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Biology of Cabbage Aphid Under Laboratory Conditions

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Abstract.-Biology of cabbage aphid, *Brevicoryne brassicae* L. was studied on canola leaves under laboratory conditions at Multan, Pakistan. Biological parameters were studied by feeding canola leaves to aphid in 50 ml capacity plastic vials. Food was changed daily. Aphid had a pre-reproductive and reproductive period of 2.34 and 6.25 days, respectively. Number of nymphs produced per female was 30.79 and reproductive rate was 3.85 nymphs per female per day. Longevity of reproductive females' was 9.0 days. Nymphs completed three instars in 9.09 days. Mean duration of first, second and third instar was 2.09, 3.50 and 3.50 days, respectively. Mortality of nymphs during development was 67.85, 17.85 and 14.30 percent in first, second and third instar, respectively. Out of the total nymphs produced 54.16% reached the reproductive stage.

Key words: *Brevicoryne brassicae* L., *Brassica napus* L., development, mortality, Pakistan

Rapeseed/mustard and canola are important oilseed crops in Pakistan after cotton (PARC, 2007). These crops were cultivated on 402.86 thousand hectares during 2006-2007 and seed production from these crops was 377,000 tonnes. Pakistan produces 27 percent of its total requirement of edible oils and 73 percent is met through imports (Anonymous, 2008). In Pakistan, the yield of oilseeds per unit area is much lower as compared to that in other countries. Major reasons are low

yielding varieties, poor management practices, and severe attack of pests (Khattak and Hamed, 1993). Aphids are among the important insect pests attacking these crops (Rehman *et al.*, 1987). Predominant aphid species infesting these crops are cabbage aphid (*Brevicoryne brassicae* L.) green peach aphid (*Myzus persicae* (Sulzer)) and turnip aphid (*Lipaphis erysimi* (Kalt.)). Cabbage aphid is a major pest in southern Punjab area of Pakistan (Aslam *et al.*, 2007). High populations of these species of aphids cause wilting and yellowing of leaves and stunting of the plants (Jamaya and Ronald, 1998).

Sound knowledge of fecundity, development, survivorship and longevity is essential for aphid management (Halbert *et al.*, 1981). A number of workers have studied the biology of cabbage aphid on different host plants in different parts of the world. Hughes (1963) reported that cabbage aphid reproduction was 3.46 to 5.37 nymphs per day for Australian species. Adults of European and Australian species lived for 18.5 and 11.2±0.7 days, and 12.2 and 7±0.4 days at 18.2°C at 23.8°C, respectively. According to Raworth (1984) cabbage aphid produced 40.7 nymphs per female. Kashayp and Sharma (1994) found four instars and a life span of 37.25 to 42.25 days of cabbage aphid on vegetative stage of Indian mustard. They also reported that nymphal duration was 11-13 days on flowering stage and 12-14 days on vegetative stage of the crop Hines and Hutchinson (1997) reported that cabbage aphid developed in 8-12 days from first instar nymph to adult. Jamaya and Ronald (1998) reported that duration of the life cycle of cabbage aphid, depending on the temperature, ranged from 16-60 days. It has been reported by Bargg and Burns (2001) that cabbage aphid female can produce 10 nymphs per day, whereas Paula *et al.* (1998) reported 1.25 offspring per female per day. Williamson (2001) reported that cabbage aphid female can live up to one month and give birth to 60-100 nymphs in this duration. Webb (2002) reported that cabbage aphid nymphs matured in 7-10 days. Satar *et al.* (2005) reported a development period of 12.5 days, and 16.3 days longevity of females of cabbage aphid at 15°C, whereas reproductive period of 6 days and female longevity of 9.8 days was reported by them at 30°C. They also

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reported reproduction of 47.1 nymphs/ female at 25°C and 1.5 nymphs/ female at 30°C. According to them mean generation time was 22.6 days at 15°C and 11.3 days at 30°C. According to Ulusoy and Olmez-Bayhan (2006) total development time of cabbage aphid was 8.9 days on cauliflower and 10.4 days on cabbage and mortality of immatures was 16% on cabbage and 88% on turnip.

No work on the biology of this pest has been reported from southern Punjab, Pakistan. Keeping in view, the importance of canola as an oilseed crop in the country, its yield loss and aphid infestation the present research was undertaken to study the development, fecundity and survival of cabbage aphid on canola (*Brassica napus* L.) under laboratory conditions at Multan.

Materials and methods

The biology of cabbage aphid was studied on canola leaves in the laboratory at room temperature at Multan (32.2° N, 71.45° E), Pakistan. Minimum and maximum temperature during the study period was 12.4-16.3 and 22.4-28.5°C, respectively. Wide mouthed (3.5 cm) transparent plastic vials of 50 ml capacity, having screw lid, were used in the study. Twenty newly-moulted apterous cabbage aphid females, collected from canola, were put in the plastic vials. The vials were labeled from 1 to 20 for keeping track of the females during data recording. All the females were fed on fresh fifth leaf from the top of canola plants. The leaves were removed from plants and cut into pieces of about 25cm² and one piece was placed in each vial. The leaves were washed with tap water and air dried before feeding to the females. Leaves were replaced daily and vials were cleaned. Observations were recorded after every 24 hours for pre-reproductive, reproductive, post-reproductive period, mortality and fecundity. The experiment was repeated three times.

Females of cabbage aphid collected from canola and reproducing on canola leaves in laboratory in plastic vials were chosen for studies on the development of nymphs. Sixty nymphs, born on the same day, were put into plastic vials separately. Similar vials were used for nymphs as for females. Food to nymphs was also provided in similar manner as described for females. Data were recorded after every 24 hours for determining for

number of instars, duration between two moults, mortality rate and developmental time.

Results and discussion

Cabbage aphid had an average pre-reproductive period of 2.34 days and mean reproductive period was 6.25 days (Table I). Reproduction was 30.79 nymphs per female in eight days (Tables I, II). These results are different from those of Williamson (2001), who reported 60 to 100 nymphs per female and Raworth (1984), who reported an average of 40.7 nymphs per female. Fecundity in our study was lower as compared to that recorded by earlier workers. Maximum reproductive period was eight days in the present study (Table II) whereas, Hines and Hutchinson (1997) reported 30+ days and Williamson (2001) reported 30 days reproductive period.

Table I.- Biological parameters of *B. brassicae* on canola leaves under laboratory conditions at Multan, Pakistan.

Reproduction in females (n=20)		
Average pre-reproductive period		2.34 days
Average reproduction		30.79 nymphs/female
Average reproductive period		6.25 days
	Development period of nymphs (days)	Nymph mortality (%)^a
1 st Instar	2.09	67.85
2 nd Instar	3.50	17.85
3 rd Instar	3.50	14.30
Average	9.09	-

^aCalculations based on nymphs dying out of 60, i.e., 27.5.

Mean number of nymphs produced per female per day was 3.85. Reproduction gradually increased to third day of reproduction and thereafter decreased to a minimum on eighth day. Maximum reproduction (22.1% of the total nymphs produced) was noted on third and minimum (0.6%) on eighth day. Nymphs produced in first four days were 73.5% of the total. In the present study number of nymphs per female per day was close to that recorded by Hughes (1963), who reported 3.46 to 5.37 nymphs per female per day, but was lower as compared to that reported by Hines and Hutchinson

(1997), who found 5 to 6 nymphs per female per day. Bragg and Burns (2001) reported that a female produced 10 nymphs per day. This reproductive rate is also higher than that found in our study. The difference in results of the present and previous studies could be due to different experimental conditions and different host plants. Most of the earlier workers used vegetables as hosts, whereas we used canola leaves as food for aphid. Reproductive females lived for a maximum period of nine days. No female mortality was noted in first three days of reproductive period. Maximum mortality (35.09%) was recorded on eighth day. It is important to note that 73.5% nymphs had been produced when significant mortality of females started to occur.

Table II.- Reproduction, female mortality and nymphal mortality of *B. brassicae* on canola leaves under laboratory conditions at Multan, Pakistan.

Days	Reproduction (Nymphs per female per day) ^a	Female mortality (%) ^b	Nymphal mortality (%) ^c
1	4.97	0.00	0.00
2	5.50	0.00	22.72
3	6.79	0.00	13.64
4	5.37	2.47	18.18
5	3.62	9.80	9.09
6	2.49	22.49	0.00
7	1.87	23.26	4.55
8	0.18	35.09	9.09
9	-	6.82	9.09
10	-	-	13.64

^an = 20 females, ^bCalculation based on 20 females,

^cCalculation based on number dying out of 60 nymphs, i.e. 27.5.

Three nymphal instars were observed in mean nymphal development period of 9.09 days (Table I). Mortality of nymphs was 67.85% in the first, 17.85% in the second and 14.30% in the third instars. Maximum mortality was recorded on second day during the development period. Most of the nymphs (63.6%) died in first five days and mortality decreased in later days. Higher mortality in early days might have resulted during handling of very young nymphs, which were moved with a camel hair brush when manipulating in the laboratory. Out of the total nymphs produced 54.16% reached the

reproductive stage.

Detailed study on biology in Australia proved that duration of the nymphs of *B. brassicae* collected from different hosts ranged between 38.7-69.2, 36.6-54.4 and 37.9-58.7 hours at temperature range of 18 to 24°C. It may be noted that nymphs were reared on leaf discs (Hughes, 1963). The results confirm the results reported by Kashyap and Sharma (1994), who also reported three nymphal instars and duration of nymphal period between 10 to 14 days on different brassica species. Duration of nymphs of another species of aphid *L. erysimi* was observed 6.5-8.9 days on different species of the brassica that is close to the observed duration in our study (Rana, 2005). Therefore, leaf disc method seems to be reliable to study the biology of aphids. This method has also been employed by Hughes (1963). However feeding on alive plant may prove better to study biology of aphids due to preference for feeding on plant parts.

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A Study on Serum Biochemistry and Hematological Profiling of Blue Rock Pigeon (*Columba livia*) in Multan (Punjab, Pakistan)

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Abstract.- Thirty wild blue rock pigeons (*Columba livia*) were caught from Multan city (Pakistan). Hematological values were established for total red blood cells, total white blood cells, packed cell volume, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and differential leukocyte count. Serum bio chemistry values were determined for glucose (294±95.1), urea (17.9±4.9 mg/dl), cholesterol (250.3±57.2 mg/dl), creatinine (0.41±0.2 mg/dl), lactate dehydrogenase (1069±581), total protein (9.8±0.7 mg/dl), alanine aminotransferase (75.9±64.3) and aspartate aminotransferase (297.2±177) as indicator of bird health and can be used in the future as a reference values in

hematology and serum biochemistry of blue rock pigeon.

Key words: Wild blue rock pigeon, hematological values of pigeon, serum biochemistry of pigeon.

Clinical hematology and blood biochemistry are not only relevant tools in veterinary medicine for caged birds (Peinado *et al.*, 1992) but can also prove useful as physiological indicators in wildlife and conservation programs (Ferrer, 1990). Hematological and biochemical measurements may vary depending on gender, age, pregnancy, physical exercise, weather, stress and season (Kaneko *et al.*, 1997).

Using some biochemical components to determine the physiological and nutritional condition of individuals can prove extremely important in understanding and in tempering behavioral problems or abnormalities (Ferrer, 1990). Hematology and plasma biochemistry values have been used to investigate changes in nutritional state of birds (Alonso-Alvarez and Ferrer, 2001), reproductive status (Merino and Barbosa, 1997), body condition (Ewenson *et al.*, 2001), the physical condition of nestlings (Villegas *et al.*, 2002), and health status (Fargallo *et al.*, 2001).

Hematological values are important for clinicopathological diagnosis such as traumatic injury, parasitism, organic disease, bacterial septicemia and nutritional deficiencies. The clinical signs of illness in birds are frequently subtle, clinical chemistry is important to evaluate cellular changes (Ritchie *et al.*, 1994). However, literature on hematological and serum biochemical values of blue rock pigeon is limited. Pigeon hematology is reviewed by Schummer (1973). Erdöse and Fountaine (1977) presented some normal blood values for different pigeon races and Gylstroff (1983) presented values for pigeons of different ages. Sexual differences in the blood values of pigeons were investigated by Gayathri and Hegde (1994) and Pavlak *et al.* (2005). There is no baseline data available regarding blood parameters in local pigeon population of southern Punjab. Therefore the objective of this study was to establish hematological and serum biochemical values for healthy blue rock pigeon of Multan. The collected

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data provides the first information on natural composition of blood in this specie, in Pakistan, and can be used for clinical, pathological diagnosis and further studies.

Materials and methods

Thirty wild blue rock pigeons (*Columba livia*) were captured from the premises of Bahauddin Zakariya University Multan (Punjab; Pakistan) with the help of nets in early May 2006. Pigeons were kept in cages until blood was sampled, the next day. Blood samples (2ml) were collected from cutaneous ulnar vein with ml sterilized syringes. Blood for smear formation was used from syringes while remaining blood was transferred into two tubes, one with EDTA for hematological determination and the second without anticoagulant for other serum biochemical determinations. The samples were kept in an icebox and transferred to the laboratory for further analysis. Blood samples were centrifuged in microcentrifuge (Clay Adams, USA) at 12000 RPM for 10 minutes to isolate serum for further biochemical analysis.

Differential Leukocyte counts (DLC) were made on monolayer blood films, fixed and stained with Giemsa-Wright's stain. Total red blood (TRBC) and white blood cell count (TWBC) were determined by a manual method using hemacytometer (Campbell, 1995). Packed cell volume (PCV) was measured by standard manual technique using microhematocrit capillary tubes and centrifuged at 12000g for 5 minutes. Hemoglobin (Hb) concentration was measured by cyanmethemoglobin method. Erythrocyte indices such as Mean corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated by using conventional formulas. (Ritchie *et al.*, 1994).

Quantitative analysis of plasma biochemical parameters total protein, creatinine, glucose, lactate dehydrogenase (LDH), urea, cholesterol, alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) was done by using Photolab 1400.

Results

Results of hematological and serum

biochemical profile of wild blue rock pigeon are presented in Table I.

Table I.- Hematological and plasma biochemistry Reference Ranges In Blue Rock Pigeon (*Columa livia*). Values are expressed as mean \pm standard deviation.

Parameter	Mean \pm SD	Range
TRBC (μ l)	3485926 \pm 924534	860000-5320000
TWBC (μ l)	11464 \pm 8530	1100-40260
PCV (%)	49.3 \pm 6.4	17.65-58.51
Hb. Conc. (gm/dl)	15.6 \pm 2.2	12-19.2
MCV (fl)	156.8 \pm 698	90.36-456
MCH (pg)	45.9 \pm 13.7	30.65-91.9
MCHC (gm/dl)	31.7 \pm 5	21.43-42.91
Differential leukocyte count		
Heterophils (%)	55.4 \pm 9.1	28-71
Lymphocytes (%)	30.7 \pm 6.6	21-52
Eosinophils (%)	7.3 \pm 3.4	2-16
Basophils (%)	3.0 \pm 2.5	0-11
Monocytes (%)	3.7 \pm 2.6	0-12
Plasma biochemistry		
Urea (mg/dl)	17.95 \pm 4.9	10-29.5
Cholesterol (mg/dl)	250.3 \pm 57.18	22.7-346.9
Glucose (mg/dl)	294 \pm 95.1	27-404
Creatinine (mg/dl)	0.41 \pm 0.19	0.016-0.73
LDH (u/l)	1069 \pm 581	500.6-2434
ALAT (u/l)	75.9 \pm 64.3	12.2-255
ASAT (u/l)	297.2 \pm 177	92.68-693.4
Total protein (g/dl)	9.8 \pm 0.7	8.5-11

Discussion

In general, blood examination is performed as a screening procedure to access general health (Jain, 1993). Hematocrit and red blood cell counts have generally been used to evaluate the normality of the oxygen transport system (Gessaman *et al.*, 1986) or adaptation (Polo *et al.*, 1992) Since the white blood cells in the avian species, in general, serve to phagocytic function similar to their mammalian counterparts and different leukocyte count as well as H/L ratio were used as indicators of stress response. Our leukocyte values for free living blue rock pigeons (Table 1) are much higher than counts reported in various captive bird species (Puerta *et al.*, 1990; Schulz *et al.*, 2000) as well as free living pigeons from different habitats (Gayathri and Hegde, 1994; Pavlak *et al.*, 2005). In our study, the predominant leukocytes are heterophils followed by

lymphocytes, whereas eosinophils, basophils and monocytes were found in very small numbers. Similar observations were reported by Gayathri and Hegde (1994). It can be concluded from this observation that captivity diminishes the risk of infection and accordingly the number of leukocytes is smaller in captive animals than in free living ones. Higher leukocyte count in our studies can also be reaction to stress, such as clinical examination and blood sampling. Results revealed that member of same species living in different habitats do have variations in hematological profile.

Comparisons of blood plasma analysis from healthy and sick birds can provide the basis for diagnosis of various diseases. Glucose, lactate dehydrogenase, urea, cholesterol, alanine aminotransferase, and aspartate aminotransferase levels are diagnostic values for diabetes mellitus, liver disease, hypoparathyroidism, chronic hepatopathy, gout, kidney disease, chronic diarrhea and dehydration (Ritchie *et al.*, 1994). Although general guidelines exist for interpreting biochemical values in birds with unknown reference values, species specific ranges are preferred. The interpretation of plasma biochemistry values is usually more complex than evaluating a single parameter. Generally, the entire biochemistry profile is used to characterize the bird health status (Schulz *et al.*, 2000). The current study will provide a baseline data regarding hematology and serum biochemistry of the local pigeon population as no published data is available on these parameters to our knowledge.

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Abid's Trackmove: A Computer Application for Monitoring the Walking Behaviour of Insects and Other Arthropods

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Monitoring the walking speed of arthropods is an important component of insect behavior studies under a given set of biotic or abiotic environment.

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Walking speed and pattern is an indicator of arthropod's response to various physical and chemical or physiological stimuli (Mason and Hopper, 1997; Roermund, 1996).

Studies on walking behavior require the observation of movement pattern, in the presence of such stimuli, either manually or through using electronic monitoring systems. Manual recording does not provide an accurate measurement of the response, while electronic recording needs specialized apparatus (Alebeek *et al.*, 1996; Bowie, 1999).

Screenshots of the software

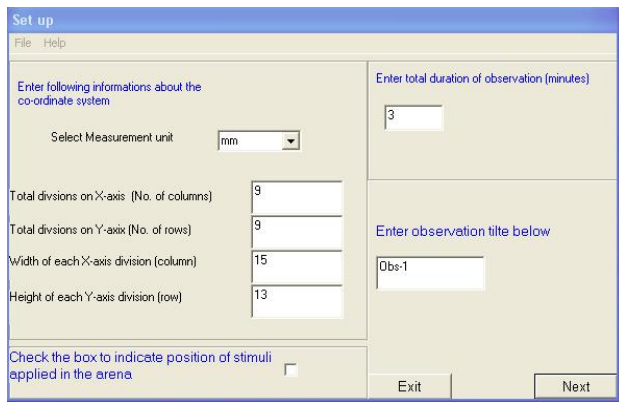


Fig. 1. Screen shot 1: Set up window.

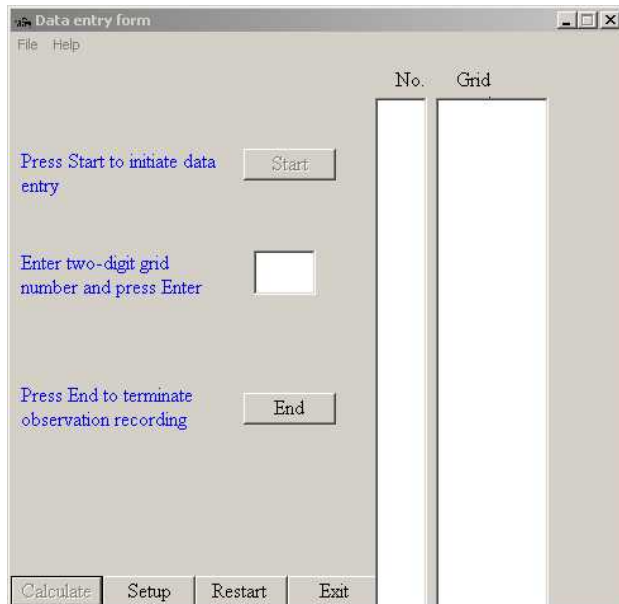


Fig. 2. Screenshot 2: Data input window.

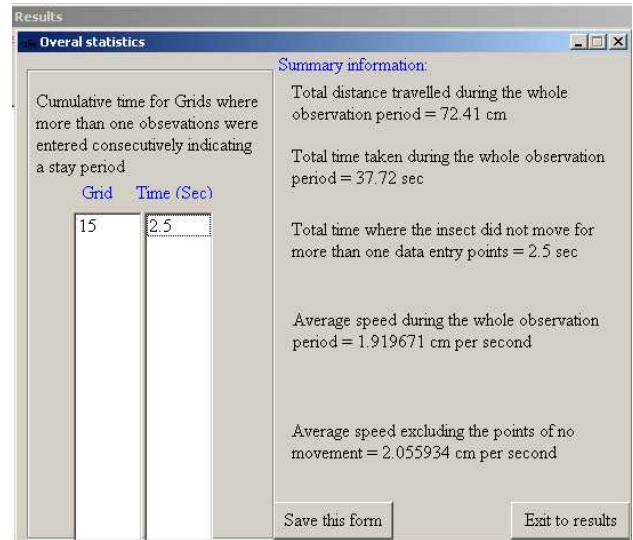


Fig. 3. Screen shot 3: Result summary window.

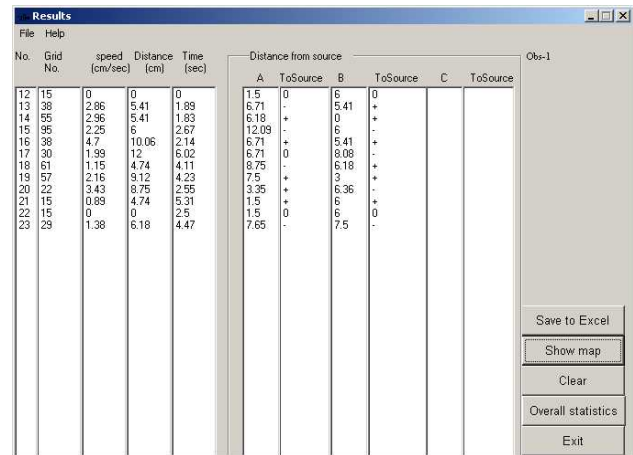


Fig. 4. Screen shot 4: Result window.

A windows-based computer application 'Abid's Trackmove' was developed for assisting the monitoring of walking speed and pattern of arthropods' movement using a simple coordinate system (Figs. 1-4). The application can be used to monitor the response of biological control agents or insect pests to chemical stimuli (attractants or repellents), comparison of laboratory-reared strains with their wild counterparts and see the mobility of insect pests at various intervals after getting infected by an entomopathogen (Lacey *et al.*, 1997) or any other situation where the study of walking behavior is important.

The software can also help to study the orientation response of an organism to a maximum of three stimuli provided on the arena. During the course of movement, the software measures the distance of organism from the source stimuli and also indicates if the organism is moving towards or away from a certain stimulus. The application was basically developed for the insects and other arthropods but can be used in general zoological studies involving other animals as well.

The experimental arena is divided into a grid which is numbered according to the given co-ordinate system. After introducing the organism into the arena, the grid position of the organism is entered as a two-digit grid number. The output includes speed of insect, distance travelled, and the pause time during the course of movement and can be transferred directly to an Excel file (The computer must have Microsoft Excel installed). At the end, movement map is drawn along with the indication of stimuli position and can be saved as a graphic file. The limitations of the programme are that it does not record the actual turning angles as the distance is approximated to the centre of grid. Also, the observations for a single individual only can be recorded at a time. Abid's Trackmove has been used successfully for studying impact of egg retention on walking behavior of *Trichogramma chilonis*. The software is available for download at <http://www.nifa.org.pk/Software.htm>.

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Response of Recently Developed and Already Cultivated Genotypes of *Brassica napus* L. and *Brassica juncea* (L.) to Two Aphid Species at Multan, Pakistan

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Abstract. Some recently developed and previously cultivated genotypes of canola, *Brassica napus* L. (BRN 07001, BRN 07002 and Shiralee) and Raya, *Brassica juncea* (L.) (BRJ 07051, RL 18 and KHN Raya) were evaluated for comparative resistance against cabbage aphid, *Brevicoryne brassicae* L and mustard aphid, *Lipaphis erysimi* (Kalt.) at Multan during crop year 2007-08. A completely randomized block design field trial was conducted by assessing aphid densities on each of the six genotypes over six week period. Densities of *B. brassicae* and *L. erysimi* were not different on all the sampling dates across the tested genotypes.

Key words: *Brevicoryne brassicae*, *Lipaphis erysimi*, *Brassica napus*, population density, genotypes, *Brassica juncea*.

Brassica juncea, *Brevicoryne brassicae* L. and turnip aphid, *Lipaphis erysimi* (Kalt.) are major insect pests of canola, *Brassica napus* L. and Raya, *Brassica juncea* (L.) in Southern Punjab, Pakistan (Aslam and Razaq, 2007). Many researchers have reported lack of plant resistance in available cultivars of *B. napus* and *B. juncea* in different districts of Southern Punjab (Aslam *et al.*, 2005, 2007, 2009; Amer *et al.*, 2009). Breeders continuously try to develop new varieties to enhance yield and the particular area. In this study we evaluate some recently developed and previously

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available other desirable traits according to requirements of genotypes of *B. napus* and *B. juncea* against *B. brassicae* and *L. erysimi* through seasonal abundance under field conditions.

Materials and methods

The research was carried out at the Experimental Farm of the University College of Agriculture, Bahuddin Zakariya University, Multan, during crop season 2007-2008. The trial was laid out in Randomized Complete Block Design in three replicates by sowing three genotypes of *B. napus* (BRN 07001, BRN 07002 and Shiralee) and *B. juncea* (BRJ 07051, RL 18 and KHN Raya) by hand drill on November 15, 2007. Each treatment consisted of four rows with row to row distance of 0.5 m and plant to plant distance of 10 cm. Each row was 5m long. Treatments and replicates were 1.5m apart. After germination, all the cultural practices were performed through out the growing season uniformly in all plots.

The aphid population was recorded at weekly intervals from the initiation of aphid attack till maturity. Six randomly selected plants, *i.e.*, three from each of two central rows were taken. Top fifteen cm of the central shoot inflorescence was beaten gently six times with a 10 cm long stick of pencil thickness. Dislodged aphids were collected on a piece of white sheet and counted. This method has been employed by Amer *et al.* (2009). The data were analyzed for ANOVA by using MSTATC computer software (MSU, 1982).

Results and discussion

Population densities of *B. brassicae* and *L. erysimi* were not significantly different across the tested genotypes on all the sampling dates. Peak populations of both the species of aphids were noted on the 8th March, 2008 (Table I). Genotypes of *B. napus*, Abaseen, CON-I, CON-II, CON-III, Dunkald, KS-75, Oscar, Rainbow, Shiralee and Wester have been reported to harbour similar populations of *B. brassicae* and *L. erysimi* during cropping season 2003 at Multan (Aslam *et al.*, 2005, 2007). Similar results were reflected for 12 genotypes (ten above mentioned and 19-H and 20-E) during 2004 at Multan and Bahawalpur,

during 2005 (Abaseen, CON-III, CON-I and KS-75) at Multan and during 2006 (Abaseen, CON-II, CON-I and KS-75) at Dera Ghazi Khan (Amer *et al.*, 2009). Genotypes of *B. juncea* BARD-1, BRS-3, P11R-1, UCD-6/10, P63R5, UCD-44/4, UCD636, P-37, RC-280, 95101/163 and 95102/51 have been reported to harbour statistically similar populations of *B. brassicae* and *L. erysimi* at Multan and Bahawalpur during, 2004. Populations of both the aphid species were observed significantly different for three sampling dates out of seven on above mentioned genotypes (except 95101/163 and 95102/51) of *B. juncea* at Multan during 2003 but no differences in densities of both the species were observed during the same cropping season at Bahawalpur (Aslam *et al.*, 2009). Previously, genotypes of the *B. napus* and *B. juncea* have been evaluated separately for seasonal abundance of aphids at Multan. Many researchers from other parts of Pakistan have reported differences among the genotypes of *B. napus* (reviewed in Amer *et al.*, 2009) in harbouring aphid densities. However, non significant difference in aphids population among different genotypes of *B. juncea* have been found in India (reviewed in Aslam *et al.*, 2009). But in present study we evaluated genotypes of both the species together and no differences were observed among the genotypes regarding population densities of *B. brassicae* and *L. erysimi*. It has been proved that level of resistance in identified sources is very low and stands danger of dilution in the process of agronomic adapted varieties (Bakhetia, 1990). Therefore, similar response of varieties of *B. napus* and *B. juncea* in this study might be due to low level of resistance or absence of resistance.

Oilseed brassicas are minor crops in Multan region. Farmers usually do not apply insecticides to reduce the damage. In previous studies application of insecticides has been recommended as available cultivars of *B. napus* and *B. juncea* lack plant resistance (Amer *et al.*, 2009; Aslam *et al.*, 2009). But the economic threshold level (ETL) of aphids has not been determined. Further research will be directed towards determination of ETL or to record the timing of application to manage aphids. *B. napus* than *B. juncea* will be the better choice for the farmers as this species is economically more important.

Table I.- Mean population of *B. brassicae* and *L. erysimi* with standard error on top 15 cm of inflorescence per plant of different varieties of *B. napus* and *B. juncea* on different sampling dates during 2008.

Varieties	Sampling dates				
	23 rd February	01 st March	08 th March	15 th March	22 nd March
<i>B. napus</i>					
BRN 07001	11.33±1.19 ^{n.s}	21.14±1.56 ^{n.s}	34.30±1.75 ^{n.s}	14.19±0.48 ^{n.s}	9.77±0.52 ^{n.s}
BRN 07002	13.11±2.35	25.15±3.50	36.94±5.67	17.84±3.36	9.22±2.22
Shiralee	18.16±3.52	29.22±5.26	48.39±6.80	28.16±3.81	18.77±2.66
<i>B. juncea</i>					
RL 18	10.56±0.55	20.88±0.58	31.39±0.49	16.22±0.98	6.39±0.38
BRJ 07051	6.72±2.25	13.33±4.15	19.33±5.54	11.05±3.75	4.61±1.88
KHN Raya	4.94±1.04	5.05±1.04	3.27±0.78	2.61±0.57	0.94±0.38
<i>B. napus</i>					
BRN 07001	21.17±2.63 ^{n.s}	38.83±3.84 ^{n.s}	60.11±6.11 ^{n.s}	44.17±6.72 ^{n.s}	20.66±3.72 ^{n.s}
BRN 07002	19.55±2.30	32.89±3.65	50.27±5.41	33.67±5.31	11.99±1.67
Shiralee	31.05±1.9	71.22±3.84	97.38±3.56	80.44±2.51	47.55±2.35
<i>B. juncea</i>					
RL 18	8.83±0.32	14.94±0.90	24.61±0.70	14.16±1.04	6.99±0.57
BRJ 07051	15.61±2.84	16.60±2.15	27.11±2.82	13.50±2.71	4.99±1.61
KHN Raya	8.44±0.83	14.77±1.88	24.77±4.40	16.33±5.48	10.38±3.45

n.s= non-significant

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Record of *Delta dimidiatipenne* (Saussure, 1852) (Hymenoptera: Vespidae: Eumeninae) From Barani Areas of Punjab Province of Pakistan

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Abstract.- *Delta dimidiatipenne* (Saussure, 1852) is recorded for the first time from barani areas of Punjab Province of Pakistan. A taxonomic note along with distribution from seven new localities in barani areas of Punjab is presented. The wasp is illustrated using micrographs which will help

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workers in Pakistan for identification in future. Host (prey) range of these wasps has also been given.

Key words: *Delta*, *dimidiatipenne*, Hymenoptera, Vespidae, Punjab, Pakistan

Genus *Delta* of Potter wasp is recognized with about 50 species and many subspecies, distributed through-out the Old World, with one species adventive in North America (Carpenter, 2008). It has been recorded from neighboring countries of Pakistan like Afghanistan to Nepal (Van der vecht and Fischer, 1972) and India with five species records (Srinivasan and Kumar, 2010) including *Delta dimidiatipenne* (Saussure, 1852). This species belongs to subfamily Eumeninae of family Vespidae (Hymenoptera). It has been reported from many parts of the world like Bhutan, Cambodia, China, Hawaii, Hong Kong, Indonesia, Malaysia, Moluccas Islands, Myanmar, Nepal, New Guinea, Sri Lanka, Taiwan, Thailand, Saudi Arabia, Egypt, Tunisia, Canary Islands and Vietnam (Van der vecht and Fischer, 1972; Lauterbach and Latsch-Lauterbach, 2007; Srinivasan and Kumar, 2010). It can be separated from other species of the genus due to distinct characters like apical half of second gastral tergite and whole of the remaining tergites black; in female, clypeus and lower half of frons red (Srinivasan and Kumar, 2010).

This species has been reported for the first time in Barani areas of Punjab Province of Pakistan. We have illustrated this species with the help of micrographs along with its distribution in Punjab which will help the future workers on these wasps in identification.

Materials and methods

A survey was conducted during 2009-2010 from barani areas of Punjab Province of Pakistan. Adult wasps were collected from various weeds grasslands, houses with the help of collection net. Wasp nests were also taken to the laboratory and nests were observed for adult wasps emergence. Nests were also broken in order to observe the larve of the prey of the wasps. Prey larvae were also identified. Wasps were killed in a cyanide killing

bottle. After setting and spreading the wasps on a setting board, pinning was done. Wasps were observed under Swift sm-80 binocular microscope. The illustrations were prepared using a Nikon microscope (SMS-1500, with 30x 1-11.25x magnification). All the studied specimens are deposited in the Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan. The morphological terminology used in this paper follows Bingham (1897) and Goulet and Huber (1993).

Delta dimidiatipenne (Saussure, 1852)

1852. *Eumenes dimidiatipennis* Saussure, Et. Fam. Vesp., Mon. Guep. Sol.:51. Female, Male, Syntypes, Type locality Arabia (Djidda), The East Indies (India), Egypt (MNHN).
1918. *Delta dimidiatipenne* (Saussure): Bequaert, Bull. Amer. Mus. Nat. Hist., 39: 59- 62.

Taxonomic note

Body 26 mm long, obscure dull red with following black markings: vertex, extending to occiput usually at the apex of antennae, propleuron, mesopleuron except two red patches, metapleuron except a red patch on upper metapleuron, mesoscutum, base of petiole, apical half of second tergite and whole of the remaining tergites. Antennal pedicel three times shorter than flagellum. Longitudinal eye diameter is about 1.9 times the tranverse eye diameter and 1.2 times the transfacial line. Ocelli forming a broad triangle. Clypeus smooth, oval, convex, anterior margin transverse. Tergite-1 about three times longer than wider. Propodeum about 1.2 times wider than longer. Wings 22 mm long, three times longer than wider, ferruginous, with apical half of forewing fuscous having a purple reflection. Metasoma smooth and shiny.

Male

Similar to female in general appearance except the clypeus and the lower half of frons yellow, and smaller.

Material examined

Rawalpindi, 28.09.09, 4♀ and 2♂; Attock, 04.10.10, 4♀ and 10♂; Bakhar, 16-10-09, 3♀ and 8♂; Layyah, 21-10-10, 5♀ and 12♂; Muzafargarh, 20-9-09, 2♀ and 14♂; Mianwali, 21-10-19, 5♀ and 10♂; Islamabad, 04.10.09, 2♀.

Remarks

Specimens collected from Pakistan were compared with diagnostic characters given by (Srinivasan and Kumar, 2010) and found to be morphologically similar excepting negligible color variations.

Wasp of this species make nests of free mud cells attached a variety of substrates like walls and roofs of houses, branches of trees and flowers. They are active during October to November in various Districts of Punjab. Mostly they are visiting various weed (unknown) during day time. Nests were found to be filled by various larval instars of *Pieris brassicae* and *Helicoverpa armigera*, seems most common prey of this species. Wasps taken about one month to come out from their nests when reared in a laboratory at ambient temperature.

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Comparative Efficacy of Intramedullary Pinning With Full Cerclage Wires and Screwing for the Repair of Mid Shaft Tibial Fracture in Dogs

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Abstract.- The present study was designed to evaluate the comparative efficacy of two different established techniques of internal implants such as intramedullary pinning with multiple full cerclage wires and cortical screws alone for the repair of tibial fracture in dogs. The study was carried out on sixteen mongrel dogs, having age ranges from two to six year, which were brought to pet centre of the university during last year for treatment of tibial fracture. Dogs were divided into two groups, Group A and group B each having eight animals. In group A fracture site was reduced and immobilized with cortical screws alone after taking lag effect, while in group B, intramedullary pins with multiple full cerclage wires were used for rigid immobilization of the fracture segments. The dogs were kept for a period of sixty days and efficacy of both techniques was judged on the basis of physical examination and radiographic evaluation. The results of the study clearly revealed that the intramedullary pinning with multiple full cerclage wires was the better technique for the repair of tibial fracture in dogs as compare with screwing alone.

Key Words: Mongrel dogs, tibial fracture, physical examination, radiographic evaluation.

Dogs are predisposed to various complications of the musculoskeletal system. As a result their working efficiency and quality of life may thus be greatly affected. This necessitates that surgery procedures be improved for better results. Tibia is currently the most commonly fractured long

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bone in the body (Alho *et al.*, 1992). Open fractures of the tibia are common because there is little soft tissue covering its craniomedial aspect. Tibial fractures have the highest rate of non-union after those of the radius (25% and 60% of all non-unions, respectively) (Glyde and Arnett, 2006).

Almost 16% of long bone fractures occur in tibial diaphysis and 62% of tibial diaphyseal fractures are simple or long oblique. In long bones, tibia is second to femur, the bone most frequently fractured (Harasen, 2003). Jones (1987) reported that fixation with plates and screw is biomechanically the strongest fixation available for fractures of small bones of hands. With appropriate pre-operative planning and surgical application, it is considered the most reliable technique when early motion is desired after open reduction and internal fixation of the bone of the hand.

Keeping in view the quality of various precious breeds of dogs, its usefulness, human attachment with them and the fact that intramedullary pinning with full cerclage wires and screwing neutralizes all the forces acting on fracture site (Archibald, 1974; Harrason, 2003). The present study was designed to evaluate the comparative efficacy of two established techniques of internal fixation, *viz.*, intramedullary pinning with full cerclage wire and screwing in the repair of long oblique mid shaft tibial fractures. The efficacy of both the internal implants was compared on the basis of various parameters to assess healing which included, Physical and Radiographic examination of the animals. The basic objective of the study was to provide guidelines to the veterinary surgeon as to which treatment regime should be under taken while treating mid shaft fracture of the shin bone in valuable animals where quality is more important to the owner than quantity.

Materials and methods

Sixteen mongrel dogs with an average body weight of 20 kg and 2-6 years old were used for the study. The animals were admitted after the consent of the owners. The animals in Group A were numbered from one to six and in Group B, from seven to sixteen for identification and post-operative evaluation purposes.

Prior to the initiation of the experiment, the

animals were dewormed with Albendazole (™Zentel;Galaxosmithkline) tablets @ 10 mg/Kg body weight orally and was also vaccinated against rabies using Rabisin (Merial Animal Health Ltd) @1 ml/dog intramuscular. Acepromazine (™Sedastress; Farvet pvt. Ltd.) at dose rate of 0.1mg/kg was administered as a pre-anesthetic, followed by Pentothal sodium (Abbott Laboratories Limited) intravenous at the dose of 15 mg /kg body weight for the induction of anesthesia.

In Group A the hole was made in the bone with the help of drill. A screw of size 3.5 mm with length 18mm was used. First of all the drill of bit size 2.7 was used in both cortex. Then the second bit of size 3.2 was used to make a gliding hole in the near cortex for lag effect. The screws were inserted by a screw driver (Fig. 1) and hence the fracture was reduced and immobilized (Whittick, 1974).

In Group B the pin of 4mm diameter was selected. The length of the pin to be inserted in the bone was measured by comparing the pin with the bone and a mark was put on the pin. The pin was put in the Jacob chuck tightly, leaving just a few millimeters out side. The stifle was flexed and a small incision over the site of pin insertion on the skin was given with the scalpel blade. The pin was inserted cranial to the medial meniscus on the medial aspect of anterior tibial crest just medial to the patellar ligament first in the proximal segment and then in the distal segment up to the point of tarsal joint capsule to avoid damage to the tarsal joint. The extra part of the pin was cut by a pin cutter and then the pin was fixed down with the help of hammer (Whittick, 1974).

After proper placing of pin, the two pieces of cerclage wire was cut by a wire cutter according to the diameter of bone. One piece was put around on the proximal end and second around the distal end of the fracture with the help of curved forceps. Then the wires were tightened by wire twister around the segments. The extra edges were cut by wire cutter and the twisted edges were turned down to avoid punching to the soft tissues and pain to the animal (Piermattei and Flo, 1997).

The dogs were kept for a period of sixty days postoperatively. During post-operative period following medicines were used. Daily dressing of incision line with polyvinylpyrrolidone solution,

and topical spray of Rifaximin (™Fatroximin; Prix Pharmaceutical Ltd.). Daily administration of Amoxycyillin trihydrate 517.5 mg (™Formox; Prix Pharmaceutical Ltd.) intramuscular for 10 days, Enrofloxacin (™ceriflox; Star laboratories Pvt. Ltd) at dose rate of 2.5mg/kg intramuscular from 11 to 17 days and flunixin meglumin (™Loxin; selmore korea) at dose rate of 2.2 mg/kg intramuscular.

Results and discussion

During the postoperative period, the dogs were practically examined and the efficacy of the two established surgical techniques were assessed on the basis of healing of skin incision, degree of lameness during walk and trot and radiographic evaluation.

In group A, the process of wound healing was very encouraging and promising in all the dogs in 1st week except dog 1, in which dehiscence of the suture line occurred due to the slippage of screws on the 4th day. But all the incision lines of all the dogs of this group were reopened one after the other in the following weeks during lameness test. It was due to the failure of screws which was displaced due to weight bearing on the same limb. The animal also got temperature and became anorectic. These results have also been documented by Bellenger *et al.* (1981) that complications develop after application of the lag screws in metacarpal fractures. Similar results have also been reported by Firoozbaksh *et al.* (1996) who declared screw techniques as the weakest method of internal fixation for oblique fracture.

In group B, the skin incision showed a good healing pattern. All the skin incisions were healed by 1st intention except in dog 8 and 11 in which the dehiscence occurred due to self mutilation. After six weeks, dog 8 developed a suppurating tract due to the development of osteomyelitis. It was managed by administering Gentamicin (™Gentaflora, Selmore Korea) at dose rate of 3mg/kg on the basis of culture sensitivity test and daily dressing with polyvinylpyrrolidone and spraying with topical spray Rifaximin (™Fatroximin, ©Prix Pharmaceutica). These results were quite in agreement with the results of Bombaci *et al.* (2004) where in they found very lower complication rate while using intramedullary nails for the repair of tibial fracture.

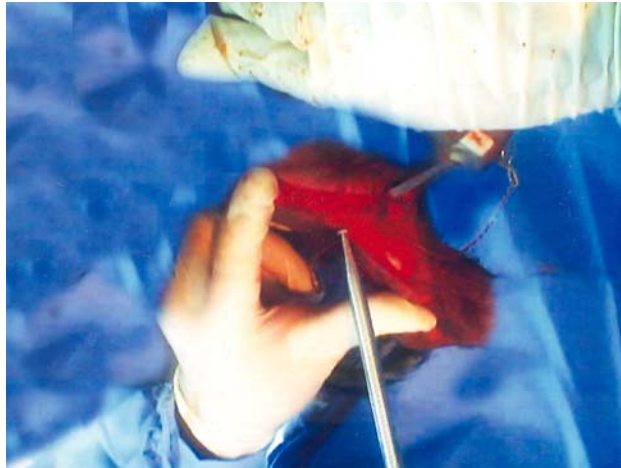
Coles and Gross (2000) in his study (in the treatment of tibial shaft fracture with plate and screw, and reamed and unreamed intramedullary pinning) found superficial infection, 9% for bone plate, 2.9% for reamed nailing and 0.5% in unreamed nailing. Mehmet and Kaya (2004) in contrast to these results found no complication at all. But the reason of controversy might be that they used cross pin fixation and that is too for the proximal fracture of tibia in dogs or they might have used more aseptic precautions than taken in the present study.

Animals of group A when tested for lameness at walk one week post-operatively and also during the following weeks, all showed marked lameness at walk, screws of this group had slipped, suture lines were ruptured due to the sharp edges of the bones and the wound inflamed, infected and some became gangrenous. These findings have also been reported by Whittick (1974), Slovenkai *et al.* (1995), Piermattei and Flo (1997) who recommended that screws should never be used as the sole method of fixation for the repair of fractures of long bones and also had opinion that some auxiliary device should always be used with screw for fracture fixation.

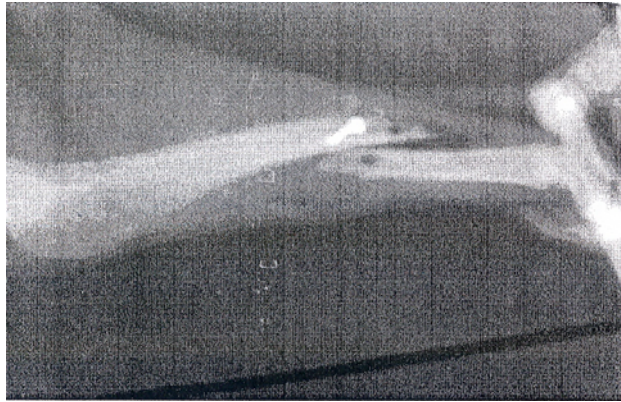
In contrast to this Matloub *et al.* (1993) recommended that single compression screw provides a satisfactory treatment of spiral fracture fixation. The reason of controversy may be due to the fact that they used screw in the spiral fracture and that too in small bones of hand, which do not bear so much weight as the shin bone has to bear. While the reason of differences with Klauze and Perren (1991) is that they used screws with plates.

All dogs in group B were found dead lame when lameness was evaluated one week post-operatively. The lameness however, improved with passage of time and after sixty days all the dogs were completely normal and they showed no lameness at all, except dog 8, who showed slight lameness. While dog 1 died due to gastroenteritis after 10 days. After the experimental period all the animals were quite normal both at walk and at trot except dog 8, who was normal at walk but showed slight lameness at trot.

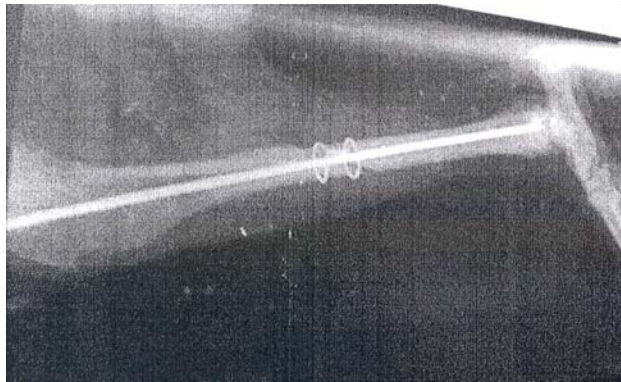
Intramedullary pinning technique with wiring used on ten tibial and femoral fractures neither



A



B



C

Fig. 1. Repair of mid shaft tibial fracture in dog; A, medial view of insertion of screw with screw driver in left limb; B, showing avulsed screw from their beds; C showing proper reduction and exact placement of implants.

showed deleterious effect on blood circulation nor on the healing process (Hinko and Rhinelander,

1975). Because of better preservation of periosteal circulation and lower complication rates, the locked intramedullary pins are more appropriate in comminuted fracture of tibial diaphysis (Bombaci, 2004).

Radiographic evaluation of the dogs in Group A revealed proper reduction of the fracture segments in immediate x-rays but displayed and over ridden segments were seen when the radiographs were taken after two weeks (Fig. 1B). These results are also documented by Bellenger *et al.* (1981) and Firoozbaksh *et al.* (1996) who reported that complication develops after the application of lag-screws and it was found to be the weakest technique.

The radiography of group B showed ideal reduction of the fracture segments and return to normal function as evidenced by callus formation and complete bone union (Fig. 1C) except in dog 8 which showed a big callus formation and delayed union.

The findings of Mehmet and Kaya (2004) are also in consistent with our finding that depending on pin size, healing period extends up to 5.5 months with no complications.

Conclusions

The analysis of the results made it clear that the intramedullary pinning with multiple full cerclage wires is better internal method of fixation for the repair of long oblique mid shaft tibial fracture in dogs than screwing alone.

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