Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
e-ISSN: 2395-3454

Vol. 16 | No. 4 | pp. 1189-1194 | 2017

Original Research Paper | Open Access

Alterations in Haematological and Biochemical Profile of Freshwater Fish, *Cirrhinus mrigala* (Hamilton) Exposed to Sub-lethal Concentrations of Chlorpyrifos

Anita Bhatnagar†, Navneet Cheema and Abhay Singh Yadav
Department of Zoology, Kurukshetra University, Kurukshetra, 136 119, India
†Corresponding author: Anita Bhatnagar

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 05-03-2017
Accepted: 05-05-2017

Key Words:
Chlorpyriphos
Dose dependent response
Haematological parameters
Cirrhinus mrigala
Toxic effects

ABSTRACT

Pesticide pollution in aquatic ecosystems is an environmental issue throughout the world. Chlorpyrifos is the most widely used organophosphate pesticide against pests in agricultural farms, orchards of fruit trees and household purposes. Herein, we investigated the impact of sublethal toxicity of this organophosphate on some haematological and biochemical parameters of freshwater fish *Cirrhinus mrigala*. The acute median lethal concentration (LC_{50}) value of chlorpyrifos calculated by probit analysis was found to be 0.44 mg L^{-1}. On the basis of LC_{50} value, the fingerlings were exposed to three sublethal concentrations (1/20th, 1/10th, 1/5th) of chlorpyrifos. During the prolonged treatment, haematological parameters including RBC, Hb and Hct values were found to decrease, whereas WBC count was found to increase in pesticide treated fishes. Similarly, indices like MCH and MCHC were significantly lower and MCV was significantly higher when compared to control. In the biochemical study, values of plasma glucose showed a gradual increase, whereas plasma protein levels, albumin and globulin showed a gradual decrease with increase in dose of chlorpyrifos at the end of experimental period. Results of the study clearly revealed that chlorpyrifos can disrupt the haematological and biochemical parameters of *Cirrhinus mrigala* and these can be used as non specific biomarkers in pesticide contaminated aquatic ecosystems.

INTRODUCTION

Organophosphorus pesticides (OP) have fully replaced the persistent chlorinated pesticides in 1970's and in the beginning of 1980's. The main advantage of OP pesticides is their low cumulative ability and short term persistence in the environment (Banaee et al. 2008). Chlorpyrifos (O, O-diethyl O-3, 5, 6-trichlor-2-pyridyl phosphorthioate) is a wide ranging organophosphate pesticide used to control the agricultural and household pests (Gluszak et al. 2006) and is commonly reported as contaminant of surface waters and groundwater in many countries. There are various ways by which chlorpyrifos can be distributed throughout the aquatic ecosystems. In fact, runoff from agricultural farms and irrigation waters are the main cause of entry of these contaminants into aquatic environment where they worsen the quality of water and alter the chemical composition of aquatic environment and can affect the freshwater fauna, particularly fish (Wild 1975). The chlorpyrifos can be absorbed through gills, skin and digestive system of fish and distributed in blood and different tissues via blood. Due to lipophilic property of this pesticide, it accumulates mainly in the fatty tissue. Chlorpyrifos is known to inhibit the acetylcholine esterase (AchE) enzyme and affects the nervous system of aquatic organisms thus influencing their physiology and also interact with the DNA (Banaee et al. 2013, Gluszak et al. 2006, Snieszko 1960, Svoboda 2001). The exposure of fish to various chemicals may induce changes in these haematological parameters. So, haematology has been widely used for detection of physiopathological alteration following different stress conditions (Harff 1983, Svoboda et al. 2001).

Various authors have reported toxic effects of various organophosphate pesticides on growth parameters, biochemical, haematological and physiological achievements of different fish species. However, the current status of toxicological effects of chlorpyrifos on *Cirrhinus mrigala*, one of the most important cultured fish species with high economical value, are meagre to reach any conclusion. Thus, the purpose of the present study was to investigate the effect of sub-lethal chlorpyrifos concentration on haematological and biochemical parameters of *Cirrhinus mrigala*. This study would also supplement the current knowledge on pesticide toxicity and will help in the effective management of freshwater systems with respect to the input of chlorpyrifos from agricultural fields.

MATERIALS AND METHODS

Collection and maintenance of experimental fish: Healthy fish *Cirrhinus mrigala* (weight 15.1±0.3 g and 9.5±0.7 cm)

were brought from a local fish farm near Kurukshetra, India and acclimatized at 28°C in large sized plastic tubs disinfected with potassium permanganate and washed thoroughly to prevent the fungal infection for 20 days prior to experimentation. During laboratory conditions, fish were fed with diet containing 40% protein (dietary ingredients (g kg^{-1}): groundnut oil cake: 650 g, rice bran: 42 g, processed soyabean: 276 g, wheat flour 32 g, mineral mixture: 10 g) @ 4% BW in two instalments a day. Tap water free from chlorine was renewed every day with these physicochemical characteristics (temperature 32±0.02°C, pH 7.3±0.33, DO 5.5±0.33 mg/L).

Pesticide: The pesticide for the study, technical-grade chlorpyrifos (50% EC), CAS No. 2921-88-2.1 with product name Classic Super (manufactured by M/s Cheminova, India Ltd., Gujarat) was purchased from the local market and used for further experimentation.

Preparation of stock solution and determination of LC_{50} value of chlorpyrifos: A stock solution of chlorpyrifos was prepared by dissolving 1 mL of chlorpyrifos (50%) in appropriate 1 L of normal tap water. Fish were randomly selected from stock and exposed to different concentrations of chlorpyrifos (0.10 mg L^{-1}, 0.20 mg L^{-1} and 0.30 mg L^{-1}) in different 50 L plastic tubs. Fifteen fish were introduced into each aquarium and water was replaced daily with fresh chlorpyrifos mixed water to maintain a constant level of chlorpyrifos during the exposure period. For each concentration, three replicates were maintained. A control was also maintained in three different aquaria under identical conditions. The mortality of fish was recorded at the end of 24, 48, 72 and 96 h concentration at which 50% mortality was taken as a median lethal concentration. The experiment was repeated thrice to obtain the LC_{50}- 96 h value of the test chemical for the fish. The concentration at 96 h causing 50% of death was calculated by probit analysis (Finney 1952) in SPSS Software version 11.5. The LC_{50}- 96 h value of chlorpyrifos was determined as 0.44 mg L^{-1} for *Cirrhinus mrigala*.

Chronic toxicity tests: To determine the prolonged effect of this pesticide, three sublethal doses were selected i.e., 1/20th of LC_{50} value (0.02 mg L^{-1}), 1/10^{th} of LC_{50} value (0.04 mg L^{-1}) and 1/5^{th} of LC_{50} value (0.08 mg L^{-1}) along with the control (no pesticide). One hundred eighty fingerlings were randomly selected from the stock and divided into 4 groups (TC, T1, T2 and T3). The experiment was conducted for 90 days. All the treatments were maintained at a constant concentration of toxicant after renewal of the same volume of water. No mortality was observed during the experimental period.

Collection of blood sample: The experiments and handling of the test organisms were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Blood from control as well as treated fish was drawn from caudal puncture using disposable plastic syringe fitted with 26 gauge needle, earlier moistured with heparin, (beparin, heparin sodium, IP 2000 IU mL^{-1}, derived from beef intestinal mucosa containing 0.15% W/V cholesterol IP preservative) an anticoagulant manufactured by Biological E Limited, Hyderabad, India. Pooled blood samples were transferred in heparinized vials and placed on ice. The whole blood was used to estimate the RBC, WBC and Hb. Remaining blood samples were centrifuged for 30 minutes at 10,000 rpm and plasma was separated for estimation of total glucose and protein.

Heamatological analysis: The haemocytometer (Neubauer's counting chamber) was used for total erythrocyte count and total leucocyte count following the method of Dacie & Lewis (1971). Haemoglobin (Hb) concentration was estimated by Sahil's method. Haematocrit (Hct) was determined by microhaematocrit method. Other erythrocyte indices like MCV, MCH and MCHC were calculated using standard formulae.

Mean Corpuscular Volume (MCV)

$$= \frac{\text{Haematocrit (\%)} \times 10}{\text{RBC count in millions}} \quad ...(1)$$

Mean Corpuscular Haemoglobin (MCH)

$$= \frac{\text{Haemaglobin (gdL} - 1) \times 10}{\text{RBC count in millions}} \quad ...(2)$$

Mean Corpuscular Haemoglobin Concentration (MCHC)

$$= \frac{\text{Haemaglobin (gdL} - 1) \times 10}{\text{Haematocrit(\%)}} \quad ...(3)$$

Biochemical analysis: Plasma glucose and protein were analysed following the method of Henry et al. (1974) and Lowry et al. (1951) respectively. Albumin estimation was done according to bromocresol method (Gordon et al. 1967) and globulin content was calculated using standard formulae:

Globulin = Total protein - Albumin

Statistical analysis: Significant differences among treatment groups were tested by analysis of variance (ANOVA) followed by Duncan's multiple range tests for the experiments. Statistical significance was settled at a probability value of $P<0.05$. All statistics were performed using SPSS version 11.5 for Windows.

RESULTS

Sublethal Chronic Tests

Effects of chlorpyrifos on haematological parameters: The calculated LC_{50} value of chlorpyrifos of *Cirrhinus mrigala* fingerlings was 0.44 mg L^{-1}. The selected sublethal concentrations for toxicity testing of chlorpyrifos were equivalent to approximately $1/20^{th}$, $1/10^{th}$, $1/5^{th}$ of 96 h LC_{50} value. Physico-chemical characterization of the water was carried out according to the standard methods. The values of various water quality parameters were as pH 7.5±0.05, alkalinity 43.4±0.77 mg L^{-1}, hardness 350±1.15 mg L^{-1}, chloride 37.1±0.7 mg L^{-1} and calcium 26.21±0.26 mg L^{-1}. No mortality was recorded in fish exposed to sublethal concentrations of chlorpyrifos and control group during the experimental periods. Certain behavioural changes as lethargy, loss in balance, irregular opercular movement, loss in appetite, increased mucus secretion, swimming at surface of water and swimming vertically were observed in fish exposed to higher doses of pesticide. The changes in the profile of haematological parameters are presented in Table 1.

During the sublethal treatments, values of RBC/TEC, Hb and Hct values showed a significant decrease ($P<0.05$) from the control (TC) to treatment T3 registering a direct relation with concentration, whereas significant ($P<0.05$) increase in WBC count was observed in fish from treatment TC to treatment T3. Other haematological indices like MCH and MCHC also showed significant ($P<0.05$) decrease with increase in dose of chlorpyrifos, whereas no significant difference in value of MCV was observed between treatment TC, T1 and T2, T3; however, values were found to increase but no clear trend was observed.

Effects of chlorpyrifos on biochemical parameters: Variations in biochemical parameters such as glucose, total protein, albumin and globulin levels are given in Table 2. Glucose (mg 100 mL^{-1}) is an important diagnostic tool of carbohydrate related disorder as it is reliable endocrine and physiological indicator of relative severity of stress. It is evidently clear from the data (Table 2) that there is significant ($P<0.05$) concentration dependent response showing maximum value in treatment T3 (in which fishes were exposed to 0.08 mg L^{-1} of chlorpyrifos) and minimum in control TC. Level of total serum protein content (μg mL^{-1}) was also affected by the prolonged exposure to pesticide. The values clearly revealed significant ($P<0.05$) decrease in all treated groups (T1, T2 and T3) as compared to control (TC). Also, the mean values of total albumin showed significant ($P<0.05$) decrease as the concentration of chlorpyrifos increased from treatment T1 to T3. Similar trend was observed in globulin content.

DISCUSSION

Haematological characteristics have been considered as tools for screening pathological stress. These constitute a good indicator of physiological responses (Blaxhell 1972) because they provide information about internal environment of organisms (Lazzari et al. 2006). The clinical symptoms in terms of behavioural responses observed during chlorpyrifos exposure of *Cirrhinus mrigala* corresponds to the toxicity of organophosphate on this fish species. The main breakdown product of chlorpyrifos in the environment and in organisms is chlorpyrifos-oxon, which disrupts AChE activity (Chawanrat 2007, Oruc 2010) and hence the negative effects on the functioning of locomotory, respiratory and digestive systems of fish. Similar types of behavioural alterations were also studied by various authors on different fish species exposed to different organophosphates (Dutta 1995, Dutta & Choudhary 1996, Venkata & Nagaraju 2014, Jindal & Kaur 2015). In the present study, exposure of sublethal concentration of chlorpyrifos in fingerlings of *Cirrhinus mrigala* showed remarkable alteration in haematological and biochemical parameters. There was a significant ($P<0.05$) decrease in red blood cell count, haemoglobin (Hb) content and packed cell volume (PCV) or the haematocrit (Hct) whereas, a significant increasing trend in WBC was observed with each increase in the dose of pesticide. Other red blood cell indices such as mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) also showed appreciable decrease whereas mean corpuscular volume (MCV) in exposed fish has been observed to show increasing trend when compared with the control.

The total RBC count, haemoglobin (Hb) and haematocrit (Hct) showed a decreasing trend with increasing concentration and exposure time of chlorpyrifos which might be attributed to the decreased erythropoietic activity. In most vertebrates, including fishes, erythropoietic activity is regulated by erythropoietin produced in the kidney (Gluszak et al. 2006). This erythropoietin further promotes erythropoiesis by inducing haemopoietic stem cells to differentiate into erythroblasts (which form RBCs). Erythropoietin also activates pyridoxal phosphate in development of RBCs, inducing haemoglobin synthesis (Oruc 2010). Some histopathological observations have also revealed progressive dystrophic changes in kidney tubules when exposed to organophosphates. Kidney damages cause a decrease in erythropoietin level, which in turn decreases RBC production and Hb synthesis under hypoxic condition. The decrease in all these parameters may be indicator of anaemia that might be due to destructive action of organophosphate on cell membrane. Also, decline in RBC count, Hb concentration and haematocrit value presumably reflected erythrocyte haemolysis and/or irreparable damage of kidney functions. The decrease in Hb concentration might be

Table 1: Changes in haematological parameters of chlorpyrifos exposed to fishes in comparison to control.

Haematological Parameters	Chlorpyrifos Treatments			
	TC(control)	T1(0.02 mg L^{-1})	T2(0.04 mg L^{-1})	T3(0.08 mg L^{-1})
TEC (RBC) (10^6 mm^{-3})	1.99 ± 0.01^{A}	1.82 ± 0.02^{B}	1.52 ± 0.02^{C}	1.34 ± 0.01^{D}
Haematocrit value (%)	28.98 ± 0.04^{A}	26.58 ± 0.29^{B}	25.35 ± 0.19^{C}	22.64 ± 0.31^{D}
Haemoglobin value (%)	9.4 ± 0.14^{A}	7.3 ± 0.11^{B}	6.0 ± 0.05^{C}	4.86 ± 0.08^{D}
MCV (μm^3)	145.15 ± 1.25^{B}	145.84 ± 2.62^{B}	166.85 ± 2.85^{A}	169.10 ± 4.34^{A}
MCH (pg)	47.40 ± 0.55^{A}	40.03 ± 0.45^{B}	38.68 ± 1.15^{C}	36.32 ± 0.67^{D}
MCHC (%)	48.40 ± 0.77^{A}	40.03 ± 0.45^{B}	38.67 ± 1.15^{BC}	36.32 ± 0.67^{C}
TLC (10^3 mm^{-3})	13.34 ± 0.30^{D}	14.13 ± 0.14^{C}	15.55 ± 0.23^{B}	18.49 ± 0.22^{A}

All values are mean ± S.E of mean; Means with different letters in the same row are significantly (P<0.05) different; (Data were analyzed by Duncan's Multiple Range test); TEC- Total Erythrocyte Count, MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Heamoglobin, MCHC- Mean Corpuscular Haemoglobin Concentration, TLC- Total Leucocyte Count

Table 2: Changes in plasma glucose level and protein level of chlorpyrifos treated fishes in comparison to the control.

Biochemical Parameters	TC(Control)	T1(0.02mg L^{-1})	T2(0.04mg L^{-1})	T3(0.08mg L^{-1})
Blood glucose (mg 100 mL^{-1})	74.36 ± 0.32^{D}	82.47 ± 0.30^{C}	107.13 ± 1.00^{B}	143.38 ± 1.58^{A}
Total protein (µg mL^{-1})	3.28 ± 0.16^{A}	2.98 ± 0.04^{A}	2.40 ± 0.21^{B}	1.03 ± 0.04^{C}
Albumin (µg mL^{-1})	2.06 ± 0.04^{A}	1.92 ± 0.02^{B}	1.19 ± 0.02^{C}	0.66 ± 0.02^{D}
Globulin (µg mL^{-1})	1.30 ± 0.26^{A}	1.05 ± 0.05^{A}	1.15 ± 0.19^{A}	0.37 ± 0.05^{B}

All values are mean ± S.E of mean; Means with different letters in the same row are significantly (P<0.05) different; (Data were analyzed by Duncan's Multiple Range test)

due to an increase in the rate at which Hb is destroyed or a decrease in the rate of its synthesis (Ural 2013). Further, the decrease in Hct in fish exposed to pesticide was due to decrease in RBC count, which in turn might be due to the effect of pesticide on blood forming organ as explained above (Saha & Kaviraj 2009). Similar results were obtained by some authors showing effects of sub-lethal exposure of lindane on haematological and biochemical responses of freshwater teleost *Cyprinus carpio* resulting in decreasing trend in RBC, Hb and Hct values (Ural 2013). The present findings are same to earlier reports on freshwater fish *Channa punctatus* after acute exposure to diazinon (Anees 1978, Srinivasan & Radhakrishanamurthy 1988). Erythrocyte level was also found to be depressed in fishes subjected to stressful conditions. Inhibition of erythropoiesis and increase in the rate of erythrocyte destruction in haematopoietic organs are the cause of decrease in RBC count as stated by Johnson & Larsson (1978).

Further, changes in blood cell indices like MCH, MCH, MCHC and MCV were also observed in the present study. This may be due to the fact that these are very sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices directly correspond with the values of RBC count, haemoglobin concentration and packed cell volume. The present findings are similar to the earlier reports (Balini et al. 1995, Svoboda et al. 2001, Das & Mukherjee 2003).

Leucocytes are involved in the control of immunological function and the changes in the WBC counts after exposure to various toxicants may indicate a decrease in non specific immunity of the fish (Saha & Kaviraj 2009) and the significant increase in the count may be a protective response in fish under stress. In general, increased WBC count in fish exposed to the sub-lethal doses indicates leucocytosis such as heterophilia and lymphophenia, which are characteristic of leucocytic response in animals exhibiting stress (Johnson-sjobeck & Larssob 1978, Ahmad 2011). Stimulation of lymphopoiesis and/or enhanced release of lymphocytes from lymphomyeloid tissue under toxic stress may lead to an increase in WBC number (Svobodova et al. 1997). The observed increase suggests that body tried to enhance the TLCs on a protective mechanism against stress at lower concentration and tendency of the body to enhance the defence mechanism against stress.

Among the biochemical profiles, plasma glucose has been extensively used as a sensitive indicator of environmental stress in fish (Nemcsok et al. 1982), because carbohydrates are the primary and immediate source of energy. Marked significant increase in glucose concentration with increase in concentration has been seen in the present study, thus clearly demarcating the action of chlorpyrifos as the manifestation of stress to the test fish. Chronic sublethal treatments of the pesticide have increased the levels of plasma glucose due to the gluconeogenesis to provide en-

ergy for the increased metabolic demands imposed by the pesticide chlorpyrifos. Similar results were given by various other authors suggesting that glucose concentration increases as a general response of fish to acute pollutant effects, including organophosphate (Mishra 1983, Sastry 1998). Banaee et al. (2011) reported an increase in blood glucose of fish exposed to the chlorpyrifos may reflect the increased need for energy to counteract the effects of stress caused by chlorpyrifos toxicity.

The protein content, albumin and globulin in fish showed a significant ($p<0.05$) reduction on exposure to toxicant. Reduction of proteins might be due to the blocking of protein synthesis, protein denaturation or interruption in the amino acid synthesis. Also, the low protein contents in chlorpyrifos exposed fish may be due to the reduction in feed intake and further breakdown of these molecules as energy substrates to cope up with chlorpyrifos induced stress support (Cheema et al. 2014). Similar findings have been made in different fish by other workers viz., *Channa punctatus* exposed to quinalphos (Sastry et al. 1998), *Cyprinus carpio* exposed to deltamethrin (Svoboda et al. 2001) and *Oncorhynchus mykiss* exposed to bifethrin (Velisek et al. 2008). The decrease in protein level observed in freshwater *Channa marulius* exposed to the lattices of *E. royleena* and *J. gossypifolia* has been attributed to the destruction or necrosis of cells and subsequent impairment in protein synthesis (Singh & Singh 2003). Possible reason for lowered amount of albumin and globulin levels of fish exposed to chlorpyrifos may be due to the depletion in total protein level in plasma. A significant ($P<0.05$) low serum protein albumin and globulin levels in treated fish exposed to chlorpyrifos may also be attributed to the stress mediated mobilization of these compounds to fulfil an increased demand for energy by fish to cope up with the detrimental conditions exposed by the toxicant. Decreased protein levels may be seen in starvation and malabsorption and malnutrition.

CONCLUSION

From the present investigation, it can be concluded that exposure of *Cirrhinus mrigala* fingerlings to the sublethal concentrations of 96h-LC_{50} caused significant alterations in haematological and biochemical parameters. The alterations in these parameters may provide early warning signals for the determination of acute and sublethal toxic levels of pesticides used in the field and its deterioration into the nearby ponds.

ACKNOWLEDGEMENTS

The authors are grateful to The Chairman, Department of Zoology, K.U.K. for providing facilities for carrying out the work. The financial support in terms of Senior Research fellowship by University Grant Commission under the scheme of Maulana Azad National Fellowship to Navneet Cheema is highly acknowledged.

REFERENCES

Ahmad, Z. 2011. Acute toxicity and haematological changes in common carp (*Cyprinus carpio*) caused by diazonin exposure. African Journal of Biotechnology, 10: 13852-13859.

Anees, M.A. 1978. Haematological and abnormalities in a freshwater teleost, *Channa punctatus* (Bloch) exposed to sub-lethal and chronic levels of three organophosphorus insecticides. International Journal of Ecology and Environment Science, 4: 53-60.

Balini, T., Szegletes, T., Szegletes, Z., Halasy, K. and Nemcsok, J. 1995. Biochemical and subcellular changes in carp exposed to the organophosphorus methidathion and the pyrethroid deltamethrin. Aquatic Toxicology, 33: 279-295.

Banaee, M., Mirvagefei, A.R., Rafei, G.R. and Majazi-Amiri, B. 2008. Effect of sub-lethal diazonin concentrations on blood plasma biochemistry. International Journal of Environment Research and Public Health, 2: 189-198.

Banaee, M., Sureda, A., Mirvaghefi, A.R. and Ahmadi, K. 2011. Effects of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*). Pesticide Biochemistry and Physiology, 99: 1-6.

Banaee, M., Haught, B.N. and Ibrahim, A.T.A. 2013. Sublethal toxicity of chlorpyrifos on common carp, *Cyprinus carpio* (Linnaeus, 1758): biochemical response. International Journal of Aquatic Biology, 1: 281-288.

Blaxhall, P.C. 1972. The haematological assessment of the health of freshwater fish. Journal of Fish Biology, 4(4): 593-604.

Chawanrat, S., Voravit, C., Chutarat, S., William, F. and Beamish, H. 2007. Variability in acetylcholinesterase upon exposure to chlorpyrifos and carbaryl in hybrid catfish. Science Asia, 33: 301-305.

Cheema, N., Bhatnagar, A. and Yadav, A.S. 2014. Changes in growth performance and biochemical status of fresh water fish *Cirrhinus mrigala* exposed to sublethal doses of chlorpyrifos. International Journal of Agriculture and Food Science Technology, 2: 619-630.

Dacie, J.V. and Lewis, S.H. 1963. Practical Hematology. J & A Churchill Ltd., London.

Das, B.K. and Mukerjee, S.C. 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences. Comparative Biochemistry and Physiology C., 134: 109-121.

Dutta, H.M. 1995. A composite approach for evaluation of the effects of pesticides on fish. In: Fish Morphology Horizon for New Research, Dutta J.S. and Dutta H.M. (Eds.), Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, pp. 249-271.

Dutta, M.J. and Choudhary, S. 1996. Ecology of *Heteropneustes fossilis* (Bloch): Air breathing Catfish of South East Asia. Narendra Publishing House, New Delhi.

Finney, D.J. 1952. Probit Analysis. Cambridge University Press, London/New York.

Gluszak, L., Santos, M.D., Crestani, M., da Fonseca, M.B., de Araujo Pedron, F., Duarte., M.F. and Vieira, V.L.P. 2006. Effect of glyphosate herbicide on acetylcholinestrase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens)*. Ecotoxicology and Environment Safety, 6: 237-241.

Gordon, A.S., Goper, G.N. and Zaryani E.D. 1967. The kidney and erythropoiesis. Sem. Haemat., 4: 337-343.

Harff, G.A. 1983. Albumin determination with bromocresol green: Imprecision, comparison of methods and quality control. Journal of Clinical Chemistry, 21: 679-682.

Henry, R.J., Cannon, D.C. and Winkelman, J.W. 1974. Clinical Chemistry Principles and Techniques, 11th ed., Harper and Row, New York, p. 1629.

Jindal, R. and Kaur, M. 2015. Ultrastructure alterations in scales of *Ctenopharygodon idellus* (Cuvier and Valenciennes) induced by chlorpyrifos: A promising tool as bioindicators of pesticide pollution. International Journal of Fisheries and Aquatic studies, 2(3): 58-62.

Johnson-Sjobeck, M.L. and Larsson, A. 1978. The effect of cadmium on the hematology and on the activity of delta-amino leverlinic acid dehydratase (ALA-D) in blood and hematopoietic tissues of the flounder, *Platichthys flesus* (L.). Environmental Research, 21: 129-131.

Lazzari, R., Duarte, M.F., Morsch, V.M., Pippi, A.L. and Vierira, V.P. 2006. Effects of clomazone herbicide on hematological and some parameters of protein and carbohydrate metabolism of silver catfish *Rhamdia quelen*. Ecotoxicology and Environmental Safety, 65: 48-55.

Lowry, O.H., Rosenberg, N.J., Farr, A.L. and Randal, R.J. 1951. Protein measurement with folin phenol reagent. Journal of biochemistry, 193: 265-75.

Mishra, J. 1983. Malathion induced hematological and biochemical changes in the Indian catfish *Heteropneustes fossilis*. Environmental Research, 30: 393-398.

Nemcsok, J., Benedeczky, I., Boross, B., Asztalos, B. and Orban, L. 1982. In vivo effects of deltamethrin on some biochemical parameters of carp (*Cyprinus carpio* L.). Acta Biologica Szegediensis, 27: 9-15.

Oruc, E.O. 2010. Oxidative stress, steroid hormone concentrations and acetylcholinestrase activity in *Oreochromis niloticus* exposed to chlorpyrifos. Pesticide Biochemistry and Physiology, 96: 160-166.

Saha, S. and Kaviraj, A. 2009. Effects of cypermethrin on some biochemical parameters and its amelioration through supplementation of ascorbic acid in freshwater catfish *Heteropneustes fossilis*. Chemosphere, 74: 1254-1259.

Sastry, K.V., Siddiqui, A.A and Singh, S.K. 1998. Alterations in some biochemical and enzymological parameters in the snake head fish, *Channa punctatus* exposed clinically to quinalphos. Chemosphere, 11: 12-21.

Singh, D. and Singh, A. 2003. Effect of stem bark extract of some common plants on non-target fresh water fish *Channa marulius* (Ham.). Indian Journal of Fisheries, 50(4): 525-531.

Snieszko, S.F. 1960. Microhaematocrit as a tool in fishery research and management. Special Scientific Report of United States, Fish and Wildlife Serial No: 341

Srinivasan, R. and Radhakrishanamurthy, R. 1988. Effect of dietary intake of hexachlorocyclohexane isomers on some hematological parameters. Journal of Food Science and Technology, 20: 322-325.

Svoboda, M., Luskova, V., Drastichova, J. and Ziabek, V. 2001. The effect of diazonin on hematological indices of common carp (*Cyprinus carpio* L.). Acta Veterinaria Brno., 70(1): 457-465.

Svobodova, Z., Groch, M., Flajshans, M., Vyukusova, B. and Machova, J. 1997. The effect of long term therapeutic bath of malachite green on common carp *Cyprinus carpio* L. Acta Veterinaria Brno., 66: 111-117.

Ural, M.S. 2013. Chlorpyrifos-induced changes in oxidant/antioxidant status and haematological parameters of *Cyprinus carpio* ameliorative effect of lycopene. Chemosphere, 90: 2059-2064.

Velisek, J., Svobodova, Z. and Piackova, V. 2008. Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*). Veterian Medicina, 54: 131-137.

Venkata, V.R. and Nagaraju, B. 2014. Acute toxicity and histopathological changes in freshwater fish *Cirrhinus mrigala* exposed to chlorantraniliprole. Journal of Zoological Studies, 1: 23-30.

Wild, D. 1975. Mutagenicity studies on organophosphorus insecticides. Mutation Research, 32: 135-150.