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Comparative Toxicity of Acetamiprid and *Azadirachta indica* Leave Extract on Biochemical Components of Blood of *Labeo rohita*

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Abstract.- *Labeo rohita* was exposed to different concentrations of acetamiprid (10, 11, 12, 13, 14 and 15mg/L) and *Azadirachta indica* leaves extract (250, 500, 750, 1000and 1050mg/L) to determine their LC_{50}. The LC_{50} of *A. indica* leaves extract and acetamiprid were 785.4 and 11.62 mg/L, respectively. Fish were exposed to 20% of LC_{50} of acetamiprid and *A. indica* leaves extract for 28 days to evaluate changes in the biochemical component of fish blood. A significant decrease in serum calcium, phosphate and albumin, and a significant increase in urea was recorded when fish were exposed to acetamiprid. In contrast, no significant alterations were observed in blood parameters of fish revealed to *A. indica* leaves extract. The results, therefore, suggest that *A. indica* leaves extract can be used safely for aquatic pests since it is non-toxic to non-target organisms.

Key words: LC_{50}, biopesticide, calcium, phosphate, urea, albumin.

INTRODUCTION

Pesticides are used excessively all over the world to control plagues. In particular, the use of chemical pesticides is considered as an economic strategy for plague control; however, such synthetic substances are extremely hazardous to non-target species in the environment (Gaafar *et al.,* 2010). After their application, pesticides make their way into the various aquatic ecosystems, affecting non-target inhabitants (Moraes *et al.,* 2011) or causing ecological imbalances (Velisek *et al.,* 2012). These toxicants may cause changes in a single cell or in a whole organism; they also produces stressful conditions with consequent alterations in cells via biological magnification in aquatic animals such as fish, and ultimately cause damages for humans through the food chain (Giari *et al.,* 2008).

Acetamiprid is a chemical pesticide which is related to neonicotinoid family (Si *et al.,* 2005) and is extensively utilized in agriculture for pest management in various countries (Jiao *et al.,* 2011). Pesticides enter into aquatic ecosystems by agricultural run-off and may cause in physiological abnormalities, in aquatic organisms (Siddique and Wanule, 2010).

Many plant derived products are being used to control insects (Dubey *et al.,* 2010). In particular, *Azadirachta indica* (neem tree) leaves, are used for pest management. Azadirachtin is the most important active substance in neem plant which is mostly present in seed, leaves, and in other parts of the neem tree (Senthil *et al.,* 2008).

Labeo rohita is an edible fish and it is cultured in various farms of Indo-Pak sub-continent. This fish has significant economic value as it is a source of livelihood to numerous people (Akhtar *et al.,* 2011). The use of growth promoters and pesticides has considerable affected the production of *L. rohita* in aquaculture systems and in wild fish populations all over the world. Additionally, the species has been considered as a model for physiology/stress studies (Ficke *et al.,* 2007).

Blood parameters are considered as physiological indicators of animals undergoing stressful conditions such as the presence of toxicants, because blood acts as a pathophysio-logical reflector of the whole body (McDonald *et al.,* 1989; Velisek *et al.,* 2012). Particular ions such as calcium and phosphate are vital components for a broad range of biological activities in organisms. Calcium plays a role in cell adhesion, muscle contraction, acid-base balance and helps in

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triggering endocrine activities in vertebrates. Phosphate plays a crucial role in enzyme/protein components, membrane structure, metabolism and genetic information (Kumar *et al.*, 2011a). Urea is the foremost osmolyte in fish and plays a major role in acid-base balance (Hazon *et al.*, 2003). Albumin is the essential component in serum protein (Kovyrshina and Rudneva, 2012), it regulates blood osmotic pressure and has a role in the transportation of endogenous metabolites (bilirubin, fatty acids and hormones) and exogenous compounds (drugs) (Baker, 2002).

The present study was performed to evaluate the effect of *A. indica* leaves extract and acetamiprid on the biochemical parameters (calcium, phopshate, urea and albumin) and behaviour of the experimental fish *L. rohita*.

MATERIALS AND METHODS

Collection and acclimatization of experimental fish

Live specimens of fresh water fish (*Labeo rohita*) (weight 35±7.90 g) were collected from the ponds of University of Veterinary and Animal Sciences (UVAS) Pattoki. Fish samples were maintained in glass aquaria containing70L of tap water; the organisms were acclimatizated to laboratory environment and maintained at room temperature for 15 days. Continuous aeration was provided and fish were fed daily with a balanced diet. Average values of water quality parameters of tap water used during experimentation were temperature 18.25±1.48°C; DO 9.02±0.14 mg/L; pH 7.52 and EC (Electrical Conductivity) 342.9±0.23 µS. Fish were exposed to natural photoperiod. Dead fish and feces (if any) were removed from glass aquaria.

Pesticides

Acetamiprid was obtained from Abdullah Haseeb Group of Companies (pH 9.39), whereas *Azadiracta indica* (biopesticide) leaves were locally collected from garden and cleansed with dechlorinated tap water in order to remove dust. Washed leaves were air dried at room temperature, chopped with the help of grinder, and suspected for 48h at room temperature (Farah *et al.*, 2006). The mixture was filtered after 48 h and the filtrate was used as biopesticide.

Determination of LC_{50} values

For determination of lethal concentration (96 h LC_{50}) of acetamiprid and *A. indica* leaves extract in *L. rohita* (APHA, 2005), five acetamiprid concentrations 10, 11, 12, 13, 14mg/L and five *A. indica* leaves extract concentrations 250, 500, 750, 1000 and 1250 mg/L were used. Test was conducted in duplicates and each concentration considered 10 fish in 70 L of water; a control treatment without pesticide was performed simultaneously. Fish were deprived of food 24 h prior and during the trial. Test aquaria were examined regularly and the mortality percentage was observed at 24, 48, 72 and 96 h. Experimental data were analyzed by probit analysis method (Finey, 1971). Chi-square test exhibited that all values were appropriate at 0.05 probability levels.

Evaluation of blood parameters

For sub-lethal toxicity test, *L. rohita* were maintained in glass aquaria containing 70 L of water (10 fish per aquarium). There were one control and two experimental groups. Treatments were also performed by duplicate. Fish were fed daily *ad libitum*. In experimental groups, fish were exposed to 20% LC_{50} at 96 h of acetamiprid (2.2 mg/L) and *A. indica* leaves extract (150 mg/L) for twenty eight days. Temperature, dissolved oxygen (DO), pH and electrical conductivity (EC) were monitored weekly. Media (water) was replaced on alternate days and to renew the media, continuous aeration was provided with air pumps (Qayyum *et al.*, 2005).

Blood collection and analysis of blood components

Blood of fish was collected directly from the fish's heart with non-heparinized syringes at 7^{th}, 14^{th}, 21^{st} and 28^{th} days of exposure from both control and experimental groups. Blood samples were centrifuged at 3000 rpm for 15min using a microcentrifuge (Model: Scan speed mini, 2010, Denmark) (Monteiro *et al.*, 2006; Moraes *et al.*, 2011). Serum was used to analyze the biochemical parameters (calcium, phosphate, albumin and urea).

Analysis of biochemical parameters

Calcium (Analyticon Fluitest CA CPC, Germany), phosphate (Fluitest Phos, Germany), urea (Crescent diagnostics catCS612, Saudi Arabia) and albumin (Crescent diagnostics, catCS60, Saudi Arabia) were analyzed by using commercial kits following the manufacturers specifications. Blanks, standards and samples were prepared for biochemical analyses and a spectrophotometer was used to measure the absorbance (Model: spectro-scan 80D UV-VIS Spectrophotometer).

Statistical analysis

SPSS software 16 was used to analyze the data. The entire data were expressed as means ±S.E. One way ANOVA (Analysis of Variance) was used to find out the significances between different multiple groups. Results of Tukey Duncan post hoc test showed that significance level was 0.05.

RESULTS

LC_{50} value of *A. indica* leaves extract (785.4 mg/L) was 67.59 fold-times higher than acetamiprid (11.62 mg/L). Results of biochemical parameters of the control and experimental fish (*L. rohita)* demonstrated that acetamiprid significantly (*p*<0.001) enhanced the serum urea level but lowered serum calcium, phosphate and albumin levels compared to the control group. *A. indica* leaves extract did not result in any significant changes (*p*>0.05) in biochemical blood parameters of *L. rohita* (Fig.1).

Fish exposed to acetamiprid registered significant decreases (p<0.05) in serum calcium, phosphate and albumin concentration at all sampling dates compared to the control, but the most drastic diminution occurred at 28th day of exposure to the synthetic pesticide, in which calcium levels decreased by 84%, phosphate level decreased by 90.6%, albumin level decreased by 84.3% compared to the control (Fig. 1). Fish exposed to acetamiprid registered a significant increase (p<0.05) in serum urea level and a 56% increase occurred at 28th day of exposure. No significant differences for calcium, phosphate, urea and albumin levels were detected among fish treated with *A. indica* and the control at any of the sampling dates.

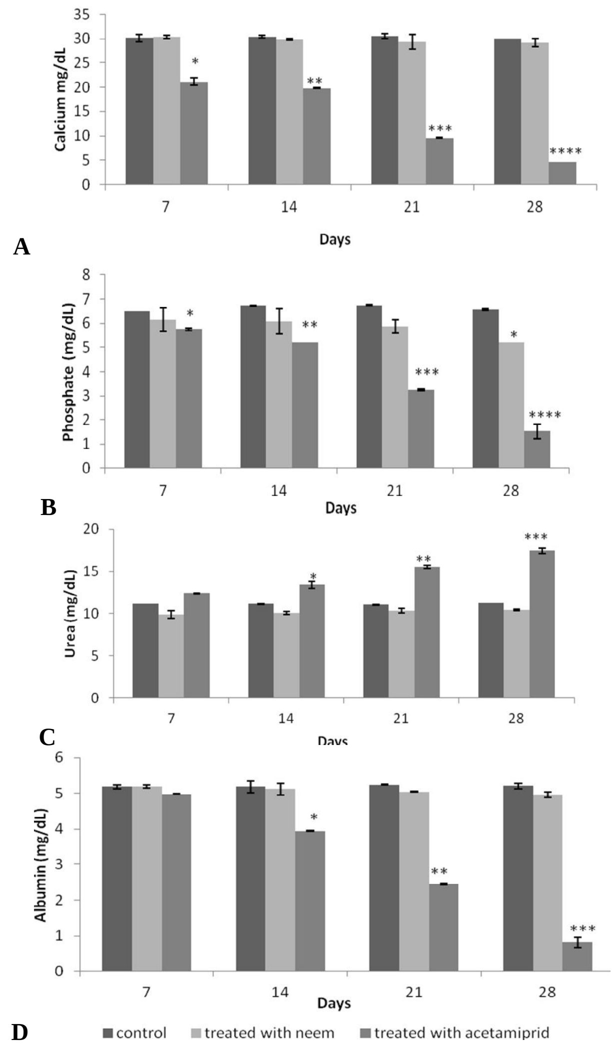

Fig. 1. Effect of acetamiprid (1 mg/dl) and *Azadirachta indica* leaves extract ((1 mg/dl) on serum calcium (A), phosphate (B), urea (C) and albumin (D) level in blood of *Labeo rohita.* Values are mean ± SE. Asterisk indicates significant differences (P < 0.05) from control. * p<0.05, **p<0.01, *** p<0.001, **** p<0.0001.

DISCUSSION

Biopesticides are gaining momentum as they are environment friendly and can replace some of the chemical pesticides (Israel *et al.*, 2008). Biopesticides are not much harmful to the environment (Ghimerya *et al.*, 2009). In the present study, 96 h LC_{50} value of *A. indica* leaves extract was higher than acetamiprid. This illustrated that biopesticides are less hazardous to non-target organisms as compared to the chemical pesticide. Similarly, Israel *et al.* (2008) evaluated 96 h LC_{50} of *A. indica* based pesticide azadirachtin for *Poecilia reticulate* and found that LC_{50} value has higher than that of the chemical pesticide deltamethrin.

The estimation of biochemical parameters in fish has become an imperative approach to understand normal and pathological activities of aquatic organisms as well as toxicological impacts (Sudova *et al.,* 2009). Pesticides have potential to induce oxidative stress in fish (Monteiro *et al.*, 2006). Siddiqui and Wanule (2010) illustrated that exposure of acetamiprid increased the leucocyte concentration of *Channa punctatus,* which induced further tissue damage. Bhatekar and Dhande (2000) explained that exposure of furdan (chemical pesticide) caused alterations in hematology of *L. rohita.*

In the present study, acetamiprid decreased serum calcium contentwhich caused a subsequent hypocalcemia in *L. rohita,* while *A. indica* leaves extract did not cause significant changes. Earlier studies have also demonstrated the decrease in calcium value of fish exposed to cypermethrin (Mishra *et al.,* 2005) and deltamethrin (Srivastav *et al.,* 2010). Velisek *et al.* (2012) also illustrated decline in calcium level in common carp which is exposed to simazine and decrease in calcium cause hypocalcemia in fish. Hypocalcemia has been noticed in other species exposed to high concentrations of pesticides such as *Clarias batrachus*, *Heteropneustes fossilis* and *Oreochromis mossambicus* (Singh and Srivastava, 1998). Calcium plays a vital role in blood coagulation and regulates the permeability of the cell membrane to inorganic ions and water. It also plays a role in the development of action potential in nerves and muscles and also maintained the membrane potential (Kumar *et al.,* 2011b). Pesticides such as organophosphates, carbamates and neonicotinoids inhibit the enzyme acetylcholinestrase that causes skeletal muscle paralysis and death from asphyxiation in the organisms exposed to these insecticides (Singh *et al.,* 1996). Thus, the decrease in calcium in *L. rohita* exposed to the chemical pesticide could be evidence of disturbance in the physiological function of fish.

In the present study, acetamiprid significantly decreased the serum phosphate in *L. rohita,* causing hypophosphatemia. This represents the toxic effects of acetamiprid to *L. rohita.* In contrast, there was non-significant decline in phosphate value of *L. rohita* exposed to *A. indica* leaves. The inorganic phosphate is the main source of energy exchange and it plays a crucial role as a chief cytoplasmic buffer. Both hypo and hyperphosphatemia have been observed in fish treated with several chemical pesticides (Singh and Srivastava, 1998) signifying that pesticides are lipophilic in nature and they access easily through the membrane of mitochondria and restrain the enzymes occupied in electron transport chain which affects the phosphorylating capability of mitochondria.

The chemical pesticide caused hypophosphatemia and hypocalcaemia in catfish, affecting the homeostasis of calcium and phosphate. Calcium and phosphate are the importations for the vitellogenin production, which is a lipophosphoprotein mainly documented in females; thus chemical pesticides severely affect the fish's reproductive physiology (Srivastav *et al.,* 1997).

The significant urea increase in fish treated with acetamiprid, suggests that the pesticide may have caused physiological damage in kidney tissue. Patnaik (2010) reported a considerable increase in urea level of *Clarias batrachus* exposed to sevin. Gaafar (2010) illustrated that increase in urea level affects the kidney tissue with the chronic treatment of ediphenphos in Nile tilapia. Organs such as intestine, gill and kidney are primarily responsible to regulate the water and ionic movement between external and internal environment of fish and these organs are greatly vulnerable to poisonous effects of pesticides (Hazon *et al.,* 2003).

The significant albumin decrease in fish treated with acetamiprid, suggests that the pesticide may have cause renal disease and liver insufficiency. Prusty (2011) also observed the decrease in albumin level of *L. rohita* which is treated with chemical pesticide fenvalerate and noticed that under stress situation, numerous animals mobilize protein as an energy source during the oxidation process of amino acids. Patnaik (2010) also noticed significant decreases in albumin levels and protein levels, probably because of tissue repair, increased energy cost of homeostasis, impaired food intake and detoxification mechanisms during stress. The other reasons for the decrease of protein and albumin level are the liver cirrhosis or kidney nephritis and inhibition of protein synthesis. Velisek *et al.* (2012) described significant decrease in albumin after the exposure of simazine in common carp. The decline in albumin content signifies that chemical pesticide could have caused malnutrition, renal disease, liver inefficiency and may induce harmful impacts on immune system. The pesticide caused stress in fish which degrades the protein. Non-significant decrease in fish treated with *A. indica* leaves extract, proves that biopesticide is safe for non-target organisms.

In the present study, analysis of biochemical parameters such as calcium, phosphate, urea and albumin revealed significant difference in fish treated with acetamiprid as compared to control and fish treated with *A. indica* leaves extract had non-significant changes alterations in its blood parameters and behavior. This suggests that biopesticides are less hazardous and safer to environment as compared to toxic chemical pesticides (Ansari and Ahmad, 2010).

In the present study, fish *L. rohita* exposed to acetamiprid exhibited extreme behavioral changes. Fish displayed stress throughout the experiment and attempted to escape the aquaria. Irregular, rapid movement and loss of balance of fish was observed throughout the experiment. Tiwari *et al.* (2012) noticed similar behavioral alterations in *L. rohita* treated with cypermethrin. Velisek, (2008) also observed similar behavioral alterations such as movement loss and increased rate of respiration in rainbow trout and common carp exposed to metribuzin. Fish treated with *A. indica* leaves extract were exempted from such stressful situation. Fish moved in groups in aquaria and sometimes they reached to water surface to consume air.

Comparison of toxicity potential of chemical and biopesticide shows that *A. indica* leaves extract can be utilized as eco-friendly plant based pesticide to restrain pests on crop fields as an alternative of detrimental and harmful chemical pesticides. Acetamiprid in contrast, should be mentioned in list of toxic pesticide as it brings changes in biochemical parameters such as calcium, phosphate, albumin and urea of aquatic ecosystem inhabitants such as fish.

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REFERENCES

AKHTAR, M.S., PAL, A.K., SAHU, N.P., ALEXENDAR, C. AND MEENA, D.K**.,** 2011. Dietary pyridoxine enhances thermal tolerance of *Labeorohita*(Hamilton) fingerlings reared under endosulfan stress. *J. Thermal. Biol.*, **36:**84–88.

ANSARI, B.A. AND AHMAD, M.K., 2010. Toxicity of synthetic pyrethroid lambda-cyhalothrin and neem based pesticide neem gold on zebra fish *Daniorerio* (Cyprinidae). *Global. J. environ. Res.*, **4:**151-154.

APHA, 2005. *Standard methods for the examination of water and waste water* (19 ed.). American Public Health Association, American Water Works Association and Water Pollution Control Federation, New York, USA.

BAKER, M.E., 2002. Albumin, steroid hormones and the origin of vertebrates. *J. Endocrinol.*, **175:** 121-127.

BAHATAKAR, N.V. AND DHANDE, R.R., 2000. Furadon induced hematological changes in the freshwater fish *Labeo rohita*. *J. Ecotoxicol. environ. Monitor.*, **10:** 193–196.

DUBEY, N.K., SHUKLA, R., KUMAR, A., SINGH, P. AND PRAKASH, B., 2010. Prospects of botanical pesticides in sustainable agriculture. *Curr. Sci.*, **98:**479–480.

FARAH, M.A., ATEEQ, B. AND AHMAD, W**.,** 2006. Antimutagenic effect of neem leaves extract in freshwater fish, *Channa punctatus* evaluated by cytogenetic tests. *Sci. Total Environ.*, **364**: 200-214.

FICKE, A. D., MYRICK, C. A. AND HANSEN, L. J., 2007. Potential impacts of global climate change on freshwater fisheries. *Rev. Fish Biol. Fish.*, **17:** 581-613.

FINNEY, P.J., 1971. *Probit analysis*, 3rd edition, Cambridge University Press.333-337.

GAAFAR, A.Y., MANAKHLY, E.M., SOLIMAN, M.K., SOUFY, H., MONA, S.Z., SAFINAZ, G.M. AND SHAHENAZ, Ms 2010.Some pathological, biochemical and hematological investigations on Nile tilapia (*Oreochromis niloticus*) following chronic exposure to edifenphos pesticide. *J. Am. Sci.*, **6:** 542-551.

GHIMERAY, A.K., JIN, C., GHIMIRE, B.K. AND CHO, D.H**.,** 2009. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica*, a Jussgrown in foothills of Nepal. *African J. Biol.*, **8:**3084-3091.

GIARI, L., SIMONI, E., MONERA, M. AND DEZFULI, B.S., 2008. Hysto-cytological responses of *Dicentrarchus labrax* (L.) following mercury exposure. *Ecotoxicol. environ. Safe.,* **70:**400–410.

HAZON, N., WELLS, A., PILLANS, R.D., GOOD, J.P., ANDERSON, W.G. AND FRANKLIN, C.E., 2003. Urea based osmoregulation and endocrine control in elasmobranch fish with special reference to euryhalinity. *Comp. Biochem. Physiol.,* **36:**685–700.

ISRAEL, S.S., KIRUBA, S., SAM, S. AND MANOHAR, D., 2008. A comparative study on the toxicity of a synthetic pyrethroid, deltamethrin and a neem based pesticide, Azadirachtin to *Poecilia reticulate* Peters 1859 (Cyprinodonti formes: Poeciliidae). *Turk. J. Fish. aquat. Sci.,* **8:**1-5.

JIAO, Z., Y, W., YANG, Z. H., MENG-XUE, L., WEN-HAO, L., KAI-GE, M., ZHONG, W.X. AND JIA-HUA, Z., 2011. Oxidative stress: role in acetamiprid-induced impairment of the male mice reproductive system. *Agric. Sci. China*, **10:**786–796.

KOVYRSHINA, T.B. AND RUDNEVA, I.I., 2012.Comparative study of serum albumin levels in round goby *Neogobius melanostomus* from black sea and Azov Sea. *Int. J. Advan. Biol. Res.,* **2:**203–208.

KUMAR, A., SHARMA, B. AND PANDEY, R.S., 2011a.Cypermethrin induced alterations in nitrogen metabolism in freshwater fishes. *Chemosphere,* **83:**492–501.

KUMAR, A., MANIRAM, P., DIWAKAR, M., SUNIL, K.S. AND AJAI, K.S., 2011b. Botanical pesticide, azadirachtin attenuate blood electrolytes of a freshwater catfish *Heteropneustes fossilis*. *Pestic. Biochem. Physiol.,* **99:**170-173.

MCDONALD, D.G., READER, J.P. AND DALZIEL, T.R.K., 1989.The combined effects of pH and trace metals on fish ionregulation. *Acid. Toxic. Aquat. Anim.,* **4:** 2212–2241.

MISHRA, D., SUNIL, K.S. AND AJAI, K.S., 2005. Effects of the insecticide cypermethrin on plasma calcium and ultimobranchial gland of a teleost, *Heteropneustes fossilis*. *Ecotoxicol. environ. Safe.,* **60:**193–197.

MONTEIRO, D.A., ALMEIDA, J.A., RANTIN, F.T. AND KALININ, A.L., 2006. Oxidative stress biomarkers in the freshwater characid fish, *Bryconcephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). *Comp. Biochem. Physiol.,* **143:** 141–149.

MORAES, B.S., CLASEN, B., LORO, V.L., PRETTO, A., TONI, C., AVILA, L.A., MARCHESAN, E., MACHADO, S.L.O., ZANELLA, R. AND REIMCHE, G.B., 2011. Toxicological responses of *Cyprinus carpio* after exposure to a commercial herbicide containing imazethapyr and imazapic. *Ecotoxicol. environ. Safe.,* **74:**3283–35.

PATNAIK, L., 2010. Biochemical alterations induced by sevin in *Clarias batrachus*. *Asian J. exp. biol. Sci.,* **1:**124-127.

PRUSTY, A.K., KOHLI, M.P.S., SAHU, N.P., PAL, A.K., SAHARAN, N., MOHAPATRA, S. AND GUPTA, S.K., 2011. Effect of short term exposure of fenvalerate on biochemical hematological responses in *Labeo rohita* (Hamilton) fingerlings. *Pestic. Biochem. Physiol.,* **100:**124-129.

SENTHIL, N.S., CHOI, Y.M., YUL, S.H., PAIK, H.C., KALAIVANI, K. AND DUK, K.**J.,** 2008. Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown plant hopper *Nilapar vatalugens* (Stal). *Ecotoxicol. environ. Safe.,* **70:** 2442–2450.

SI, S.S., LIU, X.M. AND WU, R.F., 2005. Efficacy of several insecticides on the control of *myzuspersicae* and *Lipaphiserysimi*. *Pestic. Sci. Admin.,* **26:**121–7.

SIDDIQUE, M.S. AND WANULE, D.D., 2010.Acetamiprid induced changes in total leucocytes and erythrocytes count in a freshwater fish, *Channa punctatus* (Bloch). *Int. Quart. J. Life. Sci.,***5:**163–164.

SINGH, N.N. AND SRIVASTAVA, A.K., 1998. Formothion induced biochemical changes in blood and tissues of freshwater catfish, *Heteropneustes fossilis*. *J. appl. Biol.,* **27:**39-43.

SINGH, N.N., DAS, V.K. AND SINGH, S., 1996. Effect of aldrin on carbohydrate, protein, and ionic metabolism of a freshwater catfish, *Heteropneustes fossilis*. *Bull. environ. Contam. Toxicol.,* **57:**204-210.

SRIVASTAV, A.K., SRIVASTAVA, S.K. AND SRIVASTAV, S.K., 1997. Impact of deltamethrin on serum calcium and inorganic phosphate of freshwater catfish, *Heteropneustes fossilis*. *Bull. environ. Contamin. Toxicol.,* **59:**841-846.

SRIVASTAV, A.K., SRIVASTAVA, S.K., MISHRA, D. AND SRIVASRTAV, S.K., 2010. Deltamethrin induced alterations in serum calcium and prolactin cells of a freshwater teleost, *Heteropneustes fossilis*. *Environ. Toxicol. Chem.,* **92:**1857–1864.

SUDOVA, E.K., PIACKOVA, V., KROUPOVA, H., PIJACEK, M. AND SVOBODOV, Z., 2009.The effect of praziquantel applied on selected hematological and biochemical indices in common carp (*Cyprinus carpio* L.). *Fish Physiol. Biochem.,* **35:**599-605.

TIWARI, S., TIWARI, R. AND SINGH, R.A., 2012. Impact of cypermethrin on fingerlings of common edible carp *(Labeo rohita)*. *J. scient. World.,* **12:** 1-7.

VELISEK, J., STARA, A., MACHOVA, J. AND SVOBODOVA, Z., 2012. Effects of long-term exposure to simazine in real concentrations on common carp (*Cyprinus carpio*). *Ecotoxicol. environ. Safe.,* **76:** 79–86.

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