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Effect of Endosulphan and Dimethoate Pesticides on Haematological Parameters of Fresh Water Fish *Channa punctatus*

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Research Article

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ABSTRACT

Fish hematology is one of the most recent branches of physiology to assess the status of general health. Blood is the transport medium, a defense system, and an acid/base buffer system. Circulating blood is the common denominator of health and illness and alteration in its chemical or cellular illness and composition can indicate hematological and non-hematological diseases. Erythrocytes occupy the largest fraction of the formed elements of the blood. For hematological observations of the fish *Channa punctatus,* blood from control and experimental fish was drawn directly from the ventricles after exposing the heart, with the help of a syringe fitted with 20 gauze needle. The blood without anti-coagulant was directly used for the total erythrocyte count (TEC), total leucocytes count (TLC) and smear preparation. The number of erythrocytes per cubic millimetre of blood was calculated with the help of a hemocytometer using a Neubaur's counting chamber. The method given by Dacie and Lewis was followed to count the number of leucocytes. Blood was drawn up to 0.5 mark of the WBC pipette and diluted up to 11 mark with Turk's fluid and the number of WBC in corner most four squares, whose area is 1 sq. mm were counted. For the preparation of blood smear standard glass slides washed in 100% ethanol were used. Blood film was made immediately after the blood was drawn from the fish. From each fish, four blood smears were prepared using Leishman's, Gimsa, Wright's and Field stains. The morphology and staining property of all types of blood corpuscles were studied. For differential leucocytes counts, at least 100 leucocytes were observed and counted for each blood smear at a time, and differentiated into various types: monocytes, lymphocytes, neutrophils, eosinophils and basophils.

Differential leucocytes count also was altered in fish exposed to endosulfan and dimethoate. Basophils are quite scanty in normal circulating blood. Basophils decreased in all the exposures, increase in eosinophils on exposure to dimethoate and decrease on exposure endosulfan for 96 hr was observed in the present findings. In the present study increase in neutrophils number was recorded upto 72.0% in fish exposed to endosulfan. In the present study both the pesticides decreased the number of basophils, eosinophils, monocytes, lymphocytes, neutrophils and RBC in *Channa punctatus* after 96 hr, except in few observations. Number of eosinophils, monocytes and lymphocytes in endosulfan exposures; only eosinophils and lymphocytes count in dimethoate exposure was increased after acute exposure.

Our health and environment have become almost synonymous today; it is equally true for all biotic organisms, and fishes are thus no exception. Fishes are one of the most primitive groups of vertebrates and their environment i.e., hydrosphere is everything for them. The impairment of behavioral and physiological functioning due to polluted ambient habitat could result in gradual reduction in the adaptation capacity of a species leading to decrease in the survival capacity and gradual fall in population

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density. Fish hematology is one of the most recent branches of physiology to assess the status of general health. Blood is the transport medium, a defense system, and an acid/base buffer system. Circulating blood is the common denominator of health and illness and alteration in its chemical or cellular illness and composition can indicate hematological and non-hematological diseases [1]. Erythrocytes occupy the largest fraction of the formed elements of the blood. In normal functioning of the body, the blood counts remain stable, but environmental as well as pathological conditions can alter the erythrocyte count. Variations in either direction outside the designated range of normal counts usually indicate erythropoietin dysfunction. Hemoglobin is the respiratory protein contained within erythrocytes. It has a multtude of functions in the circulatory system including transport of O_2 and CO_2 and its action as the most important buffer in the blood, etc. It is a representative of the globular proteins. The body has a great capacity for conservation; it relinquishes very little of those resources that may be reused. The destruction and reutilization of hemoglobin is a good example of this conservation. Lysis of erythrocytes in the blood stream is called intravascular hemolysis and when it occurs due to phagocytosis or sequestration by the reticuloendothelial system is called extravascular hemolysis [2,3].

Leucocytes (white blood cells) constitute an important part of the body's defense system. The number of white cells in the blood, compared to the number of erythrocytes is quite small. A temporary increase in the white blood cells in the peripheral circulation is called leukocytosis and decrease in number of white cells is leucopenia. There are several varieties of white blood corpuscles, each type possessing characteristic morphology and staining property [4]. Determination of the percentage of different varieties of leucocytes is known as the differential count of white blood corpuscles. White blood corpuscles or leucocytes can be divided into two main categories: granular leucocytes or granulocytes, and agranular leucocytes or agranulocytes. Granulocytes are produced in the bone marrow and are of three types: neutrophils or polymorphs, eosinophils and basophils. Agranulocytes produced in the lymph nodes and spleen are of two types: lymphocytes and monocytes [5,6].

Several attempts have been made to study the effect of number of pesticides on different parameters of the blood of fishes. Verma [7-9] showed increase in Hb, RBC, WBC and PCV but decrease in ESR and CT in Saccobranchus fossilis after intervals of 15,30,45 and 60 days of chlordane exposure. Bansal reported that exposure to different concentrations of chlorinated hydrocarbons for varying periods bring about increase in TEC, TLC, haemoglobin and packed cell volume. studied the fact of Aldrin on RBC count, hemoglobin content and hematocrit value, and white blood cells.

Goel reported anemia, decrease in RBC count, hemoglobin and hematocrit value in Heteropneustus fossilis after malathion exposure. Occurrence of necrosis of erythrocytes resulting in hypochromic microcytic anemia has been reported in Tilapia, possambica exposed to thiodon. Lindane is reported to cause increase in TEC, Hb, PCV, and TLC in Heteropneustes fossilis. Significant decrease in MCI-IC has been observed by Ciarias batrachus exposed to Rogor. Chakarabarty [10] have studied variations in blood parameters of the fish Channa punctatus exposed to organophosphorus pesticides. Exposure of Channa punctatus toendosulfan for 30 days decreased TEC, TLC, Hb and PCV [11] studied the effect of sublethal toxicity of sevin on blood parameters in Ciarias batrachus. Exposure of the carp Cyprinus carpio to malathion caused decrease in Hb and RBC and increase in leucocyte count. Srivastava and Singh [12] studied hematological changes in Heteropneustes fossilis following exposure to Aldrin. examined the effect of chlordane and malathion on certain haematological parameters of a freshwater teleost [10], Notopterus notopterus [13]. studied hematological changes in the freshwater catfish, Mystus vittatas exposed to sublethal concentration of phosphamidon.

A survey of literature revealed that several types of leucocytes present in the peripheral blood varies from species to species. classified leucocytes into lymphocytes, thrombocytes, monocytes, macrophages, neutrophils, eosinophils and basophils, in the marine teleost Gerres filamentosus, while in Elops saurus, large and small lymphocytes, large and small thrombocytes, monocytes, ~acrophages, neutrophils, eosinophils, and basophils have been recorded. Ref. [12] distinguished monocytes, large lymphocytes~ small lymphocytes, monocytes, neutrophis, polymorphs and basophils in circulating blood of Channa punctatus. Prasad and Baneriee identified leucocytes as large and small lymphocytes, monocytes, neutrophils, eosinophils and basophils in Catla catla, Labea rohita and in Cirrhhina mrigala except eosinophils, all other leucocytes were found. Banerjee et al. [3] reported large and small lymphocytes, monocytes neutrophils and eosinophils, and basophils in Mystus vittattus. classified seven types of leucocytes namely small and large lymphocytes, monocytes, neutrophils, eosinophils, basophils and thrombocytes in Anabas testudineus. studied the toxicity of sublethal concentration of some heavy metal salts on hematology of Channa punctatus.

MATERIALS AND METHODS

Exposure of Test Fish

Healthy living specimens of the fresh water teleost fish *Channa punctatus* collected from the local ponds of Rohtak or purchased from fish market were quickly transported to laboratory. The fish were maintained in glass aquaria and fed twice daily with pelleted diet (prawn powder, fish powder, and minced liver in 2:2:1 ratio. The water in aquaria was continuously aerated in order to maintain the dissolved oxygen concentration above 7 ppm.

Individual Exposure

Eighty fishes divided into 4 groups of 20 each were exposed to LC_{50} of zinc (18.62 mg/l), copper (0.56 mg/l) and cadmium (11.8 mg/l) separately. The fourth group of 20 fishes was kept in metal free water served as control for each experimental group. After 96 hr from each of the experimental and control groups 4 surviving fishes were dissected and blood was drawn.

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Group Exposure

Sixty fishes were divided into 3 groups of 20 fishes each and were exposed to LC_{50} of zinc (18.62 mg/l), copper (0.56 mg/l) and cadmium (11.8 mg/l) separately. After 96 hr from each of the experimental and control groups blood was collected from surviving fishes.

Preparation of Fish for Blood Sampling

For collection of blood, a fish was taken out from aquarium with the help of a hand net and immediately immersed in 2% paraldehyde solution. The fish was taken out from this solution when the opercular movements ceased, but movement of the gills still persisted. Immediately the fish was soaked dry with clean towel or filter paper.

Blood for Haematological Studies

For hematological observations of the fish *Channa punctatus,* blood from control and experimental fish was drawn directly from the ventricles after exposing the heart, with the help of a syringe fitted with 20 gauze needle. The blood without anti-coagulant was directly used for the total erythrocyte count (TEC), total leucocytes count (TLC) and smear preparation.

Total Erythrocyte Count

The number of erythrocytes per cubic millimetre of blood was calculated with the help of a haemocytometer using a Neubaur's counting chamber as given by Dacie and Lewis.

Total Leucocytes Count

The method given by Dacie and Lewis was followed to count the number of leucocytes. Blood was drawn up to 0.5 mark of the WBC pipette and diluted up to 11 mark with Turk's fluid and the number of WBC in corner most four squares, whose area is 1 sq. mm were counted.

Differential Leucocytes Count

Preparation of blood smear: For the preparation of blood smear standard glass slides washed in 100% ethanol were used. Blood film was made immediately after the blood was drawn from the fish. From each fish, four blood smears were prepared using Leishman's, Gimsa, Wright's and Field stains.

Study of blood smear: The morphology and staining property of all types of blood corpuscles were studied. For differential leucocytes counts, at least 100 leucocytes were observed and counted for each blood smear at a time, and differentiated into various types: monocytes, lymphocytes, neutrophils, eosinophils and basophils.

RESULTS

The acute toxic effects of endosulfan and dimethoate pesticides has been examined on some blood parameters. The parameters selected were total erythrocyte count, total leucocytes count and differential leucocytes count.

Effect of Endosulfan

Endosulfan is very toxic to fish. It produced marked alteration in the blood arameters of group as well as individual fishes after acute exposure, as are describe d in the following account **(Table 1 and Figure 1).**

Table 1. Alteration in haematolological parameters in *Channa punctatus* exposed to Endosulphan for 96 hr. individually and group.

Parameter	Control fish	Individual exposed fish				Group exposed
		1st	2nd	3rd	4th	
TEC (× 10^6/mm^3)	2.12 ± 0.062	1.95 ± 0.02***	1.87 ± 0.02***	1.98 ± 0.05***	1.96 ± 0.03***	1.97 ± 0.03***
TLC (× 10^4/mm^3)	6.12 ± 0.22	7.15 ± 0.23***	7.64 ± 0.22***	6.99 ± 0.25***	7.14 ± 0.02***	7.26 ± 0.22***
Basophils (%)	4.11 ± 0.30	3.10 ± 0.18***	2.96 ± 0.12***	3.14 ± 0.14***	3.24 ± 0.24***	3.17 ± 0.12***
Eosinophils (%)	5.25 ± 0.42	2.82 ± 0.22***	2.90 ± 0.05***	2.64 ± 0.08***	2.75 ± 0.09***	2.80 ± 0.22***
Monocytes (%)	6.25 ± 0.36	4.25 ± 0.34***	5.81 ± 0.07***	7.58 ± 0.21***	5.81 ± 0.14***	6.48 ± 0.12***
Lymphocytes (%)	22.25 ± 0.56	17.68 ± 0.08***	18.12 ± 0.92***	17.46 ± 0.84***	17.28 ± 0.72***	17.60 ± 0.48***
Neutrophils (%)	62.14 ± 0.36	72.15 ± 1.42***	72.12 ± 1.78***	70.84 ± 0.84***	74.74 ± 1.24***	72.01 ± 0.98***

TEC=Total Erythrocyte Count; TLC=Total Leucocyte Count; Values are Mean ± SD; n=6; *P<0.05; **P<0.01; ***P<0.001

Total Erythrocyte Count

From the result shown in **Table 1**, it is evident that the number of erythrocytes in group of fishes exposed to endosulfan was significantly decreased as compared to control fish. The percentage of alteration was 0.1%. In Individual exposure, all the four fish showed significant decrease which varied from 6.6% to 11.7%.

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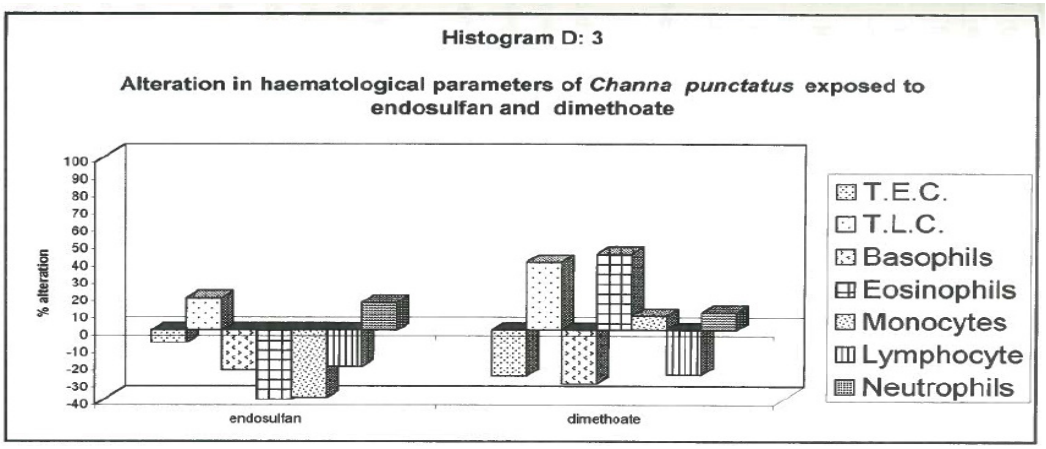

Figure 1. Histogram D: 3.

Total leucocytes count Significant increase was recorded in total number of leucocytes in group exposed fishes. The percent of alteration was 18.6%. Although there was significant increase of white blood corpuscle in individual fish, the increase varied from 14.2% to 24.8%.

Differential Leucocytes Count

Basophils: Exposure of group of fishes to endosulfan for 96 hr decreased the number of basophils by 22.8% and the decrease was significant. There was variation in the number of basophils in four individually exposed fishes and decrease in each fish was statistically significant.

Eosinophils: The number of eosinophils of experimental fish was low than the control fish showing an decrease of 55.2% that was statistically significant. All the four fish exposed separately also showed significant decrease which ranged between 53.6% to 57.8%.

Monocytes: The present experiment revealed that the number of monocytes in group of fishes significantly decrease by 39.0%. In individual exposure, decrease of monocyte in four fish varied from 35.4% to 43.7% and the decrease was also statistically significant.

Lymphocytes: The number of lymphocytes of group of fishes was also significantly lower in endosulfan exposure as compared to control fish. The percentage of decrease was 20.9. There was also variation in the number of lymphocytes in four individually exposed fishes and the decrease in each fish was statistically significant which varied from 18.5 to 22.3%.

Neutrophils: Exposure to endosulfan for 96 hr increased the number of neutrophils by 15.9% in group exposure and the increase was significant. In individual exposure, fish No. 4 show highest increase by 20.3% and lowest increase was 14.0% for fish No 3.

Effect of Dimethoate

Dimethoate is an organophosphate pesticide which produced marked alteration in the blood parameters of group exposed as well as individual exposed fishes after acute exposure which are described in the following account **(Table 2).**

Table 2. Alteration in haematolological parameters in *Channa punctatus* exposed to Dimethoate for 96 hr. individually and in group.

Parameter	Control fish	Individual exposed fish				Group exposed
		1st	2nd	3rd	4th	
TEC (× 10^6/mm^3)	2.12 ± 0.062	1.51 ± 0.02***	1.46 ± 0.18***	1.58 ± 0.03***	1.54 ± 0.03***	1.56 ± 0.05***
TLC (× 10^4/mm^3)	6.12 ± 0.22	8.29 ± 0.20***	8.74 ± 0.02***	7.86 ± 0.03***	9.02 ± 0.04***	8.52 ± 0.44***
Basophils (%)	4.11 ± 0.30	2.86 ± 0.52***	3.12 ± 0.18***	2.74 ± 0.12***	2.92 ± 0.14***	2.84 ± 0.16***
Eosinophils (%)	5.25 ± 0.42	7.67 ± 0.70***	7.20 ± 0.52***	8.12 ± 0.46***	7.50 ± 0.64***	7.56 ± 0.34***
Monocytes (%)	6.25 ± 0.36	5.74 ± 0.42***	5.84 ± 0.10***	5.94 ± 0.46***	4.98 ± 0.34***	5.74 ± 0.32***
Lymphocytes (%)	22.25 ± 0.56	28.84 ± 0.16***	27.74 ± 1.25***	28.12 ± 0.79***	26.72 ± 0.88***	27.98 ± 0.14***
Neutrophils (%)	62.14 ± 0.36	54.79 ± 1.42***	56.10 ± 0.84***	52.08 ± 0.92***	57.88 ± 0.65***	55.88 ± 0.97***

TEC=Total Erythrocyte Count; TLC=Total Leucocyte Count; Values are Mean ± SD; n=6; *P<0.05; **P<0.01; ***P<0.001

Total Erythrocyte Count

In group exposed fishes significant decrease was recorded in the total number of erythrocytes. The percentage of decrease was 26.4 and the decrease was significant. In individual exposure all the four fish showed significant decrease which varied from 25.5% to 31.1%.

Total Leucocytes Count

The number of leucocytes in group of fishes exposed to dimethoate was significantly increased by 39.2% as compared to

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control fish. There was also significant increase of white blood corpuscle in individual fishes and the increase varied from 28.4% to 43.4%

Differential Leucocytes Count

Basophils: Exposure of group of fishes to dimethoate for 96 hr decreased the number of basophils by 30.9% and the decrease was significant. There was variation in the number of basophils in four individually exposed fishes and the increase in each fish was statistically significant.

Eosinophils: In group exposed fishes significant increase was noticed in the number of eosinophils. The percentage of alteration was 44.0 and the increase was significant. All the four fish exposed separately also showed significant increase which ranged between 42.8% to 54.6%.

Monocytes: The number of monocytes of experimental group of fishes was lower than the control fish showing an decrease of 8.2% and the decrease was significant. Decrease of monocytes in four individually exposed fishes varied from 4.9% to 20.3%.

Lymphocytes: Acute exposure to dimethoate produced significant 25.7% increase in lymphocytes number in group exposure. In individual exposure, all the four fish showed significant increase which varied from 22.2% to 29.6%.

Neutrophils: The number of neutrophils of group of fishes decreased significanztly by 10.1% in dimethoate exposed fish. Fish No. 1 of individually exposed fish show highest decrease of 11.6% and lowest decrease was 6.8% for fish No. 4.

DISCUSSION

Endosulfan produced significant decrease in the number of erythocytes but increase the PCV level. The general tendency of increased in the PCV points at RBC swelling, which must be persistent because PCV does not decrease even when RBC count falls. Swelling of RBC's has been related to hypoxia [14]. It may also be in response to methemoglobin- induced hypoxia or a result of adrenaline stimulation of the membrane bound Nat/K ATPase [15]. This contention is further supported by the increase in mean cell volume and decrease in mean cell hemoglobin concentration.

The present observations clearly indicate that TEC decreased significantly after 96 hr of exposure to dimethoate indicating the anaemic state of fish, which in turn may be due to decrease in erythropoietic activity or increased destruction of blood cells. According to Chakarbarty [3] decrease in Hb indicates poor oxygen transport by blood caused by damage or due to increased accumulation of CO_2 in blood. According to decrease in RBC may also be as a result of decreased iron uptake by intestine or due to the damage of intestinal villi and mucosa a) resulting in defective intestinal absorption of iron and other substances essential for intestinal absorption of iron. A number of workers have earlier reported similar haematelogical alterations produced by different toxicants. Anees [12] also observed decrease in Hb content and TEC in *Channa punctatus* exposed to three organoph sphate insecticides namely diazinon, dimethoate and methyl parathion. The occurance of erythrocytic necrosis resulting in hypochromic microcytic anaemia with possible increase in the accumulation of methehaemoglobin and there by reducing the oxygen carrying capacity of blood has been reported by Koundilya and Ramamurthi in Tilapia mossambica exposed to sumithion and Sevin. Ramesh and Manavalaramanujam [14] reported decrease in Fib and TEC in malathion exposed fresh water fish Cyprinus carpio and Hb, TEC and HCT in sumithion exposed Ciarias batrachus respectively. Sexena and Chauhan also reported regular fall in erythrocytes and leucocytes number in *Channa punctatus* due to forced exercise. Reduced RBC count was also observed in Garragotyla, *Channa punctatus* and Channa striatus exposed to BHC by Sahai and Thakur [15].

Differential leucocytes count also was altered in fish exposed to endosulfan and dimethoate. Basophils are quite scanty in normal circulating blood. Basophils decreased in all the exposures,. In the present study the observed low percentage of basophils and eosinophils supports the findings of Srivastva [16] who observed very low percentage of basophils and eosinophils in *Clarias batrachus*, *Hetropneustes fossilis* and *Amphipnous Cuchia* and did not found a single basophil and eosinophil in Ophioephalus punctatus.

Increase in eosinophils on exposure to dimethoate and decrease on exposure endosulfan for 96 hr was observed in the present findings. According to Joshi [16] the unusual occurrence of eosinophils in circulating blood may be the index of gome physiological demand of the fish, warranted by the internal and ecophysiological conditions.

In the present study increase in neutrophils number was recorded upto 72.0% in fish exposed to endosulfan, which is supported by the findings of Sharnia and Joshi [16], who observed an increase from 20.8% to 26.5% in Tar putitora. Impact of toxic substances on neutrophil number has been reported by Ref. [17] in air breathing fish *Anabas testudinetis* exposed to detergent.

CONCLUSIONS

In the present study both the pesticides decreased the number of basophils, eosinophils, monocytes, lymphocytes, neutrophils and RBC in *Channa punctatus* after 96 hr, except in few observations. Number of eosinophils, monocytes and lymphocytes in endosulfan exposures; only eosinophils and lymphocytes count in dimethoate exposure was increased after acute exposure. This is in contradiction to the previous findings.

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REFERENCES

1. Banerjee S and Chaudhary S. Haematological monitoring of a bioindicator fish, *Heteropneustes fossilis* on exposure to copper toxicity. J Ecol. Environ. 1990;42:46-51.
2. Adhikari S, et al. Ayyappan S Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, **Labeo rohita** (Hamilton). Ecotoxicol Environ Safe. 2004;58:220-226.
3. Chakrabarty C, et al. Effect of sublethal toxicity of three organophosphorus pesticides on the peripheral hemogram of the fish Channa punctatus. Environ And Ecol. 1988;6:151-158.
4. Chandrasekar S and Jayabalan N. Hematological responses of the common carp, *Cyprinus carpio L*. exposed to the pesticide endosulfan. Asian Fish Sci. 1993;6:331-340.
5. Dhanapakiam D, et al. Changes in the level of transaminases in Indian corp, *Labeo rohita* exposed to sublethal concentration of tannery and distillery effluent. J. Environ. Biol. 2006;27:567-570.
6. Dharam S, et al. Impact of copper on haematological profile of freshwater fish, *Channa punctatus* Journal of Environmental Biology. 2008;29:253-257.
7. Garg VK, et al. Haematological parameter in fish *Channa punctatus* under the stress of manganese. Environ. Eco. 1999;7:752-755.
8. Jaya S, et al. Comparative study on the haematological effect of synthetic and plant origin pesticides on fish *Channa punctatus*. Indian journal of Natural products and Resourses. 2013;4:48-53.
9. Nath R and Banerjee V. Effects of various concentrations of lead nitrate on haematological parameters of an air breathing fish, *Clarias batrachus.* J. Freshwater Biol. 1995;7:267-268.
10. Pandey K and Shukla JP. A textbook of Fish and Fisheries. Rastogi Publications, Meerut. 2012.
11. Sastry KV and Gupta A. Haematological study on the effects of cadmium and dimethoate alone and in combination on the fresh water teleost fish, *Channa punctatus*. Environ. Pollut. 1994;1:133-139.
12. Sastry KV and Sachdeva S. Studies on the individual and combined effect of cadmium, endosulfan and surf on the haematology immunology and metabolism of teleost fish *Channa punctatus*. Thesis submitted to MDU, Rohtak. 1996.
13. Shukla V, et al. Bioaccumulation of Zn, Cu and Cd in *Channa punctatus*. J. Environ. 2007;28:395-397.
14. Singh M. Haematological responses in a fresh water teleost, *Channa punctatus* to experimental copper and Cr poisoning. J. Environ. Biol. 1995;16:339-341.
15. Svobodova ZB, et al. The effects of pollutants on selected haematological and biochemical parameters in fish. In: Sub lethal and chronic effects of pollutants on freshwater fish. *Müller R, Lloyd R* (eds.), Fishing new books, London. 1994.
16. Tripathi PK, et al. Toxic effects of dimethoate (organophosphate) on metabolism and enzyme system of freshwater teleost fish *Channa punctatus*. Asian Fish Sci. 2003;16:349-359.
17. Velisek J, et al. Effects of acute exposure to metribuzin on some hematological biochemical and histopathological parameters of common carp *Cyprinus carpio L*. Bull Environ Contam Toxicol 2009;82:492-495.