Zoology, King Abdullah Campus, University of Azad Jammu and Kashmir, Muzaffarabad*

²*Department of Livestock and Dairy Development,*

Azad Government of the State of Jammu and Kashmir, Muzaffarabad

*Corresponding Author: beenish.shahid@ajku.edu.pk

Lumpy skin disease (LSD) is an infectious disease in cattle resulting from the lumpy skin disease virus (LSDV) belongs to the family Poxviridae. The aim of the present study was to provide evidence about LSDV because no study has been conducted in Azad Jammu and Kashmir (AJK). Data was collected from farm owners or individuals responsible for livestock care through in-person interviews, using a structured questionnaire that comprised both closed- and open-ended questions. The first clinical case of LSD was reported on 10 June 2022 in the Khila (Muzaffarabad city) of AJK among cattle that were kept on pastures. Out of 545,239 cattle, 20268 cases were detected and reported in 2022. Among them 257,568 animals (47.23%) were vaccinated immediately to stop the spread of disease and to minimize cattle production losses caused by outbreaks. The disease affected almost all districts of the Azad Jammu and Kashmir. Overall morbidity, fatality and mortality rates were 3.72%, 5.85% and 0.21% respectively. The study revealed that the most common clinical signs were fever (91.82%), nodules on skin (84.40%), nasal discharge (81.65%), anorexia (64.22%). While oedema (52.29%) and mastitis (40.36%) were less common in animals affected with LSD infection. Milk production in cattle with LSD was significantly ($P < 0.0001$) reduced by 6 L (75%) during the severe phase and 25% once cattle recovered from the disease. The decrease in body weight was also observed which was 10% ($P < 0.0001$) illustrating the marked and protracted adverse impact of LSD. In financial loss analysis, the average loss per case was 102,784.35 PKR (368.86 US \$). The direct losses were financial loss due to reduced milk

profit and mortality while the indirect losses were extra-management, treatment and vaccination cost. It is concluded that the disease results in chronic debilitation, diminished milk production, weight loss, damaged skin, fatality and mortality in cattle. Its economic impact on the livestock industry is significant.

PREVALENCE OF HAEMONCHUS CONTORTUS IN SINDH, PAKISTAN, AND ITS BIOLOGICAL CONTROL USING BACILLUS THURINGIENSIS SPORE CRYSTAL SUSPENSION AND PURIFIED CRYSTAL PROTEINS AGAINST ADULT HAEMONCHUS CONTORTUS IN VITRO

Sana N. Panhwar^{1,3}, Javaid A. Gadahi³, Qihui Luo¹, Chao Huang¹, Sehrood A. Siddique², Lanlan Jia¹, Shaista Jalbani³, Irum Sehnaz² and Zhengli Chen^{1*}

¹ *Laboratory of Animal Disease Model, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China*

² *Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam*

³ *Department of Fisheries and Aquaculture, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand*

*Corresponding Authors: jalbanishaista@gmail.com, dr.sananoor409@gmail.com, jagadahi@sau.edu.pk, lqhbiology@163.com, huangchao@sicau.edu.cn, xiayu113bvs@163.com, jialanlan@sicau.edu.cn, chzhli75@163.com

This study investigated the prevalence of *Haemonchus contortus* infection in goats and observed the efficacy of Bio-anthelmintic on adult worms. The environmental conditions of interior Sindh Pakistan are suitable for raised species of small ruminants, especially goats; purposefully, we surveyed five districts of Sindh. The overall prevalence of *Haemonchus contortus* in goat species was 52.4%. As concerned with Age, 8-12 months goats were at high risk with an infection percentage of 66.11%, followed by less than >8 months, 47.22%, and more than a year has 43.51% infection, with a confidence interval (CI) an odd ratio (OR) and p-value (95% CI), (1.1757 to 1.8995), 1.4944, P = 0.0010. Moreover, regarding gender, males are at higher risk 60% compared to females 49.14% infected, respectively CI, OR, and p-value (95% CI), (0.4896 to 0.8476), 0.6442, P = 0.0017. A seasonality check concludes that the summer season plus the monsoon supports the worm population 64.44% as compared to winter 21.42% infection recorded with CI, OR, and p-value, (95% CI), (4.8081 to 9.1860) 6.6458, P < 0.0001, above study concluded that different districts of Sindh significantly infected with *Haemonchus contortus*. Additionally, *Haemonchus contortus* produces resistance to chemical-based anthelmintic compounds; due to this, the researchers drove concentration toward biological control; the present study focused on biological control with the environment-friendly bacterium *Bacillus thuringiensis*. We applied *Bacillus thuringiensis* purified crystal proteins and spore crystal mix separately to the adult worm of *H. contortus*. Adult *H. contortus* were collected from monospecific animals and poured into a 12-multiwell plate, and the motility rate was checked after 12, 24, and 48 hrs. of treatment. Experiment results show that purified crystal protein at the concentration of 2mg/ml diluted in normal saline can control worm mobility within 12 hrs. 50% followed by 24 hrs. 66.66 % and 48hrs 83.33%. Likewise, Spore crystal suspension at the concentration of 1x 10⁸ declined worm motility in 12 hrs. 16.66 %, followed by 24 hrs. 25% and 48 hrs. 66.66% compared to the control group other concentrations of both treatments are non-significant. The findings of the above study concluded that purified crystal toxins of *Bacillus thuringiensis* are used as bio-anthelmintic to control worm burden in small ruminants.

SEROPREVALENCE STUDY OF PESTE DES PETITS RUMINANTS (PPR) IN SHEEP & GOATS OF AZAD JAMMU & KASHMIR

Maryam Bibi¹, Beenish Shahid^{1*} and Muhammad Ijaz Khan²

¹*Department of Zoology, King Abdullah Campus, University of Azad Jammu and Kashmir, Muzaffarabad*

²*Department of Livestock and Dairy Development, Azad Government of the State of Jammu and Kashmir, Muzaffarabad*

*Corresponding Author: beenish.shahid@ajku.edu.pk

Peste des petits ruminants (PPR) disease is a highly contagious and economically important viral infection in small ruminants causing high morbidity and mortality. Illness and death rate can be as high as >90%. It is caused by a *morbillivirus* from the *paramixoviridae* family. The aim of the present study was to provide evidence about LSDV because no study has been conducted in Azad Jammu and Kashmir (AJ&K). In the present study 360 blood samples were taken from the jugular vein of the apparently healthy and diseased goats and sheep with common symptoms of PPR. Serum samples were tested for seroprevalence using PPR antibody ELISA kit manufactured by ID. Vet Innovative Diagnostic 310 rue Louis Pasteur 34790 Grables - Franc. The overall rate of PPR seroprevalence in AJ&K was 38%. District-wise highest seroprevalence was recorded in Muzaffarabad whereas lowest in Neelum. Goats were significantly more affected (41%) than sheep (23%). In gender-wise study no significant difference was observed. Adults (48%) and kids (45%) were significantly more affected than young animals (33%). The results were statistically significant at $P=0.05$ with 95% confidence interval. Our results revealed that the PPR seroprevalence was high in AJ&K and preventive proceeding is needed to control and eradication of the disease in this region.

SURVEY OF INTENSITY AND ASSOCIATED RISK FACTORS OF LUMPY SKIN DISEASE IN SARGODHA

Sawera Sehar and Sana Ashraf*

Department of Zoology, The University of Lahore, Lahore

Corresponding Author: sanaashrafdr@gmail.com

Lumpy skin disease (LSD) is a contagious and transmissible cattle disease that spreads by lumpy skin disease virus (LSDV). This disease resulted in great economic losses in Pakistan. A cross sectional one year study based on questionnaire survey was conducted in Sargodha. The climatic conditions of the study area showed the average temperature between 30-40 degree Celsius (53.7%). Almost all of the herd owners were male between the age 30-50 and have almost education no more than primary level. Result showed 57% herd level prevalence, mortality rate was 4.5%. female cattle showed greater prevalence (60%), as compared to male cattle (48%) due to pregnancy and lactation pressure in female. Animals between the age of 2-5 years showed the highest prevalence (64.7%), as compare to other age groups. Disease showed more prevalence in summer season (74.2%). Disease was characterized by nodular lesions on different parts of body as face, body and other parts. Disease has no effect on humans and other animals.

4. MICROBIOLOGY

ENTEROTOXIGENIC METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: A CHALLENGING ISSUE FOR DAIRY ANIMALS AND OCCUPATIONAL WORKERS

**Muhammad Ijaz¹, Arslan Ahmed¹, Muhammad Umar Javed¹, Hamza Rasheed¹,
Muhammad Jawad Sabir¹ and Ali Abdullah Jabir¹**

¹*Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore*

*Corresponding Author: Address: mijaz@uvas.edu.pk

Staphylococcus aureus (*S. aureus*) is considered a significant pathogen of ubiquitous nature due to its key involvement in dairy animal mastitis and is also considered the leading cause of food-borne maladies among humans. Enterotoxin producing methicillin resistant *S. aureus* is considered as the main culprit of escalating food poisoning issues among humans. This paper reports the prevalence of significantly increased enterotoxigenic MRSA pathogen among bovines and dairy occupational workers along with antibiotic-resistant pattern by *in-vitro* technique. For this purpose, 384 bovine (n=192 cattle, n=192 buffalo) milk samples and 100 nasal or skin swab samples from dairy workers were collected and the prevalence of *S. aureus*, MRSA, *spa*, and enterotoxin genes (*seb*, *sec*) was measured genotypically by PCR. Moreover, the prevalent enterotoxin *seb* gene sequence from bovines, workers, and other sources was compared and analyzed by phylogenetic analysis. The study revealed 49.79%, 29.46%, and 66.80% overall prevalence of *S. aureus*, MRSA, and *spa* genes among bovine and human samples collectively while the enterotoxigenic *S. aureus* was found 16.18% prevalent in bovine and human samples. The phylogenetic analysis revealed a significant resemblance of the bovine *seb* gene with the human gene sequence except for minor differences. Moreover, the commonly used antibiotics showed resistance against enterotoxigenic MRSA. The study concluded the increased prevalence and antibiotic resistance of enterotoxigenic MRSA isolates among dairy occupational workers of south Punjab that can be transferred from dairy animals to occupational workers.

SCREENING AND CHARACTERIZATION OF ANTIBIOTIC RESISTANT BACTERIA FROM BUFFALO DUNG

Iqra Riaz, Rimsha Riaz, Tasleem Akhtar and Saiqa Andleeb

¹*Microbial Bioremediation and Vermi-technology laboratory, Department of Zoology,
University of Azad Jammu & Kashmir, King Abdullah Campus, Chattar Kalass, Muzaffarabad*

²*Department of Zoology, Government College University, Faisalabad*

⁴*Department of Zoology, Hazara University, Khyber Pakhtunkhwa*

*Corresponding Author: drsaiqa@ajku.edu.pk; drsaiqa@gmail.com

Excessive application of antibiotics and medicines as preventive measures in animal farms releases antimicrobial resistance genes into the atmosphere. Therefore, the purpose of the current study are to screen the antibiotic resistant bacteria from the buffalo dung and to characterize them using various techniques. Bacteria were isolated and identified by standard microbiological and biochemical techniques. Results showed that twenty-three bacteria were isolated from the buffalo dung, and screened through different nutritional media. On MacConkey agar media, isolated microbes 19b, 20b, 21b, 22b, 23b

indicated fermentation of lactose while Mannitol salt agar medium showed that bacterial isolates of 13b, 14b, 17b, 18b, 19b, 20b, 22b, 23b indicates fermentation of mannitol. Staining techniques indicates that 1b, 2b, 3b, 4b, 13b, 14b, 15b, 16b, 17b, 22b are gram-positive bacteria while 4b, 5b, 7b, 9b, 10b, 18b, 20b are non-endospore forming bacteria. Biochemical characterization revealed that all buffalo dung inhibiting bacteria do not produce oxidases and ureases, proteolytic test indicates that 4b, 5b, 6b, 7b, 8b, 9b, 10b, 11b, 12b, 15b, 16b, 17b, 18b, 19b, 20b, 21b, 22b, 23b produces proteases while lipolytic test showed that 7b, 8b, 15b, 16b, 18b, 23b produces lipases, hemolytic results indicated that all bacterial isolates are pathogenic and Methyl red test showed that 1b, 2b, 3b, 4b, 5b, 6b, 9b, 10b, 11b, 12b, 13b, 14b, 15b, 21b, 22b, 23b produced fermented products. Antibiotic sensitivity of bacteria inhibiting buffalo dung against eleven antibiotics was evaluated by Kirby-Bauer disk diffusion method. Tobramycin (TOB), Streptomycin (S10), Piperacillin (PIP), Nalidixic Acid (NA), Norfloxacin (NOR) and Streptomycin (S3) showed maximum sensitivity against several bacterial isolates while other antibiotics Azithromycin (ATM), Piperacillin-Tazobactam (TZP), Ampicillin (AMC), Cefixime (CXM), Amoxicillin (AML) showed resistance against bacteria. Resistogram analysis revealed that Cadmium (Cd) showed zone of inhibition against 1b, 2b, 4b, 7b, 9b, 11b, 12b while Lead (Pb) showed maximum zones of inhibition against 1b, 2b, 7b, 8b, 9b, 10b, 11b, 12b. The findings of this study will assist veterinarians in developing and refining novel therapy techniques against bacteria inhibiting buffalo dung that cause zoonotic infections.

BACTERICIDAL EFFECT OF ORGANOMETALLIC-BASED SYNTHETIC COMPOUNDS AGAINST NOSOCOMIAL PATHOGENS

Kiran Maqbool¹, Saiqa Andleeb^{1*}, Kaleem Imdad² and Muhammad Siraj ul Haq³

¹Microbial Biotechnology lab, Department of Zoology, University of Azad Jammu and Kashmir, King Abdullah Campus, Chattar Kalas, Muzaffarabad

²Bioscience Department, COMSATS University, Park Road, Chak Shahzad, Islamabad

³Department of Chemistry, University of Science & Technology, Bannu, KPK

*Corresponding Author: drsaiqa@gmail.com; drsaiqa@ajku.edu.pk

Infectious diseases caused by various multidrug resistance pathogenic bacteria and in recent scenario organometallic-based synthetic compounds are used as innovative antimicrobial agents. The aim of current research was to evaluate the bactericidal effect, biofilm inhibition and cell proliferation inhibition of synthesized organometallic compounds against nosocomial pathogens i.e., *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. Agar well diffusion methods were used to assess the antimicrobial activity. The agar disc diffusion method was used for antibiogram analysis to check the sensitivity of standard antibiotics against bacterial pathogens, and activity index was performed to compare the efficacy of organometallic-based compounds with standard antibiotics. The effect of organometallic-based compounds on pathogenic bacteria for biofilm formation and the viability of bacterial cells were examined using the crystal violet assay and MTT assay. Results revealed that compounds of Msn and Bsn series indicated significant results against nosocomial pathogens. The highest sensitivity showed by Msn-20, Msn-24, Msn-19, and Bsn-19 (15.0 ± 0.0 mm), (11.0 ± 0.0 mm), (14.0 ± 0.0 mm) and (13.3 ± 0.47 mm) against *Streptococcus pyogenes*. Msn-20, Msn-24, Bsn-15, Bsn-16, Bsn-21, Bsn-23, Bsn-35, Bsn-36, Bsn-37 showed maximum sensitivity (15.6 ± 0.47 mm), (11.3 ± 0.47 mm), (11.0 ± 0.0 mm), (15.0 ± 0.0 mm), (15.0 ± 0.0 mm), (16.0 ± 0.0 mm), (11.3 ± 0.47 mm), and (10.6 ± 0.47 mm) of *Staphylococcus aureus*. Msn-24, Bsn-15, Bsn-14, Bsn-21, Bsn-35 showed maximum inhibition (14.0 ± 0.0 mm), (11.0 ± 0.0 mm), (11.0 ± 0.0 mm), (11.0 ± 0.0 mm) and (11.0 ± 0.0 mm) of *Pseudomonas aeruginosa*. Whereas Msn-20

showed maximum inhibition of *Klebsiella pneumonia* (12.0 ± 0.0 mm). Activity index revealed that tested organometallic-based synthetic drugs were more effective against bacterial pathogens compared to Oxy-tetracycline, Tetracycline, and Amoxicillin. Crystal violet assay and cell proliferation inhibition assay supported the outcomes of agar well diffusion method. Current findings concluded that organometallic-based synthetic compounds have potential bactericidal effects against infectious pathogens and could be used as forthcoming antibacterial agents.

BACTERICIDAL EFFECT OF SPIROGYRA EXTRACT AGAINST NOSOCOMIAL PATHOGENS

Neelofar Tahir¹, Saiqa Andleeb^{1*} and Kaleem Imdad²

¹*Microbial Biotechnology Lab, Department of Zoology, University of Azad Jammu and Kashmir, King Abdullah Campus, Chattar Kalas, Muzaffarabad*

²*Bioscience Department, COMSATS University, Park Road, Chak Shahzad, Islamabad*

*Corresponding Author: drsaiqa@gmail.com ; drsaiqa@ajku.edu.pk

In the present study, 13 nosocomial pathogens both antibiotic resistant and non-resistant were chosen to check the anti-bacterial efficacy of spirogyra extract. The efficient bactericidal effect of Spirogyra algae has been recorded against nosocomial pathogens via different techniques. Fresh algae and boiled extract of spirogyra were prepared. The objectives of the current study were 1) to evaluate the bactericidal effect of Spirogyra extracts against nosocomial pathogens through agar well diffusion method, 2) to screen the bioactive compounds of Spirogyra extracts through thin layer chromatography, 3) To evaluate the bactericidal effect of bioactive compounds associated with Spirogyra extracts against nosocomial pathogens through direct bioautography. Results revealed that all the 13 microbes shown the significant zone of inhibition when fresh algae were applied. Thin layer chromatography showed the presence of bioactive compounds in spirogyra which also possessed antibacterial efficacy against tested bacterial strains. In bioautography two solvent systems are used. TLC plates of both solvent systems showed the inhibition of tested bacteria. Spirogyra possessed bioactive compounds having antibacterial effect. Bioactive compounds could be synthesized and modified according to multidrug resistant developing microbes. It was concluded that bioactive compounds would have great importance in the field of pharmaceutical industry and could be used as potential therapeutic agent against bacterial infections.

LOCALLY ISOLATED BIOFILM FORMING BACTERIAL STRAINS SHOWED BIOREMEDIATION OF HEAVY METALS

Noor Muhammad*, Iram Liaqat* and Waiza Ansar

Microbiology Lab, Dept. of Zoology, GC University, Lahore

*Corresponding Author: iramliaq@hotmail.com, noormuhammadkhan000@gmail.com

Environmental pollution by heavy and toxic metals because of tanning, metallurgic processes, and other chemical industries is a worldwide problem distressing both human health and the environment. Heavy metals are present in the soil, but higher concentration of these elements are damaging to plants, animals, and humans. The aim of this study was to examine the synergistic outcome of bacterial biofilms on the bioremediation of Zn, Co, and Cu from contaminated soils. Total 2 biofilm forming strains (AF1 & 5A) were isolated. The isolated strains were characterized morphologically and biochemically. Ribotyping confirmed that *Lysinibacillus* sp. AF1 belongs to *Lysinibacillus sphaericus* (OP589134), and *Bacillus* sp. 5A belongs to

Bacillus sp. (OP586601). Both strains showed mature biofilm formation on 5th day of incubation. Using biofilm of *L. sphaericus*, *Bacillus* sp. and a mixture of both strains remediated Zn up to 1.46, 0.51 and 0.42 mgmL⁻¹, respectively. Likewise, *L. sphaericus*, *Bacillus* sp. and a mixture of both strains remediated Co up to 0.37, 0.35 and 0.47 mgmL⁻¹, respectively. *L. sphaericus*, *Bacillus* sp. and consortia remediated Cu up to 0.43, 0.78 and 0.48 mgmL⁻¹, respectively. This study suggested that the tanneries effluent of Kasur, can be a source of heavy metal remediating microbial flora. In this study, both isolated bacteria strains showed excellent metals (Zn, Cu and Co) bioremediation activity. These findings indicated that these bacterial isolates have the ability to degrade heavy metals such as Co, Zn and Cu suggesting as potential biological tools of bioremediation.

TRANSMISSION OF *STREPTOCOCCUS PNEUMONIAE*: NASOPHARYNGEAL CARRIAGE IN CHILDREN IN URBAN AND PERI-URBAN REGIONS OF QUETTA, PAKISTAN

**Niamatullah Kakar^{*1}, Irfan Shahzad Sheikh², Ramsha Tariq², Habib ur Rehman³,
Mujeeb ur Rehman⁴ and Jameel ur Rehman⁵**

¹Department of Natural and Basic Sciences, University of Turbat, Kech

²Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta

³Department of Microbiology, University of Balochistan, Quetta

⁴Dr. Ishrat-ul-Ebad Khan Institute of Oral Health Sciences, Dow University of Health Sciences, Karachi

⁵Bolan Medical College, Bolan University of Medical and Health Sciences, Quetta

*Corresponding Author: Niamatullah.kakar@uot.edu.pk

Streptococcus pneumoniae (SP) resides in the upper respiratory tract of humans. Pneumococcal carriage is the first step towards pneumococcal infection, which may lead to invasive and non-invasive infections in children under the age of 5 years. The purpose of this study was to evaluate the prevalence of pneumococcal carriage, transmission patterns, virulence gene determination, and growth behavior. Nasopharyngeal samples were taken from the upper respiratory tract. The pneumococcal diagnosis was carried out by cultivating the sample on blood agar plates and the growth was determined by using classical and standard tests. The results showed (72%) pneumococcal growth on culture plates. A significant (29.67%) increase in carriage rate was observed at an early age of 2-24 months. The genes associated with virulence, colonization, and glutamine and arginine regulatory genes were determined from the isolates. Pneumococcal isolates showed varying growth behavior. The isolates exhibited varying degrees of antibiotic resistance to trimethoprim (42%), amoxicillin (28%), and oxacillin (21%). Close living conditions such as living in crowded houses, and attending school were identified as risk factors for pneumococcal carriage. Household air pollution, passive smoking, and the use of coal, wood, and dung for cooking and heating were contributing to pneumococcal carriage. In conclusion, considerable pneumococcal carriage was found in children under 5 years. Notably, genes implicated in pneumococcal colonization in the nasopharynx and pathogenicity in humans, as well as variations in growth patterns, reflect the predominance of pneumococcal serotypes that cause disease in the community.

RANDOM MUTAGENESIS OF *CORYNEBACTERIUM GLUTAMICUM* FOR ENHANCED PRODUCTION OF L-LYSINE

Amjed Hussain^{1*}, Hamid Mukhtar², Umar Farooq Gohar², Faraz Akrim¹, Adeena Anwar¹, Sehrish Nazir¹ and Muhammad Taimoor Chisti¹

¹Department of Zoology, University of Kotli-11100, Azad Jammu and Kashmir

²Institute of Industrial Biotechnology, GC University, Lahore

*Corresponding Author: amjedbiotech@yahoo.com

Corynebacterium glutamicum is a versatile biotechnological working horse, producing L-lysine in multi-million tons per year. In present study wild type L-lysine producing strain of *C. glutamicum* IIB646 was selected. By using suitable and optimized fermentation medium wild strain produced 5.5 g/L L-lysine in 250 ml of shaking Erlenmeyer flask. Then this wild strain was subjected to ultraviolet (UV) treatment. For this purpose, fresh wild strain was plated on nutrient agar plates and exposed to a suitable concentration of UV light for different time intervals i.e. 10 min to 45 min, until 90% death rate observed after incubation of 24 hrs to 72 hrs in nutrient agar plates. Out of 30 homoserine auxotroph mutants, only three mutants (IIB646^{UV9}, IIB646^{UV15} and IIB646^{UV24}) showed higher amount of L-lysine production as compared to wild strain. Auxotrophic mutant IIB646^{UV9} produced 5.7 g/L L-lysine and IIB646^{UV24} showed 5.6 g/L L-lysine production. Maximum 5.9 g/L L-lysine was produced by auxotrophic mutant IIB^{UV15} in the optimized fermentation medium. Furthermore, wild strain of *Corynebacterium glutamicum* was also subjected to chemical mutagenesis. For this purpose, chemical mutagens *N*-ethyl-*N*-nitrosourea (ENU), ethyle methane sulfonate (EMS) and nitrous acid (NA) were used in different concentrations for different time intervals, until 90% death rate was observed on nutrient agar plates after incubation of 24 hrs to 72 hrs. Eight homoserine auxotroph mutants obtained by NA treatment. Out of these eight homoserine auxotrophs only two mutants i.e., IIB646^{NA11} and IIB646^{NA21} showed increased L-lysine production i.e. 5.8 g/L and 5.6 g/L respectively. By using ENU three mutants namely IIB9^{ENU12}, IIB9^{ENU20} and IIB9^{ENU45} showed higher L-lysine production as compared to wild strain. IIB9^{ENU12} was found to produce 6.0 g/L, IIB9^{ENU45} produced 6.1 g/L and IIB9^{ENU20} produced 6.3 g/L of L-lysine. Two mutants IIB9^{EMS18} and IIB9^{EMS22} obtained by EMS treatment, showed enhanced production of L-lysine and maximum 7.3 g/L L-lysine was produced by IIB9^{EMS18} in suitable fermentation medium.

CHARACTERIZATION OF POLYCLONAL ANTIBODIES FOR CHLORAMPHENICOL DETERMINATION IN ANIMAL-DERIVED FOOD EMPLOYING RADIOTRACER TECHNIQUES

Shumaila Ameer, Uzma Maqbool, Ismail Chughtai, M. Yasin, Mehwish Mumtaz and Bilal Aslam

Nuclear Institute for Agriculture and Biology (NIAB-C) PIEAS, Faisalabad

^{*}International Atomic Energy Agency (IAEA), Vienna, Austria

*Corresponding Author: sh.cvas@gmail.com

Chloramphenicol (CAP) is a cheap and effective broad-spectrum antibiotic, used as therapeutic and prophylactic agent in animals. However, in humans it may cause many side effects like irreversible aplastic anaemia, hypersensitivity reactions and Grey baby syndrome in neonates. Consequently, it has been banned in food-producing animals since 1994. The EU set its Reference

Point of Action (RPA) 0.15 µg/Kg for all food products. In present study, efforts were made to develop and validate antibodies in ELISA and CHARM techniques for detection of CAP in milk and tissue samples. By using carbodiimide protocol, CAP was coupled with human serum albumin (HSA), bovine thyroglobulin (BTG) to prepare immunogens (CAP- HSA and CAP-BTG) and with horseradish peroxidase (HRP) to prepare enzyme tracer (CAP-HRP). Polyclonal antibodies were produced in rabbits. Different dilution levels of antibodies (Ab) and enzyme tracer were evaluated for better sensitivity through checkerboard and the optimal dilutions @ 1:2K (Ab) and 1:10K (tracer) were selected. Different standards including 0, 0.1, 0.2, 0.4, 0.8 and 1.6 ppb were used for preparation of standards curve. From the calibration curve, IC₂₀ was calculated 0.1 ppb and IC₅₀ 0.15 ppb. The concentration of residues in unknown samples was calculated by interpolating the relative absorbance (%) in calibration curve. Total 20 samples bovine milk, collected from District Jhang (Punjab), Pakistan was analyzed. Results indicated that 04 milk samples were found positive with maximum concentration 0.52 ppb, by following the EU RPA for all food matrices 0.15 ppb.

MICROBES IN AQUACULTURE: NEW TRENDS AND APPLICATION IN FRESHWATER FISH CULTURE

Muhammad Younis Laghari*

Department of Fisheries and Aquatic Sciences, University of Sindh, Jamshoro

*Corresponding Author: laghariyounis@yahoo.com

Microbial community play most important role in aquatic ecosystem. These microbes has great role in fish growth and aquaculture production. Unfortunately, the farmers are unaware from these useful creatures. Nowadays, the trend of fish farming is developed to re-circulatory aquaculture system (RAS) to increase production and reduce the investment/management cost to increase the profit. However, sometimes, it has been observed that even the growth of fish is decreased in RAS without apparent changes in water quality. There is a great importance of microorganisms in aquaculture, where they occur naturally. However, they can be added artificially by applying different roles. Even, these microbes play an important role in the degradation of organic matter and recycling nutrients, along with nutritional support to fishes. Even some microorganisms may protect fish and larvae against diseases. But if not managed/utilized properly, they may cause to infect or kill the fish and their larvae. However, manipulating the microbes and monitoring them in aquaculture systems hold great potential to assess and improve the water quality as well as to control the development of microbial infections. While there is an utmost need for research to determine the microbiomas of healthy aquaculture systems, we also need to develop authentic methods for the successful manipulation of microbes as well as engineer these microbiomas. Hence, we should develop a plan to utilize and get full advantage from these microbial interactions for the successful management of aquaculture through advanced research and technology.

FUNGUS GROWING TERMITES AND TERMITOMYCES AS A SYMBIONTS: AN ALTERNATIVE HIGHLY NUTRITIOUS FOOD SOURCE UNDER CLIMATE CHANGE

Maid Zaman*, Abid Fareed, Rashid Azad and Ahmad Faraz

¹Department of Entomology, The University of Haripur, Off Hattar Road, Haripur

*Corresponding Author: Maidzaman@uoh.edu.pk

Termites are eusocial insects which are known as a pest in the urban areas and ecological engineers in the wild but it also has a symbiotic relation with the fungus. It grows Termitomyces fungi in their combs which is essential for the breakdown of plant matter inside the mounds and a highly nutritious food source. In

present study we have investigated the available knowledge about the termite's species associated with fungi. Continent Africa has the highest number of symbiotic termite's species (26 species) while continent North America with least number of species (02). Similarly, Nigeria has 11 fungus growing termite's species followed by India (10 species) and Pakistan (04 species) while Sri Lanka, Indonesia and Zimbabwe has the least number of species (01 species) in which only 08 reference sequence and 05 COII sequence is available for the reported symbiotic species. Overall higher number of fungi growing termites belong to the genera *Acanthotermes*, *Odontotermes*, *Macrotermes*, respectively along with 08 other genera. Out of 26 known fungus species only 04, 03 and 01 are present in Pakistan, India and China with 24 available Mycobank numbers only. Termites associated with fungus are poorly known in Pakistan and needs further exploration.

EFFECT OF PROBIOTIC SUPPLEMENTATION ON PERFORMANCE, HEMATOLOGICAL INDICES AND ANTIBODY TITER LEVEL AGAINST NEWCASTLE DISEASE IN VACCINATED BROILER CHICKENS

Samina Younas^{1*}, Dil Ara Abbas Bukhari¹, Abdul Rehman², Zuhra Bibi¹ and Arif Ullah

¹*Microbiology Lab, Zoology Department, GC University, Lahore*

²*Institute of Microbiology and Molecular Genetics, University of Punjab, Lahore*

*Corresponding Author: saminazain363@yahoo.com

The current study aimed to analyze the effect of probiotics on body weight gain, hematological Indices, biochemical parameters and immune response against Newcastle disease. A total of 90 chickens were assigned in nine groups (n=18) in a random fashion. The four groups (G1, G2, G3 and G4) were vaccinated with the Newcastle vaccine (LaSota strain), except G5 which act as a control. Vaccinated groups were supplemented with two laboratory isolated probiotic strains GCU-DAB- S-11 (*Bacillus cereus*, OR563784.1) and GCU-DAB- S-18 (*Pediococcus acidilactici*, OR563798.1). The control group was fed with only basal diet for 42 days of trial. The results showed that vaccinated groups of chicken had higher titer values in G3 followed by G2. Body weight and relative weight of the bursa of Fabricius, thymus and spleen had significantly increased in first three groups as compared to G4 (only vaccinated). Liver enzymes (ALT, AST, ALP) level was reduced as compared to the control. Some biochemical indices also improved such as the increase in RBC count, level of hemoglobin and HDL values as compared to G4 (only vaccinated) and G5 (control). Cholesterol levels decreased significantly in probiotic treated groups. In conclusion, probiotics in vaccinated chickens enhance the immune system by producing antibodies, which provide the better safety level against pathogens.

EFFECT OF PROBIOTICS ON HEMATOLOGY AND HISTOPATHOLOGY OF ORGAN ASSOCIATED WITH IMMUNE SYSTEM IN MALE AND FEMALE WISTAR RATS

Zuhra Bibi¹, Dil Ara Abbas Bukhari¹, Abdul Rehman², Naureen Fatima, Muhammad Qadeer Sarwar, Arif Ullah, Samina Younas and Tayyab Manzoor

¹*Microbiology Lab, Department of Zoology, GC University, Lahore*

²*Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore*

*Corresponding Author: zuhrabibi@gcu.edu.pk

In the present study, probiotics were isolated from dairy products and characterized on a molecular basis. Male and female (16+16, n=4) Wistar rats were randomly assigned to 5 groups to revise the effects of isolated probiotics on the gut immune system: 0-day, negative control, positive control

(commercial product *Lactobacillus acidophilus*-14), and laboratory isolated strains Pro-3 (*Weissella confusa* MZ735961) and Pro-4 (*Lactoplantibacillus plantarum* MZ727611). Aspartate-aminotransferase, Interleukin-6, bilirubin, and Alanine-transaminase values in both genders were expected, but AST and creatinine values had little increase in the female group compared to the male probiotic treatment group. Cellular blood count level of mean corpuscular hemoglobin in male groups showed no considerable differences ($p > 0.95$), while there were differences ($p < 0.00$) in female groups. The level of mean corpuscular hemoglobin concentration (MCHC) showed distinct changes ($p < 0.02$) in male groups, while these values were insignificant changes ($p > 0.05$) among female groups. Histopathological results revealed no damage to the thymus, liver or colon. The total thickness of the colon wall, crypt depth, external muscle and mucosa were significantly ($p < 0.05$) increased in rats given probiotics. Overall, probiotic supplementation stimulates the immune system and may be able to protect the intestinal mucosa by strengthening the gut's immune system and promoting intestinal efficiency. *L. plantarum* can therefore offer a reliable, affordable, and safe treatment for functional gastrointestinal illnesses.

INVESTIGATING THE ANTIBACTERIAL AND BIOFILM INHIBITORY POTENTIAL OF MUSHROOM CONJUGATED SILVER NANOPARTICLES

Isbah Fatima* and Iram Liaqat*

Microbiology Lab, Department of Zoology, Government College University, Lahore

*Corresponding Author: dr.iramliaqat@gcu.edu.pk, isbahwahla555@gmail.com

Nanotechnology is a rapidly growing field which has a variety of applications in biomedicine, food and engineering due to the exemplary characteristics of nanoparticles such as biocompatibility, high productivity, speed of production, cost effectiveness and safety. Silver nanoparticles (AgNPs) are considered potent nano-weapons that can be used as active antimicrobial agents in the extermination of many types of commonly occurring bacteria. The present study demonstrates an economical and eco-friendly method for the synthesis of AgNPs using the enoki mushrooms. The synthesized mushroom conjugated silver nanoparticles (M-AgNPs) were characterized and confirmed by UV- visible spectroscopy and fourier transform infrared spectroscopy (FTIR) analysis. The UV-vis absorption spectrum of the M-AgNPs gave a characteristic peak at 432nm. FTIR spectrum of M-AgNPs showed peaks at 3206 cm^{-1} , 2948 cm^{-1} , 1408 cm^{-1} and 1033 cm^{-1} indicating. The broadband at 3206 cm^{-1} was due to the -NH₂ and O-H stretching. The peak at 2948 cm^{-1} was attributed to the stretching vibration of the C-H groups. Peaks at 1408 cm^{-1} and 1033 cm^{-1} were ascribed to the O-H bending vibration and stretching vibration, respectively. The potential impacts of M-AgNPs on food safety and control will be evaluated by determining the antimicrobial activity of the synthesized M-AgNPs against different bacteria isolated from wound samples. The findings of current study will highlight the potential of M-AgNPs for use in various biomedical applications including controlling the growth of different bacteria isolated from wounds.

EVALUATION OF PLASTIC BIODEGRADATION BY MUSHROOM CONJUGATED SILVER NANOPARTICLES

Asad Ali and Iram Liaqat*

Microbiology Lab, Department of Zoology, Government College University, Lahore

*Corresponding Author: dr.iramliaqat@gcu.edu.pk, aleeasad84@gmail.com

Nanoparticles have been proven more beneficial in the field of environmental remediation. Silver nanoparticles (AgNPs) are being used for various applications such as removal of harmful or toxic

substances and fabrication of environmental sensors. The current study is focused on the mushroom conjugated silver nanoparticles (M-AgNPs)-based degradation of polyethylene. Using enoki mushroom, AgNPs were synthesized and checked for the degradation ability of high-density polyethylene (HDPE) and low-density polyethylene (LDPE). The M-AgNPs were characterized using UV-visible spectroscopy and fourier transform infrared spectroscopy (FTIR). The M-AgNPs gave a characteristic peak at 432nm at the UV-vis. FTIR spectrum of M-AgNPs showed peaks at different positions. The peak at 3206 cm^{-1} indicated $-\text{NH}_2$ and O-H stretching, 2948 cm^{-1} was attributed to the stretching vibration of the C-H groups, 1649 cm^{-1} was due to the amide, 1408 cm^{-1} and 1033 cm^{-1} were ascribed to the O-H bending vibration and stretching vibration, respectively. The $-\text{NH}_2$ and $-\text{OH}$ are most likely related to mushroom extract. Plastic biodegradation findings showed average weight loss of 11.6% in HDPE and 26.3% in LDPE after 4 weeks. The FTIR analysis of HDPE and LDPE degraded products showed the characteristic peaks representing the alcohols, phenols and other smaller components which indicated the breakdown of the plastic. FTIR, SEM and GC- MS analysis will be done in future to check the plastic degradation potential of M-AgNPs. This method has been successful in the biodegradation of plastic by using M-AgNPs. Findings of this study are expected to be ecofriendly and safe option for bioremediation.

ZINC OXIDE CHITOSAN BASED NANOMATERIALS AS ANTIMICROBIAL FOOD PACKAGING

Shiza and Iram Liaquat*

Microbiology Lab, Department of Zoology, Government College University, Lahore

*Corresponding Author: dr.iramliakat@gcu.edu.pk, shizaasif314@gmail.com

Food insecurity and economic loss are the major problems caused by microbial contamination of food. Globally, food waste is an environmental problem. Subsequently, good packaging is essential to protect the food from spoilage and contamination. Usually, plastic packaging is used which is rarely recyclable, bad for the environment and causes serious health hazards. Bio-based materials are becoming popular for food packaging as they are biodegradable, renewable and have small impact on the environment. Hence, the current study aimed to synthesize zinc oxide chitosan based nanoparticles (ZnO-Ch-NPs) as biodegradable food packaging material. For this purpose, ZnO-Ch-NPs were synthesized and characterized using ultraviolet-visible (UV-VIS) spectrophotometry, fourier transforms infrared spectroscopy (FTIR), x-ray diffraction (XRD), scanning electron microscope (SEM), transmission electron microscopy (TEM) and zeta potential. Following that, ZnO-Ch-NPs were impregnated using chitosan film leading to the formation of ZnO-Ch-film. Next, 05 bacterial strains were isolated from spoiled chicken and characterized up to genus level based on morphological, biochemical and differential media growth. Biofilm time kinetics of all isolates were determined using the crystal violet (CV) test tube staining method. Antibacterial and antibiofilm activities of ZnO-Ch-film were determined at various concentrations. UV- spectroscopy showed the strongest peak at 360nm (narrow bandgap) indicating that the ZnO-Ch-NPs were absorbing light with a wavelength corresponding to that value. FTIR results showed characteristics peaks (1539, 1432, 1003 and 782 cm^{-1}) associated with the presence of functional groups i.e. $-\text{OH}$, $-\text{NH}_2$ and $-\text{COOH}$. The findings of current study will highlight the potential of ZnO-Ch-film for used in food packaging including controlling the growth of different bacteria isolated from spoiled chicken.

CD⁺² RESISTANCE MECHANISM IN *CANDIDA TROPICALIS* 3Aer: A POTENTIAL TOOL TO ERADICATE TOXIC METAL IONS FROM THE ENVIRONMENT

Abdul Rehman

Department of Microbiology and Molecular Genetics, University of the Punjab, New Campus, Lahore

*Corresponding Author: rehman.mmg@pu.edu.pk

The present study examines the bioremediation potential and cadmium-induced cellular response on a molecular level in *Candida tropicalis* 3Aer. Spectroscopic analysis clearly illustrated the involvement of yeast cell wall components in biosorption. Cadmium bioaccumulation was confirmed by TEM, SEM, and EDX examination. TEM images revealed extracellular as well as cytoplasmic and vacuolar cadmium nanoparticle formation, further validated by presence of *ycf1* gene and increased biosynthesis of GSH under cadmium stress. Fourteen proteins exhibited differential expression and during cellular redox homeostasis are found to involve in nitrogen metabolism, nucleotide biosynthesis, and carbohydrate catabolism. Interestingly, *C. tropicalis* 3Aer is equipped with nitrile hydratase enzyme, rarely been reported in yeast. It has the potential to remove nitriles from the environment. The Cd⁺² toxicity not only caused growth stasis but also upregulated the cysteine biosynthesis, protein folding and cytoplasmic detoxification response elements. The present study suggests that *C. tropicalis* 3Aer is a potential candidate for bioremediating environmental pollution by Cd⁺².

SURVEILLANCE OF MULTI DRUG RESISTANT *marR* GENE IN HOSPITAL SEWAGE DISCHARGE IN SOUTH PUNJAB, PAKISTAN

**Kiran Ashiq^{1*}, Muhammad Tariq Mahmood¹, Huma Naz¹, Firassat Hussain¹,
Santosh Kumar¹ and Tanveer Ahmed²**

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Life Sciences, Khwaja Fareed University of Engineering and
Information Technology, Rahim Yar Khan

Corresponding Author: kiranashiq820@gmail.com

The increasing prevalence of multidrug-resistant bacteria poses a significant threat to public health. Hospital sewage discharge has been identified as a potential reservoir for antibiotic resistance genes, including the *marR* gene, which confers multidrug resistance. This study aimed to investigate the surveillance of the multidrug-resistant *marR* gene in hospital sewage discharge in South Punjab, Pakistan. Sewage samples were collected from six district hospitals in South Punjab, and DNA extraction protocols, agarose gel electrophoresis, and DNA quantification methods were employed to prepare DNA sequencing libraries. Bioinformatics analysis using assembly-based and alignment-based approaches was conducted, and the Antibiotic Resistance Database (CARD) was used for identification. Descriptive statistics were used to analyze the data, allowing for the determination of the prevalence of the *marR* gene and antibiotic resistance patterns. The results showed variations in the presence of the *marR* gene among the sampled sites. The Bahawalpur BVH site had no instances of the *marR* gene, while Deara Ghazi Kahn DHQs had 2,304 instances, resulting in *marR* percentages of 0% and 1% from ARGs, respectively. Lodhran DHQs and Multan DHQs sites also had no instances of the *marR* gene, while Muzaffargarh DHQs had 2,479 instances, resulting in *marR* percentages of 0% and 2% from ARGs, respectively. The Rahim Yar Khan Sheikh Zayed hospital sites either had no instances or extremely low numbers of the *marR* gene. The data highlighted the varying prevalence of the *marR* gene in hospital sewage discharge and its potential as a reservoir for antibiotic resistance.

ANTIMICROBIAL PEPTIDES: INNOVATIVE THERAPEUTIC AGENTS IN FISH EPIDERMAL MUCUS

Iqra Zulfiqar^{1*}, Huma Naz¹, Nimra Zahid¹, Amna Marium², Tanveer Ahmed³

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, The Government Sadiq College Women University, Bahawalpur

³Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

*Corresponding Author: iqrach1028@gmail.com

Antimicrobial peptides (AMPs) are small, usually cationic, and amphiphilic molecules generally considered as an essential component of innate immunity, thereby providing the first line of defence against wide range of pathogens including bacteria, fungi, yeast, protozoa, and viruses. They can also kill the pathogens which are generally resistant to number of antibiotics, thereby considered alternatives to classical antibiotics and may become an excellent candidate for tackling antimicrobial resistance. Fish epidermal mucus is a great source of these peptides, as it contains all of the major classes of AMPs, including defensins, cathelicidins, hepcidins, histone-derived peptides, and a fish-specific class of the cecropin family, called piscidins. These AMPs primarily target the membranes of pathogens, disrupting their integrity and some also have immunomodulatory effects, influencing the host's immune response. These peptides exhibit broad-spectrum antimicrobial activity, killing both fish and human pathogens. This review provides an overview of the structures, functions, and putative mechanisms of major families of fish AMPs and highlighted how fish AMPs can be used as a novel therapeutic tool which is the theme of future research in drug development.

TOXICITY OF LOCAL ISOLATES OF *BACILLUS THURINGIENSIS* AND CRY2AB PROTEIN AGAINST LEPIDOPTERAN PESTS

Saddam Hussain and Munawar Saleem Ahmad*

Department of Zoology, The University of Swabi, Anbar-Swabi

*Corresponding Author: saleemsbs@gmail.com, saleemsbs@uoswabi.edu.pk

Vegetables are a vital source of nutrients and are frequently consumed as food. Many lepidopteron species behave as pests and have an impact on crop yield. *Bacillus thuringiensis* isolates were obtained from 15 soil samples taken in the Swabi KPK district. The isolate was further characterized morphologically and biochemically. The goal of the current study was to evaluate the biocidal efficacy of local isolates of *Bacillus thuringiensis* in district Swabi and a reference strain (cry2Ab) against common lepidopteran pests (*Spodoptera litura* and *Helicoverpa armigera*). Vegetable plants were grown in a small field with narrow trails that were covered in field. Similarly, in order to obtain the most colonies for the formulation of spore/crystal mixtures with varied concentrations (100 µg/ml to 1000 µg/ml), *Bacillus thuringiensis* (Kurstaki species) bearing active cry2Ab were grown under control laboratory conditions using L.B media. Additionally, several pests' larvae were reared in lab settings under strict controls. The findings of the investigation of comparative potencies showed that the local isolate SH-BT-7 exhibits significant potency, causing 90% death in *H. armigera* at 300 µg/ml and *S. litura* at 500 µg/ml. The LC₅₀ of *Bacillus thuringiensis* cry2Ab endotoxins against *S. litura* were 277.27 µg/ml and *H. armigera* were 138.95 µg/ml. The overall findings showed that local isolates of *BT* might effectively and sustainably reduce crop yield losses by eradicating lepidopteran pests. According to the results of our study, *BT* isolate biocidal activity against several lepidopteran pests indicates promising results for pest control in pest management.

COLIFORM CONTENTS OF AIR CURRENTS GENERATED BY TRAINS AT LAHORE RAILWAY STATION

Kaukab Malik and Javed Iqbal Qazi

Institute of Zoology, University of the Punjab, Lahore

*Corresponding Author: kokabmalik95@gmail.com

The growth of Coliform bacteria was observed. Samples of above bacteria were taken (Three trains; Taiz Gham, Green Line and Jaffer Express) grown on Levine EMB agar plates in February of 2020 (8-2-20,10-2-20,13-2-20). Morphology of each colony was observed, colong designation was also done on nutrient agar, again morphology was observed. The final designation of pure culture of L-EMb agar G. vials as G+ive bacteria namely Escherichia Coli, Klebsiella Pneumoniae and Bacillus cereus (colorless) and Salmonella Typhimurium G+ive. The Final designation of pure culture on (L-EMB agar G. vials) named as 1A1a, 1Aa2, 1Aa3, 1B2b, 1B3b, 1C1c, 1C3c, 2A1a, 2A2a, 2A3a, 2A4a, 2B1b, 2B2b, 2B3b, 2C1c, 2C3c, 3A1a, 3B1b, 3B2b, 3C1c, 3C2c. Growth on slant is +ive. Antibiotic resistance/sensitivity test were done (above 07) named as 1A1a, 2C3c, 2A3a, 3A1a, 2C1c, 3B1b, and the zone of inhibition were measured.

PREVALENCE OF COLIFORM BACTERIA IN DIFFERENT SAMPLES OF STREET FOOD FROM LAHORE

Fouzia Tabssum* and Iram Yousaf

Department of Zoology, University of Education Bank Road Campus, Lahore

*Corresponding Author: fouzia.tabssum@ue.edu.pk

The objective of this study is to estimate the presence of Coliform bacteria in ready to eat food obtained from different streets of Lahore, Pakistan. Coliforms were isolated from five different food samples by spread plate method using eosin methylene blue agar medium for enumeration. Bacterial colonies were further streaked on nutrient and Selective media (EMB) to obtain pure culture. Presence of coliforms were further confirmed by various bio-chemical reactions. Antibiotics susceptibility was analyzed using antibiotics' discs of different concentration. Result showed that all the samples were contaminated with coliform bacteria with 3.8×10^4 C.F.U./ml, 9.6×10^4 C.F.U./ml, 380×10^4 C.F.U./ml, 15.5×10^4 C.F.U./ml and 400×10^4 C.F.U./ml for Gol Gappy, Dahi Bhaly, Beef Pulao, Chana Chat and Suji Halva respectively. *E. coli* 57.14%, *Enterobacter* 14.2%, *Klebsiella* 14.2%, *Salmonella* 7.1% and shigella 7.1% was detected in these food samples. Out of 14 isolates of coliforms 78.5% were resistant to Cefoperazone (CFP30) while 71.4% were susceptible to Chlorpromazine (CPZ30). It is necessary to spread awareness among the food sellers and consumers about importance of hygienic conditions of food so that any pathogenic illness can be avoided.

SPATIO-TEMPORAL VARIABILITY OF PLANKTONIC BACTERIA IN COASTAL WATERS OF ORMARA, BALOCHISTAN

Anis Fatima*, Pirzada J. A. Siddiqui, Seema Shafique and Zaib-un Nisa Burhan*

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: zaib.burhan@uok.edu.pk, anisfatima.ku@gmail.com

Bacteria are key component of microbial food web of marine ecosystems. They are significant precursor for the functioning of biogeochemical cycle in all marine habitats and comparatively well

understood worldwide. However, very little is known from northern Arabian Sea bordering Pakistan. This is a first attempt to examine variability in bacterial abundance and its relationship with anthropogenic alterations and fluctuations of environmental condition at two distinct stations (St. 1 and St. 2) at Ormara coast, Balochistan, Pakistan (Northern Arabian Sea). In general, findings of the present study revealed substantial difference in bacterioplankton abundance and environmental conditions on spatio-temporal scale. The average total bacterial abundance was 0.93×10^9 and 0.44×10^9 cells L⁻¹ at St. 1 and St. 2, respectively. Average values for water temperature were found closely resembled (24.8 °C and 24.5 °C) at St. 1 and St. 2 respectively. While, salinity was same (37.7 PSU) at both stations. Concentration of dissolved oxygen were recorded on average 7.2 mg L⁻¹ at St. 1 and 6.17 mg L⁻¹ at St. 2. According to statistical analysis, Pearson's correlation showed a strong relationship of total bacterial cell abundance mainly with Chlorophyll a (Chl a) at both stations. Overall, findings of the present study shown that St. 1 is more prone due to anthropogenic influence because of fishing activities of boats as compared to St. 2 where less human activities was observed. Further studies are recommended for better understanding of microbial communities at the base of microbial food web to envisage a healthy coastal ecosystem.

5. PHYSIOLOGY

PREVALENCE OF MIGRAINE AND ITS RELATION WITH MENSTRUATION AMONG THE STUDENTS OF LCWU, LAHORE

Shagufta Naz*, Saima Sharif, Akasha Anser and Farzana Rashid

Zoology department, Lahore College for Women University, Jail Road, Lahore

*Corresponding Author: shagufta6@gmail.com

The frequency of migraine is disproportionately higher in women than in men, especially throughout the reproductive years. Many women remark that their migraine attacks coincide temporally with their menstrual cycles, suggesting that hormonal changes may contribute to the etiology of migraine. The present study aimed to assess the prevalence of migraine and its relation with menstruation among the students of LCWU, Lahore. A cross-sectional study was conducted, consisting of 210 samples. A self-reported questionnaire consists of socio-demographic information, questions on menstruation, triggering factors of menstrual migraine, and family history. The age range of the sample was 18-24. Chi-square (χ^2) was used to test the association between two qualitative parameters using Statistical Package for the Social Sciences (SPSS), version 20. The results showed that 44.3% (n=85) had tension-type headaches, 30.2% (n=58) had migraine headaches, 24.5% (n=47) had cluster-type headaches and 1% (n=2) had unknown headaches. Out of 58 female migraineurs, 39% had migraine attacks before menstruation referred to as peri menstrual migraine, 15.3% had migraine attacks during menstruation referred to as pure menstrual migraine, 11.9% had migraine attacks after menstruation referred to as late menstrual migraine and 33.9% never had migraine changes with menstruation. Premonitory symptoms of menstrual migraine were more prevalent in the perimenstrual migraine than in the pure menstrual migraine and late menstrual migraine. There was a significant relationship between menstrual migraine and body mass index with a p-value of 0.010. Migraine and menstrual migraine were related to the family history. The study focuses on the triggering synergies and interrelation of triggers that precipitate menstrual migraine so a better understanding can be developed for the prevention, diagnosis, and treatment of menstrual migraine and non-menstrual migraine.

IMPACT OF IRON OVERLOAD ON HEMATOLOGICAL PARAMETERS AND HEPATIC VIRAL INFECTION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS

Dildar Ali Solangi^{1*}, Nasreen Memon^{1*}, Naseem Aslam Channa² and Aneel Kapoor³

¹*Department of Pathology, Liaquat University of Medical & Health Science, Jamshoro*

²*Institute of Biochemistry, university of Sindh, Jamshoro*

³*Department of Biochemistry PUMHS Shaeed Benazirabad*

Corresponding Authors: dildar.solangi@lumhs.edu.pk, nasreen_kousarbks@hotmail.com,
draneelrathi@gmail.com

Thalassemia is chronic genetic disorder worldwide, which causes disturbance in blood hemolysis and anemia (destruction of red blood cells). For the management of hemoglobin level frequent blood transfusion affect the biochemical parameters in thalassemia patients. In order to investigate the

haematological and biochemical variables (iron overload and hepatic damage) that affect TDT patients in Hyderabad and adjacent regions, the current study was conducted. A total of 142 individuals were interviewed throughout the research period, comprising 71 TDT patients and 71 control subjects (age and gender matched with no personal or family history of blood disorders). Serum ferritin, hepatitis C and B, blood groups, haematological parameters, complete blood count and liver function test (LFT) have been compared with control groups. When comparing TDT patients to the control group, we discovered that their serum ferritin level, total bilirubin, alkaline phosphatase ALP, urea, and creatinine were all considerably higher ($p < 0.05$). In contrast, this was not the case for haemoglobin (HB), mean corpuscular value (MCV), or mean corpuscular haemoglobin (MCH). When compared to control participants (males and females), TDT patients' blood ferritin level, total bilirubin (T-Bil) and SGPT were considerably higher ($p < 0.05$) in both genders. In contrast to male control participants, red blood cells (RBC), HB, HCT, and MCV were shown to be considerably increased ($p < 0.05$) in male patients and dramatically decreased ($p < 0.05$) in both genders. When compared to male control participants, there was an increase in urea and a decrease in ALP ($p > 0.05$) in both genders among TDT patients. Age-wise comparisons between TDT patients and control participants revealed substantially higher ($p < 0.05$) levels of blood ferritin, T-Bil and SGPT, across all age groups.

**EFFECTS OF WET CUPPING ON CLINICAL & LAB PARAMETERS IN PATIENTS
PRESENTING WITH MIGRAINE HEADACHES AT DR RUTH K. M PFAU
HOSPITAL, KARACHI**

Zeba Hussain Soomro^{1*} and Naseem Aslam Channa^{2*}

¹*Department of Neurology, Dr Ruth K M Pfau, Civil Hospital Karachi*

²*Institute of Biochemistry, University of Sindh Jamshoro*

*Corresponding Author: zeba.soomro10@gmail.com, nachanna2000@gmail.com

Cupping therapy is a formal treatment in Chinese hospitals and in some European countries. Middle East and Eastern Europe regions. Migraine is a chronic, paroxysmal and neurovascular disease. The Prophet (PBUH) said, "The best medicine with which you treat yourselves is Hijama, or it is one of the best of your medicines" (Reference: Al Bukhari, 5371). Cupping has also been widely used in the treatment of various major neurological disorders, like, headache, stroke & epilepsy. Hence, the present study was undertaken to study the effects of wet cupping on the serum lipid profile in migraine headache patients treated at Dr Ruth KM Pafau, Civil Hospital, Karachi. Total 102 patients of migraine headache with age range of 15 to 70 years were selected after signing in the written informed consent form. Among them 32 were males and 70 were females. The intravenous samples of each patient was collected first and then all patients were undergone the cupping therapy. After that the blood of cupping therapy was collected from each patient. The lipid profile of before cupping samples and cupping samples were analyzed by autoanalyzer. All the lipid profile were significantly ($p < 0.05$) increased in cupping samples as compared to before cupping samples, though remained in normal ranges. In gender-wise comparison, we get significant variation of lipid profile in before cupping samples as well as in cupping samples. Age-wise comparisons we did not find significant variations among all age groups of migraine headache patients in both group of before cupping and cupping samples. In conclusion, the extra lipids of peripheral tissues drained in cupping samples and keep the person healthy by not storing the extra lipids in his or her body. Cupping may have a relieving effect on migraine headache patients and clinicians may suggest this treatment to decrease their suffering and get them back to a healthy life.

CONTRIBUTION OF MITOCHONDRIA TO NEUTROPHIL BEHAVIORS AND RESPONSES

**Sehrish Gohar Ali, Amna Falak, Mehreen Tariq, Nouman Habib,
Fazeela Zahoor, and Muhammad Rizwan Alam***

Department of Biochemistry, Quaid-i-Azam University, Islamabad

*Corresponding Author: mr Alam@qau.edu.pk

Neutrophils are the primary immune cells that combat foreign stresses by effector responses like ROS formation, degranulation, cytokine production, phagocytosis, and through the release of neutrophils extracellular traps (NETs). Studies have reported that agonists-mediated changes in cytoplasmic calcium (cytCa^{+2}) levels contribute to exocytosis of antimicrobial proteins from neutrophils and NETs formation that orchestrates several defence mechanisms. However, the impact of mitochondrial calcium (mitCa^{+2}) homeostasis remains poorly explored in such host defence mechanisms of neutrophils. This study was aimed to investigate the role of mitCa^{+2} efflux in behaviours and phenotypes of neutrophils during host defence. The mitCa^{+2} efflux was pharmacologically blocked before stimulation of neutrophils. We employed bright field microscopy for morphological characterization of activated neutrophils and fluorescence microscopy for measuring release of DNA from stimulated neutrophils. The exocytosis of myeloperoxidase (MPO), phagocytic responses and post-stimulation viability of neutrophils were also evaluated. Our data demonstrated that inhibiting the mitCa^{+2} efflux distresses stimulant-induced neutrophil activation. This was shown by changes in neutrophil morphology, altered exocytosis of MPO, disturbed phagocytic response, and more release of DNA upon blocking mitCa^{+2} efflux in stimulated neutrophils. Thus, we can speculate that elevated levels of mitCa^{+2} in the matrix augment agonist-induced neutrophil activation. Our study highlights the pivotal role of mitCa^{+2} homeostasis in neutrophil physiology and antimicrobial functions.

EVALUATION OF EGG QUALITY, FERTILITY AND HATCHABILITY IN KASHMIRI RHODE ISLAND RED CHICKEN

Maira Ihsan¹, Beenish Shahid^{1*} and Muhammad Ijaz Khan²

¹*Department of Zoology, King Abdullah Campus, University of Azad Jammu and Kashmir, Muzaffarabad*

²*Department of Livestock and Dairy Development, Azad Government of the State of Jammu and Kashmir, Muzaffarabad*

*Corresponding Author: beenish.shahid@ajku.edu.pk

The fertility and hatchability of the eggs are traits of economic importance in poultry production. Kashmiri Rhode Island Red (RIR) breed maintained under intensive management system by Government poultry farm Muzaffarabad, was assessed for the study of productive performance. The birds were provided with a standard formulated chick mash throughout the brooding period, grower ration for an additional period and layers mash from 21 weeks onwards. The results revealed that up to week 40, Kashmiri RIR males and females had an average weight of 2.74 ± 0.05 kg and 2.02 ± 0.04 kg respectively. The mean age at sexual maturity was found to be 148.0 ± 1.22 days. The overall egg production percentage was 72.21 ± 2.40 with mean egg weight 52.94 g, shape index 77.90%, shell weight 4.94 g, shell thickness 0.30 mm, albumen weight 28.88 g, and yolk weight 16.26 g. The albumen and yolk height were 6.51 and 16.26 mm respectively. The fertility was 88.60% while hatchability was 93.48% based on fertile eggs. Significant positive correlations were observed between age/fertility (0.712), age/hatchability of fertile eggs (0.561), age/hatchability of all eggs (0.681), fertility/hatchability of fertile eggs (0.857), fertility/hatchability of all eggs (0.982) hatchability of fertile eggs/hatchability of all eggs (0.938). Whereas significant negative correlations were assessed between traits like age/dead in germ (-0.748),

shape index/dead in shell (-0.798), dead in germ/fertility (-0.748), dead in germ/hatchability of fertile eggs (-0.505) and dead in germ/hatchability of all eggs (-0.540). This study concluded that Kashmiri RIR breed is an improved dual purpose exotic breed acclimatized in local environment with better productive and reproductive performance.

PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF KASHMIRI BLACK AUSTRALORP CHICKEN

Sana Imtiaz¹, Beenish Shahid^{1*}, Muhammad Ijaz Khan² and Zeeshan Anjum²

¹*Department of Zoology, King Abdullah Campus, University of Azad Jammu and Kashmir, Muzaffarabad*

²*Department of Livestock and Dairy Development, Azad Government of the State of Jammu and Kashmir, Muzaffarabad*

*Corresponding Author: beenish.shahid@ajku.edu.pk

Kashmiri Black Australorp (KBA) breed after more than 200 successive generation were maintained under intensive management system, assessed for productive and reproductive performance. The birds were fed standard formulated chick mash throughout the brooding period, grower ration for an additional period and layers mash from 21 weeks onwards. At 40 weeks of age, the mean weight of roosters and hens was recorded as 2.68 kg and 2.32 kg respectively. Sexual maturity in hens was achieved at an age of 145.16 ± 1.08 days, with a body weight of 1.64 ± 0.02 kg. The overall egg production percentage recorded for 60 weeks was $63.18 \pm 2.10\%$. The study analyzed various egg quality characteristics, such as egg weight, length, width, shell weight, shell thickness, shell colour, shape index, albumen height, yolk height, weight of albumen and yolk, yolk colour, and Haugh unit to evaluate the relationships among them. The analysis revealed significant positive correlations between various characteristics, including a correlation coefficient of 0.90 between age and body weight, 0.74 between age and egg weight, 0.79 between body weight and egg weight, and 0.96 between egg weight and yolk weight. Moreover, fertility and hatchability percentages were found to be $89.56 \pm 0.55\%$ and $84.25 \pm 0.73\%$, respectively. The study also noted significant positive correlations between age and fertility (0.511), and between fertility and hatchability of all eggs (0.975), as well as between the hatchability of fertile eggs and the hatchability of all eggs (0.895). Conversely, significant negative correlations were assessed between traits like fertility and dead in germ (-0.741), dead in germ and hatchability of fertile eggs (-0.989), dead in germ and hatchability of all eggs (-0.868). This study concluded that KBA breed is an improved dual-purpose breed acclimatized in local environment with better productive and reproductive performance in intensive management system.

COMPOSITION AND PHYSICOCHEMICAL PROPERTIES OF MILK IN INDIGENOUS AND F₁ CROSSBRED COWS OF AZAD JAMMU & KASHMIR

Ramla Komal¹, Beenish Shahid¹, Fayzan Sultan Awan² and Muhammad Ijaz Khan²

¹*Department of Zoology, King Abdullah Campus, University of Azad Jammu and Kashmir, Muzaffarabad*

²*Livestock Development Research Centre, Department of Livestock & Dairy Development, Muzaffarabad*

*Corresponding Author: beenish.shahid@ajku.edu.pk

Milk composition is a fiercely disputed topic among the nutrition scientists, so it's crucial to know if crossbreeding of indigenous cows has an impact on milk composition. The present study was

conducted to evaluate and compare the milk yield, physicochemical properties and colostrum composition between indigenous cows and indigenous \times Jersey (F_1) crossbred cows of Azad Jammu and Kashmir region. A total of 43 lactating cows were selected, and milk analyses were performed by using Farm Eco lactoscan milk analyzer. Statistical analysis indicated that crossbreeding of indigenous cows with imported frozen thawed semen of Jersey bull significantly ($P < 0.001$) increased the daily milk yield and lactation length in F_1 hybrid cows (9.28 ± 0.34 liters and 320 ± 133.7 days respectively) compared to indigenous cows (1.80 ± 0.06 liters and 150.90 ± 5.81 days respectively). In indigenous cows, higher values ($P < 0.001$) of total solids (TS), fats, solids-not-fat (SNF), proteins and temperature were observed in milk at early lactation, but the freezing point and density were lower. At mid stage of lactation, only the level of TS, fats, proteins, salts and temperature were observed significantly higher ($P < 0.0001$) whereas during late lactation all the contents and properties of milk in indigenous cattle were significantly higher ($P < 0.0001$) except freezing point compared to F_1 crossbred cows. Comparison of colostrum composition between two breed groups indicated that percentages of TS, proteins, lactose, and temperature in indigenous cattle were significantly higher ($P < 0.01$) compared to F_1 crossbred cows. As the age advanced, the milk proteins decreased significantly ($P < 0.05$) at mid lactation stages and TS and protein decreased significantly ($P < 0.05$) during late lactation in indigenous cows. On the other hand, in F_1 cows, the level of SNF and lactose increased significantly ($P < 0.05$) while the temperature decreased significantly ($P < 0.05$). During winter season, level of all the milk constituents except fats decreased significantly ($P < 0.0001$) in indigenous cattle. But in F_1 crossbred cows the levels of fats, SNF and freezing point were significantly higher ($P < 0.0001$) during winter season while the levels of protein, temperature and density decreased ($P < 0.0001$) significantly. It is concluded that crossbreeding decreased the constituents of milk, however, milk yield and lactation length increased in F_1 crossbred cows.

EFFECT OF OBESITY ON FEMALE INFERTILITY IN DISTRICT HYDERABAD, SINDH, PAKISTAN

Nighah Fatima, Bakhtawar Soomro*, Shakeel Ahmed Memon and Saheefa Soomro

Department of Zoology, Government College University Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

Obesity can have an impact on maternal health and is a recognized modifiable risk factor for female infertility. Obesity are considered an epidemic on a global scale. There is strong evidence that obesity has an impact on women's fertility as well as the health of expectant mothers, fetuses, and newborns. Around (30 to 47 %) of obese women experience irregular menstruation. During the present studies, it was observed that there is a correlation between obesity and infertility of women, and it is considered as risk factor for women due to this female are more prone to be infertile. Currently, more than (100) samples were studied out that (59) samples were found with `infertility. Randomly samples were collected on the questionnaire basis. Current research was intent to examine the effects of obesity that leads to infertility and the alterations in the endocrine system and the hypothalamic-pituitary-ovarian (HPO) axis in obese women that ultimately effect the female reproductive system. Higher levels of insulin and androgens are also linked to obese women, and the latter can enhance the former's effects. The effects of these two abnormalities, which include poor follicular growth, premature luteinization, atresia of ovarian cells and follicles, and poor endometrial development, are the main causes of infertility.

PREVALENCE OF ANEMIA IN PREGNANT WOMEN IN DISTRICT HYDERABAD, SINDH, PAKISTAN

Arslan Sadq, Iqra Babar, Bakhtawar Soomro and Saheefa Soomro

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

A disorder known as anemia occurs when the body does not produce enough healthy red blood cells. The body's tissues receive oxygen from red blood cells. Various forms of anemia consist of: anemia brought on by a lack of vitamin B12. Anemia brought on by a lack of folate, or folic acid. This means more iron and vitamins are needed to make more red blood cells. During the current studies, the highest intensity of anemia was observed in Hyderabad district. Due to lack of sufficient iron, it causes anemia. Symptomatic appearance occur which includes, high mortality rate in pregnancy, abnormal baby development, and risks increases in delivery, death chances also increase in both mother and baby. Samples were collected on survey basis. Current studies need more attention and awareness programs in order to cure this disease or decrease the rate of mortality rate.

RISK FACTOR AND PREVENTION OF BREAST CANCER AT DISTRICT HYDERABAD, SINDH, PAKISTAN

Khadija Ansari, Bakhtawar Soomro*, Shakeel Ahmed Memon, Saheefa Soomro and Dhani Bux Mishori

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

Breast cancer is a multi-step process that involves several cell types, and it is still difficult to prevent globally. One of the most effective ways to stop breast cancer from spreading is to diagnose the disease early. Because of early prevention, the 5-year relative survival rate for patients with breast cancer is above 80% in several developed nations. Both our knowledge of breast cancer and the advancement of prevention measures have advanced significantly in the last ten years. Your risk of breast cancer is influenced by several variables. Certain things, like being a woman, getting older, and acquiring a DNA mutation connected to breast cancer, are unchangeable. However, by adopting healthier lifestyle choices, you can alter other risk factors including being overweight, not exercising frequently, smoking cigarettes, drinking alcohol, or consuming unhealthy food. The identification of breast cancer stem cells provides insight into the etiology and tumour drug-resistant processes, and numerous genes linked to the disease have been identified. In order to improve patients' quality of life, biological prevention has recently been created, while people currently have more pharmacological alternatives for chemoprevention of breast cancer. Present studies, highlights significant research on breast cancer pathophysiology, associated genes, risk factors, and preventive measures in this review, which spans several years. In the lengthy battle against breast cancer, these findings are but a minor step forward. The illness known as breast cancer is caused by aberrant breast cells that proliferate and develop into tumours. Tumours have the potential to grow throughout the body and become lethal if ignored. The milk ducts and/or the breast's milk-producing lobules are where breast cancer cells first proliferate. There is no risk to life from the early form (in situ). It is possible for cancer cells to invade neighbouring breast tissue. Tumours produced by this result in thickening or lumps. Metastasis is the process by which invasive tumours move to neighbouring lymph nodes or other organs. One can die from metastasis. Treatment is determined by the patient, the

cancer's type, and its extent of dissemination. Treatment consists of radiation therapy, surgery, and medicine. The strongest risk factor for breast cancer is female gender. Men are affected by breast cancer in a range of 0.5–1%. The care of breast cancer in men is based on the same concepts as in women. Ageing, obesity, heavy alcohol consumption, radiation exposure history, family history of breast cancer, reproductive history (including age at first pregnancy and menstruation onset), tobacco use, and postmenopausal hormone therapy are some of the factors that raise the risk of breast cancer. During the present research more than 65 sample were collected from the different localities of District Hyderabad, it has been observed that the majority of the cases most prevalent at (over 40 age) and females were prone to be infected with breast cancer. Therefore, this research must be focused and awareness program should be introduced.

DEFICIENCY OF VITAMIN D IN CHILDHOOD AND ADOLESCENT'S IMPACT AND ITS TREATMENT AT DISTRICT HYDERABAD, SINDH, PAKISTAN

Mahnoor, Bakhtawar Soomro*, Shakeel Ahmed Memon, Saheefa Soomro and Dhani Bux Mishori

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

Vitamin D insufficiency may be more common due to new lifestyle choices, the global "epidemics" of childhood and teenage obesity, and other avoidable risk factors. Apart from the effects on the skeleton, hypovitaminosis D has been linked to the emergence of severe non-skeletal health issues in children, such as autoimmune diseases and atopy. This is due to growing concerns about the detrimental effects of vitamin D deficiency on global health. The main aspects of childhood hypovitaminosis D are covered in this review along with the shortcomings of the methods used to date to treat the ailment. It also takes into account recent information. Worldwide, children and adolescents are extremely susceptible to vitamin D insufficiency. With mounting evidence linking vitamin D insufficiency to the pathogenesis of other chronic diseases beyond rickets, such as cancer, cardiovascular disease, and autoimmune disorders, the high rates of vitamin D deficiency in kids are extremely relevant to public health. Thus, early detection, intervention, and prevention of vitamin D insufficiency may have significant long-term health implications. We go over the definitions, clinical implications, epidemiology, and management of vitamin D insufficiency in children and adolescents in this review. Present research were conducted to fill the gaps of District Hyderabad. This research is conducted on the questionnaire basis. Out of 200 samples, around more than 50 sample were collected regarding the deficiency of vitamin D in children and 30 samples were collected associated with adults. Present studies, reflects that majority of the vitamin D deficiency were observed in children. Therefore, this research should be focused and different awareness program should be introduced in the hospital, schools, colleges, and Universities so that Futuristic children would be protected.

HYPERTENSION AND CORONARY ARTERY DISEASE IN DIABETIC PATIENTS AT DIFFERENT HOSPITALS DISTRICT HYDERABAD, SINDH, PAKISTAN

Hafsa and Bakhtawar Soomro*

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

Diabetes Mellitus type 2 is considered for the wide variety of cardiovascular disease, which is the most common reason of mortality in Diabetics patients. The most important reason for the morbidity and mortality in diabetics is intensified by Hypertension. The objectives of our study are to discuss evidences

and relationship between hypertension and coronary artery disease. This is a summary based observational study performed on diabetes type 2 patients. During the current studies, a total number of study subject was 120, who are suffering in diabetes type 2. The patient's demographic information had been calculated. The systolic and diastolic blood pressure had been measured by using automatic sphygmomanometer. The lipid profile of patients had been analyzed. The result declared that out of 120, (60%) patients suffered in hypertension, about (50%) were obese, and (55%) had dyslipidemia and (20%) patients suffered in coronary artery disease. The hypertension and coronary artery disease is a well-known risk factor for Diabetes type 2. Therefore, life style changes and treatment adherence are important for the proper prevention of both diseases.

PREVALENCE OF BLOOD GROUP O (+VE) AMONG THE INDIVIDUALS OF GOVERNMENT COLLEGE, UNIVERSITY, HYDERABAD, SINDH, PAKISTAN

Ghulam Fatima and Bakhtawar Soomro*

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

O positive blood group, emphasizing its prevalence and crucial role in medical contexts. Delving into the genetic basis, compatibility for transfusions, and health implications, it sheds light on the significance of this blood type. The piece encourages blood donation, especially from O positive individuals, highlighting their universal donor status. Additionally, it touches on the importance of the Rh factor, its relevance in pregnancy, and offers a glimpse into historical perspectives. The abstract provides a concise overview of the comprehensive exploration of O positive blood, appealing to a broad audience with varied interests in health and genetics. In this insightful article, we embark on a journey into the world of O positive blood, unraveling its genetic intricacies and prevalence among the population. The piece navigates through the compatibility maze, shedding light on the vital role O positive blood plays as a universal donor for Rh-positive recipients during transfusions. Beyond the laboratory, the article delves into the potential health implications associated with this blood type, exploring susceptibility or resistance to certain diseases. A call to action resonates throughout, urging readers, especially those with O positive blood, to consider the impactful act of blood donation. During the current studies, camp was arranged at mega level within the Government College, University, and Hyderabad. Around 200 samples were collected, out of that, (07 samples) were found to be (O positive) having (3.5%) percentage. Determination of blood group is observed by the following method by using different material viz: slides, gloves, lab coat, three types of serum (A, B, and AB), needle, toothpick, cotton, alcohol swab, pen, register. Therefore, this abstract encapsulates a comprehensive overview, promising readers an engaging exploration of genetics, medical significance, and historical relevance related to O positive blood group.

DETERMINATION OF A POSITIVE BLOOD GROUP AMONG THE PERSONNEL OF GOVERNMENT COLLEGE UNIVERSITY, DISTRICT HYDERABAD, SINDH, PAKISTAN

Faiza* and Bakhtawar Soomro

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: qazifaiza76@gmail.com

A positive (A+) blood contains type (A) antigens with the presence of a protein called the rhesus (Rh) factor. Antigens are makers on the surface of a blood cell. A positive blood from male's donors can also

be made into special medicines to treat conditions such as Dry eye syndrome. Platelets from A positive donations are also important. During the current studies camp was arranged at mega level within the Government College, University, and Hyderabad. Around 200 samples were collected, out of that, (07 samples) were found to be (A positive) having (3.5%) percentage. Determination of blood group is observed by the following method by using different material viz: slides, gloves, lab coat, three types of serum (A, B, and AB), needle, toothpick, cotton, alcohol swab, pen, register. Therefore, it is observed that this finding shed light on the frequency of (A Positive) under the umbrella of Government College, University and Hyderabad. The present research reflects vitality of further research among the demographs and exploration of genetics that actually plays vital role in transmission of characters from parents to offspring.

PREVALENCE OF BLOOD GROUP B+ INDIVIDUALS AMONG THE GOVERNMENT COLLEGE UNIVERSITY, HYDERABAD, SINDH, PAKISTAN

Eisha Aqeel* and Bakhtawar Soomro

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: eishabhatti182@gmail.com

The (B+ blood group) is characterized by the presence of B antigens and Rh factor(positive) on red blood cells. Individuals with this blood type can receive blood from B+ and O+ donors, while they can donate to B+, AB+, AB-, and O+. Nature of blood groups lies in their classification based on specific antigens present on red blood cells, influencing transfusion, compatibility and medical considerations. About 9% of the population have B positive blood group. Positive red blood cells can be given to both B positive and AB positive patients. B positive patients can receive blood from B positive, B negative, O positive and O negative donors. The concept of blood group B+ primarily refers to specific blood type. Scientifically, blood typing is crucial for medical purposes, determining compatibility for blood transfusions and organ donations. Beyond medical considerations, some popular but unverified theories, like the blood type personality theory, suggest certain behavioral traits might be associated with this blood type, such as being outgoing, empathetic, or adaptable. During the current studies, camp was arranged at border level within the Government College, University, and Hyderabad. Around 200 samples were collected, out of that, (64 samples) were found to be (B positive) its highest sampling was recorded among all having (32 %) percentage. Recorded blood group is observed by the following method by using different material viz: slides, gloves, lab coat, three types of serum (A, B, and AB), needle, toothpick, cotton, alcohol swab, pen, register. Therefore, it is observed that this research spread light on the highest frequency of the (B Positive) within the Government College, University, and Hyderabad. Present results showing that further research should be carried out among the individuals and exploration of genetics that how it plays role in human life.

DETERMINATION OF RARE BLOOD GROUP (B NEGATIVE) AMONG THE PERSONNEL OF GOVERNMENT COLLEGE, UNIVERSITY HYDERABAD, SINDH, PAKISTAN

Avinash Kumar, Bakhtawar Soomro*, Shakeel Ahmed Memon and Saheefa Soomro

Department of Zoology, Government College University, Hyderabad

* Corresponding Author: bakhtawarsoomro145@gmail.com

Blood type is determined by genes inherited from your parents. Whether your blood type is rare, common are somewhere in between, your donations are vital in helping save and improve lives. As B- is

one of the most extraordinary blood classifications, it is difficult to come by new givers and to guarantee we in very cause gather sufficient blood. We generally need more B- givers. B negative blood group is one of the rarest blood type as just 2% of our blood donors have it. Around 1 in 8 people can receive red blood cells from B negative donors. During the current studies camp was arranged at mega level within the Government College, University, and Hyderabad. Around 200 samples were collected, out of that, (07 samples) were found to be (B negative) its lowest sampling was determined having (3.5%) percentage. Determination of blood group is observed by the following method by using different material viz: slides, gloves, lab coat, three types of serum (A, B, and AB), needle, toothpick, cotton, alcohol swab, pen, register. Therefore, it is observed that this finding bloom light on the rarity of the (B negative) within the Government college, University and Hyderabad. The outcome showing vitality of further research among the demo graphs and exploration of genetics at broader level.

ASSESS AND IMPROVE THE NUTRITIVE VALUE OF STRAWS TARGETED AT CLIMATE-SMART ANIMAL PRODUCTION

Neelma Irfan, Abdul Shakur and Hafiz Noubahar Hussain*

*Animal Sciences Division, Nuclear Institute for Agriculture and Biology College (NIAB-C),
Pakistan Institute for Engineering and Applied Sciences (PIEAS), Faisalabad*

*Corresponding Author: hnoubahar@yahoo.com

Improving the nutritive value of low-quality feed resources through chemical methods can be a valuable strategy in promoting climate-smart animal production. The nutritional value of non-conventional feed resources was determined through a method elaborated for proximate nutrient composition. The nutrient values of soybean straw, barley straw, gram straw, masoor straw, mung bean straw, wheat straw, rice straw was analyzed for dry matter, crude protein, crude fiber, and ash using standard analytical methods. The dry matter content of straws ranged from 91.44 % to 96.57% while the crude protein was 1.75 % to 12.48 %. The protein content in soybean straw, barley straw, gram straw, masoor straw, mung bean straw, wheat straw, and rice straw were 4.65, 1.75, 8.9, 12.48, 8.35, 3.32, and 5.08 %, respectively. The ADF (acid detergent fiber) values were 28% to 54 %, while the NDF (neutral detergent fiber) was 48 % to 80 % in the straws. The protein content in hydrolysate was 77.88 %, 76.45 %, and 68.41% respectively. These straws may serve as a valuable source for microbial cultivation and processing for poultry and livestock feeding. It is concluded that cereal and legume straws can successfully be utilized for feeding poultry and livestock with processing and nutritive assessment.

EFFECT OF LONGER PHOTOPERIODS ON THE BLOOD PARAMETERS AND REPRODUCTIVE EFFICIENCY OF JAPANESE QUAILS (*COTURNIX JAPONICA*)

Saba Suleman¹, Tanveer Ahmed^{1*}, Huma Naz^{2*}, Basharat Ali³ and Khalid Abbas⁴

¹*Department of Life Sciences, Khwaja Fareed University of Engineering and
Information Technology, Rahim Yar Khan*

²*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

³*Department of Agriculture Engineering, Khwaja Fareed University of Engineering and
Information Technology, Rahim Yar Khan*

⁴*Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad*

*Corresponding Author: dr.humanaz98@gmail.com, tanvirahmeduaf@gmail.com

Photoperiod is the ratio of light to darkness over a 24-hour period. The physiological reactions, behaviour, growth, development, production, and immunity of birds are significantly influenced by lighting conditions.

The present study was conducted to see the effects of longer photoperiod on blood parameters and reproductive efficiency of Japanese quails (*C. japonica*). For this, a total of 27 (9 males and 18 females) *C. japonica* were divided into three groups as Control (C) and experimental (T₁ and T₂). Each group comprises 3 males and six females. 1 male and 2 females *C. japonica* were kept in each cage with the same basal diet and *ad libitum*. During the experiment, the Control group (C) were exposed to natural photoperiod i.e., sunset to sunrise; 14hL:10hD, T₁ were given 16hL:8hD treatment and T₂ were given 18hL:6hD treatment. The total duration of the experiment was remained 50 days. The results showed that hemoglobin, RBCs, WBCs, lymphocytes and monocytes rate, total protein, triglycerides and phosphorus concentration were significantly higher ($p \leq 0.05$) in Control group. Hematocrit rate, MCV, MCH, uric acid concentration, weight gain of *C. japonica*, egg weight, egg length, egg width, egg shell weight, egg-yolk weight and egg-white weight were significantly higher ($p \leq 0.05$) in T₁ group. MCHC, platelets, heterophils rate, H/L ratio, cholesterol and egg production of *C. japonica* were significantly higher ($p \leq 0.05$) in T₂ group. It is concluded that 14 hours of light was effective for the majority of blood parameters. The reproductive efficiency of *C. Japonica* was improved by 16 hours of light. For optimal egg production, 18 hours of light should be used. Furthermore, 18 hours of light induced stress in these birds in addition to other effects.

CATEGORIZING ANIMAL FEED RESOURCES FOR NUTRITIVE VALUE USING PORTABLE NEAR INFRARED REFLECTANCE SPECTROSCOPY AND CHEMOMETRICS

Muhammad Usman Mazhar*, Shumaila Yousaf, Abdul Shakur and Hafiz Noubahar Hussain

Animal Sciences Division, Nuclear Institute for Agriculture and Biology (NIAB), Jhang Road, Faisalabad

*Corresponding Author: E-mail: usmandvm@gmail.com

The current application and future potential of near infrared spectroscopy (NIRS) in the evaluation of food and feed is of great importance. Adaptation of NIRS for food and feed samples requires calibration and model development. The aim of this study was to develop library of local feed resources. A total of (n=20) samples were collected and analyzed for its nutrient profile for protein, fat, fiber and ash contents. These samples were scanned on NIR spectrometer and prediction model was developed for Spectra-viz software using (solo model builder, USA). The local feed resources were categorized on basis of high protein and low protein content. High protein containing feed resources included *Vigna aconitifolia* (moth), *Vigna radiata* (mung bean residues), *Glycine max* (soybean grains), *Lens culinaris* (masoor), *Brassica napus* (rapeseed cake), masoor residues, *Linum usitatissimum* (Linseed), and Nutrilick feed supplement. Nutrients ranged from 7.9-27.2%, 1.6-11.3%, 1.8-20.3% and 1.1-18.2% with respect to protein fat, fiber, and ash content respectively. The low protein profile feed resources included grams, wheat middlings, maize, kaghani mixture, wheat bran etc. Nutrients ranged from 5.7-13.1%, 1.7-5.6%, 3.4-26.0% and 1.1-8.5% with respect to protein fat, fiber, and ash content respectively. The developed Linear regression model for high protein samples by using partial Least Squares calculated with the SIMPLS algorithm using Preprocessing: 2nd Derivative (order: 2, window: 15 pt, incl only, tails: weighted) resulted in root mean square error for calibration (RMSEC): 0.742, 0.791, 1.219, 0.251 and root mean square error for cross validation (RMSECV): 1.705, 1.341, 2.513 and 0.476 for protein, fat, fiber, and ash content, respectively. The regression for calibration (R^2 Cal) was found to be 0.95, 0.95, 0.94 and 0.99 for protein, fat, fiber, and ash content, respectively. Similarly, regression for cross validation (R^2 CV) was found to be 0.77, 0.87, 0.79 and 0.98 for protein, fat, fiber, and ash content, respectively. For low protein samples R^2 Cal was found to be 0.97, 0.91, 0.98 and 0.94 while R^2 CV to be 0.96, 0.76, 0.95 and 0.88 for protein, fat, fiber, and ash content, respectively. The results showed that tremendous potential of portable Near Infra-red spectroscopy for nutritive assessment especially in local feed resources for optimum livestock productivity.

NUCLEAR AND CYTOPLASMIC MATURATION OF *IN VITRO* MATURED DENUDED OOCYTES IN THE PRESENCE OF GONADOTROPHINS, OESTRADIOL AND INSULIN IN NILI RAVI BUFFALO

Beenish Shahid^{1*} and Muhammad Ijaz Khan²

¹Department of Zoology, King Abdullah Campus, University of Azad Jammu and Kashmir, Muzaffarabad

²Department of Livestock and Dairy Development, Azad Government of the State of Jammu and Kashmir, Muzaffarabad

*Corresponding Author: beenish.shahid@ajku.edu.pk

The lower recovery of competent oocytes in buffalo species limits the commercialization of *in vitro* embryo production technology in field conditions. The present study aimed to improve the cytoplasmic and nuclear maturation of *in vitro* matured oocytes of Nili Ravi buffalo in the presence of hormones. The denuded oocytes (DOs) obtained by repeated pipetting were collected from 2-8mm follicles. A series of experiments were conducted to evaluate the effects of oestradiol (2 µg/ml), recombinant human follicle stimulating hormone (0.05 IU/ml), human chorionic gonadotrophin (2 IU/ml) and insulin (0.12 IU/ml) alone or together in different combinations at intervals of 8, 16 and 24 h of incubation period during *in vitro* maturation on buffalo oocyte. The supplementation of TCM-199 with E₂+rhFSH showed a highly significant increase in the diameter and maturation of oocytes (P<0.0001), as a greater number of the oocytes progressed to the metaphase II stage, and a lower proportion of the oocytes became degenerated (P<0.0001) after 24 h of incubation. The supplementation of medium with E₂+rhFSH+hCG or E₂+rhFSH+hCG+insulin also showed a significant increase in the meiotic maturation rate after 24 h (P<0.01 and P=0.04 respectively) and a significant decrease in the degeneration of the oocytes (P=0.001). The addition of insulin was not found to be effective for *in vitro* maturation. It is concluded that the addition of E₂+rhFSH in culture media was found to be the best combination of hormones for *in vitro* maturation of denuded buffalo oocytes.

PREVALENCE OF THINNESS, OVERWEIGHT, AND OBESITY AMONG SCHOOL CHILDREN IN DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

Atta Ullah¹, Saira^{1*}, Sonia Ali¹, Abdul Shafi Qadri¹, Naqash Khan², Bilal Hassan¹, Muhammad Armaghan¹, Abdul Wajid¹ and Munsif Ahmad¹

¹Department of Zoology, University of Swabi

²Department of Biology, University of Haripur, Haripur

Corresponding Author: ahsan@uoswabi.edu.pk

Overweight is defined as having a higher body weight in relative to height as equated to some standard or desirable weight norm. Being overweight can be initiated by an increase in fat in body or an increase in slender muscle. Obesity is frequently defined as a disorder characterized by unexpected or excessive fat buildup in adipose tissue to the point that health is jeopardized. It becomes clear that various kinds of childhood malnutrition, ranging from short stature (SS) and underweight to overweight, are rising worldwide health concerns that affect both industrialized and developing countries. In Swabi, the prevalence of obesity, overweight, and thinness remained unknown. The perseverance of this observance was to look at the prevalence of overweight, obese, and underweight children in Swabi on a nationwide scale. This cross-sectional survey was carried out between January and May of 2022. Body mass index (BMI) was used to calculate obesity and other weights. Bray's (1978) technique was used to compute BMI. BMI = Weight in Kilograms/ (Height in Meters)² =

kg/m². Our survey covers 303 kids from Tehsil Topi, 300 students from Tehsil Swabi, and 287 students from Tehsil Lahore. Various parameters were used, such as food, outdoor activities, family income, domicile, and so on. In district Swabi, the means of overweight kids were 36.16%, underweight pupils were 35.34%, and obese students were 30.20%. Obesity, overweight, and thinness accounted for 33.9% of all students in district Swabi. Obesity was higher in Tehsil Topi (36.3%) than in Tehsil Swabi (29%) and lower in Tehsil Lahore (18.755%).

ANALYSIS OF ORAL MICROBIOMES IN PATIENTS WITH DIABETES MELLITUS TYPE II AND THEIR COMPARISON WITH THE NORMAL INDIVIDUALS

Muhammad Aftab* and Anam Aftab

Department of Biological Sciences Virtual University of Pakistan

*Corresponding Author: muhammadaftabali533@gmail.com

Diabetes is a disorder of endocrine system caused due to high blood glucose level. Diabetes caused by the difference in insulin secretion level. It can be either type I (insulin dependent) or type II (insulin independent). Oral health and diabetes are related by some researchers. Diabetic patients have more chances to develop periodontal disease than normal individuals. There are large colonies of microorganisms in the human mouth and these microbes play a decisive role in oral disease. The oral microbiomes include Streptococci, Lactobacilli spp and Candida spp. There can be variation in these biomes depending upon the severity of diabetes and also in normal individuals. The main object of the study was to understand the oral micro-biome, their effect on human health especially in Diabetes type II and to give suggestions about how to avoid oral micro- biome that may lead to many diseases i.e. fungal infections, xerostomia etc. For this study, oral samples of diabetic and normal individuals were observed under microscope by growing them on various media using Gram's staining and other chemical tests .i.e. catalase test. During the observation, the normal subjects' samples showed normal growth and behavior during morphological characterization and also in biochemical tests. The diabetic samples were observed with abnormal fungal growth and variations in growth were also observed. These findings showed that oral cavity contains both pathogenic and non-pathogenic bacteria and can cause harmful effects in oral cavity such as fungal infections, dental caries and other effects. Oral hygiene, type of food and genetics are the factors which can impact on oral micro biome presence. The difference in the oral microbiome can also be due to some other potential factors such as smoking, use of antibiotics and overall health of the individual. To avoid the adverse effect of microbiome one should follow a balanced diet, maintain good oral hygiene and check for the oral health regularly.

SEMEN CRYOPRESERVATION AND ITS SIGNIFICANCE

**Fayaz Hussain, Qudratullah Kalwar, Muhammad Mohsen Rahimoon, Iqrar Uddin,
Taj Muhammad, Sajad Ali and Abdul Razzaque**

*Department of Theriogenology, Shaheed Benazir Bhutto University of Veterinary and
Animal Sciences, Sakrand*

*Corresponding Author: fayazhussainbanglani01@gmail.com

Semen cryopreservation is a complex process and is a technology for preservation of biological materials at extreme low temperature typically in liquid nitrogen at -196°C (-321°F). It involves balancing many factors in order to obtain satisfactory results. It is an important method used in reproductive

biotechnology to preserve male semen and store it in a bank for future use. This process is very important for transferring valuable traits thus genetic improvement can be achieved. Various genetic properties can be transferred across generations to share economically important traits. Various semen collection techniques can be used but use of artificial vagina is mostly preferred. The steps involved in semen cryopreservation are semen collection, evaluation, dilution, cooling, equilibration, filling the straws, freezing and storage in liquid in nitrogen gas at -196°C , and thawing for A.I. or other procedure. There are two primary techniques for semen storage that are Chilling and Cryopreservation, meanwhile two methods used for semen cryopreservation: slow freezing and verification. Semen cryopreservation success depend upon various important factors such as extender choice, cooling and freezing rate, proper storage and handling etc. For this process, various agents known as cryoprotectants are used which directly and indirectly effect this process and sperms life. Cryoprotectants are added in cryopreservation medium to reduce the physical and chemical stresses of sperm cells important for its motility and life. Cryopreserved semen is thawed to use for various process including sperm evaluation, A.I etc. This method also provides experimental knowledge about cryopreservation method. A.I is mostly used breeding methods and is performed through various processes. Recto vaginal and speculum methods are mostly used and genetic information encoded in sperms is transferred. Adequate semen collection, maintenance of cold chain during cryopreservation and optimum thawing methods with A.I techniques is challenge which make cryopreservation successful.

CRYOPRESERVATION OF POULTRY SEMEN

**Iqrar Uddin*, Qudratullah Kalwar, Muhammad Mohsin Rahimoon, Fayaz Hussain,
Taj Muhammad, Sajad Ali and Abdul Razzaque**

*Department of Theriogenology, Shaheed Benazir Bhutto University of Veterinary and
Animal Sciences, Sakrand, Shaheed Benazirabad*

*Corresponding Author: iqrarlashari094@gmail.com

The poultry industry is a significant part of Pakistan's agriculture sector, contributing 1.3% to the national GDP. It's also one of the country's fastest growing industries, with a 10–12% annual growth rate. Poultry semen is a white or pearly white fluid highly concentrated, containing 3–8 billion spermatozoa per milliliter for broiler fowl. Semen storage is discussed in terms of Cryopreservation, which allows long-term storage of spermatozoa at -196°C , the temperature of liquid nitrogen and liquid semen storage, usually at lower than ambient temperatures above 0°C for up to 24 or 48 hours. Two methods of cryopreservation are used in poultry slow freezing and rapid freezing (verification). Both methods have similar results, with sperm fertility rates not exceeding 40%. Successful cryopreservation of sperm requires, selection of proper diluent, selection of the best cryoprotectant, determination of freezing and thawing rates for optimum retention of fertilization potential and, removal of any materials deleterious to fertility (e.g glycerol) before insemination. Semen is preserved without loss of fertility for up to about 24 hours in the medium. Spermatozoa that were the first to be frozen using glycerol, other additives that can be used to improve sperm quality after bird semen cryopreservation are cholesterol and cyclodextrins. Extenders can be defined as buffered salt solutions used to prolong the viability of good-quality semen. Extenders used in poultry cryopreservation, Beltsville Poultry Semen Extender (BPSE) Used as a semen extender with a high fertility rate of 88% for white leghorn chickens. Lactated ringer's-egg yolk (LR-EY) used for semen cryopreservation of Indonesian indigenous chickens maintaining a sperm viability rate of 48-49%. Dimethyl formamide. Found to be the most suitable extender for the proportion of morphologically normal sperm and in vitro viability rate of cryopreserved sperm samples. N-methylacetamide (NMA) Successfully studied for the cryopreservation of rooster semen. Cryopreservation of poultry semen has tested to be a viable method for long term storage, maintain the viability and fertility of sperm. In this conclusion this technique enhance the poultry breed, program, genetic

diversity and commonly used cryoprotectants include glycerol, N-methylacetamide (NMA), dimethylacetamide, and dimethyl sulfoxide.

DETERMINATION OF SECRETOR AND NON-SECRETOR STATUS IN THE POPULATION OF CITY RAWALAKOT CONCERNING THE ABO BLOOD GROUP SYSTEM

Amna Beenash, Muhammad Shahbaz*, Lubna Rashid and Badra Younis

Department of Zoology, Women University, Azad Jammu and Kashmir, Bagh

Corresponding author: shahbazchattha_pk@yahoo.com

The present study was designed to find out the status of secretors and non-secretors in the study area, City Rawalakot. A total of 200 individuals were selected for study. 142 (71%) individuals were secretors while the remaining 58(27%) were non-secretors. Blood and saliva were collected from all individuals. Secretor and non-secretor status were determined by Hemagglutination inhibition method. During this study status of secretor and non-secretor has been observed concerning gender, caste, and ABO blood group. It has been observed that females were more secretors as compared to males. People of different castes Mughal, Chadury, Sudhan, Qazi, Syed, and Hashmi were observed. The highest percentages of secretors were observed in Mughal and the lowest in Hashmi. Blood group B was more found and it was observed that more secretors were seen in blood group B individuals while lower in Blood group A. This study revealed that secretors were more found in Rawalakot than non-secretors. The variation in percentages may be due to racial and regional customs.

THE COUNT OF WHITE BLOOD CELLS AND BODY MASS INDEX IN THE SMOKER POPULATION OF TEHSIL, DHIRKOT, DISTRICT BAGH

Badra Younis, Muhammad Shahbaz*, Amna Beenish, Lubna Rashid and Shamsa Manzoor

Department of Zoology, Women University Azad Jammu and Kashmir, Bagh

Corresponding author: shahbazchattha_pk@yahoo.com

This study was carried out in the population of Tehsil Dhirkot, to Count Body mass index and White blood cells in the smoker population. A total of 145 blood samples were collected from individuals aged between 16-65 years. Among them 110 were smokers and 35 were non-smokers. Samples were taken in an evacuee container having Ethylene Diamine Tetra acetic Acid (EDTA). Leucocyte count was done by using a hemocytometer and body mass index (BMI) was measured by using the formula $BMI = \text{kg/m}^2$. Among the smokers 53(48%) individuals have normal BMI and out of the normal BMI 47 (89%) individuals have normal BMI and normal WBCs, but 3(6%) smokers had normal BMI but less leucocytes count. Similarly, 3 (6%) smokers have normal BMIs but higher leucocyte counts. In the same way, 22 smokers have higher BMI and leucocyte count. However, 12 have increased BMI with normal WBC numbers. It has been also found that 17 individuals were underweight with normal leucocyte count, Similarly, 3 individuals had low BMI as well as WBC count but 3% of smokers had decreased BMI but increased leucocytes, In nonsmokers 31 (86%) had normal BMI. Among them, 29 with normal BMI as well as WBCs but 11 have normal BMI with increased WBC count. However, 4 individuals were found overweight with normal leucocyte count. During this study, professions were also observed concerning the Body mass index and WBC count. Professions were classified into three categories Students, Government Employees, and Private Employees. It is worth noting that profession has a significant relation with Body mass index and no

significant relation with TLC. This study also observed that the persons whose BMI has been increased also have an increase in several different leucocytes such as neutrophils, lymphocytes, and monocytes. Data was analyzed by using an independent sample t-test and Chi-square test.

DEVISING AND VALIDATING PEN-SIDE HEMATOLOGICAL FORMULAE FOR CHOLISTANI LIVESTOCK SPECIES AS AN ON-FIELD DIAGNOSTIC AND PROGNOSTIC APPROACH

**Umer Farooq^{1*}, Musadiq Idris¹, Mushtaq Hussain Lashari², Nouman Sajjad¹,
Zobia Perveen¹ and Shahbaz Ahmad²**

¹*Department of Physiology, The Islamia University of Bahawalpur*

²*Department of Zoology, The Islamia University of Bahawalpur*

*Corresponding Author: umer.farooq@iub.edu.pk

In human medical practice, while using hematological attributes for diagnosis, a 'rule of three' is implied for RBC Count, Packed Cell Volume (PCV) and Hemoglobin (Hb) to ensure the correctness of deduced hematological parameters. This traditional 'rule of three' implies that a) PCV (%) = Hb (g/dL) $\times 3$; b) Hb (g/dL) = RBC Count (without the count of 3) $\times 3$; and c) PCV = RBC Count (without the count of 3) $\times 9$. However, there is a paucity of literature regarding such hematological formulae and validations for veterinary medical sciences. The Post-graduate Laboratory of the Department of Physiology, The Islamia University of Bahawalpur has lately embarked upon devising and validating such hematological formulae for various Cholistani livestock species (Funded under Natural Sciences Linkage Program of Pakistan Science Foundation, Project No. PSF/NSLP/P-IUB-931). This work will present the progress made up till now regarding hematological formulae attained from the data of Beetal goats (n=10), Cholistani cattle (n=364), Cholistani camels (n=200). The hematological formulae devised and validated can be used by the veterinary practitioners, laboratory technicians, researchers, academicians, personnel of the Livestock Department and other stakeholders as on-field diagnostic/prognostic tools for assessing anemia.

PHYSIOLOGICAL DYNAMISM OF VARIOUS SERUM CARDIAC BIOMARKERS IN APPARENTLY HEALTHY SIPLI SHEEP OF PAKISTAN

Mushtaq Hussain Lashari^{1*}, Madiha Sharif¹, Umer Farooq², Musadiq Idris² and Aisha Mahmood²

¹*Department of Zoology, The Islamia University of Bahawalpur*

²*Department of Physiology, The Islamia University of Bahawalpur*

*Corresponding Author: mushtaq.hussain@iub.edu.pk

The present work is the first record of deducing normal reference intervals (RIs) for various serum cardiac biomarkers namely cardiac troponin I (cTnI), alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase-myocardial band (CK-MB), sodium (Na⁺), potassium (K⁺) and total protein (TP), and their interrelationship in apparently healthy indigenous Sipli breed of sheep (n=141) from Pakistan. Apropos to aseptic blood collection and serum extraction, the attributes were deduced using commercially available kits. For the sake of analyses, the studied biomarkers were attributed as independent (Na⁺, K⁺, and TP) and dependent (LDH, ALT, AST, cTnI and CK-MB) variables. The RIs were determined through Reference Value Advisor Version 2.1 as per gender and age keeping in view the guidelines provided by American Society of Veterinary Pathology (ASVCP). Results revealed that the RIs for cardiac serum

biomarkers included in the present study were generally within the reference range provided for sheep with slight variations which might be attributed to physiological adaptation to harsh desert climate. The Na⁺ significantly ($P \leq 0.05$) predicted AST (adj. r-square= 0.976) and CK-MB (adj. r-square= 0.477). Similarly, K⁺ significantly ($P \leq 0.05$) predicted LDH (adj. r-square= 0.626) and ALT (adj. r-square= 0.897). The TP had highest adjusted r-square of 0.770 with AST however it was statistically non-significant ($P \geq 0.05$). The results of the study present a baseline data about these cardiac biomarkers which may be utilized for cardiac assessment of the sheep. It is recommended that the serum cardiac biomarkers ascertained in this study may be studied along with electrophysiology and ultrasonography of sheep heart for confirmed diagnosis/prognosis of cardiomyopathies.

PREVALENCE AND CAUSES OF REFRACTIVE ERRORS AMONG GENERAL POPULATION OF DIVISION MUZAFFARABAD AZAD JAMMU AND KASHMIR

Taneya Almas^{1*}, Zahid Latif¹, Tehzeeb Fareed Alvi¹ and Sajawal Mir²

¹*Department of Zoology, Faculty of Science, The University of Azad Jammu and Kashmir, Muzaffarabad*

²*Department of Ophthalmology, Abbas Institute of Medical Sciences, Muzaffarabad*

Correspondence Author: taneyaalmas@gmail.com

The purpose of this study was to determine the prevalence and causes of refractive errors among general population of division Muzaffarabad, Azad Jammu and Kashmir, Pakistan. This was a population based descriptive cross-sectional study conducted among participants who were attending the outpatient department at Al-Shifa Trust Eye Hospital, Abbas Institute of Medical Sciences, Combined Military Hospital and camps organized by ASTEH for eye care services from December 2021 to May 2022. A total of 1100 participants aged 3 to 90 years were selected. Presenting visual acuity was measured by using Snellen's chart and logMAR tumbling E-chart. The data was collected by using questionnaire. Collected data was then analyzed using Statistical Package for Social Sciences software. Chi-square and multivariate logistic regression analysis was used to determine the relationship between socio-demographic factors and refractive errors. The overall prevalence of hyperopia, myopia and astigmatism was 55.5%, 37.9% and 6.6% respectively. There were (53.4%) females and (46.6%) males. R.Es were the most prevalent among participants with higher body mass index. There positive association was found between age (p - value = <0.001), gender (p - value = 0.026), increased body weight (p -value = <0.001), height (p -value = <0.001), marital status (p -value = <0.001), occupation (p -value = <0.001), family income (p -value = <0.001), exposure to sunshine (p -value = <0.001) and refractive errors. The prevalence of R.Es among participants of rural areas was high (87.7%) (p -value = <0.001). The main causes of R.Es were inherited R.Es (66.2%), blood pressure (16%), screen usage (9.9%), eye surgery (5.5%) and trauma (1.5%) and aging (0.9%) (p -value= <0.001). A focus on optical treatment/corrections will help to reduce the burden of R.Es among the general population.

AMELIORATIVE EFFECTS OF PLUMBAGIN ON CYCLOSPORINE-INDUCED HEPATIC DAMAGES

Aqila Manzoor, Muhammad Zaid Salar, Muhammad Umar Ijaz*, Ali Akbar, Muhammad Faisal Hayat and Ghulam Mustafa

Department of Zoology, Wildlife & Fisheries, University of Agriculture, Faisalabad

*Corresponding author: umar.ijaz@uaf.edu.pk

Cyclosporine (CYS) is an immunosuppressive agent that may induce toxic effects in various organs particularly, liver. Plumbagin (PLU) is a naturally present naphthoquinone that exhibits diverse

pharmaceutical properties including anti-oxidant, anti-inflammatory and anti-apoptotic activities. Therefore, the present study was designed to evaluate the therapeutic role of PLU against CYS induced hepatotoxicity. Forty-eight rats were randomly distributed into 4 groups i.e., control, CYS (20 mgkg⁻¹) treated, CYS + PLU (20 mgkg⁻¹ + 50 mgkg⁻¹) co-treated and only PLU (50 mgkg⁻¹) treated group. After 30 days of treatment, it was revealed that CYS intoxication reduced the activities of catalase (CAT), glutathione-S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidases (GPx) glutathione reductase (GSR), glutathione S-transferase (GST) and glutathione (GSH) level, whereas elevated the levels of ROS and MDA. Moreover, CYS also increased the levels of liver serum marker enzymes such as alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST). Furthermore, the level of inflammatory makers i.e., nuclear factor-kappa B (NF-κB), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β) and cyclooxygenase-2 (COX-2) activity was increased following the CYS exposure. The intoxication of CYS elevated Caspase-3, Bax and Caspase-9 levels, while reducing the Bcl-2 level. Furthermore, the exposure of CYS induced significant histopathological damages in hepatic tissue of rats. However, the supplementation of PLU considerably improved the CYS induced damages due to its hepatoprotective, anti-inflammatory, anti-apoptotic and anti-oxidant nature.

THERAPEUTIC POTENTIAL OF PHLORETIN AGAINST PARAQUAT INSTIGATED CARDIAC TOXICITY IN RATS

**Muhammad Tariq, Ali Akbar, Muhammad Umar Ijaz*,
Muhammad Faisal Hayat and Muhammad Zaid Salar¹**

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Paraquat (PQ) is an effective herbicide however, due to its adverse effects on different organs including heart, it is considered as highly toxic to human beings. Phloretin (FLN) is a plant-based flavonoid with conspicuous pharmacological properties. This experiment was executed to evaluate the palliative actions of FLN on PQ prompted cardiac toxicity in rats. Twenty-four rats were apportioned into 4 equal groups which were designated as control, PQ treated group (5 mgkg⁻¹), Co-treated (PQ 5 mgkg⁻¹ + FLN 25 mgkg⁻¹) and FLN (25 mgkg⁻¹) treated group. After 30 days of treatment, PQ intoxication resulted in a remarkable reduction in antioxidant enzymes activities which include, glutathione reductase (GSH), glutathione S-transferase (GST), catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and glutathione disulfide reductase (GSR), whereas an elevation was observed in reactive oxygen species (ROS), & malondialdehyde (MDA) as well as hydrogen peroxide (H₂O₂) level. Furthermore, concentrations of cardiac injury markers, creatinine phosphokinase (CPK), creatine kinase-myoglobin binding (CK-MB), & lactate dehydrogenase (LDH), as well as troponin I were increased in response to PQ treatment. Moreover, inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), nuclear factor-kappa B (NF-κB), and interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and cyclooxygenase-2 (COX-2) levels were augmented in PQ intoxicated group. PQ exposure reduced the gene expression of cardiac anti-apoptotic markers (Bcl-2), but the gene expression of apoptotic marker (caspase-9, caspase-3 and Bax) was increased. Histopathological damages were also observed in toxicant (PQ) exposed group. However, the administration of FLN significantly palliated PQ induced aforementioned disruptions. In the light of these findings, it is concluded that FLN is a promising bioactive compound that may be used as a curative agent against PQ instigated cardiac damage due to its antioxidant, anti-apoptotic, anti-inflammatory as well as cardioprotective potential.

PROTECTIVE ROLE OF RHAMNAZIN AGAINST PULMONARY DAMAGE INDUCED BY PFOS IN MALE ALBINO RATS

Amara Tahir, Rabia Azmat, Muhammad Umar Ijaz*,
Muhammad Sakandar Majid and Muhammad Zaid Salar

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Perfluorooctane sulfonate (PFOS) is a widespread environmental contaminant that is detected in the lung of mammals. The mechanisms underlying PFOS-induced pulmonary toxicity remain unclear. Rhamnazin is a natural flavonoid and known for its antioxidant, anti-inflammatory and anti-apoptotic properties. Therefore, the current study was designed to determine the mitigative role of rhamnazin against PFOS induced pulmonary damage. 24 adult male albino rats were randomly separated into four groups: control, PFOS (20 mgkg⁻¹), PFOS + Rhamnazin (20 mgkg⁻¹ + 5 mgkg⁻¹) and Rhamnazin (5 mgkg⁻¹) treated groups. The findings of this study revealed that PFOS exposure decreased the activities of antioxidant enzymes i.e., catalase (CAT), glutathione reductase (GSR), superoxide dismutase (SOD), glutathione S-transferase (GST), and glutathione (GSH), while escalated the levels reactive oxygen species (ROS) and malondialdehyde (MDA). Moreover, PFOS treatment noticeably increased the levels of inflammatory markers, including tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 β (IL-1 β), nuclear factor kappa-B (NF- κ B), and cyclooxygenase-2 (COX-2) activity. PFOS intoxication diminished the level of the anti-apoptotic protein (Bcl-2) while increasing the levels of apoptotic markers (Bax, caspase-3 and caspase-9). Furthermore, PFOS treatment also caused considerable histological damage in pulmonary tissues of rats. However, rhamnazin treatment potently alleviated all the aforementioned impairments in lungs. Conclusively, our results demonstrate the promising free-radical scavenging activity of rhamnazin, a novel flavonoid, against the PFOS-instigated pulmonary toxicity.

EFFECT OF PROTEIN FROM DIFFERENT SOURCES (PLANT AND ANIMAL) ON GROWTH AND BODY COMPOSITION OF *CIRRHINUS MRIGALA*

Rehana Iqbal

Institute of Zoology, Bahauddin Zakariya University, Multan

*Corresponding Author: rehanaiqbal82@gmail.com

The mrigal carp, *Cirrhinus mrigala* also known as the mrigal and the white carp, is a species of the carp family. It is widely aqua farmed and introduced population exists outside its native range. Mrigala is popular as a food fish and an important aquaculture freshwater species throughout South Asia. It is widely farmed as a component of a polyculture system of three Indian major carps, along with *L. rohita* and the *Catla catla*. *Cirrhinus mrigala* is the most preferred farmed fish species in the India and Pakistan because of its fast growth and higher acceptability to the consumers. The aim of the proposed research was to prepare low cost quality fish feed using different locally available cheaper protein sources (plant and animals) for *Cirrhinus mrigala*, to observe the effect of these feeds on growth and survival and estimate the minimum protein requirement for the maximum growth of *Cirrhinus mrigala*. A series of six experiments were conducted to evaluate the effect of prepared feeds. Seventeen experimental diets were formulated which are described separately according to experiments. From the analyzed data it was observed that Chicken Waste (CW) feed group showed higher growth performance and among the CW feed group CW30 showed best results for the experimental group. As far as stocking density was concerned it was observed that treatment II (15fish/cisterns) is best stocking density for this fish. Overall

the rate of growth in all experimental fishes was increased with increase in protein contents in the feed. This increase is observed in body growth rate up to an optimum level and then decreased.

ACCUMULATION OF ALBUMIN IN TUMOR INTERSTITIUM IN RESPONSE TO ELEVATED CAVEOLIN-1 EXPRESSION IN GYNECOLOGICAL CANCERS

**Sadaf Azad Raja^{1*}, Sehar Chughtai¹, Asma Gul², Fazila Kakar¹, Sabir Hussain¹,
Saima Jabeen⁴ and Syeda Batool Mazhar³**

¹*Department of Biosciences, COMSATS University, Islamabad*

²*Department of Biological Sciences, International Islamic University, Islamabad*

³*Department of Gynecology, Pakistan Institute of Medical Sciences, Islamabad*

⁴*Department of Zoology, PMAS Arid Agricultural University, Rawalpindi*

*Corresponding Author: sadafazad_raja@comsats.edu.pk

The expression of Cav-1 is associated with the progression in various cancers. In gynecological cancers, its expression remains variable. Serum composition alterations are related to cancer progression, and low albumin levels are the factor. Transcytosis of the albumin by Cav-1 might be responsible for its decreased concentration in plasma. In this study, we quantified the Cav-1 expression in all five gynecological cancers through RT-PCR and quantified the albumin concentrations using BCG, in the patient's serum and correlated the serum albumin and the tumor interstitial fluid albumin concentrations with the Cav-1 expression in selected gynecological cancers. The expression was observed variable in selected cancers. Ovarian and uterine cancer was having high expression of Cav-1 (7.9-fold, 5.8-fold respectively) ($P < 0.0001$). Whereas in cervical, vulvar and vaginal cancers the expression was low, (0.2-fold, 0.5-fold, and 0.1-fold respectively) ($P < 0.001$). We found that the tumors with raised Cav-1 expression have a significant increase in albumin concentration in tumor interstitial fluids (8.2 ± 1.02 , 6.9 ± 1.07 mg/mL, 10mg/ml standard serum albumin concentration) ($P < 0.0001$) and extremely low in their corresponding serum samples. Conversely, the cancers with downregulated Cav-1 have less accumulated albumin in their tumor interstitial fluids (4.1 ± 0.02 , 4.5 ± 0.7 mg/ml, 10mg/ml standard serum albumin concentration) and slightly below average concentration in their respective serum samples. In conclusion, Cav-1 expression-based cancer progression is also affected by the Cav-1-mediated endocytosis and transcytosis of albumin through epithelial membranes. Therefore Cav-1 might be included among other factors responsible for the variable albumin levels in serum and tumor interstitium in gynecological cancers.

6. TOXICOLOGY

PROTECTIVE EFFECTS OF *MORINGA OLEIFERA* LEAF EXTRACT (MOLE) AGAINST SILVER NANOPARTICLES AND ARSENIC INDUCED HEPATOTOXICITY IN RATS

Faiza Bashir, Saima Sharif^{1*}, Farkhanda Manzoor, Shagufta Naz and Farzana Rashid

Department of Zoology, Lahore College for Women University, Lahore

*Corresponding author: ssharif1978@yahoo.com

This study was conducted to ascertain whether concurrent exposure to *Moringa oleifera* modified the changes caused by interactive exposure of silver nanoparticles and arsenic in various parameters of hepatotoxicity. Sixty-three Sprague Dawley rats were assigned into seven groups (n=9) each. Group I was a control. Group II and III comprised of *Moringa* M(L) 2% and M(H) 3 % doses. Group IV (AgNps+As)L consisted of low doses of silver nanoparticles (100 mg/kg) and arsenic (10.25mg/kg). Group V (AgNps+As)H embraced high doses of silver nanoparticles (150 mg/kg) and arsenic (16.4mg/kg). Group VI (AgNps+As+M)L and VII (AgNps+As+M)H comprised doses of chemicals as described above. Doses were administered orally for three months. Three rats from each group were euthanized and evaluated for various parameters after the 4th, 8th and 12 weeks of the experiment. Results revealed that AgNps and As intoxication at low and high dose levels induced severe *necrotic histopathological changes* in liver tissues accompanied by remarkably escalated levels of ALT, AST and ALP and oxidative stress marker (TBARS) in each successive month. Meanwhile, albumin, total protein SOD, CAT, GPX and GSH levels were significantly depleted manifesting suppressed activity of antioxidant enzymes. Interestingly, concurrent treatment of silver nanoparticles and arsenic-intoxicated rats with MOLE prevented tissue injury by improving the *cellular integrity of the liver*, corrected liver markers, and inhibited oxidative stress by activating the detoxifying enzyme system. Therefore, MOLE administration demonstrated a therapeutic role against the hepatotoxic effects of silver nanoparticles and arsenic, which may be related to its anti-inflammatory and antioxidant capabilities.

IN VIVO INDUCTION OF ANTIOXIDANT RESPONSE AND OXIDATIVE STRESS IN GOLD FISH (*CARASSIUS AURATUS*) DUE TO HEAVY METALS EXPOSURE AT DAMAS LAKE, FORT MUNRO, PAKISTAN

Saima Naz^{1*}, Ahmad Manan Mustafa Chatha^{2*} and Nimra Amjad¹

¹*Department of Zoology, Government Sadiq College Women University, Bahawalpur*

²*Department of Entomology, Faculty of Agriculture and Environment,
Islamia University of Bahawalpur, Bahawalpur*

*Corresponding Author: saima.naz@gscwu.edu.pk; manan.chatha@iub.edu.pk

Seasonal estimations were conducted to evaluate the water quality and concentrations of heavy metals (Cu, Ni, Pb, Cd, and As) in Damis Lake-Fort Munro, located in Punjab, Pakistan. Additionally, samples of *Carassius auratus* fish were gathered from three distinct sites: upstream, midstream, and downstream. This was undertaken to analyze how the water quality and concentrations of heavy metals might impact fish enzymes, histopathology, and hematology. Utilizing a Bulk Scientific Atomic Absorption Spectrophotometer, the levels of Ni, Cu, Cd, As, and Pb were determined in the liver, gills, and muscles of

the Goldfish (*Carassius auratus*) from Damis Lake-Fort Munro. Concurrently, the activities of enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were measured. The accumulation trend of these metals in the organs was observed as follows: Ni > As > Cd > Pb > Cu. Furthermore, the concentration sequence of these metals in the organs was: Liver > Gills > Muscle. Outcomes illustrated significant rises in oxidative stress, indicated by shifts in enzyme activity and increased reactive oxygen species. The study concluded that pollution caused by studied heavy metals had a significant adverse effect on the quality of water as well as well-being of *C. auratus* at the study site.

INDIVIDUAL AND COMBINED EFFECTS OF LEAD, COPPER AND CHROMIUM ON HEMATOLOGICAL INDICES AND DNA DAMAGE IN ROHU (*LABEO ROHITA*)

Nizar Ali¹, Dua Saleem^{2*} and Munawar Saleem Ahmad¹

¹Department of Zoology, University of Swabi, Swabi

²Department of Pharmacy, University of Swabi, Swabi

*Corresponding Author: saleemsbs@gmail.com, saleemsbs@uoswabi.edu.pk

The present study deals with individual and combined effects of lead, copper and chromium on hematological indices and DNA damage in *Labeo rohita*. The LC₅₀ values of Lead nitrate Pb(NO₃)₂, Copper Sulphate (CuSO₄) and Chromium sulphate Cr₂(SO₄)₃ for *Labeo rohita* were found to be 35 mg/l, 5.5 mg/l and 42mg/l respectively. For each and every chemical fifty (50) fish specimens were grouped in five groups ten in each group. Group 1 was considered as control and kept as toxicant free and the rest four (EG1, EG2, EG3 and EG4) were exposed to sub-lethal concentration of Lead, Copper and chromium. When fish specimens were exposed to Lead nitrate, Copper sulphate and Chromium sulphate, various damages were reported in hematological parameters of *Labeo rohita*. RBCs, Hb, Hct and PLT level shows a significant decrease while WBCs number shows a significant increase when exposed to sub-lethal concentration of Lead nitrate, Copper sulphate and Chromium sulphate. Results show that combined concentration of Lead, Copper and Chromium has severe effects and causes severe damage to hematological indices of the treated groups. Results also show that exposure of these heavy metals causes DNA damage in experimental groups. DNA damage was studied through micronucleus assay, through which micronuclei formation were reported in experimental groups which confirms that heavy metals exposure caused DNA damage in *Labeo rohita*.

EVALUATION OF ATRAZINE AND METHOMYL AGAINST HEMATOLOGICAL INDICES AND DNA DAMAGE IN ROHU (*LABEO ROHITA*)

Abdul Waheed¹, Nehafta Sabir², and Munawar Saleem Ahmad^{1*}

¹Department of Zoology, University of Swabi

²Govt Girls Degree College, Mansehra

*Corresponding Author: saleemsbs@gmail.com, saleemsbs@uoswabi.edu.pk

The present study is carried out to study individual and combined effects of atrazine and methomyl on hematological indices and DNA damage in *L. rohita*. The LC₅₀ values for, atrazine and methomyl were found to be 37mg/l and 8.5mg/l to *L. rohita* respectively. For each and every chemical fifty (50) fish specimens were grouped in five groups of ten each. Group 1 was considered as control and kept as toxicant free. EG1 were exposed to sub-lethal concentrations of atrazine and methomyl for 24hrs with the concentrations of 15 and 4.5mg/l. EG2 were also exposed to the sub-lethal concentrations of atrazine and

methomyl for 48hrs with the concentrations of 20 and 5mg/l. EG3 were also exposed to the sub-lethal concentrations atrazine and methomyl for 72hrs with the concentrations of 25 and 5.5mg/l. The EG4 were exposed to the sub-lethal concentrations of atrazine and methomyl for 96hrs. When fish specimens were exposed to atrazine and methomyl various damages were reported in hematological parameters of *L. rohita*. RBCs, Hb, Hct and PLT level shows a significant decrease while WBCs number shows significant increase when exposed to sub-lethal atrazine and methomyl Results show that combined concentration of atrazine and methomyl has severe effects and causes severe damage to hematological indices of the treated groups. Results also show that exposure of these herbicides causes DNA damage in experimental groups. DNA damage was studied through micronucleus assay, through which micronuclei formation were reported in experimental groups which confirms that atrazine and methomyl exposure cause DNA damage in *L. rohita*.

BIOACCUMULATION OF HEAVY METALS IN FRESHWATER AND MARINE FISH SPECIES OF PAKISTAN

Alisha Fatima, Jaweria Aqeel and Hina Jabeen*

Department of Biological Sciences, Virtual University of Pakistan

*Corresponding Author: hina.jabeen@vu.edu.pk

Fish is a crucial source of protein for human health due to low saturated fat, and high omega-3 fatty acid content, and its consumption has quickly expanded across the world. Fish have a higher likelihood of surviving under hypo toxic conditions when exposed to heavy metals, but they can accumulate in humans' brain, liver, kidney, lungs, and muscles after consumption, posing a significant health risk. It is known that the heavy metals are released into aquatic environments on purpose or accidentally. The main sources of heavy metal pollution in the aquatic environment are anthropogenic activities, such as industry and agriculture. The primary objective of this work was to measure the concentration of heavy metal concentrations (Pb, Fe, Cu, Cr, and Ni) in the tissues of different marine and freshwater fish species. A total of 10 individuals each of three species (*Pampus* sp., *Wallagu attu* and *Scomberomorus guttatus*) were collected from River Chenab, River Indus and Karachi coastal region. Atomic absorption spectrophotometer (AAS) analysis was done on fish samples to determine the presence of heavy metals. The overall findings showed that the iron had higher concentrations, followed by copper, chromium, lead and nickel. The average values of these heavy metals' bioaccumulation (BAF) were discovered in the following decreasing order: Fe > Cu > Cr > Pb > Ni. With the exception of Pb and Cr, all metals in this investigation were determined to be within acceptable limits. Aside from iron toxicity, eating this species' muscles is healthy for human health. Additionally, the calculated averages and standard deviations offered crucial information for understanding the potential environmental impact and guiding future conservation efforts in the freshwater and coastal regions of Pakistan.

TOXIC EFFECTS OF CHLORPYRIFOS ON HEMATOLOGICAL PARAMETERS IN PIGEONS (*Columba livia domestica*)

Shakeel Ahmed Memon*, Bakhtawar Soomro* and Saheefa Soomro

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: shakeelahmed.memon@gcu.edu.pk; bakhtawarsoomro145@gmail.com

Pesticide use has greatly increased in modern agriculture as a means of controlling vector-borne diseases. There is ample evidence that bird populations are declining globally, and one of the main causes of

this loss is pesticide poisoning. In addition to being an important component of ecosystems, birds are profitable as a source of food. The current study was carried out to determine the alteration in hematological parameters due to pesticide toxicity in birds. For this, 80 healthy pigeons (*Columba Livia domestica*) were kept in hygienic conditions. Throughout the study, the pigeons were fed a meal consisting of 22% crude protein and were given access to unlimited water. Following a 30-day acclimation period, 20 pigeons in each of four (A, B, C and D) groups were randomly assigned for the exposure to chlorpyrifos insecticide, while group A kept as control. The Chlorpyrifos 40EC insecticide oral doses of 1/25th, 1/20th and 1/15th of LD50 (1.3mg, 1.6mg and 2.1 mg/kg body weight/day) were administered to each test groups (B, C and D) of birds for 30 consecutive days suspended in 1 ml corn oil. The 30-day investigation involved monitoring cumulative/average feed consumption, body weight as well as clinical and physical problems. Blood drawn from the experimental pigeons to examine hematological parameters such as Hemoglobin concentration (Hb.), total Erythrocytes Count (TEC), total Leukocytes Count (TLC), Packed Cell Volume (PCV%) and Platelets. In the present study birds of group (B, C, and D) showed prominent clinical signs throughout the experiment including ruffled feathers, lethargic, marked depression and dullness, open mouth breathing, salivation, reduced intake of food, tremors, inactive and decreased motor activities, isolated, decreased frequency of mating and watery diarrhea. Exposures of chlorpyrifos exhibited significant ($P < 0.05$) decrease in body weights in group B, and highly significant ($P < 0.01$) in pigeons of group C and D. The pigeons of control group (A), had hemoglobin mean levels (17.2 g/dl) and notable significant decline were observed in the mean values of (Hb.) 13.85 g/dl, 12.2 g/dl and 10.50 g/dl respectively in pigeons of group B, C and D. Whereas, birds of subgroups B, C, and D also showed significant alterations in findings of total erythrocytes count (TEC), total leucocytes count (TLC), packed cells volume (PCV%), platelets in comparison to the birds of control group. Significant variations in blood glucose levels were also seen when compared to control group pigeon. Pigeons exposed to chlorpyrifos exhibited hematological alterations. This study demonstrates that the abandoned use of pesticides impairs ecosystems and poses a substantial health risk to birds and ultimately to human beings.

ASSESSMENT OF BIOCHEMICAL INDICATORS OF HEPATOTOXICITY IN PIGEONS ON EXPOSURE TO BIFENTHRIN INSECTICIDE

Saheefa Soomro, Bakhtawar Soomro* and Shakeel Amed Memon

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

Pesticides affect the human, environment and wildlife including birds. Evidences suggest that, there is widespread decline in bird's population. Pesticide toxicity is considered as a major contributing factor for these declines. Therefore, present study was aimed to evaluate the extent of bifenthrin insecticide toxicity on liver parameters of pigeons (*Columba Livia domestica*). This was a difficult to estimate because birds may die away from the place of poisoning. To evaluate the biochemical indicators of hepatotoxicity, 60 healthy Pigeons (*Columba Livia domestica*) were used and reread in clean wooden cages under similar condition. After acclimatization for 30 days, pigeons were randomly divided in to three main groups respectively having 20 pigeons in each group, while group A kept as control. The oral doses of bifenthrin insecticide of 1/25th and 1/20th of LD50 (10 mg and 20mg./kg. /day) were administered to each test group (B and C) of birds for 30 consecutive days suspended in 1 ml corn oil. In the present study birds of subgroups B and C showed prominent clinical signs throughout the experiment including ruffled feathers, lethargic, marked depression and dullness, open mouth breathing, salivation, reduced intake of food and diarrhea. Exposures to bifenthrin

insecticide exhibited significant ($P < 0.05$) decrease in body weights in group B and C. The mean values of absolute liver and relative weights to body weights were observed significant ($P < 0.05$) increase in group B and C. Serum biochemical analyses regarding (AST), (ALT) and (ALP) levels reflected as significant ($P < 0.05$) increase in Mean \pm SD levels as compared in control group pigeons. It is concluded that exposure to bifenthrin insecticide exerted biochemical alterations of liver parameters in pigeons. This indicates that, eco toxicological hazards to the birds and non-target species.

RECOGNITION OF BIOMARKER FOR HG POLLUTION IN KIDNEY, LIVER AND SKIN OF *EUPHLYCTIS CYANOPHLYCTIS* FROM SELECTED AREAS OF THATTA DISTRICT, SINDH, PAKISTAN

Raheema Tahir^{1*}, Ghazala Yasmeen¹ and Noor Us Saher²

¹Department of Zoology, University of Karachi, Karachi

²Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: raheematahir@hotmail.com

The present study is to report the hazardous concentrations of Hg in kidney, liver and skin of *Euphlyctis cyanophlyctis* (common skittering frog) by determining the pollution biomarkers from urban and agricultural areas. Concentration of Hg was also evaluated in the water from the habitat of frogs to calculate the correlation between the tissues and environment. Kidney, liver and skin of *E. cyanophlyctis* showed positive and significant correlation with water. This indicated that Hg increases in the tissues with an increase in the environment. Moreover, no particular pattern was detected in the concentration of Hg in all four seasons throughout the year. Thus, kidney, liver and skin of *E. cyanophlyctis* can be used as potentially good biomarkers for Hg pollution. This research will not only benefit in developing conservation plans for amphibians but will also be helpful for other aquatic fauna.

PROTECTIVE ROLE OF DIETARY POMEGRANATE PEEL AGAINST BIFENTHRIN INDUCED TOXICITY IN ENDANGERED GOLDEN MASHSEER: A MULTIPLE BIOMARKER BASE ASSESSMENT

Shehzad Ghayyur^{1*}, Rimsha¹ and Shahryar Ghayyur²

¹Department of Zoology, Hazara University Mansehra

²Department of Physics, Govt Postgraduate College Haripur

*Corresponding Author: sgmzoologist1989@gmail.com

The current research was aimed to investigate the hazardous effects of bifenthrin on hematological, antioxidant, and immunological biomarkers of Endangered Golden Mahseer (*Tor. potitura*) along with the protective role of pomegranate peel powder to mitigate the toxic effect induced by bifenthrin. Fish were divided into six groups (n=10) in triplicates as follows: first group (T₁) served as control having no pesticide and feed with normal diet, the second and third groups (T₂ and T₃) were feed with 3% and 6% pomegranate peel supplementation, respectively, the fourth group (T₄) was treated with 2ppb of bifenthrin based on calculated LC₅₀ value and feed with normal diet, while fifth and sixth groups (T₅ and T₆) each were treated with 2ppb of bifenthrin and feed with 3% and 6% pomegranate peel supplementation, respectively for 1-week. The results displayed that bifenthrin exposure alone caused significant reduction in RBCs, Hb, HCT with increment in WBCs, MCH and Platelets in contrast to control group. Similarly,

significant reduction in immunological parameters i.e., IgM, lysozyme and respiratory burst were reported in bifenthrin exposed fish. Additionally, bifenthrin exposure significantly incline in hepatic and gills MDA and GSH levels and marked reduction in hepatic and gills CAT and SOD levels contrast to control group. Dietary supplementation of pomegranate peel powder significantly attenuated the bifenthrin induced alterations in studied biomarkers and this improvement was more pronounced in fish feed with 6% pomegranate peel powder. It is concluded that dietary supplementation with pomegranate peel powder has beneficial effects and was able to antagonize bifenthrin induced toxicity in *Tor. potitura*.

TOXICOLOGICAL IMPACTS OF NICKEL OXIDE NANOPARTICLES (NiO-NPs) ON HEMATO-BIOCHEMICAL PROFILE AND TISSUE HISTOLOGY OF FRESHWATER FISH SILVER CARP

Warda Naz* and Shehzad Ghayyur

Department of Zoology, Hazara University, Mansehra

*Corresponding Author: wardanaz669@gmail.com

NiO-NPs are considered one of the most common transition metal oxide nanoparticles excessively used in variety of industrial and commercial goods, can have several detrimental consequences on both human and environmental health. The goal of present investigation was to evaluate the effect of NiO-NPs on *Hypophthalmichthys molitrix*. A total 250 specimens were collected from fish farm and transported to the Zoology laboratory, Hazara University Mansehra. After calculating LC50 value 40 fish specimens were randomly divided into four different groups (n=10). Group-I served as a chemical-free control group, while the other three groups were treated with three different doses of NiO-NPs based on the calculated LC50 value. The results of hematological parameters revealed significant ($p < 0.05$) concentration dependent reduction in RBCs, Hb, HCT, and platelets with significant ($p < 0.05$) rise in TLC, MCV and MCH in NiO-NPs treated groups. Similarly, NiO-NPs exposed fish showed a significant ($p < 0.05$) increase in serum glucose, uric acid, urea, bilirubin, Na, K, Cl, and Fe while decrease in total protein, triglyceride, Ca, and P in contrast to control. In addition, the histopathological investigation also revealed remarkable alterations in the gills, liver and intestine tissues of fish exposed to NiO-NPs. It is concluded that NiO-NPs have a strong potential to change histo-architecture and hemato-biochemical profile which may contribute to the decline in population of Silver Carp in natural environment.

TOXICOLOGICAL EFFECTS OF CADMIUM ON LABEO ROHITA: A HISTOPATHOLOGICAL ANALYSIS

Muhammad Nadeem¹* Syed Qaswar Ali Shah¹ and Murtaza Manzoor¹

¹*Department of Zoology, Cholistan of Veterinary and Animal Sciences, Bahawalpur*

*Corresponding Author: nadeemray53@gmail.com

In aquatic life, cadmium is a non-essential metal that serves no biological purpose. Current study aims to understand the toxic effects of cadmium on *Labeo rohita*. *L. rohita* fingerlings were exposed to sub lethal concentrations of cadmium for a period of 28 days. Histopathological alterations and biomarker levels were assessed in gills. The gills showed notable histopathological alterations when exposed to cadmium. These changes include increase in primary lamella length, secondary lamella length, primary lamella width and secondary lamella width in treated group as compared to control. After metal exposure to fish histological alterations included hyperplasia, edema, curling of secondary lamella and epithelial

lifting was observed in gills as compared to control group. Additionally, catalase activity were increased. The results reveal that cadmium exposure can cause serious damage to gill tissues of *L. rohita*, posing a threat to fish health and the overall aquatic ecosystem.

AMELIORATIVE EFFECT OF *NIGELLA SATIVA* SILVER MEDIATED SILVER NANOPARTICLES AGAINST HEXAVALENT CHROMIUM INDUCED TESTICULAR TOXICITY IN MICE

Tooba Nauroze^{1*}, Shaukat Ali², Shagufta Andleeb¹ and Chaman Ara³

¹Department of Zoology, University of Education, Lahore

²Department of Zoology, Government College University, Lahore

³Institute of Zoology, University of the Punjab, Lahore

*Corresponding Author: dr.shaukatali@gcu.edu.pk, tooba.nauroze@ue.edu.pk

The present study was planned to find harmful effects on the reproductive system caused by Cr (VI) and the ameliorative effect of *Nigella sativa* and *Nigella sativa* mediated AgNP on male mice (*Mus musculus*). In the study of known infertility medicine, clomiphene citrate is also used as a positive control. The main objective of the present study was to assess the ameliorative potential of oral administration of a dose of 50 mg/Kg B.W clomiphene citrate (control), AgNP via chemical synthesis, *Nigella sativa* seed extract, and *Nigella sativa* mediated AgNP against the Cr (VI) at the dose of 1.5 mg/Kg B.W from K₂Cr₂O₇ orally induced toxicity over eight weeks on the reproductive performance of male albino mice. *Nigella sativa* mediated AgNPs were characterized by UV, SEM, FTIR, and XRD. The histological analysis, smear study, antioxidant capacity test, and hormone analysis were conducted by blood samples of albino mice. Cr exposed groups showed a significant decrease in sperm size, LH, testosterone, SOD, CAT, GSH, and no of spermatogonia and spermatocytes. However, administration of *Nigella sativa* and *Nigella sativa* mediated AgNPs reduced the toxicity.

SUB-LETHAL EFFECTS OF CHROMIUM+CADMIUM MIXTURE ON FISH, *LABEO ROHITA*

Ghanwa Maryum^{1*}, Huma Naz^{1*}, Tanveer Ahmed², Syed Qaswar Ali Shah¹, Sajid Abdullah³, Muhammad Umer Ijaz¹, Muhammad Usman⁴, Zulfiqar Khursheed¹ and Murtaza Manzoor¹

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

³Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

⁴ Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding author's e-mail: ghanwamaryam@gmail.com, dr.humanaz98@gmail.com

Fish in particular are increasingly at risk of illness due to heavy metal-induced aquatic pollution. The purpose of the study was to assess the impact of chromium and cadmium mixture on gills histology and enzyme (CAT) activity of *Labeo rohita*. For 15 days, fish were exposed to Cr+Cd mixture. Fish were dissected after the trial and the organs (gills, brain, heart, and muscles) were isolated to check the activity of the enzyme. The histological study of gills showed curling of lamellae, degeneration of epithelium, necrosis and fusion of secondary lamellae in exposed fish as compared to control group. Results of this study showed that the activity of catalase (CAT) enzyme in organs (gills, brain, Heart and muscles) was increased as compared to control group. In the conclusion, the exposure of Cr+Cd mixture

caused important alterations in gills histology and catalase activity of fish. Hence, biomarkers such as histology and CAT activity could serve as useful tools for identification of heavy metals contamination in water and to monitor the health of aquatic fauna. These heavy metals can cause the adverse health effects in organisms. There is no denying that industries are necessary for development, but on the other hand they are also creating heavy loss to the livelihood of humans and aquatic fauna.

STUDIES ON THE BIOCHEMICAL AND HISTOLOGICAL ALTERATIONS IN *OREOCHROMIS NILOTICUS* EXPOSED TO CEFOTAXIME

Muhammad Ahmad^{1*}, Huma Naz¹, Tanveer Ahmed², Syed Qaswar Ali Shah¹, Muhammad Adeel Hassan³, Sajid Abdullah⁴, Muhammad Usman⁵ and Murtaza Manzoor¹

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

³Department of Parasitology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

⁴Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

⁵Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: jamafanali125@gmail.com, dr.humanaz98@gmail.com

This study was assessed the chronic effects of antibiotic (cefotaxime) on biochemical and histological alterations of Nile tilapia (*O. niloticus*). The fish, tilapia was be exposed to 1/3rd value of LC₅₀ of cefotaxime for 30-day. After completion of exposure period, fish was be dissected and organs like heart, brain, intestine and muscles were isolated to observe the biochemical parameters viz., superoxide dismutase, catalase and peroxidase. The histological changes in gills of tilapia were also assessed. The statistical analysis showed the activities of, SOD, CAT and POx in heart, brain, intestine and muscles of cefotaxime-treated *O. niloticus* were significantly higher at end of the 30 days trial when compared with control. The comparison among tissues showed that SOD activity was higher in muscles followed by heart, brain and intestine. The observed histological disorder in gills of cefotaxime treated fish were hyperplasia, curling of secondary lamella, edema and epithelial.

CHRONIC EFFECTS OF METAL (Cd) TOXICITY ON LIVER OF *OREOCHROMIS NILOTICUS*

Mariam Toufiq^{1*}, Huma Naz¹, Tanveer Ahmed², Syed Qaswar Ali Shah, Sajid Abdullah³, Tahreem Asad⁴, Iqra Zulfiqar³, Muhammad Usman⁵ and Murtaza Manzoor¹

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

³Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

⁴Department of Poultry Sciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

⁵Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: maryamtoufiq6@gmail.com, dr.humanaz98@gmail.com

Aquatic pollution due to heavy metal causes toxic effects to fish and other organism. This study was designed to determine the chronic effects of cadmium on hepatosomatic index and liver histology of tilapia (*O. niloticus*). The fish were exposed to sub-lethal concentration (18.67mg/L) of Cd for 15 days. The fish was dissected every three days, and hepatosomatic index was calculated. There was an increase in HSI in control group and decrease in HSI in treatment group. The liver of Cd treated *O. niloticus* revealed severe damages,

including hemorrhage, irregular shaped hepatocytes (loss of their polygonal shape), enucleated cells, vacuolization and necrosis as compared to control group. This study has confirmed the toxic effects of cadmium to *O. niloticus* when introduced as a water pollutant. Therefore, we recommend the authorities not to dispose sewage and industrial waste into surface water or at least to treat these effluents before dumping them.

CHRONIC EFFECTS ON PB+CD MIXTURE ON BIOCHEMICAL AND HISTOLOGICAL PARAMETERS OF TILAPIA

Aqsa Batool^{1*}, Huma Naz¹, Tanveer Ahmed², Sajid Abdullah³, Muhammad Usman⁴, Adnan Ahmed Qazi¹, Zahid Manzoor⁵, Muhammad Adeel Hassan⁶ and Murtaza Manzoor¹

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

³Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

⁴Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur,

⁵Department of Pharmacology and Toxicology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

⁶Department of Parasitology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: aqsabatool809@gmail.com , dr.humanaz98@gmail.com

The study was planned to evaluate effect of cadmium (Cd) and lead (Pb) mixture on various health indices of *Oreochromis niloticus*. The fish was exposed to 1/3rd of LC₅₀ 180.349 mg/L of Pb+Cd for 15 days. After trial, fish was dissected and its organs (brain, heart, muscles and intestine) were isolated to check the catalase (CAT), peroxidase (POx) and superoxide dismutase (SOD) activities. The histological alterations in gills and liver were also observed in both control and treated fish. The activity of CAT, SOD and POx was significantly ($P>0.05$) increased in Pb+Cd exposed fish. The histological results showed that the diameter of hepatocytes in Pb+Cd treated fish was significantly less than control fish. Moreover, width of sinusoids in Pb+Cd mixture treated fish was significantly greater than control group. Some other histological disorders in liver were also seen in Pb+Cd mixture treated fish viz. haemorrhage, irregular shape hepatocytes (loss also of their polygonal shape), enucleated, eccentrically situated nuclei, vaculation and necrosis. The histological disorders in gills were seen in Pb+Cd treated *O. niloticus* include fusion of secondary lamella, curling filaments of secondary lamella, aneurysm, hyperplasia in secondary lamella, epithelial lifting and destruction in both lamella.

ASSESSMENT OF ARSENIC METAL IN FOOD CHAIN AT JINNAH BARRAGE

Muhammad Abrar, Sana Ashraf* and Muhammad Zohaib

Department of Zoology, The University of Lahore

Corresponding Author: sanaashrafdr@gmail.com

This study was designed to investigate the transfer of arsenic at various trophic levels in food chain at Jinnah Barrage, Mianwali. For this purpose, ten samples at each trophic level were selected. Different mean concentrations (ppm) of arsenic were observed in water (0.0215 ± 0.013), phytoplankton (*Spirogyra pratensis*) (0.047 ± 0.042), herbivores fish (*Labeo rohita*) (0.046 ± 0.003), omnivores fish (*Cyprinus carpio*) (0.028 ± 0.014), carnivores fish (*Wallago attu*) (0.026 ± 0.017) and kingfisher (*Halcyon smyrnensis*) (0.0125 ± 0.012). So, arsenic concentration at Jinnah Barrage was lower than WHO permissible value.

INVESTIGATE THE TOXIC EFFECT OF MORTEIN ON VITAL ORGANS**Iqra Ismail and Sana Ashraf***Department of Zoology, The University of Lahore, Lahore*Corresponding Author: sanaashrafdr@gmail.com

The study was conducted on one of the most widely used insecticidal Mortein in Pakistan as mosquito killer. The present work aims to investigate the effect of Mortein on liver tissue due to inhalation exposure. The study conducted on 30 mature Swiss albino laboratory male mice and equally divided into three groups. The control without any exposure but other two groups was fumigated as 10 ml Mortein/ 12 hr. for consecutive 15 days in an inhalation chamber. The histopathological changes in liver reveal that Mortein induce lipogenesis, hypercholesterolemia, necrosis, fibrosis, and megacheroitic generation of hepatocytes. The Mortein significantly ($p < 0.05$) increase cholesterol, triglyceride.

EFFECT OF TITANIUM DIOXIDE NANOPARTICLES ON GROWTH PARAMETERS OF GRASS CARP (*CTENOPHARYNGODON IDELLA*)**Sana Ashraf* and Sobia Nawaz***Department of Zoology, The University of Lahore, Lahore*Corresponding Author: sanaashrafdr@gmail.com

Titanium dioxide nanoparticles (TiO₂-NPs) are widely produced and released into the environment, posing a serious threat to fish and human health. Four million TiO₂ nanoparticles are consumed annually as pigments in a wide range of products, including food coloring, cosmetic industry, paper products, ink, and plastic products. This study was conducted to evaluate the effect of TiO₂-NPs on the growth parameters of grass carp. Fishes with average weight of 25-30g were used in a 60-day feeding experiment to evaluate the effect of TiO₂-NPs on growth parameters. Titanium dioxide nanoparticles at different concentrations of T1(0.5mg/L of TiO₂-NPs), T2(1mg/L of TiO₂-NPs), T3(1.5mg/L of TiO₂-NPs) T4(2 mg/L of TiO₂-NPs) were incorporated in a fish diet during 8-weeks period. Total three hundred fishes (20 per each treatment) were divided into five treatments (T₀, T1, T2, T3, T4) and each treatment has three replicates. Fish length (cm) and weight (g) were measured at 15th, 30th, 45th and 60th day. High mortality rate was observed after the exposure of T4 (high concentrations of TiO₂-NPs). A gradual increase in body weight (33.6 ± 1.23) and length of the fish was observed (21.5 ± 0.52) in the control group. A decrease in body weight and length was observed in all treatments until the 8th week. The results showed that high concentrations of TiO₂-NPs have an adverse effect on the growth. Therefore, TiO₂-NPs in natural water bodies may affect the aquaculture industry.

ASSESSMENT OF AFLATOXIN'S CONTAMINATION IN RICE SAMPLES**Sadia Amjad and Roheela Yasmeen****Department of Biology, Lahore Garrison University, Lahore**Corresponding Author: roheelayasmeen@lgu.edu.pk

Rice is a primary food crop for more than 50 % of the world's population and about 500 million tons of milled rice are produced each year at a global level. A lot of food wastage occurred due to

storage incapability. Approximately 1.6 million dollars economical loss is only related to the rice industry due to aflatoxins. These aflatoxins are also potent to produce health risks in humans. Although, various research practices are also going to find way for better storage and to reduce aflatoxin contamination. In the present study white and brown rice samples were selected to analyze aflatoxin levels and further treated for detoxification. A total of 50 rice samples of which white rice (30) and brown rice (20) were collected from different areas in and around Lahore, Pakistan. The quantification of aflatoxins in collected rice samples was carried out using Thin Layer Chromatography. Total 36% contamination was found in white rice samples and 23% were contaminated beyond permissible levels set by European Union. For brown rice 40% samples were contaminated and 20% were beyond the permissible levels. Hence brown rice were found more contaminated. Highest level $16.45 \pm 0.06 \mu\text{g/kg}$ of aflatoxins was found in brown rice sample. It was concluded aflatoxins as a source to spoil rice so, there is need to find appropriate ways to improve storage conditions.

EVALUATING THE THERAPEUTIC POTENTIAL OF *ALLIUM SATIVUM* CONJUGATED SILVER NANOPARTICLES IN DIETHYLNITROSAMINE-INDUCED HEPATOCELLULAR CARCINOMA IN MICE

Arooj Fatima, Shaukat Ali*, Nazish Mazher Ali, Muhammad Summer and Hafiz Muhammad Tahir

*Applied Entomology and Medical Toxicology Laboratory, Department of Zoology,
Government College University, Lahore*

Corresponding Author: dr.shaukatali@gcu.edu.pk

Hepatocellular Carcinoma is the most common and highly aggressive form of liver cancer, with high incidence and mortality rate. Medicinal plants like *Allium sativum* (Garlic) have anti-cancer properties due to their active biochemical components. Particularly, *Allium sativum* conjugated silver nanoparticles have been shown to have effective cytotoxic activity against hepatocellular carcinoma (HCC) cells. Current study was designed to evaluate the anticancer potential of *Allium sativum* conjugated silver nanoparticles against hepatocellular carcinoma in mice. Male albino mice were divided into 11 groups (n=5 in each group). Diethylnitrosamine (DEN) carcinogen was used to induce HCC. The treatment groups G1, G2A, G2B, G2C, G2D, G3, G4, G5, G6, G7, and G8 were treated with Saline, DEN, Cisplatin (T), *A. sativum* extract (T), *A. sativum* AgNPs (T), Cisplatin, *A. sativum* extract, *A. sativum* AgNPs, DEN+ Cisplatin, DEN+ *A. sativum* extract, DEN+ *A. sativum* AgNPs, respectively. The biochemical tests and liver histology were determined to analyze HCC status in mice. Results showed that the level of biomarkers AFP, AST, ALP, ALT, LDH, GGT, MDA, bilirubin, GSH, Catalase were significantly restore to its normal in all treatment groups but highest results was shown by ASNPs (T) (150mg/kg) as followed; AFP ($57.8 \pm 1.5 \text{ ng/mL}$), AST ($299.2 \pm 8.0 \text{ U/L}$), ALT ($142.8 \pm 3.9 \text{ U/L}$), ALP ($239.6 \pm 4.1 \text{ U/L}$), LDH ($546.8 \pm 6.9 \text{ U/L}$), GGT ($35.2 \pm 1.1 \text{ U/L}$), MDA ($6.7 \pm 0.2 \text{ mmol/l}$), CAT ($159.0 \pm 3.2 \text{ mmol/l}$), GSH ($3.9 \pm 0.1 \text{ umol/l}$), and bilirubin ($6.6 \pm 0.2 \text{ mg/dl}$). The histopathological study of liver tissues showed destruction in the DEN treated group, that were restored in all treatment groups after given treatment to them. The effective and encouraged results was showed in ASNPs (T) group. It can be concluded that, *Allium sativum* (bioactive components i.e., allicin, S-allyl cysteine, diallyl disulfide) conjugated AgNPs could be used against cancer by improving their liver histology and enhancing antioxidant defense systems.

EXPLORING THE ANTICANCER PROPERTIES OF *LACTOBACILLUS* SPP. PROBIOTICS IN DIETHYLNITROSOAMINE-INDUCED HEPATOCELLULAR CARCINOMA IN MICE

Muhammad Hammad, Shaukat Ali*, Nazish Mazhar Ali and Muhammad Summer

*Applied Entomology and Medical Toxicology Laboratory, Department of Zoology,
Government College University, Lahore*

Corresponding Author: dr.shaukatali@gcu.edu.pk

Hepatocellular carcinoma is the third leading cause of mortality and account for one million deaths annually, globally. Chemotherapy and radiotherapy are the conventional treatments with multiple side effects. In this regard, current study was aimed to investigate the anti-cancerous potential of probiotic *Lactobacillus reuteri* against hepatocellular carcinoma (HCC). Mice (60) were divided into 08 groups. Group 2 was administered with DEN for one month to induce HCC and then mice (20) were further divided into four subgroups 2A, 2B, 2C and 2D, treated with *Lactobacillus reuteri*, cisplatin, cisplatin+*Lactobacillus reuteri* and DEN, respectively. The biochemical analysis showed higher level of biomarkers i.e., AFP (97.8 ± 3.1 ng/mL), ASAT (528.4 ± 9.7 U/L), ALAT (249.2 ± 5.1 U/L), ALP (394.0 ± 6.0 U/L), LDH (968.8 ± 10.2 U/L), GGT (58.7 ± 1.7 U/L), bilirubin (11.1 ± 0.5 mg/dL), MDA (12.2 ± 0.3 mmol/L) and lower level GSH (2.4 ± 0.2 umol/L) and Catalase (91.8 ± 3.3 mmol/L) was observed in the DEN administered group that indicated the induction of HCC in mice. However, lower level of biomarkers i.e., AFP (53.0 ± 1.2 ng/mL), ASAT (285.4 ± 6.9 U/L), ALAT (152.4 ± 4.2 U/L), ALP (235.2 ± 1.8 U/L), LDH (575.2 ± 14.1 U/L), GGT (32.2 ± 1.2 U/L), Bilirubin (6.2 ± 0.2 mg/dL), MDA (6.8 ± 0.2 mmol/L) and higher level of biomarkers i.e., GSH (3.9 ± 0.1 umol/L) and Catalase (159.0 ± 3.2 mmol/L) was observed in treatment group with *Lactobacillus reuteri* + cisplatin. The histopathological analysis of liver from DEN treated group showed damaged central vein, liver hemorrhage with chronic inflammation. However, in the treatment group *Lactobacillus reuteri* + cisplatin, mild inflammation, normal central vein and activation of kupffer cells was observed. It is concluded that *Lactobacillus reuteri* has great anti-cancer potential in the prevention and treatment of hepatocellular.

PLUMBAGIN PROTECTS POLYETHYLENE MICROPLASTICS MEDIATED PULMONARY TOXICITY VIA ATTENUATION OF OXIDATIVE STRESS, INFLAMMATORY RESPONSE, APOPTOSIS AND HISTOPATHOLOGICAL DAMAGES

**Ali Akbar, Muhammad Umar Ijaz^{1*}, Muhammad Faisal Hayat¹, Muhammad Zaid Salar¹,
Rabia Azmat¹, Naila Ghafoor¹, Mehrab Khalil¹ and Muhammad Tariq¹**

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

Corresponding Author: umar.ijaz@uaf.edu.pk

Polyethylene microplastics (PEMPs) is a ubiquitous antineoplastic medicine that has been recognized to have sever toxic effects on several organs including lungs. Plumbagin (PBN) is a therapeutic compound with significant pharmacological activities. The present study was designed to evaluate the protective effect of PBN against PEMP induced pulmonary toxicity. 24 rats were randomly divided into 4 groups: control group, PEMP treated group (1.5 mgkg⁻¹), PEMP + PBN treated group (1.5 mgkg⁻¹ + 20 mgkg⁻¹) and PBN supplemented group (20 mgkg⁻¹). Following 30 days of administration, our results showed that PEMP treatment decreased the activity of antioxidant enzymes such as glutathione (GSH), glutathione reductase (GSR), glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) while elevated the level of malondialdehyde (MDA) along with reactive oxygen species (ROS). Furthermore, levels of inflammatory cytokines involving interleukin-6 (IL-6), nuclear

factor-kappa B (NF- κ B), interleukin-1 beta (IL-1 β), tumor necrosis factor alpha (TNF- α) and cyclooxygenase-2 (COX-2) were escalated. Besides, treatment with PEMP_s enhanced the activities of apoptotic proteins i.e., Bax, caspase-9 along with caspase-3 while reduced the activity of Bcl-2. Additionally, the histopathological examination revealed significant pulmonary tissue impairments in the PEMP_s exposed group. Nonetheless, PBN treatment considerably ($P < 0.05$) recovered the abovementioned PEMP_s - induced toxic effects. Therefore, the current research demonstrated that PBN may be used as a promising ameliorative agent to cure PEMP_s-instigated pulmonary damages due to its antioxidant, anti-inflammatory, antiapoptotic and histo-protective properties.

MITIGATIVE EFFECTS OF SCPOLETIN ON POLYSTYRENE MICROPLASTICS INDUCED RENAL DAMAGE

Muhammad Umar Ijaz*, Muhammad Zaid Salar

Department of Zoology, Wildlife & Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Polystyrene microplastics (PS-MPs) are one of the most prevalent environmental contaminants due to their ability to instigate impairments in different organs including kidney. Scopoletin (SPO) is a naturally occurring compound which belongs to coumarin family and obtained from many plants. SPO possess various biological and pharmacological activities such as anti-oxidant, anti-inflammatory and anti-apoptotic and anti-cancerous. Therefore, the present research was conducted to evaluate the mitigative potential of SPO against PS-MPs prompted renal toxicity in albino rats. Forty-eight albino rats were randomly divided into 4 groups, such as control group, PS-MPs treated group (0.01 mgkg⁻¹), PS-MPs + SPO co-treated group (0.01 mgkg⁻¹ and 20 mgkg⁻¹ respectively) and only SPO treated group (20 mgkg⁻¹). After 30 days of treatment, it was revealed that the exposure of PS-MPs significantly reduced anti-oxidant enzymes levels such as, glutathione peroxidase (GPx), glutathione reductase (GSR), catalase (CAT), glutathione-S-transferase (GST), superoxide dismutase (SOD) and glutathione (GSH). However, in PS-MPs treated rats malondialdehyde (MDA) and reactive oxygen species (ROS) contents were significantly elevated. PS-MPs exposure also increased the serum level of urea, and creatinine while reducing creatinine clearance levels. Furthermore, KIM-1 and NGAL levels were also increased in PS-MPs intoxicated rats. Moreover, inflammatory indices i.e., nuclear factor kappa-B (NF- κ B), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) interleukin-1 β (IL-1 β) and cyclooxygenase-2 (COX-2) activity was increased in PS-MPs treated rats. Additionally, it also escalated the expression of pro-apoptotic indices (Bax and Caspase-3) while decreasing the expression of antiapoptotic marker (Bcl-2). The exposure of PS-MPs also instigated significant histopathological damages in renal tissues. Nonetheless, SPO supplementation recovered all these damages due to its anti-apoptotic, anti-oxidant and anti-inflammatory nature.

PALLIATIVE POTENTIAL OF BACUCHIOL AGAINST CISPLATIN PROMPTED HEPATIC DAMAGES IN RATS

Muhammad Faisal Hayat and Muhammad Umar Ijaz*

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Cisplatin (CP) is a ubiquitous chemotherapeutic agent which is used to treat various sorts of malignancies. CP intoxication is known to be associated with various clinical complications

including hepatotoxicity. Bacuchiol (BAK) is an active meroterpenoid that exhibits a broad range of biological as well as pharmacological activities. This study was designed to determine the hepato-protective potential of BAK against CP instigated hepatotoxicity in rats. Forty-eight rats were distributed into four groups such as control group, CP-treated group (10 mg/kg), CP (10 mg/kg) + BAK (2 mg/kg) exposed group and BAK (2 mg/kg) only supplemented group. It was revealed that CP exposure reduced the activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSR), glutathione peroxidase (GPx), Heme- oxygenase-1 (HO-1) & glutathione (GSH) content. Furthermore, the levels of reactive oxygen species (ROS) & malondialdehyde (MDA) were increased. In addition, CP significantly increased the hepatic serum enzymes including alkaline phosphatase (ALP), aspartate transaminase (AST), & alanine transaminase (ALT) along with inflammatory biomarkers levels such as tumor necrosis- α (TNF- α), nuclear factor- κ B (NF- κ B), interleukin-6 (IL-6), interleukin 1beta (IL-1 β), & cyclo-oxygenase-2 (COX-2) activity. CP intoxication increased the expressions of pro-apoptotic markers i.e., Bcl-2-associated X protein (Bax) & Cysteine-aspartic protease-3 (Caspase-3) while reducing the expression of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2). Furthermore, CP intoxication prompted various histopathological impairments. However, the co-administration of BAK significantly improved the abovementioned hepatic damages induced by CP. The present study indicated that BAK may be an effective therapeutic candidate to mitigate CP provoked hepatic impairments due to its anti-apoptotic, antioxidant & anti-inflammatory properties.

THERAPEUTIC POTENTIAL OF GERANIOL TO COUNTERACT POLYETHYLENE MICROPLASTICS INSTIGATED HEPATIC DAMAGES IN RATS

**Syeda Sania Zahara, Muhammad Faisal Hayat, Muhammad Umar Ijaz*,
Ali Akbar and Muhammad Zaid Salar**

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Polyethylene microplastics (PEMPs) are environmental contaminants which pose adverse effects to the health of humans as well as their ecosystem. Geraniol (GN) is plant-based isoprenoid which is well-known for its pharmacological potential. The current study was conducted to evaluate the therapeutic potential of GN against PEMP-induced liver damage in albino rats. Twenty-four rats were divided into 4 groups such as control, PEMP (1.5 mg/kg) treated, PEMP (1.5 mg/kg) + GN (50 mg/kg) exposed and only GN (50 mg/kg) supplemented group. It was revealed that PEMP exposure significantly reduced the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GSR) & glutathione (GSH) contents while escalating the levels of reactive oxygen species (ROS) and malondialdehyde (MDA). Furthermore, administration of PEMP elevated the levels of alanine transaminase (ALT), aspartate transaminase (AST) & alkaline phosphatase (ALP). Besides, the levels of nuclear factor- κ B (NF- κ B), tumor necrosis- α (TNF- α), interleukin-6 (IL-6), interleukin 1beta (IL-1 β), & cyclo-oxygenase-2 (COX-2) activity were increased in response to PEMP treatment. Moreover, PEMP administration instigated various histopathological impairments in hepatic tissues. However, supplementation of GN substantially restored aforementioned irregularities owing to its antioxidant, anti-inflammatory as well as hepatoprotective potential.

AMELIORATIVE EFFECTS OF SINAPIC ACID AGAINST - NONYLPHENOL INDUCED RENAL TOXICITY IN RATS

**Mehrab Khalil, Muhammad Umar Ijaz*, Syeda Sania Zahara, Muhammad Faisal Hayat,
Ali Akbar and Muhammad Sakandar Majid**

Department of Zoology, Wildlife & Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Nonylphenol (NP) is a ubiquitous environmental toxicant which instigate adverse effects on multiple organs including kidneys. Sinapic acid (SA) is a phenolic compound belongs to class of hydroxycinnamic acids, which exhibits a wide range of pharmacological properties. The current investigation was planned to assess the protective effect of SA against NP-induced renal toxicity in albino rats. 48 albino rats were separated into four groups: control group, NP administrated group (50 mg/kg), NP + SA treated group (50 mg/kg+ 25 mg/kg) and only SA administrated group (25 mg/kg). Following 30 days of administration, results showed that NP reduced the antioxidant enzymes i.e., glutathione (GSH), glutathione reductase (GSR), glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) activities, besides elevated malondialdehyde (MDA) along with reactive oxygen species (ROS) level. Moreover, level of inflammatory cytokines involving nuclear factor-kappa B (NF- κ B), interleukin-6 (IL-6), interleukin-1 beta (IL-1 β), tumor necrosis factor alpha (TNF- α) & cyclo-oxygenase-2 (COX-2) were escalated ($p < 0.05$) by NP intoxication. Furthermore, treatment with NP increased the level of apoptotic proteins comprising of Bax, caspase-9 along with caspase-3 besides lessened the level of Bcl-2. In addition to this, NP reduced the level of albumin, creatinine clearance & augmented the level of creatinine, urea, urobilinogen, urinary protein, kidney injury molecules-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL) as well as instigated various histopathological damages. However, SA treatment substantially ($p < 0.05$) recovered NP-induced damages & histopathological impairments. Therefore, it can be presumed that SA may be used as a therapeutic agent to palliate NP-induced kidney damages.

ASSESSMENT OF PROTECTIVE EFFECTS OF FORMONONETIN ON NONYLPHENOL INSTIGATED HEPATOTOXICITY IN RATS

**Naila Ghafoor, Muhammad Umar Ijaz*, Muhammad Faisal Hayat,
Syeda Sania Zahara and Ali Akbar**

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Nonylphenol (NP) is a ubiquitous environmental toxicant that is known to cause hepatotoxicity via generation of reactive oxygen species (ROS). Interestingly, a plant-extract named formononetin (FN) is found to possess promising pharmacological activities, including cellular neuroprotection. The current study was intended to ascertain the hepatoprotective role of FN against NP-generated liver toxicity in rats. 48 male rats were distributed into four groups. viz. Control, NP-intoxicated group (50 mg/kg), NP + FN-treated group (50 mg/kg + 50 mg/kg) and FN-treated group (50 mg/kg). All the doses were administered orally. Our findings indicated that the NP intoxication upregulated the alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) level. Moreover, it brought down the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GSR) and glutathione-S-transferase (GST), as well as the glutathione (GSH) content. Conversely, the levels of malondialdehyde (MDA) and ROS were escalated. Besides, the levels of nuclear

factor-kappa B (NF- κ B), tumor necrosis factor-alpha (TNF- α), interleukin-1beta (IL-1 β) and IL-6 as well as the cyclooxygenase-2 (COX-2) activity were elevated due to the NP administration. In addition to it, histopathological assessment displayed the prominent morphological alterations in hepatic tissues of rats. Nevertheless, treating the rats with FN significantly abated all the NP-instigated liver damages in rats. Therefore, it was evinced that FN could be used as a hepatoprotective agent against the NP-prompted liver toxicity owing to its antioxidant and anti-inflammatory properties.

AMELIORATIVE EFFICACY OF WEDELOLACTONE AGAINST BISPHENOL AN INDUCED CARDIOTOXICITY IN RATS

Rabia Azmat, Muhammad Umar Ijaz*, Muhammad Faisal Hayat, Ali Akbar and Muhammad Zaid Salar

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Bisphenol A (BPA), which is extensively used in the production of polycarbonate plastics and the incidence of cardiovascular diseases. This association is most likely caused by the BPA's ability to disrupt multiple cardiac mechanisms. Wedelolactone (7-methoxy-5, 11, 12-trihydroxy-coumestan, WED), the natural active compound derived from *Ecliptae Herba*, has been reported with valuable bioactivity for heart protection. Nevertheless, the effect of WED on BPA induced cardiotoxicity remains unexplored. Therefore, the present study was designed to evaluate the protective effects of WED against BPA induced cardiotoxicity. Twenty-four male albino rats were randomly divided into 4 different groups viz. control group, BPA (100 mg/kg), BPA+WED (100 mg/kg + 10 mg/kg) group, and WED (10 mg/kg) group. BPA intoxication substantially reduced the activities of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSR), glutathione S-transferase (GST), and glutathione (GSH), whereas an elevation was observed in reactive oxygen species (ROS), malondialdehyde (MDA) along with hydrogen peroxide (H₂O₂) level. Moreover, BPA treatment also increased the concentrations of cardiac injury markers, creatinine phosphokinase (CPK), creatine kinase-myoglobin binding (CK-MB), lactate dehydrogenase (LDH), and troponin I. The findings indicated that BPA substantially increased the inflammatory markers, including nuclear factor kappa B (NF- κ B), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) levels and cyclooxygenase-2 (COX-2) activity and histopathological damages. However, the administration of WED significantly reduced BPA induced aforementioned disruptions. In the light of these findings, it is concluded that WED is a promising bioactive compound that may be used as a protective agent against BPA instigated cardiac damage owing to its antioxidant, anti-apoptotic, anti-inflammatory as well as cardio-protective potential.

METHANOLIC EXTRACT OF *FICUS BENJAMINA* VAR. *COMOSA* AS AN AMELIORATIVE AGENT AGAINST ARSENIC INSTIGATED TESTICULAR DAMAGE IN MALE ALBINO RATS

Muhammad Zaid Salar, Muhammad Umar Ijaz*, Muhammad Faisal Hayat, Ali Akbar, Naila Ghafoor, Mehrab Khalil and Rabia Azmat

Department of Zoology, Wildlife & Fisheries, University of Agriculture, Faisalabad

*Corresponding author: umar.ijaz@uaf.edu.pk

Arsenic (AR) is one of the most toxic environmental pollutants that induce oxidative stress (OS) in various body organs, including testes. *Ficus benjamina* var. *comosa* (FBC) is a plant

belonging to family Moraceae with potential antioxidant, antimicrobial, antiviral and hemolysis properties. Therefore, the present study was designed to evaluate the mitigative potential of FBC against AR prompted testicular toxicity in rats. Forty-eight adult male albino rats were randomly distributed into 4 groups: control, AR administered (8 mgkg⁻¹), AR (8 mgkg⁻¹) + FBC (75 mgkg⁻¹) co-treated and FBC (75 mgkg⁻¹) only treated group. After 56 days of treatment, it was revealed that the activities of anti-oxidants i.e., catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSR), hemeoxygenase-1 (HO-1) and glutathione peroxidase (GPx) were reduced, besides malondialdehyde (MDA) and reactive oxygen species (ROS) contents were increased significantly following the AR exposure. Moreover, AR exposure significantly reduced the sperm motility, viability and count, whereas considerably increased the dead sperm number and sperm structural anomalies. Furthermore, AR remarkably decreased steroidogenic enzymes and Bcl-2 expression, while increasing the expression of Caspase-3 and Bax. AR exposure significantly reduced the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, whereas inflammatory indices were increased. AR exposure also induced significant histopathological damages in the testes. Nevertheless, FBC supplementation significantly abrogated all the damages induced by AR. The findings of our study demonstrated that FBC could significantly attenuate AR instigated OS and testicular toxicity, due to its anti-oxidant, anti-inflammatory, androgenic and anti-apoptotic potential.

STUDY OF ECOTOXICOLOGICAL EFFECTS OF BIOPESTICIDES ON *LUMBRICUS TERRESTRIS* AND *PHERETIMA POSTHUMA*

Zoha Manzoor, Warda Mustfa, Nazia Ehsan*, Muhammad Tahir and Iqra Bibi

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: nazia.ehsan@uaf.edu.pk

Earthworms play a significant role in soil fertility and organic matter decomposition. They increase soil fertility by burrowing behavior. In present study the ecotoxicological effects of biopesticides on earthworms *Lumbricus terrestris* and *Pheretima posthuma* were investigated. The present experiment was designed to study the effects of biopesticides on earthworm's growth, survival, cocoon production and DNA damage. Earthworms were collected from fish farm of Zoology, Wildlife and Fisheries Department, University of Agriculture Faisalabad and treated with biopesticides in biomonitoring laboratory. Leaves extract of neem and datura were taken as biopesticide. There were total seven groups, one with control and six treatment groups with different concentrations of biopesticides. Group T0 was control, Group T1 and Group T2 were treated with low dose of neem and datura (150mg/kg), Group T3 and Group T4 were treated with high doses (250mg/kg) of neem and datura, Group T5 was treated with the mixture of low doses and Group T6 was treated with the mixture of high doses of both biopesticides. After the exposure of high concentration, weight of *Pheretima posthuma* and *Lumbricus terrestris* was significantly decreased. In treated groups weight and length decreased and no cocoon production and mortality also occur. In comparison to control group there is no reduction in weight and length was observed. Comparison of control group and treated groups were analyzed by Tukey HSD test. There is a significant difference was observed between all treatments. But T5 and T6 show more significant differences both in weight and length. DNA damage was analyzed by Comet Assay technique for both species at different concentrations of biopesticides. There was slight damage observed in DNA when high doses were applied.

ASSESSMENT OF PROTECTIVE EFFECTS OF FORMONONETIN ON NONYLPHENOL INSTIGATED HEPATOTOXICITY IN RATS

Rimsha Eman, Naila Ghafoor, Muhammad Umar Ijaz* and Muhammad Sakandar Majid

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Nonylphenol (NP) is a ubiquitous environmental toxicant that is known to cause hepatotoxicity via generation of reactive oxygen species (ROS). Interestingly, a plant-extract named formononetin (FN) is found to possess promising pharmacological activities, including cellular neuroprotection. The current study was intended to ascertain the hepatoprotective role of FN against NP-generated liver toxicity in rats. 48 male rats were distributed into four groups. viz. Control, NP-intoxicated group (50 mg/kg), NP + FN-treated group (50 mg/kg + 50 mg/kg) and FN-treated group (50 mg/kg). All the doses were administered orally. Our findings indicated that the NP intoxication upregulated the alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) level. Moreover, it brought down the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GSR) and glutathione-S-transferase (GST), as well as the glutathione (GSH) content. Conversely, the levels of malondialdehyde (MDA) and ROS were escalated. Besides, the levels of nuclear factor-kappa B (NF- κ B), tumor necrosis factor-alpha (TNF- α), interleukin-1beta (IL-1 β) and IL-6 as well as the cyclooxygenase-2 (COX-2) activity were elevated due to the NP administration. In addition to it, histopathological assessment displayed the prominent morphological alterations in hepatic tissues of rats. Nevertheless, treating the rats with FN significantly abated all the NP-instigated liver damages in rats. Therefore, it was evinced that FN could be used as a hepatoprotective agent against the NP-prompted liver toxicity owing to its antioxidant and anti-inflammatory properties.

EVALUATION OF RENOPROTECTIVE EFFECTS OF ISOQUERCITRIN AGAINST DOXORUBICIN INDUCED KIDNEY DAMAGE BY MITIGATION OF OXIDATIVE STRESS

Ghulam Mustafa, Muhammad Umar Ijaz*, Rabia Azmat and Muhammad Sakandar Majid

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Doxorubicin (DOX) is an anticancer agent that is generally used to treat wide range of malignancies. Isoquercitrin (quercetin-3-O- β -d-glucopyranoside) is a flavonoid that exhibits antioxidant, anti-inflammatory and anti-apoptotic activities. However, it is unclear whether isoquercitrin has protective effects on kidney damage. Therefore, this study was designed to evaluate the curative potential of isoquercitrin on DOX induced renal damage in rats. 24 male albino rats were distributed into four groups that include control group, DOX (3 mg/kg) treated group, DOX + isoquercitrin (3 mg/kg + 40 mg/kg) co-treated group and isoquercitrin (40 mg/kg) only treated group. The results of the experiment showed that DOX exposure induced a considerable reduction in enzymatic activities of antioxidants including glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GSR), catalase (CAT), glutathione-S-transferase (GST) as well as glutathione (GSH) along with an increase in the levels of reactive oxygen species (ROS) and malondialdehyde (MDA). Similarly, DOX administration provoked rise in the urea, kidney injury molecule-1 (KIM-1), creatinine, in addition to neutrophil gelatinase-associated lipocalin (NGAL) level, on the other hand prompting a notable decline in creatinine clearance level. DOX induction

brought a significant escalation in interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), nuclear factor kappa-B (NF- κ B) and cyclooxygenase-2 (COX-2). In addition, DOX intoxication showed a considerable elevation in Bax, Caspase-3, and Caspase-9 levels, while lowered Bcl-2 level. DOX treatment also prompted histomorphological impairments in kidney tissues. However, isoquercitrin co-treatment significantly alleviated DOX induced kidney damage in rats. In conclusion, isoquercitrin has the potential to significantly mitigate DOX-induced nephrotoxicity because of its antioxidant, anti-inflammatory, and anti-apoptotic activities.

HEPATOPROTECTIVE EFFECTS OF SQUALENE AGAINST POLYSTYRENE MICROPLASTICS INDUCED HEPATIC DAMAGE IN MALE ALBINO RATS BY MODULATING BIOCHEMICAL AND HISTOLOGICAL PROFILE

Muhammad Makhdoom Jalal, Muhammad Umar Ijaz* and Ali Akbar

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Polystyrene microplastics (PSMPs) are environmental pollutants that induce oxidative stress (OS) in multiple organs particularly, liver. Squalene (SQN) is a naturally present flavones that shows diverse pharmaceutical properties i.e., anti-oxidant, anti-inflammatory and anti-apoptotic. Therefore, the present study was designed to evaluate the therapeutic role of SQN against PSMPs induced hepatotoxicity. 48 rats were distributed into 4 groups i.e., control, PSMPs (0.01 mgkg⁻¹) treated, PSMPs + SQN (0.01 mgkg⁻¹ + 30 mgkg⁻¹) co-treated and only SQN (30 mgkg⁻¹) treated group. PSMPs intoxication reduced the activities of catalase (CAT), glutathione-S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidases (GPx) glutathione reductase (GSR) and glutathione (GSH) level, whereas increased the levels of ROS and MDA. Additionally, PSMPs increased the levels of liver serum marker enzymes i.e., alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST). Moreover, the level of inflammatory makers such as nuclear factor-kappa B (NF- κ B), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β) and cyclooxygenase-2 (COX-2) activity were increased following the PSMPs exposure. The intoxication of PSMPs elevated Caspase-3, Bax and Caspase-9 levels, while reducing the Bcl-2 level. Furthermore, the exposure of PSMPs induced significant histopathological damages in hepatic tissue of rats. However, the supplementation of SQN considerably improved the PSMPs induced alterations due to its hepatoprotective, anti-inflammatory, anti-apoptotic and anti-oxidant nature.

CURATIVE POTENTIAL OF VANILLIN AGAINST PFOS INSTIGATED HEART DAMAGE IN RATS

Qurat-Ul-Ann Sattar, Naila Ghafoor, Muhammad Sakandar Majid and Muhammad Umar Ijaz*

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Perfluorooctane sulfonate (PFOS), a persistent organic pollutant, has been demonstrated to cause multiple toxicities. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a naturally occurring phenolic compound is found to possess promising pharmacological activities. In this study, the role of Vanillin was explored in alleviating PFOS induced heart toxicity in rats. 48 male rats were distributed into four groups. viz. Control, PFOS-treated group (20 mg/kg), PFOS + Vanillin treated group (20 mg/kg + 40 mg/kg) and

Vanillin treated group (40 mg/kg). All the doses were administered orally. Furthermore, concentrations of cardiac injury markers, creatinine phosphokinase (CPK), creatine kinase-myoglobin binding (CK-MB), & lactate dehydrogenase (LDH), as well as troponin I were increased in response to PFOS treatment. Moreover, it brought down the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GSR) and glutathione-S-transferase (GST), as well as the glutathione (GSH) content. Conversely, the levels of malondialdehyde (MDA) and ROS were escalated. Besides, the levels of nuclear factor-kappa B (NF- κ B), tumor necrosis factor-alpha (TNF- α), interleukin-1beta (IL-1 β) and IL-6 as well as the cyclooxygenase-2 (COX-2) activity were elevated due to the PFOS administration. In addition to it, histopathological assessment displayed the prominent morphological alterations in cardiac tissues of rats. Nevertheless, treating the rats with Vanillin significantly abated all the PFOS-instigated cardiac damages in rats. Therefore, it was evinced that Vanillin could be used as a cardioprotective agent against the PFOS-prompted heart toxicity owing to its pharmacological properties.

MITIGATIVE ROLE OF B-AMYRIN AGAINST PARAQUAT INDUCED RENAL TOXICITY IN RATS

Maryam Jameel, Muhammad Faisal Hayat, Muhammad Umar Ijaz*, Syeda Sania Zahara, Ali Akbar and Muhammad Zaid Salar

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Paraquat (PQ) is an agriculture herbicide which is used to control the growth of unwanted herbs. β -amyryn (β -Amy) is a triterpenoid which is derived from various medicinal herbs and possesses a wide range of biological as well as pharmacological potential. The present study was designed to assess the ameliorative ability of β -Amy against PQ instigated renal toxicity in albino rats. Twenty-four rats were apportioned into four equal size groups including control, PQ (5 mg/kg), PQ (5 mg/kg) + β -Amy (50 mg/kg) and only β -Amy (50 mg/kg) treated group. It was revealed that activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GSR) & glutathione (GSH) contents were reduced while the levels of reactive oxygen species (ROS) and malondialdehyde were escalated in response to PQ exposure. Moreover, PQ administration upregulated the levels of KIM-1 and NGAL. Similarly, PQ treatment increased the levels of urea and creatinine while reducing the levels of creatinine clearance. Additionally, the levels of inflammatory biomarkers nuclear factor- κ B (NF- κ B), tumor necrosis- α (TNF- α), interleukin-6 (IL-6), interleukin 1beta (IL-1 β), & cyclo-oxygenase-2 (COX-2) activity were escalated due to PQ exposure. Besides, administration of PQ elevated the levels of Bax and caspase-3 while downregulating the levels of Bcl-2. Furthermore, PQ exposure disrupted the normal architecture of renal tissues. Nevertheless. β -Amy supplementation revoked abovementioned impairments via regulating antioxidant, inflammatory and apoptotic biomarkers.

7. VIROLOGY

ESTIMATES OF BURDEN OF COVID-19 AT CIVIL HOSPITAL SUKKUR, SINDH, PAKISTAN

Urooj Zainab, Ghulam Murtaza Golo, Sapna Waheed, Khadim Hussain Memon*

Department of Zoology, Shah Abdul Latif University, Khairpur

*Corresponding Author: khadim.memon@salu.edu.pk

This study was conducted on the prevalence of COVID-19 disease at the Civil Hospital, Sukkur, Sindh, Pakistan based on secondary data. The data of 836 Covid patients were provided by Civil Hospital Sukkur from the period of March 2020 to December 2021. According to present research work the first Covid case was registered in March 2020 at civil hospital Sukkur. This study found that out of 836 Covid patients the 2.5% patients were in age group of (Below 20 years), 7.05% in (21-30), 13.63% in (31-40), 20.09% in (41-50), 24.1% in (51-60), 19.97% in (61-70), 9.21% in (71-80) and 3.34% in (81 to above). The maximum covid patients were found in age group of (51-60) and minimum in age group of (Below 20 years). The males 59% (495) were more affected than females 41% (341). But the mortality rate was high in females (34%) as compared to males (32%). In males the mortality rate below the age group of 20years was (20%), 21 to 30 (27%), 31 to 40 (26%), 41 to 50 (28%), 51 to 60 (29%), 61 to 70 (44%), 71 to 80 (43%), 81 to above (28%). In females the mortality rate below the age group of 20years was (9%), 21 to 30 (16%), 31 to 40 (22%), 41 to 50 (40%), 51 to 60 (40%), 61 to 70 (42%), 71 to 80 (36%), 81 to above (30%). The total 154 (18.4%) patients were admitted in the year of 2020, of which 1.9% patients were admitted in March, 7.7% in April, 13.6% in May, 22.7% in June, 12.9% in July, 8.4% in August, 1.2% in September, 1.2% in October, 11.6% in November, and 18.1% in December 2020. The total 682 (81.5%) patients were admitted in the year of 2021, of which 6.1% were admitted in January, 2.9% in February, 1.7% in March, 11.4% in April, 23.3% in May, 5.1% in June, 7.6% in July, 12.1% in August, 13.7% in September, 5.7% in October, 5.5% in November and 4.3% in December 2021. Out of 836 patients, 559 (66%) patients successfully recovered while 277 (33%) patients expire. The case Fatality ratio (CFR) of our study was 33%. Ratio of the patients was increased in 2021 as compare to 2020.

8. CANCER BIOLOGY

EVALUATING SOCIODEMOGRAPHIC FACTORS INFLUENCING CERVICAL CANCER AND DETECTION OF CERVICAL LESION BY SCREENING METHODS; VISUAL INSPECTION WITH ACETIC ACID (VIA) AND PAPANICOLAOU (PAP) SMEAR AMONG WOMEN IN A TERTIARY CARE HOSPITAL

Amama Rani^{1*} and Fariha Hasan²

¹*Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad*

²*Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad*

*Corresponding Author: amama.rani@yahoo.com

Cervical cancer is the most prevalent gynaecological malignancy in low to middle-income countries. In Pakistan, cervical cancer ranks as the 3rd leading cause of death in females. The purpose of this study was to determine the socio-demographic factors influencing cervical cancer screening and to assess the role of screening test for early detection of cervical carcinoma in the Pakistani female. Cervical smears were taken from married females with gynaecological problems visiting Pakistan Institute of Medical sciences. Pap smears were also obtained from Armed forces institute of Pathology, Rawalpindi. In the present study, a total of 118 cervical smears were screened using Pap smear test. All patients were also subjected to visual inspection with acetic acid (VIA). The mean age of the female patients was 35.78 ± 9.8 years. There were 42(35.5%) normal Pap smear, 33(27.9%) inflammatory, 11(9.3%) LSIL, 5(4.2%) HSIL and 18(15.2%) abnormal Pap smear. Acetic acid (VIA) was positive in 27 (22.8%) patients. An early Pap smear test can be effective in the diagnosis of premalignant lesions of cervix. However, visual inspection of the cervix with acetic acid is an effective method for immediate detection of cervical malignancies in low resource countries like Pakistan. Nationwide programs should be established to increase knowledge of cervical cancer and its screening to decrease late presentation and rate of mortality.

THE PROBLEMS RELATED TO PREVENTIVE, DIAGNOSTIC AND TREATMENT STRATEGIES OF CANCER IN PAKISTAN

Durr-e-Samin Tahir

Department of Zoology, Abdus Salam School of Sciences, Nusrat Jahan College, Rabwah

*Corresponding Author: durre.samin@njc.edu.pk

Cancer is a growing health concern in Pakistan, and the country faces several challenges related to the preventive, diagnostic, and treatment strategies in this regard. Nineteen million new cancer cases were recorded in 2020 alone in Pakistan. Among children, the prevalent types of cancers for both sexes encompass Hodgkin lymphoma, acute lymphoblastic leukemia, non-Hodgkin lymphoma, osteosarcoma, and retinoblastoma. In the case of adult females, the most frequently observed cancers include breast cancer, ovary and uterine adnexa cancers, lip and oral cavity cancers, cervix uteri cancer, and colorectum cancers. For adult males, the top five most common malignancies comprise colorectum cancer, prostate cancer, lip and oral cavity cancer, non-Hodgkin lymphoma, and liver and intrahepatic bile duct cancers. The country grapples with significant risk factors influencing cancer pathogenesis, including food adulteration, the use of gutkha/pan, smoking, and nutritional deficiencies. These factors collectively

contribute to the complex landscape of cancer in Pakistan. Lack of awareness and education, limited access to healthcare facilities, inadequate screening programs, high treatment costs, shortage of oncologists and cancer treatment facilities, stigma and sociocultural factors, insufficient government initiatives are some of the key problems that contribute towards the above-stated figures.

BERGENIA CILIATA AND SILK FIBROIN NANOPARTICLES: A NOVEL APPROACH TO TARGETING BREAST CANCER IN MICE

Faiza, Shaukat Ali^{*}, Muhammed Summer and Hafiz Muhammad Tahir
*Applied Entomology and Medical Toxicology Laboratory, Department of Zoology,
Government College University, Lahore*

*Corresponding Author: dr.shaukatali@gcu.edu.pk

Breast cancer is the most common fatal disease among women. The conventional treatments raised concerns about reduced bioavailability, poor cellular uptake, emerging resistance, and unwanted toxicity. *Bergenia ciliata* has drawn increasing attention in the treatment of carcinoma due to its remarkable pharmacological activities. In this research, *Bergenia ciliata*-loaded silk fibroin nanoparticles were developed for controlled drug delivery to overcome drug resistance and toxicity. Silk fibroin, due to its distinct attributes such as biodegradability, biocompatibility, and exceptional mechanical properties, is a promising vehicle for delivering therapeutic drugs. In this study, breast cancer was induced by cadmium chloride (25 mg/kg) in mice. After breast cancer induction, the groups were treated with Tamoxifen (25 mg/kg), *Bergenia ciliata* (200 mg/kg), FNP (25 mg/kg), and BCFNP (25 mg/kg) for 1 month. At the end of the trial, the serum levels of various pro-inflammatory cytokines, such as TNF- α , IL-6, and IL-10, and various metabolic enzymes, namely LDH, ASAT, ALAT, GSH, ALP, and MDA, were analyzed from the blood serum of all groups. However, the best result was exhibited by the group treated with *Bergenia ciliata* loaded fibroin nanoparticles (BCFNP) exhibiting the minimum level of pro-inflammatory cytokines, i.e., TNF- α (31.7 ± 1.4 pg/ml), IL-6 (20.2 ± 0.9 pg/ml), and IL-10 (25.4 ± 1.9 pg/ml), as compared to the CdCl₂-treated group, i.e., TNF- α (57.0 ± 2.8 pg/ml), IL-6 (39.8 ± 1.6 pg/ml), and IL-10 (63.0 ± 2.8 pg/ml). Moreover, the BCFNPs treated group also showed favourable enzymatic level results, close to the control group. Histological analysis of BCFNPs treated group revealed normal mammary tissue structure. The UV spectra of FNPs and BCFNPs showed maximum absorption at 252nm and 292 nm, respectively. In conclusion, *Bergenia ciliata* loaded fibroin nanoparticles exhibit effective potential to treat tumors through controlled drug delivery.

RAPHANUS SATIVUS CONJUGATED SILVER NANOPARTICLES: A PROMISING ANTICANCER AGENT IN DIETHYLNITROSAMINE-INDUCED HEPATOCELLULAR CARCINOMA IN MICE

Tooba Naveed, Shaukat Ali^{*}, Muhammad Summer and Hafiz Muhammad Tahir
*Applied Entomology and Medical Toxicology Laboratory, Department of Zoology,
Government College University, Lahore*

*Corresponding Author: dr.shaukatali@gcu.edu.pk

Originating in the liver, often linked to chronic disease, liver cancer poses a substantial global health threat. The primary form, hepatocellular carcinoma (HCC), accounts for 90% of cases. However, available treatments like transplantation, chemotherapy, and radiotherapy have limitations

in terms of efficacy, cost, and side effects. Cruciferous vegetables like *Raphanus sativus* (red radish) possess antitumor properties due to their bioactive components. Particularly, *R.sativus* conjugated silver nanoparticles (AgNPs) exhibit cytotoxic effect against cancers *in vitro*. Current study was designed to evaluate its anticancer potential against HCC *in vivo*. Diethylnitrosamine (DEN) was employed for HCC induction in mice. *R.sativus* conjugated AgNPs were synthesized using green synthesis method and characterized using UV-Vis spectrophotometry and FTIR Analysis. Swiss albino mice were divided in 11 groups (n=5 in each group) and treated with saline, DEN, Cisplatin (Treatment), *R.sativus* extract (Treatment), *R.sativus* conjugated AgNPs (Treatment), Cisplatin, *R.sativus* extract, *R.sativus* conjugated AgNPs, DEN+Cisplatin (Prevention), DEN+*R.sativus* extract (Prevention) and DEN+*R.sativus* conjugated AgNPs (Prevention) respectively. The biochemical parameters were evaluated to analyze the HCC status in mice. The histopathology of liver was also examined. The outcomes of the present study highlight that the application of the treatment resulted in significant amelioration in levels of serum biomarkers and an elevation in antioxidant levels in contrast to the DEN-treated group. *R.sativus* conjugated AgNPs (150mg/kg) showed the most significant results as follows: AFP (47.8±1.5), AST (284.6±6.0), ALT (126.2±5.2), LDH (522.6±7.9), ALP (225.0±6.7), MDA (5.2±0.1), GGT (30.0±1.2), bilirubin (5.7±0.1), GSH (2.8±0.1) and CAT (139.0±3.2). The histopathological study of liver tissues showed alteration in liver architecture in the DEN-treated group but restoration in the treatment groups. In conclusion, the anticancer potential of *R.sativus* conjugated AgNPs was demonstrated by their ability to reinstate the normal liver structure, serum biomarker and oxidative marker levels and hence could be used as an effective treatment against HCC.

TARGETING THE ANTIOXIDANT SYSTEM OF THE CELL FOR ROS MODULATION

**Areeka Naveen, Muhammad Faisal Maqbool, Sameena Gul,
Amara Maryam and Muhammad Khan***

Cancer Research Lab, Institute of Zoology, University of the Punjab, Lahore

*Corresponding Author: mkhan.zool@pu.edu.pk

Elevated levels of Reactive Oxygen Species (ROS) induce oxidative stress, precipitating cell death upon surpassing a critical threshold. Maintaining a delicate balance between ROS and antioxidant defenses is crucial for cell survival. This study employed a computational approach, virtual screening, to identify natural compounds capable of inhibiting key antioxidant enzymes. Three compelling candidates emerged: alantolactone, brevilin A, and lathyrol. Alantolactone displayed the most potent binding affinities, interacting with all targeted enzymes (catalase, SOD, HO1, and NRF2) with binding energies ranging from -8.5 to -6.7 kJ/mol. Brevilin A followed closely, showcasing binding energies within a similar range (-9.0 to -7.0 kJ/mol). Lathyrol exhibited strong binding potential as well (-9.0 to -6.7 kJ/mol). Further analysis revealed favorable ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles for all three compounds, indicating good gastrointestinal absorption and adherence to Lipinski's rule of five for drug-likeness. Additionally, their calculated Log P values (2.61, 3.35, and 1.99 for alantolactone, brevilin A, and lathyrol, respectively) suggest optimal membrane permeability. Detailed analysis of the binding interactions revealed the formation of crucial hydrogen bonds between the identified compounds and key amino acid residues within the target enzymes. Alantolactone, for instance, formed promising hydrogen bonds with catalase (Arg111 and Arg364), NRF2 (Glu518), and HO1 (Lys86), suggesting its potential as a potent antioxidant system inhibitor. Similarly, brevilin A and lathyrol exhibited favorable interactions with all target proteins, including hydrogen bonds and hydrophobic interactions. These

findings unveil the exciting potential of these plant-based compounds as natural alternatives to target the antioxidant defense system in cancer cells. Further in vitro and in vivo studies are warranted to validate their efficacy and explore their potential as novel therapeutic agents in the fight against cancer.

IDENTIFICATION AND CHARACTERIZATION OF NATURAL NOVEL STAT3 INHIBITORS TARGETING STAT3 DIMERIZATION BY BINDING TO STAT3 SH2 DOMAIN

**Fozia Bakhtawar, Muhammad Faisal Maqbool, Sameena Gul,
Amara Maryam and Muhammad Khan***

Cancer Research Lab, Institute of Zoology, University of the Punjab, Lahore

*Corresponding Author: mkhan.zool@pu.edu.pk

The signal transducer and activator of transcription 3 (STAT3) is a pivotal player in cellular processes regulated by cytokines and growth factors. Its overexpression, often triggered by phosphorylation, fuels tumor progression, and worsens clinical outcomes across diverse cancers. Consequently, inhibiting STAT3 holds immense promise for developing effective anti-cancer therapeutics. This study aims to discover and characterize natural compounds capable of acting as innovative STAT3 inhibitors. The focus lies on disrupting STAT3 dimerization by targeting its crucial Src homology 2 (SH2) domain. A multi-pronged approach utilizing molecular docking software like Autodock Vina, Instadock, MGL Tools, and PyRx is employed to achieve this. Our investigation delves into the inhibitory potential of distinct bioactive molecules: solasodine, oroxylin A, dalbergiodine, and cryptotanshinone. Excitingly, all four candidates engage with a crucial residue, GLU-616, highlighting their shared potential as SH2 domain inhibitors. Solasodine emerges as the frontrunner, exhibiting impressive binding energy (-9.6 Kcal/mol) and a low inhibition constant (7.11 μ M), achieved through hydrophobic and van der Waals interactions. Furthermore, it engages with key residues GLU-616, LYS-642, LYS-574, ASN-567, and ASP-567. While oroxylin A, dalbergiodine, and cryptotanshinone demonstrate slightly lower binding energies (-7.6, -7.4, and -7.5 Kcal/mol, respectively) and inhibition constants (5.45, 5.43, and 5.5 μ M), they retain promising interactions with GLU-616 and other crucial residues. Notably, cryptotanshinone forms a hydrogen bond with ASP-335, potentially adding another layer to its inhibitory potential. Beyond their inhibitory prowess, all four compounds exhibit favorable toxicity and drug-like characteristics, paving the way for their potential use in human therapeutics. Further in vitro and in vivo investigations are crucial to solidify the promise of these natural molecules as potent STAT3 inhibitors, bringing us closer to harnessing the power of nature in the fight against cancer.

REDESCRIPTION AND INTENSITY OF TREMATODE PARASITES (FAMILY: DICROCOELIIDAE) IN COMMON MYNA *ACRIDOTHERES TRISTIS* (PASSERIFORMES: STURNIDAE) OF DISTRICT LARKANA, SINDH, PAKISTAN

Iqra Babar and Bakhtawar Soomro*

¹*Department of Zoology, Government College University, Hyderabad*

*Corresponding Author: bakhtawarsoomro145@gmail.com

During present studies, a total of 240 Common myna (*Acridotheres tristis*) were collected from District Larkana, Sindh, Pakistan and examined for the presence of the trematode parasites. Out of 240,

hosts examined only six host birds were found positive hosting the trematode parasites in their gallbladder. All trematodes collected belonging to the genus *Conspicuum* and identified as *C. murtazi*, *C. quratulainae* and *C. aliraazi*. It was found that the highest intensity of *C. murtazi* was followed by the *C. quratulainae*, whereas lowest intensity was exhibited by *C. aliraazi*.

CREATION AND EVOLUTION OF HUMANS IN THE LIGHT OF SUPREME SCIENCE

**Rajput Muhammad Tariq, Masarrat Yousuf, Farzana Yousuf, Ghazala Yasmeen,
Sumera Farooq, Samreen Mirza and Rizwana A. Ghaffar**

Department of Zoology, University of Karachi, Karachi

*Corresponding Author: tariqbrc@yahoo.com

We are in the age of modern science, which is the science of matter but this is an all-agreed fact that this universe in which we are living on the Earth planet, basis not only on matter but also on non-matter. About matter the chemist scientists described a law, the law of conservation of mass, which states that, the matter cannot be created and the matter cannot be destroyed. Here the question arises in our minds, then who created this matter, which we are observing in this our universe, especially on earth planet. The most common, all agreed and un-refutable answer is / will be “The Creator” created this matter. The other part of this universe especially of earth planet is non-matter, which is all agreed, the most important fact and un-challengeable truth ignored by modern science and scientists. Examples of non-matter may be given as soul (basis of life & death), the angels created from noor, the Jinns (giants), created from the flame of fire, and the eye sight which get pass from glass/glasses, whereas the drop of water, which could not pass the same glass/glasses why?, because the water drop is matter, as it occupies the place and have the mass, on the other hand the sight (Nazar=Basarat) does not occupies place (volume) and does not have any mass, as it is non-matter. Thus, the non-matter cannot be measured, weighed, touched or observed by common sense organs / equipment of modern science, due to the same reason the modern science is unable to consider the non-matter, but the scientist who accept the reality of non-matter, they step forward from matter science to non-matter science and the combination of matter science and non-matter science is the supreme science. In clear words, the acceptance of creation of matter & non-matter by the creator, the almighty Allah and the evolution (changes) in the created creatures by the command of creator, with accordance to environment for the requirement (need) of creatures is supreme science. The biological science is unable to develop a living cell, even the exact composition of a living cell of plant / animal / human could not be determined / calculated uptill now, because a living thing (organism) requires life (Rooh / Soul), which is not in the hands of science and scientists but the rooh / soul is the spiritual order and command of the creator, i.e. Qulirrooho Min Amr-e-Rabbi. (Al-Quraan). The creation and evolution of human beings in the light of supreme science may be described as follows. The 1st human Aadam (identical male) on the earth planet was created in the heaven by creator naturally, without any sample & example, then through natural cloning / budding of somatic cells, the second human Hawwa (Eve, identical female Alaiha Assalam) was evolved from the body of pre-existing 1st human, the Aadam Alaihissalam, then all the men & women were produced and spread through this pair (Tariq, 2013, 2018). Therefore, in the all books & journal of science, the common ancestors of all present living human beings is described but the name of their ancestors is not shown / given which is Hazrat Aadam Alaihissalam & Hazrat Bibi Hawwa Alaiha Assalam. (Tariq et al. 2023). Hazrat Aadam Alaihissalam and Hazrat Bibi Hawwa Alaiha Assalam, were landed on Earth Planet, on Aadam’s Peak in Sri Lanka, Aadam Alaihissalam, the Jadd-e-Amjad means father, grandfather (nana/dada) and Hazrat Bibi Hawwa Alaiha Assalam was landed in Jiddah (جده), means mother, grandmother

(nani/dadi). Both Aadam's peak in Sri Lanka & the most international city Jiddah in Saudi Arabia are still existing & present. Their remains and grave may be visited today. In this sense the Human beings are the Alians, as they came from another planet. The grave / Mazar of the son of Hazrat Aadam Alaihissalam, the Habeel Razi Allah-ho-Taaala Anho may be seen and visited in Damishk / Shaam (Syria), murdered by Qabeel (Al-Quraan). The other son of Hazrat Aadam Alaihissalam was Hazrat Sheedth (شيث) (Alaihissalam). The Aadam-e-Sani is known as Hazrat Nooh Alaihissalam. Since Hazrat Aadam Alaihissalam to Hazrat Nooh Alaihissalam ten thousand years have been passed, whereas from Hazrat Nooh Alaihissalam to Hazrat Eessa Alaihissalam about (more or less) five thousand years have passed. At present it is 2024 from Hazrat Eessa Alaihissalam, which means from Hazrat Aadam Alaihissalam to present about 15-17 thousand years have been passed, in which 1446 years are included from the event of Hijrat of Muhammad Sallal-lahu-Alaihi-Wasallam. Evolution in human beings is recorded as (1) Hight from 90 ft decreased to 9 fts., (Robert Wadlow was 8.11" from USA) (2) The age of human beings is decreased from 1000 years to 100 (average) years. (3) The progeny started with dizygotic, which is also continued uptill now, but monozygotic progeny, binary fission & / multiple fission is now a common trend. (4) The language from Ibrani/Suryani to many now a days (about 300 languages in human beings) (5) Hazrat Aadam Alaihissalam has A blood group due to N-acetyl-D-galactos amine, whereas Hazrat Bibi Hawwa Alaiha Assalam received type B blood group from Hazrat Aadam Alaihissalam due to alpha-D-galactose, and now the all progeny of this pair have A,B,AB and O blood groups (6) From Hazrat Aadam Alaihissalam to present 2024) after about 15-17 thousand years only 0.03% change has been recorded in Human gene. Which is negligible. (7) Difference color of skin in human on the basis of melanin (darker in Africans to albino). Now recently in 2023 human beings are described in Kingdom: Humania, separated from Kingdom Animalia.

IMPACT OF IRON DEFICIENCY ON SERUM CORTISOL LEVEL AND ITS ASSOCIATION WITH STRESS, ANXIETY AND DEPRESSION IN YOUNG FEMALES

Rabia Shereen, Faseeha Azmat, Zarrish Rubab and Muhammad Rizwan Alam

Department of Biochemistry, Quaid-i-Azam University, Islamabad

*Corresponding Author: mr Alam@qau.edu.pk, rabiashereenkhan@gmail.com

Anxiety, stress & depression (ASD) are mental health disorders which are present worldwide with an increasing tendency in youth. These are multifactorial disorders with several etiologies including iron deficiency. Currently, there are no clinical biomarkers for depression, but cortisol is often prescribed to assess the acute conditions. This study was aimed to investigate the impact of iron deficiency on serum cortisol levels and determine its association with ASD in young females. Young females with iron deficiency were recruited along with healthy controls. A combination of DASS (Depression, Anxiety, Stress) questionnaire and experiment-based approach was followed. Hemoglobin was measured using Drabkin's assay while serum ferritin and cortisol levels were analysed using ELISA. We infer from our results that nearly half of the females were found iron deficient with no significant increase in the number of anemic individuals. Interestingly, there was a negative correlation between DASS score and ferritin. Whereas an increasing trend was observed between serum cortisol and ferritin levels. Notably, the comparison of stress score with iron status revealed that iron deficient individuals were slightly more stressed. Our study concludes that individuals with low ferritin level were more stressed as compared to those with a normal serum cortisol level.

CLIMATE OF MILITARIZATION IN IIOJK: AN IN-DEPTH EXPLORATION OF EDUCATIONAL RAMIFICATION

Abida Rafique and Saba Ghulam Nabi

The Center for International Strategic Studies Azad Jammu & Kashmir (CISS-AJ&K), Muzaffarabad

*Corresponding Author: abi186@yahoo.com

The Kashmir is a prolonged protract conflict centered in South Asia. The human ramifications of this prolonged discord are substantial, as the civilian populace frequently becomes entangled in the hostilities, where educational landscape in IIOJK is also significantly affected by these multifaceted challenges arising from the conflict. The research aims to bridge the existing gap in understanding the long-term consequences of the militarized climate on the educational infrastructure of Indian Illegally Occupied Jammu and Kashmir (IIOJK). The constant presence of armed forces in the region is analyzed to understand its influence on the sense of safety and security, mental health, and academic performance of individuals within the educational structure. The theoretical framework guiding this research is the "Human Security" paradigm, which represents a paradigm shift from traditional security concerns to safeguarding individuals and communities from various insecurities, encompassing economic, security, identity security and societal challenges. Applying the Human Security framework to the context of IIOJK involves a thorough examination of how militarization affects the fundamental well-being and dignity of individuals within the education system. The Human Security framework identifies several dimensions crucial to understanding the impact of climate of militarization on the education in IIOJK. These dimensions include economic security, environmental security, identity security, and community security. By adopting the Human Security framework, this research provides a comprehensive analysis of the multifaceted challenges posed by militarization within the educational environment of IIOJK, which will fill the gap and highlight the context within academic discourse.

OPTICALLY TRIGGERED GOLD NANOPARTICLES AS MULTIFUNCTIONAL CARRIERS FOR COMBINED CHEMO-PHOTOTHERMAL THERAPY OF HEPATOCELLULAR CARCINOMA

Uzma Azeem Awan^{1,2*}, Abida Raza^{3*}, Steven Hira² and Shaukat Ali⁴

¹*Department of Biological Sciences, National University of Medical Sciences (NUMS), Islamabad*

²*Laser Dynamics Laboratory, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia, United State*

³*University Institute of Biochemistry and Biotechnology, PMAS-Arid Agriculture University Rawalpindi*

⁴*Medical Toxicology Lab, Department of Zoology, Government College University Lahore, Lahore*

*Corresponding Author: uzma.awan@numspak.edu.pk

Hepatocellular carcinoma (HCC) is a common cancer worldwide, despite significant advances in cancer therapeutics, limited efficacy and severe dose-related side effects of anticancer drugs remain the leading causes of cancer-related morbidity and mortality. The challenges associated with conventional chemotherapeutic includes poor solubility, narrow therapeutic index and non-selective site of action that lead to drug resistance and side effects. To overcome these challenges, we have developed the combination of anticancer drug with photothermal nano-carriers that suppressed cancer development through synergistic effects and reversal of drug resistance. The current study aimed to fabricate biocompatible multifunctional drug-loaded nano-moieties for synergistic therapy (chemo-photothermal). We have developed an effective near infrared (NIR)-stimulus system, polyelectrolyte-poly (sodium-4-styrenesulfonate) coated gold nanorods (GNRs) containing doxorubicin (DOX) (PSS-GNRs-DOX). Near infra red (NIR 808 nm, power density = 1.5 W/cm² for 2 min) laser

irradiation cause hyperthermia due to GNRs, which enhanced drug release rate to the cancer cells. The synthesized nano-complex was found to be biocompatible, thermo-stable and exhibited high drug loading capacity. Cumulative DOX release significantly increased after NIR laser exposure compared to non-irradiated samples ($p < 0.05$). In vitro cytotoxicity testing revealed that the nano-complex with NIR laser irradiation appears more efficient in cell inhibition than without laser exposure and drug alone. The targeted, photothermal and antitumor synergetic effect of the synthesized nano system could be a safe and promising therapeutic approach for HCC management.

ROLE OF THYMOQUINONE IN REGULATION OF ADHESION MOLECULES IN HYPERLIPIDEMIC MICE

Muhammad Awais^{1*}, Shafaat Yar Khan^{1,2*}, Muhammad Khalid Mukhtar¹, Ahmad Khan¹, Ambreen Khalid¹ and Hira Fatima¹

¹Department of Zoology, University of Sargodha, Sargodha

²Department of Zoology Government College University, Lahore

*Corresponding Author: mohammadawais100@gmail.com, shafaatyarkhan@gcu.edu.p

Thymoquinone (TQ) is a major component of *Nigella sativa* which is abundantly used to treat several disorders across the globe. The current study was designed to investigate the protective role of TQ against the high-fat diet induced endothelial dysfunction evaluated through expression of cellular adhesion molecules in serum of hyperlipidemic mice model. Mice were fed for 12 weeks on a cholesterol and cholic acid rich high fat diet (HFD) to establish hyperlipidemia induced vascular inflammation. In the treated group, mice were fed on a high-fat diet along with an oral dose of 20 mg/kg body weight of TQ dissolved in corn oil for the same period, and the control group mice were fed only on the normal feed. In all three mice groups, lipid profile and serum level of cellular adhesion molecules (ICAM-1, VCAM-1 and E-selectin) were estimated and compared. High-fat diet on one side caused an increase in serum concentration of cholesterol, LDL, triglycerides, and VLDL with decreased serum concentration of HDL, while on the other side led to a significant rise in the levels of cellular adhesion molecules i.e. ICAM-1, VCAM-1 and E-selectin. However, TQ administration prevented the serum increase in triglycerides, cholesterol, LDL, VLDL, ICAM-1, E-selectin and VCAM-1 concentration along with an increase in serum HDL concentration. Our results suggest the protective and effective role of TQ in preventing the hyperlipidemia induced endothelial dysfunction by the decrease in cellular adhesion molecules (ICAM-1, E-selectin and VCAM-1) through NF- κ B pathway inhibition as these cell adhesion molecules are regulated by transcription factor and TQ may have contributed in preventing hyperlipidemia and its associate endothelial dysfunction and resultant atherosclerosis.

EXPOSURE OF ALBINO MALE MICE TO BUPROFEZIN-INDUCED REPRODUCTIVE HORMONAL CHANGES AND THE PROTECTIVE EFFECT OF VITAMIN C AND CURCUMIN AGAINST IT: AN IN VIVO AND IN SILICO STUDY

M. Ikram Khan and Irfan Zia Qureshi*

Department of Zoology, Quaid-i-Azam University, Islamabad

*Corresponding Author: irfanzia@qau.edu.pk

Buprofezin regulates insect development by inhibiting the formation of chitin, a vital component of the exoskeleton. It interrupts the process of insect ecdysis, mimics their hormones, and inhibits

nymph molting, resulting in insect death. Vitamin C and curcumin are promising therapeutics for testicular damage. Therefore, the present study explored the protective effect of vitamin C and curcumin (100mg/kg/bw each) against reproductive damage in male mice faced with 28 days of exposure to buprofezin doses. Mice were given the following treatments: control, buprofezin 30%, and 60% of LD50, vitamin C, Curcumin, and their combination along with a buprofezin 60% of LD50. The results displayed the protective effect of vitamin C and curcumin against buprofezin-induced damage. The antioxidant enzyme activity increased ($p < 0.01$), whereas oxidative stress indicators decreased ($p < 0.01$). Significant increases were seen in relative organ weight ($p < 0.01$), seminal fructose ($p < 0.001$), serum testosterone ($p < 0.01$), and improved sperm parameters ($p < 0.001$). The preventive action of vitamin C and curcumin against testicular tissue damage significantly decreased lactate dehydrogenase concentration. The negative effect of buprofezin on the male primary reproductive hormone testosterone was explored further using an In-silico technique, molecular docking, which yielded -6.1 energy, an excellent binding energy for the androgen hormone receptor. This work demonstrated both an in-silico technique and experimental method for the protective effect of vitamin C and curcumin against buprofezin-induced seminal fructose, lactate dehydrogenase, antioxidant enzymes, sperm parameters, and reproductive hormonal alterations.

EFFECT OF A FOOD ADDITIVE ASPARTAME ON HEMATOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL PARAMETERS OF A CYPRINID FISH (*CYPRINUS CARPIO*) AND REMEDIAL EFFECT OF VITAMIN C AND FOLIC ACID

Sumaira Khan and Irfan Zia Qureshi*

Department of Zoology, Quaid-i-Azam University, Islamabad

*Corresponding Author: irfanzia@qau.edu.pk

Food additives are regularly added to several food items as artificial sweeteners or preservatives. Untoward effects of food additives have been reported in some studies. However, the data are scant. Since fish are very delicate animals as show quick responses if faced with chemical insults. In the present study, a local cyprinid fish, *Cyprinus carpio*. Fish were exposed to an artificial sweetener called aspartame in low (40 mg. L⁻¹) and high doses (120 mg. L⁻¹). High dose aspartame group was further supplemented with Vitamin C (2.3 g per 20 fish) and folic acid (2.2 g per 20 fish) and a combination of Vitamin C and folic acid (2.8 g per 20 fish) in fish feed. The experiment lasted for 28 days. Blood and tissues were recovered after 28 days. Results were analyzed statistically. The results demonstrated significantly ($P < 0.001$) increased WBCs, decreased RBCs, HB, Ht and related parameters. Smear preparation showed increased heterophils, lymphocytes and MID percent. With respect to lipid profile, significant increase occurred in total cholesterol and triglycerides ($P < 0.001$), but a decreased occurred in the HDL cholesterol ($P < 0.001$). Total protein content was also altered. Histology of brain, heart, gills, liver, kidney and skin demonstrated cellular alterations, necrosis, significant damage to neurons, gill filaments, hepatocytes and kidney tissue. Cells demonstrated abnormalities of structure. On the other, where fish were treated with either Vitamin C or folic acid most of the alterations reverted to normal. Similarly, the hematological parameters and lipid concentrations became normal with vitamin C and folic acid. However, pronounced remedial effect was observed where a combination of vitamin C and folic acid was administered to fish. The present study demonstrated that fish when exposed to an artificial sweetener exhibit altered behavior, mortality at high dose, alterations in blood, lipid and histological parameters. Further research is required to explore in depth dangerous effects of such kind of sweeteners which are commonly used by people in their daily diet or in food products.

AN IN VIVO AND IN SILICO STUDY ON BUPROFEZIN-INDUCED DISRUPTION OF REPRODUCTIVE HORMONES AND THE PROTECTIVE EFFECTS OF VITAMIN C AND CURCUMIN IN FEMALE MICE LABORATORY

Taimoor Ahmed and Irfan Zia Qureshi*

Department of Zoology, Quaid-i-Azam University, Islamabad

*Corresponding Author: irfanzia@qau.edu.pk

Buprofezin (BPFN), classified as a type-1 chitin synthesis inhibitor insecticide, is frequently used to control hemipteran insects. It functions as an insect growth regulator and has found extensive application in managing the brown planthopper (BPH), *Nilaparvata lugens*, a highly destructive pest that causes significant damage to rice crops in Asia. While it is generally considered safe for humans, its persistent nature may pose health risks with prolonged exposure. Nonetheless, there is a scarcity of research regarding the effects of BPFN on mammals. In this study, we investigated the effects of BPFN on reproductive hormones and explored the protective role of vitamin C and curcumin in female mice using both in vivo and in silico approaches. Adult female mice were intraperitoneally treated with varying doses of BPFN: a low dose (30% of LD50) and a high dose (60% of LD50). Vitamin C and curcumin and their combination were administered orally, with doses adjusted based on average body weight over a 28-day period. Statistical analyses revealed a significant decrease ($P < 0.05$) in body weight and organ mass index of the ovary. Variations in levels of reproductive hormones, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol, were observed. While LH and FSH levels showed non-significant decreases, estradiol levels significantly decreased in the high-dose group. Ovarian activities of reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS) increased significantly ($P < 0.05$). Concomitantly, tissue total protein, antioxidant enzymes (superoxide dismutase, catalase, and peroxidase), and non-enzymatic reduced glutathione in the ovary decreased significantly. Histomorphological analysis revealed substantial tissue damage in ovarian tissues. Molecular docking using AutoDock Vina indicated strong binding affinity between BPFN and LH, FSH, and estrogen receptors. This binding affinity also predicted the toxic nature of BPFN concerning reproductive toxicity. Our findings suggest that BPFN poses a high toxicity risk to mammalian reproductive tissues. Further investigations, including biochemical, molecular, and cellular studies, are warranted to comprehensively understand its effects on non-target species.

PREVALENCE OF HYPERANDROGENIC HIRSUTISM IN ADOLESCENCE TO ELDERLY WOMAN IN THE AREA OF HYDERABAD DISTRICT

Khanam Zubaida, Tahira Jabeen Ursani and Jawaid Ahmed Khokhar

Department of Zoology University of Sindh, Jamshoro

*Corresponding Author: Zubaida.khanam.abbasi@gmail.com

The hormonal disease known as hyperandrogenic hirsutism causes excessive terminal hair, which often appears in a masculine pattern, to grow in strange places on the female body where vellus hair would normally grow, giving the illusion of baldness. The term "hyper androgenic condition" refers to the bodily repercussions of excessive testosterone levels in females. From July 2021 to July 2022, this research project was carried out in private practices and government hospitals in the District of Hyderabad using a well-developed cross-sectional questionnaire that was adopted after discussion with the project supervisor and the certified gynecologists, dermatologists, and endocrinologists of the relevant hospitals. This research work followed the WHO method to collect data and conducted a systematic overview to find the

prevalence of hyper androgenic hirsute patients. An ultrasound of the pelvis is performed to assess ovarian health. Females between the ages of 12 and 65 were found to have reported cases. 68 female hirsute patients were examined during this research among them 8 patients (12%) were ranging 12 to 19 years old, 28 patients (41%) were 20 to 30 years old, 19 patients (28%) were 31 to 40 years old, 9 patients (13%) were 41 to 50 years old and 4 patients (6%) were 51 to 65 years old. The other finding of study was that which parts of the body have terminal hairs by using Ferriman Gallway scoring scale method, during this study 27 patients (40%) had severe coarse hair on upper lips, chin, inner thighs, 16 patients (23%) had coarse hairs on lower abdomen side burns of face, and back of body, 15 patients (22%) had thicker hairs on upper arms and shoulders and 10 patients (15%) had on full face and lower body. From all the 68 HAH female patients, 25 (37%) were unmarried, 29 (43%) were married, 6 (9%) were divorced, and 8 (11%) were widow. In reported cases the literacy rate of 68 female patients is also evaluated that among them 5 (7%) females were post graduated, 20 (29%) were graduates, 23 (34%) were intermediate, 12 (18%) were primary, and 8 (12%) were not literate. The data about the prevalence of hyperandrogenic hirsute females have further examined according to the type of diet, out of 68 HAH female patients 15 (22%) consume balanced diet, 19 (28%) consume moderate diet, and 34 (50%) consume imbalance diet. The sign and symptoms of the HAH female patient out of 68, 33 (48%) females had symptoms like oily skin, acne on face and body, amenorrhea, masculinization, 15 (22%) experience obesity, deepening of voice and temporal baldness, 6 (9%) had virilization such as decreased breast size and enlargement of clitoris, 8 (12%) had Oligomenorrhea, infertility, mood swings and severe depression and 6 (9%) females had random nonspecific sign and symptoms. The females suffering from hyperandrogenic hirsutism have to face depression, physiological and psychological pain, and lack of confidence. Females have to compromise their physical wellbeing, mental peace and social life at various aspects.

STUDY OF INHERITANCE PATTERN AND CLINICAL MANIFESTATION OF MUSCULAR DYSTROPHY IN FAMILIES FROM DISTRICT HAVELI, AZAD JAMMU AND KASHMIR

Muhammad Kamran Ali¹, Muhammad Touseef², Ali Muhammad and Nausheen Irshad¹

¹*Department of Zoology, University of Poonch, Rawalakot 12350, Azad Jammu and Kashmir*

²*Department of Zoology, Faculty of Natural and Applied Sciences,*

Mirpur University of Science and Technology Mirpur, Azad Jammu and Kashmir

*Corresponding Author: nousheenirshad@upr.edu.pk

Mutations in human genome cause many human genetic disorders. Among these, Muscular Dystrophy (MD) is more common neuromuscular disorder, with global incidence of 1 in 2000 live births annually. MDs are classified into nine categories on the basis of their clinical characteristics, age of onset and mode of inheritance. The primary purpose of this research was to report various types of MDs, their modes of inheritance, their phenotypic characterization and correct diagnosis by using standard genetic and medical data bases from district Haveli, Azad Jammu and Kashmir. After obtaining informed consent, personal information about the family, clinical history and phenotypic details of affected subjects were collected. Radiological data of affected subjects (where required) were also taken. Detailed pedigrees of affected families were drawn by standard methods after interviewing the elders of the family. All the data was collected using standardized Proforma. Creatine Kinase (CK) tests of affected subjects were done for the conformation of MDs. All the data were compared with existing published literature and analyzed with the help of (genetic database) OMIM. Six families with different type of MDs were ascertained from study area. On the basis of survey of OMIM database, comprehensive literature review, effective status, detailed clinical presentations and specific mode of inheritance, two families (Family 1 and Family 2) were diagnosed

as Duchenne Muscular Dystrophy (DMD), two families (Family 3 and Family 4) were diagnosed as Limb-Girdle Muscular Dystrophy Type 2 (LGMD2), one family (Family 5) was diagnosed as Facioscapulohumeral Muscular Dystrophy (FSHD) and one family (Family 6) was diagnosed as Merosin-Deficient Congenital Muscular Dystrophy (MDCMD). It is recommended to conduct molecular genetics analyses for all six families.

MOLECULAR IDENTIFICATION OF SMALL KASHMIR FLYING SQUIRREL FROM RAWALAKOT, AZAD JAMMU AND KASHMIR

Sana Khurshid, Nausheen Irshad, Nasim Ahmed and Ali Muhammad

Department of Zoology, University of Poonch, Rawalakot 12350, Azad Jammu and Kashmir

*Corresponding Author: nousheenirshad@upr.edu.pk

The Small Kashmir Flying Squirrel (*Eoglaucomys fimbriatus*) is a rodent species belongs to family Scuridae. Flying squirrels present worldwide and are categorized into 15 genera, approximately 38 to 45 species. In Pakistan, only two species i.e., *Petaurus tapetaurista* and *E. fimbriatus* have been reported. The member of genus *Petaurus* consist of Giant red or Indian giant flying squirrels while the genus *Eoglaucomys* (previously known as *Hyloppetes*) comprised of small Kashmir flying squirrel. The Small Kashmir Flying Squirrel considered as “Vulnerable” in Pakistan. The Small Kashmir Flying Squirrel is morphologically recognized on the molecular/genetic level, it has not been characterized till now from Azad Jammu and Kashmir (AJ&K). The main aim of the current study was molecular characterization of the Small Kashmir Flying Squirrel collected from AJ&K region based on DNA marker gene (e.g., cytochrome b). For this purpose, two samples of small Kashmir flying squirrel (based on their physical appearance) were collected from different areas of Rawalakot AJ&K. Their genomic DNA was extracted and used as template in PCR (polymerase chain reaction) with primers for full length cytb gene amplification. The PCR products were amplified and sequenced. Sequenced reads were assembled and analyzed. The BLASTn (Basic local alignment searching tool) results showed 99% sequence similarity of the current isolates with *E. fimbriatus* previously reported from different regions. Further the phylogenetic and SDT (sequence demarcation tool) analysis explored the evolutionary relationship and pairwise sequence identity (PSI) scores with other related sequences respectively. Furthermore, in the current study, molecular data of tribe Pteromyini were retrieved from NCBI (National Center for Biotechnology Information) database and analysed for global scenario of tribe Pteromyini and proper classification based on molecular 2 data. Furthermore, phylogenetic and SDT analysis were also performed to determine the evolutionary relationship and sequence identity scores among different species and genera respectively. The cutoff values for species and genus demarcation were determined using different marker genes. Moreover, a masterlist was prepared consisting of NCBI accession number, organism name, isolate name, country name, year of reporting, DNA marker used and its size etc. Absolute value of each item was determined that showed its overall distribution in the tribe Pteromyini. This organized Masterlist and phylogenetic and SDT analysed data will provide reference list and reference trees and cutoff values for proper classification of different isolates belonging to tribe Pteromyini. Further the importance of the current study is discussed.

PREVENTATIVE EFFICACY OF SOLUBLE DIETARY FIBERS AGAINST NSAID-INDUCED ENTERIC INJURY IN MOUSE MODEL

Soumble Zulfiqar, Aimen Karim and Aiman Sahar

School of Biological Sciences, University of the Punjab, Lahore

*Corresponding Author: soumblesbs@pu.edu.pk

The prolonged and necessary use of non-steroidal anti-inflammatory drugs (NSAIDs) to alleviate joint pain often results in the development of enteric ulcers, for which available therapeutic options are limited. Soluble dietary fibers have shown promising effects on intestinal health. During this study, the preventive efficacy of dietary fibers against intestinal ulcers was investigated and compared with that of lansoprazole, a known drug. Mice were divided into nine groups. Enteropathy was induced in six experimental groups by administering indomethacin (an NSAID) after feeding them diets supplemented with various soluble dietary fibers, including psyllium, germinated barley, guar gum, gum Arabic, pectin, and beta-glucan, for two weeks. The remaining three groups (fed a regular diet) were designated as control, injury (indomethacin administered), and Lansoprazole (indomethacin and Lansoprazole administered) groups. Gross examination of the intestine, assessing the presence of lesions and edema, as well as measuring intestinal length, along with histopathological analysis, revealed that the adverse effects induced by indomethacin treatment were mitigated in mice fed the dietary fibers, with psyllium showing the most promising results. Additionally, quantitative PCR analysis of marker genes associated with inflammation (*Tnf- α* , *Cox-2*, *eNos*), oxidative stress (*Gpx-2*), and mucosal integrity (*Muc-2*) demonstrated the reversal of indomethacin-induced adverse effects by the dietary fibers.

DETERMINATION OF HYDROLYZING AND ETHANOLIC POTENTIAL OF CELLULOLYTIC BACTERIA ISOLATED FROM FRUIT WASTE

**Areeba Shehzadi^{1*}, Asma Chaudhary¹, Ayesha Aihetasham², Nageen Hussain³,
Sumaira Naz⁴, Tariq Aziz⁵, and Abdullah F. Alasmari⁶**

¹*Department of Zoology, University of Education, Township, Lahore*

²*Institute of Zoology, University of the Punjab, Lahore.*

³*Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore*

⁴*Department of Biochemistry University of Malakand, Chakdara*

⁵*Laboratory of Animal Health, Food Hygiene and Quality, Department of Agriculture,
University of Ioannina, 47132 Arta, Greece;*

⁶*Department of Pharmacology and Toxicology, College of Pharmacy,
King Saud University, Riyadh, Saudi Arabia*

*Corresponding Author: areeba7799b@gmail.com

Fruit wastes containing cellulose can be made valuable by cellulolytic bacteria in biofuel production. This study aimed to assess the potential of isolated cellulolytic bacteria to produce bioethanol and its fermentation efficiency. Seven out of 16 bacterial isolates were selected on the basis of their cellulose-degrading potential by providing cellulose as the only carbon source. Their potential to degrade cellulose was determined by different biochemical tests. All strains produced bubbles as indicators of carbon dioxide production in Durham tubes. The maximum hallow on Congo red staining was shown by CA2, CG2, as 54, 40 mm with cellulolytic index 16.3 and 19. Cellulose degradation was evaluated as light pink to maroon color in triphenyl tetrazolium in all strains except CA4 and CB1. Cellulose (2%) added medium was provided to the isolated strains for the period of 10 days to allow fermentation. CG2 and CA2 yielded

maximum ethanol as 0.42 ± 0.005 and 0.43 ± 0.011 (g of ethanol/ g of reducing sugar consumed), respectively. Their percent fermentation efficiency was observed as $79.71 \pm 0.059\%$ and $75.58 \pm 0.011\%$ correspondingly. All strains showed cellulose activity, and the maximum was seen in CA2 and CG2 as 0.265 ± 0.05 , 0.27 ± 0.011 $\mu\text{mol}/\text{min}/\text{L}$. Molecular characterization suggested that the CA2 and CG2 strains were *Bacillus manliponensis* CA2 and *Bacillus* sp. CG2 (Accession Nos. ON324120 and OM974175). This study elaborates on the capability of bacteria to produce bioethanol by degrading cellulose.

ISOLATION AND CHARACTERIZATION OF CADMIUM-RESISTANT BACTERIA FROM INDUSTRIAL AND SURROUNDING ENVIRONMENTAL SAMPLES FOR BIOREMEDIATION PURPOSES

Sadia Mushtaq^{1*}, Iqra Shahid¹, Abdul Rehman², Farhan Ul Haque¹ and Soumble Zulfiqar¹

¹School of Biological Sciences, University of the Punjab, Lahore

²Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore

*Corresponding Author: sadiamushtaq501@gmail.com

Cadmium pollution in industrial areas presents significant environmental and health problems. This study aims to explore bacteria with possibly best potential for cadmium removal from the environment. Sampling was performed from industrial and surrounding areas in Lahore, Pakistan. Cadmium-resistant bacteria were isolated through enrichment culturing using specific media followed by the assessing their cadmium resistance levels and optimal growth conditions. The selected strains, identified by ribotyping, included *Proteus mirabilis*, *Proteus penneri*, *Providencia stuartii*, *Klebsiella pneumoniae*, *Cupriavidus gilardii*, and *Cupriavidus taiwanensis*. Biochemical assays revealed urease and catalase positivity, gram-negative nature, and starch hydrolysis for the majority of strains. The bioremediation potential of these selected strains was evaluated in the presence of varying cadmium concentrations (1, 3, and 5mM) in the medium. Notably, *P. mirabilis* exhibited superior cadmium removal capabilities, achieving removal rates between 50% and 65% within eight hours of incubation, underscoring its effectiveness in cadmium bioremediation. These findings suggest *P. mirabilis* a promising candidate for addressing environmental cadmium pollution, given its intrinsic cadmium tolerance, rapid growth, and potential synergistic interactions, thus emphasizing its importance in future bioremediation endeavors.

DRUG RESPONSE OF UNTYPABLE ISOLATES TO CURRENT DIRECTLY ACTING ANTIVIRAL (DAA) REGIMEN IN PAKISTAN

Madiha Khalid^{1,3*}, Abdul Rauf², Sadia Mumtaz³, Aisha Siddique⁴ and Zeeshan Anjum¹

¹Department of Biotechnology, Women University of Azad Jammu and Kashmir, Bagh AJK

²Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad

³Department of Biotechnology, University of Azad Jammu and Kashmir, Muzaffarabad

⁴Department of Microbiology, Women University, Mardan

*Corresponding Author: madihaakhalid11@gmail.com

An increased prevalence of diagnostically untypable HCV isolates have been reported for the past few years in Pakistan and the efficacy of DAA based treatment regimens can be significantly affected by HCV genotypes and subtypes. Current study was aimed to evaluate the drug response of diagnostically untypable isolates to existing DAA based treatment regimens in Pakistan. Drug response was evaluated by an observational study including 192 patients infected with untypable genotype with 101 patients receiving

Sofosbuvir-Daclatasvir (SOF-DCV) and 91 receiving Sofosbuvir-Ribavirin (SOF-RBV) for 12 weeks and by monitoring Rapid virologic response (RVR) at 4 weeks of initiating therapy, end of treatment (EOT) response at 12 weeks and sustained virologic response at 12 weeks (SVR12) after EOT. Overall, SVR12 of untypable genotypes against current treatment regimen was 83.3% (160/192). Untypable genotype responded significantly well to SOF-DCV as compared to SOF-RBV with an SVR12 rate of 91.0% and 74.7% respectively. Proportion of non-responders to SOF-DCV (6.9%) was significantly less as compared to non-responders to SOF-RBV (19.7%). Also relapse rate were significantly high in the group treated with SOF-RBV (5.4%) as compared to SOF-DCV (1.9%) ($p < 0.05$). Efficacy of both regimens varied significantly with age ($p > 0.05$) but did not vary with gender. Pangenotypic SOF-DCV treatment regimen has been found to be an effective regimen for these circulating untypable genotypes.

THYROID DYSFUNCTION AND PREVALENCE OF BOTH CLINICAL AND SUBCLINICAL FORM OF HYPOTHYROIDISM AND HYPERTHYROIDISM IN DISTRICT SWABI, KHYBER PAKHTUNKHWA, PAKISTAN

Madeeha¹, Mehreen Riaz^{2*} and Abdul Wahab³

¹⁻²*Department of Zoology, University of Swat*

³*Doctor Khan Shaheed Govt. Degree College Kabal, Swat*

*Corresponding Author: drmehreen1986@gmail.com

Thyroid dysfunction is one of the most prevalent endocrine abnormalities, varies with age and gender. The study objective is to find thyroid dysfunction and the occurrence of clinical and subclinical hypothyroidism and hyperthyroidism in people of district Swabi, Khyber Pakhtunkhwa, Pakistan. Blood samples were taken from 104 people, and information was gathered via questionnaire. CLIA kit, Auto Bio Diagnostics LTD, China was used to detect TSH, T3, and T4 quantitatively by using Chemiluminescence Immunoassay (CLIA). Out of 104 individuals, 64 were females and 40 were males. A total of 24 people (23.04%) were affected by thyroid dysfunction, with 7 (17.5%) men and 17 (26.5%) women suffering from clinical and subclinical forms of hypothyroidism and hyperthyroidism, respectively. The occurrence of subclinical hypothyroidism was found to be the highest, with 10 cases (9.6%) recorded, followed by subclinical hyperthyroidism with 7 cases (6.7%), clinical hyperthyroidism with 4 cases (3.84%), and clinical hypothyroidism with 3 cases (2.9%). The study also revealed that the highest incidence of thyroid dysfunction occurred among individuals aged 31-40, with 6.73% affected, followed by those aged 41-50, with 5.8% affected. The women were more probable than men to have thyroid dysfunction in the area of District Swabi, and older people were more probable to have it than younger people. Iodine deficiency is the most typical cause, whereas in females, breastfeeding and pregnancy were the typical reasons.

PREVALENCE OF DIABETIC NEPHROPATHY IN ADULTESCENT PATIENTS OF TYPE 1 DIABETES MELLITUS (T1DM) FROM HOSPITAL OF HYDERABAD AND JAMSHORO

Fahmida Channa^{1*}, Tahira Jabeen Ursani¹, Jawaid Ahmed and Tahira Channa²

¹*Department of Zoology, University of Sindh, Jamshoro*

²*Department of Forensic Biology, Liaquat University of Medical and Health Sciences, Jamshoro*

*Corresponding Author: fahmidachanna1056@gmail.com

Diabetic nephropathy is a type of diabetic microvascular complication (MVC) characterized by kidney damage. One of the biggest challenges in healthcare is diabetic nephropathy. It is a major cause of end-stage

renal disease (ESRD) and affects up to 50% of people with diabetes (DM). A clinical diagnosis of Diabetic nephropathy in Type 1 diabetes mellitus can be made when there is persistent moderate or severe albuminuria or a persistent decline in eGFR to $<60 \text{ mL/min/1.73 m}^2$, happening at least 5 years following the onset of diabetic mellitus (DM). The purpose of this study was to assess the prevalence of type 1 diabetes mellitus patients with diabetic nephropathy in Hyderabad and Jamshoro. To assess the influence of diabetic nephropathy on quality of life (QOL) and the severity of the condition. To determine the frequency of clinical manifestation in diabetic nephropathy patients. This was a cross-sectional study carried out at an institution. 25 patients with ESRD who had received a new diagnosis between July and December 2018 were among the 125 (30.5%) Diabetic nephropathy patients that we examined, out of 410 T1DM patients who came from OPD / the outpatient departments of Hospitals of Hyderabad and Jamshoro. We used a standardized questionnaire to collect information about demographic factors, prior history of type 1 diabetes, length of illness, routine blood pressure checks, blood glucose readings, and urine examinations. Our findings suggested that males have a higher prevalence of diabetic nephropathy than females. Patients with diabetic nephropathy appear to have higher rates of proteinuria. Our current study suggests that patients with diabetic nephropathy had less confusion or difficulties focusing on their work. Patients suffering from diabetic nephropathy commonly exhibit micro- and macro-albuminuria. Diabetic nephropathy patients have poor quality of life, which is characterized by confusion, swelling of the hands and feet, swelling of the eyes, and proteinuria.

EFFECT OF DIETARY SUPPLEMENTATION ON LIVESTOCK AND FOOD ALLERGIES FROM LIVESTOCK PRODUCTS

Aneela Zameer Durrani, Muhammad Aftab, Muhammad Rizwan, Muhammad Zahid Iqbal

Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore

*Corresponding Author: aneela@uvas.edu.pk

This review seeks to provide valuable information for researchers, veterinarians, and livestock producers on the effects of dietary supplementation to livestock. The review will high light food allergies from livestock products. Zinc is a crucial trace element that plays a vital role in various physiological functions, including immune function, growth, and reproduction in livestock. Zinc deficiency can lead to various health problems in livestock, such as decreased growth rates, impaired reproductive performance, and increased susceptibility to diseases. In contrast, supplementing livestock diets with zinc can promote health and enhance microbiome balance, leading to improved animal welfare and productivity. While the homeostatic regulation of zinc levels in the body is usually sufficient to avoid deficiencies in livestock provided dietary zinc, certain conditions can lead to deficiencies or toxicities. Dietary zinc generally has a beneficial effect on gastrointestinal and microbial health in many livestock species when supplemented at recommended levels. However, environmental contamination with zinc and the selective effect of zinc on antimicrobial resistance has led to the need to critically re-evaluate current levels of zinc in the diets of livestock. For both food allergy and food intolerance, the symptoms do not appear immediately after consuming the food. It always takes some time for you to experience the symptoms. though most food allergies cause relatively mild and minor symptoms, some food allergies can cause severe reactions, and may even be life-threatening. There is no cure for food allergies. Strict avoidance of food allergens and early recognition and management of allergic reactions to food are important measures to prevent serious health consequence.

ANTIMICROBIAL RESISTANCE PATTERN OF BACTERIAL ISOLATES FROM BURN PATIENTS

Sadia Mumtaz^{1*}, Kanwal Rauf¹, Nazia Khatoon² and Madiha Khalid¹

¹Department of Biotechnology, Faculty of Science and Technology,
Women University of Azad Jammu and Kashmir Bagh

²Prime Institute of Health Sciences, Islamabad

*Corresponding Author: sdiamumtaz93@yahoo.com; sdiamumtaz@wuajk.edu.pk.

Burn infection is still a serious concern on a global level and is particularly damaging in developing nations. Infection associated complications account for over 75% of deaths after burning. The present study aimed to characterize the bacterial profiles of burn wound infections and their patterns of antimicrobial resistance against the commonly used antibiotics in a tertiary care hospital of Pakistan. The wound cultures of diverse clinical samples taken from 1169 patients with burn injuries were investigated from September 2021 to February 2022. The isolated microorganisms were identified and characterized on the basis of morphological features, Gram-staining reactions and biochemical tests. The identified bacteria were subjected to antimicrobial resistance profiling against commonly used antibiotics by Kirby-Bauer Disc Diffusion Method. A total of 65 bacterial species were isolated from burn patients. Among which 41 (63%) were gram negative bacteria while 24 (37%) were gram positive bacteria. The most prevalent among Gram-negative bacteria was *Pseudomonas aeruginosa* (53%) followed by *Klebsiella pneumoniae* (20%), *Acinetobacter baumannii* (15%) and *Enterobacter cloacae* (12%). Out of total 41 (63%) gram-negative bacteria, 26 (64%) were multi-drug resistant. Among Gram-positive isolates, *Staphylococcus aureus* (71%) was the most prevalent followed by *Staphylococcus epidermidis* (29%). Out of 24 (37%) Gram-positive bacterial isolates, 22 (91%) were multi-drug resistant. A varying pattern of antibiotic resistance was observed among the isolated gram-negative bacteria towards antibiotics tested. As *K. pneumoniae* showed 100% resistance against ticarcillin, cephalothin, cefuroxime and gentamicin. *E. cloacae* were found to be 100% resistant to ampicillin, piperacillin and cephalothin. *A. baumannii* showed 100% resistance against ceftazidime. Similarly, 95% of *P. aeruginosa* was also resistant to ceftazidime. However, all the gram-negative strains were found to be highly susceptible to imipenem and colistin. Grampositive bacteria showed highest resistance against penicillin, oxacillin and gentamicin, however, they were found susceptible to nitrofurantoin and vancomycin. Hence, it is concluded that imipenem and colistin are considered as drugs of choice for gram-negative bacteria and nitrofurantoin and vancomycin for Gram positive bacteria. Owing to the wide variety of bacterial species that can cause burn wound infection, it is recommended to routinely isolate bacteria from burn wound infection and screen for their patterns of resistance in order to find out the suitable antibiotic regimen for appropriate and effective treatment.

DETERMINATION OF BRCA1 AND BRCA2 GENE MUTATIONS IN BREAST CANCER PATIENTS OF SOUTHERN PUNJAB, PAKISTAN

Asia Bibi and Yusra Samad

Department of Zoology, The Women University, Multan

*Corresponding Author: drasia@wum.edu.pk

Breast cancer (BC) is one of the most common forms of cancer worldwide. Small extent of BC belongs to familial cancer syndromes but majority of patients have sporadic cancer. Breast cancer is the most

common carcinoma in Pakistani females and its incidence is becoming high day by day. Many different factors are involved in BC development including genetic and non-genetic factors. Among the genetic factors, mutations in BRCA1 and BRCA2 genes are the most important contributing factors for BC development. There is no data available regarding BRCA1 and BRCA2 genes in southern Punjab population of Pakistan. Keeping in view the above mentioned facts, the present study was designed to find out the frequency of most commonly occurring mutations of BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) in Southern Punjab population and to determine association of clinicopathological characteristics with BC. DNA was extracted from blood samples according to standard protocols by salting out method. In order to test the quality and quantity of DNA, spectrophotometry and Agarose gel electrophoresis were done. A simple method to detect mutations of BRCA1 and BRCA2 genes was used to study all of the three mutations in a single PCR reaction, the method is named as mutagenically specific PCR (MS-PCR). The exon 22 and 28 regions of BRCA1 and BRCA2 were amplified by MS-PCR. Nine different primers were used for detection of these three mutations i.e, two mutations of BRCA1 (185 del AG and 5382 ins C) and one mutation of BRCA2 (6174 del T). Statistical analyses were performed by using SPSS software. Association between BRCA1 and BRCA2 gene mutations and demographic and clinico-pathological features of patients were found by the χ^2 test. No significant association was found with any of the features including age, diet, smoking habit, marital status, socio-economic status, family history of cancer/breast cancer, type of carcinoma, histological differentiation, tumor stage and metastasis etc.

ASSOCIATION OF *SFTPB* GENE POLYMORPHISMS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) SUSCEPTIBILITY IN THE POPULATION OF SOUTHERN PUNJAB, PAKISTAN

Sadia Shahnawaz¹, Asia Bibi^{1*}, Tania Ahmed Shakoori² and Sajid Malik³

¹*Department of Zoology, The Women University, Multan*

²*Department of Physiology, University of Health Sciences, Lahore*

³*Human Genetics Program, Department of Zoology, Quaid-i-Azam University, Islamabad*

*Corresponding Author: drasia@wum.edu.pk

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality globally and its prevalence is increasing in Pakistan. COPD has heterogeneous etiologies ranging from environmental causes to genetic factors. Single nucleotide polymorphisms (SNPs) in *SFTPB* gene have been shown to be associated with pathogenicity of COPD. This study was aimed to examine the SNPs in *SFTPB* gene along with some demographic and clinical parameters in a cohort of COPD ascertained from Southern Punjab, Pakistan, in a case-control study. Three hundred subjects (150 cases and 150 controls) were recruited and four SNPs rs3024791, rs1130866, rs2118177 and rs2304566 were genotyped through ARMS-PCR. Results showed that cigarette smoking and pulmonary function parameters were significant risk factors of COPD in Southern Punjab, Pakistan. Two SNPs, rs1130866 and rs2118177, were significantly associated with pulmonary function. Two of the four studied SNPs, rs1130866 and rs2304566, were found to be significantly associated with COPD in different inheritance models. Haplotype analysis showed that three haplotypes “CCCC”, “CATT” and “TCTT” at the four studied SNPs were significantly associated with reduced risk of COPD and statistically significant linkage disequilibrium was found between two *SFTPB* gene SNPs rs2118177 and rs2304566. Collectively, our findings show that SNPs in *SFTPB* may be utilized as predictor of COPD in the study population and may help guide in the prospective personalized medicine.

PROXIMATE COMPOSITION OF *CYPRINUS CARPIO* REGARDING BODY SIZE DURING SUMMER SEASON FROM HEAD ISLAM, HASILPUR, PAKISTAN

Dua Asghar¹, Asia Bibi^{1*} and Muhammad Naeem²

¹Department of Zoology, The Women University, Multan

²Institute of Zoology, BZU, Multan

*Corresponding Author: drasia@wum.edu.pk

The proximate composition of fish is a method of analyzing the percentage composition of the four chief components including proteins, ash, lipids and water. It also includes carbohydrates but they are present in lesser amounts and thus can be neglected. Multiple variables, such as dietary composition, food intake behaviors, consumption pace, age, sexual activity, habitat, ecosystem, genetic makeup, seasons and migration can affect a fish body chemical makeup. A total of 29 samples of *Cyprinus carpio* were analyzed and proximate body composition of all samples collected from the Head Islam, Hasilpur, Pakistan during summer season were evaluated. Before the water content was measured, every fish specimen was wrapped in foil made of aluminium that had been pre-weighed and then dried at 60 to 70 degrees Celsius in a Memmert oven until the constant weight is attained. For the estimation of the ash content, dried fish specimens were then grounded into a homogenized powder by using a grinder and then one gram powder of each sub-specimen was put in pre-weighed China crucibles. All the crucibles are then arranged in the muffle furnace at 450-500°C for about one day. The difference was then computed by deducting both the initial and terminal weights, in order to determine the ash content of fish. For the estimation of lipid content, a chemical combination of methanol and trichloromethane with the ratio of 2:1 was executed and about ten milliliters of this amalgam were added in a test tube with one gram of sub specimen and stirred with a glass rod. After being left overnight, the mixture was poured into glass bottles that had been previously weighed and left at 40-50 degrees Celsius in oven until the lipid residue was left beyond. Using the difference approach, the protein content was then determined by deducting the ash and lipid content from the dry weight of fish. The water content in Common carp ranges from 63.53% – 82.48% while the mean and the standard deviation percentage value for water comes as 72.94 ± 0.79 . In the wet body weight of Common carp, other constituents including ash, fat, protein and organic contents ranges from 3.77%-8.21%, 2.80%-7.42%, 10.44%-25.53% and 13.31%-29.90%, respectively, and the mean and standard deviation percentage values comes out to be 6.07 ± 0.18 , 4.75 ± 0.25 , 16.25 ± 0.62 and 20.99 ± 0.74 , respectively. Similarly, in the dry body weight of the fish, ash, fat, protein and organic contents ranged from 17.00%-35.00%, 12.00%-25.00%, 49.00%-70.00% and 65.00%-83.00%, respectively. And the mean and standard deviation percentage values in the dry weight of fish comes as 22.72 ± 0.76 , 17.48 ± 0.69 , 59.79 ± 0.95 , and 77.28 ± 0.76 , respectively. Understanding the proximate composition of aquaculture species is essential for assessing the quality of raw materials, for applying various technological procedures, understanding the post-mortem traits of species, and determining the reproductive phases of the species.

GENETIC ASSOCIATION STUDY OF FAS GENE SNP (RS1800682) WITH POLYCYSTIC OVARIAN SYNDROME

Qurat ul Ain, Asia Bibi* and Sadia Shahnawaz

Department of Zoology, The Women University, Multan

*Corresponding Author: drasia@wum.edu.pk

Polycystic ovarian syndrome is a complicated heterogeneous endocrinopathy in females of fertile age. One out of fifteen female was affected by this disease, with a prevalence of approximately 4% to

20% worldwide, making it a serious global health threat. Its clinical manifestations have been associated with psychological morbidity and fertility in reproductive age of women. The current study was designed to investigate the association of FAS gene SNP (rs1800682) with PCOS in the women of Southern Punjab, Pakistan. Moreover, the association of this polymorphism with other risk factors including anthropometric parameters, socio-demographic factors, psychotic morbidity, obstetric parameters was also analyzed. A total of 145 clinically diagnosed cases according to Rotterdam criterion and 100 controls were recruited after having approval from the University Research Ethics Committee (UREC) and the Institutional Ethical Review Board (IERB), Nishtar Medical University, Multan and the required data was collected through a pre-designed questionnaire along with blood samples. Single nucleotide polymorphism (rs1800682) of FAS gene was amplified by Tetra-ARMS-PCR following DNA extraction from blood samples. The SNP analysis showed a strong relation between the genetic variant rs1800682 with the susceptibility to PCOS. Allele A's frequency was higher in those with PCOS, while the frequency of G allele was higher in control samples. The statistical analysis revealed a significant difference in the frequency of genotypes and alleles ($p < 0.001$). Moreover, other risk factors including Anthropometric parameters, Sociodemographic data and psychological factors were also found to be associated with the disease.

PREDICTIVE ROLE OF MMP7 GENE VARIANT RS1943779 IN BREAST CANCER PATIENTS OF SOUTHERN PUNJAB, PAKISTAN

Sumaiya Miraj¹, Asia Bibi^{1*}, Sadia Shahnawaz¹ and Rubina Mukhtar²

¹*Department of Zoology, The Women University, Multan*

²*Multan Institute of Nuclear Medicine and Oncology, Multan*

*Corresponding Author: drasia@wum.edu.pk

The prevalence of breast cancer has been surging over the past few decades. The development of breast cancer is a multistep process and there are a lot of genetic and non-genetics risk factors for breast cancer. Worldwide, there are about a million cases of breast cancer recorded in women each year. The World Health Organization (WHO) study from 2020 states that the incidence of the top ten cancers that affect women, breast cancer has the highest global rate at 34.4%. According to the same research, 28.7% of Pakistani women had breast cancer. Therefore, it becomes necessary to look into the other associated risk factors due to the elevated risk of breast cancer incidence and related death. There is a lack of knowledge among Asians about the breast cancer risk factors. MMP7 genes play an important role in the development of different types of cancer. This gene has polymorphisms that may be linked to susceptibility to breast cancer. The SNP studied in this research was rs1943779. Blood samples was collected from healthy people and breast cancer patients. First, DNA was extracted and then genotyping of this polymorphism was performed by TETRA ARMS PCR. In this study, different risk factors like menarche age, smoking, breast feeding were studied. The association of age, educational status and breast feeding with breast cancer was significant while diet, smoking and menarche age were not significantly associated with breast cancer. Our findings concluded that rs1943779 was associated with breast cancer risk. This will, as far as we currently know, the first study that evaluates MMP7 rs1943779 SNP in the Pakistani population.

ANALYZING THE ROLE OF GENETIC VARIANT OF MDM2 GENE RS937283 IN BREAST CANCER PATIENTS OF SOUTHERN PUNJAB, PAKISTAN

Sunaina Altaf¹, Asia Bibi^{1*}, Sadia Shahnawaz¹ and Rubina Mukhtar²

¹*Department of Zoology, The Women University, Multan*

²*Multan Institute of Nuclear Medicine and Oncology, Multan*

*Corresponding Author: drasia@wum.edu.pk

Breast cancer is a multifactorial and complicated illness that develops as a result of the interaction of hereditary and epigenetic dysregulation of crucial genes that affect crucial cellular pathways. Excessive and uncontrollably growing cancerous cells in the breast tissue is the hallmark of breast cancer. It is a diverse disease, with invasive ductal and lobular carcinomas being the most common types. Nearly a million new cases are reported globally each year. These days, it is increasing rapidly in Asian population. Annually, about 51.7 out of 100,000 cases have been reported in Pakistan. The study was done to evaluate the association of *MDM2* SNP rs937283 with breast cancer. There were 190 participants in the study, including 90 controls and 100 Breast cancer patients. Data from all research participants regarding age, familial history of breast cancer, menopause age, use of oral contraceptives, ethnicity, was collected along with blood samples for genetic analysis. DNA was extracted and *MDM2* gene polymorphism rs937283 was genotyped by tetra arm PCR. The findings indicated that the rs937283 polymorphism is associated with a risk of breast cancer in Pakistani women. The age range for patients and controls was 20 to 70 year and 40-50 being the mean age for BC patients. The predominant form of carcinoma observed in patients with breast cancer was invasive ductal carcinoma (91%). Additionally, the rs937283 polymorphism is strongly linked to an increased susceptibility to breast cancer in patients who were ER positive, PR positive and HER2 negative.

INTRONIC SNPS OF THE PIK3CA GENE AND THEIR IMPLICATIONS IN HEAD AND NECK CANCER POPULATION OF PUNJAB, PAKISTAN

Aafia Rabbani, Asia Bibi^{1*} and Sajid Malik²

¹*Department of Zoology, The Women University, Multan*

²*Human Genetics Program, Department of Zoology, Quaid-i-Azam University, Islamabad*

*Corresponding Author: drasia@wum.edu.pk

This case control study was designed to investigate the single nucleotide polymorphisms (SNPs) in the *PIK3CA* gene and various demographic parameters (Age, Gender, Family history of cancer, smoking habits, smoking frequency, chewing habits, sun exposure and location), in a cohort of head and neck cancer (HNC) patients from Punjab, Pakistan. A total of 640 individuals, comprising of 320 cases and 320 controls were included in the study and data regarding demographic factors was collected through a questionnaire. Blood samples from all of the study participants were collected and DNA was isolated. Three SNPs of *PIK3CA* gene (rs7621329, rs6443624 and rs7640662) were genotyped using tetra ARMS-PCR. The findings revealed that age, smoking, chewing habits and smoking frequencies were significantly associated with HNC in Punjab, Pakistan. Furthermore, genotypes of all three SNPs, rs7621329, rs6443624 and rs7640662 were significantly associated with HNC whereas only two SNPs (rs6443624 and rs7640662) showed significant allelic association with HNC.

ROLE OF *ANOXYBACILLUS* SP. COPPER PROCESSING GENE IN THE REMOVAL OF COPPER FROM WASTEWATER

Maria Fayyaz and Bushra Muneer and Ikram-ul-Haq

Institute of Industrial Biotechnology, Government College University, Lahore

*Corresponding Author: bushramuneer11@yahoo.co.uk

Resistant thermophilic strain was isolated from waste water and recognized as *Anoxybacillus rupiensis* on the basis of 16S rRNA gene sequence analysis. It was grown in LB media at 60°C with pH 7.0. It showed typical growth pattern except for the lag phase extended in the presence of copper because of resistance against metal. An efflux system plays an important role in pumping out of metal. Therefore, the effects of Cu resistance have been thoroughly investigated. SEM and FT-IR were used to analyze changes in the surface macrostructure and functionality of this strain showing interaction with Cu. The ability to adsorb and absorb Cu²⁺ inside their cells was evaluated by AAS, which showed that bacterial cell had the ability to adsorb 72% of the Cu²⁺ and absorb 28% Cu²⁺ inside them. Expression of copper resistant gene was quantified through PCR by adding primers. The result of purified PCR product demonstrated that copper resistant gene was functional. This is the first research to identify *copA* gene in thermophilic *A. rupiensis*. It is concluded that copper-resistant gene *copA* proving that bacteria could make much more efficient inoculum for remediation of copper in contaminated industrial waste water.

NORMALIZATION OF HUMAN-BEHAVIOR THROUGH HOMEOPATHIC MEDICINES

G.M.A. Ghani and Slaman-ur-Rahman

Pakistan homeo-clinic, Abubakr Town Dera Ghazi Khan, Pakistan

*Corresponding Author: s.khattak300@gmail.com

Human mind possesses special centers that control specific activity. Any derangement in a mind center's activity creates a related change in one's behavior. Specific Homeopathic medicine can normalize the physiology of related specific portion of our mind and in turn, normalization of such behavior is observed. As regards the normalization of human behavioral activity, the Homeopathy gives miraculous and prompt results. Though each and every homeopathic medicine possesses its effects over patient's mind but some elucidate rather pronounced results, I may quote a few instances in this aspect of human issues.

- a) A lady of round about fifty was weeping while explaining her health problem. I enquired the cause of her misery as to why she was weeping she didn't disclose any sound cause of her sorrow leading to weep. I gave her a single dose of *Pulsatilla pratensis* 200, after few minutes she started smiling while discussing her same health hazards.
- b) A child of about eleven years died, naturally, his mother went unconscious owing to this psychosomatic trauma. She was provided *Ignatia amara* 30. After few minutes she was behaving quite normal.
- c) An adolescent of 16 was to go mad for suicide, he was duly prescribed *Aurum metallicum* 30 and he reclaimed his normality within one month.
- d) The most interesting and extremely useful use of a homeopathic medicine for foolish passion of so-called frantic romance of -teen agers. I successfully treated over a thousand boys and girls, with the hundred percent successes, through a single homeopathic medicine named *NATRIUM MURIATICUM* 200

or Sodium Chloride 200 (medicine prepared from table Salt) that normalized their behavior regarding foolish passion of crazy-love of youngsters. This medicine gives miraculous results whenever the person is under the stress of any sort of this psychological problem. In this regards an interesting example can explain the miraculous aspect of the action of this homeopathic medicine. A usual victim of ailing customs in the sub-continent was coerced to suffer the pangs of infructuous marital life. A newly married life maiden was denied the merriment of the first night by no one else but the bridegroom himself who whimsically deserted her for good. She had to lead a melancholic life of a condemned spinster. A magician of a homeopath helped restore her spirits and developed an angelic lust for life through a wonder drug *Natrium muriaticum* 200 on symptomization of her other health problem without knowing her long standing marital issue. The wronged bride after over a decade of frustration, demanded deliverance from conjugal yoke and zestfully made preparations for another chess of locating joyous tactility-more than the good-luck to the victim and boosting tribute to the efficient prescriber, the talismanically efficacious prescription ought to be eulogized to do justice in the matters of cause and effect.

SECTION – I I

PESTS AND PEST CONTROL

EFFECT OF CHITIN SYNTHESIS INHIBITORS ON FITNESS AND REPRODUCTIVE TRAITS OF *CHILO PARTELLUS* (SWINHOE) (LEPIDOPTERA: CRAMBIDAE)

Khalid Hussain Dhilloo, Ali Ahmed, Kaneez Fatima and Aneeta Lashari

¹Department of Entomology, Sindh Agriculture University Tandojam*Corresponding Author: khddhilloo@sau.edu.pk

Laboratory experiments were conducted to evaluate the effects of lufenuron and pyriproxyfen on the fitness and reproduction of *C. partellus* in the laboratory of pesticide toxicology and application technology at Sindh Agriculture University Tandojam. The maize stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is one of the most harmful polyphagous insect pests throughout the world, causing great losses and threat not only for fodders but also for several cultivated crops. During the last few decades, using the insect growth regulators (IGRs) is considered as one of the possible alternative ways for controlling *C. partellus*. In contrast to the classical insecticides, IGRs are not directly toxic, but act selectively on the development, metamorphosis, or reproduction of the target insect species. In present study, effect of different concentrations such as, 0.02, 0.04, and 0.08ml of lufenuron and pyriproxyfen on developmental duration of larval to adult stages, survival percent of larval and pupal, fitness such as larval and pupal length and weight and reproductive traits such as longevity, fecundity, and fertility were carried out. Results showed that (16.22±0.62, 11.56±0.59, 9.00±0.19 and 10.78±0.48) duration in days of larval, pupal, adult male and adult female, respectively was recorded in control and these numbers reduced to maximum level (9.33±0.19) in larval (6.33±0.19) in pupal (4.00±0.51) in male adult and (4.00±0.19) in female on 0.08 of lufenuron as compared to rest of the concentrations of both lufenuron and pyriproxyfen (9.33±0.19, 7.56±0.40, 5.22±0.29 and 4.22±0.29) of larval, pupal, adult male and adult female duration days, respectively. Lowest percent larval survival (52.44%, 55.78%) were recorded when *C. partellus* larvae were tested with 0.08 concentration of lufenuron and pyriproxyfen, respectively and highest (90.56%) was recorded in control. However, minimum pupal survival (44.67%, 48.89%) were recorded with 0.08 concentration of lufenuron and pyriproxyfen, respectively and highest (90.56%) was recorded in control. The fitness results declared that overall minimum larval lengths (5.22 ± 0.29, 7.78 ± 0.29), pupal length (4.00 ± 0.51, 6.11 ± 0.29), larval weight (9.89 ± 0.73, 7.78 ± 0.29) and pupal weight (29.22 ± 0.91, 31.89 ± 0.48) were recorded after 72 hours with 0.08 concentration of lufenuron and pyriproxyfen. Concentration with 0.08 of lufenuron significantly reduced the longevity (3.22±0.29, 4.67±0.19) of adult male and female *C. partellus*, followed by pyriproxyfen (5.33±0.19, 6.00±0.19) and the maximum adult male longevity (8.78±0.48) and female (11.89±0.40) days were recorded in control. The highest fecundity 860.33±3.56 was recorded in control, which declined to 290.67±19.00 in lufenuron treated with 0.08 concentration and 515.33±13.53 when treated with pyriproxyfen with 0.08 concentration. Fertility percent 39.67±0.84 was recorded with 0.08 concentration of lufenuron and 51.22±1.74 when treated with pyriproxyfen, while maximum fertility was noted in control (78.78±1.13) of *C. partellus*. Finally, the obtained results emphasized that lufenuron and pyriproxyfen are promising IGRs and suitable for IPM programs directed against lepidopteran pests.

**EFFICACY OF PLANT OILS AGAINST COTTON MEALYBUG,
PHENACOCUSSOLENOPSIS TINSLEY (HEMIPTERA: PSEUDOCOCCIDAE) IN FIELD**

**Aneeta Lashari*, Bhai Khan Solangi, Sajjad Hussain Rind, Muhammad Yousif Rahimoon,
Arfan Ahmed Gilal and Khalid Hussain Dhilloo**

¹*Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam*

²*Plant Protection Research Institute, Agriculture Research Centre. Tandojam*

*Corresponding Author: anlashri@yahoo.com

Cotton crop is the main source of fiber industry in Pakistan and is infested by huge number of climatic contents such as, insect pests, diseases, and abiotic factors and among insect pests, *P. solenopsis* has been described as a serious and invasive pest of cotton and other crops in Pakistan and neighboring countries. Heavy application and indiscriminate uses of chemical pesticides against *P. solenopsis* management has led to the advancement of resistance in all classes of insecticides, so plants offer a different and alternative source of insect biocontrol agents, as they contain a wide range of bioactive materials, and many of such compounds are selective or have no harmful effects on nontargeted organisms as well as the environment, unlike synthetic insecticides. Therefore, three different kinds of locally available plant oils were tested to observe their efficacy on reduction of *P. solenopsis* from cotton field. Statistical results for overall mean and reduction percent showed highly significant difference for *P. solenopsis* population reduction among various botanicals with reference to their effect on different days when compared with control (DF= 3, 23; F= 194.42; P= 0.0000) at (P<0.05). Results obtained that after first spray, highest overall mean *P. solenopsis* reduction percent 61.26 ± 10.69 was recorded in cotton plot sprayed with neem oil, followed by 50.11 ± 10.94 , and 38.30 ± 8.39 sprayed with castor oil and mustard oil, respectively. Results after the second spray showed that the highest overall mean *P. solenopsis* reduction percent 70.01 ± 9.26 was recorded in cotton plot sprayed with neem oil, followed by 50.51 ± 8.90 , and 40.06 ± 8.74 sprayed with castor oil and mustard oil, respectively. The results for overall mean and reduction percent for *P. solenopsis* population in both sprays of different plant oils showed that the overall mean population of *P. solenopsis* ranged between (94.75-121.88) in pre-treatment data collection, after spray, the results for neem oil reduced highest *P. solenopsis* population 84.45% on third day, with overall 65.64 ± 9.94 percent reduction, second highest *P. solenopsis* population reduction 68.96% with overall 50.31 ± 9.9 percent reduction was recorded in plot sprayed with castor oil, followed by mustard oil with *P. solenopsis* population reduction 54.05% with overall 39.18 ± 8.55 percent reduction after third day of spray. The results further declare that efficacy of neem oils remained effective till third day after spray while on seventh day *P. solenopsis* started creeping up gradually. Finally, the obtained results emphasized that synthetic insecticides may be replaced with locally available plant oils which may prove safe for the environment and non-target organisms.

**DEVELOPMENT AND EVALUATION OF NANO-FORMULATION OF SOME SELECTED
PHYTOEXTRACTS AGAINST FALL ARMYWORM *SPODOPTERA FRUGIPERDA*
(LEPIDOPTERA: NOCTUIDAE)**

Muhammad Zeeshan Majeed¹*, Umair Abbas¹ and Muhammad Luqman²

Department of Entomology, College of Agriculture, University of Sargodha, 40100, Sargodha

Department of Agricultural Extension & Rural Studies, College of Agriculture, University of Sargodha

*Corresponding Author: zeeshan.majeed@uos.edu.pk

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is one of the destructive pests of maize and other agricultural crops. Synthetic insecticides are predominantly used against this pest but extensive use

of these agrochemicals is causing pest resistance, environmental contaminations and health hazards. This study evaluated nanoformulated aqueous extracts of some promising local plant species against 3rd instar larvae of *S. frugiperda* under laboratory and field conditions. Initial screening bioassay results showed the highest larval mortality by 20% extracts of *Nicotiana tabacum* L. (66.67%), followed by *Azadirachta indica* A. Juss. (53.33%), *Withania somnifera* L. (46.67%), *Melia azedarach* L. (40%) and *Dodonaea viscosa* Jacq. (33.33%). The two most effective plant extracts (i.e. *A. indica* and *N. tabacum*) were further nanoformulated with silver nitrate (AgNO₃) and were bioassayed against *S. frugiperda* larvae using different concentrations (i.e. 80, 40, 20, 10 and 5%). Results showed that nanoformulated *A. indica* and *N. tabacum* extracts caused significant larval mortality with LC₅₀ and LT₅₀ values of 37.36 and 28.21% at 72 h and 52.19 and 33.25 h at 80% concentration, respectively. Field experiment showed maximum larval reduction by nanoformulated *A. indica* extract (48%) followed by *N. tabacum* extract (36%) while 80 and 20% larval reduction was noted in case of positive (Super Lock®: emamectin benzoate and tebufenozide) and negative (water) control, respectively. Furthermore, characterization of both silver nanoparticles-based plant extract formulations was done by using ultraviolet-visible (UV-vis) spectroscopy, Raman spectroscopy and scanning electron microscopy (SEM) techniques which confirmed formation of silver nanoparticles. It is concluded that nanoformulated plant extracts can be effective alternative to synthetic pesticides combatting *S. frugiperda* and other lepidopterous pests.

DEVELOPMENT OF BIOLOGICAL CONTROL FOR THE MANAGEMENT OF FRUIT FLIES, *BACTROCERA* SPP. THROUGH THE USE OF X-RAY IRRADIATION

Zain-Ul-Aabdin Abro^{1*}, Naheed Baloch¹, Raza Muhammad Memon² and Niaz Hussain Khuhro²

¹Department of Zoology, University of Sindh, Jamshoro

²Nuclear Institute of Agriculture (NIA), Tando Jam

*Corresponding Author: zainabro128@gmail.com

Fruit flies belonging to family (Tephritidae-Diptera) are the most injurious fruit pests of the fruits and vegetables. Biological control is an environmentally friendly control method that has been used against pest fruit flies. Parasitoids have been one of the most widely used groups of natural enemies. In these studies, field investigations were made on monitoring the population fluctuations of *Bactrocera zonata* and *Bactrocera dorsalis* in mango and guava growing areas of two different climatic regions using methyl eugenol baited traps. We have also surveyed the biological control agents from two different zones of Sindh. The field locations were Hyderabad and Larkana districts from where infested fruits were collected from guava (*Psidium guajava* L.) and mango (*Mangifera indica* L.) orchards during 2018 and 2019. Furthermore, the laboratory strains of the *B. zonata* and *B. dorsalis* were reared on natural diet (Guava and Mango) and artificial diet and pupae were subjected to different X-ray doses (20, 40, 60, 80, 100 Gy) to examine effect of different doses on development of both fruit fly species. Additionally in another laboratory studies comparative biological parameters of *D. longicaudata* and *T. daci* reared on larvae of *B. zonata* and *B. dorsalis* were observed. Moreover, field investigations were conducted in mango and guava experimented orchards by releasing *D. longicaudata* and *T. daci* along with mass trapping of fruit flies in district Larkana and Hyderabad. The population fluctuations of *Bactrocera* fruit flies on mango and guava orchard plants in parts of two districts (Hyderabad and Larkana) of Sindh Province were monitored using methyl eugenol traps from January to December 2018 and 2019. The male lure traps were erected at three meter height and replenished on fortnightly basis. Results revealed that significantly higher population of *B. zonata* flies per trap were recorded in the month of June in mango orchards of district Hyderabad and Larkana as (658.6±20.26, 623.50±14.76, 601.90±25.38, 588.80±20.62) respectively followed by *B. dorsalis* (484.5±29.11, 440.90±24.59, 447.40±18.35, 400.40±11.86) respectively at both studied sites. Moreover,

($P < 0.05$) significantly highest monthly population of *B. zonata* (1020.70 ± 96.87 , 1009.30 ± 100.64 , 989.40 ± 75.24 , 943.60 ± 70.36) followed by *B. dorsalis* (776.40 ± 39.07 , 708.70 ± 21.80 , 416.80 ± 19.84 , 389.20 ± 16.58) respectively were recorded in guava orchards of Hyderabad and Larkana regions in the off seasoned guava in the month of August during 2018-19. Lowest number of fruit flies of both species were collected in the month of January from guava and mango fields of both regions in year wise data. Population of both *Bactrocera* spp. were positively correlated with temperature ($^{\circ}\text{C}$) and negatively related with RH % in both climatic regions. Results of the present investigation would be helpful in developing sustainable male annihilation technique (MAT) which is an important component in integrated pest management program (IPM) being in practice for fruit fly monitoring and control in orchard agro-ecosystems in Sindh. In these studies we have surveyed the biological agents from two different zones of Sindh. The field locations were Hyderabad and Larkana districts from where infested fruits were collected from guava and mango orchards during 2018-19. The results revealed that significantly ($P < 0.05$) higher number of larval cum pupal parasitoid *Trybliographa daci* (62.20 ± 3.03 , 55.60 ± 4.52 and 50.20 ± 4.45 , 46.40 ± 5.47) respectively emerged from guava infected fruits collection of both districts during entire period of survey. Furthermore, maximum number of *Bactrocera zonata* infestation were recorded (395.6 ± 4.50 , 288.00 ± 11.57 and 381.00 ± 10.85 , 259.40 ± 16.95) respectively from guava orchards of both districts. This study established that Larval/pupal parasitoid *Trybliographa daci* proved most promising bio-agent in limiting the population of fruit flies in guava and guava observed most susceptible host for fruit flies in terms of infestation. In present investigation the laboratory strains of the Peach fruit fly and Oriental fruit fly were reared on natural diet (Guava and Mango) and artificial diet than pupae were provided with different X-ray doses (20, 40, 60, 80, 100 Gy) to examine effect of irradiation on adult emergence. Results shown that ($P < 0.05$) a significantly greater number of deformed adults (Males and Females) of *B. zonata* (27.20 ± 1.43 , 29.20 ± 1.80) respectively were recorded from un-irradiated pupae reared on artificial diet followed by *B. dorsalis* (22.60 ± 0.51 , 28.20 ± 0.86) respectively while minimum number of deformed adults (Males and Females) of both fruit fly species were recorded when reared on mango and provided with 100 Gy X-ray dose. Significant ($P < 0.05$) maximum normal adult (Males and Females) emergence of both *Bactrocera* spp. (22.80 ± 0.86 , 21.40 ± 0.68 and 34.60 ± 1.33 , 29.20 ± 2.20) respectively were observed in non-radiated pupae reared on mango. Reduced number of normal emerged adults (males and Females) were recorded when maggots of both fruit fly species were provided with artificial diet and pupae irradiated with 100 Gy X-ray dose. Furthermore, ($P < 0.05$) significantly higher partial pupal emergence of *B. zonata* and *B. dorsalis* (20.60 ± 1.21 , 19.60 ± 0.51) respectively were recorded when larvae of fruit flies were reared on artificial diet of un-irradiated medium. Interestingly greater number of pupae did not emerge when larvae of both fruit fly species were reared on artificial diet and pupae supplied with 100 Gy dose of X-rays and higher number of emergence was observed in control with artificial diets. From above results it's determined that *B. zonata* is more tolerant to different irradiation doses. During comparative biology *D. longicaudata* and *T. daci* reared on larvae of *B. zonata* and *B. dorsalis* increased longevity period (days) were observed in virgin females of *D. longicaudata* and *T. daci* (37.40 ± 1.96 , 31.80 ± 1.59) respectively reared on *B. zonata*. However, no any significant results were recorded among mated adults (Females and Males) of both parasitoids reared on *B. zonata* and *B. dorsalis*. Furthermore, the females of *D. longicaudata* and *T. daci* reared on *B. zonata* oviposited for ($P < 0.05$) significantly maximum days (27.80 ± 0.86 , 22.80 ± 0.77) respectively when compared with females reared on *B. dorsalis*. Interestingly ($P < 0.05$) maximum number of eggs laid by per female of *T. daci* (145.00 ± 4.63) were observed when reared on *B. zonata*. In addition we have observed superparasitism in *T. daci* up to 4 eggs on *B. zonata* larva while up to 2 eggs in *B. dorsalis* whereas no superparasitism were recorded in *D. longicaudata*. Field studies were conducted in mango and guava experimented orchards by releasing *D. longicaudata* and *T. daci* along with mass trapping of fruit flies in Larkana and Hyderabad districts. Results revealed significantly ($P < 0.05$) greater parasitization of *T. daci* (342.00 ± 16.26 , 320.00 ± 14.85) respectively in guava treated blocks at Hyderabad and Larkana. Whereas, lower parasitization of both parasitoids were observed in the untreated blocks of mango at discrete regions. Furthermore, significantly ($P < 0.05$) reduced

number of *B. dorsalis* (510.00 ± 118.57 , 558.40 ± 75.86) followed *B. zonata* (611.80 ± 109.38 , 680.00 ± 40.50) respectively were found in mango treated blocks of Larkana and Hyderabad using mass-trapping technique for fruit flies. While higher number of both species of fruit flies were recorded in untreated blocks of guava at both experimental sites. The present investigations would be helpful to develop bio-control base IPM model for *Bactrocera* spp. on area-wide basis.

THE RELATIONSHIP OF FRUIT FLIES *BACTROCERA* SPECIES (DIPTERA: TEPHRITIDAE) WITH ASSESSMENT OF INFESTED MANGO CULTIVARS AND INFLUENCE OF BIOTIC AND A BIOTIC COMPONENT IN DISTRICT NAUSHAROFEROZE SINDH

Aiman Amur^{*1}, Nasreen Memon², Abdul Manan Shaikh¹, Bhojo Mal¹, Kamal Abro² and Abdul Waheed¹

¹Department of Zoology Government College University, Hyderabad

²Department of Zoology University of Sindh, Jamshoro

*Corresponding Author: amuraiman@gmail.com

In two orchards of the Nausharoferoze District, fruit flies that are primarily associated with mango orchards were studied between 2015 and 2016 using traps enticed with methyl eugenol, terpinyl acetate, and torula. Variations in the population were examined in connection to environmental variables such as rainfall, relative humidity, and air temperature in relation to various mango. During the two harvest seasons, mangoes were tested every two weeks to gauge the extent of these isolated pests' infestation on ten foremost varieties. The diverse group of carefully examined fruit flies that accompanied the mango orchards of Nausharoferoze included native species of *Bactrocera* and a newly described novel foreign species, *Bactrocera dorsalis*. During the dry season, species were present, with *Bactrocera dorsalis* being the primary species that peaked towards the conclusion of the dry season. During the rainy season, from the end of May to the end of July, *Bactrocera dorsalis* populations rose gradually after being rare in March and May. Regression studies revealed that the primary climatic parameters influencing fly populations were rainfall, relative humidity, and (minimum–maximum) temperature. The component that had the largest positive link with *B. dorsalis* population was seasonal precipitation. Another crucial element affecting the population variations was the host plant. In early wet season a steady population growth of *B. dorsalis* seriously harmed late season and mid-season cultivars. In this upper region of Sindh the primary mango cultivars fruiting seasons corresponded with a seasonal increase in *B. dorsalis* population. However, the availability of mangoes only had an impact on the population of this new aggressive species once the rains arrived. Average mango damage during the course of two seasons.

INVESTIGATING THE EFFECT OF PHYTOSANITARY IRRADIATION TREATMENTS IN THE CONTROL OF COTTON MEALY BUG *PHENACOCCLUS SOLENOPSIS* TINSLEY (HEMIPTERA: PSEUDOCOCCIDAE)

Inamullah Khan*, Afaq Ahmad, Muhammad Humayoon and Muhammad Salman

Nuclear Institute for Food and Agriculture NIFA, Peshawar

*Corresponding Author: inamullah17@gmail.com

World trade of agriculture commodities continue to grow. This may result introduction and establishment of exotic pests into new areas where these pests are absent. The phytosanitary irradiation (PI) treatment of food commodity is residue free, environment friendly technique with

uniform penetration into the commodity and helpful in killing and inactivating the contaminating microorganisms, food borne pathogens and insect pests. Studies were conducted on the PI treatment of *Phenacoccus solenopsis*, a destructive mealy bug that was colonized in the laboratory at NIFA under ($28 \pm 2^\circ\text{C}$), $65 \pm 5\%$ RH and 14:10 LD. Batches of neonates and other nymphal stages at 2nd, pre-oviposition 3rd stage ♀ and adults were brushed over pumpkins using a camel hair brush to establish cohorts of similar age. Nymphs ~200 in each stage were transferred to individual okra pieces and confined in Pettery dishes and then exposed to gamma irradiation at 50,100,150, 200 and 250 Gy in a Cobalt 60 irradiator. Each dose was replicated 4 times on individual infested okra fruit. In order to minimize the dose uniformity ratio, pieces of okra were positioned in the middle of the canister. The absorbed dose was measured using Fricke dosimeters. Post irradiated insects were enclosed in Pettery dishes and confined in small cages (30x30x30 cm), maintained at 28 ± 2 ; $65\% \pm 5$ RH and examined twice per week to determine mortality and the number that had developed to the next stage according to the formula; mortality (%) = (no. 1st stage - no. 2nd stage) ÷ no. 1st stage × 100. The number of neonates produced by the irradiated mealy bugs was recorded from the survivors within the same stage. Untreated controls for each stage were held under identical conditions and examined similarly. All doses affected the survival of the mealy bugs. The mortality rate increased with increasing radiation dose. The 3rd instar nymph irradiated at 50 Gy produced the highest number of F1 neonates (112 ± 7) followed by 100Gy dose. The 3rd stage nymphs and adults exposed to 150 Gy and higher dose could not produce any neonates or develop further. All stages in the control reproduced as usual to complete the cycle to the next generation. Thus the 150 Gy gamma radiation dose is sufficient to control all stages of *P. solenopsis* and therefore, recommended as PI treatment for *P. solenopsis*.

STUDY OF THE TOXIC EFFECT OF DIFFERENT INSECTICIDES AGAINST *CAMPONOTUS COMPRESSUS*

Naheed Bano^{1*}, Sadia Maalik², Sajida Mushtaq²

¹Faculty of Veterinary & Animal Sciences, MNS-University of Agriculture, Multan

²Department of Zoology, GCWU, Sialkot

*Corresponding Author: naheed.bano@mnsuam.edu.pk

Carpenter ant species play a significant ecological role and are widely prevalent. Ants contribute to safeguarding plants from sap-sucking pest insects through natural biological control mechanisms. The current study was conducted to collect ants from Block B farms at Muhammad Nawaz Sharif University of Agriculture, Multan (MNSUAM), Pakistan. In current study, various insecticides (such as Imidacloprid, Fipronil, Methoxyfenozide, Emamectin benzoate, and Flubendiamide) were formulated at different concentrations (including 160, 80, 40, 20, 10, 5, and 0 ppm) for evaluation against carpenter ants. Notably, Fipronil and emamectin benzoate exhibited heightened toxicity, even at lower doses of 5 ppm and 10 ppm, resulting in the mortality of nearly all worker ants within 24 hours. In contrast, Imidacloprid displayed no distinct preference, as most worker ants remained alive even after 72 hours. These findings indicate that Fipronil and emamectin benzoate may serve as effective active ingredients for controlling carpenter ants. The data was meticulously recorded and subjected to rigorous statistical analysis using specialized software.

ASSESSING THE TOXICITY OF *BEAUVERIA BASSIANA* AGAINST NYMPHAL STAGES OF SOME GRASSHOPPER PESTS

Amna Hamid^{1*}, Santosh Kumar^{1*} and Riffat Sultana²

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: amnaahamid2234@gmail.com , santoshkumar@cuvas.edu.pk

Beauveria bassiana, an entomopathogenic fungus renowned for its capacity to produce various toxins and enzymes that infect and eliminate insects, has emerged as a promising biocontrol agent against various insect pests, including grasshoppers. This study specifically focused on evaluating the toxicity of *B. bassiana* against the nymphal stages, preferably stages 3-5, of grasshoppers responsible for significant crop losses in Punjab. The results exhibited promising outcomes, with mortality rates ranging from 75% to 95%, depending on the grasshopper stage and treatment. This research underscores the considerable potential of *B. bassiana* as an effective biocontrol agent for managing grasshopper populations during their nymphal stages. Further research and field trials are needed to optimize its use in integrated pest management strategies for sustainable agricultural systems.

EVALUATING THE EFFECTIVENESS OF *BEAUVERIA BASSIANA* AGAINST ADULT HOPPERS (ACRIDIDAE: ORTHOPTERA)

Sadia Tariq^{1*}, Santosh Kumar^{1*} and Riffat Sultana²

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: sadiatariq249@gmail.com, santoshkumar@cuvas.edu.pk

Beauveria bassiana, a soil-dwelling fungus known for causing white muscardine disease in insects, serves as a biological insecticide against various pests, including termites, thrips, whiteflies, aphids, and beetles. This study aimed to assess the efficacy of *B. bassiana* against adult hoppers, a type of grasshopper damaging crops and pastures. Results indicated that *B. bassiana* effectively reduced hopper populations and activity, with higher concentrations demonstrating increased efficacy. The impact varied among hopper species, showing susceptibility and resistance. The study identified a temperature-dependent relationship, highlighting optimal efficacy at around 30°C. These findings contribute valuable insights for developing effective biological control strategies using *B. bassiana*.

OBSERVATIONS ON SEASONAL DIVERSITY OF COTTON PESTS IN DERA GHAZI KHAN

Faqiha Syed¹, Santosh Kumar¹ and Riffat Sultana²

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: faqihasyed6@gmail.com , santoshkumar@cuvas.edu.pk

Cotton (*Gossypium hirsutum* L.), considered the 'white gold' of Pakistan, stands as one of the most ancient and important cash crops in Pakistan, playing a significant role in the Pakistani national economy. It is grown on a large scale across all provinces, with Sindh and Punjab being its major producers.

Unfortunately, it is attacked by a number of pests, including cotton leafhopper, cotton bollworm, pink bollworm, american bollworm, whitefly, jassid, mealybug, thrips, aphid, and red spider mite. These pests not only affect its quality but also reduce its yield. The major pests attacking cotton are aphid, jassid, thrips, whitefly, mealybug, and mite. For their control, the use of insecticides at planting has been a common crop management practice in cotton for several decades. It was felt necessary to conduct a comprehensive survey to observe the seasonal diversity of its major and minor pests so that any biofriendly planning would be recommended in the future.

ONGOING FALL ARMYWORM (FAW) INVASION IN MAIZE CROPS IN HYDERABAD AND ADJACENT AREAS

Fatima Anum* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: fatimaanum.leo@gmail.com, Riffat.Sultana@usindh.edu.pk

Recent study was framed to highlight the status of fall armyworm, an invasive larval stage of *Spodoptera frugiperda* on maize growing fields in district Hyderabad and its surrounding agricultural areas. Present study was restricted to monitor its occurrence and possible invasion status in the above-mentioned region. Consequently, weekly extensive surveys were carried out during the months of May 2021 to Feb 2022. Its occurrence was first detected in the month of October 2021 from maize crop where the most prevailing months of FAW infestation were recorded by November 2021 to Feb 2022. Morphological characteristics of pest were used as a tool of identification as it retains considerable resemblance with other armyworm species. During surveys maize growing areas were heavily infested with the pest at every growing stage of the crop, particularly its vegetative stage. The 6th larval stage of the invasive pest was observed to be the most destructive stage of FAW causing serious damage to the crop. FAW as a polyphagous species is a serious concern for other agricultural crops for the reason that if appropriate analysis of FAW prevalence would not be detected in other agricultural crops and no action would be taken on the pretentious areas then it will eventually lead to an extensive monetary loss to other valued crops.

IDENTIFYING CRICKETS (GRYLLIDAE: ORTHOPTERA) AS AGRICULTURAL CROP PESTS IN SINDH, PAKISTAN

Naila Bhangar^{1*}, Riffat Sultana^{1*}, Naheed Baloch¹ and Santosh Kumar²

¹*Department of Zoology, University of Sindh, Jamshoro*

²*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

*Corresponding Author: nailaqurban89@gmail.com, riffat.sultana@usindh.edu.pk

The representatives of Gryllidae are considered major pests of many agricultural crops such as *Tritium aestivum* (Wheat), *Oryza sativa* (Rice), *Sacharum officinarum* (Sugarcane), and *Dactyloctenium aegyptium* (Common lawn grass). Currently, there are many species which affect the agricultural crops, from which major species are the field crickets species of Gryllidae, namely *Acheta domesticus* (Linnaeus, 1758), *Gryllus* (*Gryllus*) *bimaculatus* De-Geer, 1773, *Gryllus* (*Gryllus*) *campestris* Linnaeus, 1758, *Teleogryllus* (*Brachyteleogryllus*) *occipitalis* (Serville, 1838). The occurrence of many species has extended, show casing a diversified range across the country. These species have been known to cause damage to crops in different regions of Pakistan. It is important for

farmers and agricultural authorities to monitor and manage these pest populations to minimize crop losses. Furthermore, it is worth mentioning that there may be other cricket species or pests that affect agricultural crops in Pakistan beyond the ones mentioned here. The occurrence of many species indicates the diversified range of pests across the country, posing challenges for effective pest management strategies.

EFFICACY AND POTENTIAL PERSISTENCE OF *METARHIZIUM ANISOPLIAE* AGAINST VARIOUS SPECIES OF LOCUSTS

Faria Abbasi* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: fariaabbasi12@gmail.com, riffat.sultana@usindh.edu.pk

Locust outbreaks can cause significant damage to crops and vegetation, leading to economic losses for farmers and communities. The effectiveness of *Metarhizium anisopliae* as a biological control agent against locusts was evaluated in this study. The rate of mortality in the treated group was shown to be higher as compared to the un-treated group, having a mortality rate of 96% in treated group and 52% in the control group. The results suggest that Green Guard (*M. anisopliae*) has a significant lethal effect on adults of *Schistocerca gregaria*, *Locusta migratoria*, *Nomadacris succinta*, and *Anacridium aegyptium* also, the mortality rate increased gradually over the seven-day period, indicating a cumulative effect of the fungal infection on the locusts. These findings highpoint the potential of *M. anisopliae* as an active implementation in controlling populations of *Nomadacris succinta* and other locusts. These natural enemies are predators and pathogens including fungi. Fungal control is widely used all across the world as it one of the most promising types of biological control. At present study fair numbers of specimens were captured and identified into 3 species i-e *Schistocerca gregaria* (Forsskål, 1775) *Locusta migratoria* (Linnaeus 1758) and *Anacridium aegyptium*, . However, other observations are under way. Fungi (*Metarhizium anisopliae*) are non-harming to humans and lethal for insects. The behavioral changes observed in infected insects included the cessation of feeding, problem in coordination, shaky movements, extra grooming, less orientation, confusion at the time of mating, limited mating and dropping eggs. These findings have implications for the use of pathogens as means of biological control for insect pests.

TOXICITY OF NATURAL INSECTICIDES ON DIFFERENT LIFE STAGES OF OXYINAE RICE PESTS (ACRIDIDAE: ORTHOPTERA)

Mohsin Ali* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: mohsinmoin30@gmail.com, riffat.sultana@usindh.edu.pk

The long-term and extensive use of synthetic insecticides has resulted in accumulating residues in food, milk, water, and soil, leading to adverse health effects on humans and ecosystems. Natural insecticides exhibit different toxicological effects on pests, depending on their mode of action. This study employed natural insecticides to assess their toxic effects on various developmental stages of Oxya species, the biggest threat to rice crops in Pakistan. The research aimed to collect data from the Hyderabad district to evaluate the toxicity of different natural insecticide extracts on various life stages of rice pests. At 24 hours, 48 hours, and 72 hours of treatment against three species of the genus Oxya, adults and nymphs in their first to sixth nymph stages were compared between various treatments. After three days of

treatment, average mortality rates were noted at 60% to 70%. The highest mortality was observed with *Azadirachta indica*, at 84%, while the lowest mortality rate was recorded with *Piper nigrum* at 62%. When compared to the untreated control group, the mortality rate was noted at 5% to 6%. During treatment, some behavioral changes affected feeding, reproduction, and ecdysis for metamorphosis. All developmental stages experienced significant mortality due to natural insecticides. To prevent this damage, the use of natural insecticides was considered more convenient and cheaper than other chemical pesticides, as it is less harmful to the environment. This information will assist farmers and control planners working on implementing Integrated Pest Management (IPM) techniques."

SURVEY AND EXPLORATION OF *SCELIO* PARASITOIDS AS BIOCONTROL AGENTS FOR DESERT LOCUST MANAGEMENT

Muhammad Muqem Jakhrani^{1*}, Riffat Sultana^{1*} and Santosh Kumar²

¹*Department of Zoology, University of Sindh, Jamshoro*

²*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

*Corresponding Author: muqem.jakhrani@scholars.usindh.edu.pk, riffat.sultana@usindh.edu.pk

During the present study, approximately 125 specimens of desert locusts were collected from different localities where rice, sugarcane, wheat, maize, millet, and fodder were predominantly cultivated. Unfortunately, a recent swarm of *Schistocerca gregaria* caused significant losses to these crops in desert areas, posing a substantial threat to the local agricultural practices. As part of the current study, an extensive survey was initiated to document the emergence of locusts, including the presence of the egg-parasitoid wasp *Scelio*, with the aim of developing potential implementation strategies for future locust control. Some egg pods were collected from the arid zones of Baluchistan; some were found to be fully parasitized by the wasp, while others were only partially parasitized. A detailed study on this biocontrol agent is currently underway. It is important to highlight that biological control methods, such as utilizing *Scelio*, not only prove to be effective but also play a crucial role in being environmentally friendly. As the population of *Scelio* increases, it has the potential to significantly reduce the number of viable desert locust eggs. The present work provides valuable insights for managing the destructive population of desert locusts through parasitoids, especially *Scelio*. The grant received from HEC (NRPU Project No.14787) is highly acknowledged.

SPATIOTEMPORAL DYNAMICS OF CITRUS RUST MITES (*PHYLLOCOPTRUTA OLEIVORA* ASHMEAD) IN GUANGDONG'S CITRUS ORCHARDS: A COMPREHENSIVE QUARTERLY STUDY

Syed Usman Mahmood, Runqian Mao and Xiao-Duan Fang*

Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Institute of Zoology, Guangdong Academy of Sciences, Guangzhou, 510260, Guangdong, China

*Corresponding Author: fangxiaoduan@163.com

The citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), plays a crucial role as a notable pest in citrus cultivation areas globally. It is well-established that this mite significantly affects the quality and yield of citrus crops, especially under favorable conditions leading to population surges. This study investigates the spatiotemporal distribution of the citrus rust mite in citrus orchards (*Citrus reticulata*

Banco) in Guangdong, China. The study conducted quarterly sampling from April 2022 to December-2022 in three citrus fields, selecting 15 trees per field. Sampling involved the systematic collection of 11 leaves from each cardinal direction (East, South, North, West), both inner and outer portions, as well as top, mid, and bottom sections. Temperature and relative humidity were also recorded of each direction. Collected leaves were brought to the lab, where rust mites were counted under a microscope. Maximum mean rust mites found in field 1 on 26-April were 7.5 per leaf. Highest density of citrus rust mites was recorded on the leaves in the southern portion of the tree. Temperature and humidity have no significance difference in all directions but was found less in southern direction. This sampling method systematically assesses citrus rust mite distribution in citrus fields, providing precise maps and ecological insights. Our findings reveal a distinctive distribution pattern of the mite within the citrus orchards. This research contributes valuable insights into understanding the ecological dynamics of citrus rust mites, aiding in the development of targeted pest management strategies for citrus cultivation in the region. Additionally, it supports long-term monitoring, aiding in the evaluation of interventions and enhancing overall citrus crop management.

IMPACT OF COTTON PLANT DENSITY ON INSECT PESTS' PHENOLOGY UNDER CHANGING CLIMATE SCENARIO

**M. Sohail Qamar Zaman¹, M. Ishtiaq^{1*}, Mirza Abdul Qayyum¹, Nadeem Ahmed¹,
M. Akram², M. Rafiq Shahid², M. Zeghum Ali¹ and Umer Sharif¹**

¹*Institute of Plant Protection, Muhammad Nawaz Sharif University of Agriculture, Multan*

²*Cotton Research Institute (CRI), Multan*

Corresponding Author: m.ishtiaq@mnsuam.edu.pk

Agriculture is an important source for the economy of Pakistan. In agriculture, cotton is an important cash crop and also the main source of raw materials for Pakistan textile industry. Pakistan is the world's fifth-largest producer of cotton, after China, United States, Brazil and India. Cotton consumption has increased in response to a significant increase in global population. Unfortunately, cotton production is decreasing day by day in Pakistan due to the changes in environmental conditions, pest outbreaks, insecticide resistance and pest resurgence. To improve cotton cultivation and production research was planned to check the response of different insect pests on various planting densities at Cotton Research Institute, Multan. The data of whitefly, thrips, jassid, dusky cotton bugs and pink bollworm were recorded at various plant densities on weekly basis. Moreover, population of natural enemies and metrological data were also recorded during whole cotton crop season at different plant densities. A registered cotton variety MNH-1020 was sown using Randomized Complete Block Design (RCBD) with five treatments T₁, T₂, T₃, T₄, and T₅ on the basis of different plant densities i.e., 6, 9, 12, 15 and 18 inches respectively with three replications of each treatment were applied in this experiment. The results revealed that the population of whitefly, jassid, thrips, and dusky cotton bug were statistically different at different plant densities when compared with each other. Highest population of sucking insect pests (whitefly 5.87, jassid 2.34, thrips 1.98 and dusky cotton bug 3.86) were recorded in T₁ (P-P = 6 inches) and lowest populations (whitefly 4.02, jassid 0.11, thrips 1.14 and dusky cotton bug 2.90) were recorded in T₅ (P-P = 18 inches). The population of pink bollworm in all tested treatments was non-significant at different densities. The population of ants, green lacewing, lady bird beetle, and spiders was significant while the population of *Geocoris* and *Orius* bug was non-significant in all treatments at all tested plant densities. In T₁, the population of ants, green lacewing, lady bird beetle, and spiders was highest and it was lowest in T₅ at different densities. The results concluded that cotton crops could be grown at 6 inches to increase the production and minimize the population of insect pests by natural enemies. However, this study could also be helpful in integrated pest management (IPM) for the control of pest population.

SEASONAL ACTIVITY OF FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (SMITH) IN MAIZE AGROECOSYSTEM OF SOUTH PUNJAB MULTAN, PAKISTAN

M. Ishtiaq^{1*}, Saleem Riaz¹, Fawad Z. A. Khan^{1,2}, Mirza Abid Mehmood¹ and M. Zeghum Ali¹

¹Institute of Plant Protection, Muhammad Nawaz Sharif University of Agriculture, Multan

²Department of Outreach and Continuing Education, Muhammad Nawaz Sharif University of Agriculture, Multan

Corresponding Author: m.ishtiaq@mnsuam.edu.pk

Maize (*Zea mays* L.) is an important cereal crop of Pakistan. Fall armyworm (*Spodoptera frugiperda*) is considered as one of the key pests which reduces the maize quality and yield. We investigated the dynamics of FAW moth populations and the harm the larvae inflicted on maize crops during Fall 2022 and Spring 2023. Significantly higher mean trap catches moths/trap/week in Fall season 2022 were noticed in September (42.13) followed by October (33.5) and higher moth catches were recorded in March (40.25) followed by April (35.88) in the Spring season 2023. Moth catches increased gradually from the emergence of the crop, peaked during the vegetative growth of the crop (V8 to VT) till reproductive stage and after that starts to decrease from the start of reproductive stage till maturity to harvesting stage (MH) of the crop during both seasons. Similarly, significantly higher FAW percent damage was recorded in September (36%) followed by October (18%) during Fall 2022 and March (33%) followed by April (18%) during Spring 2023. Also, higher FAW percent damage was recorded during vegetative growth of the crop (V8 to VT) and lower during the reproductive stage (R1 to R6), maturing to harvesting (MH) during the both seasons. Correlation analysis of moth catches showed the significant positive correlation with relative humidity (morning) and rainfall whereas maximum temperature showed a significant negative correlation in both years. Results of present study could be helpful in timely management practices on specific time and specific crop stage of maize crop to avoid big losses in yield.

INVESTIGATING SOME BIOLOGICAL PARAMETERS AND HOST SELECTION OF COTTON MEALYBUG (*PHENACOCCLUS SOLENOPSIS*) IN CONTROLLED LABORATORY ENVIRONMENT

Muhammad Salman*, Inamullah Khan and Muhammad Hamayoon Khan

Plant Protection Division, Nuclear Institute for Food and Agriculture (NIFA), G.T. Road, Peshawar

*Corresponding Author: sufisaib@gmail.com

Cotton mealybugs, recognized as *Phenacoccus solenopsis* (Tinsley), represent pervasive pests causing widespread infestations across diverse plant species globally. Through a series of experiments, we aimed to uncover their preferences for hosts, developmental stages, and reproductive behaviors across different hosts. Our study encompassed No-Choice and Free-Choice tests, examining host preferences, while thoroughly investigating the durations of instar stages, adult stages, and the total production of crawlers per female across distinct hosts. Both tests were replicated thrice in Completely Randomized Design. In the Free-Choice Test, it was evident that okra emerged as the favored host, attracting significantly the highest number of mealybug crawlers (126.3), followed by pumpkin (35.2), potato (17.7), and applegourd (12.8). Similarly, the No-Choice Test reinforced okra's dominance as the preferred host across various exposure times. Analysis of instar stage durations revealed that okra notably extended the third instar stage duration (ranging from 10.12 to 12.67 days). Additionally, okra showcased the highest reproductive output, yielding the most crawlers per female (195.4), emphasizing its suitability for mealybug reproduction. Conversely, among the hosts, pumpkin demonstrated the lowest reproductive output (48.04). These findings underscore the variations in

cotton mealybug behavior and reproductive success, reliant on the host plant. Okra emerged as the most appealing and conducive host for reproduction, while other hosts displayed varying degrees of suitability.

INVASION ALERT: INCIDENCE OF FALL ARMY WORM (SPODOPTERA FURGIPERDA) IN AZAD JAMMU & KASHMIR, PAKISTAN

Naila Nazir*, Iraj Yaseen, Khalid Mahmood, Muhammad Saqlain Shad, Mujtaba Ahmed Salik and Mohsin Maqbool

Department of Entomology, University of Poonch Rawalakot, Azad Jammu & Kashmir

*Corresponding Author: nailanazir@upr.edu.pk nailanazir9@gmail.com

Fall Army Worm (FAW) *Spodoptera furgiperda* (Noctuidae: Lepidoptera) is an invasive notorious pest of maize and other cereal crops worldwide and expanding its host range very swiftly. To check the incidence of FAW in Azad Jammu & Kashmir, this study has been planned during 2022-2023 in Mazie growing areas of AJ&K. During this study FAW population was recorded from all the selected localities of study site. A Correlation of temperature and other weather condition to FAW population emergence was also assessed. This study will be a baseline for further development of management strategies of this pest.

EVALUATION OF INERT MATERIALS FOR SLOW RELEASE OF METARHIZIUM SPORES FOR MOSQUITO CONTROL

Hafiza Aliza Sajjad*¹, Unsar Naeem-Ullah¹, Mirza Abdul Qayyum¹, Shafia Saba^{1,2}, Muhammad Asif Farooq¹, Khadija Azam¹, Muhammad Ahmad¹, Rana Zain Khizar¹ and Saba Iftikhar¹

¹*Institute of Plant Protection, Muhammad Nawaz Sharif University of Agriculture, Multan*

²*District Health Authority, Multan*

*Corresponding Author: alizasajjad777@gmail.com

Mosquitoes transmit deadly diseases and their control is important for public health. Chemical insecticides are widely used but pose environmental and health risks. The entomopathogenic fungus *Metarhizium anisopliae* naturally infects and kills several insect species including mosquitoes. Its field application requires frequent re-application as spores wash off rapidly. This study aims to evaluate inert materials that can adsorb and slowly release *Metarhizium* spores for extended mosquito control. The controlled dispersal of this beneficial fungus can help in controlling larval stage of mosquitoes for longer period of time. Materials like zeolite, sodium alginate and perlite were tested for their ability to adsorb *Metarhizium* spores from solution. The spore loaded materials were submerged in aquatic ecosystem. Their spore release kinetics in water counted through periodic spore counts. Mosquito larviciding assays were determine the longevity of larval mortality caused by spores released from these materials. The adsorption capacities and release profiles were indicating suitable slow-release formulations. Field studies were employed the best formulations in habitats containing mosquito larvae. Periodic larval counts and fungus re-isolation were evaluated persistence and activity. This study revealed that cost-effective, environment-friendly carrier materials to facilitate the use of *Metarhizium* fungi as a natural mosquito control alternative to chemicals. Suitable formulations were shown to reduce costs and labour while extending protection time compared to direct spore applications.

COMPARATIVE EFFECTIVENESS OF PHYTOPHAGOUS INSECTS TO BE USED AS BIOCONTROL AGENTS AGAINST *PARTHENIUM HYSTEROPHORUS* L

Muhammad Ahmad*, Unsar Naeem-Ullah, Hafiza Aliza Sajjad, Muhammad Ishtiaq, Mudssar Ali, Rana Zain Khizar and Saba Iftikhar

Institute of Plant Protection, Muhammad Nawaz Sharif University of Agriculture, Multan

*Corresponding Author: mahmadent@gmail.com

Parthenium hysterophorus L., commonly known as parthenium weed, is considered one of the top noxious weeds of the world. It poses a major threat to agricultural lands, fallow fields, grasslands and natural ecosystems in many parts of the world. Chemical and mechanical control methods have shown limited success in managing this invasive weed due to its prolific seed production and regenerative ability. Biological control offers a safer, sustainable and cost-effective alternative. The biocontrol agents including suppressive plants (*Crotalaria juncea* and *Cassia tora* etc.), insect (*Epiblema strenuana* stem galling moth, *Zygogramma bicolorata* Leaf feeding beetle and *Listronotus setosipennis* stem boring weevil) and pathogen (*Puccinia abrupta* var. *partheniicola*) help in reducing the growth and spread of parthenium weed. In Australia, stem-galling moth *E. strenuana* was successfully established in the late 1980s and provides good control. In India, the foliage-feeding beetle *Z. bicolorata* was introduced in the early 1990s and is now well established, reducing weed density and biomass. *E. strenuana* act as synergist with suppressive plants e.g., buffel grass. Across countries, the rust fungus *P. abrupta* var. *partheniicola* has been particularly effective. These three insect biocontrol agents combined with rust fungus reduced parthenium population to 60-90% in Queensland, Australia. The overall objective is to directly compare the impacts of these biological control agents in different locations. to significantly reduce the population of parthenium weed in agricultural fields and natural ecosystems in a sustainable manner.

EFFICACY AND REPELLENCY OF SOME INSECTICIDES AGAINST HOUSE FLY, *MUSCA DOMESTICA* L. (DIPTERA: MUSCIDAE)

Mohsin Ashfaq¹ Muhammad Saleem Afzal² Rana Zain khizar³ and Saim Ibtesam⁴

Department of Entomology, Institute of Plant Protection, Muhammad Nawaz Sharif University of Agriculture, Multan

*Corresponding Author: mmohsanm215@gmail.com

The house fly *Musca domestica* L. (Muscidae: Diptera) is a harmful insect pest on both large farms and households globally. It is a major source of many dangerous food borne infections in human environments, which represent its synanthropic characteristics. House fly significantly spread the cholera, salmonellosis, and other severe foodborne diseases. Dairy farming is important to Pakistan's economy as it provides milk, meat, wool, hide, blood, bones, and farm animal dung. Pakistan is an agricultural country. The dairies usually play a significant role in the breeding of flies due to poor hygiene conditions in the form of farm manures, poor disposal, and open defecation places. All such places are recognized as potential feeding and breeding places for house flies. The control of house flies using sanitation, screening procedures, traps, insecticides, biological control and integrated house fly control have been put into place. Chemical control is the most commonly approach because it is simple and quick to implement. Lufenuron, pyrifluquinazon, fipronil, afidopyropen, chlorpyrifos and dinotefuron insecticides were used. The mortality of 2nd instar larvae (80–90%) of dinotefuron is higher than other insecticides in the treated diet. Lufenuron was less effective (38% mortality) than other insecticides. Dinotefuron and pryfluquinazone were strongly repellent (84 and 78%, respectively) for the larvae of house flies. For adult house flies

pyrfluquinazone and afidopyropen were more repellent (80 and 75%, respectively) than other insecticides in the treated diet. Dinotefuron and fipronil were higher repellent (70 and 60%, respectively) than lufenuron and chlorpyrifos (40 and 30%, respectively). To check the repellency of adult house flies bioassay test was used. This study shows that dinotefuron was the most effective product to control house flies at the larval stage and pyrfluquinazon was effective for adult repellency.

COMPARISON OF BIORATIONAL AND CHEMICAL MANAGEMENT TO CONTROL FALL ARMYWORM IN EARLY AND LATE SOWN MAIZE IN MULTAN, PAKISTAN

Muhammad Aizaz¹, Fawad Zafar Ahmad Khan^{1,2}, Mudssar Ali¹ and Babar Farid³

¹*Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan*

²*Department of Outreach and Continuing Education, Muhammad Nawaz Shareef University of Agriculture, Multan*

³*Institute of Plant Breeding and Biotechnology, Muhammad Nawaz Shareef University of Agriculture, Multan*

*Corresponding Author: fawad.zafar@mnsuam.edu.pk

Fall armyworm *Spodoptera frugiperda* has emerged as an important pest of maize in Pakistan. Most of the farmers are only relying on chemical control for the quick management of this pest, however, pesticides have adverse effects on non-target animals. However, there is a need to explore non-chemical options for managing this pest. The current study aimed to compare combination of plant powders to manage fall armyworm in early and late sown maize. The live observations of the beneficial arthropods were also made on all treatments. The results showed that the infestation level of fall armyworm was lowest in synthetic pesticide treatment as compared to the bio-rational treatments in early and late planted maize. Moreover, abundance of natural enemies was higher in bio-rational treatments as compared to pesticide treatment. Among the plant powder mixtures with sand, the mixture of sand, ash, neem and turmeric was the successful treatment against the fall armyworm. The result shows the potential of the non-pesticide options for managing the fall armyworm in maize.

BIOPESTICIDES TOXICITY ON *SITOPHILUS ORYZAE*

Jawed Iqbal Khan¹, Sobia Khwaja², Rubina Mushtaq², Ambreen Akram² and Aasia Karim³

¹*Assistant Entomologist, Department of Plant Protection, Malir Halt, Karachi*

²*Department of Zoology, Federal Urdu University of Arts Science and Technology, Karachi*

³*Department of Zoology, Sardar Bahadur Khan Women University, Quetta*

*Corresponding Author: sobia.khwaja@fuuast.edu.pk

Experiments were conducted to determine the insecticidal properties of three essential oils derived from indigenous plants viz., *Datura stromonium*, *Ocimum basilicum*, and *Mentha spicata*. As *Sitophilus oryzae* (Rice weevil) is an important pest of rice in Pakistan and is regarded as an economic threshold insect and capable of destroying stored grain goods, in a laboratory conditions at 32±3°C and 59±5% relative humidity, stored grain pest received treatments with different amounts of 3, 4, 5, 6, and 7 mL with extract of ethanol in every combination of concentration levels and contact times three extracted essential oils were shown to have a substantial impact on mortality. Concentration and exposure time were found to be related to higher death as results indicate that toxic substances made from the pure oils of the studied plants are effective for preventing insect pests out of products that are kept in storage. In conclusive

remarks the essential oils of three different plants i.e. *Datura stromonium*, *Ocimum basilicum*, and *Mentha spicata* extract with 99.9% pure ethanol has the greater repellency and contact toxicity against stored grain pest rice weevil whereas, *Datura spp.*, is higher toxicity affinity against stored commodity pest.

EVALUATION OF LIQUID FORMULATIONS OF ENTOMOPATHOGENIC FUNGI AGAINST *BACTROCERA ZONATA*

Umer Sharif*, Mirza Abdul Qayyum, Shafqat Saeed, Akhtar Hameed, Unsar Naeem Ullah, M. Ishtiaq¹, Hasan Taha¹ and Aiman Khalid¹

Institute of Plant Protection, MNS-University of Agriculture, Multan

*Corresponding Author: umersharif173@gmail.com

Fruit flies are global to economy and food security because they are causing losses billions of dollars annually. Tephritidae is a large family of fruit flies having more than 4700 species and 500 genera. At least 250 species are of most economic importance. In Pakistan, especially in Punjab, the fruit fly *Bactrocera zonata* (Diptera: Tephritidae) is a common pest. Currently chemical insecticides are used for control of fruit fly. Environmental concerns related to use of insecticide demand for sustainable Integrated Pest Management (IPM). Entomopathogenic fungi (EPF) such as *Beauveria bassiana* and *Metarhizium anisopliae* are best alternative of pesticide. These fungi have certain limitation regarding their efficacy in regions with high temperature. Low shelf life is another limitation in commercialization of its products. Current research was conducted to find out certain liquid carriers which can maintain the viability of the conidia of entomopathogenic fungi. Conidia both EPF were embedded in vegetable oils such as Corn oil, Groundnut oil, Sunflower oil, Sesame oil, Coconut oil and Soyabean oil along with the aqueous suspension. All formulations were prepared on the same day. Their efficacies were evaluated on 1st day, 15th day and 30th day after preparation of formulation against two stages (Pupae and adult) of *B. zonata*. Adult emergence of the pupae and mortalities of adult were recorded. Aqueous suspension of conidia performed better on 1st day of preparation of formulation but lost its efficacy on 15th and 30th day. According to study sesame oil and corn oil were found to be more effective in maintaining the viability of the conidia of EPF even up to 30th day. Sesame oil and Corn oil recommended for preparing liquid formulation of Entomopathogenic fungi.

COMPARATIVE ANALYSIS OF THREE MANGO CULTIVARS TO ASSESS THE FITNESS OF *BACTROCERA ZONATA*

Aiman Khalid, Mirza Abdul Qayyum, Muhammad Nadir Naqqash, Hasan Riaz and Umer Sharif

Institute of Plant Protection, MNS University of Agriculture, Multan

Corresponding Author: aimankhalid523@gmail.com

Bactrocera zonata (Diptera: Tephritidae) is one of the major pests of fruits and vegetables, with a wide host range. Annually, an estimated loss of \$200 million is recorded in Pakistan due to this insect pest. To manage *B. zonata* through integrated practices, life history traits should be understood. Each cultivar possesses the physiochemical, and morphological properties which influence the host acceptance. The effect of different mango cultivars on growth and reproduction can be investigated using the TWO SEX MSChart. Life table analysis is a precise tool to calculate the life history of pests. Fajri, Langra, and Alphanso were exposed to the *B. zonata* population. The results revealed that *B. zonata* could complete its life cycle on all the tested cultivars. The finite rate λ , intrinsic rate r , mean generation time T , gross

reproductive rate GRR, and mean longevity were significantly higher on Fajri. The lowest growth rates were observed on Alphanso. Among all three varieties, the highest survival rate was observed on Alphanso, and maximum growth parameters were observed on Fajri. It is concluded that the host adaptability of pests influences their oviposition behavior.

EFFICACY OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* AGAINST WHEAT APHID SPECIES

Iqra Saif^{1*}, Irfan¹, Muhammad Sufyan², Hamid Majeed¹, Rida Nosheen Saif², Muhammad Adnan², Muhammad Junaid Iqbal², Ahsan Shafique¹ and Muhammad Jabbar¹

¹*Cholistan university of Veterinary and Animal Sciences, Bahawalpur*

²*University of Agriculture, Faisalabad*

Corresponding Author: iqrasaif0201@gmail.com

In the recent study, the entomopathogenic fungus, *Beauveria bassiana* and *Metarhizium anisopliae* against wheat aphid species in field conditions to check the insecticidal effect of entomopathogenic fungi were used to evaluate their pathogenicity against adults of different aphid species i.e., *Schizaphis graminum*, *Rhopalosiphum padi*, *Brevicoryne brassicae* and *Lipaphis erysimi*, and their natural enemies of crops. *Beauveria bassiana* and *Metarhizium anisopliae* were found effective at all concentrations i.e., 1×10^6 , 1×10^7 and 1×10^8 cfu/ml on all aphid species, but the uppermost concentration (1×10^8 cfu/ml) provided maximum control within a short period of time. *B. bassiana* concentration (1×10^8 cfu/ml) proved to be lethal to wheat aphid species after the 3rd day. Mortality of wheat aphids were observed at maximum highest concentration of 1×10^8 cfu/ml were in range of 100% at 8th day of treatment for different aphid species treated at various concentrations. *M. anisopliae* (1×10^8 cfu/ml) showed excellent mortality 85% and 84% at 5th and 6th day respectively, against wheat aphid species. In conclusion, the use of *Beauveria bassiana* and *Metarhizium anisopliae* as biopesticides in agriculture is a sustainable and environmentally friendly approach to control insects' pests.

LIFE TABLE ANALYSIS OF *BACTROCERA CUCURBITAE* ON DIFFERENT HOSTS

Iqra Maryyam*, Mirza Abdul Qayyum, Farrukh Baig, and H.M. Usman Aslam

Institute of Plant Protection, MNS, University of Agriculture, Multan

*Corresponding Author: iqramarram@gmail.com

Bactrocera cucurbitae is a quarantine insect pest of agriculture crops worldwide and affect the quality of fruits and vegetables by direct feeding. It causes significant losses on host crops in quantitative and qualitative terms. The study of development of life table is an integral component for understanding the behavior of *B. cucurbitae*. Life table study focused on biological and population parameters. This study was performed under laboratory condition on multiple hosts Cucumber, Pumpkin, Ridge gourd, Brinjal, Bitter gourd, Luffa and Sponge gourd with no-choice test for the study of biological and population parameters. Results show that the Cucumber, Pumpkin and Bitter gourd was most preferable hosts having highest intrinsic rate (r), net reproductive rate (R_0), mean generation time (T) and finite rate of increase (λ). The intrinsic rate (r) was higher in Cucumber and Pumpkin 0.13 and Bitter gourd was 1.16, finite rate of increase (λ) was 1.14 on Cucumber, Pumpkin and Bitter gourd. This study was helpful for understanding the development and population parameters on different hosts for successful integrated pest management strategies.

ROLE OF CULTURAL CONTROL AND INSECTICIDES APPLICATION IN MANAGEMENT OF MANGO FRUIT BORER, AND IMPROVEMENT OF EXPORTABLE MANGO FRUITS QUALITY

Asifa Hameed*, Abid Hameed Khan and Abdul Ghaffar Grewal

Mango Research Institute, Multan

Corresponding Author: asifa_hameed_sheikh@yahoo.com

Mango fruit borer is an important emerging threat to mango industry in southern Punjab Pakistan. This pest was previously reported in Australia, Indonesia and India; however, this pest was not reported causing extensive damage in Pakistan until 2017. In 2020, very serious losses to qualitative and quantitative mango production were reported. The immature fruits were dropped, mango fruit clusters were badly affected and caterpillars of this pest were feeding on mango fruits during early ripening stage. The exportable quality of mango fruit could not be attained. Hence, in 2021, we designed an experiment to manage this pest through adopting cultural control practice and insecticides application. In module 1, five insecticides viz., chlorantraniliprol (coragen 20SC), chlorantraniliprole + thiamethoxam (valium flexi), Lunfenuron (Match 50EC), Flubendiamide (Belt 480 SC), and Emamectin benzoate (Proclaim 19EC) were compared for their effectiveness against mango fruit borer. In module 2, the thinning was done to provide an environment unfavorable for the pest outbreak. Data was taken 24hrs, 48hrs, 72 hrs, 7 days, 10 days, 20 days and 40 days after insecticide application and thinning practice. At each location 2 orchards were selected. Overall, we found that insecticidal application was better than thinning. Insecticide coragen was most effective in controlling mango fruit borer population. Reduction in pest population was higher in insecticide treated plots compared to thinning treatment. Mango fruit yield was higher in sprayed plots compared to the plots where thinning practice was done. Farmer income was higher in sprayed plots compared to experimental plots where thinning practice was done.

COMPLIANCE OF QUARANTINE REGULATORY LAWS: A PREREQUISITE FOR BIOSAFETY ESPECIALLY IN DEVELOPING COUNTRIES

**Khadija Azam*, Unsar Naeem-Ullah, Shafqat Saeed, Naeem Iqbal,
Muhammad Saim Ibtesam and Aliza Sajjad**

Institute of Plant Protection, MNS University of Agriculture, Multan

*Corresponding Author: khadeejazam11@gmail.com

Biodiversity loss is a global challenge faced due to unchecked transport of goods and individuals. It disturbs the ecological balance, economic stability and societal well-being. Biodiversity conservation depends upon strict adherence to quarantine regulatory laws that are devised to protect species and ecosystems. The National Biodiversity Strategy and Action Plans (NBSAP) is multi-sectoral, participatory tool for national biodiversity planning. It is a process by which nations identify and mitigate the risks to their biodiversity and resources related to it to plan for the conservation of biological diversity, the sustainable use of its constituent parts, and the just and equitable sharing of the benefits resulting from the use of genetic resources. Observing these laws reduces the anthropogenic pressure and helps in mitigating the negative impacts of alien species on indigenous biodiversity. Strict enforcement discourages destructive behavior and promotes sustainability by fostering accountability and openness in environmental governance. By being decisive, this strategy deters criminal activity and protects endangered species and hotspots for biodiversity. Nevertheless, obstacles including institutional

constraints, insufficient funding, and corruption exist for optimal compliance. Building capacity, being transparent, and giving local communities more influence are all part of strengthening government. In order to promote environmental stewardship, public involvement and awareness are essential. Encouraging ecological literacy gives people the ability to actively participate in conservation. For example, the recent increase in agricultural goods from outside and tourists owing to Korea's economic expansion and globalization has led to increase in invasive alien insects setting in Korea. Among these alien insect species, 171 are classified as invasive. For the previous 40 years, an average of 0.85 foreign insect species have got established every year, with 76.6% of them being economic agricultural pests. Despite not being the ultimate solution, regulatory compliance is an essential component of successful conservation. Maintaining the planet's ecological richness requires strong procedures, clear norms, and responsibility for quarantine checks. For biodiversity protection to continue to be effective, governance, public participation, and constant legislative framework modifications are necessary.

BIOINSECTICIDAL EFFICACY OF THE VENOM OF PARASITIC WASP (*AENASIUS ARIZONENSIS*) FOR SUSTAINABLE MANAGEMENT OF SUCKING INSECT PESTS OF COTTON

Hasooba Hira¹, Zain ul Abidin^{1*}, Syeda Eisha tu Razia¹, Hafiz Muhammad Tahir²

¹*Department of Entomology, University of Agriculture, Faisalabad*

²*Department of Zoology, GC, University, Lahore, Lahore*

*Corresponding author: zainentomology@uaf.edu.pk

One of the most promising alternatives to chemical pesticides involves using biocontrol methods and natural products derived from organisms like parasitic wasps. These wasps target and suppress important pest insects, offering a more targeted solution compared to broad-spectrum chemical pesticides. This approach, known as "bioinsecticides" helps to reduce negative impacts on food safety, non-target organisms, and the environment. This preliminary study investigated the host regulation factors involved in host-parasitoid relationship Cotton mealybug (*Phenacoccus solenopsis*), a significant pest of cotton in Pakistan, and its parasitoid, *Aenasius arizonensis* (Girault). The goal was to explore whether the venom blend injected by female parasitic wasps into their host insects could serve as a source of insecticidal bioactive genes. This research aimed to develop new insect control methods using integrated biotechnological approaches. The study identified bioactive genes responsible for causing castration, paralysis, and developmental arrest in the host insects. This initial investigation lays the groundwork for further analysis of bioactive genes using functional, molecular, genomic, and proteomic approaches to study bioactive peptides. The experimental approach employed in this study has provided valuable new insights that could pave the way for more sustainable insect control programs, marking a significant advancement in this field.

INCIDENCE OF RICE LEAF FOLDER *CNAPHALOCROCIS MENDINALIS* (LEPIDOTERA: CRAMBIDAE) IN WHEAT FIELDS OF CENTRAL SINDH, PAKISTAN

Mansoor Ali Shah*

Department of Zoology University of Sindh, Jamshoro

*Corresponding Author: mansoor.shah@usindh.edu.pk

Rice leaf folder is usually insect pest of Paddy crop in rice cultivated areas of Pakistan. Every years Rice leaf folder badly effect the rice production. Present study was conducted in Matiari and Shaheed

Banazeerabad Districts of Sindh, Pakistan from December 2023 to January 2024. Both districts situated center of province and very famous due to their fertile land and variety of crops. Infestation of leaf folder found mostly fields of study areas but in some areas infestation reached economic injury level. It was observed growers were totally unaware because this pest usually found in rice crop and these districts are included in cotton belt. Main reason behind this infestation is growers cultivated rice crop in some pockets of study area and change in weather patterns especially humidity was recorded very high in these months.

SECTION – I I I

E N T O M O L O G Y

BIODIVERSITY OF SHORT HORNED GRASSHOPPERS ACRIDIDAE FROM MIDDLE SINDH, PAKISTAN

Asif Nazeer Memon^{*1}, Naheed Baloch², Riffat Sultana², Sidratul Muntaha² and Shamshar Unar²

¹Department of Zoology, Govt Ustad Bukhari Degree College, Dadu

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: memonasifnazeer@hotmail.com

Middle Sindh includes three Districts namely District Dadu, Shaheed Benazirabad and Naushero Feroze. These above said Districts possess agriculture field, their temperature is suitable for rapid multiplication of insects, like short horned grasshoppers belonging to family **Acrididae** and long horned grasshoppers family **Tettigonidae**. They have economic importance to consider pest of different crops in these districts of Middle Sindh, so that proper diagnosis can be made, because locust is notorious member of the above said family and is a major pest of the various cash crops. We have collected **2416 specimens** from different localities of Districts of Middle Sindh, namely village Qazi Arif, Village Ghulam Hussain Gadhi, Village Phaka, Village Muhammad Ibrahim Panhwar and Village M. Bachal Bouk of district Dadu, village Walidad Zardari, village Bux Ali Dahri, village Bandhi, Village Baharo Khan Mari and village Sardar Khan Rind of District Shaheed Benazirabad and Village Puran, Gh; Hyder Jesar, Aayal Khan Tunio, Halani and Tharu Shah of District Naushero Feroze. We have collected the following 17 species namely *Oxya hyla hyla*, Serville 1831, 7.28%, *Oxya fuscovittata*, Marshall 1836 7.98%, *Hieroglyphus perpolita*, Uvarov 1832 7.78%, *Aiolopus thalassinus thalassinus* Fabricius 1781 9.47%, ***Aiolopus thalassinus tamulus*, Fabricius 1798 9.97%**, *Acrotylus insubricus*, Scopoli 1786 7.16%, *Acrotylus fischeri*, Azam 1901 5.83%, *Locusta migratoria*, Linnaeus 1758 5.75%, *Sphingnostus savingnyi*, Saussure 1884 4.75%, *Trilophidia anulata*, Thunberg 1815 2.11%, *Truxalis eximia eximia* Eichwald 1830, 2.35%, *Acrida exaltata*, Walker 1859 5.54%, *Hilethera aeolopoides* Uvarov 1922 5.50%, *Gonista rotundata* Uvarov 1933 4.51%, *Anacridium rubrispinum* Bie Benkio 1948 4.47%, *Oxya velox*, Fabricius 1787 8.27%, *Oxya japonica*, Thunberg 1815 1.20%.

INTERACTION OF ABIOTIC AS WELL AS BIOTIC COMPONENTS WITH POPULATION OF HOVERFLIES IN TWO CLIMATIC REGIONS OF SINDH, PAKISTAN

Attaullah Ansari^{1*}, Nasreen Memon² and Mansoor Ali Shah²

¹GHSS Saeed Khan Laghari, Near Bhanoth, District Matiari

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: ansariatta@gmail.com

Hoverflies provide two vital services in ecosystem as bio control agent and plant pollinators. They are also known as syrphid flies or flower flies and belong to large family Syrphidae containing about six thousand species worldwide. Currently a detailed survey was conducted in major agricultural field of two climatic regions of Sindh i.e. Central and Lower Sindh during January to October 2023 in order to monitor

the interaction as well as effect of biotic and a-biotic factor with population of Hoverflies. These flies were trapped by means of two standard methods i.e. Hand sweeping insect net and Malaise trap. As a result a total of 1240 specimens of syrphid flies belonging to 8 species of four genera were collected from the randomly selected crops of studied areas such as brassica, wheat, rice, corn, fruits, fodder and vegetables from different studied localities of Central and Lower Sindh. The results of this study revealed that both biotic factors i.e. host plants and aphid population had positive correlation as well as well as impact on Syrphid population. Among a-biotic factors humidity, rainfall and cloudiness had positive while temperature had negative correlation as well as impact on the population of syrphid hoverflies.

TAXONOMIC STUDIES ON CHEWING LICE (PHTHIRAPTERA: INSECTA) *CHELOPISTES MELEAGRIDIS* (LINNAEUS, 1758), COLLECTED FROM TURKEY FOWLS (ISCHNOCERA: PHILOPTERIDAE) FROM HYDERABAD, SINDH, PAKISTAN

Farheen Shaikh*, Saima Naz and Nadir Ali Birmani

Department of Zoology, University of Sindh, Jamshoro, Sindh

*Corresponding Authors: farheen.shaikh@usindh.edu.pk, farheenshaikh1578@gmail.com

The chewing lice (Phthiraptera: Insecta) *Chelopistes meleagridis* (Linnaeus, 1758) is commonly known as turkey louse. It was slow moving philopterid and most of the time was remain attached to furry and fluffy feathers of host bird *Meleagris gallopavo* (Linnaeus, 1758) Turkey fowl and causes hindrance, madness, irritation and insanity in hosts when high in numbers. It was large sized. It was broad and pale yellow pigmented louse. It was host specific and causes parasitism. It was peculiar species with head prolonged far behind the occipital margin. It was most common in only one type of galliform birds of family Phasianidae *Meleagris gallopavo* (Linnaeus, 1758) Turkey fowls. This is reported first time as new locality record from Hyderabad, Sindh, Pakistan. The data was collected from study area from January 2020 to March 2021. The type host of *Chelopistes meleagridis* (Linnaeus, 1758) is *Meleagris gallopavo* (Linnaeus, 1758) Turkey fowl. The species *Chelopistes meleagridis* (Linnaeus, 1758) is closely related to *chelopistes. Karachiensis* in head tempora lmargin; charaters of antennae; articulation of segments; pterothoracic lateral margin, structure of abdomen; tergal and pleural sclertization and female abdominal terminal segment. Twenty-one *Meleagris gallopavo* (Linnaeus, 1758) Turkey fowls were examined for investigation of chewing lice. The samples were examined from all parts of the body of the turkey fowls, like head, abdomen, belly and feathers. The powder pyrethroid (anti-lice powder) was sprinkled on whole body parts during collection of chewing lice. The main purpose of the study was to find out the maximum number of species of chewing lice and described morphotaxy with special reference to its genitalia of both sexes from the study area.

DIVERSITY AND DISTRIBUTION OF THE GRYLLINAE (GRYLLIDAE: ORTHOPTERA) FROM CHOLISTAN DESERT

Santosh Kumar^{1*} and Riffat Sultana²

¹*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

²*Department of Zoology, University of Sindh, Jamshoro*

*Corresponding Author: santoshkumar@cuvas.edu.pk, drsantosharshi@gmail.com

Cholistan Desert is considered one of the largest deserts in Punjab, occupied by a variety of fauna and flora. Its climate is characterized as an arid and semi-arid tropical desert, with very low annual humidity. The mean temperature in Cholistan is 28.33 °C (82.99 °F), with the hottest month being July, recording a

mean temperature of 38.5 °C. Due to this diversity, the desert harbors various insects in different seasons. In order to understand the diversity and distribution pattern of Gryllinae, comprehensive surveys were conducted in Bahawalpur, Rahim Yar Khan, and Bahawalnagar from March 2023 to November 2023. During this survey, about 329 specimens were collected at different intervals. The expedition revealed six genera encompassing 10 species, including *Acheta domesticus* (Linnaeus, 1758), *Acheta meridionalis* (Uvarov, 1921), *Acheta hispanicus* Rambur, 1838, *Gryllus* (*Gryllus*) *multipulsator* Weissman, 2009, *Gryllodes sigillatus* (Walker, 1869), *Gryllodes supplicans* (Walker, 1859), *Callogryllus ovilongus* Saeed, Saeed & Yousuf, 2000, *Callogryllus saeedi* (Malik, et al., 2013), *Callogryllus bilineatus* (Bolívar, 1900), and *Phonarellus* (*Phonarellus*) *minor* (Chopard, 1959), reported from different geographical zones of Cholistan. This study suggests that more frequent surveys are needed to discover the hidden wealth of this arid region.

SYSTEMATIC STUDY OF EARWIGS (DERMAPTERA) FROM SAHIWAL DISTRICT

Ayesha Iqbal¹, Santosh Kumar^{1*} and Riffat Sultana²

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: santoshkumar@cuvas.edu.pk , drsantosharshi@gmail.com

Earwigs are free-living, flat, elongated insects characterized by a pair of forceps-like cerci at the end of their abdomen. They are often challenging to observe due to their nocturnal and secretive nature, preferring to hide in small cavities. Nevertheless, this taxonomic group is ubiquitous. A preliminary survey was undertaken in Sahiwal District, and the following species were recorded: *Anisolabis gestri* Borelli, 1907, *Labidura riparia* (Pallas, 1773), *Forficula auricularia* Linnaeus, 1758, and *Spongiphora lacustris* Linnaeus, 1758. The study found that *A. gestri* and *L. riparia* were the most widely distributed earwig species, while *F. auricularia* and *S. lacustris* were the most restricted. The study also suggests that further research is needed to understand the evolutionary history and biogeography of earwigs, as well as their role and impact in the ecosystem.

BIODIVERSITY OF OEDIPODINAE (ACRIDIDAE: ORTHOPTERA) FROM BAHAWALPUR

Muhammad Ahmed Rameez^{1*}, Santosh Kumar¹ and Riffat Sultana²

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: muhammadahmedrameez147@gmail.com , santoshkumar@cuvas.edu.pk

Grasshoppers, perhaps the most conspicuous of all insect pests, are abundant in dry grasslands and deserts, posing a constant threat to pastures and a variety of crops in both irrigated and rain-fed areas of Pakistan. The Oedipodinae group stands out among short-horned grasshoppers due to their diversity and occurrence in agricultural crops, hilly areas, and desert-like plains. Commonly known as geophiles and phytophiles, they thrive in the diversified habitat and ecosystem of sandy deserts interspersed with hillocks and sandy and gravel plains. In the arid region, the life of grasshoppers in these stretches of sand is remarkably rich in contrast to other deserts around the world. As a result of numerous surveys conducted in 2023, a total of 239 specimens of Oedipodinae were collected, including 178 immature and 61 adult grasshoppers. The captured material was categorized into three tribes: *Acrotlyini* Johnston, 1956, *Epacromiini* Brunner von Wattenwyl, 1893 and *Locustini* Kirby,

1825 encompassing eight species, namely *Acrotylus longipes longipes* (Charpentier, 1845), *A. longipes subfasciatus* (Bey-Bienko, 1948), *Aiolopus thalassinus thalassinus* (Fabricius, 1781), *A. thalassinus fabricius* (Fabricius, 1781), *Hilethera aeolopoides* (Uvarov, 1922), *Locusta migratoria* (Linnaeus, 1758) and *Oedaleus sengalensis* (Krauss, 1877).

CHECKLIST OF THE SCARABAEIDAE (COLEOPTERA) FROM CHOLISTAN DESERT

Muhammad Waseem^{1*}, Santosh Kumar^{1*} and Riffat Sultana²

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: dr.waseemcivas@gmail.com, santoshkumar@civas.edu.pk

The Scarab beetles, belonging to the Suborder Polyphaga and Family Scarabaeidae, are a significant group of beetles that have various structures ornamenting their large bodies. The goal of this study is to fill in the knowledge gaps about Scarabaeidae fauna. The specimens collected belonged to the 3 sub families, 6 genus and 6 species. The subfamily Scarabaeinae Latreille, 1802 contains genus *Onitis* is represented by the species *Onitis philemon* Fabricius, 1801, while *Scarabaeus* Linnaeus, 1758, is characterized by *Scarabaeus gangeticus* (Castelnau, 1840). *Ontherus* Erichson, 1847, is associated with the species *Ontherus aphodioides* (Mannerheim, 1829), Subfamily Melolonthinae Leach, 1819 consisted of the genus *Amblonoxia* Reitter, 1902, is represented by the species *Amblonoxia palpalis* (Horn, 1880) and genus *Phyllophaga* Harris, 1827, includes the species *Phyllophaga micans*, Leach 1801. In the subfamily Dynastinae MacLeay, 1819, the genus *Pentodon* Hope, 1837, is identified with the species *Pentodon algerinus* (Fuessly 1788). These taxonomic details offer a comprehensive insight into the specific beetles inhabiting the Cholistan desert, contributing to a deeper understanding of the region's Scarabaeidae biodiversity.

FIRST INVENTORY OF CONOCEPHALINE (TETTIGONIIDAE: TETTIGONIDEA: ENSIFERA) FAUNA IN BAHAWALPUR DIVISION

Muhammad Irfan^{1*}, Santosh Kumar¹ and Riffat Sultana²

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: irfanabbasi0062@gmail.com, santoshkumar@civas.edu.pk

Subfamily Conocephalinae, described by Kirby & Spence in 1826 and commonly known as meadow katydids, belongs to the phytophagous insects known as Tettigoniodea. Some species within this subfamily are significant pests of agricultural crops, while many others are ecologically associated with forest biocenoses, causing damage to trees, flowers, fruit orchards, berry herbs, shrubs, and grasses. This research aims to create the first comprehensive catalog of Conocephaline fauna in Bahawalpur Division. The study involves identifying different species, understanding their distribution, and potentially exploring their behavior and habitats. The identified species include *Euconocephalus incertus*, Walker 1869, *E. pallidus*, Redtenbacher, 1891, and *E. indicus* (Redtenbacher, 1891).

A COMPREHENSIVE STUDY ON OVIPOSITION BEHAVIOR IN ACRIDIDAE (CALIFERA: ORTHOPTERA)

Muhammad Zain ul Abedin¹, Santosh Kumar¹ and Riffat Sultana²

¹*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

²*Department of Zoology, University of Sindh, Jamshoro*

*Corresponding Author: jamzain037@gmail.com, santoshkumar@cuvas.edu.pk

The family Acrididae poses a threat to numerous crops, including cotton, wheat, rice, sugarcane, maize, and millets, among others. In the current study, species will be collected from various fields and then brought to the laboratory for rearing. The following observations were noted: morphological description of egg-pods and arrangement of eggs in the pods. Additionally, sexual maturity, pre-oviposition, oviposition, and fecundity of various collected species will be recorded from the date of the last molting. The morphological description of egg-pods proved to be very helpful in identifying pest species in the field. Throughout this study, evidence of relationships among various species, based on similarities and differences in egg patterns, has been investigated, yielding unique results. Egg pods used in this research were collected in two different ways: 1. Direct survey in the field by digging the soil to search for egg-pods. 2. Rearing of adult gravid females under laboratory conditions to obtain egg-pods. A detailed account of all oviposition behavior was presented in this study.

A STUDY OF ORTHOPTERAN DIVERSITY IN THE CHOLISTAN DESERT, BAHAWALPUR DIVISION

Sana Iqbal¹, Santosh Kumar¹, Riffat Sultana²* and Muhammad Mazhar Ayaz¹

¹*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

²*Department of Zoology, University of Sindh, Jamshoro*

*Corresponding Author: iqbalsana.uaf@gmail.com , santoshkumar@cuvas.edu.pk

Caelifera, commonly known as grasshoppers and locusts, are cosmopolitan insects inhabiting grasslands, meadows, and other open habitats. They are exclusively herbivorous and likely represent the oldest group of chewing herbivorous insects. A field survey was conducted to estimate the distribution pattern of Caelifera (Orthoptera) in the Cholistan Desert, Bahawalpur. Samples were collected using insect nets and brought to the laboratory. Mounting was performed according to the standard described method, and photographs were taken for visualization using a camera. Species identification was carried out with the assistance of relevant literature. This study aims to illuminate the faunistic pattern of Caelifera in the vast and distinctive desert ecosystem of Cholistan, playing a crucial role in the local food chain.

STUDY ON THE DIVERSITY OF BUSH CRICKETS (PHANEROPTERINAE: TETTIGONIIDAE) FROM DISTRICT MULTAN

Amna¹*, Santosh Kumar¹ and Riffat Sultana²

¹*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

²*Department of Zoology, University of Sindh, Jamshoro*

*Corresponding Author: amnakafeel66@gamil.com , santoshkumar@cuvas.edu.pk

During the present study, bush crickets were collected from six different habitats, including maize, sugarcane, cotton, mangoes, guavas, and vegetables, using various methods. The collected

specimens were identified as *Trigonocorypha unicolor* (Stoll, 1787), *Trigonocorypha angustata* Uvarov, 1922, *Phaneroptera spinosa* Bei-Bienko, 1954, *Phaneroptera roseate* Walker, 1869, and *Holochlora japonica* Brunner von Wattenwyl, 1878. The study revealed that cotton and sugarcane had the highest number of bush crickets collected, while mangoes and guavas had the lowest number. Additionally, *Phaneroptera roseate* Walker, 1869 exhibited the highest richness of all species in the cotton habitat, while *Holochlora japonica* Brunner von Wattenwyl, 1878 displayed the lowest richness in all habitats. Furthermore, *Trigonocorypha unicolor* (Stoll, 1787) and *Trigonocorypha angustata* Uvarov, 1922 showed a preference for moderate temperature and humidity. *Phaneroptera spinosa* Bei-Bienko, 1954 appeared less adapted or tolerant to abiotic factors. *Phaneroptera roseate* Walker, 1869 preferred high temperature and low humidity, while *Holochlora japonica* Brunner von Wattenwyl, 1878 exhibited a more specialized or restricted habitat preference. This study suggests that bush crickets play a diverse and crucial role in grassland ecosystems. Their distribution and population dynamics are influenced by various biotic and abiotic factors.

DIVERSITY OF SHORT-HORNED GRASSHOPPERS (ACRIDIDAE: ORTHOPTERA) IN MUZAFFARGARH

Shazia Batool¹, Santosh Kumar¹ and Riffat Sultana²

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: shazia6628@gmail.com , santoshkumar@cuvas.edu.pk

Grasshoppers, belonging to the suborder Caelifera within the order Orthoptera, encompass around 12,000 species distributed across 26 subfamilies globally. This study aimed to conduct a survey on the diversity and distribution of grasshoppers (Acrididae) in various agricultural zones of Muzaffargarh district, alongside identifying their food habits and host plants. A total of 286 specimens were collected, representing four species of Acrididae. Among these, *Acrida exaltata* (Walker, 1859) emerged as the most abundant, while other species included *Acrida turtica* (Linnaeus, 1758), *Truxalis gigantea* (Herbst, 1786) and *Aiolopus thalassinus* (Fabricius, 1798), indicating a diverse array of grasshopper species. Further, study is needed to explore its diversity.

EFFECT OF CLIMATE ON DISTRIBUTION OF OXYINAE (ACRIDIDAE: ORTHOPTERA) FROM HYDERABAD DISTRICT

Sadaf Soomro* and Riffat Sultana

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: sadafsoomro137@gmail.com, riffat.sultana@usindh.edu.pk

Due to favorable climatic conditions, Hyderabad division is highly diversified, and a variety of valuable crops are cultivated. An extensive survey was carried out to evaluate the distribution of Oxyinae in different cultivated and uncultivated areas of Hyderabad. From December 2021 to September 2023, a total of 911 specimens were captured and identified into 5 species: *Oxya hyla* (Serville, 1831), *O. velox* (Fabricius, 1787), *O. fuscovittata* (Marschall, 1836), *O. nitidula* (Walker, 1870), and *Oxyina sinobidentata* (Hollis, 1971), belonging to 2 genera, *Oxya* and *Oxyina*. Oxyinae grasshoppers have a survival temperature range between 18 °C to 36°C ± 1°C. Slight changes in temperature lead to an increase or decrease in their population and developmental stages. All these factors contribute to the expansion of the

geographical ranges of Oxyinae. Due to their migratory action and reproductive abilities, these hoppers are also considered major pests. Naturally, they are phytophagous, causing damage to valuable and economically important crops such as sugarcane, wheat, maize, cotton, and rice. During random data collection, it was observed that the maximum population of *Oxya hyla hyla* was 35.6%, followed by *O. velox* with 21%, *O. fuscovittata* with 14%, *O. nitidula* with 4%, and the minimum Oxyina sinobidentata with 1.2%. Additionally, we provide some major factors and climatic changes that highly affect the distribution of Oxyinae in the Hyderabad Division.

TAXONOMIC STUDY ON THE PYRGOMORPHINAE (PYROMORPHIDAE: CAELIFERA) IN SINDH, PAKISTAN

Seema Perveen Memon, Riffat Sultana* and Barkat Ali Bughio

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: riffat.sultana@usindh.edu.pk

The Pyrgomorphidae family is known for its vibrant colors among the Orthoptera fauna. Pyrgomorphs inhabit bushes, herbage, grasses, sedges, as well as soil and sand. While many species exhibit camouflage and adapt their body shapes to resemble twigs, grasses, leaves, etc., some are striking examples of aposematic coloration. The species within the Pyrgomorphidae family are considered major pests in various crop fields, including cotton, wheat, sugar cane, aak plant, cowpea, and maize. In the current study, a total of 287 specimens were collected from different localities in district Tando Muhammad Khan, Sindh Province, between July and November 2023. The collected material was categorized into five species belonging to four genera under the subfamily Pyrgomorphinae. These species are *Chrotogonus* (*Chrotogonus*) *trachypterus trachypterus* (Blanchard 1836), *Chrotogonus* (*Chrotogonus*) *homalodemus homalodemus* (Blanchard 1836), *Tenuitarsus orientalis* (Kevan, 1959), *Poekilocerus pictus* (Fabricius, 1775), and *Atractomorpha sinensis sinensi* (Bolivar, 1905). The genera *Chrotogonus* and *Tenuitarsus* belong to the tribe Chrotogonini Bolívar, 1904, while *Poekilocerus* is associated with the tribe Poekilocerini Burmeister, 1840, and *Atractomorpha* with the Tribe Atractomorphini. The analysis revealed that the highest ratio within the subfamily Pyrgomorphinae was observed in *C. (Chrotogonus) t. trachypterus* compared to other species.

CONSERVATION OF ORTHOPTERAN BIODIVERSITY FROM NARA DESERT

Versha Kumari*, Riffat Sultana* and Barkat Ali Bughio

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: versha.makhija@gmail.com , riffat.sultana@usindh.edu.pk

The Nara Desert, a unique arid ecosystem, is home to a diverse range of flora and fauna, with a particular emphasis on Orthopterans – a group of insects that includes grasshoppers, crickets, and locusts. These insects play a crucial role in maintaining ecological balance, serving as both prey for various predators and as pollinators for select plant species. However, the delicate balance of this biodiversity is increasingly threatened by anthropogenic activities and climate change. During the present study fair numbers of Acrididae Gryllidae Gryllotalpidae Pyrgomorphidae Tettigoniidae (orthoptera) were collected many of them have rare status some are declared endangered by IUCN in this study many groups of Orthoptera will be present along with their status so that conservation planning could be possible. As we

navigate the intricacies of Orthopteran populations in this study, we will uncover the specific groups present and their respective conservation statuses. This comprehensive exploration serves as a vital resource for conservationists, researchers, and policymakers alike, facilitating the development of effective strategies to safeguard the endangered Orthopteran species within the Nara Desert.

AN OBSERVATION OF FASCINATING BEHAVIORS IN PRAYING MANTIDS

Aneela Chandio* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: anilalibra83@gmail.com riffatsultana@usindh.edu.pk

Mantids are one of the imperative groups of predacious insects of diverse agro-ecosystems because of their preying capability entitled them as preying mantids. Mantids exhibit remarkable speed, flexibility, and agility, often blending into their surroundings with cryptic behaviors. Their flying ability, propelled by weaponized front legs, is astonishing, covering significant distances. While adept at perceiving movements up to sixty feet away, their efficiency diminishes at night, leading to most of their hunting during the day. Camouflage is a key survival strategy for insects, and mantids excel in this art. They mimic elements like leaves, sticks, flowers, and even bird droppings, becoming virtually invisible. Some species adapt to the arid season by molting into a dusky black color, blending seamlessly with post-brush fires. Notably, flower mantids use wild ornamentation to deceive unsuspecting insects seeking nectar. The Mantis religiosa species, a skilled predator, employs martial arts-like moves and specific colors, shapes, and behaviors for successful prey capture. Despite their exceptional skills, mantids often go unnoticed due to their sedentary and cryptic lifestyle, resembling twigs, flowers, and tree bark. Praying mantis diversity is observed during August and September, with effective camouflage making them elusive in other months. Occasional captures occur when perched on tree trunks, grasses, or twigs, offering glimpses into their fascinating lives in the natural world.

BIODIVERSITY ASSESSMENT OF GRASSHOPPERS (CAELIFERA: ORTHOPTERA) OF UPPER SINDH

Abdul Aziz Babar¹, Riffat Sultana^{1*} and Santosh Kuma²

¹*Department of Zoology, University of Sindh, Jamshoro*

²*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

*Corresponding Author: riffatsultana@usindh.edu.pk

During the present study about 1023 of specimens were collected and sorted as *Acrida willemsei*, Dirsh, 1954, *Oxya hyla* Serville, 1831, *Sphingonotus savignyi*, Saussure, 1884, *Truxalis eximia eximia*, Eichwald, 1830, were collected which belong to family Acrididae from the different habitats and host plants such as Alfalfa-losan (*Medicago sativa*), Barley (*Hordeum vulgare*), White bark acacia (*Acacia leucophloea*), Neem (*Azadirachta indica*), Desert acacia (*Acacia jacquemontii*), Kandi (*Prosopis cineraria*), Tamatisk (*Tamarix articulata*), Salvadora (*Salvadora oleoides*), Iron wood (*Tecomella undulata*), Indokh (*Anogeissus rotundifolia*) and Indian plum (*Ziziphus mauritiana*), Bermuda grass (*Cynodon dactylon*), Indian bdellium (*Commiphora wightii*) and Caperberry (*Capparis deciduas*), Ziziphus (*Ziziphus nummularia*), Euphorbia (*Euphorbia nerifolia*) and etc. at present seasonal distribution, taxonomic keys, measurements, color-morphism, seasonal occurrence, body size, morphological variations, zoogeographical distribution, natural habitats of species were recorded to note

actual pest status of species in the upper Sindh province. In addition to this, biodiversity Index, Simpson Index of Biodiversity and Species Richness were also done to know the diversity of species. Among the various taxon distance summaries within species, genus was noted with the lowest value distance was 0.3%, average mean distance was 1.18%, and distance variance 0% - 0.35% however, within families, it was noted that the minimum and maximum distances were both 0% - 0.5%, and SE distance 1.25% - 5.23% was analyzed maximum.

AN OBSERVATION ON ANT-HONEYDEW INTERACTION AMONG LEAFHOPPERS IN THARPARKAR DISTRICT"

Azra Soomro* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: soomroazranaz5@gmail.com, riffat.sultana@usindh.edu.pk

The Cicadellidae family, encompassing over 22,000 global species of leafhoppers and sharpshooters, exhibits diverse forms and colors, thriving in various environments, including deserts. Many species within this family secrete honeydew, a sugary substance that serves as a nutritional attractant for ants. This study, centered on the Tharparkar desert, reveals a direct correlation between honeydew production by Cicadellidae, the presence, and intensity of ants, influenced by climate conditions and leafhopper diversity. The Thar region's arid subtropical climate, characterized by temperature extremes and low rainfall, significantly impacts the peak agricultural season, fostering leafhopper activity and honeydew production. The dynamics of ant-hemipteran interactions are further nuanced by the types of ant species, distinguishing between invasive and native varieties. Invasive ants may adversely affect plant health, while native ants contribute positively by preventing issues such as sooty mold accumulation and deterring other herbivores attracted to honeydew. This study sheds light on the intricate relationships within this ecological system.

PRELIMINARY STUDY ON PAMPHAGIDAE IN PALAEARCTIC REGION OF PAKISTAN

Babul Mahar*, Riffat Sultana* and Barkat Ali Bughio

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: babulmahar786@gmail.com, riffatsultana@usindh.edu.pk

The present work aims to study the distribution of Pamphagidae in the Palaearctic regions of Pakistan. For this, a survey was initiated in many arid regions of Baluchistan, and we have succeeded in reporting its single species, *Eremopeza cinerascens aurantipes* (Uvarov, 1933). These insects inhabit dried, barren lands and rocks near hilly mountainous landscapes with sparse vegetation, characteristic of the Palaearctic regions. They are characterized by a remarkable sexual dimorphism, with males generally being winged, while females are apterous. Adults, especially females, rarely jump; they appear torpid and clumsy and can be easily captured by hand. The family Pamphagidae includes some remarkably spiny, well-camouflaged insects that closely resemble stones or branches, usually only discovered when they move. Some species resemble short, squat creatures, earning them the common name of "toad grasshoppers." They are diurnal, fast-moving, and well-developed herbivores. Their color and behavior play important roles in their recognition and survival in their habitat. They are widely distributed in the Palaearctic regions of Europe, Africa, and Asia, as well as some cold regions in the north and south. However, to gather more material and study additional species, further research is underway.

TAXONOMY OF DARKLING BEETLES (COLEOPTERA: TENEBRIONIDAE) IN LOWER SINDH, PAKISTAN

Farheen Deeba Soomro* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: farheensoomro23@gmail.com , riffat.sultana@usindh.edu.pk

Darkling beetles cause economic damage to several agricultural crops mostly on Wheat, Guava and Mustard due to their common prevalence. This study has been designed because still no detailed study carried out on taxonomy in lower Sindh, Pakistan. This research was done to investigate the taxonomic status of two sub-families Tenebrioninae (Latreille, 1802) and Pimeliinae (Latreille, 1802) of family Tenebrionidae. Species of subfamily Tenebrioninae and Pimeliinae were collected from Hyderabad, Tando jam, Jamshoro and Badin of lower Sindh with the help of pitfall trap, light trap, and hand-picking methods from 2018 to 2020. The most sources of collection for these species were the soil surface of diverse ranches, beneath the shaken and mountain regions, the stockpiles area/godown, and the soil surface of the Wilderness range. During the present study 300 individuals of Tenebrionidae were accumulated and classified into two subfamilies: Pimeliinae which consisted of *Trachyderma phalistinia* (Reiche and Saulcy, 1857), *Pimelia capito* (Krynicky, 1832), species, and Tenebrioninae which consisted of *Gonocephalum hispidocostatum* (Fairmaire, 1883), and *Pseudoblaps Javana* (Wiedemann, 1819) species. However, *Pimelia capito* (Krynicky 1832) discovered from desert and mountain areas and the abundance of *Trachyderma phalistinia* (Reiche and Saulcy, 1857) were found almost all region of lower Sindh. While, both species were recorded for the first time from Sindh province. It has been observed that several of the larger species were flightless and unable to do high flight. i.e., *Pseudoblaps Javana*. However, this object is under inspection and requires further research in the future.

STUDY ON SCELIO (SCELIONIDAE: HYMENOPTERA) PARASITIZATION ON EGGS OF SCHISTOCERCA GREGARIA (ORTHOPTERA: ACRIDIDAE)

Furqan Al Hussain* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: furqanalhussain28@gmail.com, riffatsultana@usindh.edu.pk

The desert locust, *Schistocerca gregaria*, is the most destructive migratory pest, continually causing extensive damage to large areas of cropland and pastures in many countries, including Pakistan, resulting in millions of dollars in annual losses. Currently, expensive chemical insecticides are being used to control desert locusts. However, due to the harmful effects of conventional insecticides on human health and the environment, as well as the emergence of insecticide-resistant insects, alternative pest management programs are needed. These programs should involve the use of natural enemies and pathogens to control pests. Among these alternatives, wasps Scelio, (Scelionid: Hymenoptera), play a crucial role in reducing the field and agricultural population of grasshoppers and locusts. These obligate endoparasitoids have a significant impact on the biocontrol of grasshopper eggs (Orthoptera: Acrididae). To reach their host eggs, females attach themselves firmly to female grasshoppers, allowing them to be transported to the oviposition sites of their hosts. Female Scelio oviposit into host eggs, primarily those located in the soil, and their progeny develop as internal parasitoids. The host egg becomes turgid, opaquer, and darker than unparasitized eggs. During pre-natal development, the host embryo is disturbed and killed by the second larval instar of the parasitoid. After the completion of the pupation period, adults emerge from the host egg by chewing an irregular hole. Once the parasitoid attains maturity, it becomes responsible for keeping

locust plagues in check. This study is financially supported by the Higher Education Commission, Islamabad, under the Research project (HEC NRP Research Project: No. 14787).

BIODIVERSITY OF ACRIDOMORPHA IN DIFFERENT VEGETATION IN MEHRANO WILDLIFE SANCTUARY, KHAIRPUR MIRS, SINDH

Muhammad Irfan Bozdar^{1*}, Riffat Sultana^{1*} and Fakhra Soomro²

¹*Department of Zoology, University of Sindh, Jamshoro*

²*Department of Zoology, Shah Abdul Latif University, Khairpur*

*Corresponding Author: irfanbozdar786@gmail.com, riffat.sultana@usindh.edu.pk

Mehrano forest is situated in the Khairpur District of the Pakistani province of Sindh, encompassing agricultural land, forests, and hunting areas, serving as a habitat for diverse flora and fauna. Despite its rich insect fauna, particularly Orthoptera, the area has remained largely unexplored, with no prior surveys conducted to document and unveil the hidden wealth of this desert ecosystem. Therefore, the present study aims to address this gap, employing fortnightly surveys to explore the orthopteran diversity within Mehrano. The survey results yielded a total of 580 specimens, categorized into three superfamilies: Acridoidea, Pyrgomorphae, and Tetrigoidea, corresponding to three families: Acrididae, Pyrgomorphidae, and Tetrigidae. Nine species were identified, including *Phlaeoba tenebrosa* (Walker, 187), *Locusta migratoria* (Linnaeus, 1758), *Heteracris littoralis* (Rambur, 1838), *Heteracris adspersa* (Redtenbacher, 1889), *Gonista bicolor* (Haan, 1842), *Atractomorpha acutipennis* (Guerin-Meneville, 1844), *Hedotettix gracilis* (Hann 1843), and *Paratettix cingalensis* (Walker, 1871). In addition to the taxonomic study, biodiversity indices such as the Simpson Index, Biodiversity Index, and Species Richness were employed to assess the variety of species present. The study also highlighted color morphism observed in several species. Furthermore, the collected data from various ecosystems and locations underwent a comprehensive analysis.

INCIDENCE OF SCELIO (SCELIONIDAE: HYMENOPTERA) ON LOCUST EGGS IN THE THAR DESERT SINDH

Jeram Das* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: jeramdas28@gmail.com, riffat.sultana@usindh.edu.pk

The genus *Scelio* (Latreille 1805) is known to occur worldwide as obligate endoparasitoids of the eggs of locusts and grasshoppers. They are considered important natural enemies, regulating populations of acridids in both agricultural and natural habitats. Locusts and grasshoppers are regarded as pests of crops and pastures. This study aims to investigate the occurrence and importance of *Scelio* species as biocontrol agents to combat locust and grasshopper pests in the Thar Desert. Various field trips were conducted to collect *Scelio* species from July 2022 to June 2023. During field surveys, 117 specimens of *Scelio* and 11 affected egg pods of locusts and grasshoppers were collected. It was observed that *Scelio* species had paralyzed and buried many locusts and grasshoppers in the soil. In the field, it was also noted that *Scelio* species were engaged in laying eggs in the eggs of locusts and grasshoppers. This study revealed that *Scelio* species are associated with a relatively spatially static declining host population in the field. Therefore, *Scelio* may be proposed as an excellent candidate with high biotic potential to combat the severe issue of locusts, as an alternative to conventional chemical control. Eco-friendly strategies for

insect-pest management should be considered, or targeted approaches can be adopted. This study also includes the generic diversity and DNA molecular characterization of *Scelio*, which is being planned to understand the potential applicability of this taxon in the near future.

FEEDING HABITS AND HABITAT PREFERENCES OF PHANEROPTERA BIVITTATA UNDER CONTROLLED LABORATORY CONDITIONS

Syeda Laraib* and Riffat Sultana

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: syedalarai542@gmail.com, riffat.sultana@usindh.edu.pk

The Phaneropterinae subfamily, known as bush katydids or shrub crickets, comprises a diverse and phytophagous group within the Ensifera order. Some species are pests that cause damage to both forest trees and agricultural crops. In our laboratory experiments, we focused on *Phaneroptera bivittata* (Bei-Bienko 1965) to assess its fecundity, fertility, and dietary preferences. A total of 113 Phaneropterinae individuals from various regions in the Hyderabad district were tested on different diets, including *Cynodon dactylon* (Bermuda grass), *Helianthus annuus* (Sunflower), *Oryza sativa* (Rice), *Pennisetum tergidum* (Desert grass), *Medicago sativa* (Alfalfa), *Sorghum vulgare* (Jowar), and *Poa tenella* (Grass). Among these, *Pennisetum tergidum* proved to be the most favorable diet, as individuals showed a preference for it, while other diets were either rejected or consumed in smaller quantities. This study provides valuable insights into the dietary preferences of *Phaneroptera bivittata*, offering potential applications in pest management, such as the implementation of crop rotation strategies to mitigate its impact on agricultural yields.

STUDY ON THE REPRODUCTIVE ACTIVITY AND LIFE CYCLE OF SCHIZODACTYLUS MONSTROUS (SCHIZODACTYLIDAE: ENSIFERA)

Maleeha Jamil and Riffat Sultana

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: maliha4848@gmail.com, riffat.sultana@usindh.edu.pk

Schizodactylidae members, commonly known as dune crickets or splay-footed crickets, are predominantly found in Asia and southern Africa, thriving in desert and sandy habitats. These insects exhibit primarily predatory behavior, with species displaying vigorous carnivorous tendencies. Besides this, in the genus *Schizodactylus*, energy utilization in female reproductive activities exceeds that of males, making them more cannibalistic in nature. Additionally, females are larger in size compared to their mates. Although female and male *Schizodactylus* exhibit morphological similarities, prominent reproductive structures have been observed upon dissection. Adult females were found to have a bunch of 23-28 eggs in their abdomen. To study the life cycle of these insects, weekly visits were arranged to the study sites to collect as many specimens as possible. The entire life cycle of these insects typically spans more than one year. This insect undergoes nine nymphal instar stages before becoming an adult, with each nymph stage being a burrow maker. Some nymphs were collected from the field, while most were captured from burrows at night. Capturing views from a boroscopy camera indicated that females hatched 20-23 eggs at a time. The first-hatched nymph feeds on the other eggs, and sometimes the female also nourishes herself with eggs. The grant received under the Indigenous Ph.D Fellowship 5000 Phase II from HEC vide NO: HEC(FD)/2022/14482 is highly acknowledged.

SYSTEMATIC STUDY OF HIEROGLYPHUS (HEMIACRIDIDAE: ACRIDIDAE: ORTHOPTERA) WITH THE DISCOVERY OF A NEW SPECIES FROM SINDH PAKISTAN

Muzamil Ali Jakhrani¹, Riffat Sultana¹, Santosh Kumar² and Naheed Balouch¹

¹Department of Zoology, University of Sindh, Jamshoro

²Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: muzamilali637@gmail.com, riffat.sultana@usindh.edu.pk

The study focuses on the species identification and classification of Hieroglyphus based on morphological and molecular characteristics. During the investigation, 1854 specimens were collected from different ecological habitats in Sindh, with a special focus on paddy crops and the banks of the Indus River basin due to their diverse habitats. The specimens were identified as five species, namely *H. oryzivorus* (Carl 1916), *H. banian* (Fabricius 1798), *H. nigrorepletus* (I. Bolivar 1912), *H. perpolita* (1932), and *H. concolor* (Walker, 1870). The last species was reported for the first time in Pakistan. Additionally, a new species was discovered: *H. sultanesis*. With these discoveries and a new report, Pakistan has become richer in the diversity of Hieroglyphus than other zoogeographical regions, hosting 7 species, similar to India. The *H. concolor* species is very closely related to the Indian species *H. oryzivorus*. These two species are separated from the rest by the shape of the posterior margin of the pronotum and the shape of the male cercus. However, these characteristics are not sufficiently important to warrant placing these species in separate genera. Investigation of the phallic complex also confirms that they all belong to the same genus, while *H. sultanesis* is identified as new to science.

A STUDY ON THE CAMOUFLAGE BEHAVIORAL TACTICS OF CICADAS (HEMIPTERA)

Nazish Gull Khattak¹, Riffat Sultana¹ and Imran Khatri²

¹Department of Zoology, University of Sindh, Jamshoro

²Department of Entomology, Sindh Agriculture University, Tandojam

*Corresponding Author: riffat.sultana@usindh.edu.pk

Cicadas are the most wondrous group of insects. They have unique characteristics. They are easily recognized by their loud noise. Besides this, cicada species have a great sense to detect danger from predators; 02 developed compound eyes and 03 ocelli help them to fly away from the predator. In adulthood, cicada species, for survival, can rely on different defense mechanisms. From the nymph stage, playing dead behavior is probably a carryover; however, it is not seen in all species of cicadas. Male Tibicen cicadas produce a loud squawk when startled or handled. However, it is unfortunate that this behavior is not exhibited in females because they lack tymbals. During this study, its defense mechanisms - predator foolhardiness, playing dead, alarm squawk, and mortality factors - were observed and discussed.

COMPARATIVE MORPHOLOGICAL ANALYSIS OF SPERMATHECA IN TWO GENERA OF OXYINAE (ACRIDIDAE: ORTHOPTERA)

Nuzhat Soomro* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: nuzhat.fatima2k12@gmail.com, riffatumer@hotmail.com

A comparative study on Spermatheca was carried out in six species of subfamily Oxyinae viz: *Oxya hyla* Serville, 1831, *O. velox* (Fabricius, 1787) and *O. fuscovittata* (Marschall, 1836) 01 new record *Oxya*

nitidula (Walker, 1870) , 01 sp. nov *Oxya Kashmorensis* and *Oxyina bidentata* Willemse, 1925. An attempt has been made to describe and illustrate the structures of spermatheca in *Oxya* and *Oxyina* species has been carried out. The structure of spermatheca in *Oxya* and *Oxyina* species was investigated microscopically. In *Oxya* species it is observed that spermatheca with long sinuous pre-apical and short sac reminiscent of apical diverticulum. spermatheca ends distally in an apical tube, long and slender and a pre-apical diverticulum in the form of curved sac which is bent inward at the tip. However, in *Oxya hyla* it was observed that spermatheca with apical diverticulum long, bearing a small protuberance as its apical one-fifth; pre-apical diverticulum broad and curved, thrice the width of apical diverticulum. In *Oxya velox* spermatheca medium size, apical diverticulum bent downwards and pre-apical diverticulum narrow, more or less straight and coiled anteriorly. Pre-apical diverticulum broadly tubular and bent, as long as apical diverticulum. In *Oxya fuscovitatta* spermatheca tube like rounded, short, apical diverticulum short and pre-apical diverticulum is double the size of apical diverticulum and forms an inverted 'L' shaped loop. While, in *Oxya nitidula* Spermatheca with apical diverticulum narrow slightly dilated apically, preapical diverticulum tubular. There is significant variation in the form of spermatheca occurring in the same species of this genus, while in sp.nov *Oxya Kashmorinses* spermatheca elongated. While in the species of genus *Oxyina* there is significant difference, In *Oxyina bidentata* spermatheca is curved.

HOST PLANT INFLUENCE ON MATING BEHAVIOR IN ATRACTOMORPHA SINESIS SINESIS (ORTHOPTERA: PYRGOMORPHIDAE)

Raheela Shah* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: raheelashah90@gmail.com , riffat.sultana@usindh.edu.pk

This study was designed to investigate the influence of host plants on the mating behavior of grasshoppers, aiming to elucidate the intricate interplay between plant-insect interactions and reproductive strategies. Grasshoppers are crucial components of terrestrial ecosystems, playing vital roles in nutrient cycling and serving as prey for numerous predators. The old-world tropics and subtropics are home to the easily recognized pyrgomorphid genus *Atractomorpha*, which is extensively spread. We conducted experiments with grasshoppers (*Atractomorpha sinesis sinesis*: Orthoptera, Pyrgomorphidae) to examine mating behavior. They feed exclusively on *M. sativa*; therefore, a considerably larger number of matings were observed on *M. sativa*. The copulation lasted much longer (54.28 ± 1.19) hrs. when fed on *S. bicolor*. The lifespan was significantly higher on *M. sativa* in both sexes (10.14 ± 1.12 ♂ and 13.0 ± 0.99 ♀) days. We believe that this finding contribute to a deeper understanding of the ecological dynamics within terrestrial ecosystems, emphasizing the importance of considering plant-insect interactions in the formulation of effective conservation and management strategies."

A STUDY ON THE REPRODUCTIVE ACTIVITIES OF FIELD CRICKETS (GRYLLIDAE: GRYLLOIDEA ENSIFERA)

Shamshad Ali Talpur* and Riffat Sultana

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: shamshadtalpur@gmail.com

The Gryllidae family encompasses over 2400 known species of cricket. Among these, the courtship rituals involve males "singing" by rubbing their forewings together, a behavior shared by various cricket

species. Interestingly, each species possesses its unique song, prompting females to exhibit a preferential response, directing their migration toward the source. This highlights the significant role of these acoustic signals in mate detection, potentially forming the basis for behavioral distinctions between species. Remarkably, at a rearing temperature of around 28 °C, many crickets, including *G. bimaculatus*, display the ability to lay eggs and breed throughout the year. Under these conditions, the reproductive cycle lasts between 40 and 50 days, with each female producing an impressive tally of over 1000 eggs throughout her lifetime.

STUDY ON THE REPRODUCTIVE BEHAVIOR OF *TRIGONOCORPHA UNICOLOR* (ENSIFERA: TETTIGONIIDAE) UNDER LABORATORY CONDITIONS

Muhammad Siddique Dayo* and Riffat Sultana*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: siddiquedayo@gmail.com, riffatumer2@gmail.com

Mating structures hold the interest of a broad spectrum of biologists because, in many taxa, these structures display incredible diversity and wide-ranging characteristics. Studying the biology of *Trigonocorpha unicolor* is crucial as it can offer insights into the ecological and evolutionary processes shaping the diversity of these insects. During the present study, *Trigonocorpha unicolor* was reared under laboratory conditions, and observations were made on copulation, oviposition, along with the examination of immature stages. Copulation duration was noted to range from 25 to 45 minutes in *Trigonocorpha unicolor*. Oviposition duration varied between 25 to 56 minutes, while the egg disposition ratio was recorded as 18 to 23. For hatching, the eggs were kept in incubation at 25±2°C. These species exhibit parental care, where the female may provide additional protection for the eggs. This can include covering the eggs with plant material or secreting substances that offer some level of protection against predators or environmental conditions. We believe that this study on *Trigonocorpha unicolor* underscores the importance of investigating these behaviors within a broader evolutionary context.

ORTHOPTERAN (ENSIFERA) RESPONSE TO MACROCLIMATE CHANGES

Surriya Sanam¹* and Riffat Sultana²*

¹*Centre for Agriculture and Bioscience International, Regional Bioscience Centre Pakistan*

²*Department of Zoology, University of Sindh, Jamshoro*

*Corresponding Author: suraiya.sanam@gmail.com, riffat.sultana@usindh.edu.pk

The climate has direct effect on insects through the precipitation, seasonal changes and temperature whereas indirectly, the climate can affect insects' presence, causes exploitation of disease-causing insect species hence their important characteristics i.e., predators, changes in profundity and its vegetation. Some characteristics such as insects' density and distribution may cause by the changes in climatic conditions. Orthopterans are considered as the good indicators that face the macroclimate changes in the environment. The variations caused by the change in global climate that have minor effects on the bodily and behavioral characteristics including variations in phenotypical characteristics of insects. Although, DNA barcode gives us strong evidence of identification as compare to the taxonomic identification regarding the fauna of Orthopteran species. DNA sequence showed us strong evidence of variation in Orthopteran species, rather we identified them as a species of other group on the basis of morphological characteristics. Behavioral changes like color variation in morphs is an attribute caused by ecological changes in

association which forms the similarity among orthopteran species. Macroclimate changes may cause an increase result in thermophilic species.

SPATIO-TEMPORAL VARIATION IN ABUNDANCE AND DIVERSITY OF NECROPHAGOUS INSECTS IN BAHAWALPUR, PAKISTAN

Haseeb Ahmed Lalika*

*Department of Entomology, Faculty of Agriculture and Environment,
The Islamia University of Bahawalpur*

*Corresponding Author: haseebahmedlalika@gmail.com

Necrophagous insects are important in the decomposition of cadavers. This study was performed to monitor variation and biodiversity of necrophagous insects in Cholistan area of district Bahawalpur. Spatio-temporal variability and preferences of baits were studied by performing a survey on the diversity of necrophagous species in three types of landscapes; planted forests, agriculture and desert. Adult flies were collected by using suspended bottle traps (carion traps) installed 1.5-meter high from ground surface in different study areas at least 100 meter apart. These traps contained decomposing animal tissues (chicken liver and chicken meat) as bait. A diverse assemblage of necrophagous was recorded in all landscapes i.e., 10 species in 5 families; Calliphoridae, Fanniidae, Muscidae, Phoridae and Sarcophagidae. These families were identified under stereo microscope. Calliphoridae and Sarcophagidae were the most abundant families. The forest and agricultural landscapes had highest species richness and abundance. The types of baits did not significantly influence the number of species captured. Calliphoridae was the characteristic feature of desert whereas Sarcophagidae were the most abundant in agricultural landscape.

SYSTEMATIC STATUS OF DRAGON FLY (*ANISOTEPA*) BELONGS TO (INSECT: ANISOPTERA FAMILY) DISTRICT HYDERABAD, SINDH, PAKISTAN

Aisha Alladino, Bakhtawar Soomro*, Shakeel Ahmed Memon and Saheefa Soomro

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

Dragonflies are fascinating creatures that captivate our imaginations with their delicate beauty and unique behaviors. These enchanting insects belong to the order Odonata and are known for their striking colors, intricate wing patterns, and agile flight. Dragonflies have been around for millions of years and can be found in various habitats around the world, from ponds and lakes to forests and meadows. With their large compound eyes, dragonflies have exceptional vision, allowing them to spot prey and predators with remarkable accuracy. During the present studies, dragonflies were collected from various localities of District Hyderabad and brought to the laboratory of Government College, University, Hyderabad for examination and identification. The morphological identification of dragonflies revealed the occurrence of nine (09) species.

PRESERVATION AND ECOLOGY OF GRAY WALL JUMPER (*MENEMERUS BIVITTATUS*) IN DISTRICT HYDERABAD SINDH PAKISTAN

Saheefa Soomro, Najis Fatima, Bakhtawar Soomro*, Shakeel Ahmed Memon, and Iqra

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: bakhtawarsoomro145@gmail.com

Gray Wall jumper (*Menemerus bivittatus*) is a spider belongs to the *Salticidae* family commonly known as the Jumping spiders. It is usually found on the walls of buildings or on tree trunks where it stalks its prey, mainly flies and small insects. Grey wall jumper is commonly found on the walls of home. During the present studies, 10 species were recorded which are preserved in the laboratory, Department of Zoology, Government College, University, Hyderabad. This study was held to know the ecology. During this study, physical and biological parameters were recorded. Therefore, this species would be focused for the further research.

BEEKEEPING IN PUNJAB: PROBLEMS & SOLUTIONS

Muhammad Rizwan

Beekeeping and Hill Fruit Pests Research Station, Shamsabad, Rawalpindi

*Corresponding Author: ranarizwanjabbar@yahoo.com, entobkrwp@yahoo.com

This study was designed to formulate a road map and policy for beekeeping promotion to enhance honey production in Punjab, Pakistan. All aspects were studied and stakeholders were interacted to find out the constraints and formulate policy to minimize these limitations to improve beekeeping business in Punjab. The data were collected from beekeepers, science community involved in beekeeping, and stakeholders involved in sale, import and export of honey. The main aspects discussed are beekeepers, trainings, market, technical, and environment. It was suggested to address low productive & non-productive hives, IPM training to beekeepers including soft and hard adulteration, low return of investment, problems in apiary movement, data assemblage of beekeepers of Punjab, and minimal financial support. Training to beekeepers on IPM on biotechnical, soft and hard chemical approaches for insect pests and diseases management. Adulteration includes soft and hard adulteration. Different tests are accepted at local and international level for adulteration testing. Owing to reduced flora, competition has increased for nectar collection that has put a pressure on honey production. There is no policy regarding placement of bee farm and maintenance of distance among farms. Moreover, pollen is being imported from Iran and China to feed bee colonies in pollen dearth season. Wax foundation sheet is prepared manually and pore size is not uniform which develops more drone population. Moreover, pure bee wax sheet is not common and paraffin mixed wax sheets are at large in the local market which do not provide optimal conditions for colony working. The genetic vigor of the lastly imported *Apis mellifera ligustica* has not been maintained and improved with the passage of time. So, it is very important to import fresh colonies of the *A. mellifera* to replace less productive strain at beekeepers' farm.

ENTOMOPHAGY, AN EMERGING TREND TO COPE WITH FOOD SHORTAGE

Rana Zain Khizar*, Saba Iftikhar, Hafiza Aliza Sajjad and Muhammad Ahmad

Department of Entomology, Institute of Plant Protection, Muhammad Nawaz Sharif University of Agriculture, Multan

*Corresponding Author: zaynzko@gmail.com

World population has been increasing day by day and may reach 9.7 billion in 2050 according to the United Nations. According to World Food Program, almost 828 million people go to bed hungry every night. Moreover, during the COVID-19 pandemic, millions of people in the world faced a severe food shortage. According to the Global Report on Food Crisis, 135 million people were affected by food insecurity in 55 different countries, of which 36 were from Africa. In 2020, 78 million people were unable to afford a good diet in Asia. Efforts are required to find alternate food sources that are not only sustainable but also compete with the global food shortage. According to the World Research Institute, by 2050, there will be a 50% increase in global food demand, with a 70% increase in demand for meals derived from animals. Farmers need to produce almost 60% more crop calories and use 593 million hectares more land in order to meet the rising demand for food. Scientists have suggested that replacing at least 25% of the protein in livestock with alternative, more sustainable sources of protein could allow for the reforestation of agricultural land and cut greenhouse gas emissions from agriculture by at least 4%. Entomophagy, the practice of eating insects, is an emerging trend in many countries. It is estimated that between 25-50% of Native American populations in the United States eat insects. Insects are becoming more and more popular as alternate sources for the production of food and feed because of their high fecundity, short life cycle, and, more recently, health benefits. The primary components of insect-based diets include protein, which includes all essential amino acids, lipids, chitin, and minerals such as magnesium, calcium, potassium, and phosphorus. However, legislation pertaining to food is a hurdle when it comes to edible insects because sufficient scientific validation is required to resolve safety concerns before market approval is granted. Legislative regulations controlling their manufacturing and commercialization must also be established, as heat-resistant thiaminase, found in the pupae of African silkworms (*Anaphe* spp.), has been linked to multiple occurrences of thiamin insufficiency in Nigeria. Moreover, chitin present in the insect exoskeleton needs to be a concern as it links with urinary stone formation in some individuals if not properly digested. Besides all this, entomophagy proves an alternative protein source in situations of severe food insecurity.

FIRST REPORT OF FEATHER MITES GENUS *TROUESSARTIA* (ACARI: ANALGOIDAE) FROM SINDH, PAKISTAN WITH NEW HOST ASSOCIATION

Shaila Khaskheli, Saima Naz and Aziz Ahmed Ujjan

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: Shaila.khaskheli@scholars.usindh.edu.pk

Advanced Parasitology Research Laboratory, Department of Zoology, University of Sindh, Jamshoro-70608, Sindh, Pakistan Institute of Plant Sciences, University of Sindh, Jamshoro-70608, Sindh, Pakistan During the present investigation, the parasitic mites from Jungle Babbler, *Argya striatasindiana* Dumont, 1823 (Passeriformes: Leiothrichidae) were collected from Hyderabad, Sindh, Pakistan. A total of 300 specimens of mites of genus *Treoussartia* were recovered from 11 hosts, with the prevalence of 36.6%. Previously, *T. Pygoceras* were reported from various hosts of the Passeriformes and Pelecaniformes; at present it was isolated from *A. striatasindiana* for the first time with a new locality record.

SPATIO-TEMPORAL BEHAVIOUR OF *BOMBUS SIMILLIMUS* (HYMENOPTERA: APIDAE) FROM TOLIPIR NATIONAL PARK, AZAD JAMMU AND KASHMIR, PAKISTAN

Umer Ayyaz Aslam Sheikh*, Junaid Rahim and Muhammad Imran

Department of Entomology, University of Poonch Rawalakot, AJK

*Corresponding Author: umerayaz@upr.edu.pk

From the last decades use of commercial bumblebee species for crop pollination in managed cropping systems is increasing and on other hand *Bombus* pollinators are in decline, becoming endangered species around the globe. *Bombus simillimus* is native bumblebee species of Azad Kashmir and Northern Pakistan and this study was designed to explore their ecological behavior like seasonal distribution, habitat preference and floral host range from Tolipir National Park Azad Jammu and Kashmir, Pakistan. Results explore that hibernating queens of *B. simillimus* emerged in April and start of May, workers recorded from July to last of September and sexual (male and daughter queens) were found in September and October. Maximum population was observed in August and there was no population from November to March. Queens of *B. simillimus* mostly preferred the forest boundaries for nesting place and their least preference was forest area to build their nests. Individuals of *B. simillimus* were recorded on 33 floral host plants during the foraging activities and Asteraceae plant family found with maximum foraging plants of *B. simillimus*. This study will be helpful in domestication of this bumblebee species for crop pollination as alternative to imported species. It will also helpful to develop conservation strategies for this important native bumblebee pollinator in future.

EXPLORING ANTIBIOTIC RESISTANCE IN *ESCHERICHIA COLI* ISOLATES FROM FISH AND ENVIRONMENTAL SOURCES IN TWO PUNJAB DISTRICTS, PAKISTAN

Shahzad Ali^{1*}, Nauman Faisal¹ and Anam Iftikhar²

¹Wildlife Epidemiology and Molecular Microbiology Laboratory (One Health Research Group), Discipline of Zoology, Department of Wildlife & Ecology, University of Veterinary and Animal Sciences, Lahore, Ravi Campus, Pattoki

²Department of Biological Sciences, University of Veterinary and Animal Sciences, Lahore, Ravi Campus, Pattoki

*Corresponding Author: shahzad.ali@uvas.edu.pk

Escherichia (E.) coli belongs to the bacterial family known as Enterobacteriaceae and is primarily found in the intestines of animals, fish, and humans. This bacterium has the capability to transfer antibiotic resistance genes to other bacteria. The presence of *E. coli* in fish intended for human consumption is a matter of significant concern. To investigate the prevalence of *E. coli* and its antibiotic resistance patterns, we collected a total of 96 fish samples and 48 environmental samples (water) from various markets and farms in the Lahore and Kasur districts of Punjab, Pakistan. Swabs from both the fish and the environment were utilized for *E. coli* isolation. Isolation and identification of *E. coli* were performed using MacConkey agar and Eosin methylene blue agar (EMB). Bacterial identification involved observing colony morphology on different media and conducting biochemical tests following standard procedures. Antibiotic sensitivity testing was conducted through the disc diffusion method on Muller Hinton agar, employing various antibiotics. The zone of inhibition was measured, and observations were made to determine whether the isolated strains were susceptible or resistant to each antibiotic. The prevalence of *E. coli* in different fish and water samples was 43 (45%) and 15 (31%), respectively. Out of the 57 isolates from fish, 19 (33%) were obtained from the intestine, 17 (29%) from the environment, and 21 (36%) from

the muscles of different fish species. All 57 isolates were confirmed as *E. coli*, exhibiting gram-negative, catalase-positive, coagulase-negative, and oxidase-negative characteristics. The colonies were smooth and rounded, and the isolates appeared as gram-negative rods with a pink color after gram staining. On MacConkey agar, they displayed a pink color, and on Eosin methylene blue agar, a green color with a metallic sheen. Regarding antibiotic resistance in fish isolates, the highest rates were observed against Penicillin (88%), tobramycin (77%), ampicillin, and cefuroxime (77%), followed by Ampicillin and Ceftriaxone (61%), and Amikacin (44%). In the case of *E. coli* isolates from water, the highest rates of antibiotic resistance were against Tobramycin (66%), followed by Penicillin (50%), Ampicillin (50%), Amikacin (44%), and Ceftriaxone (38%). The prevalence of *E. coli* in fish and water samples from Punjab, Pakistan, raises concerns about its potential impact on human consumption. The identified antibiotic resistance patterns, particularly against commonly used antibiotics like Penicillin and Tobramycin, underscore the urgent need for monitoring and intervention in the food and environmental sectors to mitigate public health risks.

DIVERSITY AND SPATIAL DISTRIBUTION OF TICKS COLLECTED FROM WILD ANIMALS IN PUNJAB PAKISTAN

Ahmad Hassan, Shahzad Ali* and Muhammad Rizwan

¹*Wildlife Epidemiology and Molecular Microbiology Laboratory (One Health Research Group), Discipline of Zoology, Department of Wildlife & Ecology, University of Veterinary and Animal Sciences, Lahore, Ravi Campus, Pattoki*

²*Department of Biological Sciences, University of Veterinary and Animal Sciences, Lahore, Ravi Campus, Pattoki*

*Corresponding Author: shahzad.ali@uvas.edu.pk

A total of 320 wild animals were captured for tick examination, comprising 200 black rats, 70 long-eared hedgehogs, and 50 Indian monitor lizards. Among them, black rats showed no tick infestation, while 25.7% of long-eared hedgehogs and 30% of Indian monitor lizards were found to be infested with ticks. Gender analysis revealed insignificant differences, with a chi-square value of 0.59 ($p > 0.05$) between males and females. However, when considering age, juvenile animals exhibited a higher infestation rate at 46.7%, whereas adults and young animals had significantly lower infestation rates. Body condition was also found to have a significant impact on tick infestation. At the tehsil level, Bahawalpur exhibited the highest infestation rate at 17.6%, followed by Okara (12.5%), Chichawatni (12.1%), Arifwala (10.9%), Depalpur (10.3%), Renala (8.3%), and Sahiwal (4.2%) (Table 14). A total of 281 ticks were collected from wildlife animals, including 160 *Amblyomma gervaisi* ticks (66.2% male, 33.8% female) from Indian monitor lizards and 121 *Rhipicephalus turanicus* ticks from long-eared hedgehogs (69.4% male, 30.6% female) (Table 15). Additionally, 50 tick samples were collected from the environment using methods such as dragging, hand picking, and dry ice. Among these samples, 52% were identified as *Hyalomma anatolicum*, 40% as *Rhipicephalus microplus*, and 8% as *Rhipicephalus turanicus*. Regarding the spatial distribution of *Rhipicephalus turanicus* in long-eared hedgehogs, a prevalence of this tick species was observed in all individuals captured across four districts. A similar pattern was noted in the spatial distribution of *Amblyomma gervaisi* ticks infesting Indian monitor lizards, as depicted in Figures 59 and 60. In the case of ticks collected from the environment, the spatial distribution of *Hyalomma anatolicum* was evident across all four districts, while *Rhipicephalus microplus* was found in only three districts, specifically Sahiwal, Okara, and Bahawalpur. *Rhipicephalus turanicus*, on the other hand, was exclusively observed in district Pakpattan.

**POLYSTYRENE BIODEGRADATION BY *ALPHITOBIOUS DIAPERINUS* (PANZER)
(TENEBRIONIDAE: COLEOPTERA)**

M. Shakeel Abbas and Unsar Naeem Ullah

*Department of Entomology, Institute of Plant Protection, Muhammad Nawaz Sharif University of
Agriculture, Multan*

*Corresponding Author: shakeelabbas278@gmail.com

Polystyrene is a synthetic polymer used in the packing of lab instruments and electronic accessories. Polystyrene is persistent in nature and survive long period of time due to hardness, hydrophobic nature and chemical composition, and causes pollution in the environment. The lesser mealworm, *Alphitobius diaperinus* is a cosmopolitan stored products pest in many countries and used in the biodegradation of polystyrene. It is a vector and competent reservoir of several poultry pathogens and parasites. It is also used as food for humans, poultry, dead or sick birds and fish. in many countries. Various studies revealed that temperature has important role in the development of lesser mealworm. In controlled environmental conditions commercial level rearing of mealworm is beneficial for its culture. These results showed that in the treatment that contains packing polystyrene, pigeon diet and utensil polystyrene high amount of feeding was observed in packing polystyrene and utensil polystyrene. No role of bacteria was observed in degradation of polystyrene. Mostly consumption was done on packing polystyrene and pigeon diet while there was minimum consumption observed on utensil polystyrene. There was also cannibalism observed in packing polystyrene while in utensil polystyrene and pigeon diet least cannibalism was observed. Highest pupation was observed in packing polystyrene as compared to other diets. This study reveals that *A. diaperinus* reduced the economic value of poultry houses. Residues on poultry product cause diseases in human beings. The larvae of lesser mealworm have potential to degrade polystyrene without any harmful effect on environment. It was observed that there was no difference in antibiotic treated and untreated population of lesser mealworm.

**STUDIES ON THE EFFECTIVENESS OF SELECTED TRAPS FOR INSECTS DIVERSITY AND
DISTRIBUTION IN TEHSIL BAFFA PAKHAL, MANSEHRA**

Bilal Ahmad*, Zakir Ullah, Shehzad Ghayyur and Rehan Ullah

Hazara University, Mansehra

*Corresponding Author: bilalsw33@gmail.com

The present research work was conducted to investigate the effectiveness of Pit Fall, Flight interception and Light trapping system for insect diversity and distribution in Tehsil Baffa Pakhal, District Mansehra K P. The surveys for insect trapping was carried out from April 2022 to September 2022. There were three were trapping system used in four selected localities i.e. Hazara University, Baffa, Dhodial and Shinkiari. The application of trapping system for insect study is the first documented reports in Tehsil Pakhal, District Mansehra. A total of 553 specimens were trapped and identified and yielded into 15 families, 21 Genera and 26 species. *Parcoblatta Americana* (Scudder, 1900), *Pheropsophus africanus* (Dejean, 1825), *Scarities subterraneus* (Fabricius, 1775), *Stromatium barbatum* (Fabricius, 1775), *Anegleis cardoni* (Say, 1824), *Adalia decempunctata* (Linnaeus, 1758), *Hybosorus orientalis* (Westwood, 1845), *Mylabris phalerata* (Pallas, 1781), *Mylabris pustulata* (Thunberg, 1821), *Adoretus ictericus* (Laporte, 1840), *Anomala bengalensis* (Blanchard, 1851), *Copris repertus* (Walker, 1858), *Heteronychus artor* (Fabricius, 1775), *Musca domestica* (Linnaeus, 1758), *Eurydema ornate*

(Linnaeus, 1758), *Apis florea* (Fabricus, 1787), *Apis mellifera* (Linnaeus, 1758), *Camponotus vagus* (Scopoli, 1763), *Polistes olivaceus* (DeGeer, 1773), *Polistes rothneyi* (Cameron, 1900), *Polistes wattii* (Cameron, 1900), *Polistini canadensis* (Linnaeus, 1758), *Vespa oreintalis* (Linnaeus, 1771), *Vespa tropica* (Linnaeus, 1758), *Junonia coenia* (Hubner, 1822) and *Gryllotalpa orientalis* (Burmeister, 1838) were trapped during the present study. The resulted orders Coleoptera and Hymenoptera were found most abundant in respect of number of specimens. Whereas orders Diptera Hemiptera Lepidoptera and Orthoptera were yielded less abundant regarding number of specimens. Flight interception trap was found more effective than pit fall and light trap, light trap was resulted more effective than pit fall trap and less effective than flight interception trap. These trapping systems potentially may be used for pest control.

CYROMAZINE AFFECTS BIOLOGY OF *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE)

Hafiz Azhar Ali Khan

Institute of Zoology, University of the Punjab, Lahore

*Corresponding Author: azhar.iags@pu.edu.pk

Cyromazine is a triazine insect growth regulator insecticide that is recommended for control of *Musca domestica* worldwide. Cyromazine is highly effective in causing mortality of *M. domestica*; however, some aspects of its lethal and sublethal effects on the biology of *M. domestica* are still unknown. The present study explored lethal and sublethal effects on several biological traits and population parameters of *M. domestica*. Concentration–response bioassays of cyromazine against third-instar larvae of *M. domestica* exhibited sublethal and lethal effects from concentrations of 0.03 (LC₁₀), 0.06 (LC₂₅), and 0.14 (LC₅₀) µg/g of a larval medium. Exposure of *M. domestica* larvae to these concentrations resulted in reduced fecundity, survival, longevity and oviposition period, and delayed development of immature stages (i.e., egg hatch time and larval and pupal durations) in the upcoming generation of *M. domestica*. The values of population parameters such as intrinsic rate of increase, finite rate of increase, net reproductive rate, age-specific survival rate and fecundity, and age–stage life expectancy and reproductive value, analyzed using the age–stage and two-sex life table theory, were significantly reduced in a concentration-dependent manner in comparison with the control group. In conclusion, the study highlights the significant effects of cyromazine on the biology of *M. domestica* that could help suppress its population in cases of severe infestations.

KEY TO THE SPECIES OF SCHIZODACTYLUS BRULLE, 1835 (SCHIZODACTYLINAE: SCHIZODACTYLIDAE: ENSIFERA: ORTHOPTERA), OCCURRING IN THE RIVER INDUS, SINDH, PAKISTAN

Sabir Ali Channa and Muhammad Saeed Wagan

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: channasabir61@gmail.com

Specimens of remarkable genus *Schizodactylus* Brulle, 1835 were collected 6 km from the Jamshoro by pass on the left bank of River Indus near Sahrish Nagar, Hussainabad, and Latifabad, No.4 Hyderabad, Sindh, during 2022-2023. The material was collected on moderate slopes or on the level in deep layers of loose and fine sand, with or without thin semi-desert vegetation, or in places to which layers of fine soil were deposited by wind. The collected material was sorted out into four species namely: *Schizodactylus monstrosus* (Drury 1773), *Schizodactylus minor* Ander (1938), *Schizodactylus hesperus* B.Bienko 1967

and *Schizodactylus sindhensis* Channa and Wagan 2022. A simplified taxonomic key based on the easily recognizable characters is presented. Such as the structure of male sub genital plate, titillators epiproct, spurs of hind tibia and body size. Photographs and illustrations are also provided.

NOTES ON SPECIES OF THE GENUS *LAMPRIGERA* MOTSCHULSKY (COLEOPTERA: LAMPYRIDAE) FROM PAKISTAN

Zubair Ahmed

Department of Zoology, Federal Urdu University of Arts, Sciences & Technology, Karachi

*Corresponding Author: Zbrahmed36@gmail.com

The Himalaya-Karakorum region is predominant of firefly genus *Lamprigera* Motschulsky which extended to South East Asia, with 17 species of the genus described from that of area. One of species described from Chitral, KPK, Pakistan with close allied *L. minor* (Olivier, 1885) on emphasis of body structure (pronotum coloration with disc and circum); elytral shoulder dilation, light organs as well ventrites and male genitalia. The species assigned here as *Lamprigera chitralensis* sp.n with extension range of Palaearctic series of mountain.

EUCALYPTUS OIL EFFECTS ON HONEY BEE'S COLONY

Rubina Mushtaq¹, Sohail Khan¹, Sobia Khwaja¹, Ambreen Akram¹, Aasia Karim²

¹*Department of Zoology, Federal Urdu University of Arts Science and Technology, Karachi*

³*Department of Zoology, Sardar Bahadur Khan Women University, Quetta*

*Corresponding Author: rubinamushtaq@fuuast.edu.pk

Aromatic herbs and oils now provide considerable benefits to farm animal growth performance, digestive system management, and disease resistance. Many synthetic medications are used in beekeeping to combat bee illnesses and pests. Medicinal aromatic herbs and oils with documented positive properties are utilized in beekeeping to address the aforementioned concerns naturally. Several studies have found that thyme, clove, mint, lemongrass, cinnamon, grapefruit, rosemary, marigold, laurel, eucalyptus, and tea tree essential oils and herbs have fatal effects on mites, germs, and fungus. Aromatic oils have no harmful effects on bee colonies and have no detrimental impact on colony formation when applied in proper dosages. Furthermore, the use of medicinal aromatic herbs and oils in beekeeping promotes organic production while reducing residual issues in bee products (honey, beeswax, and so on).

SAND FLIES AVAILABILITY WITH DOMESTICATED AND UN-DOMESTIC ANIMALS

Syed Haroon Masood Bokhari* and Unsar Naeem-Ullah

*Institute of Plant Protection, Department of Entomology,
Muhammad Nawaz Shareef University of Agriculture, Multan*

*Corresponding Author: haroonbukhari0@gmail.com

One of the insects that poses a threat to human life is found in the Diptera order of insects. More than a thousand species of sandflies belonging to various genera have been identified as of the documented record. However, within the described species, species belonging to *Phlebotomus* and *Sergentomyia*

genera are recognized to cause public human diseases including Cutaneous, Visceral and Mucocutaneous Leishmaniasis. Leishmaniasis is present in over 98 countries in both the Old and New Worlds. Therefore, it is crucial to understand what biotic elements contribute to the propagation of the deadly vector. As a result, the study was planned, and sandflies were attracted by randomly placing A4-sized sticky notes on the chosen locations. The results demonstrated that domesticated and un-domesticated animals play its important role. As of results, it was depicted that Mongoose was harboring more of the sand flies as compared to other animals.

DIVERSITY AND DISTRIBUTION OF TWO SLAVE-MAKING ANTS, *FORMICA SANGUINEA* LATREILLE, 1798 AND *F. TRUNCORUM*, FABRICIUS, 1804 FROM PAKISTAN

Abdul Hafeez Soomro, Ghulam Mujtaba Lakho and Imran Khatri*

Department of Entomology, Sindh Agriculture University Tandojam

*Corresponding Author: imrankhatri.agri@gmail.com, ikhatri@sau.edu.pk

Ants (Hymenoptera: The suborder Formicidae) are very important in terrestrial ecosystems shaping soil conditions, assisting in seed dispersal and pest control. The genus *Formica* Linnaeus, 1758, contains a large number of species that can have various ecological roles. In fact, this one is a widely distributed genus in almost every temperature area where it can be commonly found in many parts. This big Holarctic clade accounts for many well-known species, such as 279 living ones and 68 fossil ones. They are also called timber ants, mound ants, thatching ants and ground ants. It is a fragmented taxonomy into many smaller subdivisions comprising both subgenera and species groups. For the purpose of the current study, collections of ants from various areas and habitats within Pakistan were carried out using a number of techniques including aspirator, baiting, pitfall trapping, leaf litter sifting subterranean sampling as well as beating low vegetation. The ants were collected during day or night in accordance with the caste of ants involved. Specimens of *Formica sanguinea* Latreille, 1798 and *F. truncorum* Fabricius, 1804 were collected and identified from various localities in Pakistan, covering three zoogeographical regions: Palearctic, Ethiopian, and Oriental. There were significant differences in morphology, coloration and pubescence of both species; also there were notable distinctions in their preferences of habitats and the habits of nesting. The distribution maps and high-quality photographs of these two species are also offered so that the diversity and biogeography of these insect groups in Pakistan can be comprehensively compared.

CAMPONOTUS ANGUSTICOLLIS (JERDON, 1851) AND *C. SYLVATICUS* BASALIS SMITH, 1878: TWO ANT SPECIES WITH DIFFERENT DISTRIBUTION PATTERNS AND ECOLOGICAL NICHES IN PAKISTAN

Ali Raza Bhatti, Ghulam Mujtaba Lakho and Imran Khatri*

Department of Entomology, Sindh Agriculture University, Tandojam

*Corresponding Author: imrankhatri.agri@gmail.com, ikhatri@sau.edu.pk

Ants are insects from the Formicidae family are beneficial insects in ecosystems, having a broad spectrum of species and playing different ecological functions. The genus *Camponotus* Mayr, 1861 is recognized for its carpenter ants. It has a wide morphological and ecological variety at the global level. *Camponotus* is a genus which is very large and complex with more than 1000 species and nearly 500 subspecies of them distributed in about 45 subgenera. Revisionary studies on *Camponotus* are usually carried out either in the context of species groups or specific geographical regions because these ants occupy great

numbers and types of habitats. The genus poses a real threat for biological characterization due to its high areas of occurrence, with nests built inside the ground, rotten branches or twigs and sometimes living wood (Bolton 1973). Most species are highly generalist feeders. The taxonomy of *Camponotus* species is intricate due to the remarkable diversity of these species, considerable intraspecific and geographic variation and polymorphism. In this study, we collected ants of this genus from different regions and habitats in Pakistan using different techniques such as the aspirator, baiting, pitfall trapping, leaf litter sifting subterranean sampling and beating low vegetation. Depending on the caste, day or night collection activities were carried out. In the present paper we provide diagnosis of two species *Camponotus angusticollis* (Jerdon, 1851) and *Camponotus sylvaticus basalis* Smith, 1878 from various localities of Pakistan. We also observed significant differences in the appearance, coloration, hairiness, habitat preferences and nesting habits of these two species. The study features high-resolution images and distribution maps of these species, which allows for a comprehensive comparison of their diversity and biogeography in Pakistan.

RECOGNITION AND DIVERSIFICATION TWO ANT SPECIES, *LASIUS ALIENUS* FOERSTER, 1850 AND *L. BRUNNEUS*, FOERSTER, 1850 IN PAKISTAN

Asad Ali Gil, Ghulam Mujtaba Lakho and Imran Khatri*

Department of Entomology, Sindh Agriculture University Tandojam

*Corresponding Author: imrankhatri.agri@gmail.com, ikhatri@sau.edu.pk

Ant genus *Lasius* Fabricius, 1804 is a well-known group, but since the eighteenth century, especially in Reaumur's time Wheeler 1926, it has received much attention to its spectacular nuptial flights. It has also been highlighted as one of the rare ant genus that have remained largely unaltered since the longer period. Currently representing 133 known species, the *Lasius* group has a large Holarctic range comprising northern Scandinavia, the Baikal region of Siberia, North Africa and Iraq's north up to the Himalayas in southern areas. *Lasius* is limited by temperate vegetation at higher elevations and they are typified as common Holarctic ant genera (Wilson 1955). We further put forward the Himalayas as another hub of diversity for this genus. The *Lasius* includes species adapted to an underground mode of life, living under the bark and in trees. In this research, we attempted to collect the ants from different areas and habitats all over Pakistan through various methods such as aspirator use, baiting; pitfall trapping; leaf litter raking test; soil sampling, and beating of low growth vegetation. Collections were made both in the day and the night, again based on what caste of ant was involved studies will be presented on two different species of *Lasius alienus* Foerster, 1850, and *Lasius brunneus* Foerster, 1850. It was noticed during morphology that there were certain distinctions in both the species. Morphological images of both species necessary for their identification would be presented, and distributional map of studied species is generated for Pakistan.

MORPHOLOGICAL CHARACTERS AND DISTRIBUTION PATTERNS OF *MONOMORIUM INDICUM* FOREL, 1902 AND *M. LONGI* FOREL, 1902, (HYMENOPTERA: FORMICIDAE: MYRMICINAE): TWO INVASIVE ANT SPECIES, IN PAKISTAN

Ghulam Mujtaba Lakho¹, Imran Khatri^{1*} and Zubair Ahmed²

¹*Department of Entomology, Sindh Agriculture University Tandojam*

²*Department of Zoology, Federal Urdu University of Arts, Science & Technology, Karachi*

*Corresponding Author: imrankhatri.agri@gmail.com, ikhatri@sau.edu.pk

The subfamily Myrmicinae (Hymenoptera: Formicidae) encompasses a diverse and ecologically significant array of ants. Myrmicine ants exhibit considerable size variation, ranging from 1 mm to 10

mm. Their ecological diversity is also notable, with species serving as generalist predators, specializing in specific soft-bodied invertebrates like Collembola, or playing a key role as seed harvesters. *Monomorium*, a large and highly diverse genus with around 400 species, approximately half of its species in the Afrotropical region. Predominantly inhabiting the Old World, especially the tropics, *Monomorium* workers display remarkable morphological diversity between groups. Despite being globally successful species, little is known about the natural history of most *Monomorium* species. Although feeding habits appear generally generalist, some species are granivorous (Ettershank, 1966). In this study, ants were gathered from diverse regions and habitats across Pakistan utilizing various methods such as an aspirator, baiting, pitfall trapping, leaf litter sifting, subterranean sampling, and beating low vegetation. Collection activities occurred during either the day or night based on the caste of the ants. Samples of *Monomorium indicum* Forel, 1902, and *M. longi* Forel, 1902 were acquired and identified from various locations of Pakistan, spanning three zoogeographical regions: Palearctic, Ethiopian, and Oriental. Substantial variations were noted in the morphology, coloration, and pubescence of these two species, along with differences in their habitat preferences and nesting behaviors. The provision of high-quality images and distribution maps facilitates a thorough examination of their diversity and biogeography in Pakistan.

BARCODING THE ANT FAUNA OF PAKISTAN: CHALLENGES AND OPPORTUNITIES

Syeda Maira Jafery, Ghulam Mujtaba Lakho and Imran Khatri*

Department of Entomology, Sindh Agriculture University Tandojam

*Corresponding Author: imrankhatri.agri@gmail.com, ikhatri@sau.edu.pk

Ants being one of the most diverse and ecologically significant groups of insects, still have major gaps in taxonomy and biogeography knowledge especially in countries like Pakistan. This study examines the possibilities and obstacles of barcoding, an identification technique based on DNA for organisms, in determining the ant fauna of Pakistan. Barcoding provides opportunities for species discovery, biodiversity assessment and ecological tracking. As such, barcoding in Pakistan is benefited by the revelation of ant diversity, clarification of taxonomic uncertainties. Secondly, during our journey for Bar coding of ants we encountered challenges and limitations, which are to be addressed here. It requires a significant number of resources necessitating funding equipment personnel and time, which most times in Pakistan is very scarce or unavailable. Facing difficulties in conducting extensive and representative ant sampling throughout Pakistan due to diverse landscapes, political and security issues as well as limited access and permits. Challenge of standardized process for obtaining, preserving, processing, sequencing ant specimens because different methods and materials have been used. Dealing with the lack of a well-equipped and updated reference database of ant barcodes due to few studies conducted on the ants of Pakistan. Suffers from inability to interpret and validate barcode data owing to intraspecific variation, interspecific similarity in ants. Facilitates impediments in collaboration and communication among stakeholders, such as researchers, taxonomists, curators, policy makers and local communities due to their lack of coordination, cooperation and trust. Barcoding the ant fauna of Pakistan is a promising potential supplement to the current taxonomy, serving as an opportunity for further enhancing our knowledge about the diversity and distribution of ants. However, there are also challenges that need to be addressed in order to make a meaningful contribution towards scientific knowledge, conservation efforts and awareness at the societal level in Pakistan.

INSECT DIAGNOSIS THROUGH TIME: PAST, PRESENT, AND FUTURE TRENDS**Imran Khatri***Department of Entomology, Sindh Agriculture University Tandojam*

*Corresponding Author: imrankhatri.agri@gmail.com, ikhatri@sau.edu.pk

Insect diagnosis is the process of identifying and classifying insects based on their characteristics. It has evolved over four eras of entomology: classical, modern, molecular, and digital. In this study, we review the history and development of insect diagnosis methods and techniques, from Aristotle's *Historia Animalia* to the latest 3D models of insects. One of the earliest surviving insect diagnosis and classifications of the natural world is that of Aristotle (384–322 BC). Aristotle's *Historia Animalium* is the earliest surviving work in which a comprehensive organization of insects is to be found, he described bees, wasps, ants, butterflies, flies and beetles. In seventeenth century Carl von Linné known as the "father of modern taxonomy" Published a book *Systema Naturae* in 1758 and is still the acceptable format for the use of scientific names commonly known as binomial nomenclature. Edward Baker in 1878 first reported the value of the concealed male genital structure in differentiating species, thus the method followed by subsequent workers is now well established and is the basis of modern Taxonomy. Paul Hebert, researcher in 2003 at the University of Guelph in Ontario, Canada, proposed "DNA barcoding" as a way to identify species. Rather, DNA barcoding can serve a dual purpose as a new tool in the taxonomists toolbox supplementing their knowledge as well as being an innovative device for nonexperts who need to make a quick identification. The field of artificial intelligence has advanced rapidly in the last ten years, and the use of digitization for object detection has enabled the creation of auto id platform for insect diagnosis, which many modern agricultural forms utilize. The audience will learn about the history, current state, and future outlook of insect diagnosis.

**IDENTITY OF BIG-EYED BUG *GEOCORIS OCHROPTERUS* (FIEBER, 1844)
FROM TANDOJAM, SINDH PAKISTAN**

Abida Parveen Soomro¹, Imran Khatri^{1*} and Zubair Ahmed²¹*Department of Entomology, Sindh Agriculture University, Tandojam*²*Department of Zoology, Federal Urdu University of Arts Science & Technology, Karachi*

*Corresponding Author: imrankhatri.agri@gmail.com, ikhatri@sau.edu.pk

Big-eyed bugs (*Geocoris* spp.) are important predators of various pests, especially thrips, in many crops. In this study, we report the occurrence and identity of *Geocoris ochropterus* (Fieber, 1844) from Tandojam, Sindh, Pakistan. The genus *Geocoris* Fallén, 1814 with its 147 valid species is the largest and most diverse taxon of subfamily Geocorinae (Hemiptera: Heteroptera: Lygaeoidea). Representatives of the genus are distributed in most of the biogeographic regions with warm and moderate climates. This species is one of the most widely distributed and economically significant members of the genus in the Oriental region. Big-eyed bugs are small, oblong to oval shaped; head broader than long, prominent eyes, curve backward, overlap the front of the pronotum; stylus with a longitudinal groove; very short or absent claval commissure. This study contributes valuable insights into the taxonomy and characteristics of *G. ochropterus*, enhancing our understanding of its distribution and ecological significance in the given region. We provide detailed morphological descriptions and illustrations of the male and female specimens collected from different crops. We also discuss the distribution and taxonomic status of *G. ochropterus* and its related species in the subfamily Geocorinae. Our findings contribute to the knowledge of the diversity and ecology of big-eyed bugs in South Asia.

OCCURRENCE AND DESCRIPTION OF *LEPISIOTA SERICEA* (FOREL, 1892) IN PAKISTAN

Masaud Ali Gandahi, Ghulam Mujtaba Lakho and Imran Khatri*

Department of Entomology, Sindh Agriculture University Tandojam

*Corresponding Author: imrankhatri.agri@gmail.com, ikhatri@sau.edu.pk

This study focuses on the occurrence and description of *Lepisiota sericea* (Forel, 1892) in Pakistan. The ant genus *Lepisiota*, Santschi, 1926 belonging to the Formicinae subfamily, comprises 88 described species and 47 valid subspecies, distributed across the southern Palearctic, Afrotropical, and Indomalayan regions. Members of this genus are known for their generalist foraging behavior, *Lepisiota* species inhabit diverse environments, including decayed wood, soil, and living trees where they attend to sap-sucking sternorrhynchan insects. The genus is characterized by antennae with 11 segments, well-developed eyes, and distinct features on the mesosoma and petiole, *Lepisiota* ants exhibit a unique morphology. Collections for this study were conducted in various regions and habitats in Pakistan using multiple techniques such as aspirator, baiting, pitfall trapping, leaf litter sifting, subterranean sampling, and beating low vegetation. The specimens of *L. sericea* were specifically identified from different localities in Pakistan. The study aims to provide distribution maps and high-quality photographs of *L. sericea*, offering insights into the diversity and biogeography of this ant species group in Pakistan. The audience will gain a comprehensive understanding of the occurrence and characteristics of *L. sericea*, contributing to the broader knowledge of ant fauna in the region.

LUCILIA CAESAR (LINNAEUS, 1758): A BLOW FLY SPECIES WITH DIVERSE OCCURRENCE PATTERNS IN PAKISTAN

Hira Mannan, Imran Khatri* and Bhai Khan Solangi

Department of Entomology, Sindh Agriculture University, Tandojam

*Corresponding Author: imrankhatri.agri@gmail.com, ikhatri@sau.edu.pk

The common greenbottle is the popular name for the blow fly species *Lucilia caesar* (Linnaeus, 1758), a member of the Calliphoridae family. This study investigates the various distribution patterns of *L. caesar* in Pakistan. Adult *L. caesars* are distributed in Europe, Asia, and North Africa, and their larvae typically feed on pollen and nectar. The gathering of anthophilous fly specimens from diverse flowers in Tando Jam and its neighbouring areas was part of our research. These specimens were methodically identified at SAU Tandojam's Insect Systematics Laboratory, Department of Entomology. *L. caesar* has a striking look, usually as a gleaming metallic green. This study adds to our understanding of *L. caesar*'s ecological and geographical dynamics in Pakistan, offering light on its adaptability and prevalence in a variety of habitats.

EXPLORING THE TAXONOMIC DIVERSITY AND GEOGRAPHIC DISTRIBUTION OF CRICKETS (ORTHOPTERA: GRYLLIDAE) IN JACOBABAD DISTRICT, SINDH, PAKISTAN

Afaque Ahmed Sarki*, Waheed Ali Panhwar and Naheed Baloch

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: afaqueahmedofficial@gmail.com

A vast array of crickets inhabits our world, showcasing remarkable diversity influenced by several key factors. One pivotal factor is their role as significant pests affecting agricultural crops, vegetables,

ornamental plants, and floor or soil-stored grains, as well as household items. Collectively known as crickets, insects belonging to the Gryllidae family exhibit a relatively flattened and smaller size, with rounded vertex fastigium and smaller, triangular ocelli. Their subquadrate pronotum, long spines, and light hairs on the hind femora contribute to their distinctive features, along with a needle-like ovipositor and somewhat cylindrically elongated cerci. Field crickets maintain an omnivorous diet, with some displaying herbivorous tendencies, feeding on fruits, young leaves, and flowering plants. Those residing in gardens prefer grasses, leaves, plant shoots, and seeds. In this study, a total of 104 specimens were collected, revealing six genera and seven species, including *Miogryllus itaqueinisi*, *Acheta domesticus*, *Acheta supplicans*, *Gryllus bimaculatus*, *Acheta hispanicus*, *Grylloides siggilatus*, and *Callogryllus ovilongus*. *Miogryllus itaqueinisi* was initially discovered in Pakistan. *Acheta supplicans* exhibited the highest reported population at 22%, followed by *Grylloides siggilatus* at 19.2%, and *Callogryllus ovilongus* at 14.4%. *Miogryllus itaqueinisi* displayed the lowest population at 6.7%, succeeded by *Gryllus bimaculatus* at 7.6%, and *Acheta hispanicus* at 12.5%. The study includes species descriptions and digital images, providing a comprehensive reference for future research endeavors. The outcomes of this investigation stand as a valuable benchmark for subsequent studies in the field.

PRELIMINARY STUDY ON BEES (HYMENOPTERA) FROM VICINITY OF LARKANA

Ashfaq Abdul Khalique, Waheed Ali Panhwar and Naheed Baloch

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: achandio140@gmail.com

The wasps, ants, bees, and sawflies are all members of the order Hymenoptera. roughly 150,000 currently existing species and roughly 2,000 extinct species are known to exist in the Hymenoptera order. In nature, many organisms are parasitic. Typically, females deposit their eggs into hosts or other inaccessible locations using a special ovipositor. Hymenoptera are divided into two groups: the Symphyta, which have no waist, and the Apocrita, which have a narrow waist. Bees are most significant group of pollinators for agricultural and wild plants. Such as crop production of more than \$15 billion is achieved by the managed honey bee pollination, the commonly managed crop pollinator. Similarly, a wild bee contributes the worth of 3,251 US dollars per hectare to the agricultural production of crops throughout the world which is almost equivalent to managed honey bees. A total of 145 samples of bees were captured from the different localities of Larkana and sorted into five species, single family Apidae, and three tribe Apini, Anthophorini, Xylocopini under three genera i-e: *Apis* Linnaeus, 1758, *Amegilla* Friese, 1897 and *Xylocopa* Latreille, 1802. Beside this, description of species and digital images are provided.

TAXONOMY OF TENEBROINIDAE (COLEOPTERA) FROM DISTRICT SUKKUR, SINDH

**Abdul Manan Shaikh¹ Badar Alam Samejo², Waheed Ali Panhwar³,
Fakhra Soomro² and Sajjad Ali Larik²**

²*Department of Zoology, Government College University Hyderabad Sindh*

¹*Department of Zoology, Shah Abdul Latif University Khairpur Mirs Sindh*

³*Department of Zoology, University of Sindh, Jamshoro*

*Corresponding Author: dr.manan.shaikh@gcu.edu.pk

Darkling beetles are the popular name for members of the Tenebrionidae family. Following Staphylinidae, Carabidae, Curculionidae, and Chrysomelidae, they are one of the top five largest families. It has 96 tribes and between 18000 to 20,000 species divided into 10 subfamilies. Tenebrio, which means

"one who loves darkness" in Latin, is the etymological source of the family name Tenebrionidae. Darkling beetle species are nocturnal in both their natural environment and behaviour. Tenebrionids are classified as omnivore, herbivorous, carnivorous, and detritivores in accordance with their dietary habits. Identification of darkling beetles is crucial for pest control since they are a pest organism of the family Tenebrionidae that harms the economy. In the current study, 210 specimens from the district of Sukkur were divided into 7 species and 05 genera, all of which belong to the family Tenebrionidae. The species were, *Tribolium destructor* (Uyttenboogaart, 1934), *Tribolium confusum* (Jacquelin, 1863), *Tribolium castenum* (Herbst 1797), *Tenebrio molitor* (Linnaeus, 1758), *Alphitophagus bifasciatus* (Say, 1824), *Thriptera kraatzi* (Haag-Rutenberg, 1876) and *Trachyderma hispida* (Forsk. 1775). The three species in the genus *Tribolium* with the largest population were *Tribolium confusum* (Jacquelin, 1863) (28.57%), *Tribolium confusum*, and *Tribolium destructor*, whereas *Thriptera kraatzi* had the lowest population at 0.47%. In addition, descriptions of genera and species, as well as digital photos, were offered to make species identification simple. The current study is a proactive step towards increasing the diversification of the darkling beetle fauna in the Sukkur district.

INVESTIGATING THE DIVERSITY OF ACRIDIDAE (ORTHOPTERA) FROM HYDERABAD AND ITS ADJOINING AREAS

Naheed Baloch

Department of Zoology, University of Sindh, Jamshoro

Corresponding Author: naheed.baloch@usindh.edu.pk

Grasshoppers exhibit a widespread distribution across diverse ecological systems, holding significant economic importance due to their detrimental impact on various types of green vegetation. In the course of this investigation, an extensive survey was conducted to assess the prevalence of grasshoppers within the family Acrididae in Hyderabad and its adjacent areas. The findings revealed the presence of 25 grasshopper species, representing 10 genera and five sub-families, namely Oedipodinae, Acridinae, Hemiacridinae, Gomphocerinae, and Truxalinae. The study underscored the dominance of the Acridinae subfamily, followed by Oedipodinae and Gomphocerinae, while Truxalinae and Hemiacridinae were identified as the least common subfamilies in the surveyed localities of Hyderabad and its surroundings. Furthermore, a systematic examination was conducted on the collected material from diverse habitats and locations. The acquisition of Acridid fauna from various habitats and host plants enhances the material's scientific value for future references. Comprehensive identification keys were developed, incorporating easily distinguishable morphological features and detailed illustrations of concealed genitalia. These keys cover the various subfamilies, genera, and species of Acrididae, complemented by distribution maps for a more thorough understanding.

STUDIES ON THE IMMATURE STAGES OF ACRIDINAE (ORTHOPTERA: ACRIDIDAE) FROM SINDH, PAKISTAN

Sajjad Ali Larik^{1*}, Riffat Sultana², Imran Khatri³, Waheed Ali Panhwar², Fakhra Soomro¹

¹*Department of Zoology, Shah Abdul Latif University Khairpur Mirs, Sindh*

²*Department of Zoology, University of Sindh, Jamshoro*

³*Sindh Agriculture University, Tandojam*

*Corresponding Author: sajjad.larik@salu.edu.pk

The economically significant crops suffer severe damage from acridid hoppers. The Acridinae family of short-horned grasshoppers is widely distributed in Sindh. In the world, including Pakistan, the slant

hoppers appeared to be a pest of rice, sugarcane, wheat, maize, cotton, and various types of cereals, vegetables, orchards, and pastures. They are herbivorous by nature. These insects are active during the day. Their expanding population obliterates healthy agricultural crops. Due to their inability to use their wings and their incessant consumption of crops, hoppers appear to be more widespread than adults. These hoppers are typically found along neighbouring sides of waterways, in fields, marshy areas, wet pastures, and on common grasses. As a consequence of this work, 8067 adults and nymphs in total were gathered and divided into two genera. *Acrida* and *Truxalis* is a member of the Acridinae subfamily, which also includes the species *Acrida gigantea* Herbest 1786, *Acrida exaltata* Walker 1859, *Truxalis fitzgeraldi* Drish 1951, and *Truxalis examia examia* Eichward 1830. The collected material was taken to the lab and preserved using a standardized entomological procedure. Identification keys for images, drawing lines, measurement tables for various body parameters of numerous species, as well as host plants, were provided. In this study, the occurrence of different nymphal stages was emphasized from the field, in addition to the preferred host plants of different species, and it was discovered that nymphs eat more as compared to adults.

WHISPERS IN THE DUNES: UNDERSTANDING THE DIVERSITY AND POPULATION PATTERNS OF ORTHOPTERA FAUNA IN NARA DESERT, SINDH, PAKISTAN

Waheed Ali Panhwar*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: waheed.panhwar@usindh.edu.pk

Orthoptera is a large order of insects found in grasslands. Orthopterans are found all over the world in different physiographic zones, and their frequency is impacted by the plantations found in meadows, forests, and fields. In grassland environments, Orthoptera especially katydids, crickets, and grasshoppers are essential herbivores that play a major role in the food chain of many other creatures, including birds, spiders, and reptiles. They so have a crucial role in both the ecosystem and the economy. Herbivorous in nature, they serve as a critical element in food webs, supplying a rich supply of sustenance to a range of species such as lizards, raptors, and birds. The ecological systems of Sindh are made up of a variety of ecosystems, including marshes, savannas, agricultural areas, and dry areas. It is commonly acknowledged that orthoptera significantly contribute to the biodiversity of grasslands and agricultural areas. Orthoptera are of special relevance while researching semi-natural grasslands for a variety of reasons. From March 2019 to March 2022, a study on the diversity of Orthoptera from the Nara desert was carried out. The study's field sites included Nara proper, Choondiko, Khehwari, Kot-jabo, and Sikandarabad. The soil types at these locations varied, with some having sandy, loamy soil and others having medium-fertile soil that was used for cultivation. In the present study, a total of 23 species were identified, including *Duroniella laticornis* (Krauss, 1909) *Truxalis eximia eximia* Eichwald, 1830, *Acrida exaltata* (Walker, 1859), *Acrida gigantea* (Herbst, 1786), *Anacridium rubrispinum* Bey-Bienko, 1948, *Anacridium aegyptium* (Linnaeus, 1764) *Heteracris littoralis* (Rambur, 1838), *Acrotylus humbertianus* Saussure *Spathosternum prasiniferum* (Walker, 1871), 1884, *Aiolopus thalassinus thalassinus* (Fabricius, 1781), *Acrotylus longipes longipes* (Charpentier, 1845), *Schistocerca gregaria* (Forskål, 1775), *Oxya velox* (Fabricius, 1787), *Cyrtacanthacris tatarica tatarica* (Linnaeus, 1758), *Gryllodes supplicans* (Walker, 1859) *Gryllodes sigillatus* (Walker, 1869), *Acheta domesticus* Linnaeus, 1758, *Gryllus bimaculatus* De Geer, 1773 and *Turanogryllus histrio* Saussure, 1877 *Turanogryllus lateralis* Fieber in 1853, *Conocephalus* (Anisoptera) *maculatus* (Le Guillou, 1841), *Eucrietix montanus* (Hancock, 1912) and *Bolivaritettix nilgircus* (Hebard, 1930), have been recorded. In addition to an in-depth examination of the genitalia, this work includes a comprehensive overview of species descriptions and a corresponding species identification guide. Additionally, effect of climate change on population of Orthoptera has been documented.

TAXONOMY AND DISTRIBUTION OF GENUS *MELOLONTHA* FABRICIUS 1775 IN SINDH, PAKISTAN

Mehtab Ali Mahar^{1*}, Waheed Ali Panhwar² and Abdul Manan Shaikh³

¹*Department of Zoology, Shah Abdul Latif University, Khairpur Mirs*

²*Department of Zoology, University of Sindh, Jamshoro*

³*Department of Zoology, Government College University, Hyderabad*

*Corresponding Author: mehtabmahar87@gmail.com, waheed.panhwar@usindh.edu.pk

The current definition of the family Scarabaeidae includes more than 30,000 species of beetles from all over the world that are commonly referred to as scarabs or scarab beetles. There have been a lot of shifts in thinking about how to categorize this family in recent years. Scarabaeid beetles are an important pest of both farm and wild vegetation. The fauna of beetles in Sindh Province was surveyed and collected from a variety of habitats. *Melolontha indica* Hope, 1831 and *Melolontha fuscicauda* Ancy, 1881 were two of the 46 specimens collected and grouped into the Genus *Melolontha* Fabricius, 1775 of the subfamily Melolonthinae. In addition, the presence of *Melolontha fuscicauda* in Sindh Province of Pakistan is recorded for the first time, and a new regional record for *M. indica* in Pakistan is built. In addition, for the first time, digital photos and a description of the species' range are supplied. Future studies interested in this population will hopefully benefit from the solid groundwork provided by the present study.

FROM TAXA TO TRACKS: NAVIGATING ORTHOPTERA RESEARCH IN PAKISTAN FOR CONSERVATION INSIGHTS

Samiullah Soomro* and Riffat Sultana

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: samiqau373@gmail.com

With an emphasis on four main areas: taxonomy, diversity, ecology, and genomics—this study provides a comprehensive assessment of the state of orthopterology research in Pakistan today. Using a variety of sources, we have assembled an extensive library of research publications to evaluate the depth of studies conducted in each area. After the data is classified, a quantitative study of the proportion of research in the orthopteran order's taxonomy, diversity, ecology, and genomics may be done. Prioritizing topics that need further investigation and identifying research gaps are the main goals. With this model, we want to shed light on the existing state of knowledge on orthopterology in Pakistan by drawing lines on the distribution of research efforts. This will hopefully reveal trends that guide future study efforts and contribute to the conservation of orthopteran species. The results of this study have important ramifications for Pakistan's biodiversity protection. We may direct future research to fill in important knowledge gaps by identifying regions with less study coverage. The suggested model acts as a road map for successful conservation initiatives in addition to facilitating a comprehensive understanding of orthopteran species. This model supports an all-encompassing strategy to protect Pakistan's rich orthopteran diversity by encouraging cooperation between scientists, politicians, and conservationists. Ultimately, this model contributes to worldwide efforts to conserve biodiversity.

MORPHOLOGICAL AND MOLECULAR ANALYSIS OF WEEVILS (CURCULIONIDAE) FROM KHAIRPUR, SINDH

Shahnoor Suhriani¹, Waheed Ali Panhwar^{2*}, Abdul Manan Shaikh³ and Sajjad Ali Larik¹

¹Department of Zoology, Shah Abdul Latif University, Khairpur Mirs

²Department of Zoology, University of Sindh, Jamshoro

³Department of Zoology, Government College University, Hyderabad

*Corresponding Author: waheed.panhwar@usindh.edu.pk

The order Coleoptera, which includes beetles, is the biggest group of insects. Weevils are members of the family Curculionidae. Weevils are a common pest that feeds on vegetation that is otherwise healthy. Since 1980, the most common pests of date palms in the Khairpur district have been scale insects and red palm weevils. Khairpur's date palm industry is crucial to the city's economy. Numerous additional weevils are a common grain storage pest. They pose a threat to stored grains like corn, wheat, and rice. A great many beetle species are still undiscovered. Distinguishing these pests molecularly and identifying them morphologically is necessary for using the control strategies to halt their spread. The goals of this study are to (1) identify and assess the weevil fauna of the Khairpur area of Sindh, Pakistan, and (2) investigate the morphological and molecular variations between different species of weevils. The research was conducted at Shah Abdul Latif University (SALU), Khairpur, at the Entomology Laboratory within the Department of Zoology. Over the course of 2019–2022, researchers collected 1018 specimens from the family Curculionidae at various sites across the Khairpur district. The samples were characterized morphologically and genetically to determine their identities. *Rhynchophorus ferrugineus*, *Sitophilus oryzae*, *Sitophilus zeamais*, *Sitophilus granarius*, *Tanymacus khaipuresnsis* **sp.nov**, *Pachyrhinus lethierryi*, *Sphenophorus parvulus*, *Phylobius pomaceus* and *Phylobius ferrugineus* were also among the species identified. Three of these species are new to science: *Pachyrhinus lethierryi*, *Sphenophorus parvulus* and *Phylobius pomaceus*. One species, *Tanymacus khaipuresnsis* **sp. nov** is being proposed to the scientific community as a new species. Furthermore, molecular evidence supports the existence of species variation and suggests additional species.

TAXONOMY OF VESPIDAE (HYMENOPTERA) FROM SHIKARPUR, SINDH, PAKISTAN

Shah Rukh Soomro^{1*}, Waheed Ali Panhwar², Abdul Manan Shaikh³,
Fakhra Soomro¹ and Sajjad Ali Larik¹

¹Department of Zoology, Shah Abdul Latif University Khairpur Mirs

²Department of Zoology, University of Sindh, Jamshoro

³Department of Zoology, Government College University, Hyderabad

*Corresponding Author: srksoom65@gmail.com, waheed.panhwar@usindh.edu.pk

Wasps are members of the family Vespidae. The family Vespidae, which is part of the order Hymenoptera and contains more than 5,500 described species, is divided among 250 genera and six subfamilies. Vespidae (wasps) are beneficial in terrestrial ecosystems due to the predatory nature of their larvae on caterpillars and other insects. These wasps play a crucial role in the pollination of many different types of fruit trees and vegetable crops. Most of their homes can be found in woods, farms, and orchards. Wasps are well-known for their importance in global ecosystems; in fact, some species are utilized as insecticides in agriculture and beekeeping. This research examined the varied wasp fauna in the Shikarpur Sindh area of Pakistan. The samples were from four different talukas in the Shikarpur area. Approximately 236 samples were taken and analyzed. There was a total of eight different species named:

the *Vespa orientalis* *Polistes indicus* *P. wattii* *P. olivaceous* *P. flavus* *P. associus* *Delta dimidiatipenne* and *D. pyriforme*. Four of these species are new records for Sindh Pakistan: the *Polistes flavus* *Polistes associus* *Delta dimidiatipenne* and *Delta pyriforme*. While other species in the region are re-described. Most of the species are *P. flavus* (28.1%), then *P. wattii* (25.8%), and *P. indicus* (18.2%). *D. pyriforme* has the lowest population at 1.2%, followed by *P. associus* at 1.6% and *D. dimidiatipenne* at 2.1%. In addition, we provide digital photos and morphological features. Without a doubt, this research will serve as a benchmark for scholars to come.

**DIVERSITY OF GENUS *SCOPULA* SCHRANK, 1802
(LEPIDOPTERA: GEOMETRIDAE) FROM DISTRICT JAMSHORO, SINDH**

Zaryab Gul*, Mansoor Ali Shah, and Naheed Baloach

Department of Zoology, University of Sindh, Jamshoro

Corresponding: zaryabguldayo@gmail.com

Moths, primarily nocturnal and phytophagous, play diverse roles as pests in agriculture, night pollinators, and potential bioindicators. This study marks the first attempt to furnish information on the diversity, richness, and abundance of moths belonging to the genus *Scopula* in Jamshoro, Sindh, Pakistan. Moths were collected using various techniques, including light traps, alight sheets, and insect nets. To assess the diversity (species richness, evenness, and abundance) of the moth fauna, Simpson's Diversity Index (D0) and Shannon Diversity Index (H0) were calculated. A total of 406 specimens were collected over 8 months, representing two species: *Scopula pulchellata* and *Scopula minorata*, belonging to two genera within one subfamily and one family. The species *Scopula minorata* was the most prevalent, comprising 228 specimens, followed by *Scopula pulchellata* with an abundance of 178 specimens.

**EFFECT OF DIFFERENT MULBERRY VARIETIES ON THE LARVAL AND COCOON
PARAMETERS OF SILKWORM (*BOMBYX MORI*).**

Fiza Asif*, Nasra Ashraf*, Nuzhat Shafi, Saba Khalid, Rizwanullah Khan and Raja Hanif

Department of Zoology University of Azad Jammu and Kashmir, Muzaffarabad

*Corresponding Author: fizaasif830@gmail.com, nasra.ashraf@yahoo.com

Bombyx mori is generally known as mulberry silkworm and it feeds on mulberry leaves. The ability to switch plant proteins from mulberry leaves to silk proteins is one of the most important characters of the *Bombyx mori*. The growth and development of the larvae and production of silk is determined by the nutritional value of mulberry leaves. Present study was conducted at sericulture research center Pattika. Objective of the study was to check the effect of different mulberry varieties on two different varieties of silkworm. Eggs were hatched and larvae were reared under suitable environmental conditions of temperature and humidity, as described by Harizanis, (2007). Levene's t-Test for Equality of Variances was performed to compare the results of two seasons. Seasonal comparison of the growth and silk production was also done. Different economic and growth parameters (larval weight, cocoon weight, shell weight, effective rate of rearing and adult emergence) of silkworm were noted down feeding on leaves from different mulberry varieties. Larval weight of C102 was maximum at Punjab I (4.44g) during spring while during autumn season at Punjab I (3.04g). Statistically there was a significant difference of larval weight of C102 during two seasons (t (df =10) =20.41, $p < 0.5$). During spring rearing Larval weight of Jam120 was maximum at control group (4.80g) whereas during autumn season at control group (2.49g). There was a significant difference of larval weight of Jam120 between two seasons (t

(df= 10)= 5.10, $p=0 <0.05$). Larval duration was same at all treatments during both seasons. Pupal weight of C102 was maximum at NARC local (1.35g) during spring rearing whereas during autumn rearing it was maximum at Punjab II (1.12g). Pupal weight of C102 was significantly different between two seasons (t (df =10) =4.782, $p=0.001<0.05$). During spring season pupal weight of Jam120 was maximum at Punjab I (1.72g) while during autumn season at Punjab II (1.01g). Statistical analysis showed that there was a significant difference of pupal weight of Jam120 during two seasons (t (df= 8)=7.8, $p=0.001<0.05$). During spring season cocoon weight of C102 was maximum at *Morus latifolia* Sri Lanka (1.94g) whereas during autumn season cocoon weight was greater at Punjab II (1.47). Statistically there was a significant difference of cocoon weight of C102 during both seasons (t (df=10) =3.062, $p= 0.012<0.05$). During both seasons Maximum shell weight was same at Punjab II (0.38g). Cocoon weight of Jam120 was maximum at Punjab II (1.89g) during spring season while during autumn season maximum cocoon weight was at Punjab II(1.39g). Statistically there was a significant difference of cocoon weight of Jam120 during both seasons (t (df =10)=10.499, $p=0<0.05$). Maximum shell weight was observed at Punjab II (0.38g) and during autumn season greater shell weight was observed at Punjab II (0.35g). Adult emergence of C102 was highest at Punjab I (100%) during spring season while during autumn season at NARC local (91%). There was a significant difference of adult emergence of C102 between two seasons (t (df=10)= -3.912, $p=0.003<0.05$). Adult emergence of Jam120 during spring season was maximum at Gumgi Sri Lanka, NARC local and control group (100%) while during autumn season it was highest at control group (91%). There was a significant difference of adult emergence of Jam120 during two seasons (t (df =10)= 4.09, $p=0.002<0.05$). ERR of C102 during spring rearing season was highest at Gumgi Sri Lanka (100%) while during autumn season it was maximum at *Morus latifolia* Sri Lanka (95%). There was not a statistical difference of ERR between two seasons (t (df =10)= -1.629, $p=0.134 >0.05$). ERR of Jam120 was highest at Punjab I, NARC local and control group (100%) while during autumn rearing season it was highest at NARC local. There was a significant difference of ERR of Jam120 during two seasons (t (df =10) =3.97, $p=0.003<0.05$). It was concluded from the results of the present study that different mulberry varieties vary in their nutritional contents. Punjab II showed best results for most of the parameters under study. As far as seasons are concerned results of the spring rearing were best. So maximum portion of the Punjab II should be included in the silkworm diet and local farmers should rear the silkworm during spring season to get good results.

PRELIMINARY STUDY ON HOUSEFLY (*MUSCA DOMESTICA*) AS MECHANICAL VECTOR OF VARIOUS PATHOGENS FROM LARKANA DISTRICT, SINDH PAKISTAN

Haneef Sajjad, Fakhra Soomro, Zulfiqar Malik and Toheed Fatima

Department Of Zoology, Shah Abdul Latif University, Khairpur

*Corresponding Author: haneefsajjadgopang786@gmail.com

Housefly (*Musca domestica*) is the notorious mechanical vector belongs to the order diptera and is a well-known human and animal disease spreader. It is the most prevalent species and can be found in homes, hospital and public space where it contaminates food. In addition to being on annoyance, houseflies can carry pathogens. The present study was carried out during the year 2022 from April to July from following localities i.e. talukas Larkana, Bakrani, Dokri, Retodero of district Larkana. Survey was carried out to analyze the population of houseflies with relation to prevalence of vector borne diseases. Methodology was based on two experiments 1. Population fluctuation of houseflies and prevalence of vector borne diseases 2. Identification of pathogens through biochemical test. The population of *Musca domestica* remains highest in the month of April (i.e 2433) at the relative optimum temperature and humidity (temp 36-39 humidity 20-40). These finding are the conformity with the Aubuchon, 2006. Housefly population was significantly lowest in the month of July (i.e 1546). The maximum patients

reported in the study area were belongs to the age group (1-12 years) and minimum to the age group (19 and onwards). The susceptibility of most affected age is due to immunity or resistant power. Socio-economic status was observed that poor people were most affected (i.e. 1891 out of total 2827) due to lack of awareness regarding hygiene. Total 6 pathogens were identified (i.e. *Klebsiella* species, *Proteus* species, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*). The most frequent isolated organism of gram negative was *Escherichia coli* followed by *Klebsiella* species, *Pseudomonas aeruginosa* and least was *Proteus* species while gram positive most frequent was observed *Staphylococcus aureus* followed by *staphylococcus epidermidis*. Highest number of disease was Diarrhea and least was Food poisoning. Houseflies therefore, may act as vector of potentially pathogenic bacteria in hospital and environment outside of hospital.

SONICATION AND HEAT-MEDIATED SYNTHESIS, CHARACTERIZATION, AND LARVICIDAL ACTIVITY OF SERICIN-BASED SILVER NANOPARTICLES AGAINST DENGUE VECTOR (*Aedes Aegypti*)

Muhammad Summer, Hafiz Muhammad Tahir and Shaukat Ali*

*Laboratory of Applied Entomology and Medical Toxicology, Department of Zoology, GC University
Lahore*

Corresponding Author: dr.shaukatali@gcu.edu.pk

Fabrication, characterization, and evaluation of the larvicidal potential of novel silk protein (sericin) based silver nanoparticles (Se-AgNPs) were the prime motives of the designed study. Furthermore, investigation of the sericin as a natural reducing or stabilizing agent was another objective behind the current study. Se-AgNPs were synthesized using sonication and heat. Fabricated Se-AgNPs were characterized using a particle size analyzer, UV spectrophotometry, FTIR, and SEM which confirmed the fabrication of the Se-AgNPs. The size of sonication-mediated Se-AgNPs was smaller (7.49nm) than heat-assisted Se-AgNPs (53.6nm). Being smallest in size, sonication-assisted Se-AgNPs revealed the significant highest ($F_{4,10}=39.20$, $p=0.00$) larvicidal activity against 4th instar lab and field larvae ($F_{4,10}=1864$, $p=0.00$) of Dengue vector (*Aedes Aegypti*) followed by heat assisted Se-AgNPs and positive control (temephos). Silver (without sericin) showed non-significant larvicidal activity, which made silver's temperature stability debatable. Furthermore, findings of biochemical assays (Glutathione-S transferase, esterase & acetylcholinesterase) showed the levels of resistance in field strain larvae. Above mentioned findings of the study suggested sonication as the best method for the synthesis of Se-AgNPs while the larvicidal activity is inversely proportional to the size of Se-AgNPs i.e. smallest the size, highest the activities. Conclusively, the findings of the present study have endorsed the status of sericin as a natural reducing/stabilizing agent.

ESTIMATION OF ABUNDANCE AND DIVERSITY OF INSECTS IN CITRUS ORCHARDS DISTRICT LAYYAH, PUNJAB

Nazia Ehsan*, Muhammad Tahir, Aqsa Riaz, Warda Mustfa and Nosheen Fatima

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: nazia.ehsan@uaf.edu.pk

Insects are the most diverse and largest group of organisms. The aim of study was to determine quantity, abundance, diversity and feeding level of insects in the ecological condition of district Layyah.

The insect fauna was collected by using sweep net, forceps and handpicking along with glass jars containing solution of formalin and glycerin. The specimen was collected at morning time from 9:00 am to 11:00 am in the fields. For determine the biomass of collected fauna, electronic balance was used. After sorting, the samples were placed into vials for further identification. Various approaches were utilized to identify insects, including the naked eye, hand lens, microscope, and taxonomic literature. For statistical analysis, the Shannon diversity index and regression analysis were utilized to evaluate the diversity component and data significance, respectively. R.A was noted at the order level out of 8 orders, highest R.A was result out for the highly abundant order was Lepidoptera 18.2896(N=34), succeed by Orthoptera 24.43%(N=32). However least was Neuroptera 0.54%(N=1). The highest family recorded was Coccinellidac 22.97%(N=34) followed by Libellulidae 23.88% (N=10), Syrphidae 21.62% (N=32) and least abundant families were Tineidae, Geometridae, Lophophidae, Chrysopidae, Gryllidae, Calliphoridae. The highest R.A was for genus Coccinella Septempunctata (Coocinelladac: Coleoptera) 29.77% (N=39), Papilio machaon (Papilionadea:Lepidoptera) 8.59%(N=11) Episyrphus balteatus (Syrphidae: Diptera) 20.959% (N=31), Musca domestica (Muscidae: Diptera) 16.42% (N=12). Bombylius major (Bombylidac: Diptera) 6.889%(N=- 11) and least abundant was Amara similata, Pyrrilla perpusilla, Euodynerua megaera, Pieris brassica, Chrysoperla carnea. The highest feeding status recorded for Predator in citrus orchard was recorded up to 2.67% (N=35). It was detected that populace means of verified taxa between groups and within the groups in citrus fields indicated significance outcomes ($F=2.7579$; $P=0.001585$).

USING DNA BARCODES TO INVESTIGATE APHIDS DIVERSITY (HEMIPTERA: APHIDIDAE AND ADELGIDAE)

**Sheikh Muhammad Usamma, Abdul Manan Shaikh, Aiman Amur*, Bhojoo Mal,
Muhammad Tayyab, Vikiram Kumar and Baboo Mal**

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: amuraiman@gmail.com

Identification of aphid species and connection analysis are complicated by phenotypic plasticity resulting from host and environmental conditions, as well as a trend towards loss of taxonomically relevant features. It is challenging to reliably link routinely obtained field samples with specific species classifications due to the existence of distinct physical forms of a single species on diverse hosts and at different seasons of the year. It has been suggested that the standard method for characterising living forms is DNA barcoding. We examined how well the common 658-bp barcode fragment from the 5' end of the mitochondrial cytochrome c oxidase 1 gene (COI) could distinguish between various aphid and adelgid species. Results are shown for an initial investigation into the use of DNA barcoding, where around. It is challenging to reliably link routinely obtained field samples with specific species classifications due to the existence of distinct physical forms of a single species on diverse hosts and at different seasons of the year. It has been suggested that the standard method for characterising living forms is DNA barcoding. We examined how well the common 658-bp barcode fragment from the 5' end of the mitochondrial cytochrome c oxidase 1 gene (COI) could distinguish between various aphid and adelgid species. The findings of an initial investigation into the use of DNA barcoding are reported. Approximately 3600 specimens representing 568 species and 169 genera of the major aphid and adelgid subfamilies have been.

RISK FACTORS OF SECONDARY INFERTILITY IN WOMEN

Mansoor Anwar Sahito, Naheed Shah, Bhojoo, Mal and Abdul Manan Shaikh

Department of Zoology, Gov. College University, Hyderabad

Corresponding Author: amuraiman@gmail.com

Secondary infertility is growing now days. Secondary infertility is the inability to conceive for one year after having conceived at least once before. FSH and LH are major reproductive hormone that play a vital role in the normal reproductive cycle and in maintaining reproductive health among women. Disturbance in these two hormones is one of the risk factors for secondary infertility. A disturbing level of TSH is also a risk factor for secondary infertility age plays an important risk factor for secondary infertility. Survey-based research is carried out in the civil hospital in Hyderabad. We formed a questionnaire to collect the required data from the patient. The major symptom found among most of the female was an abnormal menstrual cycle, skin dryness, weight loss, stress (50%), miscarriage (50%), and hair loss (60%), Most patients have a low literacy rate (90%), The main cause of secondary infertility from these results is the disturbing level of FSH, LH, and TSH as the normal levels of these hormones are necessary for maintaining reproductive health.

THE REPRODUCTIVE AND FEEDING BEHAVIOURS OF CENTROTUS CORNUTUS (Horned TREE-HOPPER) ON KAKAR TREE/ BABUR ACACIA NILOTICA (L.)

Baboo Mal, Aiman Amur*, Abdul Manan Shaikh, Muhammad Tyyab and Vikiram Kumar

Department of Zoology, Gov. College University, Hyderabad

Corresponding Author: amuraiman@gmail.com

Insects that live on different habitat with different diversity, such as Tree hoppers and thorn bugs are belong to family Membracidae a group of insect be similar to the cicadas and leaf hopper. About 3200 species of tree hoper, one of Centrotus cornutus (Horned tree hopper) species were collected from Hyderabad district during August to December 2023. The present species of representatives of Heteroptera and Membracidae observed during this study, This species, explained with numbers of specimens collected, ranges of collection dates (seasonality), and available material on their hosts, habitats, and ranges, is presented. The reproductive behavior observed on common and local tree Acacia, it was observed that female ovipositor saw like, which slits cut in to stem or bark of tree, with her egg laying in less or more straight position on the bark of kankar (Acacia tree) with an oval dark colored egg. Eggs are may be parasitized by wasps such as the tiny fairy flies. The Membracid family (female) mostly sit over their eggs to protect them from predator and parasites. Some species are extroverted to protect eggs of each other. It is minor pest of many ornamental plants and Acacia tree. This tree hoper perforate plants stalks and shoots with their beak and feed upon sap. The larvae can habitually be found vegetation bushes and grasses while the adults survive hard wood tree species. These insects' mutualism ant and wasp species. There is inadequate information documented on this pest, but this research work will be contributed important role towards this pest.

**STUDY OF MORPHOLOGICAL APPEARANCE AND ENVIRONMENTAL EFFECTS ON
GROWTH OF HIEROGLYPHUS ORYZIVORUS (CARL 1916) (ACRIDIDAE: ORTHOPTERA)
IN DISTRICT HYDERABAD SINDH**

**Vikram Kumar, Bhojoo Mal, Abdul Manan Shaikh, Aiman Amur*,
Muhammad Tayyab, and Baboo Mal**

Department of Zoology, Gov. College University, Hyderabad

Corresponding Author: amuraiman@gmail.com

A significant rice pest in Hyderabad, Carl is the oryzivorous Hieroglyphus. It is also known, though, that wheat, sugarcane, millets, and corn act as its alternate hosts. Three deep transverse sulci split the pronotum, and the posterior sulcus bears a central bow shape. The superior plate is the same length, the male appendix is called appendicle, and the face and mouthpart are yellowish-green rather than dark green. Brachypterous (short-winged) and macropterous (long-winged) are the two forms of this pest; the latter is more likely to form swarms. After a fortnight in the paddy field, hoppers often appear in the field between the months of August through September. While the hoppers aggressively fed on *Cynodon dactylon*, the adults preferred to eat the just emerged leaves of the rice plant. The adult population peaked in September and November, after which it rapidly declined in the field.

**DIVERSITY OF PIERID BUTTERFLY (LEPIDOPTERA: PIERIDAE)
IN DISTRICT HYDERABAD, SINDH**

Alisha Solangi, Abdul Manan Shaikh, Bhojo Mal, Aiman Amur* and Abdul Waheed

Department of Zoology, Gov. College University, Hyderabad

Corresponding Author: amuraiman@gmail.com

The present study was carried out during January to December, 2023, in Hyderabad. Throughout this study period 665 specimen of pierid butterflies were collected with the help of insect net and hand pickling method from the different localities of Hyderabad Sindh in the group of these specimen of pierid butterflies belonging to 3 genera (*Anaphies*, *Colotis*, and *Eurema*) of two subfamilies (*Pierinae* and *coliadinae*) were identified. This was the first record of pierid butterfly in Hyderabad.

BIOLOGY AND MORPHOMETRIC OF *PAPILIO DEMOLEUS*

Abdul Waheed, Aiman Amur*, Abdul Manan Shaikh and Bhojo Mal

Department of Zoology, Gov. College University, Hyderabad

Corresponding Author: amuraiman@gmail.com

Papilio demoleus L, commonly called Citrus lime, it is found in the Asia, Africa, Middle East of Europe and Australia. *Papilio demoleus* L., is medium sized butterfly. Its Head, thorax and abdomen is black, Wings have black ground colour, with white and red spots, and the inner side of red spot are black. The host plant of this butterfly is *Citrus limon* where they reproduce. The study was carried out on the biology of *Papilio demoleus* L, done in the month of June 2023. The eggs were collected from the host plant (*Citrus limon*) and put in the plastic jars at the home on the natural diet under control condition (average temperature between 30°C to 37°C). The eggs hatched after 3-5 days, the larvae feed on the leaves provided them regularly. The larvae make cocoon and convert pupa after 6 to 10 days and attached

itself on underside of the cap of bottle. The pupae development takes 16 to 22 days to become adult. It was observed that there was some variation in the development of the larva due to fluctuation in the temperature. The purpose of the study is to know the life cycle of the *Papilio demoleus* L, under controlling at home.

MORPHOLOGICAL CHARACTERISTICS OF SEVEN SPOTTED LADYBIRD BEETLE (COLEOPTERA, COCCINELLIDAE) OF DISTRICT HYDERABAD SINDH, PAKISTAN

Arisha Ayaz, Bhojoo Mal, Abdul Manan Shaikh and Aiman Amur*

Department of Zoology, Gov. College University, Hyderabad

Corresponding Author: amuraiman@gmail.com

Coccinella septempunctata the seven spotted lady bird beetles is native to Eurasia, and it is said to be the most common ladybug in Europe. It was intentionally introduced into North America in the middle of 20th century to combat aphid pests, lady bird beetles are the important group of beetles due to important universal predatory and occupies important place in biological control. It is stereotypical lady beetle, the current study revealed that *Coccinella septempunctata* is a reddish with black spots, has a shiny, round, domed body, underside flat, possess tiny segmented antennae, identify this species by the presence of a white spots on either side of the head, and possess seven black spots on the Elytra (a shell like wing cover). It is the complex of species or the same species throughout the Palearctic region. The numerical data of *Coccinella septempunctata* reveals that these species are highly abundant and were collected from different localities of Hyderabad region. As adults, these beetles continue their role as effective aphid predators, contributing to natural pest control in ecosystems. The seven-spotted ladybird beetle's successful introduction to various regions for biological pest management highlights its adaptability and ecological importance. Its morphological traits, including the distinctive spots and compact body, make it a recognizable and valuable ally in maintaining a balanced ecosystem.

BIODIVERSITY AND GUILD STRUCTURE OF SPIDER (ARACHNIDA: ARANEA) FROM BANANA PLANTATION OF DISTRICT MATIYARI, SINDH HYDERABAD, PAKISTAN

Tahira Jabeen Ursani, Shadab Kaka, Samina Malik. and Jawaid A. Khokar

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: tjchandio65@gmail.com

Pakistan is rich in spider fauna and has diverse habitats. Spiders are an ancient and successful group of invertebrate animals and are known as poisonous arthropods. Spiders belong to phylum Arthropoda, subphylum chelicerate, class Arachnida, and order Aranea. They may feed on adults, sub-adults, nymphs, and even caterpillars though feeding on eggs is not observed yet. This quality makes them the most suitable potential bio-control agents for regulating the population of insect pests in different agro-ecosystems and necessitates their extensive study. No proper and detailed research work has been done on the biodiversity and guild structure of spiders from different localities of Districts Hyderabad, Shaheed Benazir Abad, Mirpurkhas and Matiyari. Therefore, present work is focused on the biodiversity of spiders from banana Agro-ecosystem of District Matiyari, Sindh, Pakistan. District Matiyari is located in the central part of Sindh and is the part of Hyderabad division. For data collection of Specimens in a large number from Banana plantations, to conduct and check the

guild structure and its different natural inhabitants living style on basis of different web and non - web spiders regions of Districts Matiyari, to identify the collected specimens up to families level, to calculate the diversity and distribution ribution of vario of various Family of spiders and determine the species richness in the field of Banana plantation. Banana crops i is one of the most fertile agricultural crops are being grown here. Banana (*Musa Paradidica* L.) belongs to the family Musaceae. Banana is a major fruit crop of Pakistan that mainly grown in Sindh province where the soil and climate conditions are favorable for its successful cultivation. Banana is grown in Pakistan over more than 349,000 hectares cultivation and 90% of this land lies in the Sindh province. Banana crops are infected by the numbers Pest names. of the pests) The farmer of Sindh, Pakistan generally uses chemical pesticides to protect their crops. Chemicals pesticides create many problems such as diseases, environmental pollution and kill the harmfui pests as well as beneficial living organisms. Use of the biological control is fundamental approach for reduce the pest's density with in an effective integrated pest management (IPM) program. Biological control refers to the use of natural enemies against the pest population to reduce the pest's density. It is one of the safe methods to control the pests because it is not toxic, pathogenic and injurious to human beings. Biological control has the advantage of being self-established and usually does not harm non-targeted organisms in the environment. During the present study, Spider biodiversity exploration, the first activity executed in selected areas in August 2022 yielded 950 specimens made up of spiderlings, suhadults and adults. The composition represented 06 families of spiders were collected. These six families namely Araneidae, Lyconsida Oxyopidae, Tetragnathidae, Salticidae and Gnaphosidae. All the sorted families are initially studied at the family level and classified on the basis of their web-building and predation methods. On the basis of web-building, Oxyopidar, Saricidae and Gnaphosidae, Lycosidae and are classified as non-wee builders. Tetragnathidae and Araneidae, are classified as orb-web builders. On the basis of predation method, Lyconidae and Gnaphosidae are identified as ground runners and Araneidae, Oxyopidae. Tetragnathidae and Salticidae are classified as stalkers. It has been observed that during flood season month of many families as most abundant in decreasing order, namely: Oxyopidae Araneidae Tetragnathidae Thomisidae, Lycosidae and Salticidae. In terms of family the immatures of the Oxyopidae and orb-web, Araneidae were most dominant keystone species that were likely to have exerted strong ecological services in the Banana environment by preying on planthoppers, leafhoppers and leaf folder moths. Across sites, the trend in total spider abundance.

BIOTYPE DETERMINATION OF *NILAPARVATA LUGENS* (STÅL) (HEMIPTERA: DELPHACIDAE) USING PCR FROM VARIOUS RICE GROWING REGIONS OF THE PUNJAB, PAKISTAN

**Arshed Makhdoom Sabir^{1*}, Bilal Atta¹, Amara Nasiba¹, Muhammad Ijaz¹, Muhammad Sabar¹,
Muhammad Usman Saleem¹, Hira Sehr¹, Fariha Shahzadi¹,
Tahir Hussain Awan² and Faheem Azher³**

¹*Rice Research Institute, Kala Shah Kaku, 17 Km G.T. Road, Lahore*

²*Punjab Agricultural Research Board, Lahore*

³*Sultan Group Pakistan, Lahore*

*Corresponding Author: amsabir@yahoo.co.uk

Rice (*Oryza sativa* L.) is consumed by more than one-third of the total human population across the globe. Brown planthopper (BPH) *Nilaparvata lugens*, is one of the economically important insect pests and cause more than 60% economic yield loss under favourable environmental conditions throughout Asia. Many chemical insecticides successfully control BPH, but their injudicious use, destroy the

beneficial fauna responsible to keep its population below economic injury level. To develop rice varieties resistant to BPH and their effective management, it is imperative to know the biotype of BPH. This study describes the use of Polymerase Chain Reaction (PCR) to determine the presence of BPH Biotype 4 in specimens collected from different regions (Mandi Bahaudin, Sialkot, Narowal, Gujranwala, Sheikhupura, Hafizabad, Gujrat, Lahore, Faisalabad, Sahiwal, Multan, D.G Khan, Jhang, Bahawalnagar and Sargodha) of the Punjab, Pakistan. The Gel electrophoresis results indicated that all BPH specimens showed the presence of the specific band at the expected size (positive results) under UV image visualizer, indicating the presence of BPH Biotype 4. This finding will further facilitate the BPH resistance rice breeding program more effectively and efficiently in the future.

SECTION – I V

P A R A S I T O L O G Y

***LOPHOSICYADIPLOSTOMUM BILQEESAE* SP.N. (TREMATODA: DIPLOSTOMIDAE POIRIER, 1886) FROM THE INTESTINE OF *PHALACROCORAX CARBO* (LITTLE CARMORANT) IN LARKANA, SINDH, PAKISTAN**

**Abdul Saeed Hulio*¹, Sanjota Nirmal Das¹, Aly Khan² Tasmeena Laghari¹,
Seema Mahesar and Mahek Hulio³**

¹Department of Zoology, University of Sindh, Jamshoro

²Agricultural Research Council, University of Karachi

*Corresponding Author: abdulsaeedhulio@gmail.com

During present studies on the helminth parasites of birds, fourteen *Phalacrocorax carbo* (Little cormorant) were collected from District Larkana, Sindh, Pakistan. The birds were anaesthetized, autopsied and examined for helminth parasitic infections. Out of fourteen, nine were found infected. The worms were preserved with alcoholic series, stained with Mayer's carmalum and mounted in Canada balsam. After detailed study the specimens were identified as *lophosicyadiplostomum bilqeesae* sp.n belonging to family Diplostomidae (Poirier, 1886) and Subfamily Diplostominae (Poirier, 1886) and to the genus *Lophosicyadiplostomum* (Dubois, 1936). The new species is characterized by having: bipartite body, Fore body broader, elongated and hind body much smaller than fore body and balloon shaped; Oral sucker is elliptical to truncate conical shaped; Acetabulum round-shaped, located towards the middle of body and smaller than oral sucker; Holdfast organ present in the fore body is rounded, median and postacetabular; Testes tandem, unequal post-ovarian located in anterior of the hind body, Anterior testis oblique and touches the ovary while posterior testis bi-lobed with deep median groove; Ovary rounded, sub median, posteriorly touches the anterior testis; Seminal vesicle is reniform in shape and present in the posterior end of hind body; Vitellaria starts below the intestinal bifurcation, follicle in shape, scarcely dispersed in lateral fields and in the middle of fore body and reaching to posterior end of the hind body. Bursa reduced, wide without sucker with terminal opening and eggs are few in number.

TAXONOMIC STATUS OF NEW SPECIES OF *DIPLOTRIAENA FAHMIDAE* N.SP. (NEMATODE: FILARIIDAE) FROM JUNGLE MYNA (*ACRIDOTHERES FUSCUS*) WAGLER; 1872 (PASSERIFORMES: STURNIDAE) IN DISTRICT LARKANA, SINDH, PAKISTAN

Bakhtawar Soomro*, Shakeel Ahmed Memon¹, Saheefa Soomro and Dhani Bux Mishori

Department of Zoology, Government College University Hyderabad, Sindh

*Corresponding Author: bakhtawarsoomro145@gmail.com

A new nematode from *Diplotriaena fahmidae* n.sp was recorded from the body cavity of the Jungle myna *Acridotheres fucus* in Larkana district, Sindh, Pakistan. A total of 30 nematodes (♂♂) were recorded. This nematode reflects diversification in the following characteristics compared to congeners nematodes which includes: Shape; size and shape of the trident. The shape of the tridents, spicules and the presence of

20-27 pairs of caudal papillae. Due to these morphometric changes, this species; *Diplotriaena fahmidae* can be treated as a new species. This species was named in honor of my late mother, Miss Fahmida Soomro. During the present studies, 70 specimens of Jungle mynas (*Acridotheres fuscus* Wagler, 1827) were collected from the various locations of Larkana District, Sindh, Pakistan. Larkana is at a latitude of 27.563993 and a longitude of 68.215134. Its GPS coordinates are 27° 33' 50.3748" N and 68° 12' 54.4824" E. All samples brought to the parasitological Laboratory Department of Zoology. All the specimen of the birds were autopsied and analyzed for the helminthes parasites. Around 30 (♂♂) specimens of nematodes were recovered from the body cavities of hosts belonging to the genus *Diplotriaena* Railliet and Henry, 1909. Live specimens were killed in hot 70% ethanol, cleared in a solution of lacto phenol and glycerol, and kept in an alcohol-glycerol solution. Photographs were made with the assistance of camera Lucida Garcia and Ash 1979. Photos were taken with the assistance of camera DP12. The units of measurement are in millimeters (mm).

ASSESSMENT OF KNOWLEDGE, ATTITUDES, AND PRACTICES OF CARTHORSE OWNERS REGARDING TICK-BORNE DISEASES, PAKISTAN

Syed Faizan Ali Shah, Muhammad Ijaz*, Arslan Ahmed, Muhammad Umar Javed, Hamza Rasheed, Muhammad Jawad Sabir and Ali Abdullah Jabir

¹Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore

Corresponding Author: mijaz@uvas.edu.pk

Over the past few years, vector-borne diseases (VBDs) have been emerging and reemerging, affecting the welfare and health of equines. This study undertook a Knowledge, Attitudes, and Practices (KAP) assessment among 264 carthorse owners. The investigation aimed to elucidate the understanding, beliefs, and behaviors concerning ticks and TBDs. The findings revealed a conspicuous deficit in knowledge regarding tick-borne disease transmission and zoonotic implications associated with tick-infested equines. Although participants recognized seasonal tick prevalence, awareness of equine susceptibility to TBDs was alarmingly low, with a majority (60.60%) unaware of this vulnerability. Despite acknowledging the endemicity of ticks (89.77%), a vast majority (88.25%) remained unaware of the concurrent infectious diseases. Remarkably, despite instances of tick bites, no respondents sought medical consultation. Attitudes expressed concern for equine health yet displayed misconceptions surrounding antibiotic treatments and suboptimal tick removal practices. Challenges in implementing effective tick control measures surfaced, driven by concerns over repellent-associated adverse reactions and practical hurdles. These findings underscore an urgent need for targeted educational interventions to rectify knowledge gaps and enhance TBD prevention practices among carthorse owners in Pakistan.

MOLECULAR DETECTION OF BARTONELLA IN RODENTS AND ECTOPARASITES: INSIGHTS INTO PREVALENCE AND IDENTIFICATION

Mariam Munir* and Shahzad Ali

Department of Wildlife & Ecology, University of Veterinary and Animal Sciences, Lahore, Ravi Campus, Pattoki

*Corresponding Author: mariamnoor065@gmail.com

The *Bartonella* genus encompasses numerous zoonotically relevant species, with rodents serving as reservoirs for some of these *Bartonella* strains. The primary transmission route involves ectoparasites such

as fleas, ticks, lice, and mites. In Pakistan, there is a notable lack of information regarding the prevalence and molecular identification of *Bartonella* species in animals, particularly rodents. This study was conducted in the Kasur district, located in the Punjab province of Pakistan. Eighty rodents, including black (*Rattus rattus*) and brown (*Rattus norvegicus*) rats, were captured from four tehsils in District Kasur, Punjab, and examined for *Bartonella* through the collection of fleas and blood samples. Various data, including host species, habitat types (houses, livestock farms, aviaries, grassland, and crop areas), age, gender, urbanicity (rural/urban), and the presence of ectoparasites, will be systematically gathered during the study. Molecular detection involved DNA extraction and PCR targeting *gltA* (825bp) and *rpoB* (201bp) genes, followed by sequencing *rpoB*-positive and *gltA*-positive samples. Genetic relatedness to other published *Bartonella* spp. gene sequences was explored using BLAST and phylogenetic analysis. Chi-square analysis was employed to identify associations between positive samples and risk factors. The results reveal an exceptionally high prevalence of *Bartonella* DNA in rat-associated fleas (n=16; 20%) and blood (N=10; 12.5%). Habitat and the presence of ectoparasites demonstrated significant associations with positive cases. *Bartonella* sequences from this study closely resembled those of *Bartonella* species found in various countries. In conclusion, this study sheds light on the substantial prevalence of *Bartonella* DNA in both fleas and blood samples obtained from rodents in the Kasur district, Punjab, Pakistan. The findings emphasize the importance of understanding the role of rodents and their ectoparasites in *Bartonella* transmission, highlighting significant associations with habitat types and the presence of ectoparasites. The genetic relatedness of *Bartonella* sequences to those from diverse countries underscores the global relevance of these zoonotic agents.

PREVALENCE OF METAZOAN PARASITES IN STRIPED SPINY EEL *MACROGNATHUS PANCALUS* (SYNBRANCHIFORMES: MASTACEMBELIDAE) IN DISTRICT SANGHAR, SINDH, PAKISTAN

Jemi*, Nadir Ali Birmani and Naheed Baloch

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: ja12century@gmail.com

During current study (January 2020 to the September 2023), a total of 35 hosts Striped Spiny Eel *Macrognahtus pancalus* (Synbranchiformes: Mastacembelidae) were collected from different localities of district Sanghar and brought to parasitology laboratory, department of Zoology, University of Sindh, Jamshoro for the examination of metazoan parasites. Gut contents revealed the presence of 10% acanthocephalan, 12% trematodes, 16% cestodes, 17% nematode and 45% gill parasites.

RESISTANCE OF *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA, NOCTUIDAE) AGAINST INSECTICIDES

Saif Ullah*, M. Saleem Afzal, Fayyaz Hussain and Adnan Bashir

Department of Entomology, Institute of plant protection, MNS University of Agriculture, Multan

*Corresponding Author: saifullahshafeeq786@gmail.com

In America, Africa, and Asia, the fall armyworm (*Spodoptera frugiperda* Smith) is a significant pest of maize, cotton, and other crops. The species is endemic to tropical and subtropical regions of the Americas, but it has been introduced to other parts of the world in the past few decades. The pest's host range is widespread. Fall armyworm populations in large numbers have the potential to seriously

harm crops, resulting in decreased crop quality and yields. Food security and agricultural productivity are at risk due to *S. frugiperda*'s potential for developing pesticide resistance. While insecticides are an effective means of managing populations of fall armyworms, the rise of pesticide resistance to these pesticides is posing a severe threat to many areas. This pest has demonstrated resistance to several insecticides, including as carbamates, organophosphates, and pyrethroids. In addition to using Bt crops, it's critical to take these variables into account and create integrated pest management (IPM) techniques in order to effectively manage pesticide resistance in *S. frugiperda* populations. The primary means of controlling *S. frugiperda*, the fall armyworm, is through genetically modified Bt crops. Since its invasion, insecticide use has increased and susceptibility to various insecticides used for decades to manage *S. frugiperda* has decreased, partly due to the absence of Bt crops. In order to prevent the development of resistance, this may involve the adoption of techniques like transgenic crops, biological and cultural management, and minimal insecticide use. Collaboration among farmers, researchers, and policymakers is crucial in order to devise and execute IPM approaches that minimize pesticide resistance and effectively control *S. frugiperda* populations, particularly when employing synergists. There is evidence that resistance evolution is driven by local selection for resistance. The best technique to reduce the number of *S. frugiperda* and the overuse of insecticides that leads to resistance is to combine IPM with Bt crops (high dose, pyramided strategy, and not Bt refuge crops with Bt).

TREMATOD DIVERSITY IN PURPLE HERON, *ARDEA PURPUREA* LINNAEUS, 1766 (CICONIFORMS: ARDEDAE) FROM DISTRICT SANGHAR SINDH PAKISTAN

Khalid Hussain Buriro¹, Saima Naz², Nadir Ali Birmani² and Sajid Siyal³

¹Department of Zoology, Government Girls Degree Daulatpur

²Department of Zoology, University of Sindh, Jamshoro

³Department of Wildlife Management, Shaheed Benazir Bhutto University of
Veterinary and Animal Sciences, Sakrand

Corresponding author: khalidsujamshoro@gmail.com

Helminths are mostly parasitic worms having diverse characteristics. They parasitize almost every fauna and damage their tissues by eating them or by absorbing vitamins. They are well adapted in their host. Among helminthes Trematods are successful parasites because of having special parasitic adaptations including suckers, hooks and highly developed reproduction system. Sexually trematods are hermaphrodite. Their hosts range from invertebrates to vertebrates. They like to parasitize snails, fish and birds. Their adult forms are generally prevalent in fish birds and mammals including man. The purple heron (*Ardea purpurea*, latin ardea “heron” and purpureus “purple”) is most wading bird in the heron family, Ardeidae. It is widely distributed in Pakistan. It is slightly smaller, slender and has darker plumage then that of common grey heron. Purple Heron (*Ardea purpurea* Linnaeus, 1766) locally in Chotiario Reservior, Sanghar Sindh are commonly called as “Jaho or some times Saen” and Jaho pakhi. In present investigation a total of 15 birds were examined during February 2019 to August 2020. Eleven out of fifteen birds were infected with trematodes the prevalence is 73%. Total 85 specimens were recovered sort out four species of trematodes including *Apharynogstrigea cornu* (Zedar, 1800), 19 specimens with prevalence is 22.35%, *A. ardeolina* (Vidyarthi 1937), 22 specimens with prevalence is 25.88%, *Strigea falconis* (Szidat, 1928) 16 specimens with prevalence 18.82% and *S. elongata* (Yamaguti, 1935) 28 specimens with prevalence 32.94%. This study is first time from this region making new host and new locality record.

A MESOCESTOIDES SPECIES POSSESSING PECULIAR MORPHOLOGICAL FEATURES AND DISTINCT PHYLOGENETIC POSITION WITHIN FAMILY MESOCESTOIDIDAE

**Mursaleen Khan¹, Suleman^{1*}, Mian Sayed Khan¹, Guo-Hua Liu^{2*}, Nehaz Muhammad³,
Daim Saood⁴ and Hammad Ahmad Khan⁴**

¹Department of Zoology, University of Swabi, Anbar, Swabi

²Research Center for Parasites and Vectors, College of Veterinary Medicine,
Hunan Agricultural University, Changsha 410128, Hunan, China

³Hebei Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology,
Hebei Collaborative Innovation, Center for Eco-Environment, College of Life Sciences,
Hebei Normal University, Shijiazhuang, Hebei Province, P. R. China

⁴Department of Zoology, Abdul Wali Khan University Mardan, Mardan

*Correspondence: zaheer0332@gmail.com; liuguohua5202008@163.com

Mesocestoididae (Order: Cyclophyllidea) is a family of tapeworms, parasitic in humans and domestic animals. With rare exceptions, they are parasites of homeotherms and are particularly diverse in birds and mammals, although a few species also parasitize amphibians and reptiles. During a study on the occurrence and diagnosis of *Mesocestoides* parasites of vertebrate hosts of the Khyber Pakhtunkhwa, specimens of *Mesocestoides* sp. were collected from 3 out of a total 11 diagnosed agama lizards. The diagnosed agama lizard belongs to the genus *Laudakia*. All the collected specimens were in its third larval stage, called tetrathyridia. The tetrathyridia were found encysted in liver where each cyst was containing 10 to 15 specimens of tetrathyridia. The present *Mesocestoides* sp. was different from its congeners having some peculiar characteristics including, the presences of long rostellum bearing long spines in 9 to 10 rows where each row contain 4 to 5 spines. The scolex of this species was found either invaginated or fully evaginated. Similarly, the rostellum was found evaginated in 5 to 8 specimens while the remaining specimens have invaginated rostellum. The occurrence of *Mesocestoids* in other reptilian hosts, especially lizards have been frequently recorded by authors from different countries. However, the present study is the first report from Pakistan and the first reptilian host diagnosed for helminth parasites from district Mohmand. Prior to the present study, no molecular data of 12S rDNA was available for this parasite. We sequenced partial 16S rDNA and 12S rDNA sequences (930bp) of the *Mesocestoides* sp. and it revealed 80.37% identity with corresponding sequences of *M. vogae*, 80.26% with *M. corti* and 80.2% with *Versteria mustelae* in BLASTn analyses. The systematics of *Mesocestoides* sp. is not well delineated due to their problematic morphological diagnostic features, phenotypic plasticity and pleomorphic metacestodes/larvae (tetrathyridia) which do not provide sufficient characteristic features for delineation of the species. Therefore, the DNA sequence data particularly, 12S rDNA sequences of additional *Mesocestoides* will help to solve the controversies in the classification of *Mesocestoides* and to assess the true and natural phylogenetic relationship of this group of parasites.

PREVALENCE OF ENDOPARASITES IN PALM SQUIRRELS (RODENTIA: SCIURIDAE) IN SHAHEED BENAZIRABAD, PAKISTAN

Toheed Fatima, Khadim Hussain, Nadir Birmni, Fakhra Soomro and Haneef Sajjad

Department of Zoology Shah Abdul Latif University, Khairpur

*Corresponding Author: toheedfatima110@gmail.com

The palm squirrel (*Funambulus pennantii*) belonging to the family Sciuridae. It is a species of small arboreal rodent found in northern India and in Pakistan. There are 58 genera of squirrel specie. In Asia squirrels are widespread from alpine deserts to rainforests and around the human population as well. Several species of squirrels (marmots) are known for their key role as predators and seed disperser while some smaller size species

are pollinators. The population of relatively few species living in close association with humans sometimes causes economic damage or become a threat to humans. Rodents are the primary and secondary hosts of several parasitic helminths. It is estimated that approximately one billion disease cases and millions of deaths from zoonosis occur annually. The present study has been carried out from February 2022 to March 2023. In this work an attempt has been made to identify the prevalence of endoparasites in palm squirrels from Shaheed Benazirabad, district, Sindh, Pakistan. The specimens have been collected from the parks and gardens of four talukas of Shaheed Benazirabad including Sakrand, Kazi Ahmad, Daur and main city Nawabshah. A total of 36 samples have been collected during study period. 06 species of endoparasites had been isolated from 36 samples, 05 of them were identified as Nematode species which are *Syphaciamesocieti*, *Syphaciaeutamin*, *Syphaciaspp*, *Syphaciacriti*, *Spirurasp* and one species of Trematoda was identified named *Plagiorhisproximus* in developing stage. It was observed that more parasites were isolated from female samples owing to its food habit. Squirrels, hence, may cause zoonosis and damage to human and animals.

SEROLOGICAL AND COPROLOGICAL EXAMINATION OF SHEEP FASCIOLOSIS IN DISTRICT BAGH

Syeda Samayyah Bukhari¹, Muhammad Shahbaz¹ and Ayesha Shahba²

¹*Department of Zoology, Women University, Azad Jammu and Kashmir, Bagh*

²*International Higher School of Medicine, Bishkek, Kyrgyzstan*

Corresponding author: shahbazchattha_pk@yahoo.com

A serological and coprological examination for fasciolosis in sheep was conducted in District Bagh, from September 2021 to April 2022. Fecal and blood samples of sheep were collected from a field survey for coproscopy by following and serology for *Fasciola* and were examined for the presence of any *Fasciola* in blood and ova in feces. Serum was separated and analyzed by a standard coprological sedimentation method and the seroprevalence of *Fasciola* was investigated by piloting an indirect enzyme-linked immunosorbent assay (ELISA) using excretory-secretory products from *Fasciola* as the antigen. A total of 200 fecal samples were collected from animals of the study area and brought in the laboratory for the examination of *fasciola hepatica* through the sedimentation method the prevalence rate was 18%. During this study 65 of the sheep of the study area were collected in serum samples where it was found that 22 samples were positive and 43 were negative. It was observed that 66% of serum samples were negative 34% serum samples were positive and contained *fasciola hepatica*. which are inflicting financial costs on the owners in the form of decreased milk and meat production, increased animal mortality, and damage to farmers. This study greatly contributed to the prevalence of fasciolosis in sheep from the study region, and the combination of ELISA and coprological sedimentation will be extremely helpful for demonstrating fasciolosis.

INFESTATION OF ECTOPARASITES IN EDIBLE CARNIVORE MARINE FISH *LETHRINUS NEBULOSUS*

Muhammad Farman, Syeda Nasreen, Seema Shafique* and Zaib-un Nisa Burhan

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: seema.shafique@uok.edu.pk

Parasites are integral components of all biological entities and play a pivotal role in the health of their hosts. Many commercially important marine fishes are under stress due to several reasons including parasitic diseases, overexploitation, anthropogenic influence and climate change. Present investigation was undertaken

to find out the parasitic fauna of commercially important marine carnivore fish *Lethrinus nebulosus* (Spangled Emperors) inhabiting coastal waters of Karachi, Pakistan. Fish samples were collected during the study period between August 2021 to July 2022 from largest landing site, Karachi fish harbor located at Karachi coast and examined for morphometric measurements. Their sizes were ranged between 22.1-45.7 cm (total length) and 0.2-1.4 Kg (total weight), with an average length and weight of 34.6 ± 6.9 cm and 4.4 ± 0.4 Kg respectively. A comprehensive fauna of ectoparasites were retrieved from host fishes. These parasites were recorded as Helminths (Monogeneans, Digeneans Trematodes) and Crustaceans (Copepods and Isopods). It has been noticed that parasites infecting the host fishes were only observed from the gills. Overall, the prevalence and mean abundance was recorded highest for Copepods (53.85%). Whereas, mean intensity was found at peak in Monogeneans (18.0). In the light of their prevalence and infestation intensity only on gills may questioned the status of health of marine ecosystems in coastal waters of Karachi, Pakistan. Presence of certain ectoparasites in high numbers on gills of *L. nebulosus* is needed further investigation to resolve their status as potential indicator of environmental conditions

BIOLOGY OF WARBLE FLY AND ITS CONTROL MODELS IN PAKISTAN

Munib Hussain¹, Abdul Razzaq^{2*} and Mohammad Qasim^{1*}

¹Animal Science Institute, Animal Health Program, National Agriculture Research Center

²Animal Sciences Division, Pakistan Agricultural Research Council, Islamabad

*Corresponding Author: munib.bangash@gmail.com, drqasim46@gmail.com,
abdulrazzaqazrc@gmail.com

Warble fly is an important disease of economic significance affecting ruminants especially cattle (*Hypoderma lineatum*) and goats (*Przhevalskiana silenus*), and commonly seen in hilly, semi-hilly and sandy areas of Pakistan. The warble fly larval prevalence was recorded higher in goats (66%) compared to cattle (32%) at Gilgit during autumn, followed by D.I. Khan (5-11%), Ziarat (1-8%), and Fort minro (14%) area. The life cycle trend in all the sites was comprised of warble (nodules) appearance between August-November and the dropping of L3 larvae from October to December. Telephonically Veterinarians confirmed the prevalence of warble fly in 20 districts of Pakistan, while 21 districts were reported free of warble fly during year 2022-23. In federating units animals were randomly divided into 4 groups i.e. A, B, C and D with 50 goats and 12 cattle in each group and were administered ivermectin 1% (1 ml/50 kg body weight) subcutaneously during August–September, group D was kept as negative control. In these therapeutics control models the treated groups of animals were found free of warble fly larvae while control/untreated group showed 32-66% prevalence. It is concluded that administration of ivermectin demonstrating its effectiveness in eliminating warble fly larvae as recorded in treated animals.

REDESCRIPTION OF *CLINOSTOMUM COMPLANATUM* (RUDOLPHI, 1814) BRAUN, 1899 REPORTED FROM *CHANNA STRIATUS* IN MORO, SINDH, PAKISTAN

Tasmina Leghari^{1*}, Sanjota Nirmal Das, Aly Khan², Abdul Saeed Hulo and Taseer Fatima

¹Department of Zoology, University of Sindh, Jamshoro

²Crop Diseases Research, Institute (CDRI), University of Karachi Campus, Karachi

*Corresponding Author: tasminaali45@gmail.com

In the result of helminthological studies, twelve specimens of fish species *Channa striatus* were caught from freshwater of District, Naushahro Feroze, Sindh, Pakistan. These fishes were carried to the

Parasitological laboratory, Department of Zoology, University of Sindh, Jamshoro. For the examination of helminth parasites freshwater fishes were dissected, seventeen specimens of genus *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 were recovered from the gills of 08 infected freshwater fish *Channa striatus*. After that these recovered specimens were killed and fixed in steaming 70% alcohol, the specimens were kept between two glass slides pressed lightly, unite with thread under light pressure, fixed in Formalin Acetic Acid and 70% ethanol solution for 24 hours. Then, specimens were transferred to 70% ethanol, and stained with Mayer's carmalum-stain, dehydrated in graded series of alcohol, then specimens cleared in clove oil, rinsed with xylene and permanently mounted in Canada balsam. A detail study was conducted as identify the recovered specimens belongs to the genus *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899. The specimens are characterized by having elongated body rounded at both ends. Maximum width of the body is attained at the posterior end of the body. Cuticle is thin without spines. The position of oral sucker is subterminal and smaller in size. Pre-pharynx is absent while pharynx is not prominent. Esophagus is short. Acetabulum is larger in size than oral sucker. Caecal bifurcation anterior to ventral sucker reaches up to the ends of the posterior extremity. Testes are post-acetabular, asymmetrical, tandem, intercaecal and present at the posterior side of the body. The anterior testes is larger than posterior testes. The Ovary is intercaecal small and present between the testis. Vitelline follicles are rounded and its distribution starts from ventral sucker to the posterior region of the body. Eggs are not clearly visible. Excretory vesicles opens terminally at the posterior end of the body.

**REDESCRIPTION OF *PALLISENTIS ROPARENSIS* (RANA AND KAUR) 2021
(ACANTHOCEPHALA; QUADRIGYRIDAE) IN A FRESH
WATER FISH FROM SINDH, PAKISTAN**

Aly Khan^{1*}, Sanjota Nirmal Das², Syed M Hasan Mehdi Naqvi³ and Noor un Nisa³

¹*C.D.R.I, Pakistan Agricultural Research Council, Karachi University Campus, Karachi*

²*Department of Zoology, University of Sindh, Jamshoro*

³*Vertebrate Pest Control Institute, Pakistan Agricultural Research Council,
Karachi University Campus, Karachi*

*Corresponding Author: aly.khan@hotmail.com

During a survey of freshwater fish of Keenjhar Lake, Sindh eight acanthocephala specimens were recovered from a ray finned fish *Notopterus notopterus*. The worms were deeply embedded in the small intestine of 3 fish out of 10 examined. The acanthocephalan collected were relaxed in water fixed in a F.A.A (a solution of formalin, Acetic acid and 50% alcohol (5:3:92). Stained with Mayer's carmalum. The acanthocephala recovered were 6 females and 2 males. The worms had four circles of hooks, each having 10 hooks. The anterior row hooks were largest. The collar spine had 14-16 circles of Y-shaped collar spine and body spine present up to the posterior end in females and a little anterior to posterior end to male. Cement gland had 25 nuclei, both lemnisci unequal, tubular. Neck is very short. Trunk divided into two regions. The male body length was 6.8-8.2mm maximum with at the middle of trunk region. The anterior most row of proboscis hooks was 84mm in length. The second row was 66mm, 3rd row 42mm and the last row 36mm. Proboscis 0.20-0.22 by 0.16-0.18mm. Collar spines 22mm in length. Testes two measuring 0.46-0.48 by 0.12-0.14mm. Cement gland elongated 0.32-0.34 by 0.86-0.88. Seminal vesicle posterior to cement reservoir 0.40-0.50 by 0.92-0.98mm, the Saeftigen's pouch 68-70mm in length. Bursa small 0.84mm in length. Female length 9.6-10.4mm much longer than males. Hooks and spines similar in both sexes. Uterine bell prominent 84-85µm, uterus somewhat muscular 190-200 µm, vagina opens into terminal gonospore. Eggs numerous, double walled, without polar prolongations, measuring 22-22 by 10-12 µm. Since the original worms were described from a different fish *Wallago attu* from Punjab India. The present specimens are reported as same *Pallisentis roparensis* due to similar morphological characters but from different host and locality.

***CENTRORHYNCHUS MUNIFI* SP.N. (ACANTHOCEPHALA: CENTRORHYNCHIDAE; VAN
CLEAVE, 1916) FROM THE BIRD *AQUILA NIPALENSIS* (STEPPE EAGLE)
IN LARKANA, SINDH, PAKISTAN**

**Sanjota Nirmal Das, Abdul Saeed Hulio*, Aly Khan, Tasmina Leghari,
Seema Mahesar and Mahek Hulio**

Department of Zoology, University of Sindh, Jamshoro

*CDRI, Pakistan Agricultural Research Council, University of Karachi, Sindh, Pakistan

*Corresponding Author: sanjota.bai@usindh.edu.pk

Parasitic infection is a serious health problem in the world, especially in developing countries. This study was conducted to find the Helminth parasites in *Aquila nipalensis* (steppe eagle) from District Larkana, Sindh, Pakistan. The birds were anaesthetized, autopsied and examined for helminth parasitic infections. Twenty two birds were dissected, out of them, eleven were found infected with 62 (22 males, 40 females) specimens recovered from the small intestine. The worms were preserved with alcoholic series, stained with Mayer's carmalum. Specimens were identified as *Centrorhynchus munifi* sp.n. belongs

to family *Centrorhynchidae* (Van Cleave, 1916). The new species is characterized by having: **Male:** smaller in size as compared to female showing apparent sexual dimorphism, Trunk elongate, curved or spiral, hypodermic nuclei 2 on the right side of the body and 3-4 on the left, Proboscis elongate, long, Hooks rooted, mature and equal in both sexes. Neck short, Proboscis receptacle double walled with a single nuclei at its base, lemnisci unequal, the left longer as compared to the right. Testes two almost rounded. Cement glands three elongate, tubular. Cement reservoir prominent. Bursa large with sensory plates. **Female:** Trunk with 2 hypodermic nuclei on each body side. Proboscis long, receptacle, Lemnisci unequal, in most female specimens, ovarian balls present, Genital pore sub terminal, Vagina followed by selector apparatus, uterus small, Eggs few small and oval shaped.

TRADITIONAL AND MACHINE-LEARNING BASED INTERROGATION OF NUCLEAR rDNA AND MITOGENOMIC DATA OF A NEWLY SEQUENCED NOTOCOTYLID TREMATODE FOR ESTIMATING GENETIC DIVERSITY AND PHYLOGENETIC AFFINITIES

Daim Saood¹, Suleman², Gauhar Rehman¹, Abid Ali^{1*}, Mian Sayed Khan²,
Guo-Hua Liu^{3*} and Nehaz Muhammad⁴

¹Department of Zoology, Abdul Wali Khan University Mardan, Mardan

²Department of Zoology, University of Swabi, Anbar, Swabi

³Research Center for Parasites and Vectors, College of Veterinary Medicine,
Hunan Agricultural University, Changsha 410128, Hunan, China

⁴Hebei Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology,
Hebei Collaborative Innovation, Center for Eco-Environment, College of Life Sciences,
Hebei Normal University, Shijiazhuang, Hebei Province, P. R. China

*Correspondence: drabid@awkum.edu.pk; liuguohua5202008@163.com

Notocotylidae is a family of trematode parasites classified within the superfamily Pronocephaloidea of the order Plagiorchiida. Adult notocotylid colonize the intestines of avian and mammalian hosts. In this study a specimen of notocotylid, identified as *Catatropis* sp., was recovered from the intestine of an aquatic avian host, *Charadrius dubius* in district Swabi and sequenced its 18S, ITS and 28S rDNA for molecular identification and phylogenetic affinities with its congeners. The nearly complete mitochondrial genome of the present *Catatropis* sp. was also sequenced, annotated and analyzed for gene organization, nucleotide and codon usage, genetic diversity, and phylogenetic relationship among other trematodes selected from other families. To date, no molecular study on these parasites has been published from Pakistan and the available molecular studies on *Catatropis* species from other parts of the world are only based on partial nuclear ribosomal DNA sequences. Moreover, the mitochondrial genome of only four species of the family Notocotilidae has been sequenced, characterized, and utilized for phylogenetic analyses. But, the mt genome of any member of the genus *Catatropis* has not been sequenced or characterized and analyzed yet. The 28S rDNA region of the present *Catatropis* sp. was 99.1% identical with that of *C. indicus* from Australia and *C. vietnamensis* from Vietnam. Phylogenetic tree based on 28S sequences placed the *Catatropis* sp. closed to *C. vietnamensis* in a clade uniting *C. indicus* on a separate branch which intensifies that *Catatropis* sp. and *C. vietnamensis* are closely related species. The nearly complete mitogenome sequences consist of 11 protein-coding genes (PCGs), 19 transfer RNAs, and two ribosomal RNAs. Both, Maximum likelihood and Bayesian inference (BI) phylogenetic methods based on amino acid sequences of 11 PCGs, placed our specimen of *Catatropis* sp. closed with *Notocotylus* sp. (*N. intestinalis*; MT560390) in a clade containing other notocotylids including *Ogmocotyl ailuri* and *Ogmocotyl sikae*. In PCGs, *cox1* (0.171) and *nad1* (0.175) are the most conserved and *nad2* (0.266),

nad5, (0.250) nad6 (0.219) and nad4 (0.218) are the least conserved among notocotylids. The nucleotide diversity (π), Kimura 2 parameter distance, non-synonymous/synonymous substitutions rate and relative synonymous codon usage (RSCU) was also calculated. Additionally, we predict the secondary structure, active site and ligand binding site of a selected gene atp6 using deep neural network and a machine learning based method, support vector machine (SVM) These findings may provide a foundation for further research direction, drug discovery, surveillance and effective control of helminth parasite.

SECTION - V
**FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY,
MARINE BIOLOGY**

- 1. FRESHWATER BIOLOGY AND FISHERIES**
- 2. MARINE BIOLOGY**
- 3. PALEONTOLOGY**
- 4. WILDLIFE, DIVERSITY AND CONSERVATION**
- 5. BIODIVERSITY**
- 6. ECOLOGY**
- 7. ENVIRONMENTAL BIOLOGY**

1. FRESHWATER BIOLOGY AND FISHERIES

ECOLOGICAL AND MORPHOLOGICAL STUDIES OF INDIAN PEAFOWL (*PAVO CRISTATUS*) OF KHAR CENTRE KHAR DIVISION AT KHIRTHAR NATIONAL PARK, SINDH, PAKISTAN

Mohsin Ali Brohi¹*, Ghulam Sarwar Gachal², Punhal Khan Lashari² and Sajid Siyal³

¹Sindh Wildlife Department Government of Sindh

²Department of Zoology, Department of Fresh Water Biology and Fisheries,
University of Sindh, Jamshoro

³Department of Wildlife Management, Shaheed Benazir Bhutto University of
Veterinary and Animal Sciences, Sakrand

*Corresponding Author: mohsinalibrohi143@gmail.com

The peafowl belongs to family Phasianidae and order Galliformes commonly members of this family called Game Birds. There are only three species of peafowls throughout the world, only one among of them Indian peafowl (*Pavo cristatus*) is present in Pakistan. In Pakistan, Blue peafowl occur in Narowal district Punjab and Tharparkar district Sindh and now this bird is breeding in natural habitat of Khar Centre Kirthar National Park. Kirthar National Park is being the one and only national park in Sindh. The present research work of the Ecological and Morphological studies carried out in 8 km radius of Khar Centre Kirthar National Park from July 2021 to June 2022. The estimated population of Indian peafowls were recorded 250. Among them Adult were 30%. Subadult 36% and 34% peachicks and male, female ratio is 34% and 66%. Thirty specimens of Indian peafowls were collected and different body parameters were measured. Average body length beak to cloaca was measured 64cm and 52.8cm for adult male and female respectively. While for subadults male and female it was 49.2cm and 44cm respectively. And while for peachicks it was 38.4cm and 29.6cm respectively. Average beak length was measured 2.96cm to 1.5cm in adult, subadult and peachick. Tail length varied from 146.8 cm to 29.2 cm in different categories of Indian peafowl. Wingspan length varied from 156cm to 54cm. while crest length varied from 8.2cm to 4cm in adults and in peachicks respectively. The temperature varies from 100C to 450C in study area. The water used by the Indian peafowl (*Pavo cristatus*) its pH. Salinity. Conductivity and TDS is (pH: 8.60±0.59), (Salinity: 0.60±0.28ppt), (Conductivity: 1609.33±300.57µs/cm) and (TDS: 790.67±147.64mg/l). Due to some Threats from wild cats, jackals and domestic dogs, the mortality occurs and the mortality ratio was observed 13.79% and 3.84% in males and females respectively during study period and survival ratio was observed in males and females as 86.20% and 96.15% respectively. In the present study the estimated population of Indian peafowl (*Pavo cristatus*) observed as 31 individuals/km. Though mortalities occur, but population is increasing and environment is encouraging the species ratio. The present study indicate that the Khar Centre Kirthar National Park is the suitable place for the breeding and conservation of Indian peafowl (*Pavo cristatus*). This study was carried out for the first time from inside Khar Centre Kirthar National, Sindh.

MOLECULAR AND PHYLOGENETIC ANALYSIS OF FISH FAUNA OF RIVER GOKAND DISTRICT BUNER PAKISTAN

Kausar Saeed¹ and Munawar Saleem Ahmad²

¹Department of Zoology, University of Buner; ²Department of Zoology, University of Swabi

*Corresponding Author: Kausarsaeed@yahoo.com Kausarsaeed@ubuner.edu.pk

DNA barcoding technique for fish identification is an effective, rapid and precise method as compared to the morphological method. Cytochrome c oxidase 1 gene- based DNA barcoding is

frequently used in species identification and biodiversity studies. The recent study was designed to identify the fish fauna of river Barandu with the help of DNA barcoding and phylogenetic tree analysis resulting COX1 gene sequences which were used in the construction of genetic diversity and evolutionary history of fish fauna of river Gokand district Buner Pakistan. A short template of DNA sequence of 650 base pairs (COX1) was amplified, sequenced and analyzed by using different bioinformatics tools. The pairwise distance and phylogenetic analysis by Maximum Likelihood (ML) tree based on Kimura 2 Parameter method was constructed by using MEGA11 software. The present study was conducted on molecular phylogenetic study of fish fauna of river Gokand district Buner Pakistan during July 2022 to July 2023. In the recent study we have reported seven species belonging seven genera, four orders and three families of fish. The reported species are *Schizothorax plagiostomus*, *Metacembelus armatus*, *Channa gachua*, *Puntius sophore*, *Barilius pakistanicus*, *Campostoma anomalum* and *Schistura punjabensis*. The reported species were treated molecular characterized and constructed phylogenetic tree of each species.

IMPACT OF CLIMATE CHANGE ON THE FISHES OF KOTRI DOWNSTREAM, SINDH, PAKISTAN

Dhani Bux Mishori*, Bakhtawar Soomro and Shakeel Ahmed Memon

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: ghanibuxmashori2015@gmail.com, bakhtawarsoomro145@gmail.com

During the present studies ecological assessment on the various fresh water fish's habitat has been carried out (April 2022 to September 2022) from the area Kotri downstream. The study of the aqua-Fauna reveals 17 fresh water fishes, out of which 04 were recorded as rare. The ecological assessment was focused on the water quality parameters that had impact on the fishes. All samples were collected and brought in the laboratory. All required protocols were employed as per SEQS guidelines. Under research water quality parameters indicated the ranges of temperature of water was recorded 13°C in winter and 34°C in summer. (μ 20.34642, σ 8.732125), pH of water between 8.2 to 8.9, alkalinity of water 267 to 480 mg/l, hardness of water 620 to 1150 mg/l, salinity of water from 0.22 to 27 ppt of both the freshwater. Therefore, the water quality samples indicated the suitability that supports the growth, dispersal, production and reproduction of aqua- fauna particularly fishery occurring in area Kotri downstream.

DETERMINATION OF HEMOGLOBIN CONCENTRATION OF FIVE FISH SPECIES COLLECTED FROM ROHRI CANAL NEAR DALEEL DARO VILLAGE, SAKRAND SINDH, PAKISTAN

Shaista Jalbani¹*, Sana Noor², Majid Hussain Soomro³, Sahir Odhano¹ and Bilques Baloch¹

¹Department of Fisheries and Aquaculture, Shaheed Benazir Bhutto University of Veterinary and Animal +Sciences, Sakrand

²Department of Veterinary Parasitology, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand

³Department of Veterinary Pathology, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand

*Corresponding Author: jalbanishaistagmail.com

Current study was design to determine hemoglobin concentration from of 05 fish species, *Channa striata*, *Wallagu attu*, *Cirrhinus cirrhosus*, Rita rita and *Labeo rohita* respectively. Total 75 fish samples

were collected from month of March –July 2023 from the Rohri canal near village Daleel Daro Sakrand Sindh, with the help of fisherman. Blood of fishes was collected from caudal vein and heart of fishes on the spot with the help of sterilized syringe then preserved in EDTA vials for subsequent study. Analysis of blood parameters was done in the Laboratory of Fisheries and Aquaculture and in the Laboratory of Molecular Biology University of Veterinary and Animal Sciences, Sakrand. Concentration of Hemoglobin was recorded 9.8 in *Channa striata*, 10.0 in *Wallagatu*, 10.0 in *Cirrhinus cirrocius*, 11.0 and 7.5 in *Labeo rohita* respectively. Concentration of Hemoglobin observed found to be in normal ranges. Finally concluded that fish collected from Rohri canal near Daleel Daro Village was found in healthy condition.

EFFECT OF WATER FLOW ON THE MORPHOLOGICAL CHARACTERS OF ICHTHYOFAUNA AT THE INDUS RIVER DOWNSTREAM, SINDH, PAKISTAN

Maryam Khalid and Muhammad Younis Laghari

Department of Fisheries and Aquatic Sciences, University of Sindh, Jamshoro

*Corresponding Author: maryamsheikh1947@gmail.com

Freshwater environments are disproportionately biodiverse, yet they are also under threat from human activities and environmental change, which have led in significant losses in freshwater biodiversity over the last decades. The flow rate of water is a critical environmental component for fish habitat viability. The fish life cycle consists mostly of migration, growth, feeding, and reproduction. The primary function of water flow in terms of ecological relevance is to enhance fish mating. However, in order to be moved downstream, the fish must situate themselves inside the water column and aggressively swim out of sloughs and back waters. Fish habitats are of amazing scientific value for research on river ecological control and restoration, as well as assessing aquatic creature survival circumstances. The current investigation was carried out to examine the morphological model about changes in the quantity of chromophores and pigment attention in several downstream fish species. The samples were collected from eight major touchdown zones between March 2017 and February 2021. The most recent Ichthyofaunal survey of the Indus River's downstream reveals a total of 124 species. They are members of 12 Orders and 43 families. Cyprinidae was discovered to be the leading family with 30% of the fish species detected, followed by Bagridae with 15% of the fish species. Station 1 (RM) gathered the greatest number of species (115), whereas station 8 (JS) collected the fewest (56). Morphological changes in 14 fish species were observed during the study. The results revealed that the fish habitats and morphological changes occur as a result of stagnant water and are distributed in a limited number of areas with a slow waft and complicated habitat types on both banks and a large shallow habitat location with obvious eddy modifications toward the downstream. As a result, comprehensive conservation measures are needed to maintain the normal flow of the downstream (Indus River) for increased fish conservation.

CONTRIBUTION OF SUSTAINABLE FISHERIES AND AQUACULTURE TO FOOD SECURITY IN PAKISTAN

**Nargis Naheed^{1*}, Naheed Bano¹, Asher Azeem¹, Areesha Fatima¹,
Muhammad Aatif Shahab¹ and Abida Razi¹**

¹*Department of Zoology, Wildlife and Fisheries, Muhammad Nawaz Shareef University of Agriculture, Multan*

*Corresponding Author: nargisnaheed036@gmail.com

The prominence of aquaculture and fisheries towards nutrition and food security in the present and future is influenced by a complex interplay of environmental, development, policy, and governance

challenges. At present, Pakistan is regarded as one of the prime water-stressed nations among the world. Extreme temperatures, low precipitation, inadequate arable land, the absence of productive soils, and dwindling water resources characterize the tough physical environment of the world. Rapidly growing population and excessive extraction of groundwater continue to stress the prevailing water resources. Furthermore, it is anticipated that global climate change will have adverse effects on water resources and agriculture systems that are already depleted. As a result, potential for the enhancement of agriculture sectors particularly fisheries or aquaculture production systems to meet increasing demands of foods are extremely inadequate, despite the fact that majority of the current demand is being met through imports. It is expected that by the year 2050, Pakistan will have to import all of the goods it requires. In the meanwhile, there is a wide variety of technology that can help to save land and water, and it has the ability to contribute to the production of enough energy to meet the needs of the home market. Therefore, the extension agent's role is crucial in promoting innovative technologies and educating the agricultural community so that the recommendations can be put into practice to satisfy the dietary requirements of the nation. These recommendations demonstrate the necessity to strengthen the fisheries and aquaculture production systems so as to ensure food security and combat climate change.

UTILIZATION OF PEA PEEL MEAL IN THE DIET OF JUVENILES *LABEO ROHITA* IN INTENSIVE AQUACULTURE

**Aasia Karim^{1*}, Muhammad Mudassar Shahzad², Mohammad Shoaib³,
Sobia Khawaja⁴, Brishna¹ and Shagufta Riaz³**

¹*Department of Zoology, Sardar Bahadur Khan Women's University, Quetta*

²*Department of Zoology, Division of Science and Technology, University of Education, Lahore*

³*Department of Zoology, University of Karachi, Karachi*

⁴*Department of Zoology, Federal Urdu University of Arts, Science and Technology, Karachi*

*Corresponding Author: aasiakarim@gmail.com

Vegetable protein is the cheapest protein source to replace fishmeal. It is widely used as a perfect replacement for high-quality fishmeal in many classes of aquaculture feed due to its high availability, and stable supply. *Pisum sativum* has been successfully used as an energy and protein-rich dietary feed in aquaculture feeds designed for various species of aquatic organisms. A trial was designed to find out the effects of pea peel meal on *Labeo rohita* growth performance. Fish meal was substituted with pea meal in graded levels in the diet of *Labeo rohita* juveniles. Study confirmed that 40% (DIII) fishmeal replacement by pea peel meal had considerable positive effect on growth of Rohu, which is followed by 60% (DII). Both produced very chunky fish in contrast to the control. The optimal protein requirement of *Labeo rohita* was found to be 30% protein and 25%. A decreased growth was observed with increase in fishmeal substitution (such as for 60% to 80% substitution) in DI and DII. It was also observed that pea protein could be used as an alternative protein source to minimize the inclusion level of fishmeal for *Labeo rohita* juveniles.

LEVELS OF GONADAL HORMONES IN THE FAECES OF INDIAN PANGOLIN (*MANIS CRASSICAUDATA*) FROM POTHWAR PLATEAU

**Tariq Mahmood^{1*}, Asma Bibi¹, Faraz Akrim^{2,7}, Irfan Zia Qureshi³, Muhammad Sajid Nadeem¹,
Amjad Rashid Kayani¹, Muhammad Mushtaq¹, Nausheen Irshad⁴,
Shazia Shamas⁵ and Shumaila Noreen⁶**

¹Department of Zoology, Wildlife and Fisheries, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi

²Zoology Department, University of Kotli, Kotli, Azad Jammu & Kashmir

³Department of Zoology, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad

⁴Zoology Department, University of Poonch, Rawalakot, Azad Jammu & Kashmir,

⁵Zoology Department, Rawalpindi Women University, Rawalpindi

⁶Zoology Department, Hazara University Mansehra, Khyber Pakhtunkhwa

⁷IUCN SSC Pangolin Specialist Group, c/o Zoological Society of London, Regent's Park London, England

*Corresponding Author: tariqjanjua75@uaar.edu.pk

Non-invasive techniques to monitor the gonadal functioning of a species by quantifying reproductive hormones excreted in the urine and faeces have recently been developed successfully for some target wildlife species. We estimated the concentrations of testosterone, estradiol, and progesterone, in male and female Indian pangolins (*Manis crassicaudata*) inhabiting the Pothwar Plateau, Pakistan. We analyzed scat samples collected in the field during 2021-2022 using commercially available ELISA hormonal kits. Testosterone levels were found to be elevated from May (0.59 ng/ml) until October (1.28 ng/ml). Estradiol levels were raised from May (0.59 pg/ml) until December (2.64 pg/ml). Progesterone concentrations were relatively low from January until March but increased from April (4.85 ng/ml) through December (14.0 ng/ml) with the highest concentrations (20.13 ng/ml) in September and October. Mean hormone (testosterone, estradiol and progesterone) concentrations were elevated in winter compared to summer ($p < 0.001$). These findings indicate that Indian pangolin breeds once a year with mating occurring between May and October. The gestation period peaks at the end of December when estradiol and progesterone concentrations are highest, while parturition.

DNA BARCODING OF COMMERCIALY IMPORTANT TROUT SPECIES (SALMONIDAE: SALMONIFORMES) OF KAGHAN AND SWAT, KPK PAKISTAN

Amina Qureshi, Nuzhat Shafi* and Tasleem Akhtar*

Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad

Corresponding Author: nuzhatshafi@gmail.com, tasleem.akhtar@ajku.edu.pk

In the current study, population-wise genetic analysis of rainbow trout based on CO1 gene was carried out for molecular identification. Using the Clustal W program, the total number of 40 sequences of *Oncorhynchus mykiss* was aligned with other international sequences retrieved from GenBank, generated a total length of 761 positions out of which 337 were conserved, 294 variables, 13 parism-info and 279 were singleton. CO1 gene displayed an average base composition of (23.8%) A, (16.7%) G, (30.4%) T and (29.1%) C. Total number of haplotypes were observed ($h=10$). The average haplotype diversity (hd) was 0.728 ± 0.068 , while nucleotide diversity (pi) was 0.0318 ± 0.02553 (mean \pm SD), molecular diversity index showed the average number of transitions (10.75), number of transversions (10.00), and number of substitutions (20.75) in all *O. mykiss* populations. Analysis of molecular variance (AMOVA) using CO1

showed that low genetic variance among population (31.12) and high genetic variation within population (82.23) indicated that all *O. mykiss* population were genetically identical.

BIOFILTRATION OF RIVER-BASED POLLUTED WATER SAMPLES FROM MUZAFFARABAD AZAD JAMMU AND KASHMIR

Hafsa Khalid, Nuzhat Shafi* and Tasleem Akhtar*

Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad

Corresponding Author: nuzhatshafi@gmail.com, tasleem.akhtar@ajku.edu.pk

Important issues related to water contamination can be effectively addressed using the biofiltration method. The combined effects of biological oxidation, adsorption, and filtering processes contribute to the removal of contaminants. This research aims to assess the performance of a quartz sand biofilter in eliminating various physico-chemical and biological pollutants. It also aims to compare the effectiveness of upflow and downflow biofilters and develop an empirical model for designing quartz sand biofilters for the treatment of polluted river water. The experiments involved two biofilter units constructed with plastic and equipped with pipes, which were filled with quartz sand as the filter media. The results demonstrate that quartz sand biofiltration can effectively eliminate pollutants from river water. The removal efficiencies of turbidity, total dissolved solids, total suspended solids, chemical oxygen demand, biological oxygen demand, and total coliform using the upflow biofilter were approximately 5.4-23%, 3.7-41%, 4.8-40.7%, 5.5-43%, 4-24%, and 3.04-17.2%, respectively. Meanwhile, the downflow biofilter achieved removal efficiencies of approximately 7.5-25.6% for turbidity, 6.1-43.2% for total dissolved solids, 6.9-48.3% for total suspended solids, 7.6-45.7% for chemical oxygen demand, 8-30% for biological oxygen demand, and 4.86-19% for total coliform. These results indicate that the downflow biofilter slightly outperforms the upflow biofilter in terms of removing physical, chemical, and biological parameters. The effectiveness of pollutant removal is dependent on the applied hydraulic retention time, with longer retention times yielding better biofilter performance. Additionally, the downflow biofilter has been demonstrated to be slightly more efficient than the upflow biofilter. It is important to note that this biofiltration method is cost-effective and does not introduce any harmful substances into the environment.

COMPOSITION, DISTRIBUTION AND COMMUNITY STRUCTURE OF ICHTHYOFAUNA IN THE UPPER NEELUM RIVER AND ITS TRIBUTARIES, AZAD JAMMU & KASHMIR

Rabiha Zareen, Nuzhat Shafi* and Tasleem Akhtar*

Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad

Corresponding Author: nuzhatshafi@gmail.com, tasleem.akhtar@ajku.edu.pk

The present study was designed to explore the diversity of fish fauna in the Neelum River, as well as to assess the environmental factors influencing the distribution of Ichthyofauna. Seven environmental factors, namely water temperature, pH, dissolved oxygen, total dissolved solids, turbidity, electrical conductivity, and water speed, were analyzed at selected sites along the main course of the Neelum River, its associated tributaries, and their mixing points. All parameters were found to be within the optimal range for the survival of fish fauna. A total of 4 fish species were identified in the selected study sites: *Oncorhynchus mykiss*, *Salmo trutta fario*, *Schizothorax plagiostomus*, and *Glyptosternum reticulatum*. *S. plagiostomus* was found to be abundant in the lower reaches of the Neelum River, while *S. trutta fario* was more prevalent in the upper

Neelum river sites. The research work, coupled with visual observations, indicated that the Neelum River is relatively unpolluted. However, the river's environment has been disrupted due to cloudbursts, flooding, and their subsequent consequences. When compared to previous research studies, there have been minimal changes in the physico-chemical parameters, except for a slight alteration in the pH value. Nevertheless, a significant decline in the diversity of fish fauna has been observed. This research study serves as a foundation for further investigations into the causes behind the decrease in fish diversity in the Neelum Valley.

EFFECTS OF PROBIOTICS ON GROWTH PERFORMANCE, INTESTINAL MICROBIAL FAUNA AND HISTOLOGY OF *LABEO ROHITA*

Tehniat Khaleeq^{1*}, Huma Naz¹, Tanveer Ahmed², Syed Qawar Ali Shah¹, Amjad Islam Aqib³, Sajid Abdullah⁴, Muhammad Usman⁵ Iqra Zulfiqar¹, and Murtaza Manzoor¹

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

³Department of Medicine, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

⁴Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

⁵Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

*Corresponding Author: tehniatkhalieeq@gmail.com, dr.humanaz98@gmail.com

The objective of present study was to understand the influence of probiotics on growth parameters, microbial fauna and intestinal histology of the fish (*Labeo rohita*). The fish were segregated into two distinct groups. In initial group maintain probiotics culture were used a diet enriched with probiotics supplementation, while the subsequent group, serving as the control, received the basal diet. The trial extended over a span of two months. During trail growth performance of fish was observed by measuring daily weight gain (DWG), daily length gain (DLG), specific growth rate (SGR), condition factor (CF) and feed conservation ratio (FCR) on weekly basis. The intestine of fish was also be observed for microbial fauna and histological alterations. The results showed that the probiotics feed was beneficial for overall growth and health status of the fish. Probiotics have positive effects on fish growth performance and showed significantly increased in DWG, DLG, FCR and SGR than the control group. As probiotics feed had great influences on intestinal microbial fauna of fish, probiotics caused modulation of gut microbial fauna, probiotics produced bacterial species in gut of the fish. The gut microbial fauna community showed distinct distribution, identified species were *Sachharomyces cerevisiae*, *Escherichia coli*, *Xathomonas* and *Actinomyces*. Histomorphometric paramters of intestine showed that villus height (VH), villus width (VW), crypt depth (CD), muscularis mucosa (MM) and muscularis extrna (ME) were increased significantly in probiotics treated groups as compared to the control group.

DNA BARCODING AND PHYLOGENETIC ANALYSIS OF FISH FAUNA OF RIVER BARANDU DISTRICT BUNER PAKISTAN

Kashif Ahmad¹, Kausar Saeed^{1*}, Numan Hadi¹, Zameer Alam¹ and Munawar Saleem Ahmad²

¹Department of Zoology, University of Buner

²Department of Zoology, University of Swabi

*Corresponding Author: Kausarsaeed@yahoo.com, Kausarsaeed@ubuner.edu.pk

DNA barcoding technique for fish identification is an effective, rapid and precise method as compared to the morphological method. Cytochrome c oxidase 1 gene- based DNA barcoding is frequently used in species

identification and biodiversity studies. The recent study was designed to identify the fish fauna of river Barandu with the help of DNA barcoding and phylogenetic tree analysis resulting COX1 gene sequences which were used in the construction of genetic diversity and evolutionary history of fish fauna of river Barandu district Buner Pakistan. A short template of DNA sequence of 650 base pairs (COX1) was amplified, sequenced and analyzed by using different bioinformatics tools. The pairwise distance and phylogenetic analysis by Maximum Likelihood (ML) tree based on Kimura 2 Parameter method was constructed by using MEGA11 software. The present study was conducted on molecular phylogenetic study of fish fauna of river Barandu district Buner Pakistan during July 2022 to July 2023. In the recent study we have reported 11 species belonging eleven genera, five orders and six families of fish. The reported species are *Tor putitora*, *Schizothorax plagiostomus*, *Lebio rohita*, *Cyprinus carpio*, *Metacembelus armatus*, *Channa gachua*, *Puntius sophore*, *Barilius pakistanicus*, *Hypomesus japonicus*, *Oreochromis niloticus*, and *Schistura punjabensis*. The reported species were treated molecular characterized and constructed phylogenetic tree of each species.

PHYTOGENIC FEEDS BENEFITS AND EFFECT ON THE SEX RATIO OF FISH

Nimra Zahid^{1*}, Huma Naz¹, Iqra Zulfiqar¹, Amna Marium² and Tanveer Ahmad³

¹Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

²Department of Zoology, The Government Sadiq College Women University, Bahawalpur

³Department of life Sciences, Khawaja Fareed University of Engineering and Technology, Rahim Yar Khan

*Corresponding Author: Zahidnimra1@gmail.com

Natural substances extracted from plants called phytochemicals, are added to meals to improve animal productivity. Plant-derived products have been claimed to have beneficial properties such as: growth promotion, anti-stress, anti-pathogenic, immunostimulation, and appetite stimulation properties in fish and shrimp aquaculture due to the presence of several bioactive substances such as essential oils, alkaloids, phenolics, flavonoids, glycosides, terpenoids, saponins, steroids, and tannins. Phytochemical feeds supplement contain androgens and phytoestrogens that obtain from plants and have similarities to mammalian androgen and estrogens. 17 α -Methyltestosterone is the most often utilized sex reversal drug in aquaculture. The fish fed with lettuce had more male ratio than female. Fish that received neem seed has more female ratio as compare to male. Fish fed with phytochemical feed (pawpaw seed + lettuce seed) in equal concentration produced 100% masculinization. The productivity of aquaculture is enhanced by using these tactics. This review addresses the possible impact of medicinal herbs on fish reproductive performance while concentrating on the advantageous impacts of these phytochemicals as feed additions. Interest in the impact of nutrition on the reproductive issues that are frequently observed in ruminants is widespread.

COMPARATIVE ANALYSIS OF GENOTOXIC EFFECTS OF THREE ENDOCRINE DISRUPTOR CHEMICALS; ATRAZINE, BENZO (A) PYRENE AND CARBENDAZIM IN PERIPHERAL BLOOD ERYTHROCYTES OF THE FRESHWATER FISH SPECIES *CYPRINUS CARPIO* BY USING COMET ASSAY TECHNIQUE

Ahsan Khan^{1,2}, Nazma Habib Khan² and Kevin J Parson³

¹Department of Zoology, University of Swabi, Swabi

²Department of Zoology, University of Peshawar, Peshawar

³Department of Zoology, School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, UK

Corresponding Author: ahsan@uoswabi.edu.pk

The endocrine disruptor chemicals, among other pesticides, have drawn attention due to their toxicity and detrimental impacts on fish, aquatic ecosystems, and human health. Using the Comet

assay, the present study examined the genotoxic effects of three endocrine disruptor chemicals in peripheral blood erythrocytes of the freshwater fish species *Cyprinus carpio*, also known as common carp. For this objective, fish were exposed to these endocrine disruptors at four distinct graded concentrations (5, 15, 25, and 45 ppb) for periods of 5, 15, and 30 days, respectively. Common carp exhibited considerably varying levels of DNA damage in terms of total comet score against each chemical's doses and durations. The study found that, when comparing the treated groups to the control group, statistically significant differences in DNA damage were observed based on both concentrations and exposure times. Specifically, significant comet scores ($***P=0.001$) were observed against 15 ppb of benzo (a) pyrene and carbendazim, which are 198.3 ± 27.5 and 159.7 ± 14.9 , and against 25 ppb of atrazine were 127.3 ± 20.4 after exposure of fish for 5 days respectively in comparison to exposure for 15 and 30 days. Benzo (a) pyrene was found to have a higher comet score than carbendazim and atrazine, indicating that it is more harmful in terms of toxicity. As part of environmental monitoring programmed, the Comet test may be used to assess the toxicity of various contaminants, and the resulting data validated this approach.

MANSURAL CHARACTER AND THEIR VARIATION IN SPINAL EEL , *MASTACEMBELUS ARMATUMS* FROM RIVER INDUS NEAR JAMSHORO SINDH, PAKISTAN

Bilqees Baloch and Shaista Jalbani*

Department Fisheries and Aquaculture, Shaheed Benazir Bhutto University of veterinary and Animal Sciences Sakrand

*Corresponding Author: [jalbanishaista@gmail.com](mailto:jalbanihaista@gmail.com)

Present study carried out on mensural characters of *Mastacembelus armatus* from river Indus Jamshoro. Total 140 experimental fish specimens from 28.1-51.1cm and body mass was fluctuated from 28-366g in total weight length respectively. It was noticed from results of morphometric parameters that all sexes were positively correlated in *Mastacembelus armatus* from river Indus near Jamshoro. It was testified from the results of T-test that no significant ($P > 0.05$) difference was recorded in count of meristic characters in case of *Mastacembelus armatus* fish from river Indus near Jamshoro. It was noticed from calculation of b values that ideal growth was found in male, female and mutual sexes in *Mastacembelus armatus* fish from river Indus near Jamshoro. Relationship between weight length and obesity values that *Mastacembelus armatus* from river Indus specify acceptable positive allometric growth in both male and female and mutual sexes respectively. Finally decide that there was a single homogenous stock of *Mastacembelus armatus* exists in river Indus.

IDENTIFICATION OF POTENTIAL FISHING ZONES OF PAKISTAN AND INCORPORATION OF BLOCKCHAIN TECHNOLOGY IN THE IMPLEMENTATION OF A FRAMEWORK FOR FISH EXPORT

Mubashir Ali Shah, Saima Majeed*, Sadaf Afridi, Mohammad Idrees and Asif Inam

Department of Maritime Sciences, Bahria University Karachi Campus, 75260 Karachi

*Corresponding Author: saima.bukc@bahria.edu.pk

Strong frameworks to oversee fishing licenses and locate possible fishing areas are more important than ever as global fisheries confront growing difficulties from overfishing, illicit activity, and

environmental deterioration. This study explores the utilization of blockchain technology to create a comprehensive framework for fishing licenses and to identify possible fishing grounds within Pakistan. The research aims to enhance the transparency, traceability, and accountability of fisheries management by employing the decentralized and secure characteristics of blockchains to integrate conventional ecological assessments with cutting-edge technology to create a complete system that assures sustainable fishing practices while also lowering the risks associated with illegal, unreported, and unregulated (IUU) fishing. The study uses a variety of sources, including historical fishing methods and data from ecological studies. The application process, eligibility standards, and compliance monitoring are all smoothly integrated into the comprehensive framework for fishing licenses. Using blockchain technology, the licensing process may be streamlined and protected by providing a tamper-proof ledger for the recording and confirmation of licensing transactions. Initial findings indicate that blockchain technology can effectively protect the authenticity of fishing license documents, curtail fraudulent activity, and enhance overall governance in the fisheries industry.

OBSERVATION OF BEHAVIOR OF FRESHWATER SHARK (*WALLAGO ATTU*) IN DIFFERENT CEMENTED TANKS IN CAPTIVITY

Muthar Ali and Anila Naz Soomro*

Department of Fisheries and Aquatic Sciences, University of Sindh Jamshoro

*Corresponding Author: anila.soomro@usindh.edu.pk

The study was carried out to observe the behavior of *Wallago attu* fish in small to large captive cemented tanks. The experiment was conducted on 3 cemented tanks T1, T2 and T3. T1 was Circular tank measuring 6 feet diameter and 2.5 liter water level. Water flow was 2L/min. T2 tank was rectangular in shape with 6 feet wide and 12 feet long, was filled with 4 liter water level, while T3 was 25 feet wide and 50 feet long filled with 2.5 feet water level. In all the tanks total 2 male and 1 female having weight up to 2 kg of *Wallago attu* was observed for 15 days separately with different feeding items. Starting 2 days were not fed because of shifting stress and conditioning purpose. From 3rd to 5th days fish were fed with live fishes *Oreochromis niloticus* and *Chanda nama*. On the 6th to 8th day fish was observed with pelleted floating feed 45% CP Sind Aqua feed. On the 9th and 11th day fish was observed with chicken viscera whole and chicken viscera chopped with long and short cuts. On the 12th to 14th days fish were fed with chopped live fish and fish meal. In the whole experiment physico-chemical parameters were measured as pH 7.2-7.5 salinity was 0.0007-0.00010, TDS 711-750 and temperature was 26-30°C respectively. Water inlet was common to all the treatments. In this experiment it was observed that fish from T1 and T2 were found critical in condition they did not fed on any type of feed and they fight with each other. One mortality observed in T1, mortality cause was wounds on its body. Many wounds were noted on the body of all fish in T1 and T2. Some wounds were clearly shown teeth patches which clearly indicate fish fight and when observed fish buccal cavity, some flesh and meat particles were found as well. While in T3 fish behavior was observed satisfactory, they fed on all types of feeds given to them and no wounds or scratches were found on the body.

**PARTIAL REPLACEMENT OF FISHMEAL WITH PLANT PROTEIN SOURCE THE
SOYBEAN MEAL IN THE DIETS OF BLACKFIN SEA BREAM,
ACANTHOPAGRUS BERDA JUVENILES.**

**Naveed Ahmad^{1*}, Pirzada Jamal A. Siddiqui², Amjad Ali²; Muhammad Amin³, Saima Majeed¹,
Ayesha Umer¹, Shaista Urooj¹ and Aisha Qureshi¹**

¹*Aquatic Diagnostic and Research Centre, Department of Maritime Sciences, Bahria University Karachi, Karachi*

²*Centre of Excellence in Marine Biology, University of Karachi, Karachi*

³*Toxicology Laboratory, Department of Zoology, University of Karachi, Karachi*

Corresponding Author: naveedahmad.bukc@bahria.edu.pk

Pakistan exported fishery products worth \$ 496 million in the financial year that ended June 30, 2023, which is an impressive number in terms of foreign exchange, but still far below the target \$1 billion potential of this sector. Marine fisheries are considered an important economic contributor in the country because Pakistan is blessed with a variety of aquatic resources having a long 1050 km coastline that can be utilized for coastal aquaculture, especially for floating net cage culture. In the present study, floating net cages 1.5 x 1.5 x 1.5m were installed at Soneri Beach Karachi and stocked 30 (20.31±0.1g; average initial weight) fish per cage with replicates of each. A total sixty-day feeding trial was conducted to replace fishmeal partially with soybean meal for *A. berda* optimum growth performance, feed consumption, survival, and fish final body carcass composition. Five semi-purified experimental diets were prepared to replace fishmeal with soybean meal: 0%, 15%, 30%, 45%, and 60%. Fishes were fed twice daily with 5% body weight and survival was recorded till the end of the experiment. No significant differences ($P>0.05$) were observed in specific growth rate (SGR) and final weight gain (WG) of the juvenile *A. berda* fed on 15%, 30%, and 45% diets compared to the control diet of 0%. However, a growth decline was recorded at a 60% replacement level compared to other diet groups. A poor feed conversion ratio (FCR) was recorded at a 60% replacement level. In the final body carcass composition no significant differences ($P>0.05$) were noted in total protein, ash, and moisture contents. However, little lipid content was noted as high at a 60% replacement level. In conclusion according to the obtained data results showed that 45% of soybean meal can replace fishmeal without affecting the growth performance and health condition of *Acanthopagrus berda*.

**REPRODUCTIVE SUCCESS, FECUNDITY AND SURVIVAL OF FRY OF RAINBOW
TROUT (*ONCORHYNCHUS MYKISS*) IN CAPTIVITY**

**Talha Afzal¹, Ali Muhammad^{1*}, Zeenat Jannat¹, Waqas Hammed²,
Majid Mahmood¹ and Nausheen Irshad¹**

¹*Department of Zoology, University of Poonch Rawalakot, Azad Jammu and Kashmir*

²*Directorate of Fisheries, Rawalpindi Rawal Town, Near Rawal Chowk, Islamabad*

*Corresponding Author: alimuhammad@upr.edu.pk

Rainbow trout (*Oncorhynchus mykiss*), an exotic freshwater salmonid, is one of the promising cultivable fish species in cold-water and has considerable scope for its expansion in uplands region. Rainbow trout requires cold (9-14°C), clean and highly oxygenated water (>7 mg/l) for ripening of brooder, successful breeding and hatchery activities. Rainbow trout breed during November to February and attains maturity after completion of 3rd year. In Pakistan, an annual average production of market-sized rainbow trout is 25,000 metric tons. At present, Rainbow trout is being cultivated

artificially in Murree, Madian, Upper Swat, Deer, Chitral, Shangla, Muzaffarabad, Neelum, Kohistan and KPK by Government and semi-Government institutions. But their productivity is not up to the standards of developed countries. There are many cold water resources in northern, north eastern and north western areas of Pakistan which need to be utilized. Technological innovations in aqua culture play an important role in blue economy. There are many techniques that are currently adopted in the cultivation of trout fish like artificial insemination, cryopreservation, use of Nano-technologies and captivity rearing. For captivity rearing, mesh incubators have been used for incubation of fertilized eggs in captivity. Six Group breeding with five replicated each of rainbow trout were performed, in breeding season. Very similar to the previous studies on rainbow trout, present study demonstrates same numbers of fish seed production with mesh incubating trays. In contrary to it, Steel incubators are locally manufactured; which have carrying capacity of 15000 eggs per tray and much effective for survival rate than mesh incubating trays. Further detailed experiments are required to explore the efficiency of steel incubating tray.

SHARK SPECIES IDENTIFIED IN THE LANDINGS AT KARACHI FISH HARBOR DURING THE FIELD SURVEY (2014–2023) PERIOD

Nuzhat Afsar¹, Rabiya Nasir^{2*}, and Noor Us Saher^{2*}

¹*Institute of Marine Science, University of Karachi*

²*Centre of Excellence in Marine Biology, University of Karachi*

Corresponding Author: rabiya_zoologist@yahoo.com*, noorusaher@yahoo.com

Sharks are among the largest animals on Earth and are found in open waters where they search for food and potential partners. These sharks are periodically targeted to capture animals for human consumption and occasionally caught as bycatch. Sharks are the exemplars of several families with unique and specified characters. The Karachi Fish Harbour was selected for the research location and the landings from 2014 to 2023 were studied in a range of surveys (1–5) to validate the existence of sharks. Representatives of the following families were observed at the Karachi Fish Harbour the landings from the Arabian Sea: Alopiidae, Carcharhinidae, Hemiscyliidae, Hemigaleidae, Laminidae, Sphyrnidae, and Stegostomidae. Family Carcharhinidae was the most prevalent of all the families; thirteen (13) distinct species were identified that was found in landings the most frequently.

NEMATALOSA NASUS POPULATION ANALYSIS, GROWTH MODELING AND FISHERIES IMPLICATIONS FROM PAKISTAN COAST

Muhammad Tabish*and Noor Us Saher

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: m.tabish163@gmail.com, noorusaher@yahoo.com

The population dynamics of *Nematalosa nasus*, commonly known as the threadfin shad, were investigated in coastal area of Pakistan. Key parameters including the von Bertalanffy growth model were estimated to provide insights into the species life history and population structure. The L_{∞} value, representing the asymptotic length, was determined to be 78.43 mm, indicating the maximum size that individuals of this species can attain. The K value, a measure of the growth rate, was estimated at 0.170, indicating the rapid growth of *Nematalosa nasus* in its habitat. Furthermore, the

study found a natural mortality rate of 0.43143, shedding light on the factors influencing the survival of this species. The high R-squared value of 0.8829 indicated a strong correlation between observed and predicted values, confirming the reliability of the growth model. The regression equation $Y = 3.5058x - 15.041$ was established to estimate the length of *Nematalosa nasus* at different time intervals, with 'a' equal to 3.5058 and 'b' equal to -15.041. This research contributes valuable information on the population dynamics and growth characteristics of *Nematalosa nasus*, which can be utilized for fisheries management and conservation efforts in Pakistan. Understanding the life history and growth patterns of this species is crucial for sustainable resource management and maintaining healthy aquatic ecosystems.

SEAFOOD QUALITY ASSESSMENT THROUGH MICROBIAL ANALYSIS IN STOMACH CONTENTS OF SKATES AND SHARKS OBTAINED FROM KARACHI FISH HARBOR'S

Rabiya Nasir^{1*}, Yasir Abbas², Shahzad Ali², Sadia Khalil² and Noor Us Saher^{1*}

¹*Centre of Excellence in Marine Biology, University of Karachi, Karachi*

²*Department of Microbiology, Federal Urdu Science University, Karachi*

Corresponding Author: rabiya_zoologist@yahoo.com; noorusaher@yahoo.com

Shark and rays included 22 shark species from eight distinct group shark families and 10 skate species from three different families, were discovered to be the most commonly among the landings at KFH. Sharks and Skates are regarded as a delicacy and are edible in many countries. The skate fish's main body and tail are not edible; only its "wings" are meaty. Sharks and Skate fish have a good level of nutritional content with a lot of protein. Meat from skate fish may be good for blood, DNA, and brain. Fishing is the primary anthropogenic factor affecting elasmobranch populations therefore, fishing pressure on these species has grown recently and they are commonly caught as bycatch. The concept of "micro flora" represents the minute organisms, such as bacteria, fungus, and viruses, that are found in human bodily fluids, soil, and other living things and that hamper their growth. Finding out what microbes were found in the stomach contents of skates taken from Karachi Fish Harbour was the goal of the current investigation for the safety of these sea food items. Following fish sample collection, the guts of the fish were aseptically dissected in the lab. More saline processing was done on the gut samples. In addition, the bacterial flora of heterotrophic, aerobic organisms was isolated by homogenising and plating gut samples on nutrient agar plates. According to the results of gram staining, gram positive cocci predominate among microbial cultures, yet certain gram negative cocci colonies have also been identified as coming from the gut.

POPULATION DYNAMICS AND STOCK ASSESSMENT OF OTOLITHES RUBER (BLOCH & SCHNEIDER, 1801): A COMPREHENSIVE ANALYSIS FOR SUSTAINABLE FISHERIES MANAGEMENT ALONG THE COAST OF PAKISTAN

Abdul Hameed* and Noor Us Saher*

Centre of Excellence in Marine Biology, University of Karachi

*Corresponding Author: hameedbaluch@gmail.com; noorusaher@yahoo.com

The Scianidae family, commonly known as Croakers, constitutes a significant coastal fishery resource in tropical and temperate waters. Among the Scianidae family, the *Otolithes ruber* stands out as a demersal fish frequently captured in various coastal areas of Pakistan, primarily using gillnet, seine net, and trawl net techniques. *Otolithes ruber*, commonly known as the Tiger tooth croaker, is a commercially important

fish species with significant ecological and economic implications. Understanding its population dynamics and stock status is crucial for effective fisheries management, therefore this study was designed to assess the population dynamics of *Otolithes ruber* over a one-year period to inform sustainable fisheries management strategies for this commercial species of Scianidae, balancing economic utilization with ecological conservation. Data for this study were collected over a one-year period and Analysed through Fisat, “a widely recognized fisheries stock assessment tool” and observed crucial insights into population dynamics and stock assessment of *Otolithes ruber*. With a total mortality rate (Z) of 1.80, a natural mortality rate (M) of 0.81, and a fishing mortality rate (F) of 0.99, the exploitation rate (E) was calculated at 0.55. These findings suggest a substantial level of exploitation, with the fishing mortality approaching the natural mortality. The length-related parameters indicated that the population is subject to capture at a relatively young age, with the length at first capture (L_{50}) recorded at 18.58 cm. The exploitation rates at different lengths (E_{10} , E_{50} , E_{max}) further emphasized the intense fishing pressure, particularly at larger sizes. The relative yield per recruit (Y/R) and relative biomass per recruit (B/R) were observed at 0.018 and 0.317, respectively, indicating a potential risk of overexploitation. The results highlight a comprehensive overview of the population dynamics of *Otolithes ruber* in the studied area. The calculated mortality rates, growth parameters, and exploitation rates provide a foundation for sustainable fisheries management. The observed values can serve as a baseline for future assessments and aid in the development of effective conservation and management strategies for *Otolithes ruber*. Further research and continuous monitoring are essential to ensure the long-term health of the population and the fisheries it sustains.

2. MARINE BIOLOGY

FIRST RECORD OF EDIBLE PEANUT WORM *SIPUNCULUS NUDUS* LINNAEUS, 1766 (ANNELIDA, SIPUNCULA, GOLFINGIIDA, SIPUNCULIDAE) FROM PAKISTAN AND NOTES ON OTHER SIPUNCULIDS FROM PAKISTAN

Feroz A Mangi¹, Quddusi B*, Kazmi¹ and M. Moazzam²

¹Marine Reference Collection and Resource Centre, University of Karachi, Pakistan and ²WWF_Pakistan

Corresponding Author: qbkazmi@yahoo.com

The aim of this paper is to describe *Sipunculus nudus* from the Pakistani marine waters. The knowledge of sipunculans from the Pakistani coast is fragmentary; the current study is based on the systematics and geographical distribution. This is the first report of *Sipunculus nudus* collected along the northern Arabian Sea (Pakistan coast). The species at hand is briefly describe and photographed with a note on its distribution, ecology and habitat. A check list of sipunculids already reported from Pakistan is added.

A SUPPLEMENTARY DESCRIPTION OF SOME MARINE CLADOCERA AND OSTRACODA FROM PAKISTAN (NORTHERN ARABIAN SEA)

Quddusi B. Kazmi* and M. A. Kazmi

Department of Zoology, University of Karachi, Karachi

*Corresponding Author: qbkazmi@yahoo.com

This study was carried out in order to determine the marine Ostracoda and Ostracoda fauna of Pakistan. We report hereby descriptions of newly found halocyprid ostracod *Paramollicia dichotoma* (Müller, G. W., 1906) of family Halocyprididae Dana, 1853, myodocopid and podocopid ostracod species of the families Cylindroleberididae and Cyprinididae respectively. A sarsiellid *Ancohenia robusta* (Brady, 1890) was collected by the staff of the Marine Reference Collection and Resource Centre, University of Karachi. An additional description of *Penilia avirostris*, a cladoceran of the order Ctenopoda in family Sididae based upon samples collected in the Northwestern Indian Ocean within the framework of the Office of Naval Research project (ONR: under Grant N00014-86-86-G-0230 1991), through partnerships with Pakistan academia and government, ONR coordinates and sponsors scientific research and technology development for the U.S. Navy and Marine Corps and .. All the species are described and illustrated.

ABBREVIATED DEVELOPMENT OF LABORATORY REARED SHALLOW WATERS PORCELLANID CRAB, *PACHYCHELES TOMENTOSUS* HENDERSON, 1893 (DECAPODA: ANOMURA) COLLECTED FROM KARACHI COAST

Farhana S. Ghory^{1*}, Quddusi B. Kazmi¹, Feroz A. Siddiqui¹ and M. Afzal Kazmi²

¹Marine Reference Collection and Resource Centre, University of Karachi, Karachi-75270

²Department of Zoology, University of Karachi, Karachi-75270

*Corresponding Author: farhanaghory@yahoo.com

An ovigerous female of *Pachycheles tomentosus* Henderson, 1893 was collected from intertidal zone at Buleji (Karachi, Pakistan) and kept under the laboratory conditions. The larvae hatched after 5 days and

existed within 13 days at room temperature 23°C - 25°C in filtered seawater with a salinity of 37 - 40 parts per thousand and a pH of 7.5 - 7.8. *Artemia* nauplii were used to feed the larvae. Complete larval development (four zoeal stages, a megalopal stage and crab stage) was obtained. The stages are described, illustrated and compared with earlier studies larvae.

THE STUDY OF DINOPHYTA SPECIES DIVERSITY IN THE COASTAL WATERS OF LASBELA, BALUCHISTAN

Habibullah, Azra Bano* and Muhammed Shareef

Department of Marine Sciences, Lasbela University of Agriculture Water and Marine Sciences, Uthal

*Corresponding Author: azrabano2006@yahoo.com

The diversity of phytoplankton species found in Lasbela, Baluchistan's coastal waters was investigated. Seasonal sampling was done from September 2019 to August 2020, both offshore in the surrounding open water and inshore at Miani Hor Lagoon. A total of 119 species of phytoplankton from inshore and offshore waters were identified as planktonic throughout the course of three seasons (post monsoon, pre monsoon, and monsoon seasons). During the seasonal sampling, two different groups of species of phytoplankton (Bacillariophyta and Dinophyta) were detected. During the study 64 species of the group Bacillariophyta and 55 species of the group Dinophyta have been identified. There were prominent differences in the species composition between the post-monsoon, pre-monsoon, and monsoon seasons on the basis of relative abundance of division Dinophyta. During the post-monsoon season, 38% of the species in offshore waters and 42% of the species in inshore waters were observed belonged to the group Dinophyta. During the pre-monsoon season, 51% of inshore and 43% of offshore species were pertaining to division Dinophyta species. During the monsoon season, planktonic algae known as Dinophyta were estimated to comprise 47% of species in inshore water and 45% of species in offshore water. Based on the seasonal statistics, the pre-monsoon season had the highest ratio (46%) and the monsoon season had the lowest ratio (23%), with the post-monsoon season having the lowest ratio (31%). There were 6 families belong to group of Dinophyta were found in inshore water, whereas, 7 families were explored in offshore water during the post-monsoon season. During the pre-monsoon season, 10 families of group Dinophyta were observed in inshore waters and 12 were examined in offshore waters. During the monsoon season, 19 families of division Dinophyta have been found in offshore waters, whereas, 18 in inshore waters.

NON-INVASIVE RADIOLOGICAL TECHNIQUE FOR DETERMINATION OF POLLUTANTS UPTAKE IN MARINE ORGANISMS

Nadeem Yaqoob*, Jawaria ABID, Muhammad ADIL and Muhammad Zaman Chaudhary

Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad

*Corresponding Author: nadeemyaqoob67@gmail.com

Pollution affects the quality of life in human population in negative way and the health condition deteriorates due to various chemical pollutants available in the environment. These pollutants can be in the form of heavy metals, free radicals, compounds etc. Global efforts are in progress to eradicate this problem by understanding the behavior of these pollutants and study of their pathways by which these pollutants reach the human body. These pollutants adapt various matrices like air, food etc. to ultimately accumulate in human body. As Oceans and seas are a large water body and plays an important role in accumulation of industrial pollutants in terrestrial habitats that may become life threatening. These pollutants are usually

dumped globally in the water channel like river and streams which ultimately falls into the sea or ocean. Thus, marine organisms, that are consumed world over as a main protein source, poses every chance that these contain pollutants in their edible parts due to uptake from the marine environment. To determine the uptake potential for Zinc and Chromium, marine mussel (*Perna viridis*) and a fish (*Sardinella spp.*) were kept in laboratory aquarium spiked with their radioactive isotopes for ten days. Whole animal was analyzed after defined interval under Gamma Spectrometer without any processing / dissection. It was recorded that the mussel has the potential to retain pollutants in its body for longer period than the fish. The study method involved was found to be less tedious and non-invasive than the conventional techniques used for pollution uptake studies.

MOLECULAR CHARACTERIZATION OF POMADASYS GRUNT (TELEOSTEI: HAEMULIDAE) FROM THE SEACOAST OF GWADAR BALUCHISTAN, PAKISTAN

Rabia Kausar¹, Asmatullah Kakar^{1*}, Muhammad Shafi³, Faiz Muhammad² and Asad Ullah⁴

¹Department of Zoology, University of Balochistan, Quetta

²Center of Excellence in Marine Biology, University of Karachi, Karachi

³Lasbela University of Agriculture, Water and Marine Sciences, Uthal

⁴CASVAB, University of Balochistan, Quetta

*Corresponding Author: asmardanzai@yahoo.com

In view of the phylogenetic diversity detection and measurement, it has become essential to evolve proper strategies for fish conservation and management. This study determines the phylogenetic diversity estimation of Haemulidae fish species caught from the coastal area of Gwadar, Baluchistan. The present study was conducted with the aim to estimate the phylogenetic diversity of Pomadasys marine fishes using mt DNA CO1 gene were identified. The approximation process was assessed by reconstruction of N. J. tree and employment of measuring the Kimura distance (K2P) was conducted which evaluated by employing a thousand bootstrap replicant. Results revealed five distinct species from Haemulidae sample, namely *Pomadasys argenteus*, *P. stridens*, *P. Kaakan*, *P. aheneus* and *Diagramma picta* were re-confirmed bases on strong similarity scores in NCBI nucleotide BLAST. The Haemulidae (Pomadasys) species were divided into three major clades, with bootstrap support values of 100%, 85%, and 100% respectively. The phylogenetic diversity ranged from 0.02 to 0.26. These results indicated that molecular identification and diversity of marine grunt fish species are helpful for fisheries conservation and management plan in Pakistan.

BLOOM FORMING TOXIC AND NON-TOXIC SPECIES OF DINOFLAGELLATES IN GADANI AND SANDSPIT

Tayyaba Hamid and Nafisa Shoaib

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: nafisashoaib@gmail.com; itayyabahamid@gmail.com

In the present study the Abundance and diversity of Bloom forming Dinoflagellates (Toxic and non-toxics species) in Gadani and Sandspit are reported. Dinoflagellates are primary producers, play a significant role in maintaining the ocean's health and are indicators of climate change. Dinoflagellates mostly occurs in marine habitats, but it is also found in freshwater and estuary environments. The Seawater samples were collected monthly for one year from Gadani and Sandspit to analyze

Dinoflagellates on board using Niskin bottles. The most dominant species observed in Gadani were *Ceratium*, *Noctiluca*, *Prorocentrum* and *Pyrocystis* whereas in Sandspit *Ceratium*, *Noctiluca*, *Prorocentrum* and *Scrippsiella*. In the foregoing study, toxic species of *Dinophysis caudata*, *Dinophysis miles*, *Gonyaulax spinifera*, *Alexandrium*, have been reported from Gadani and Sandspit. The abundance of *Dinophysis caudata* and *Prorocentrum micans* in Gadani and Sandspit shows an alarming situation as they cause diarrhetic shellfish poison in humans.

ASSESSMENT OF HEAVY METAL POLLUTION IN MARINE FISHES FROM COASTAL AREAS OF PAKISTAN

Nafisa Shoaib and Roomana Yasmeen

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding author e-mail: nafisashoaib@gmail.com

Marine pollution of heavy metals is increasing and has become of great concern due to the adverse effects on marine organisms. Fishes are adversely affected by lower availability of oxygen due to a rise in surface air temperatures and climate change. The industrial and domestic waste is continuously discharge into the marine environment causing heavy metals accumulation in fishes. The period of exposure and other factors are responsible for the lethality of metals and it relies on its type and quantity. The fishes were collected from the Sonmiani (Baluchistan) and Korangi (Sindh) coastal areas. Heavy metals such as Cr, Cd, Co, Ni, Cu, Pb, Mn, Fe and Zn concentrations were analyzed in fishes by atomic absorption spectrophotometer. The concentrations of metals in Fishes were in the following order Fe>Ni>Zn>Mn>Cd>Co>Pb>Cu>Cr from both locations. We examined the highest concentration of metals in fishes collected from Baluchistan as compare to Sindh. Heavy metals bioaccumulate in marine organisms by food chain. The exposure and persistence of heavy metals is a threat to fishes and humans. Changes in ocean currents, rise in temperature due to climate change will significantly alter fish breeding grounds, food supply for fish and finally the profusion of fish populations.

INDUS RIVER ESTUARY: AN ASSESSMENT OF FISH AND SHELLFISH ASSEMBLAGE IN RELATION TO ENVIRONMENTAL VARIABLES

Mushaiyada Mairaj, Sher Khan Panhwar and Wajiha Shaikh

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: mushi-91@hotmail.com

The aim of the study was to delineate diversity, abundance and distribution of amphidromous fishes wander in the Indus River Estuary. A total of 3965 individuals of fin fishes comprises of 120 Taxa and whereas 9 Taxa were recognized from 3020 shellfishes (shells, shrimps and cephalopods) shellfish. The *Esculosa thoaracata* comprises of (17.20%) finfish and *Parapenaeopsis stylifera* (25.45%) shellfish were dominated overall species diversity. Species-wise data of both groups (fin-fish and Shellfish) were used to perform linear models to assess diversity. Data of four environmental variables were used to establish Canonical Correspondence Analysis that determined salinity and DO as major factors involve in the distribution of fauna in IRE.

**BIOCHEMICAL COMPOSITION OF GONADS DURING MATURATION CYCLE IN
THE SEA URCHIN *ECHINOMETRA MATHAEI* (ECHINODERMATA: ECHINOIDEA):
SUITABILITY FOR HUMAN UTILIZATION**

Saima Siddique^{1*} and Zarrien Ayub²

¹*Lasbela University of Agriculture, Water and Marine Sciences, Lasbela*

²*Centre of Excellence in Marine Biology, University of Karachi, Karachi*

*Corresponding Author: saima_siddiq@hotmail.com

Maturation can also be assessed because of biochemical changes in the gonads of sea urchins. The concentrations of proteins, carbohydrates, and lipids were measured in the gonads of the *Echinometra mathaei* during the ovarian maturation stages. The results revealed that the proteins, carbohydrates, and lipids contents of gonads, consequently from highest to lowest, because the proximate composition of the gonads of *E. mathaei* has been influenced by its annual reproductive cycle, if they were to be utilized. The greatest quality in biochemical contents was determined to be from February to May on the coast of Pakistan when individuals with Stage I and II possess significantly higher concentrations of proteins and lipids upholds by the nutritive phagocytes. Although, in Pakistan, there is no custom to eat sea urchin roe and no sea urchin fishery exists. But, in the future when this species becomes exploited, such studies will be very important.

**THE STUDY OF DINOPHYTA SPECIES DIVERSITY IN THE COASTAL
WATERS OF LASBELA, BALUCHISTAN**

Habibullah, Azra Bano* and Muhammed Shareef

Lasbela University of Agriculture Water and Marine Sciences, Uthal

*Corresponding Author: azrabano2006@yahoo.com

The diversity of phytoplankton species found in Lasbela; Baluchistan's coastal waters was investigated. Seasonal sampling was done from September 2019 to August 2020, both offshore in the surrounding open water and inshore at Miani Hor Lagoon. A total of 119 species of phytoplankton from inshore and offshore waters were identified as planktonic throughout the course of three seasons (post monsoon, pre monsoon, and monsoon seasons). During the seasonal sampling, two different groups of species of phytoplankton (Bacillariophyta and Dinophyta) were detected. During the study 64 species of the group Bacillariophyta and 55 species of the group Dinophyta have been identified. There were prominent differences in the species composition between the post-monsoon, pre-monsoon, and monsoon seasons on the basis of relative abundance of division Dinophyta. During the post-monsoon season, 38% of the species in offshore waters and 42% of the species in inshore waters were observed belonged to the group Dinophyta. During the pre-monsoon season, 51% of inshore and 43% of offshore species were pertaining to division Dinophyta species. During the monsoon season, planktonic algae known as Dinophyta were estimated to comprise 47% of species in inshore water and 45% of species in offshore water. Based on the seasonal statistics, the pre-monsoon season had the highest ratio (46%) and the monsoon season had the lowest ratio (23%), with the post-monsoon season having the lowest ratio (31%). There were 6 families belong to group of Dinophyta were found in inshore water, whereas, 7 families were explored in offshore water during the post-monsoon season. During the pre-monsoon season, 10 families of group Dinophyta were observed in inshore waters and 12 were examined in offshore waters. During the monsoon season, 19 families of division Dinophyta have been found in offshore waters, whereas, 18 in inshore waters.

EXPLORING CRABS FOR THE NUTRACEUTICAL PROPERTIES

Munawwer Rasheed^{1,*}, Shaher Bano¹ and Mohammad Mohtasheemul Haque²

¹*Centre of Excellence in Marine Biology, University of Karachi, Karachi*

²*Department of Pharmacognosy, University of Karachi, Karachi*

*Corresponding Author: rasheed.munawwer@uok.edu.pk

Crabs (phylum Arthropoda, sub-phylum Crustacea) offered a nutritional meal. An ethnological survey of the coastal resident affirmed the biological activities related to the consumption of the crabs in the meal. Thus, two species of marine crabs, *P. pelagicus* and *P. sanguinolentus*, commonly used as the food intake, were studied systematically for their nutritional composition and biological activities. The crabs were dissected and separated into meat (~ 30%), shell (~ 46%), and visceral organs. The edible meaty composition was subjected to aqueous extraction, following the most usual form of the crab consumption was found to be its soup but without any additives (spices etc.). Fresh meat, residual meat after the aqueous extraction and aqueous extracts were lyophilised. Water content in meat was 81.40% and 76.06% in *P. sanguinolentus* and *P. pelagicus*, respectively. The powdered samples were subjected for estimating the nutraceutical contents (proteins, lipids, and carbohydrates contents) and for the anti-tussive activities as per prominent claims of the coastal community in survey. Protein content in these different samples ranges from 5.5 to 16.1%, lipid ranges from 1 to 1.3%, while carbohydrates were 9.9 to 10.1%. The calorific values for *P. pelagicus* and *P. sanguinolentus* were calculated as 93.26 and 92.29 kCal/100 g. Acute toxicity was determined administering five doses (5, 50, 300, 800 and 1000 mg/kg) in albino rats and dosage of 400 mg/kg was found safe and selected to conduct antitussive activity by inducing cough in albino mice using sulphur dioxide. The highest antitussive activity in 30 min was observed for the aqueous extract of *P. sanguinolentus*. (80% reduction) while *P. pelagicus* and standard (Hydraline) showed 72 and 33% cough reduction. The extracts were also submitted for antioxidant activity exploiting DPPH radical scavenging activity. IC₅₀ (mg/ml) for *P. pelagicus* residual meat (0.398) and aqueous extracts (0.539) was higher than *P. sanguinolentus* residual meat (0.525) and aqueous extracts (1.020). Brine shrimps' lethality assay was performed by exposing *Artemia salina* to 25, 50, 75, 100, 500 and 1000 µg / 10 mL concentration of extracts for 24 h. All extracts were found in safer ranges.

THE QUALITY ESTIMATION OF EXPORTED SEAFOOD FROM PAKISTAN: A HEALTH CONCERN WITH REFERENCE TO FOOD SAFETY

Saima Majeed*, Naveed Ahmad*, Tayyaba Asif, Aisha Qureshi, and Ayesha Umer

Aquatic Diagnostic and Research Centre, Department of Maritime Science, Bahria University Karachi, Karachi

Corresponding Author: saima.bukc@bahria.edu.pk, naveedahmad.bukc@bahria.edu.pk

The quality of seafood is one of the significant concerns to food processors, buyers, and general wellbeing specialists. This investigation attempted to research the pervasiveness of microbial quality and antibiotic chloramphenicol CAP residue in export-oriented frozen and fresh seafood to verify and control contamination for public health safety and international trade. A total of 32 frozen and fresh seafood samples were randomly collected for microbiological analysis including crabs, shrimps, and tin-packed tuna fishes, collected from 10 diverse processing plants situated at the Karachi fish harbor, Pakistan. The examination contains the assurance of total viable aerobic count (TVAC) by the standard plate count method estimated under 5×10⁵ CFU/g, E. Coli count was found below 5 MPN/g, Yeast and Mold counts were found under 1000 CFU/g, all analyzed seafood test results were observed under the average permissible limit. Explicit fish pathogens such as Salmonella typhirium, Vibrio cholerae, Vibrio

parahaemolyticus, *Lysteria monocytogene*, and *Staphlococcus aureus* were likewise inspected yet discovered missing in all the samples under examination. In conclusion, all the frozen seafood samples were judged as protected food from a microbiological perspective and met the satisfactory limit quantified by the International Commission of Microbiological Specification for Food.

EFFICACY OF AN ANTIBIOTIC IN CONTROLLING THE AQUATIC INFECTIONS OF FISH INJECTED WITH FLORESCENT COLOUR FOR ITS ADORABILITY

Ehsan Elahi Valeem* and Nadeem Ahmed Tamimi

*Laboratory for Exploration of Marine Resources (LEMR),
A-20, Al-Habib Society, Sector 8A/1, Scheme 33, Super-Highway Corridor, Karachi*

*Corresponding Author: valeem786@gmail.com

Aquarium fish industry has been using florescent colour for freshwater ornamental fish for the last three decades for glass fish (*Chanda ranga*, *Erythrinus erythrinus* (Wolf fish) and *Ambasis nama*). The technique is being introduced for the first time for marine glass fish like *Ambassis agrammus*. The glass fish have some extra value as compared to freshwater glass fish. Firstly it looks like tetra fish, secondly survive in low biological oxygen *i.e.* in an aquarium of (24"x12"x12") size 24 freshwater one inch long glass fish can be reared without aeration, while 100 marine glass fish can be kept and reared in the same size of aquarium, thirdly shoal of marine glass fish is moving that is a liking factor for the hobbyists, fourthly it needs no heater in the winter, fifthly it is omnivorous, as compared rival, as rival is strictly carnivorous *etc.* Three hundred fishes were selected to run the experiment of colouring, out of which 100 specimens were kept in control in two aquariums under the same environmental conditions. The experiment was carried out in the month of July 2023, when water temperature was ranged between 28 to 32° C. Rest of 200 specimen were kept in four aquariums having 50 each to acclimatize them into freshwater having salinity of 0.1 to 1.0 ‰. The florescent colour was administered and all 200 specimens were put into the water containing same potency of antibiotics of four different pharmaceutical industries (one in each aquarium) in order to find out best antibiotic for controlling aquatic infections. Two specimens were died in a control set A and two in control set B of 50 specimens in each aquarium. In the four sets of experimental aquariums mortality rate was 12 in set C, 08 in D, 04 in set E and 07 in set F. The lowest mortality was occurred into the set D due to best efficacy the antibiotic of particular pharmaceutical industry. All of 300specimens were on fed on flakes for 5 days. Later on the antibiotic containing water was replaced with freshwater.

ROLE AND CHALLENGES OF THE MARINE ENVIRONMENT: PERSPECTIVE INSIGHTS FROM PAKISTANI NEWSPAPERS

Najm Us Saher*¹ Noor Us Saher², Asmat Ara¹ and Farah Naz²

¹*Department of Mass Communication, University of Karachi*

²*Centre of Excellence in Marine Biology, University of Karachi*

*Corresponding Author: noorusaheer@yahoo.com

Human activities are threatening the wellbeing and provisions of the world's oceans. Over 80 percent of marine contamination is caused by activities that take place on land due to human activity. Entire marine ecosystems are altering quickly, from sea level rise to coral bleaching. Some of the biggest dangers to the marine environment are ocean dumping, land runoff, dredging, NO_x and SO_x, ocean acidification,

oil and plastic pollution, rising sea levels, and substances that deplete the ozone layer. Numerous oceanic processes and ocean chemistry are changing as a result of global warming, endangering a wide range of marine animal species that are unable to withstand rising temperatures. Another major issue in many places of the world is overfishing. In the current era, media has a ingenious effect on the Society, it is important to understand the function and significance of mass media in the field of marine environment. There is a channel via which media find the impact of the message that is transmitted to their target audience, even when some media platforms are disregarding marine-related news and coverage. The current study investigates the marine environment related news coverage by the Pakistan print news and quantified its patterns by reviewing more than 1000 news across three national newspapers in English languages (Dawn Newspaper, The News Newspaper and The Express Tribune Newspaper) and their impact on society. The objective this study was to investigate the role and coverage effect of the news content and would identify that media message transformation is productive or not. In this study time series analysis has used to analyze the data for 10 years news from 2012-2022. Time series along content analysis have used as analysis tools, whereas the units of observation were editorial, news reports, and pictures. Difference news were grouped as item and made units of data gathering and analysis. Researchers has used ARDL and unit root test for checking data level of significance.

STUDY ON THE DIVERSITY OF ZOOPLANKTON WITH RELATION TO THE PRESENCE OF MICROPLASTICS IN THE SONMIANI BAY WATERS, PAKISTAN

Zara Imdad and Noor Us Saher*

Centre of Excellence and Marine Biology (CEMB), University of Karachi, Karachi

*Corresponding Author: noorusaheer@yahoo.com

One of the most significant food sources for aquatic animals, particularly planktivorous fish, is zooplankton. Since they play a significant role in the movement of materials and energy from phytoplankton and bacterioplankton to higher trophic levels, they are vital to the biogeochemical cycles of the planet. Microplastics may be mistaken for a species' natural prey because of their similar size, or they may be passively eaten during normal feeding behavior by zooplankton. Numerous zooplankton species have been shown to ingest microplastics with dimensions ranging from 5 to 816 micrometers. For this study, samples from Pakistan's Sonmiani Bay were taken during high tide and low tide throughout the year from May 2021 to July 2022. All of the samples were transferred to the lab after being formalin-preservation. Two subsamples from the each samples were examined under a microscope to evaluate the distribution and diversity of Zooplankton. Moreover, two sub samples (R= 25 ml) from each samples were prepared through digestion for the assessment of microplastic presence and identification. The distribution of microplastics reveals the contamination of threads and beads in Sonmiani Bay's waters. Few zooplanktons groups showed a strong correlation with the Microplastic abundance.

FIDDLER CRAB *UCA IRANICA* AND *UCA SINDENSIS* AS BIOINDICATOR IN SEA SEDIMENTS OF SANDSPIT BACKWATER OF KARACHI, SINDH

Dur-e-Shahwar and Noor Us Saher*

Centre of Excellence and Marine Biology, (CEMB) University of Karachi, Karachi

Corresponding Author: noorusaheer@gmail.com

The benthic crabs have a direct interaction with the substratum. In the present study, the distribution, composition and seasonal variation of microplastics in two species of crabs were determined with

reference to their habitat sea sediment of Sandspit backwater. Sediments and two crab (*Uca iranica* and *Uca sindensis*) species were collected from three different sites of Sandspit backwater brought to lab for microplastic studies. The sediments were dried at 90°C in oven for 48 hours, after drying 50 grams of sediment were taken in beaker and digested and filtered for the available microplastic concentration. The filter papers after drying were observed under stereo microscope. The crabs were dissected and digested in (KOH) and poured at filter paper, then the filter paper dried and observed through microscope for the presence of Microplastic. *The various coloured forms of (threads, beads, films, fragment and foams) MP's were detected in the sediments and crab body. The concentration of microplastics (MPs) in sediments and tissues of the Austruca iranica and Uca sindensis showed variability and significant difference in response of season and sites. It was concluded that both species of Fiddler crabs are good bio indicators to monitor the MPs pollution at Sandspits mangrove backwater as well as to understand human impacts in coastal ecosystems of Karachi because they are continuously exposed to environmental contaminants.*

SHELL ASSOCIATED HERMIT CRABS, DISTINCTIVE MORPHOMETRY AND ITS OCCUPATION PREFERENCE ALONG THE COAST OF PAKISTAN

Altaf Hussain Narejo¹ and Noor Us Saher^{2*}

¹*Zoological Survey of Pakistan*

²*Centre of Excellence in Marine Biology, University of Karachi, Karachi*

*Corresponding Author: noorusaheer@yahoo.com

Hermit crabs are widely distributed throughout the coastline of Pakistan. The Diogenidae and Paguridae are the most prevalent families along Pakistan's coastline. There were 2149 crabs in all that were gathered from the intertidal zone of the three different coastal habitat; rocky, muddy, and sandy. Morphological studies have been conducted between the hermit crabs and its shell from Pakistan coastline. Individuals were categorized according to size, sex and shell occupation. Crabs were measured according to Total length (TL), Cephalothorax shield length (CSL), width (CSW), Chela length (CL), width (CW), and weight of crab with and without shell. Whereas, the shells were measured according to Total length (TL), width, aperture length (AL), width and shell weight (SW). For morphometric analysis; Total length of crab used as independent variable. Results reveal the significant relationship of total length between the two families; Diogenidae and Paguridae ($P > 0.05$). Among 2149 hermit crabs, 93% individuals belonged to Diogenidae acquiring 93% whereas, Paguridae acquired only 6.9%. Total 26 families of shells found hosting crabs among them Muricidae, Turbinidae, and Certhiidae found dominant in the utilization by hermit crabs. The Sex ration showed total males ($N = 1390$) with 64.68% dominated in respective coasts while females (non-ovigerous = 667) with 31.03% and females (ovigerous = 92) with 4.28%. High number of used shell species utilization by smaller individual crabs indicated that occupation is influenced by the shell availability, while larger individuals demonstrate more specialized occupation.

A MORPHOLOGICAL AND DIVERSITY ASSESSMENT OF PENAEUS SHRIMP DIVERSITY COLLECTED FROM SONMAINI BEACH (MIANIHOR) BALOCHISTAN, PAKISTAN

Noor Us Saher* and Noor Hawa

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: noorusaheer@yahoo.com

The goal of this study was to determine the variety and size range of shrimp species found in Maini Hor lagoon during a monthly collection in 2020-2022. In the study a total of 14 distinct species of shrimps were

identified and examined, indicating that the most prevalent shrimp species are found in Sonmiani waters were belonging to the Penaeidae family. The present study was carried out for two year, from October 2020 to September 2022 in Sonmiani bay Balochistan. These species were caught using gill net and beach seine during monthly regular collection. The seasonal variations were observed in the diversity, abundance and size distribution throughout the years. Most abundant species of shrimp was *Panaeus indicus* (i.e 602) was the rarest specie found in the following research. For *Peneaus merguiensis*, the descriptive statistics for the data from 2020–2022 revealed the maximum length (25 cm) and minimum length (10 cm), with mean size calculations for total length (16.554) and species weight (21.743). During the Pre-monsoon collections, 10 distinct species *Metapenaeus japonicus*, *Metapenaeus affinis*, *Panaeus indicus*, *Panaeus merguiensis*, *Panaeus monodon*, *Penaeus penicillatus*, *Parapenaeopsis hardwickii*, *Parapenaeopsis longipes*, *Parapenaeopsis sculptilis*, *Parapenaeopsis stylifera* were observed in abundance whereas North East Season has seen the fewest species observations, including *Caradian* shrimp.

NEW INSIGHTS INTO GENETIC DIVERSITY OF THE BLUE CRAB A TAXONOMIC AND CONSERVATION APPROACH

Farah Naz^{1*}, Noor Us Saher^{2*} and Mustafa Kamal³

¹Department of Zoology, University of Karachi, Karachi

²Centre of Excellence in Marine Biology, University of Karachi, Karachi

³Department of Biotechnology, University of Karachi, Karachi

Corresponding Author: farahasjil@yahoo.com, noorusaher@yahoo.com

Climate change, habitat destruction, overexploitation, and unsustainable use of resources, threaten marine species, whereas the genetic variability of species depends on the ecological, behavior, and physiological mechanisms. A molecular approach is used to measure the genetic diversity of marine species for proper management in conservation. The objective of the present study was to determine the genetic diversity of *Portunus reticulatus* and *P. segnis* by sequencing the Cytochrome oxides mitochondrial gene. The crabs were purchased from the fish harbors and extracted DNA from the leg or claw tissue and sequence and record the number of haplotypes. Likewise, the newly procured mitochondrial Cytochrome Oxidase subunit I gene haplotype data for *Portunus reticulatus* and *P. segnis* along with another haplotype in molecular databases from the Indo-West Pacific region was included in this study for phytogeography and phylogenetic relationship. The analysis involved 30 sequences of *P. reticulatus* and 29 sequences of *P. segnis* nucleotide sequences and a total of 729 and 714 sites for COI. In COI 19 haplotypes were determined out of 30 from *P. reticulatus* and 9 out of 29 from *P. segnis*. Haplotype diversity was observed at 0.96 and 0.57, whereas the nucleotide diversity was 0.012 and 0.0003 in *P. reticulatus* and *P. segnis* respectively. Highly significant *hd* was observed in *P. reticulatus* and *P. segnis* ($P \leq 0.01$, $P \leq 0.000$). Understanding the genetic diversity of an economically important species has implications for fisheries management.

CHARACTERIZATION OF CRAB AND SHRIMP SHELLS FOR THE POTENTIAL USE IN SUSTAINABLE APPLICATIONS

Abraira Mukhtiar and Noor Us Saher*

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: noorusaher@yahoo.com

The disposal of bio waste is one of the major problems facing the sea food industry and one of the main issues the food sector is dealing with is the disposal of crustacean shell waste. Even though shrimp

shells and crab shells have obvious potential, a thorough biochemical investigation of these biomaterials is necessary to realize their full potential. In order to fully realize the potential of Shrimp shells and Crab shells, a comprehensive biochemical analysis of these biomaterials is required as the yearly production of harvested crustacean shell trash worldwide is 1.44 million metric tons. Therefore, a thorough biochemical investigation of these biomaterials is necessary to fully exploit their potential. Two main components of crustacean shells, in addition to chitin, are minerals (mostly calcium carbonate) and proteins. These three closely related components account for about 90% of the dry weight of the shell. Key components such as chitin, proteins, minerals, and lipids are extracted and quantified as part of this research project with the goal of making significant contributions to the field of sustainable biomaterials. The goal of this research is to close the information gap about the intricate biochemistry of shrimp shells, and crab shells. The waste materials were collected from the Karachi fish harbour and following techniques were used to estimate the major biochemical components namely proteins, carbohydrates, lipids with special concentration to chitin. The protein content of shrimp shells varied significantly depending on their size. To gain a deeper understanding of the composition of these two marine biomaterials shrimps and crab shells—this work offers the comprehensive biochemical investigation. Important components like chitin, proteins, minerals, and lipids are separated and quantified as part of this research endeavor with the goal of making significant contributions to the field of sustainable (Food Preservatives and Bioplastic) biomaterials.

ASSESSMENT OF HEAVY METAL POLLUTION IN MARINE FISHES FROM COASTAL AREAS OF PAKISTAN

Nafisa Shoaib* and Roomana Yasmeen

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding author e-mail: nafisashoaib@gmail.com

Marine pollution of heavy metals is increasing and has become of great concern due to the adverse effects on marine organisms. Fishes are adversely affected by lower availability of oxygen due to a rise in surface air temperatures and climate change. The industrial and domestic waste is continuously discharge into the marine environment causing heavy metals accumulation in fishes. The period of exposure and other factors are responsible for the lethality of metals and it relies on its type and quantity. The fishes were collected from the Sonmiani (Baluchistan) and Korangi (Sindh) coastal areas. Heavy metals such as Cr, Cd, Co, Ni, Cu, Pb, Mn, Fe and Zn concentrations were analyzed in fishes by atomic absorption spectrophotometer. The concentrations of metals in Fishes were in the following order Fe>Ni>Zn>Mn>Cd>Co>Pb>Cu>Cr from both locations. We examined the highest concentration of metals in fishes collected from Baluchistan as compare to Sindh. Heavy metals bioaccumulate in marine organisms by food chain. The exposure and persistence of heavy metals is a threat to fishes and humans. Changes in ocean currents, rise in temperature due to climate change will significantly alter fish breeding grounds, food supply for fish and finally the profusion of fish populations.

3. PALEONTOLOGY

DIVERSITY OF INVERTEBRATE FOSSILS IN PAKISTAN

S.I.H. Jafri*

Flat No. C-5, Commercial-1, Gulshan e Shamim, Karachi

*Corresponding Author: syediftikharhusainjafri@gmail.com

Occurrence of various invertebrate fossils groups is quite variable. Largest group was that of Gastropods (22%), Bivalves were (17%), Echinoderms were (15%), Corals (13%), Cephalopods (Nautiloids, Belminites, Ammonites) were (13%), Brachiopods (10%), Crustaceans and foraminiferons were (5%) each. Fossils studied presently belong to 8 classes, 52 orders and 88 families. 136 genera are reported here from various provinces of Pakistan. 35% of these are from Sindh; 27% from KPK (Kheyber Pakhtoon Kawah); 21% from Balochistan and 17% from Punjab.

4. WILDLIFE, DIVERSITY AND CONSERVATION

THE ECOLOGICAL DIVERSITY OF DUCKS AND GEESE (AVES: ANSERIFORMES: ANATIDAE) FROM SHAHEED BENAZIR ABAD (DIVISION) IN SINDH, PAKISTAN

**Sajid Siyal^{1*}, Saima Naz², Nadir Ali Birmani², Abdullah Arij³, Zahid Iqbal Rajput¹,
Majid Ali Soomro¹ and Asma Kanwal⁴**

¹Department of Wildlife Management, Department of Animal production & Technology, Department of Veterinary Medicine, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand- Sindh

²Department of Zoology, University of Sindh, Jamshoro

³Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam

⁴Department of Zoology, Govt Girls Zubaida College Hyderabad, Sindh

*Corresponding Author: sajid.siyal333@gmail.com

This study explores the ecological diversity of Anatid birds Shaheed Benazirabad Division in Sindh, Pakistan. Situated in the southern part of the country, Shaheed Benazirabad (Nawabshah), exhibits a diverse range of habitats, including, Wetlands, arid zones and agriculture landscapes. The avian population in this region comprise a variety of resident as well as migratory species. The present study was carried out between September 2022 to October 2023. Visited time to time different habitats of anatid birds in urban and rural areas like water-bodies, villages and cultivated areas during the research period from various districts of Shaheed-Benazirabad Division including , Noushahro Feroze, Shaheed-Benazirabad and Sanghar. A total 16 species and 6 genera of ducks and geese were reported, including *Anas acuta* (Linnaeus), *Anas crecca* (Linnaeus), *Anas platyrhynchos* (Linnaeus), *Anas penelope* (Linnaeus), *Anas poecilorhyncha* (Forster), *Anas strepera* (Linnaeus), *Aythya ferina* (Linnaeus), *Aythya fuligula* (Linnaeus), *Aythya nyroca* (Guldenstadt), *Anser anser* (Linnaeus), *Anser albifrons* (Scopoli), *Anser indicus* (Linnaeus), *Marmaronetta angustirostris* (Reichenbach), *Spatula clypeata* Linnaeus), *Spatula querquedula* (Linnaeus) and *Netta rufina* (Pallas), (Anseriformes: Anatidae), These birds are game birds resident as well as migratory bird. They were available in all seasons but abundantly in winter season. This study aims to shed light on the interconnectedness between ecosystem and avian population in Shaheed Benazir Abad Division, contribute to the overall biodiversity and ecological balance of the region. This study was carried out for the first time from this region.

BREEDING ECOLOGY OF INDIAN GREY HORNBILL (*OCYCEROS BIROSTRIS*) IN PUNJAB, PAKISTAN

Saira Batool, Sangam Khalil* and Hadeeqa Habib

Institute of Forest Sciences, The Islamia University of Bahawalpur

*Correspondent: sangam.khalil@iub.edu.pk

The Indian Grey Hornbill (*Ocyrceros birostris*) is the only species of hornbill observed in Pakistan. Indian Grey-hornbill rely on old or dead as well as alive tree cavities for nesting and also secondary nesting bird. The main reason to study the Indian Grey-hornbill in the Abdul Hakeem (ABK) in district Khanewal is that Indian Grey-hornbill (IGHB) is a rare of Pakistan and I choose this

area for research because of the accessibility and presence of potential suitable habitat of Indian Grey Hornbill. The breeding data were conducted from 2022 to 2023. In 2023, I documented two nests: one on a Neem tree and the other on a Simbal tree in Abdul Hakeem. The breeding season typically starts in the middle of February and continues until June. Indian Grey Hornbill is an arborous and predominantly aerial bird, which is more partial prefer to old orchards the neighborhood of plantations and habitats with *Ficus religiosa* (Peepal) and *Ficus benghalensis* (Banyan) *Acacia nilotica* (Kikar), *Syzygium cumini* (Jaman), *Dalbergia sisso* (Shisham), *Morus alba* (Mulberry), *Bombax ceiba* (Simbal) and *Albizia lebbeck* (White Siris). The distance between one nest and another nest was 177 meters. Breeding data are divided into: Nest selection, Nesting behavior, Nesting material, Courtship, Mating, Incarceration of the Female, Feeding quality that gave to chicks and female breaking out after incarceration. I have the opportunity to witness the Male Indian Grey Hornbill engaging in dancing behavior in distinct occasion, observed during breeding period. As stated by IUCN, Indian Grey-hornbill is called a 'species of concern' as its numbers are rapidly declining so for that reason detailed study on this particular species is required. The study will help in the conservation of to be revival habitat in Pakistan.

NEW RECORDS FOR THE ECOLOGICAL DISPERSAL OF SOME BIRDS IN PAKISTAN

Z. B. Mirza*

*Board Member and Chairman, Scientific Committee, Islamabad Wildlife Management Board,
Ministry of Climate Change and Environmental Coordination, Government of Pakistan*

*Corresponding Author: zbmirza1936@gmail.com

The plains of Pakistan and the Himalayan Ranges, below Alpine heights, are covered by the Oriental Zoogeographical Region. However, avian fauna, here, has the ability to face theregularly changing abiotic ecological conditions of the seasons, through regular annual migration, both latitudinal as well as altitudinal. Another ecological dispersal of some of the sedentary birds, is the behaviour of solitary nesting, although in non-breeding season they may be roosting communally. The following observations are explained, which have not been documented in published literature: 1. The dispersal history of Pied Myna, which was recorded by the author in 1982 as an addition to the avifauna of Pakistan. 2. Behaviour of communal night roosting of some species. 3. Ecological dispersal for nesting sites. 4. Nesting types. 5. Communal nesting behaviour. 6. Nesting security. 7. Avian predation of juvenile avian prey as well as of some butterflies. 8. Autumn and spring transit migration routes of Rosy Pastor flocks. 8. New distributional records for some already recorded bird species in Pakistan. 9. Observation on extreme western Baluchistan migratory birds' flyway and their stopover at high salinity huge closed Basin Lake called 'Hamun-e-Mashkhel'. 10. How migratory waterfowl avoid collusion at night with Wind-power rotating turbines of Wind Energy Farms situated less 5 Km. away huge Khinger Lake, in district Thatta.

THE DIET OF THE INDIAN CRESTED PORCUPINE (*HYSTRIX INDICA*) (KERR 1792) IN MUZAFFARABAD DIVISION, AZAD JAMMU AND KASHMIR

Saba Khalid*, Nasra Ashraf, Nuzhat Shafi and Gulam Ali

Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad

*Corresponding Author: saba_ak01@yahoo.com

The Indian crested porcupine (*Hystrix indica*), a member of the Hystricidae family and *Hystrix* genus, is globally classified as Least Concern due to its adaptability to various environments. In Pakistan, it

thrives in tropical to temperate scrublands, grasslands, woodlands, and stony hill slopes. However, its adaptability poses a significant threat as it is considered a harmful pest to agriculture, affecting both traditional and non-traditional crops, as well as forest plantations like fruit orchards, vegetables, blooming plants, and forages. The primary objectives of this study were to ascertain the distribution range of porcupines in the Muzaffarabad division, assess habitat usage, analyze diet composition, evaluate damage caused by porcupines, and propose management measures. Diet composition was determined through micro-histological analysis of fecal pellets, collecting a total of 336 pellet groups from the study area, including 132 from Muzaffarabad (67 in summer, 65 in winter), 87 from Jhelum Valley (47 in summer, 40 in winter), and 117 from Neelum (62 in summer, 55 in winter). Results revealed varying dietary diversity across districts, with Jhelum Valley exhibiting the highest (34 dietary items), followed by Muzaffarabad (30) and Neelum (23). The average diet breadth was lower during the winter season across all study sites compared to summer. In the summer season, *Zea mays* was the most consumed plant ($B=39.24$), while *Daphne oleoides* had the least consumption ($B=1.00$). Conversely, during the winter season, *Diospyros lotus* recorded high diet breadth (23.24), with *Abies pindrow* having the lowest (3.75).

QUANTITATIVE ANALYSIS OF WILDLIFE DISTRICT PALANDRI, AJK, PAKISTAN

Amna Safdar¹, Zulfiqar Ali^{1*}, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³, Azka Shahid Khan¹, Muhammad Sarim Hassan¹, Ghulam Rabbani¹, Maryam Tanveer¹ and Farhan Anjum¹

¹Institute of Zoology, University of the Punjab, Lahore

²Department of Zoology, Lahore College for Women University, Lahore

³Center of Earth and Environmental Sciences, University of the Punjab, Lahore

*Corresponding Author: zali.zool@pu.edu.pk

Quantitative analysis of biodiversity in district Palandri was undertaken from September, 2022 to May 2023 in district Palandri, AJK to collect data on the distribution and status of its wildlife. Point count method was used to observe the species in nine different habitats in the study area viz. Agricultural Area, Barren Land, Range land, Settlements, Shrubs and Bushes, Subtropical Broad Leave Forest, Sub- Tropical Chir Pine and Water Body. A total of 1049 individuals of 126 species of amphibians, birds, reptiles and mammals are observed in the study area belonging to 23 orders and 57 families. The Aves group had the most species variety, with 87 species followed by Mammals that were the second most varied group, with 18 species. Reptiles and amphibians contained 16 and 5 species, respectively. The most abundant species were Indus Valley Toad ($n=11$), Bengal Monitor ($n=13$), Blue- cheeked Bee-eater ($n=41$) and Himalayan Wood Mouse ($n=14$) were recorded in district Palandri, AJK. 109 species were LC, 2 species were DD, 1 species was NT, 1 species was V. Calculated values of Shannon Wiener (H) and Simpson index (1-D) of the district Palandri were 4.60 and 0.99 respectively that represent relatively high diversity. Species evenness was 0.95 that showed evenly distribution of species in the district. Sorensen indices (SSI) and Jaccard indices (J) were used to compare similarities between different habitats. Among all habitats, settlement showed greater degree of overlap with shrubs and bushes (SSI 0.73; J-0.58) respectively while plantation showed least overlap with range land (SSI-0.36; J-0.22) respectively. Anthropogenic activities such as logging, Settlements, deforestation, forest fires, hunting, extensive grazing of cattle were the reasons for decline in vertebrate species. The conservation strategies such as Establishment and management of protected areas, promote sustainable logging, and restore habitats to conserve biodiversity, strengthen wildlife conservation efforts and raise awareness among local communities, are designed to protect and conserve the biodiversity of the Palandri district. By implementing these strategies, we can help ensure the long-term survival of these species and the ecosystems.

QUANTITATIVE ANALYSIS OF WILDLIFE IN HAVELI DISTRICT, AJK, PAKISTAN

Azka Shahid Khan¹, Zulfiqar Ali^{1*}, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³, Amna Safdar¹, Muhammad Sarim Hassan¹, Ghulam Rabbani¹, Maryam Tanveer¹ and Farhan Anjum¹

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

Biodiversity, the variety of life on Earth, is essential for maintaining ecosystem balance and providing crucial services for human well-being. This study was conducted in Haveli District, AJK Pakistan, from September 2022 to May 2023, with the main objective of quantitatively analyzing the biodiversity in the study area. Point count methodology was employed to determine the number of wildlife species at each sampling point, resulting in a total count of 493 individuals, representing 32 families and 58 species. Family Muscidae dominated with 9 species, followed by Phyllostomidae with 6 species. The conservation status of the recorded species varied, with 45 listed as "Least Concern," 8 as "Not Available," and one species each as "Data Deficient," "Near Threatened," and "Vulnerable." Population trends were identified for the observed species, with 30 being stable, 11 decreasing, four increasing, nine with trends not available, and four with an unknown trend. The habitats of study area supported the following species numbers Agriculture Area (34 species), Barren Land (16 species), Plantation (16 species), Range Land (19 species), Settlement (51 species), Shrubs and Bushes (19 species), Snow and Glaciers (13 species), Sub-Alpine (12 species), Sub-Tropical Chir Pine (18 species), and Waterbody (16 species). The study area exhibited a high level of species diversity, as indicated by the Shannon-Weiner Diversity Index (H-3.81) and the Simpson's Diversity Index (D-0.973). However, the species evenness value of 0.212 suggested uneven distribution within the study area. The results from Sorenson indices revealed that Agricultural Land showed the highest overlap with Sub-Tropical Chir Pine (0.580) and the lowest with Waterbody (0.333). Similarly, with Jaccard indices, the maximum similarity was observed between Agricultural Land and Sub-Tropical Chir pine (0.409), while the maximum similarity occurred between Waterbody and Plantation (0.140).

QUANTITATIVE ANALYSIS OF WILDLIFE IN RAWALAKOT DISTRICT, AJK, PAKISTAN

Bargazida Sabahat¹, Zulfiqar Ali^{1*}, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³, and Farhan Anjum¹

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

Pakistan is really a significant country as its territory harbors two important zoogeographical realms; Palearctic and Oriental, making Pakistan one of the salient territories in Eurasia with respect to biodiversity dynamics. Current study was designed to observe the diversity, status, distribution of amphibians, reptiles, avifauna and mammals in Rawalakot city, AJK, Pakistan during December 2022 to June 2023. The study area comprised eight habitats i.e. agriculture area, range land, settlement, moist temperate forest, shrubs and bushes, subtropical broad leaved forest, subtropical chir pine and water body. A sum of 2094 individuals from 210 species belonging to 76 families, 24 orders and four classes were observed. Reptiles accounted for 21 species, amphibians for 5 species, avifauna for 155

species and mammals for 29 species. The Aves group had the most species variety. The most abundant species were White-tailed Lapwing (R.A-17%), Blue-cheeked Bee-eater (R.A-8.59%), Isabelline Wheatear (R.A-7.59%) and Tree pipit (R.A=7.04%) were recorded in district Rawalakot, AJK. Data obtained was analyzed using diversity indices to get diversity of amphibians, reptiles, avifauna and mammals. The Simpson Index showed that settlement was the most diversified habitat overall (1-D = 0.993). The ecosystem surrounding a water body was less diversified (1-D = 0.965), while Shannon-Wiener Index showed moist temperate forest was the most diverse habitat ($H' = 4.813$) and waterbody had less species diversity ($H = 4.115$). Species evenness was 0 that showed evenly distribution of species in the district. Among all eight habitats, species were evenly distributed in settlement ($E = 0.959$), while in water bodies ($E = 0.892$), species were not evenly distributed. Sorensen indices (SSI) and Jaccard indices (J) were used to compare similarities between different habitats. Among all habitats, agriculture area showed greater degree of overlap with moist temperate forest (SSI=0.722; J=0.566) respectively while agriculture area showed least overlap with waterbody (SSI=0.372; J=0.229). As for their conservation, major reasons that are causing diversity decline are anthropogenic pressure, habitat loss and degradation, lack of public awareness and legislation. Conservation measures must be taken for the good of these organisms that are significant for maintenance of the ecosystem they reside in, lest they should decline.

SPECIES RICHNESS, STATUS AND DISTRIBUTION OF WILDLIFE IN PANJAL-MASTAN NATIONAL PARK AJK, PAKISTAN

Maryam Tanveer¹, Zulfiqar Ali^{1*}, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³, Sidra Shabbir¹, Ghulam Rabbani¹, Urooj Jamil¹ and Farhan Anjum¹

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

The research conducted in Panjal Mastan National Park, located in the Bagh District of AJK, Pakistan, spanned from June 2022 to May 2023, focusing on the biodiversity of eight habitats. Utilizing diverse survey methods, including Point count and Flushing techniques, the study identified a total of 105 species from 18 orders and 52 families across amphibians, reptiles, birds, and mammals. Passeriformes emerged as the most abundant avian order, demonstrating high bird diversity, particularly within the Muscicapidae family. The majority of species were Year-Round Residents, with notable proportions as Summer Breeders, Winter Migrants, and Passage Migrants. IUCN categorization revealed 82% as Least Concern, 3% as Vulnerable, and 2% as Near Threatened. Trends indicated stability in 45 species, increase in eight, and decrease in 24. Insectivorous species dominated feeding habits. Diversity indices reflected a highly diverse ecosystem, with Shannon Weiner and Simpson's indices suggesting richness and evenness. Agricultural areas stood out with 84 species, while Snow and Glaciers exhibited the least abundance with 34 species. Habitat overlap, assessed through similarity indices, emphasized the impact of anthropogenic activities, calling for conservation efforts to counter habitat loss, climate change, and pollution threats, particularly emphasizing the need to control human-generated impacts for the preservation of biodiversity in Panjal Mastan National Park.

QUANTITATIVE ANALYSIS OF WILDLIFE IN BAGH DISTRICT, AJK, PAKISTAN

Muhammad Sarim Hassan¹, Zulfiqar Ali^{1*}, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³, Amna Safdar¹, Azka Shahid Khan¹, Ghulam Rabbani¹, Maryam Tanveer¹ and Farhan Anjum¹

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

This study was conducted in Bagh district, Pakistan, which aimed to evaluate the relative abundance of various vertebrate species, to identify species in different habitats, understanding threats faced by wildlife in the region, proposing measures to address habitat loss and human interventions, to assess species richness, distribution, and status of species. Various survey techniques, such as point count, sky view, and flushing, were employed to observe different habitats and species. Diversity indices, including Simpson Index and Shannon Wiener Index, were used to measure animal diversity and their values ($1-D = 0.987$, $H' = 4.83$) indicated that the study area has high species richness and diversity. Species evenness is 0.19 which states that species are not evenly distributed in various habitats. The similarity indices like Sorensen and Jaccard were utilized to compare species composition between habitats. The study revealed a diverse range of habitats (10) supporting 178 species, comprising amphibians, avifauna, mammals, and reptiles. The research highlighted the importance of various habitats, with Rangeland and Sub-Tropical Chir Pine exhibiting the highest species diversity, while Snow and Glaciers habitat had the lowest species count due to extreme living conditions and limited area. However, the area also faces threats to its biodiversity, such as habitat fragmentation, wood logging, urbanization, and illegal hunting. To conserve the unique biodiversity of the region, the study suggests implementing protected areas, sustainable logging practices, habitat restoration, and public awareness initiatives.

PRIORITIZING CONSERVATION: EVALUATING THE VULNERABILITY OF THREATENED SPECIES IN PAKISTAN THROUGH THE HABITAT SUITABILITY INDEX

Rida Ahmad^{1,2}, Zulfiqar Ali^{2*}, Usman Ahmad³ and Komel Ahmed²

¹*Department of Zoology, Lahore College for Women University, Lahore*

²*Institute of Zoology, University of the Punjab, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

The study addresses the critical task of assessing and prioritizing conservation efforts for threatened species in Pakistan. A comprehensive list of 1,108 bird, mammal, reptile, and amphibian species was compiled, with 80 identified as globally threatened based on the IUCN Red List. The vulnerability assessment reveals that 61% of these species are categorized as vulnerable, 25% as endangered, and 14% as critically endangered, with birds being the most prominent group. Habitats vary, with waterbodies hosting the highest number of threatened species. The research utilizes the System for Assessing Vulnerability of Species tool and Habitat Suitability Index to gauge vulnerability and habitat quality. Top priority terrestrial species for conservation include the Gharial, Asian woolly-necked stork, Indian skimmer, Egyptian vulture, and Tawny eagle. The study incorporates field surveys, expert knowledge, and environmental data, including climate projections and GIS information. An integrated assessment, considering SAVS vulnerability values, HSI scores, and IUCN status, results in a comprehensive priority list. This prioritization identifies 17 high-priority species, comprising nine birds, five mammals, and four

reptiles, along with 50 medium-priority species and three low-priority bird species. The study recommends directing conservation efforts towards these high-priority species, emphasizing the importance of strategic prioritization to maximize the impact of conservation initiatives. This holistic approach, combining vulnerability assessments and habitat suitability, offers a valuable framework for effective conservation planning in Pakistan.

SPECIES RICHNESS, STATUS AND DISTRIBUTION OF WILDLIFE IN POONCH MAHSEER NATIONAL PARK AJK, PAKISTAN

Sidra Shabbir¹, Zulfiqar Ali^{1*}, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³, Maryam Tanveer¹, Ghulam Rabbani¹, Urooj Jamil¹ and Farhan Anjum¹

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

In Pakistan, the diverse ecosystems, particularly in arid regions, contribute significantly to the country's rich biodiversity, hosting 198 mammal species, 660 bird species, 13 unique reptiles, and 21 amphibian species. Despite the challenges posed by the arid environment, these organisms play crucial ecological roles and provide diverse ecosystem services. Recognizing the environmental risks and human activities threatening this biodiversity, a research study was conducted in Poonch-Mahseer National Park, AJK, Pakistan, from August 2022 to June 2023. Utilizing methods such as point count, sky view, and flushing techniques, the study covered six habitats, including agriculture areas, barren land, range land, settlements, subtropical chir pine, and water bodies. The research identified 362 individuals representing 58 species across amphibians, reptiles, birds, and mammals. Of these, 45 species were categorized as least concerned, while two were vulnerable and two near threatened. The waterbody habitat exhibited the highest species abundance, with agricultural areas and range land also supporting a significant diversity of species. Habitat-specific analyses revealed the Himalayan wood mouse, Bar-tailed treecreeper, and Common Hoopoe as prevalent in agricultural areas, while the Black Red Start dominated barren terrain. The study employed Shannon Wiener and Simpson indices to assess habitat diversity, highlighting range land as the most diverse and subtropical chir pine as the least diverse. Feeding guild analyses identified 58 species, with carnivores being the most prevalent, particularly in water bodies, while insectivores were dominant in agricultural settings. Sorensen and Jaccard indices indicated high habitat similarity between settlements and agriculture areas, contrasting with lower similarity between settlements and water bodies. The findings underscore the importance of conservation measures to safeguard the diverse habitats and species in Poonch-Mahseer National Park amid increasing environmental risks and human-induced threats.

SULEIMAN MARKHOR POPULATION STATUS AND ASSOCIATED THREATS IN TORGHAR HILLS RANGE, WESTERN CENTRAL PAKISTAN

Asmatullah Kakar^{1*}, Zulekha Hassan², and Zafar Ullah

Department of Zoology, University of Balochistan, Quetta

Department of Zoology, University of Loralai

*Corresponding Author: asmardanzai@yahoo.com

Torghar Hills (Black mountains) lies in the Zhob division, north eastern Balochistan has been an important habitat of Suleiman Markhor (*Capra falconeri gerdoni* Hume, 1875) and Afghan Urial (*Ovis*

vignei cycloceros Hutton, 1842.). Population numbers of these species were highly reduced to critical levels through indiscriminate hunting. Therefore, to reverse the rapid decline of population numbers, the local tribesmen of the area agreed on species wide hunting ban; and in 1984, with technical assistance from the US Fish and Wildlife Service, established the Torghar Conservation Programme (TCP). Several surveys on markhor occurrence and existing threats to markhor life and habitat are conducted in Torghar conservancy. The present study was conducted in the early summer (lambing seasons) in May 2022 with the aims to provide new updates of markhor occurrence and investigation of negative impacts make worsen the survival of this gallant wild goat. Due to different habitat types and tough terrains in the study area, direct (visual) count methods were applied used Binoculars with the help of game guards and local inhabitants live nearby the TCP. Among 354 total observed individuals of this endangered markhor subspecies; females were with the highest percentage (49.17%) followed by males (24.29%) including trophy size males, young (18.92%), and lambs (7.06%). These results are close to the most recent published data (January, 2021), there were an estimated 334 Suleiman markhor including females, males and trophy size males, young ones and yearlings in its entire range in Torghar Hills. The markhor subspecies are facing many threats throughout their distribution range including poaching, habitat loss, illegal hunting, livestock grazing and reduced specialized habitat for forage. Future conservation of the *Suleiman* markhor in Pakistan could include an eco-touristry program.

DRIVERS OF HUMAN-CARNIVORE CONFLICT IN NORTH-EASTERN PAKISTAN

Khalid Mehmood¹, Alina Mushtaq¹, Muhammad Taimoor Chishti¹, Zeeshan Zulfikar¹, Muhammad Sheraz¹, Hammad Bin Shoukat¹, Muhammad Raqeeb¹, Asad Aslam¹, Siddiqa Qasim², Tariq Mahmood² and Faraz Akrim^{1*}

¹Department of Zoology, University of Kotli, Azad Jammu and Kashmir

²Department of Zoology, Wildlife & Fisheries, PMAS Arid Agriculture University, Rawalpindi

*Corresponding Author: faraz.akrim@uokajk.edu.pk; farazakrim@hotmail.com

Human–carnivore conflict is one of the major conservation and rural livelihood issue since many carnivore species have been heavily persecuted due to elevated conflict levels with communities. This challenge is particularly evident in areas where people live near natural forests and protected areas. We investigated the drivers of human-carnivore conflict in northeastern Pakistan using a self-designed questionnaire to collect data from 1052 respondents residing in 115 villages from January 2020 to December 2021. We collected data on occurrence records, number of livestock holding and livestock depredation by the common leopard and Asiatic jackal from respondents. The data were also collected on environmental factors including 19 bioclimatic variables, elevation, distance from forest, NDVI, global land cover, and slope, using zonal statistics in ArcGIS version 10.3 from topographic and environmental layers of the study area. We used a generalized linear GLM with a Poisson-linked function that is a regression model in R. We performed a stepwise GLM model based on the Akaike information criterion AIC to obtain an optimal GLM model that kept only significant factors. Livestock depredations by the common leopard and Asiatic jackal were negatively associated with Bio-2 (mean diurnal range), Bio-6 (minimum temperature of the coldest month), Bio-14 (precipitation of the driest month), distance to the forest, and NDVI (normalized difference vegetation index). In contrast, it was positively associated with the number of livestock held by the respondents. More detailed studies under varying conditions are required to understand the drivers of human-carnivore conflict in the study area.

EVALUATION OF A NOVEL TECHNIQUE TO DETECT THE PRESENCE AND ATTRACTION OF WILD BOAR (*SUS SCROFA*) TOWARDS BAITING POINTS

Irfan Ahmed^{1*}, Uswah Sehar¹, Zakia Panhwar¹, Abdul Aziz Khan², and Muhammad Mushtaq³

¹*Vertebrate Pest Management Programme, Institute of Plant and Environmental Protection, National Agricultural Research Centre, Islamabad*

²*Institute of Plant and Environmental Protection, National Agricultural Research Centre, Islamabad*

³*Department of Zoology, Wildlife and Fisheries, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi*

*Corresponding Author: rajairfanahmed@gmail.com

The population of wild boars is flourishing at rapid rate and it is widely distributed in different habitats of Pakistan because of its high fecundity rate, a smaller number of predators and being not hunted for consumption. There is a need for reliable methods to attract wild boars towards baiting points and to detect their presence in newly colonized habitat and areas in which population of wild boar is low. This study was conducted in two different locations i.e. Muzaffarabad, AJK and Attock, Punjab, Pakistan. The main aim of this study was to evaluate the effectiveness of a putative attractant as a new economical tool for detecting the presence of wild boar and attracting them towards baiting points for its management. We applied waste motor oil on ground surface at 14 treated sites in both locations measuring 6x6 square feet near established bait stations where wild boars had been regularly observed. Our results indicated that wild boar activity of digging of soil surface, rolling over oily surface and fresh foot prints were found more toward treated sites compared to non-treated sites. It is possible that wild boars might be attracted towards waste motor oil due to its pungent odour and to kill its ticks and mites present on body. These results suggest that scent-based lures and other lubricants like waste motor oil is effective for detecting the presence of wild pigs in newly colonized sites, and attracting towards sites where quick assessment of population is required for control operations or the areas which have relatively low population.

NAVIGATING COEXISTENCE: CHALLENGES FACED BY COMMON LEOPARD (*PANTHERA PARDUS*) IN AJ&K, PAKISTAN

Shaista Ali^{1,3*}, Bashrat Ahmed¹, Zeeshan Khalid², Riaz Aziz Minhas¹ and Naeem Iftikhar Dar³

¹*Department of Zoology, The University of Azad Jammu & Kashmir, Muzaffarabad*

²*WWF Islamabad*

³*Wildlife and Fisheries Department, Azad Jammu & Kashmir*

*Corresponding Author: syedashaista456@yahoo.com, Sshaista086@gmail.com

Azad Jammu & Kashmir (AJ&K) is renowned for its biological and cultural diversity and covers an important part of the leopard habitat in Pakistan. Human-leopard conflict is an alarming and complex issue in AJ&K and addressing it effectively requires a multipronged approach that includes scientific research, monitoring, policy implementation and community base approaches. The present study is initial endeavor aimed to determine the extent of human leopard conflict and execution of effective conservation strategies to alleviate of detrimental effect of conflict across AJ&K. We conducted semi-structured interviews with 590 randomly selected pastoralists & local people to investigate human-leopard conflict and community perceptions towards leopard in AJK and also used official reports & data available with the wildlife department from 2014 to 2022. According to our finding the total of 1560 livestock was killed by leopards from 2014 to 2022 and in human-leopard coexistence, seven humans were injured and five died due to leopard attacks in the leopard zone. Forty-five leopards were killed by local community in retaliation to

livestock depredation and human casualties. The results revealed that the number of prevention strategies used did not have any effect on the number of livestock killed by leopards ($r^2 = 0.049$). The majority of people suggest that compensation for wildlife loss is the most effective way to alleviate human-wildlife conflicts. The majority of respondents (86.2%) dislike the leopard due to the unavailability of leopard action plan, compensation for wildlife damages, lack of awareness & economic losses. Our study recommend that the implementation of conflict mitigation plan can effectively mitigate the detrimental effects of conflict, targeting both the socio-economic well-being of local communities, as well as the conservation of leopard, raise awareness among communities & can build trust & motivate them to foster coexistence with leopard and wildlife and to achieve benefits on multiple levels. Considering the significant role of the leopard in the AJ&K region & ecosystem, we argue that more emphasis should be given to the protection of prioritized leopard populations.

FOREST RESOURCE UTILIZATION AS FUELWOOD AND TIMBER WOOD BY LOCAL POPULATION OF MUSK DEER NATIONAL PARK (MDNP) NEELUM, AZAD JAMMU AND KASHMIR, PAKISTAN

Liaqat Ali Khan¹, Basharat Ahmad¹, Riaz Aziz Minhas^{1*}, Usman Ali¹ and Muhammad Arshad²

¹*Department of Zoology University of Azad Jammu and Kashmir, Muzaffarabad*

²*Himalayan Wildlife Foundation, Azad Kashmir*

*Corresponding Author: riaz.aziz@ajku.edu.pk

An increase in human population and anthropogenic pressure imposes serious threats to biodiversity and even many species are at verge of extinction. A global goal was set to establish the protected areas (PAs) for conservation of natural resources. Pakistan is also placed among those countries where human population growing rate is high and there is severe threat to biodiversity, however, there are number of National parks are established, where conservational plans are executed. Musk Deer National Park (MDNP) Gurez the protected area (PA) located in District Neelum, Gurez valley Azad Jammu and Kashmir, Pakistan. Sum total of 29 surveys, and questionnaire (n=576) were carried out to study the anthropogenic pressure in terms of utilization of forest as fuel and timber in MDNP. Data suggests that total of n= 22784 human population settled in 2618 household during the whole year in different parts of the MDNP. Moreover, a vast number of people (n=442 families) along with their livestock from outside the park move to hilly area during summer (May-August) in different region of MDNP. These people rely on forest for burning fuel. As fuel utilization vary in different month of year so, months are categorized into three classes May-August followed by September-November and December-April. It has been estimated that single family (average of 5 members) utilize an average total of 2043 kg/capita/year. The value of wood consumption was estimated an average of 5544.822 metric tons in whole year for 3060 families inhabiting in MDNP. The average fuelwood consumption for 3060 families were recorded as 1358640 kg/capita/year (1358.64 metric ton) from May-August, followed by 3102330 kg/capita/year (3102.33 metric ton/year) for the month of December-April and 1083.852 metric tons fuel utilized by whole families (2618) during the month of September-November. Maximum average fuelwood consumption 9.3kg/capita/day was estimated among the families which living near forest less than 1km followed by 5.1 kg/capita/day those living less than 5 Km from forest. However, those families which living at distance 5 to 10 Km from forest consume minimum quantity 3.9 kg/capita/day wood as fuel. Three models social, economic and biophysical suggested that there is significant difference among dependent and independent variable. Forest uses as timber was also estimated by counting and catalogue the Buildings, out of them 114 houses, guest house (n=22) and animals shed (n=128) were constructed during the year of 2013-2018. Maximum wood (42.02m³) is consumed for guest house construction

followed by local house (22.08m³) while minimum wood (4.1m³) for construction of animal shed. Large quantity of wood is consumed for house construction (2517.12 m³) followed by guest houses (924.44 m³) and 524.8 m³ was used in animal shed.

SPATIAL DISTRIBUTION AND DIVERSITY OF PASSERIFORMES IN THE HUMAN MODIFIED LANDSCAPE OF CHITRAL

Fathul Bari, Syed Ghulam Rabbani, Ilyas Muhammad and Aitizaz Ahmad

Department of Zoology, University of Chitral

*Corresponding Author: bari@uoch.edu.pk

The current study surveyed the spatial distribution and diversity of Passeriformes in the human-dominated landscape of Chitral, Pakistan. The study area covers various habitat types, including forest, agriculture fields, and human settlements. The line transect method was used to collect field data where at least two observers counted and recorded the birds. Eleven (11) different localities in the study area were surveyed during May and September during the early hours of morning. A total 3599 individuals belonging to 27 families and 54 species were recorded during the study. Highest number of species were recorded from areas like Booni, Sor Laspur while Khursashadeh and Charun had lower diversity of species. According to local occurrence status, there were 11 species ranked as very abundant, 8 species were ranked as abundant. Among the recorded bird species more than 66% of the species were migratory, 14% resident, around 13% summer breeders in the area. Shannon - Weiner Diversity index (H') was calculated as 3.225. Six Dominant species with their Relative Abundant RAI included, *Passer domesticus* (2.19), *Acridotheres tristis*, (1.48), *Oriolus oriolus* (0.70) , *Lanius schach* (0.57), *Troglodytes troglodytes* (0.56), *Pica pica* (0.52), *Sturnus pagodarum* (0.45). Among the recorded species 16 are listed with decreasing population trend by the IUCN which highlights their conservation importance and monitoring. The area provides important breeding and staging grounds for various species of birds. However, they are facing various challenges including expansion of human population and habitat fragmentations resulting in their population decline. The current study could serve as a benchmark for future monitoring and studies.

ASSESSMENT OF AVIAN FAUNA OF DAPHAR FOREST WILD LIFE SANCTUARY

Iqra Laraib and Sana Ashraf*

Department of Zoology, The University of Lahore, Lahore

Corresponding Author: sanaashrafdr@gmail.com

The main purpose of the study was to determine the diversity of avian fauna of Daphar forest wildlife sanctuary in District Mandi-bahu-din, Punjab, Pakistan. Point count method was used for birds' diversity. Total 48 species of Birds were recorded from Daphar forest. Most of the bird's species recorded in the forest belonged to Passeriformes order. Maximum relative abundance was recorded for house crow (9.37) from birds. Evenness was calculated for Birds (0.43). Simpson diversity index was also calculated. Shannon-wiener Diversity index was also calculated for birds (3.51). During this study it is also noted that diversity of birds is decreasing rapidly due to human interactions.

IMPACT OF CLIMATE CHANGE ON WILDLIFE

**Sajad Ali, Qudratullah Kalwar, Muhammad Mohsin Rahimoon, Fayaz Hussain,
Iqrar uddin, Taj Muhammad and Abdul Razzaque**

*Department of Theriogenology, Shaheed Benazir Bhutto University of Veterinary and
Animal Sciences, Sakrand, Shaheed Benazirabad*

*Corresponding Author: sajjad00laghari@gmail.com; VetSajjadLaghari@gmail.com

Climate change also known as global warming is an increase in temperature of our environment is believed to be caused by the greenhouse effect. Variations in the abiotic factors for long time such as temperature, rainfall, snow and wind pattern refer to climate change or global warming. Human activities are basic cause of climate change, in simple words directly or indirectly humans are responsible for climate change. Global warming is emerged as one the major challenges that affects ecosystem of our planet. It creates new challenges for all living organisms specially wildlife. Wild life and their habitats are seriously impacted by the climate change and also responsible for serious effects on ecosystem and ecosystem services all over the Globe. Most common examples of climate change are cloudbursts, increased wildfires, increasing sea level, reduced agriculture yields, water diminish and health problems etc are directly or indirectly affects the wildlife. It causes disturbances in food chains, nutrient flow and health of individual species. Climate change causes the disturbances in the balance of ecosystems by reducing the resources for the wildlife such as food, water and shelter. These are the basic necessities for their survival. These affects could be severe for endangered species for their survival. This is the time to take action together on national, regional and global levels on this global issue to reduce the effects of climate change to save our planet and make it better place for all living organisms. This is just not about the better life of wildlife but is about the future and human being itself. Climate change is well known term by now but more attention and actions are needed to be done regarding the global warming. Awareness programs, seminars, workshops, social media and electronic media can play important role to create awareness about the climate change and their harmful effects.

PREVALENCE AND FEEDING HABIT OF *PTEROPUS GIGANTEUS* IN DISTRICT BAGH AZAD JAMMU AND KASHMIR

**Tabinda Iftikhar, Muhammad Shahbaz*, Iftikhar Hussain, Zakia Arif,
Sanam Majeed and Shmsa Manzoor**

Department of Zoology, Women University Azad Jammu and Kashmir, Bagh

Corresponding Author: shahbazchattha_pk@yahoo.com

The current study was carried out to investigate the prevalence and feeding habits of flying fox (*P. giganteus*) in district Bagh from October 2022 to May 2023. Faecal samples were analyzed to find out dietary contents constituents. 253 samples were collected from four roosting sites, Global positioning system (GPS) location (coordinates) was also ascertained. Faecal pellets were brought to the lab for analysis after being properly placed in zip-top bags and analyzed. It was found that bats feed on 12 species of plants (fruit, seeds) belonging to four families Moraceae (41.08%), Rosaceae (27.19%), Ebenaceae (26.4%), Myrtaceae (5.13%). In their diet, four species of the family Moraceae: namely *Ficus carica* (1.97%, n=5), *Morus nigra* (32.80%, n=83), *Ficus benghalensis* (3.55%, n=9) and *Ficus religiosa*, (2.76%, n=7) were devoured in spring. In a similar vein, five Rosaceae species: *Prunus armeniaca* (1.97%,

n=5), *Eriobotrya japonica* (11.4%, n=29) in spring *Pyrus pashia* (9.48%, n=24), *Malus pumila*, (3.16%, n=8) in autumn, *Prunus persica* (1.18%, n=3) in spring were consumed. Family of Ebenaceae included two species: *Diospyros lotus* (13.8q %, n=35), *Diospyros kaki* (12.6%, n=32) devoured in winter and Myrtaceae family including, *Psidium guajava* (1.87%, n = 2) consumed in autumn.

BIODIVERSITY OF BIRDS IN BAJWAT WETLAND AND WILDLIFE SANCTUARY

Moazma Siddique, Syeda Shazia Bokhari* and Roheela Yasmeen*

Department of Biology, Lahore Garrison University, Sector C, Phase VI, DHA Lahore

*Corresponding Author: shaziabokhari@lgu.edu.pk; roheelayasmeen@lgu.edu.pk

Bajwat wetland and wildlife sanctuary (BWWS) is an internationally important wetland that is located near Sialkot district, Punjab, Pakistan. The present study was conducted for 11 months in 2021. One to two surveys were conducted per week at dawn and dusk. Data was collected both by direct and indirect observation methods. Different indexes were also calculated for the avifauna that showed area was rich in biodiversity. A total of 107 bird species were recorded during study period. Out of 107 bird species, 49.53%, 33.64%, 16.53%, 3.73% were resident, winter, summer, and year-round visitors respectively. Although Bajwat wildlife Sanctuary was found very rich in animal fauna however, many threats such as destruction, fragmentation of habitat illegal hunting, human interference in animal's breeding sites, lack of awareness, and pollution of water due to overuse of pesticides, herbicides and insecticides were recorded. These threats might be due to careless attitude of wildlife department. It is suggested that study site should be conserved for noticed threats on priority basis by implementation and regulation of already formed wetland and wildlife sanctuaries rules.

AVIAN DIVERSITY AND ABUNDANCE DYNAMICS AT TEHSIL CHISHTIAN, PAKISTAN

Muhammad Tahir, Sana Fatima, Nazia Ehsan*, Warda Mustfa and Iqra Bibi

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: nazia.ehsan@uaf.edu.pk

Birds are the indicators of environmental pollution and ecological deterioration in different ecosystems. It has been reported that avian fauna has been declining in various parts of the world as well as in Pakistan. The current study was conducted to analyze the diversity and abundance of avian fauna in selected areas of tehsil Chishtian of district Bahawalnagar, Pakistan. The study was conducted on monthly basis from December 2021 to May 2022. Line transects, direct method and point count method was used for sampling. Combined comparison of all habitats (canal, garden, rural and urban areas) revealed population fluctuation in various habitats. Rural areas average number of birds recorded 1616 (37.94%) was greater than other sites. Shannon Diversity index rural area was 2.83 which show more diversity of birds in rural area. Urban areas average abundance was 297 (6.97%) found and have 1.23 Shannon diversity index. Moreover, among canal and garden average number of birds was found greater in garden 1212 (28.45%) as compared to canal bank site 1134 (26.62%). There was more Shannon Diversity index of birds at canal site 2.66 while garden site has 2.65 Shannon diversity index. Total 2.763 Shannon diversity index recorded with 4259 species abundance and 43 species during six-month observation. Moreover, garden area recorded 1212 and Canal site recorded 1133 numbers of birds respectively. Obtained data and results were analyzed statistically through one way ANOVA, p-esteem value of temporal diversity and abundance of six month is 0.21, indicated that there is no significant variation between six months, month wise data collection showed that the p-esteem value of

December (0.045), January (0.028) and May (0.032) is lower than 0.05. All four habitats show variation in these months. February (0.068), March (0.058) and April (0.12) is greater than 0.05, it showed and there is no variation between the two or more habitats.

SPATIO-TEMPORAL DIVERSITY AND ABUNDANCE OF BIRDS AT DISTRICT D. G. KHAN

Warda Mustfa, Nazia Ehsan*, Muhammad Junaid, Muhammad Tahir and Iqra Bibi

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: nazia.ehsan@uaf.edu.pk

Birds are a significant element of biodiversity of our environment. They perform many biological roles in their habitats as, they are bio indicators of healthy biomes. Population of some species increases and some are decreasing with the increase in urbanization. Urbanization affects the diversity and abundance of birds as well as other species. It is because most birds prefer cracks or cavities for nesting but advanced construction of houses lack cracks or cavities, prevent nesting and so avian population decreased. In rural and other areas, factors like pesticides and other chemicals not only affect the diversity of birds but also significantly affect other species. The present study was conducted to determine the diversity of birds in district D.G. KHAN. Sampling was done on monthly basis from December 2021 to May 2022. Each sampling area was almost 1 km² with four different types of habitats viz. high density populated area (urban area of D.G. KHAN), low density populated area (Notak mehmeed), rural agricultural area (Residential area) and canal road side area. Visual counting method, least count method and point count method were used for sampling. A total of 3749 specimens were observed and counted from four different sites. Out of the total specimens 819 birds were observed from urban area, 749 specimens were observed from Kot Chutta canal side area, 781 from Notak mehmeed and 1400 birds were observed from rural residential area. There were total Order (10) families (24), Genus (34) and species (42) were identified. From all selected sites the total number or values of species was 104, Abundance (N) 3749, dominance (D) 0.05279, diversity (H') 3.301 and evenness (E) 0.6459, respectively. From all the study sites, the most abundant recorded species was *Corvus splendens* belonging to the family Corvidae has 432 (11.52%) specimens, and least abundant species *M. apiaster* has 15 (0.40%) specimens belonging to the family Meropidae, respectively.

FEEDING GUILDS OF BIRDS IN KHUNJERAB NATIONAL PARK, PAKISTAN

Ansar Habib¹, Zulfiqar Ali¹, Fiza Adil¹, Syeda Nida Fatima¹, Maria Hanif¹, Muqadas Naveed¹, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³ and Rafia bibi¹

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

Khunjerab National Park (KNP) is a high-altitude protected area in northern Pakistan. KNP hosts a rich diversity of birds, including some rare and threatened species. However, the status and trend of the bird population in KNP are not well monitored or documented. Rigorous survey methods were used to examine avian feeding guilds in Khunjerab National park. The feeding guilds of the birds were identified, comprising five feeding guilds in the park; carnivore (12), granivore (3), herbivore (2), insectivore (15),

and omnivore (19). The trophic structure and function of the ecosystem was examined in the light of feeding guilds and showed how it affected the population dynamics and resilience of the birds. The results indicated that the bird population trend in KNP is generally stable or increasing, especially for birds of prey like Lammergeier vulture and Golden eagle. This is attributed to the effective conservation efforts, anti-poaching measures, and community awareness in KNP. It was concluded that KNP is a birdwatcher's paradise and a model for other high-altitude regions in the world. We suggest that the bird population trend in KNP should be regularly monitored and evaluated, and that the challenges and threats to the birds should be addressed and mitigated.

FECAL PELLETS MORPHOMETRY IS AN INNOVATIVE APPROACH FOR AGE AND SEX IDENTIFICATION OF BARKING DEER (*MUNTIACUS VAGINALIS*)

**Shahzad Aslam^{*1}, Amjad Rashid Kayani¹, Muhammad Azhar Jameel¹*, Tariq Mahmood¹,
Shiekh Marifatul Haq²*, Ajmal Khan³ and Muhammad Fiaz Khan⁴**

¹Department of Zoology, Wildlife, and Fisheries, PMAS-Arid Agriculture University, Rawalpindi

²Department of Ethnobotany, Institute of Botany, Ilia State University, Tbilisi, Georgia.

³Department of Environmental Sciences, University of Haripur

⁴Department of Zoology, Hazara University, Mansehra

*Corresponding Author: shahzad_zoologist@yahoo.com

Fecal pellet morphometrics, a non-invasive technique, offers vital data on population age classes that can be used for wild population management and conservation. The barking deer (*Muntiacus vaginalis*) has had severe declines in population throughout its range, requiring the attention of wildlife biologists and conservationists. We used morphometric analysis of fecal pellets in this study to accurately determine the age and sex of Barking deer (*Muntiacus vaginalis*) in the Margalla Hills National Park (33.7481° N, 73.0051° E), which may have ramifications for the species' conservation. Line transect sampling was employed to gather the samples, and 112 pellets were chosen at random to be analyzed. Fecal pellets from three categories of Barking deer: males (Buck), females (Does), and yearlings, were analyzed using discriminant classification coefficient function and K-mean clustering analyses. The fecal pellets exhibited a diversity of morphometric attributes, such as position and number of the tip, as well as cleavage furrow patterns. A notable disparity was observed in the shape of fecal pellets between adult males and females. The findings regarding the estimated value of the length-to-width (L/W) ratio, which was higher for males (M=2.16) in contrast to females (M=1.67) and yearlings (M=1.66). The coefficient of variation in relation to the (L/W) ratio exhibited a higher value of 3.334 for males, compared to females (2.64) and yearlings (0.121). The discriminant function correctly assigned 86.7% of adult males, 74.3% of adult females, and 98.1% of yearlings to their respective age classes. Additionally, the K-mean clustering analysis achieved a 100% accuracy in differentiating individuals. The length of the fecal pellets in adults and the volume in yearlings were good indicators and the overall results demonstrated high level of accuracy in age and sex identification. In conclusion, this study establishes fecal pellet morphometry as a reliable method for accurately determining the age and sex of Barking deer while minimizing disturbances to the wild population.

TICK TAXONOMY AND NUCLEOTIDE SEQUENCE ANALYSIS BY INTERNAL TRANSCRIBED SPACER 2 (ITS 2) IN LARGE RUMINANTS OF POTHOHAR, PAKISTAN

Farkhanda Jabeen

Department of Zoology, Wildlife & Fisheries, PMAS Arid Agriculture University, Rawalpindi

*Corresponding Author: farkhanda1980@yahoo.com

Ectoparasites (insect and arachnids) are a worldwide problem, which are responsible for up to 80% losses to the livestock economy. Among these, the Ixodids (hard ticks) are the most important and also transmit diseases like babesiosis, theileriosis and anaplasmosis. The present study was aimed to identify prevalence and distribution of the hard tick species using morphological and molecular keys in large ruminants of Pothohar region of Pakistan. Morphological investigations of ticks from 967 cattle and 665 buffaloes revealed the genera (3) and species (13) of various Ixodid ticks mainly *Rhipicephalus* (R), *Hyalomma* (Hy), and *Dermacentor* (D) comprised of *R. microplus*, *R. annulatus*, *R. australis*, *R. eversti*, *R. turanicus*, *R. decloratus*, *Hy. dromedarii*, *Hy. scupens*, *Hy. rufipes*, *Hy. truncatum*, *Hy. excavatum*, *Hy. detritum* and *D. atrosignatus*. Tick invasion was statistically higher in cattle (53.48%) than buffaloes (40.70%). Among these 10 species identifications were confirmed through morphological identification, however, molecular characterization of tick analysis (using partial ITS2 gene) confirmed prevalence of *R. microplus*, *Hy. detritum* and *D. atrosignatus*. This study provides the basic genetic identification tool for investigating the tick species and their phylogenetic relationship in tick species commonly found in humans and animals. The precise identification unquestionably can play a dynamic role in employment of appropriate control measurements, as different genera/species cause different diseases in livestock. Different tick species identified on the basis of morphological features and molecular characterization, will add value to the existing knowledge in identification of ticks in the Pakistan, as well as helpful in various disease diagnosis spread due to these hard tick species.

THERAPEUTIC EFFECTS OF ARTEMISIA BREVIFOLIA ON CISPLATIN INSTIGATED CARDIOTOXICITY IN RATS

Naila Ghafoor and Muhammad Umar Ijaz*

Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

*Corresponding Author: umar.ijaz@uaf.edu.pk

Cisplatin (CP), an effective chemotherapeutic drug, has been widely used to treat the several types of tumors. Investigations have been accomplished on numerous species of genus *Artemisia*, but the plant *Artemisia brevifolia* has hardly been used in this respect. The present study was considered to ascertain the effects of “*Artemisia brevifolia* plant extract” on CP induced heart injury in rats. Twenty-four male rats were divided into four groups equally and termed as control, CP (10 mg/kg), CP (10 mg/kg) + *Artemisia brevifolia* extract (150 mg/kg) and *Artemisia brevifolia* extract (150 mg/kg) groups. After seven days trial, rats were dissected and different parameters were analyzed. Our results revealed that treatment of CP reduced the activity of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione-disulfide reductase (GSR), glutathione S-transferase (GST) as well as glutathione (GSH) while elevate ROS and MDA levels. Furthermore, concentrations of cardiac injury markers, creatinine phosphokinase (CPK), creatine kinase-myoglobin binding (CK-MB), & lactate dehydrogenase (LDH), as well as troponin I were increased in response to CP treatment. Besides, the levels of nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β) and IL-6 as well as the

cyclooxygenase-2 (COX-2) activity were elevated due to the CP administration. In addition to it, histopathological assessment displayed the prominent morphological alterations in heart tissues of rats. Nevertheless, treating the rats with *Artemisia brevifolia* significantly abated all the CP-instigated heart damages in rats. Therefore, it was evinced that *Artemisia brevifolia* could be used as a cardioprotective agent against the CP-prompted heart toxicity owing to its antioxidant and anti-inflammatory properties.

5. BIODIVERSITY

DISTRIBUTION AND ABUNDANCE OF EARTHWORM SPECIES IN DISTRICT NEELUM, AZAD KASHMIR, PAKISTAN AND ACT AS SOIL BIO-INDICATOR

**Rozina Ghulam Mustafa¹, Saiqa Andleeb^{1*}, Fatima Jalal²,
Muhammad Umar Khan⁴ and Muhammad Fiaz Khan⁴**

¹*Microbial Bioremediation and Vermi-technology laboratory, Department of Zoology, University of Azad Jammu & Kashmir, King Abdullah Campus, Chattar Kalass, Muzaffarabad*

²*Department of Zoology, Government College University, Faisalabad*

⁴*Department of Zoology, Hazara University*

*Corresponding Author: drsaiqa@ajku.edu.pk; drsaiqa@gmail.com

The aim of current research was to investigate the diversity and richness of earthworm species of district Neelum, Azad Jammu and Kashmir, Pakistan and impact of earthworm species on soil. Earthworms were collected by hand sorting and digging procedures and study was conducted during July, 2017 to October, 2017 from the cultivated lands on the basis of ecological zones such as ice caps and glaciers (Zone I), very cold non-monsoon alpine (Zone II), and cold non-monsoon alpine (Zone III). Soil samples were collected and were analyzed for physio-chemical properties. Population density, Shannon diversity index and encounter rates for each species were calculated. Both epigeic and endogeic species i.e. *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea trapezoides*, *Aporrectodea longa*, *Apporactodea rosea*, *Bimastos parvus*, *Drawida nepalensis*, *Eisenia fetida*, *Lumbricus rubellus*, *Pheretima hawayana*, *Pheretima lignicola*, *Pheretima posthuma* were identified morphologically. It was observed that various localities especially high elevated areas including ice caps and glaciers (Zone I) decline the earthworm diversity due to snow and cold temperature, low organic matter and other contents such as phosphate 7 ppm, nitrogen 0.01 % and potassium 78 ppm as compared to other zones. Highest earthworm abundance was recorded in various villages of Kern and Athmuqam due to presence of organic matter (4.4%) while minimum in case of Kel, Arang kel up to Taobut localities due to lowest organic matter. The *Aporrectodea* species were more dominant species in all localities. The whole observation clearly indicated that the earthworm's richness.

MAPPING THE CURRENT STATE OF SNAKEBITE OCCURRENCE AND HEALTHCARE FACILITIES IN AZAD JAMMU AND KASHMIR

Amna Hameed¹, Riaz Aziz Minhas^{1*}, Attia Farooq¹, Muhammad Siddique Awan¹, and Usman Ali²

¹*Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad*

²*Department of Zoology, Mirpur University of Science and Technology, Mirpur*

*Corresponding Author: riaz.aziz@ajku.edu.pk

Snakebite is a major public health problem and millions of annual snakebite incidences and human mortalities have been documented worldwide. Thousands have suffered serious medical, psychological, and socioeconomic consequences due to snakebite primarily the remote, impoverished,

rural farming communities with weak medical facilities. In Pakistan, the annual fatality rate due to snake bite envenomation is 1.9 per 100,000 people, with the southern region of the nation being the most afflicted. However, this information about the prevalence of snakebite in Azad Jammu and Kashmir (AJK) is scarce. Most victims cannot seek hospital-based healthcare and their disease-burden statistics are therefore unrecorded and non-reportable. Consequently, underreported snakebite makes hurdles for designing effective intervention systems. So, there was a dire need to know about the prevalence of snakebite and type of treatment available across different healthcare facilities in AJK. This study aimed to collect baseline data about the prevalence of snakebite cases, availability of healthcare facilities in the form of anti-snake venom and traditional healings and long-term impacts of the snakebite on the victims in different regions of Azad Jammu and Kashmir. For this purpose, data was collected from healthcare centers (n=11), snakebite victims (n=168) and accessible traditional healers (n=56) using prescribed data sheets and semi-structured questionnaire using snowball sampling techniques. A total of 1560 incidences of snakebite were reported from different districts of Azad Jammu and Kashmir during 2019-2021. Among these, 1392 (89%) snakebite cases were recorded from the government hospitals, while other 168 (10.7%) cases were recorded from the survey of public. However, the true estimate of the prevalence of snakebite is underreporting due to the treatment seeking behavior of the victims. Males (hospital n=888, 63.7%; survey n=88, 52%) were most bitten by snakes than females (hospital n=504, 36.2%; survey n=82; 48.8%). Majority of hospitalized victims were of age group 21-30 years (n=331) followed by 11-20 years (n=290) and 31-40 years (n=285). Southern region of the state was highly affected. The highest number of snakebite cases were reported from district Bhimber (23.1%), followed by Mirpur (19.1%), Kotli (14.9%), Muzaffarabad (12.9%). The least number of snakebite cases were reported from Neelum (1.3%). The year 2020 reported the most snakebite cases from all over the state, i.e., 42.3%, during 2021, 30.1% cases occurred and 27.5% during the year 2019. The highest number (n=754, 48.33%) of snakebite cases were recorded in summer (June, July, August), while other remaining cases (n=434, 27.8%) were recorded from other months. The highest number of snakebite cases (43.5%) were reported from mid-day (noon), followed by evening (28%), night (18.5%) and in the morning (10.1%). Traditional healers and snake charmers hold a dominant position in the community regarding snakebite treatment. A total of 56 snakebite healers were interviewed. Emergency first-aid treatments were available in all tehsils (n=32) of AJK. however, the antivenom for snakebite was only available in the DHQ hospitals (n=8), Abbas Institute of Medical Sciences (AIMS) and Combined Military hospitals (n=2) located in the headquarters of districts. Majority of (51.2%) of victims availed spiritual (dam) treatment followed by spiritual treatment and traditional remedies (19%) and first-aid facilities (7.7%). Similarly, 63.1% victims had hospital treatment while 36.9% of the victims didn't avail this facility. A total of 59 (35.1%) victims availed the traditional treatments only and fully recovered, but in other 2 (1.1%) cases such victims had died. Among total of 1560 reported snakebite victims, 1,481 (94.9%) were recovered, 1353 of them were from the hospital records and 128 were reported from the survey. A total of 47 (3.0%) deaths were reported due to snakebites, while other 32 (2.0%) victims were disabled because of the incidence from 2019-2021. Hospital records reported 19 deaths, while 28 deaths were reported from survey respondents. People don't have awareness about the snake species in their areas. Only 4.8% of the victims reported that they were bitten by Indian cobra, followed by Russell's viper (1.8%), common krait (1.8%) and Asian cobra (0.6%). The majority (83.9%) of the snakebite healers had no knowledge about the snake species in their area. Most of the snakebite victims (69.6%) experienced psychological problems followed by the 25% victims who had both economical and psychological impacts of incidence. Other 2.4% had bad economic impact while 3% of the victims had no impact of the incidence. About 70.2% of the snakebite victims and other respondents had negative perceptions about snakes, while 29.8% said that snakes had positive role. In 76.2% of the cases snakes got escaped from the incidence, and in 17.9% of the cases, snakes were

killed while in 6% cases, snakes were injured but escaped. There is a need to build primary and secondary health care centres in rural areas far from cities with basic treatment facilities available. In order to treat a highly pervasive but deadly public health issue, accessibility must be improved, information must be shared, and clinical and technical skills must be distributed.

IMPACTS OF TOURISM ON BIODIVERSITY IN THE STATE BIOSPHERE RESERVE NEELUM, AZAD JAMMU AND KASHMIR

**Attia Farooq¹, Riaz Aziz Minhas^{1*}, Amna Hameed¹, Muhammad Siddique Awan¹,
Basharat Ahmad¹, Usman Ali², and Muhammad Jahangeer¹**

¹*Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad*

²*Department of Zoology, Mirpur University of Science and Technology, Mirpur*

*Corresponding Author: riaz.aziz@ajku.edu.pk

Tourism is one of the fastest growing industries in the world. It is closely linked to the biodiversity of an area especially the protected areas. However, the scale and growth rate of tourism industry present both opportunities and threats for biodiversity conservation. Tourism if not managed properly can cause the biodiversity loss by irresponsible tourists' activities. To protect the scenic beauty and globally significant biodiversity of the area, the entire Neelum valley has been notified as the State Biosphere Reserve Neelum (SBRN) by the Government of Azad Jammu & Kashmir (AJK). However, for the last few years, tourism activities have been progressing rapidly. Being newly established tourism spot, most of the tourism activities are not properly managed so far. Like many other tourist spots, it was assumed that the tourism activities might have drastic negative impacts on the local biodiversity and other natural resources of the area. In this backdrop, this study was organized to evaluate the impact of tourism on the local biodiversity, local culture, and other natural resources of the area. The data was collected by field survey, questionnaire survey and group discussions. Approximately an average of 0.600 million tourists annually visited the Neelum valley during the last 10 years (2011-2021), however, these numbers increased in 2022 to about 1.00 million. Majority (25.4%) of the local respondents and government officials believed that deforestation rate has increased up to 60% and 40% because of tourism respectively. Consumption of timber for construction of hotels and rest houses has increased many folds and most (32.6%) of the local respondents claimed that at least 3-7 big trees are required to construct an average-sized guest house. Majority of the respondents, including residents (65.9%) and departmental respondent (51.4%) believed that there was no disturbance to the wildlife due to tourism, however, a considerable number of local people (16%) and departmental respondents (23%) thought that wildlife of the area was disturbed due to increase in tourism. Angling without licenses was predominantly carried out by tourists at Sharda, Keran, Kel and Taobut. However, no case of wildlife hunting by tourists were reported. Soil degradation and erosion was found in Sharda and Keran/Upper Neelum. Mild loss of agricultural land was recorded at Sharda, Kutton, Upper Neelum, Arang Kel and Taobut. The change in local dressing was observed in Upper Neelum, Arang Kel and Taobut. Overcrowding of tourist caused disturbance to the local community at Sharda, Upper Neelum and Arang Kel. Use of drugs and drinks were also reported from some areas. Youngs of the area were increasingly adapting number of behavioral changes while observing tourists. Some have left the education and were working at the guest houses, hotels etc. The agricultural practices have been reduced as female workers felt uncomfortable to perform outdoor activities in the presence of strangers in their surroundings. Biodiversity protection and conservation, as a primary purpose of its establishment, was suggested by most reported by tourists (46.6%) and local people (44.2%). Respondents (tourists 43.6% and local 31.2%) thought that tourism should be second important purpose of the SBRN. Most of the visitors (60.2%), local respondents (55.1%) and government officials (57.1%) were willing to pay entry fee for better management of the SBRN. A large number (32.3%) of the tourists opted neutral on the level of the compliance

of biosphere regulations, whereas 27% of the local people thought that there was low level of compliance of rules and regulations in the SBRN. Contrarily, majority (31.4%) of government officials were having opinion that compliance of rules and regulations should be strictly applied. Majority of the respondents claimed that tourism has positive impact on the economy of the area. About 85% of locals and 90% of government officials reported that there was a rise in local jobs, particularly in on-season. Rise in the number of hotels, tourist lodges, restaurants, cafes, transport vehicles and their associated jobs were observed at all tourist localities in the study area. Different stakeholder departments were playing their role in relevant fields. However, they lack any proper strategic planning's to safeguard the local biological diversity with increase influx in future.

ASSESSMENT OF INDIGENOUS ADAPTIVE MEASURES BY LOCAL COMMUNITY AGAINST CLIMATE CHANGE IMPACTS ON BIODIVERSITY IN MACHIARA NATIONAL PARK

Shaista Aziz Minhas, Muhammad Siddique Awan, Riaz Aziz Minhas*

Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad

*Corresponding Author: riaz.aziz@ajku.edu.pk

One of the most important worldwide issues is climate change impacts on biodiversity, which have devastating effects on human civilizations and natural ecosystems. Among other major environmental changes, the globe is now suffering with loss of biodiversity by habitat destruction through rising sea levels, more frequent and severe extreme weather events, rising temperatures, and changing weather patterns. This study investigated the indigenous adaptive measures taken by local communities against climate change impacts in Machiara National Park, Azad Jammu and Kashmir. It also highlights the public perception about effectiveness of different adaptive measures taken by community, role of government and non-governmental agencies in mitigation of climate change in study area. The study was conducted in different villages of three Union councils (Bheri, Machiara and Sarli Sacha) within 5 km of the park's boundaries. The data was collected by field surveys, using semi-structured questionnaires and focal group discussions during 2022-2023. The study revealed that the respondents were able to articulate the effects of a changing climate on biodiversity, water resources, agricultural and horticultural production, rainfall, temperature, and glaciation. They observed declining of biodiversity, reduction in water springs, low agricultural production, declining in wildlife population and medicinal plants, as the major impacts of climate change. Most of the respondents did not take any adaptive measures against climate change impacts. The most prominent barrier to take any adaptive measure is the lack of financial resources, followed by a lack of awareness, shortage of time, difficulties in accessing necessary information and lack of support from government. Some adaptive measures taken to adapt climate change impacts includes changing their personal behavior in a response to these impacts, adopting more environmentally friendly habits, local conservation efforts, planting more plants, reducing tree cutting, reducing harvesting, actively participating in conservation efforts, increasing plantation efforts, changing the varieties of crops, installing irrigation systems, using pesticides, altering cropping time, using predators for pest control, using eco-friendly pesticides, use of crop rotation technique, mulching/composting, drip irrigation, planting native trees, reducing energy usage, reducing water usage, reducing waste production, stopping hunting, installing alternate water sources or schemes, water conservation efforts, installing water-efficient appliances, conserving rainwater, planting drought-resistant crops or plants, shifting to renewable energy sources, installing energy-efficient appliances, altering agricultural practices, soil conservation, using public transport, reduction in the use of fossil fuels. However, majority of the respondents reported negative view about the effectiveness of these adaptive measures. Some respondents found them slightly effective and few respondents were uncertain or unsure and least number of respondents answered "very effective" about the effectiveness of adaptive measures. Regarding the role of government and non-governmental, the majority of respondents reported

receiving no support from the government in their efforts to combat climate change impacts, while some respondents mentioned receiving some level of support particularly in conservation of wildlife and water resources. The findings of this study provide insight of the indigenous knowledge about climate change impacts and adaptive measures by local communities and government. It would be helpful in devising state level climate change adaptive strategies in future.

CASE STUDIES OF MALARIAL REPORTS OF PATIENTS FROM SERVICES HOSPITAL, QASIMABAD AND CIVIL HOSPITAL, HEERABAD, DISTRICT HYDERABAD

Barkat Ali Bughio*

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: barkatali2009@gmail.com

Malaria is a life-threatening parasitic disease annually causing 300-500 million clinical cases. Malaria is caused by the protozoan *Plasmodium*, transmitted to vertebrates by female Anopheles Mosquitoes. Four species of malaria parasites cause disease in humans, *P. falciparum*, *P. vivax*, *P. Malariae* and *P. Ovale*. *Plasmodium* species have remarkable genetic flexibility which lets them adapt to alterations in the environment, giving them the potential to quickly develop resistance to therapeutics such as antimalarials and to change host specificity. Whereas three species give rise to considerable malaria morbidity, only *P. falciparum* results in high mortality. According to the World Health Organization (WHO), Pakistan is one of the seven countries in the Eastern Mediterranean Region that account for 98% of the total malaria burden in the region. Around 3,00,000 peoples affected from malaria and 50,000 died yearly in Pakistan. These cases are reported from 51 districts Throughout the country with the highest number of cases reported from Dadu and Badin districts in Sindh, Kharan and Kachi as well from Baluchistan. Despite experiencing a largely dry starting months of 2023 so far, the province of Sindh faced growing numbers of malaria cases from the months of monsoon in every division, particularly lower Sindh. This study was carried from the month of January 2023 to the month of December 2023 at district Hyderabad, which mainly focuses on the effect of malarial parasite on the human being through studying the reports of malarial positive patients. The disease is characterized by fever associated with symptoms, including headaches, chills, myalgia, malaise and joint pain that can be resolved into mild attack and run an uncomplicated course. During our visits to the Services Hospital Qasimabad and Civil Hospital Heerabad, the total number of malarial positive patients were 3195 out of 15274 suspected patients throughout the year, the 21% were malaria positive and 79% were malaria negative.

ECOLOGICAL VARIATION OF BIODIVERSITY IN THE ENTIRE MARGALLAH HILLS NATIONAL PARK. SEGMENT I: 1,800 FT. (548.6 M) ELEVATION, IN DHOKE JEEVAN VALLEY OF THE SOUTH FACING ASPECT OF THE MOUNTAIN

Sakhawat Ali*, Muhammad Saeed, Muhammad Abbas, Zakir Ali and Z. B. Mirza

Islamabad Wildlife Management Board, Ministry of Climate Change and Environmental Coordination, Government of Pakistan

*Corresponding Author: sakhawat.ali@iwmb.gov.pk

The study area is a valley on the southern aspect of the Margallah Hills Range. This valley had a village called Dhok Jiwan. This was evacuated at the time of the declaration of National Park. Plant succession took place in 44 years period in the bare agricultural areas, which were prevalent from the base

to the top of the valley. In the successional flora, animal biodiversity crept in and now flourishes. This study has attempted to figure out the ecological pattern of the floral biodiversity. The relative abundance of floral species was found variable the east facing slope, sky facing bottom of the valley and the west facing slope of the valley. The general tilt or the steepness of the mountain slope was calculated to consider its impact on flora, such as moisture retaining capacity of the soil, rocky or stony soil. Number of plant species on each type of habitat was calculated and was related to the abiotic conditions of the habitats. Further investigation is proceeding elevation wise, as well as spatially.

INVESTIGATION OF SOIL BIODIVERSITY OF MARGALLAH HILLS' NATIONAL PARK, ISLAMABAD

Muhammad Saeed*, Sakawat Ali, Muhammad Abbas, Zakir Ali² and Z. B. Mirza

Islamabad Wildlife Management Board (IWMB), Ministry of Climate Change and Environmental Coordination, Government of Pakistan

*Corresponding Author: [author: saeedwildlife@gmail.com](mailto:saeedwildlife@gmail.com)

Soil biodiversity is well studied taxonomically, least understood ecologically, particularly in Margallah Hills National Park. This abstract gives the findings of soil biodiversity relative abundance in the soils of the east facing, west facing, sky facing (bottom of) Dhoke Jeevan valley, at elevation between 1,800 ft. and 2600 ft., which is located in almost middle of the National Park and from which Dhoke Jeevan village was vacated almost 44 years ago. Two valleys of villages Rumli and Shahdara, which are located in the eastern flank of the National Park, on the south facing aspect of the mountain, almost at the same elevation as that of Dhoke Jeevan valley. However, collection of soil biodiversity was made only from some areas, as these valleys are wide with flowing water keeping the bottom soil moist. Work is continued. These villages have not been vacated. Moist soil and submerged in shallow water invertebrates belonging to 29 families, 49 genera and 58 species were collected to study their ecological linkages. Further work is continued for this long-term investigation of their ecology.

IMPACTS OF LOCAL COMMUNITIES ON BIODIVERSITY AND CONSERVATION OF MARGALLAH HILLS NATIONAL PARK (MHNP), ISLAMABAD

Zakir Ali*, Muhammad Abbas, Ms. Naghmana Iram² and Z. B. Mirza

Islamabad Wildlife Management Board (IWMB), Ministry of Climate Change and Environmental Coordination, Government of Pakistan, Islamabad

*Corresponding author: zakir.ali@iwmb.gov.pk

There are some villages still not vacated within MHNP as well as adjacent to it. There is no buffer zone around the National Park. Thus, encroachments of National Park land continued with the increase in human population in these villages. The present study analyses the impacts of local communities living in MHNP and around and adjacent to it. One of the objectives is to restore original flora and fauna after it was degraded historically. New plantations were attempted mainly in the eastern and central parts of the southern aspect of the mountain and the rest was left to plant succession. Plantations were not ecological and the succession spoiled the original ecology of the whole mountain Range. Negative anthropogenic activities, including rush tourism, for which the habitats of biodiversity of the entire southern aspect of the mountain are fragmented. After identifying the issues, community awareness has been started. Some activities with women folk to reduce poverty have been started, which are detailed in the study.

BIODIVERSITY OF MACROBENTHIC FAUNA OF CLIFTON BEACH, NORTHERN ARABIAN SEA, KARACHI

Safia Khanam, Faiza Iqbal and Javed Mustaqim

Centre of Excellence in Marine Biology University of Karachi, Karachi

*Corresponding Author: safiakh14@hotmail.com

The present paper deals with the study of macrobenthic community structure of Clifton beach to assess the prevailing health condition and vulnerability of Clifton beach, Karachi. Sampling was done on the selected sites of Clifton during low tides, from June to August, 2021, by using a quadrat (30 x 30 cm) and taking out sediments by a spade up to 10 cm depth. Physical parameters of the sea water such as salinity, temperature and pH were also examined during the study to determine the environmental health of Clifton beach. The results show a number of diverse fauna of marine macrobenthic invertebrates. A total number of 98 species were recorded, with Gastropods, the most dominant group comprising of 40 species. Other fauna included 16 species of Bivalves, 20 species of Crustaceans, 15 species of Polychaetes and, 7 species of other marine groups (Poriferans & echinoderms). Statistical analyses were also performed to compute the biodiversity and to assess the ecological health of the most visited beach of Karachi.

BIODIVERSITY CONSERVATION IN AGRICULTURAL LANDSCAPES

Saba Haider¹, Syeda Fatima Bukhari¹ and Sadaf Haider²

¹*Institute of Plant Protection, MNS-University of Agriculture, Multan*

²*Lasbela University of Agriculture Water and Marine Sciences, Uthal*

*Corresponding Author: sabahaideragri34@gmail.com

Biodiversity conservation in agricultural landscapes is important for maintaining ecosystem services, supporting food production and conserving natural resources. Due to urbanization insect communities influencing their structure by selecting species able to survive the harsh condition. Significant declines in insect biodiversity will have impact across the food chain. However, there is a 3.3% decrease in grain production across the entire value chain. Establishing areas within the farmland for natural habitats like wetlands, forests, grasslands or flower strips helps to maintain biodiversity. These area act as refuges for native species, providing nesting and homes for many organisms. Combination of shrubs, herbs, grasses and flower strips within agricultural fields creates diverse microhabitats. Goal of biodiversity conservation is not only improving soil fertility and water holding but also provides habitats for insects and other wildlife. Planting a variety of crops instead of monocultures increase biodiversity. Diverse crops enhance different species of insects and other arthropods, reducing the dependency on pesticides and encourage a balanced ecosystem. Increasing the number of natural enemies like insects to control pests decrease the use of pesticides, benefiting biodiversity in agricultural landscapes. Establishing flower strips or hedgerows with flowering plants support pollinators (bees and butterflies). This process support crop pollination and enhance biodiversity. The study was helpful to highlight the role of conservation in providing a refuge to arthropods, especially to beneficial fauna.

SPECIES RICHNESS, STATUS AND DISTRIBUTION OF BIODIVERSITY IN TOLIPIR NATIONAL PARK AJK, PAKISTAN

**Urooj Jamil¹, Zulfiqar Ali^{1*}, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³,
Sidra Shabbir¹, Ghulam Rabbani¹, Maryam Tanveer¹ and Farhan Anjum¹**

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

Biodiversity encompasses all life forms and ecosystems, a living natural heritage essential for a healthy ecosystem, providing fundamental products and services, and reducing poverty. Surveys were conducted to record vertebrate biodiversity and its challenges in Tolipir National Park, Azad Jammu and Kashmir, from September 2022 to August 2023. Point count, pallet count and track count methods were used to record 118 individuals from 89 species with 16 orders and 45 families at TNP. Noteworthy 70 species categorized as least concern, while two were Near Threatened, two Vulnerable, and one Data Deficient. The study found that 42 species were stable, 19 were decreasing, five had increasing trends, six were not available, and seven had unknown trends. Among five different habitats, Shrubs and Bushes had the highest species richness (66 species, 130 individuals), while Tropical Broadleaf Forest had the greatest species abundance (57 species, 287 individuals). In contrast, Rangeland exhibited lower diversity (33 species, 59 individuals). Insectivorous were the most dominant, comprising 34 species, while scavengers were represented by a single species. The Simpson index (1-D) at 0.01 and Shannon- Wiener index (H') at 4.31 indicated high vertebrate diversity in TNP. Agriculture areas were most similar to Rangeland but least similar to Subtropical broadleaf forests. Rangeland was most similar to Shrubs and Bushes but least to Tropical Broadleaf Forest. Shrubs and Bushes were similar to Sub-Tropical Chir Pine. The biodiversity of vertebrates in aquatic and terrestrial habitats has been significantly impacted by escalating human activities. However, the park faced threats such as climate change, habitat degradation, invasive species, pollution, human-wildlife conflicts, and poaching, which are reducing vertebrate biodiversity. Strict law enforcement and wildlife conservation awareness are crucial.

ASSESSING BIODIVERSITY AND ECOLOGY AMID INFRASTRUCTURE DEVELOPMENT IN UPPER INDUS BASIN, PAKISTAN

Usman Ahmad¹, Sajid Rashid Ahmad¹, Zulfiqar Ali², Rida Ahmad^{2,3}, Komel Ahmed²

¹*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

²*Institute of Zoology, University of the Punjab, Lahore*

³*Department of Zoology, Lahore College for Women University, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

Understanding the impact of infrastructure development on biodiversity and ecology in the Upper Indus Basin, Pakistan has become requisite with ongoing infrastructure projects in the region as it may have potential consequences on diverse ecosystems. The research aims to provide a comprehensive understanding of the ecological dynamics, offering insights into the richness and diversity of fauna species of the area. The study area encompasses Upper Indus Basin, the Karakoram and Himalaya Mountain ranges, and was concentrated on the infrastructure developments Khyber Pakhtunkhwa and Gilgit-Baltistan along the Indus River. Field surveys were conducted from May 2021 to December 2022 using the point count method, focusing on the nine identified habitats. The surveyed species included birds,

mammals, reptiles, and amphibians, and data was collected using GPS, binoculars, digital cameras, spotting scopes, and field guides. The study recorded a total of 322 species, including 242 birds, 47 mammals, 21 reptiles, and 12 amphibians. The values of Margalef, Menhinick's, Simpson, Shannon and Evenness are as follows: 31.98, 2.13, 0.94, 3.65 and 0.63. Critical information on taxonomy, IUCN status, and trends for each group was analyzed. Overall, the research provides valuable insights into the biodiversity of the Upper Indus Basin, revealing high species richness, diversity, and interesting habitat associations. The findings contribute to a better understanding of the ecological dynamics in this crucial region and can inform conservation and management strategies for the diverse habitats present.

COMPARATIVE STUDY ON TADPOLES OF HAZARA FROG *ALLOPAA HAZARENSIS* (ANURA: DICROGLOSSIDAE) DURING DIFFERENT DEVELOPMENTAL STAGES

Aamina Abid¹, Sumbul Gill, Ansa Rani, Gohar Shah², Luqman, Waseem Ahmed, Isma Maqbool and Muhammad Rais*

¹*Herpetology Lab, Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University Rawalpindi*

²*University of Buner, Buner*

*Corresponding Author: sahil@uair.edu.pk

Hazara Frog (*Allopaa hazarensis*), Family Dicroglossidae, is endemic to Hindu Kush and Himalayan region. The frog species is associated with high altitude forested montane wetlands in Pakistan. The original description of the tadpoles and data on morphology and some basic morphometric measurements are available. We aimed to describe the intraspecific variation of the tadpoles of Hazara Frog (*Allopaa hazarensis*) at hind limb bud development stage (Gosner stage 25-30, n= 23), toe differentiation stage (31-39, n= 49) and metamorphic stage (40-42, n= 20) collected from the selected streams of District Buner, Province Khyber Pakhtunkhwa (KPK), Pakistan. We found that the mean body length, total length, tail length, body height, eye diameter, inter orbital distance, oral disc width and tail muscle width differed significantly among the studied stages. The body length of metamorphic stage was significantly higher than the hind limb bud development stage and toe differentiation stage. The measurements such as maximum tail height, ventral fin height, dorsal fin height, snout-nares distance, inter narial distance and tail muscle height in metamorphic stage were significantly higher than the toe differentiation stage. The correlation between ventral fin height and dorsal fin height in hind limb bud development stage, toe differentiation stage and metamorphic stage; between inter orbital distance and inter narial distance in toe differentiation stage and metamorphic stage and between snout nares distance and eye nares distance was non-significant. The correlation for all other studied measurements in the three studied stages was significant. We provide first comprehensive data on different stages of tadpoles of Hazara Frog.

ANTHROPOGENIC THREATS AND INVENTORY OF BIODIVERSITY IN SAKESAR RESERVE FOREST PUNJAB SALT RANGE SOON VALLEY

Farooq Ahmed Awan and Naseem Iqbal Butt

Conservation of Forest Potohar Circle

Corresponding Author: farooq.agri@gmail.com, nasimiqbalbutt786@gmail.com

Sakesar reserve forest consist of 04 reserve forest including Rakh Sakesar Shahpur, Mianwali, Attock and Chitta reserve forest spread over an area of 7024 Acres. Sakesar reserve forests are located at an elevation of 3500 feet to 5010 feet the highest elevation in salt range. Sakesar reserve was declared as protected forest in

1896 with no grazing rights and is one of the best protected forests in the salt range. All these forests were declared wildlife sanctuaries during 2021 Punjab Wildlife Department. These reserve forests also serve the catchment area of the watershed feeding Uchhali Wetland Ramsar site of Pakistan. Climate of the area is harsh with minimum temperature up to -2°C in the month of January, while mean maximum temperature of 42°C in the month of June. Rainfall pattern of the area is scanty and mean annual rainfall is 600mm. Present study was conducted to establish base line data on wildlife and its habitat, threats to the biodiversity of the forest and proposed measures for the conservation of the biodiversity. Appropriate scientific methods were used to observe and record floral and faunal diversity of the Sakesar range forest. During the study 09 mammals, 7 reptiles and 7 amphibian species were recorded from the reserve forest. Avi fauna diversity of the reserve forest was also studied and 47 bird species belonging to 24 different families were recorded. The relative abundance, senses index and dominant, sub-dominant and least abundant species were also studied along with avi fauna distribution. Major wildlife species of the area include Punjab Uril, Indian Wolf, Black Partridges, Green Pigeon, Chukor, Indian Pangolin and Grey Partridges. A total number of 60 plant species are identified in the study area among which dominant species included *Olea ferruginea*, *Acacia modesta*, *Zizphus nummularia*, *Justicia adhatoda*, *Dodonea viscosa* and different grasses. Major anthropogenic threats to biodiversity in area include forest fires, tree cutting, mining, illegal hunting, heavy grazing of livestock, drought, grass cutting. There is a need to protect flora and fauna from further degradation. Sustainable use of the resources may be promoted in the area through awareness raising and the entire Sakesar forest area network management plan may be prepared for better conservation of flora and fauna.

AN INTEGRATIVE ANALYSIS FOR THE RECONSIDERATION OF SPECIES BOUNDARIES IN EAST ASIAN *PELOPHYLAX* SPECIES

Hina Amin^{2*}, Siti N. Othman¹, Yucheol Shin¹, Mi-Sook Min³, Kevin R. Messenger⁴, Ali Movahedi⁵, Deyatima Ghosh¹, Dallin B. Kohler¹, Vishal Kumar Prasad¹, Xiaoli Zhang¹, Yang Yi⁶, Yikweon Jang⁷, Honghua Ruan² and Amaël Borzée^{*1}

¹Laboratory of Animal Behaviour and Conservation, College of Life Sciences, Nanjing Forestry University, 159 Longpan Rd, Nanjing 210037, Jiangsu, People's Republic of China

²Co-Innovation Centre for Sustainable Forestry in Southern China, College of Biology and the Environment, Nanjing Forestry University, 159 Longpan Rd, Nanjing 210037, Jiangsu, People's Republic of China ³Research Institute for Veterinary Science and Conservation Genome Resource Bank for Korean Wildlife, College of Veterinary Medicine, Seoul National University, Seoul 08826, Republic of Korea

⁴Herpetology and Applied Conservation Laboratory, College of Life Sciences, Nanjing Forestry University, 159 Longpan Rd, Nanjing 210037, Jiangsu, People's Republic of China

⁵Department of Biology and the Environment, College of Life Sciences, Nanjing Forestry University, 159 Longpan Rd, Nanjing 210037, Jiangsu, People's Republic of China

⁶University of Chinese Academy of Sciences, Beijing 100049, China

⁷Department of Life Sciences and Division of EcoScience, Ewha Womens University, Seoul, Republic of Korea

*Corresponding Author: hina@njfu.edu.cn, amaelborzee@gmail.com

The phylogeography of the *Pelophylax* genus in East Asia is poorly resolved and dated, partially because of the ability of the species to hybridise, the intense trade by humans, and the lack of fossils to calibrate molecular dating estimates. For the first time, we reconstructed the biogeography of *P. plancyi* (1520 bp concatenated *CYTb-POMC-TYR*; and 714 bp *12S-ND3* gene fragments) using fossil-based molecular dating, morphometry and ecological models. Our results highlight four cryptic lineages within *P. plancyi*, but also recovered two well-supported clades for *P. chosonicus*, on either side of the Yellow Sea, and recovered *P. hubeiensis* as a well-supported monophyletic clade. Additionally, our fossil-based

molecular dating analyses supported the emergence of *P. hubeiensis* circa 10.89 (6.31-16.71) Ma and *P. chosonicus* circa 1.36 (0.96-1.69) Ma. To determine the morphological differences among the four focal clades (*P. plancyi*, *P. chosonicus* “east”, *P. chosonicus* “west”, and *P. hubeiensis*), we analysed morphological data known to be variable in this clade. Our morphometric analyses showed that morphological characters differed significantly among clades. In general, the distributions of four characters related to head width, head length, eye lateral diameter, and tympanum diameter did not overlap among clades and may be used for species identification. Finally, we modelled the suitable habitat for each of the four clades to clarify their putative distributions. The highest suitability area for *P. hubeiensis* matched the currently known distribution of the species in the Yangtze River Basin. The highest suitable habitat for *P. chosonicus* “east” was located on the plains east of the Yellow Sea, and the suitable habitat for *P. chosonicus* “west” was located on the plains west of the Yellow Sea. Our phylogenetic analysis, divergence dating estimates, morphological analyses and landscape models strongly support the monophyly and species-level taxonomy of *P. chosonicus*, *P. hubeiensis* and *P. plancyi*.

6. ECOLOGY

AUTOTROPH AND HETEROTROPHS ASSORTMENT AROUND PARADISE POINT AND THEIR ASSOCIATION WITH THE ENVIRONMENTAL VARIABLES

Wajiha Shaikh*, Sher Khan Panhwar and Mushaiyada Mairaj
Centre of Excellence in Marine Biology, University of Karachi, Karachi
 *Corresponding Author: wajihazooologist@gmail.com

To determine population dynamics and environmental impact on the distribution of microplankton (zoo and phyto) inhabiting around paradise point, Karachi in different seasons of the 2016-17. Some seven diatom and twenty-nine dinoflagellates were collected, among them *Navicula parva* (1002 cells/ml) diatom followed by *Rhizosolenia fragilissima* (887 cells/ml) whereas dinoflagellate *Alexandrium affine* (9814 cells/ml) followed by *Prorocentrum minimum* (7738 cells/ml) dominated the population. A total of twenty-three taxa of zooplankton encompass Crustaceans (21%), Cheatognaths (2%), Copepodes (74%), Cnidaria (1%), Gastropods (2%) whereas Scaphopods, Tunicates, Polychaetes and fish larvae were rarely seen. Diversity profile revealed that highest value (0.9097, 2.823) of Shannon and Simpsons' diversity indices calculated in NEM that well defines a suitable season whereas zooplankton was found to be divers (1.914, 0.8084) during SIM owing to up-welled nutrient-rich water and biological activity. Multivariate approach of canonical correspondence analysis (CCA) was established to determine impact of environmental variables on the seasonal distribution and abundance of plankton communities. The variability among PCI-axis was 64.33% variability (eigenvalue 0.24957) II-axis 35.67% (eigenvalue 0.13835) deduced from five environmental variable (temperature, dissolved oxygen, salinity, conductivity, pH and total dissolved substances) for phytoplankton whereas 77.05%, eigenvalue 0.122 and 22.95%, eigenvalue 0.036 for PCI & II from loaded data sets of zooplankton. It is summarized that plankton diversity is more likely controlled by the abiotic factor (temperature) rather than biotic mechanism (Heterotrophs) in case of phytoplankton whereas heterotrophs virtually associated with primary productivity.

SPECIES RICHNESS ANALYSIS IN THE PROTECTED AREAS OF POONCH DIVISION, PAKISTAN

Ghulam Rabbani¹, Zulfiqar Ali^{1*}, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³, Maryam Tanveer¹, Sidra Shabbir¹, Urooj Jamil¹ and Farhan Anjum¹

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

The biodiversity of vertebrates is of great importance for ecological balance and has a significant impact on the functioning of the ecosystem. Vertebrates play crucial roles in various ecosystems as predators, prey, pollinators, and nutrient cycling. Study was carried out to determine the vertebrate

biodiversity of Poonch Division Azad Jammu and Kashmir, Pakistan which is surrounded by the diverse Himalayan landscapes and the temperate forests of the Western Himalayas. To assess the species diversity, monthly based surveys from May 2022 to June 2023 were carried out in different protected areas of the region. Point count method, sky view method, scats surveys and tracks surveys were used to assess species diversity of vertebrates. A total of 6919 individuals belonging to 220 species, 25 orders and 50 families were identified from 12 different habitats of study area. One sixty-one bird species, seven amphibian species, 30 mammalian species and 22 reptiles were observed. Major portion of the observed 196 species belonged to the Least concern category of IUCN status while, Himalayan Griffon, Tytler's Leaf Warbler and Burrowing Vole were near threatened species. Cheer Pheasant, Kashmir Flycatcher, Asiatic Black Bear and Panther were vulnerable species. Among each habitat Agriculture area with 156 species was most abundant followed by Moist temperate forest having 146 species and least dominance shown in Sub-alpine with 39 species. Relative abundance, species richness and evenness were calculated for each habitat. Biodiversity was determined by using Simpson's index and Shannon- Wiener's index. Results of Simpson's index ($1-D=0.9903$) and Shannon-Wiener's index ($H'=5.0645$) indicated high diversity of species in the area. The calculated results for each habitat showed the maximum diversity in moist temperate forests ($1-D=0.990$, $H'=4.709$) and lowest diversity in Sub-alpine forests ($1-D=0.964$, $H'=3.435$). Sorensen similarity index and Jaccard similarity index were used to compare the species overlapping in the different habitats. Agricultural area was closely related to settlement due to maximum overlapping between them, while least overlapping was between agriculture area and sub-alpine. The vertebrate fauna of the entire study area is not well documented and this research work is baseline study to explore the biodiversity of species.

COMPARING ECOLOGICAL INDICES ACROSS THREE NATIONAL PARKS WITH VARIED ALTITUDINAL GRADIENTS IN PAKISTAN

Komel Ahmed¹, Zulfiqar Ali¹, Rida Ahmad^{1,2} and Usman Ahmad³

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

This research explores the avian biodiversity within three geographically diverse national parks in Pakistan: Khunjerab National Park, Lal Suhanra National Park, and Hingol National Park. The study encompasses comprehensive assessments of species richness, family and order distributions, IUCN statuses, occurrence patterns, and habitat characteristics. Utilizing rigorous survey methods, including point count sampling, the research examines the altitudinal variations in avian communities and habitat heterogeneity, across these distinct National parks. The study employs ecological indices such as Pielou's, Simpson's, Shannon-Wiener, Margalef's, and Menhinick's to quantify biodiversity, evenness, dominance, and richness. Results revealed substantial differences in species composition, abundance, and ecological indices among the three national parks. Lal Suhanra National Park stands out with the highest species richness (179 spp.) and the greatest diversity in families (62) and orders (18) followed by Hingol National Park (120 spp.) and Khunjerab National Park (51 spp.). The study also includes Sorensen's and Jaccard's similarity indices to assess interspecific linkages and habitat similarities. The findings provide valuable insights into the ecological dynamics of these national parks, highlighting the need for tailored conservation strategies based on altitude-dependent avian assemblages. The research contributes to our understanding of the intricate relationships between altitude, habitat, and avian biodiversity, with implications for conservation and management practices in diverse ecosystems.

ECOLOGICAL CONSEQUENCES OF FOREST FIRES ON SOIL INVERTEBRATES IN MARGALLA HILLS NATIONAL PARK

Kiran Sahar¹, Shahzad Aslam² and Sukhawat Ali^{3*}

¹*Department of Zoology, Employee of Hagler Baily Pakistan, Islamabad, Pakistan*

²*Department of Zoology, Wildlife and Fisheries, Faculty of Sciences, PMAS-Arid Agriculture University, Rawalpindi*

³*Islamabad Wildlife Management Board, Islamabad*

*Corresponding Author: a.sakhawat77@yahoo.com

A study was conducted to investigate the impact of forest fires on soil invertebrate diversity in the Margalla Hills National Park (MHNP) near Islamabad (33°40 – 33°44 N, 72°55 – 73°20 E), situated in the Murree foothills within an elevation range of 450m to 1500m. The study area experiences a fire season from early April to mid-July annually, resulting in significant biodiversity loss. Data were collected from June 2020 to December 2020, primarily focusing on elevation ranging from 548.64 to 1219.2 m. Eight sampling sites were selected, including Shahdara Top, Ratta Hottar, Kanthla Top, Talharr Top, Trail-3 & Trail-5 Connector, Jabbri, Chera Di Gali, and Saidpur in the study area. During the fire season, a survey compared burnt and unburned areas, revealing that abundant soil invertebrate was observed only in the burnt sites due to migration of predatory soil invertebrates from unburned areas and feed on dead insects. A total of 1211 specimens representing 57 different taxa were recorded, with 302 specimens from 33 taxa in the burnt area and 909 specimens from 57 taxa in the unburned area. The Species Diversity Index was 2.88 for the burnt area (classified as 'Not good'), while the unburned area had a Species Diversity Index of 3.54 (classified as 'Good'). Similarly, species evenness was higher in the unburned area. Post-fire season surveys revealed a significant decline in soil biodiversity, attributed to Alien Invasive Plant Species, soil compaction and dormancy of invertebrates, creating unfavorable habitats for soil biota. A comparison of diversity between summer and winter seasons in the eight sampling sites with burnt areas showed variations in the sites with Trail 3 & 5 Connector exhibiting the highest diversity in summer and Chera Di Gali ranking highest in winter due to dormancy stages of invertebrates of the order Coleoptera, Geophilida and Scolopendromorpha. Common orders observed in burnt sites included Aranea, Coleoptera, Dictyoptera, Gastropods, Hemiptera, Homoptera, Hymenoptera, and Orthoptera. In contrast, unburned sites showed abundance in orders like Aranea, Coleoptera, Diptera, Geophilida, Gastropods, Hemiptera, Homoptera, Haplotoxids, Lepidopterans, Scolopendromorpha, and Zygentoma. Certain species occurred in both burnt and unburned sites, belonging to orders including Aranea, Dictyoptera, Diptera, Gastropods, Geophilida, Hemiptera, Hymenoptera, Isoptera, and Orthoptera. It is evident that forest fires pose a limitation on data collection for invertebrates, phyto biodiversity, and macrofauna. Effective conservation and management of soil invertebrate species in MHNP, Islamabad, require immediate and comprehensive preventive measures by relevant authorities such as IWMB and CDA Islamabad.

USE OF CHEAPEST BIOSOURCES TO REDUCE INDUSTRIAL WATER POLLUTION

Amna Marium^{1*}, Shakeela Parveen¹, Huma Naz², Iqra Zulfiqar² and Nimra Zahid²

¹*Departement of Zoology, Government Sadiq College Women University (GSCWU), Bahawalpur*

²*Departement of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

*Corresponding Author: amnasarwar39@gmail.com

In last few years Pakistan has undergone through industrial revolution on a large scale. Number of working industrial units had now increased to thousands. These industries had contributed to huge increase

in profits of the country but on other hand also added in pollution rates as well. Quality of water is determined on basis of its physical, chemical and biological parameters including P^H, temperature, colour, presence of total dissolved solids, ions, dissolved oxygen, and presence of microbes. Decline of water quality is reported due to mismanagement of industrial disposal and release of untreated wastes in water bodies. Saving water for future and providing clean water to citizens is top most concern of government this time. Series of physiochemical analysis tests are performed in different areas of Pakistan to check rate of pollution especially in drinking water and results have reported poor quality. While concerning the environment, peels of vegetables and fruits could be suitable choice to overcome waste treating problems. Also leaves from plants and trees can also act as active absorbents. Therefore, mimicry nature and using these bio waste sources could be best solutions to encase environment pollution issues. A simple or chemically modified adsorbent can be created from specific readily accessible plant components, such as husks, leaves, peels, stems, branches, and pods using a technique known as bio-adsorption. A bio adsorbent's primary characteristic is its affinity for hazardous heavy metal contaminants. Several attempts are being made in this area, to develop novel varieties of bio-waste absorbents. Low cost and easier budget managements is also made possible, in this regard.

IN VIVO INDUCTION OF ANTIOXIDANT RESPONSE AND OXIDATIVE STRESS IN GOLD FISH (*CARASSIUS AURATUS*) DUE TO HEAVY METALS EXPOSURE AT DAMAS LAKE, FORT MUNRO. PAKISTAN

Saima Naz^{*1}, Ahmad Manan Mustafa Chatha², Nimra Amjad¹

¹*Department of Zoology, Government Sadiq College Women University, Bahawalpur*

²*Department of Entomology, Islamia University of Bahawalpur, Bahawalpur.*

Email: saima.naz@gscwu.edu.pk*; manan.chatha@iub.edu.pk

Seasonal estimations were conducted to evaluate the water quality and concentrations of heavy metals (Cu, Ni, Pb, Cd, and As) in Damis Lake-Fort Munro, located in Punjab, Pakistan. Additionally, samples of *Carassius auratus* fish were gathered from three distinct sites: upstream, midstream, and downstream. This was undertaken to analyze how the water quality and concentrations of heavy metals might impact fish enzymes, histopathology, and hematology. Utilizing a Bulk Scientific Atomic Absorption Spectrophotometer, the levels of Ni, Cu, Cd, As, and Pb were determined in the liver, gills, and muscles of the Goldfish (*Carassius auratus*) from Damis Lake-Fort Munro. Concurrently, the activities of enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were measured. The accumulation trend of these metals in the organs was observed as follows: Ni > As > Cd > Pb > Cu. Furthermore, the concentration sequence of these metals in the organs was: Liver > Gills > Muscle. Outcomes illustrated significant rises in oxidative stress, indicated by shifts in enzyme activity and increased reactive oxygen species. The study concluded that pollution caused by studied heavy metals had a significant adverse effect on the quality of water as well as well-being of *C. auratus* at the study site.

7. ENVIRONMENTAL BIOLOGY

CONCENTRATION OF PARTICULATE MATTER AND CO₂ IN LONG TUNNELS OF KKH, PAKISTAN.

Maria Hanif¹, Zulfiqar Ali^{*1}, Fiza Adil¹, Syeda Nida Fatima¹, Ansar Habib¹, Muqadas Naveed¹, Komel Ahmed¹, Rida Ahmad^{1,2}, Usman Ahmad³ and Rafia bibi¹

¹*Institute of Zoology, University of the Punjab, Lahore*

²*Department of Zoology, Lahore College for Women University, Lahore*

³*Center of Earth and Environmental Sciences, University of the Punjab, Lahore*

*Corresponding Author: zali.zool@pu.edu.pk

This study examines the effects of vehicular emissions on the air quality in long tunnels of the Karakoram Highway (KKH) in Pakistan, a key component of the China-Pakistan Economic Corridor (CPEC) project. Using purple air sensors and carbon dioxide monitors, the study measures and analyzes the concentrations of particulate matter (PM) and carbon dioxide (CO₂) at different locations and times along the KKH route. The study identifies the spatial and temporal variations in the PM and CO₂ levels, and pinpoints the pollution hotspots and the primary sources of emissions within the tunnels. The study reveals that PM 2.5 is the dominant pollutant, and that the PM and CO₂ levels exceed the emission standards and depend on the traffic volume and the ventilation conditions of the tunnels. The study also shows that the CO₂ emission factors increase with the altitude along the KKH route due to the trade fleets and the height effect on diesel vehicle performance. The study highlights the need for improved monitoring and management of the air quality along the KKH, and urges the CPEC project to address the environmental impacts of its development.

SPIROGYRA POWERHOUSE: A 360-DEGREE APPROACH TO TRANSFORMING PAKISTAN'S ENERGY AND WATER LANDSCAPE

Muhammad Waqas^{1*}, Razia Bashir¹, Tehmina Saddique³, Haroon Ali² and Arslan Shahzad¹

¹*Department of Zoology, Division of Science and Technology, University of Education, Township, Lahore*

²*Department of Chemistry, Division of Science and Technology, University of Education, Township, Lahore*

³*Institute of Chemical Sciences, Bahauddin Zakariya University, Multan*

*Corresponding Author: waqassiddique614@gmail.com

In the face of escalating global challenges of energy security and water scarcity, Pakistan emerges as a battleground with the urgent need for transformative solutions to sustainable development. Against the backdrop of surging energy demands and depleting water resources, Spirogyra's capacity to thrive in nutrient-rich waters, including industrial polluted and brackish waters. While accumulating lipids suitable for biodiesel synthesis becomes a pivotal asset and underscores Spirogyra's economic validity and scalability. The objective of this research is to explore the potential of Spirogyra in biodiesel production and wastewater treatment. Lipids from microalgae Spirogyra were extracted using n-Hexane as a solvent by Hot Plate Magnetic Stirrer Method and Simple Solvent Extraction Method. Effects of n-Hexane to

lipids ratio, size of algal biomass and contact time on the percentage yield of extracted lipids was studied and analyzed. Through transesterification lipids were converted into biodiesel. We found that maximum amount of lipids extracted from *Spirogyra* by using a greater ratio of solvent to algal biomass (3:1), maximum contact time, and smaller algal biomass size. It was concluded that using Hot Plate Magnetic Stirrer (5 hours) we extracted more amount of lipids (0.97 g) than 24 hours of simple settlement solvent extraction method (0.82 g). This study highlights the need for creative, nature-inspired solutions to address the complex problems of energy security, water scarcity, and environmental degradation.

ADDRESSING THE CLIMATE CRISIS IN PAKISTAN: POLICY RECOMMENDATIONS

Qurat-ul-Ain Shabbir*

The Center for International Strategic Studies Azad Jammu & Kashmir (CISS-AJ&K), Muzaffarabad

*Corresponding Author: annieshabbir341@yahoo.com

The climate emergency poses existential threats to Pakistan, manifesting in extreme weather events, rising temperatures, water scarcity, and sea-level rise, with profound implications for food security, public health, infrastructure, and the socio-economic fabric. Strategic policy actions are imperative to effectively tackle this crisis, necessitating clear, measurable targets and focused resource allocation. This paper presents policy recommendations across key areas such as adaptation, diplomacy, agricultural competitiveness, food security, forestation, and institutional empowerment. Additionally, key findings underscore challenges including coordination barriers among government departments, fragmented governance structures, adverse effects of poor coordination, lack of awareness and capacity, interdisciplinary knowledge gaps, and the imperative for informed decision-making to drive effective climate policies and adaptation efforts in Pakistan.

CONTAMINATION PROFILE OF HEAVY METALS IN GILLS AND FLESH OF *ACANTHOPAGRUS ARABICUS* FROM KARACHI COAST, PAKISTAN

Shagufta Riaz*¹, Muhammad Atiqullah Khan¹, Syed Anser Rizvi¹ and Aasia Karim²

¹*Department of Zoology, University of Karachi, Karachi*

²*Department of Zoology, Sardar Bahadur Khan Women University, Quetta*

*Corresponding Author: shagirajput7@gmail.com

Many organisms including fish accumulate metals naturally found in the aquatic systems. However, rapid industrialization and advanced agricultural activities resulting in contamination of the aquatic environment with heavy metals can cause harmful effects to fish and other aquatic organisms. This work investigates the level of accumulation of cadmium (Cd), mercury (Hg), lead (Pb), iron (Fe), chromium (Cr), Manganese (Mn), and Zinc (Zn) in the gills and flesh of a commercial fish *Acanthopagrus arabicus* widely available, consumed, and exported from the country. Fresh *A. arabicus* were purchased from Karachi Fish Harbor on monthly basis for the period of one year; gills and flesh were removed, tagged, and kept frozen. Each sample was oven dried (Anton Paar Multiwave ECO Microwave Digestion System), homogenized, and treated with acid for complete digestion. Analysis of Cd, Pb, Fe, Cr, Mn, and Zn was performed by Hitachi Z-8000 Polarized Zeeman Atomic Absorption Spectrophotometer and analysis of Hg by using Hitachi Z-5000 Polarized Zeeman Atomic Absorption Spectrophotometer. Each sample was diluted to 10 ppm before run. Result showed significant difference in concentration (ppm) of metals in gills and flesh. Iron was found to be the highest i.e. 102.39 and 37.18 in gills and flesh, respectively. Iron was followed by Zinc content with 51.13 in gills and 6.71 in meat. Moreover, Chromium and Manganese were observed in close ranges in flesh i.e. 3.33 and 3.15, respectively. However, accumulation of Mn was

higher in gills (14.47) than the Cr (04). Surprisingly, metals such as Lead and Cadmium were not found in detectable ranges in this fish at multiple sample runs. Moreover, Mercury was observed in low concentration (ppb) in both gills (65.53) and flesh (136.77) of the fish. This investigation suggested that *A. arabicus* is safe for the human consumption with the emphasis on safety measures to be taken for the waste disposal into the aquatic environment.

IMPACT OF LINE OF CONTROL ON BIODIVERSITY OF JAMMU & KASHMIR

Abdul Rehman

Centre for International Strategic Studies, AJK

*Corresponding Author: abdulrehman007.ar93@gmail.com

The article delves into the ecological crisis unfolding in the Kashmir Valley, where endangered wildlife faces a silent battle for survival amidst human-drawn boundaries. Focusing on species like the Hangul, Snow Leopards, and various pheasants, it examines how the Line of Control (LoC) fence disrupts migration corridors and fragments habitats, leading to severe consequences such as increased human-animal conflicts and maiming of wildlife by landmines. The impacts extend to ground-nesting birds, exacerbating isolation and strain on their populations. The article urges a reevaluation of geopolitical boundaries and emphasizes the urgent need for awareness and collective action to safeguard Kashmir's delicate ecosystem.

MOLECULAR IDENTIFICATION OF SMALL INDIAN CIVET FROM RAWALAKOT, AZAD JAMMU AND KASHMIR

Undleeb Sajjad, Nausheen Irshad, Nasim Ahmed and Ali Muhammad

Department of Zoology, University of Poonch Rawalakot 12350, Azad Jammu and Kashmir

*Corresponding Author: nousheenirshad@upr.edu.pk

The Small Indian Civet (*Viverricula indica*) belongs to family Viverridae (civets) of order Carnivora classified under class Mammalia. The family Viverridae is further Divided into four sub-families i.e., Hemigalinae, Paradoxurinae, Prionodontinae and Viverrinae. The *V. indica* is classified in sub-family Viverrinae that consists of 23 species grouped into six genera. Species belonging to Viverrinae are broadly scattered across the continent of Asia and Africa. Previously, the *V. indica* has been reported from the state of Azad Jammu and Kashmir (AJ&K), Pakistan based on morphological identification but its molecular identification was still not explored from this region. So, the aim of the current study was its molecular characterization based on DNA marker gene (e.g., cytochrome b). For this purpose, four samples of the Small Indian Civet (based on their physical appearance) were collected from different areas of Rawalakot AJ&K. The whole genomic DNA was extracted and used as template in PCR (polymerase chain reaction) with full length cytochrome b marker gene primers. The PCR products were amplified and sequenced. The sequenced reads were assembled and analyzed. BLASTn (basic local alignment searching tool) of sequences showed maximum similarity of 98.95% with the isolate of *V. indica* (Accession # KX891751) reported from France whereas these sequences showed 100% sequence similarity among four isolates of the current study. The phylogenetic and SDT (sequence demarcation tool) analysis were performed for exploring the evolutionary relationship and pairwise sequence identity scores of the current isolates with other related sequences respectively. Furthermore, in the current study, molecular data of sub-family Viverrinae were retrieved from NCBI (National Center for Biotechnology Information) database and analyzed for global scenario of sub-family Viverrinae and proper classification based on molecular data. Furthermore, phylogenetic and SDT analysis were also performed to determine the evolutionary relationship and sequence identity scores among

different species and genera of sub-family Viverrinae respectively. The cutoff values for species and genus demarcation were determined using different marker genes. Moreover, a masterlist was prepared consisting of NCBI accession number, organism name, isolate name, country name, year of reporting, DNA marker used and its size etc. Absolute value of each item was determined that showed its overall distribution in the sub-family Viverrinae. This organized Masterlist and phylogenetic and SDT analyzed data will provide reference list and reference trees and cutoff values for proper classification of different isolates belonging to sub-family Viverrinae. Further the importance of the current study is discussed.

A COMPARATIVE MORPHOLOGIC AND CRANIOMETRIC ANALYSIS OF GRASS CARP (CTENOPHARYNGODON IDELLA) POPULATION FROM PUNJAB, PAKISTAN

Arshia Naseer*, Muhammad Imran, Abdul Majid Khan and Muhammad Ammar

Institute of Zoology, Faculty of Life Sciences, University of the Punjab, Lahore

Corresponding Author: Arshia.zool@pu.edu.pk, majid.zool@pu.edu.pk

Morpho-craniometric analysis of a fish plays a very important role in determining intraspecific variations. These characteristics are very essential for the classification and identification of fish species. Fish exhibit the ability of phenotypic plasticity and bring some necessary variations in their morphology to adapt to a changing environment. *Ctenopharyngodon idella* (Grass carp) is an exotic fish introduced from China but now it has invaded all the provinces of Pakistan. Like other alien fishes, it shows phenotypic plasticity that leads to inter and intraspecific morphological as well as craniometric variations under the influence of different environmental factors. The intraspecific variations of *Ctenopharyngodon idella* were investigated in the current study through morphological and craniometric analysis. These variations are due to the sensitivity of fish in altering aquatic habitats. A total of twenty samples of *C. idella* were collected from two different sampling sites (ten from each) named as head Balloki (River Ravi) and head Trimmu (River Chenab) and their allometric growth, morphometric characters and craniometrics characters were analyzed. The results of the present study demonstrated the clear-cut variations among the morpho and craniometric characters of different *C. idella* populations. Moreover, the populations at both sites showed negative allometric growth but it was more prominent at site head Balloki.

ROPHIC NICHE OVERLAP AMONG NATIVE AND ALIEN FISH SPECIES IN RAVI RIVER, PAKISTAN

Muhammad Imran^{1*}, Abdul Majid Khan², Iqra Rehman³, Muhammad Tahir Waseem⁴, Rana Manzoor Ahmad⁵, Muhammad Ameen⁶, Mohammed Fahad Albeshr⁷ and Eijaz Ahmed Bhat⁸

^{1,2}*Institute of Zoology, University of the Punjab, Lahore*

³*Department of Environmental Science and Engineering, Government College University, Faisalabad*

⁴*Zoological Science Division, Pakistan Museum of Natural History, Islamabad.*

⁵*Department of Zoology, Government College University, Lahore*

⁶*Department of Zoology, University of Sialkot*

⁷*Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*

⁸*Centre de Biologie Structurale (CBS), Univ. Montpellier, CNRS, INSERM, Montpellier, France*

*Corresponding Author: pinkrose034550@gmail.com

The introduction of alien invasive species is one of the major threats to the native freshwater ichthyofauna. These species introduce diseases, reduce the native population (in some cases develop

predator-prey interaction) and also disturb the physiochemical parameters of the newly invaded ecosystems. The current study was designed to disclose the trophic niche overlap among eight exotic (*Hypophthalmichthys nobilis*, *Hypophthalmichthys molitrix*, *Cyprinus carpio*, *Ctenopharyngodon idella*, *Oreochromis aureus*, *Carassius auratus*, *Oreochromis mozambicus* and *Oreochromis niloticus*) and four indigenous fish species (*Labeo calbasu*, *Cirrhinus mrigala*, *Catla catla* and *Labeo rohita*,) from Head Baloki (Ravi River), Punjab, Pakistan with the help of stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$). Non-significant differences in mean isotopic signature values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of the selected fish species were observed. Community-wide metrics (inside $\delta^{15}\text{N}$ - $\delta^{13}\text{C}$) reflecting vital trophic structure aspects were also considered. The results indicated that the same trophic level was the feeding area for all the selected alien and native fish species, whereas trophic diversity and niche size of the exotic species were greater as compared to the natives. Wide range of $\delta^{13}\text{C}$ values showed that exotic fish species have opportunistic feeding behavior (with multiple basal resources). Among the natives, *L. rohita* has lower $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and displayed an isolated dietary niche, though this specie is not in direct feeding competition with others, and this niche partitioning might be due to the feeding pressure imposed by alien fishes.

“SCALE MORPHOMETRY OF SAWTOOTH BARRACUDA AS A TOOL IN FISH IDENTIFICATION”

Musarrat-ul-Ain* and Noor-us-Saher

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: musarrat-ul-ain@hotmail.com

Basically, fish scales provide protection from parasites and from environmental factors to fish. Furthermore, it helps fisheries workers to identify fish. Present research was based on morphometry of scales of a fish *Sphyraena putnamae* commonly known as Sawtooth barracuda. The traditional method of collection of scales from fish body and preparation of slides were adopted. In which, scales were picked from the abdominal part of fish body. Scales were cleaned and dried using different grades of Alcohol. These samples of scales were placed on glass slides and studied under a compound microscope. The result of scale morphometry shows that the focus of scale was closed to the posterior part. The lateral and anterior part of scale have several radii. The absence of ctenii on the posterior margin of scale illustrates the cycloid type of fish scales.

SECTION – V I

POSTER SESSION

DIVERSITY AND ECOLOGICAL PATTERNS OF HOVERFLIES (DIPTERA: SYRPHIDAE) IN MOHAN-JO-DARO, LARKANA, SINDH, PAKISTAN

Kamal Khan*, Naheed Baloch, Mansoor Ali Shah and Attaullah Ansari

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: kamalgul143@gmail.com

The investigation focused on the diversity and seasonal fluctuations in the abundance of hoverflies (Diptera: Syrphidae) fauna across five district habitats in various locations within Upper Sindh (Mohan-jo-Daro Larkana) from March-December 2023. Adult specimens were captured using Malaise traps, Yellow pan water traps, and insect hand net. As a result total 783 specimens from eight species and six genera belonging to two sub-families were collected. Notably, *Episyrphus baltatus*, *Eristinus aneneus*, Scopoli, and *Ischiodon scutellaris* were identified as the most prevalent while *Eristalis arbustorum* was least abundant during research period. The abundance of hoverflies varied seasonally with the highest recorded in spring and the lowest in autumn. Result of Pearson's correlation coefficient indicated a very strong positive and significant correlation between the number of host plant species and the abundance of syrphid flies. Relative humidity, rainfall and cloudiness showed very strong negative correlation with hoverfly population although these correlations were not statistically. Variations in average population size species richness evenness and diversity index were calculated based on habitat and diversity while fodder crops recorded the lowest.

STUDY ON BEHAVIOR AND DISPERSAL FLIGHT OF MOLE CRICKET (GRYLLOTALPIDAE: GRYLLOIDEA) IN CHOLISTAN PUNJAB

Muhammad Abdullah², Santosh Kumar² and Riffat Sultana¹

¹*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur*

²*Department of Zoology, University of Sindh, Jamshoro*

*Corresponding Author: abdullahkharal089@gmail.com, santoshkumar@cuvas.edu.pk

Mole crickets are notorious for causing damage to various crops and understanding their behavior is crucial for developing strategies to control their populations. This study provides an overview of the behavior of mole crickets encompassing their habitat preferences feeding habits as well as flight dispersal in addition to this their burrow's excavating behavior was also noted their distinctive front legs are adopted for digging allowing them to create intricate burrows beneath the surface. These burrows serve multiple purposes including providing shelter protection from predators and a conducive environment for egg laying. We believe that understanding their habitat preferences, feeding habits and flight dispersal allows for the development of targeted strategies aiding in effective pest management and safeguarding crop yields.

**IDENTIFICATION AND CHARACTERIZATION OF MULTIDRUG REISTANT, *E. COLI*,
SALMONELLA AND *STAPHYLOCOCCUS*, ISOLATED FROM BACKYARD POULTRY
 OF DISTRICT KHUSHAB, RAWALPINDI AND ISLAMABAD**

Arsalan Ibrahim¹, Ruqia Mehmood Baig¹ and Aayesha Riaz²

¹*Department of Zoology, Wildlife, and Fisheries; PMAS- Arid Agriculture University, Rawalpindi*

²*Department of Veterinary and Animal Sciences; PMAS- Arid Agriculture University, Rawalpindi*

*Corresponding Author: arsalanibrahim178@gmail.com

As the world population has been increasing day by day, its demand for food has also increased. To meet their nutritive demands, the focus of people towards backyard poultry has been increased because of their ease of handling and management system. The annual chicken and eggs consumption has also increased in the world. But the increasing concept of backyard poultry is facing different challenges and cause serious zoonotic diseases in humans. The backyard poultry is mostly affected by microorganisms like bacteria, viruses and fungi. Among them bacterial diseases are major cause of zoonotic diseases and infections in poultry birds. The different kinds of antibiotics are used to overcome bacterial diseases. The excessive use of antibiotics for the better growth and development of poultry birds makes pathogenic bacteria resistant against these drugs. The aim of the study was to identify the pathogenic bacteria (*E. coli*, *Salmonella* and *Staphylococcus*) from the fecal and rectal samples of backyard poultry. The microscopic examination of these pathogenic bacteria was performed by using gram staining technique. Different types of biochemical tests were performed i.e. Methyl Red, Citrate test, Catalase test and Glucose utilization test for biochemical identification of the isolates. The antibiotic resistance of the isolates was performed by using Kirby Bauer Disc Diffusion test. The *E. coli*, *Salmonella* and *Staphylococcus* had been isolated from the fecal and rectal samples of backyard poultry with a percentage ratio of 68.3%, 3.3% and 13%. The isolates were susceptible against Cip-5 µg, OT-30 µg, ENR-5 µg and S-10 µg and resistant against AMX- 25µg, VAN-30 µg, P-10 µg, LS-109 µg respectively. The *E. coli* was found susceptible against Cip, ENR and CN with a percentage ratio of 100%, 80% and 82% respectively, while it showed resistance against VAN, AMX with percentage ratio of 100% and 85%. Also, the *Salmonella* was found 100% susceptible against Cip and CN-10 µg and shows resistance against AMX, VAN and P. The isolated strain of *Staphylococcus* was found 100% susceptible against CN, AMX, and VAN while, resistance against S, OT, and ENR. This study has confirmed the presence of pathogenic bacteria in backyard poultry. Limited use of antibiotics and proper biosafety measures should be adopted in backyard poultry. Through this +we can reduce the prevalence of pathogenic bacteria in backyard poultry and also reduce the risks of zoonotic diseases in humans.

**EXAMINATION OF CONNECTIVITY AMONG AMPHIBIAN HABITATS (FORESTED
 FRESHWATER STREAMS) OF MURREE TEHSIL, RAWALPINDI, PAKISTAN**

**Muzna Kashaf¹, Muhammad Rais¹, Ehtisham Wajid², Waseem Ahmed¹, Sumbul Gill¹,
 Nuzhat Naseem¹ and Unza Waqar¹**

¹*Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University, Rawalpindi*

²*Institute of Geo Informatics and Earth Observation, PMAS-Arid Agriculture University, Rawalpindi*

*Corresponding Author: muznakashaf@gmail.com

Habitat destruction and fragmentation are reported as major threats to amphibian population in the world. Connectivity among habitats has become prime concern in biodiversity conservation due to

increased fragmentation of natural habitats. We conducted the study prepare digital maps of and connectivity among amphibian habitats (forested montane freshwater streams) of Murree Tehsil, District Rawalpindi, Province Punjab, Pakistan. The area is known to provide refuge to two frog species which are endemic to Himalayan and Hindukush ranges. Digital Elevation Model (DEM) of 12.5 m resolution was used to delineate Strahler stream order of the region in ArcMap 10.8 using flow direction and flow accumulation tools. Satellite image (Sentinel 2) was used for Land Use Land Cover classification on Google Earth Engine. Least cost path analysis was performed to examine connectivity between these streams using respective tools in ArcGIS software. Data regarding permanent / temporary streams, presence / absence and breeding evidence of *Nanorana vicina* and *Allopaa hazarensis* was collected by surveying 56 streams (nocturnal stream searches). Least cost paths were constructed between the streams which provide potential corridors for movement of amphibian species. Delineated streams (390) with an average length of 79534 m were classified into 1st, 2nd, 3rd and 4th order. The studied amphibian species (*Nanorana vicina* and *Allopaa hazarensis*) inhabit the streams of Tehsil Murree situated at elevation of >1100 m. Species (*N. vicina* and *A. hazarensis*) were present in 15 out of 56 surveyed streams and breeding evidence was recorded from nine streams. *N. vicina* was recorded from 7 of the 15 streams, *A. hazarensis* was recorded from 3 of 15 streams while 5 streams supported both of these species. Supported paths for species movement were 18, having distance less than 81 m. The data on digital maps of the streams inhabiting endemic amphibian species and connectivity of these streams will be helpful to check amphibians' dispersal and if needed, strategies like management of wetlands or creation of artificial wetlands could be devised for long-term continuation of these species.

WILDLIFE HABITAT AND PUBLIC HEALTH VALUE OF RECREATIONAL PARKS IN RAWALPINDI-ISLAMABAD AREA

**Nuzhat Naseem¹, Muhammad Rais¹, Muzna Kashaf¹, Lubna Ansari²,
Sumbul Gill¹ and Waseem Ahmed¹**

¹Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University, Rawalpindi

²Department of Forestry and Range Management, PMAS-Arid Agriculture University, Rawalpindi

*Corresponding Author: nuzhatnaseem2852@gmail.com

The ecosystem and public health values of recreational parks in Pakistan are seldom studied. We examined the wildlife habitat and public health value of recreational parks in Rawalpindi (Muhammad Ali Jinnah Park and Allama Iqbal Park) and Islamabad (Fatima Jinnah Park and Rose and Jasmine Garden). The wildlife habitat was examined by documenting vegetation of the parks, collecting data on occurrence and abundance of amphibians (frogs and toads), reptiles (lizards), and birds (nuisance birds) using Visual Encounter Survey (VES) method for amphibians and reptiles and Point Count Method for birds. Close ended questionnaire was used to get public opinion about the effects of parks on physical and psychological health. The association of variables such as gender of the respondents, age, frequency of visitation and duration of visit with visitors' health was analyzed using chi-square test/ fisher's exact test ($\alpha 0.05$) in program R 4.3.0. The results showed that most of the vegetation (>50%) was exotic; reptilian species were found on the trees which provided hiding places; and nuisance birds were reported around the litter. The results showed that recreational parks were important for public physical.

MUTATIONAL PROFILE OF BRAF IN BREAST CANCER PATIENTS OF PAKISTANI ORIGIN

**Syeda Qanitah Ayesha¹, Ruqia Mehmood Baig¹, Samina Asghar Abbasi¹, Afshan Afzal^{1,2},
Nadia Bibi¹, Bushra Ghazanfar¹, Nafeesa Kainat¹ and Arsalan Ibrahim¹**

¹*Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University, Rawalpindi*

²*Institute of Metabolism & System Research, University of Birmingham, UK*

*Corresponding Author: qanitahayesha@gmail.com

Breast cancer is a major health issue affecting a significant number of women worldwide, particularly the South-Asian region faced an alarming rise in incidence. The prevalence, mortality and survival rates for breast cancer are subjected to significant variations among different nations, influenced by numerous factors such as demographics, lifestyle choices, genetic makeup, and environmental conditions. The deregulated MAPK signaling pathway is of utmost significance in carcinogenesis and metastasis, attributed to the disrupted kinase activity of mutant proteins. The B-Raf proto-oncogene (BRAF) is a RAS-regulated cytoplasmic serine-threonine kinase that plays a key role in regulating the mitogen-activated protein kinase (MAPK) signal transduction pathway. At the BRAF gene, the BRAFV600E mutation, at codon 600 of exon 15, causes glutamic acid to replace valine. This genetic mutation leads to constitutive activation of the BRAF protein, promoting uncontrolled cell proliferation, and making it a potential therapeutic target for many types of cancers. A case-control study was conducted to analyze the mutational profile of the BRAF oncogene in breast cancer patients from Pakistan. The study duration was 1.5 years, including the sampling period as well. After sampling approval from Holy Family Hospital Rawalpindi and PIMS Islamabad, the tissue samples and demographical data of breast cancer patients was collected. After DNA extraction from collected tissue samples, primers were optimized and the BRAF gene was amplified using PCR. The PCR products were confirmed through gel electrophoresis and Sanger sequencing was done. The sequenced data was analyzed by Chromas software. In the studied sample, the majority of breast cancer patients fell within the age range of 41 to 60, with ductal carcinoma emerging as the predominant cancer type. Only 18.75% of patients had a family history of breast cancer. The majority of patients were married and had four or fewer children. Most of them also had a positive history of breastfeeding and lactation. Furthermore, the study investigates various clinical features and factors (like age of menarche, oral contraceptive consumption, tumor stage and grade, and metastasis) associated with breast cancer. The studied tissue samples (n=50; normal=25, tumor=25) were wildtype for BRAF oncogene having no Adenine (A) transversion for Thymine (T) (A→T). Conclusively, in studied breast cancer patients there was no significant association of the BRAFV600E variant with the development of breast cancer. Based on the study's findings, BRAFV600E variant testing and therapy are not currently advised for Pakistani individuals with breast cancer, but the role of the BRAFV600E mutation in breast cancer progression is still not clear. These findings contribute to a better understanding of the disease and potential future treatment strategies by providing valuable insight into the genetic and clinical characteristics of breast cancer in the Pakistani population. Further research is needed to unveil the genetic basis of breast cancer, specifically on mutations linked with breast cancer subtypes among patients of Pakistani origin which is a demand of the current era. Raising public awareness about breast cancer genetics in Pakistan helps in early diagnosis, genetic testing, and personalized treatment strategies, potentially improving the outcomes and well-being of breast cancer patients.

OCCURRENCE OF TWO DISTINCT SPECIES, *HYALOMMA SCUPENSE* AND *HYALOMMA EXCAVATUM*, ON BUFFALOES OF LARKANA, SINDH, PAKISTAN

Shakira Mushtaque*, Nadir Ali Birmani and Nahid Baloch

Department of Zoology, University of Sindh, Jamshoro

*Corresponding Author: shakira.mushtaque@yahoo.com

A parasitic investigation was carried out from April 2021 to December 2021 to analyze the diversity of hard ticks parasitizing farm buffaloes of Larkana division of Sindh, Pakistan. Beside the diversity of the hard ticks, their distribution and monthly infestation, species-wise infestation rate with the variation in male and female population of the ticks were also recorded. During the present research, 450 buffaloes were observed, 226 with 50.22% were found infested with ticks. The findings of this research suggest that there is significant intensity of ticks in farm buffaloes of the Larkana division. In this study, two species of genus *Hyalomma* were recorded, *Hyalomma scupense* Schulze, 1919, and *Hyalomma excavatum* Koch, 1844.

EXPLORING THE IMPACT OF MUTATIONS IN MAPK PATHWAY GENES ON BREAST CANCER SUSCEPTIBILITY AND PROGRESSION: A COMPREHENSIVE GENETIC ANALYSIS.

Nadia Bibi¹*, Dr. Ruqia Mehmood Baig¹, Samina Asghar Abbasi¹, and Pakeeza Arzoo Shaiq²

¹*Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University, Rawalpindi*

²*University Institute of Biochemistry and Biotechnology, PMAS-Arid Agriculture University, Rawalpindi*

*Corresponding Author: nbibi3725@gmail.com

Breast cancer, a leading cause of cancer-related mortality globally, exhibits a concerning annual increase of 3.4%. Notably, the prevalence in Pakistan is 2.5 times higher than in other countries. This study focuses on the significant role of genetic factors, particularly the MAPK pathway and the MAP3K9 gene, in breast cancer development. The BRCA1 and BRCA2 genes contribute to 10-15% of breast cancer cases. However, this investigation emphasizes the involvement of the MAPK pathway, essential for cell differentiation, proliferation, gene expression regulation, and cell development. The MAP3K9 gene, a crucial component of this pathway, is explored for its association with breast carcinogenesis, specifically targeting the SNP rs11628333. Tumor samples were collected from female breast cancer patients in Pakistan, and DNA extraction was performed. Through sequencing and data analysis using Chomax software, the study aims to unveil the role of MAP3K9 (rs11628333) in the genetic landscape of breast cancer in the Pakistani population. This research contributes to our understanding of the molecular mechanisms underlying breast cancer, potentially paving the way for targeted therapies and personalized treatment approaches.

IMPACT OF FOOD PROVISIONING ON THE FORAGING ECOLOGY OF RHESUS MONKEY (*MACACA MULATTA*) AT MARGALLA HILLS NATIONAL PARK, ISLAMABAD

**Shehar Bano*, Amjad Rashid Kayani, Tariq Mahmood, Shahzad Aslam,
Muzna Kashaf, Kainat Zamir and Sajid Nadeem**

Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University, Rawalpindi

*Corresponding Author: sheharbanosaeed@gmail.com

The rhesus monkey (*Macaca mulatta*) exhibits the broadest diversity of geographic and ecological distribution among non-human primates. In the current study, we investigated the potential

influence of provisioning on the function of rhesus monkeys in seed dispersal and changes in their activity patterns in the Margalla Hills National Park Islamabad. The study area was divided into two categories; the high-provisioned area, and the low-provisioned area, the sampling sites in these areas were visited equally for 10 days each month, to reduce the bias factor in the data collection. Direct field observations, scan sampling, and focal sampling were used to observe the details of food eaten by individual monkeys in the group. Results showed that the high provisioned group (HPG) of monkeys spent, on average, 67.53% of their time in foraging at provisioned sites, significantly higher than the low provisioned group (LPG), which allocated 46.21% of their time foraging at their provisioned sites. The HPG showed significantly lower resting behavior (11.36%) compared to the LPG (22.495%) and grooming behavior was significantly lower in the HPG (9.85%) compared to the LPG (13.49%). Similarly, the HPG exhibited a significantly lower locomotory behavior (6.17%) compared to the LPG (29.62%). The study concludes that the provisioning of food by visitors has impacted the rhesus monkey behavior in terms of distribution, daily ranging pattern and foraging in the study area. These findings could help understand and manage human-wildlife interactions to ensure the conservation of this primate species and minimize the human-wildlife conflict.

PAST AND CURRENT DISTRIBUTION RANGES OF THE ASIATIC BLACK BEAR (*URSUS THIBETANUS*)

Unza Waqar, Tariq Mahmood, Muhammad Mushtaq, Muzna Kashaf, and Ayesha Sheraz

Department of Zoology, Wildlife and Fisheries, PMAS-Arid Agriculture University, Rawalpindi

*Corresponding Author: unzawaqar99@gmail.com

The current distribution of Asiatic black bear (*Ursus thibetanus*) is available on the IUCN Red List of Threatened species website; however, nothing is known about the historical extent and occurrence of this species. Therefore, we aimed to investigate the historical distribution of the Asiatic black bear, and map and estimate its total range size, for comparison with that of species current distribution. In addition, we analyzed a network of protected areas in the past and current ranges of the species. We employed Geographic Information System (GIS) software to reconstruct and measure the historical range of the Asiatic black bear, comparing past and current ranges to analyze its expected range contraction. The focus of the study was to enhance our understanding of the species' historical distribution, contributing to better conservation strategies for the present and future perspectives. The utilization of GIS tools facilitates a comprehensive exploration of the factors influencing the species' decline, ultimately aiding in more effective management and conservation efforts. We retrieved and used published records of black bear's occurrence in anywhere in history to reconstruct its historical distribution range. Results revealed that the Asiatic black bear was more widely distributed in historical times and its range spanned across approximately 15.86 million km² while its current range is limited to approximately 7.85 million km², showing a range contraction of approximately 49.5% (8.02 million km² reduced). The total protected areas in the historical range of the species were found to be N = 9933, with total size of 0.946 million km², against N = 6580 (0.667 million km²) that are present in the current range. Approximately 27.5% of the protected areas have lost the Asiatic black bear since historical times.

ADVANCES IN SYNTHETIC BIOLOGY: BUILDING AND STRENGTHENING BIOSECURITY GOVERNANCE

Shaista Urooj¹, Samreen Sarwer², Zulfiqar Ali Mirani³, and Naveed Ahmed¹

¹*Aquatic Diagnostic and Research Centre Bahria University, Karachi*

²*Health Security Partner, Washington DC*

³*Food and Marine Resources Research Centre, Pakistan Council for Scientific and
Industrial Research Laboratories Complex, Karachi*

*Corresponding Author: shaistaurooj.bukc@bahria.edu.pk

Synthetic biology is an emerging interdisciplinary field that integrates engineering principles as a supportive guideline for systematic biological research. The twofold research objectives of synthetic biology include the modification of pre-existing biological system or the construction of entirely new system. Existing biosecurity approaches and regulations mainly comprises on previous governance models based on security concerning pathogens, research related to recombinant DNA technology, and major concerns associated to genetically modified organisms. The main contributions of synthetic biology in the field of life sciences, human health, environment protection and conservation and even economic growth have been widely recognized. However, biosafety, biosecurity and ethical issues have manifested as this emerging technology is readily available, less expensive, and capable of producing fast results. Numerous biosecurity dialogues among biosecurity consultants are therefore chiefly concerned that the prospects of synthetic biology is potentially advanced and it is out pacing the prevailing biosafety and biosecurity measures that designed to mitigate the risks of accidental release, deliberate, malicious or inadvertent use of synthetic biology procedures or products. The rapidly evolving domain of synthetic biology is reconstructing the global biosecurity framework. This article proposes strategies to improve and expand biosecurity in the field of synthetic biology: security must be recognize as a strategic investment ensuring future applicability of technology; early engagement of biologists, sociologists, stakeholders and policy makers in technology development and forecasting; and the coordination between global expertise and stakeholders is crucial to safeguard acceptable levels of risk.

Highlights:

Biosecurity governance significantly deals with polices, regulations and practices for responsible conduct and oversight of the security sensitive agents and outcomes of the synthetic biology.

In the context of synthetic biology, the strategies and approaches for establishing applicable biosecurity governance framework is of high importance.

This publication comments the cutting edge research, technologies, and advancements within this field.

INVESTIGATING EFFECTIVENESS OF ULTRA-VIOLET RADIATION AGAINST BIOFILMS OF NOSOCOMIAL BACTERIAL SPECIES

Zulfiqar Ali Mirani³, Shaista Urooj^{*1,2}, Naveed Ahmed¹, Yasir Raza²

¹*Aquatic Diagnostic and Research Center, Bahria University, Karachi*

²*Department of Microbiology, University of Karachi, Karachi*

³*Pakistan Council of Scientific and Industrial Research Laboratories Complex, Karachi*

*Corresponding Author: shaistaurooj.bukc@bahria.edu.pk

The application of Ultraviolet radiation (UV) is commonly employed in healthcare setting for the sterilization purposes. In spite of this, a comprehensive assessment of the efficacy of UV sterilization

against nosocomial bacterial strains recognized for biofilm formation has yet to be undertaken. The primary focus of this investigation is to access the sterilization potency of UV irradiation against prevalent healthcare associated pathogens known for biofilm forming potential. Bacterial species such as *Staphylococcus aureus*, Methicillin resistant *S. aureus* (MRSA), *Escherichia coli*, and *P. aeruginosa* were cultivated on their respective culture mediums. Biofilm potential of bacterial species was determined through a 96 well titer plate method. The titer plates were positioned at a distance of 50 cm against UV emitter inside of Biosafety cabinet. Germicidal activity was evaluated by viability test, crystal violet assay and a scanning electron microscope. *S. aureus*, MRSA were killed within 5 min exposure of UV radiation. Whereas, *P. aeruginosa* and *E. coli* took 15 to 20 min for complete killing through UV radiation. Nonetheless, no significant difference has been found in amounts of biomass and ultrastructure of UV- subjected microbial biofilms and the controls.

MINERALS COMPOSITION AND HEAVY METALS BIOACCUMULATION IN CONSUMABLE PORTUNUS AND CHARYBDIS CRABS FROM PAKISTAN

Ayesha Umer¹, Naveed Ahmad¹, Muhammad Noman Syed², Omer Mukhtar Tarar³,
Saima Majeed¹, Tayyaba Asif⁴ and Aisha Qureshi¹

¹Aquatic Diagnostic & Research Center, Bahria University Karachi, Karachi

²Department of Biochemistry, University of Karachi, Karachi

³Food Technology & Nutrition Section, Pakistan Council of Scientific and Industrial Research (PCSIIR)
Laboratories Complex, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi

⁴Hamdard University, Madinat al-Hikmah, Hakim Mohammed Said Road, Karachi

*Corresponding Author: ayeshamer.ncmpr@bahria.edu.pk

Pakistan is endowed with marine fishery resources, where crustacean fishery is the promising export market for Korea, Indonesia, Malaysia, Singapore, Thailand, Italy and Middle East countries. The commercially valuable crab species are *Portunus pelagicus*, *Portunus sanguinolentus*, *Scylla serrata*, and *Charybdis feriatius*. Twelve specimens for each species were collected from fisheries and local market. The Atomic Absorption Spectroscopy were used to analyzed minerals composition and toxic heavy metals contents by AOAC official methods 985.35 and 999.10 (2019) respectively. The results revealed that the crab species are rich in micronutrients in the following order Na>K>Ca>Mg>Zn>Cu>Fe with the highest concentrations of sodium (1375.57 mg/kg) in *C. feriatius* and potassium (880.09 mg/kg) in *S. serrata* were found in the analyzed samples. The heavy metals contents in the species under study are recorded as under Pb>Cd>Hg>As. Lead and mercury were detected within permissible limit whereas cadmium was not detected in the analyzed crab samples except for *P. pelagicus*. These findings suggested that the commercially important crab species are rich in essential minerals contents and free from toxic heavy metals. It will also be of scientific interest for crab products development in herbal medicines, pharmaceutical, food and cosmetics industry.

EFFECT OF HYGIENIC BEHAVIOR ON THE INCIDENCE OF CHALKBROOD DISEASE (ASCOSPHAERA APIS) IN HONEYBEE (APIS MELLIFERA).

Ghazala Muzafar^{1*}, Mazhar Quayyum¹, Shamim Akhter¹, Muhammad Asif Aziz² and Shah Alam²

¹Department of Zoology, Wildlife and Fishers, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi

²Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi

*Corresponding Author: ghazalamuzafar825@gmail.com

Hygienic behavior in honey bees reflects the social immunity against parasites and diseases including chalkbrood, which is a fungal infection of honey bee larvae (*Apis mellifera* L.), caused by *Ascosphaera*

apis. The aim of this study was to evaluate the hygienic behavior (HB) of European honeybees (*A. mellifera*) and their impact on resistance to chalkbrood diseases. The degree of HB between colonies varied significantly, with 23 % of the colonies showing high HB (≥ 95 %). The overall prevalence of chalkbrood (High, moderate, low HB colonies) was found to be 62%. A highly positive and significant correlation of number of infected colonies with rainfall ($n=10$, $r=0.93$, $P=0.001$) and humidity ($n=10$, $r=0.68$, $P=0.03$) were observed. The susceptibility of larvae belongs to colonies with high, moderate and low HB, to *Ascosphaera apis* showed no significant difference but fewer number of mummies were found in the comb cells of hygienic colonies ($r=-0.43$, $P=0.04$). Highly hygienic bees showed higher efficiency ratio (76%) which made them able to timely locate and remove the infected larvae which might be the reason of having smaller number of mummies and fast recovery.

DIVERSITY OF FAMILY VESPIDAE WASPS IN HYDERABAD DISTRICT SINDH

**Bhojoo Mal, Muhammad Tayyab, Abdul Manan Shaikh, Aiman Amur*,
Vikram Kumar and Baboo Mal**

Department of Zoology, Government College University, Hyderabad

*Corresponding Author: amuraiman@gmail.com

Wasps are present throughout the world, mostly in tropical regions. The family Vespidae comprises wasps. The larvae of Vespidae (wasps) prey on caterpillars and other insects, making them an effective biological control agent in terrestrial ecosystems. These wasps are also important pollinators for a wide variety of fruit and vegetable plants. The majority of their habitats are in forests, fields of vegetables and fruit orchards. It is well known that wasps play a vital part in ecosystems around the world, and some species are used as pesticides for cultivated plants as well as for the beekeeping sector. The specimens were captured during the August to 2023 up to beginning of 2024. The present research work was conducted in Hyderabad region Sindh, Pakistan. The fauna of wasp during the research survey some species of wasps were collected from open fields, gardens and houses and are preserved in 70% ethanol and kept in Zoology Museum of Government college university Hyderabad. Family Vespidae was the most abundant in the region mentioned above. Specimens were collected and identified. Definitely this study will form a base line for future researchers. The present research survey suggests that region Hyderabad has diverse wasp fauna. Similar research study is recommended on large scale to find out the remaining wasp species in Hyderabad and its surrounded areas.

EVALUATING THE IMPACT OF PHTHALATE ESTER EXPOSURE ON DROSOPHILA

**Amatul Wadood Beenish, Tooba Nayab, Rashiqa Manan*,
Ramsha Kanwal, and Durre Samin Tahir***

Department of Zoology, Nusrat Jahan College, Chenab Nagar

*Corresponding Author: rashiqamanan.zoo20@njc.edu.pk, durre.samin@njc.edu.pk;

Phthalate esters (1, 2-benzendicarboxylates) are hazardous environmental and health contaminants commonly found in plastics, building materials, cleaning products, insecticides, children's toys, and fragrances. These chemical compounds are used to soften and improve the flexibility and durability of plastics. They are known as endocrine system disruptors in animals, with toxic effects observed in species such as rats, abalone, and freshwater rotifers. Specimens of wild *Drosophila* were procured from Chenab Nagar in vials, subsequently; they were cultured in a medium consisting of porridge, crude sugar, agar,

yeast, nipagin, propionic acid, and water. Maintained within an incubator set to 25°C, following a 15- day incubation period, from the wild caught single female cultures were raised, five female flies were individually isolated from the culture and transferred to separate vials. To create an experimental group from laboratory-cultured *Drosophila*, varying concentrations of phthalate (0.01%, 0.05%, 0.10%, and 0.20%) were introduced into the food medium. Observations were conducted on parameters including lifespan, reproductive fecundity, and physical deformities exhibited by the *Drosophila* specimen.

EXISTENCE OF ABNORMAL SEA STAR, *ASTROPECTEN INDICUS* IN THE COASTAL WATER OF PAKISTAN

Nadia Ashfaq and Noor Us Saher

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: noorusaher@yahoo.com, sknadia@live.com

Starfish is the most important and familiar marine invertebrates. It is primarily found in sub aquatic marine environments, where starfish diversity is astounding. There are about 370 genera and 1890 species among the 36 families have been identified. Sea stars are usually pentagonal, flattened and symmetrically radiating creatures with long or short arms from a central disc, these species are free-living in benthic mode but several species are thriving by being as planktotrophic. *Astropecten indicus* only formally generates five arms as per pentamerism, yet occasionally irregularities in the number of arms are noted. The present observations suggest that deviations from pentamerism are not a heritable character but are a consequence of environmental perturbations on the metamorphosis of larvae and/or abnormal regeneration of arms.

MORPHOMETRIC AND MORPHOLOGICAL COMPARISON OF OTOLITH OF TWO SPECIES OF FAMILY PLATYCEPHALIDAE

Sana Baloch and Noor Us Saher*

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: noorusaher@yahoo.com

Platycephalidae is the family to which flathead fish species belong. Their elongated bodies, together with a large mouth and a spiky, sunken head, serve as distinguishing characteristics. Often, the lower jaw is longer than the upper. Fish species and stock identification made possible by the unique shapes of otoliths, calcareous anatomical structures found in the inner ears of fish. Otolith is calcareous anatomical structure in the inner ear of fishes and preferred as taxonomic character due to their species- specific shape. Samples of flatfish species procured from three distinct landing sites along Pakistan's coast: Sonmiani, Korangi, and Karachi fish harbors. In the lab, the gathered fish species washed and identified up to genus and species. Six specimens of variable sizes of each species *Grammopolites suppositus* and *Grammopolites scaber*, selected for otolith study. The morphometric study of the collected fishes also done and no difference found in between left and right otolith length for any of the otolith pairs. The morphological characteristics of fish otolith observed highly variable between two species. The relationships between otolith size, fish size and their body mass showed positive correlation.

IDENTIFICATION AND QUANTIFICATION OF SHAPE CHANGE IN *TETRACLITA STALACTIFERA* (*BALANOIDEA*) FOUND IN ROCKY SHORE KARACHI

Arooba Nasir and Noor Us Saher*

Marine Reference Collection and Resource Center, University of Karachi, Karachi

Centre of Excellence in Marine Biology, University of Karachi, Karachi

*Corresponding Author: noorusaher@yahoo.com

The widely distributed intertidal barnacle species *Tetraclita stalactifera* commonly known as Ribbed Barnacle or Volcano Barnacles belong to the Tetraclitidae family. There are twenty members in the *Tetraclita* Genus that are currently known. The current study designed to quantify traits of evolutionary relevance by identifying changes in shape and function or evolutionary relationships, through the morphometric relationships of the *Tetraclita stalactifera* species. In its wide range, this species shows some morphological variety. They inhabit a variety of hard substrates, including piers and rocks, and their preferred water temperature ranges from 20 to 26°C. They stick to almost any surface that can be stone, wood, or another material. Barnacles of the *Tetraclita stalactifera* species were gathered from the intertidal zone of Karachi's rocky shoreline during October 2021 to November of 2022 at the Manora site. The opercular plates and shell structure were used to identify the species. The height of the carina (HTC), height of the rostrum (HTR), basal diameter of the shell, average shell wall thickness (TKC), width of the basis (WBA), length of the orifice along the carino rostral axis (LOR), and width of the orifice (WOR) were all measured on each individual's shell. Their basal diameter ranged from 10 mm-17 mm. The color may vary from white to brown. Tergum with prominent depressor muscle crests, powerful transverse growth lines, and a narrow apex. Scutum is wide, triangular, and has a curved scutal margin. The study revealed that these selected parameters have been employed as a size estimate since they offer insights into the evolutionary traits. By utilizing allometric and statistical data, the resultant morphometric measurements provided important information regarding habitat interaction and species differentiation.

ELEVATED LEVELS OF PHOSPHOPROTEIN MAP1B IN MOUSE COCHLEA CAUSES HEARING LOSS

Bint-e-Zainab^{1*}, Yan Li², Thomas B. Friedman³ and Ayesha Imtiaz¹

¹*School of Biological Sciences, University of the Punjab, Lahore*

²*National Institute of Neurological Disorders and Stroke, NIH, Bethesda, USA*

³*National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, USA*

Corresponding Author: 3410356941282@pu.edu.pk

The pathophysiology of hearing loss cause by various mutations in phosphatases remains elusive. The comparative phosphoproteomics of cochlear tissue from wild-type and CDC14A phosphatase dead mutant mice reveals that levels of a microtubule binding protein 1B (MAP1B) is raised in mutant sample as compared to wild-type. Which makes it a potential substrate of CDC14A phosphatase that is a cell division cycle protein involved in hearing loss. Cochlear tissue harvested from mutant and wild type mice are prepared and digested with trypsin to produce phosphopeptides. Phospho-peptides are labelled with TMT and iTRAQ isobar tags and phospho-enriched using titanium dioxide resin. Then, liquid chromatography-tandem mass spectrometry (LC/MS/MS) is performed. The substrate is identified by analyzing mass spectrometry data. The abundance ratio of phosphopeptides in mutants are calculated and compared with wild type samples. From the data analyses, the elevated level of MAP1B is identified. MAP1B is highly

expressed in spiral ganglion neurons in the mouse cochlea. MAP1B deficiency is previously reported to causing hearing loss in human and mouse. The phosphorylation status of crucial residues in MAP1B is important for microtubule stability and dynamics. The phosphorylation status of Ser-1400 in MAP1B, located in highly conserved domain is essential for the normal functioning of embryonic cortical neurons in cochlea. However, the phosphatases specific to MAP1B remains unknown. Our data supports that MAP1B might be dephosphorylated by CDC14A and accumulation of phosphorylated MAP1B can cause sensorineural hearing loss in mouse cochlea. Further analyses and functional studies are needed to elucidate the spatial and temporal interaction between CDC14A and MAP1B.

PHOSPHOPROTEOMICS STUDIES REVEAL CDC14A DEPHOSPHORYLATED AKAP4 SCAFFOLD PROTEIN

Alishbah Sheikh^{1*}, Yan Li², Thomas B. Friedman³ and Ayesha Imtiaz¹

¹*School of Biological Sciences, University of the Punjab, Lahore*

²*National Institute of Neurological Disorders and Stroke, NIH, Bethesda, USA*

³*National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, USA*

Corresponding Author: alishbah.sheikh95@gmail.com

Akap4 (A kinase anchor protein 4; also called fibrous sheath component 1 or AKAP 82) is the most abundant protein in the sperm fibrous. AKAPs are a conserved family of kinases, composed of around 50 scaffold proteins which compartmentalize protein phosphorylation by tethering cyclic AMP (cAMP)-dependent protein kinase (PKA). AKAPs also can serve as scaffolds for signal-transduction complexes that contain phosphatases, kinases, and other components that are believed to coordinate the phosphorylation status of target proteins. Akap4-knockout mice produce normal numbers of spermatozoa but sperm fail to show progressive motility and male mice are infertile. These sperm have shortened flagella but normal/abnormal shape. In this study we performed comparative phosphoproteomics on wildtype and phosphatase dead mutants of CDC14A. Sperms were extracted from epididymis tissue of both mutant and wild type mice and snap frozen with liquid nitrogen. The extracted protein samples were phosphor-enriched and then was subjected to LC/MS/MS. AKAP4 was one of the substrates identified during the analyses of *in vivo* comparative phosphoproteomics data. AKAP4 may be a direct phospho-substrate of CDC14A or elevated levels of phospho-AKAP4 in mouse sperm may be due to another phosphatase which interacts with CDC14A. Further studies are required to elucidate when, where and how in the cell CDC14A interacts with AKAP4. This might help to understand the biological and cellular functions of CDC14A in the reproductive system.

ZOONOTIC AND NON-ZOONOTIC HELMINTHS IN BLACK RATS OF RAIN-FED AND IRRIGATED AREAS OF SWAT, KHYBER PAKHTUNKHWA, PAKISTAN

Wali Khan^{1*}, Noor Un Nisa², Aly Khan³ and Nasira Khatoon⁴

¹*Department of Zoology, University of Malakand, Lower Dir*

²*Medical Zoology Laboratory, VPCI /SARC/PARC, Karachi*

³*Crop Diseases Research Institute (CDRI) /SARC/PARC, Karachi*

⁴*Department of Zoology, University of Karachi, Karachi*

*Corresponding Author: walikhan@uom.edu.pk

Present study was conducted to get information on helminth parasites of zoonotic importance found in black rats of district Swat, Pakistan. Two hundred and sixty nine rats were captured from agricultural

ecosystem of the district using live captured traps from 2011-2013. Captured rats were anesthetized and screened for the presence of ectoparasites, then were carefully dissected for investigation of endoparasites. Helminth parasites of 8 species were identified. Presence of parasite was noticed in 23.7% of sampled rats. The infection rates of examined rats was given in order of their infectivity as *Syphacia obvelata* 13(4.83%), *Aspicularis tetraptera* 13(4.83%), *Heterakis spumosa* 12 (4.46%), *Hymenolepis* spp. 9(3.34%), *H. diminuta* 8(2.97%), *Hymenolepis fusa* 4(1.48%), *Lutziella microacetabularae* 4(1.48%) and *Lutziella* spp. 1 (0.37%). No significant difference ($P < 0.4289$) was found in prevalence of parasites among areas, crops, crop stages and sex of the host while adult rats were found more infected than sub-adults. *S. obvelata* and *A. tetraptera* were the most common species of helminths while *Lutziella* sp., 1 (0.37%) was found only in one host. *Rattus rattus* (the black rat) was regarded as the host of helminth parasites of zoonotic importance, therefore the hidden health hazards of this rodent species needed to be considered to prevent infectivity of humans. Current study was concluded that *Rattus rattus* harbored a wide variety of helminth parasites which shows a hidden risk to inhabitants of the region. Monitoring rats' population in settle areas and educating the local community about the risk of rat borne parasitic diseases transmission through rats appears to be absolutely essential.

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