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Effect of Sub-lethal Concentration of Dipterex on the Blood Parameters of fish *Trichogaster fasciatus*

***Anand P. Singh and Surendra Singh**

Dept. of Zoology, School of Life Sciences, Khandari Campus,
Dr. B.R. Ambedkar University, Agra - 282002

Abstract : *The fishes serve as indicator of water quality and are meant for ornamental and food value. Use of pesticides dose on their lives and almost all the pesticides go to water bodies in any from and any way. Environmental pollution is a burning issue at the moment for whole world. Many factors are behind these various types of pollution and varied effects are revealed by the pollutants. Sub lethal concentration of Diptrex on certain blood parameters such as Total erythrocyte count (TEC), Total leucocyte count (TLC), Haemoglobin concentration (Hb conc.), Erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) have been studied. Dipterex is an organophoshorus insecticide and its LC_{50} has been calculated by the log-dose/probit regression line method. Sub lethal concentration of Dipterex given to fishes showed that TEC, Hb concentration and PCV decreased significantly, while TLC and ESR have been increased after 1, 7, 15 and 30 days treatment respectively.*

Keywords : *Trichogaster fasciatus,* Dipterex, TLC, ESR, Hb. Conc., TEC, PCV.

Introduction

Pesticides play an important role in improving economy of any country. Pesticides are biologically active chemicals, used for pest control to increase agricultural yields. The poisoning by pesticides from agricultural fields is a serious water pollution problem and its environmental long-term effect may result in the incidence of poisoning of fish and other aquatic life forms (Konar, 1975). Pesticides have wide variety of chemicals with great difference in their mode of action, uptake by the body, metabolism and elimination from the body and toxicity to targeted organisms. Pesticides selected have serious impacts on the non-targeted organisms (David *et al.*, 2003). Their continuous use and flow to water bodies may cause alterations in various aquatic organisms, particularly to fishes (Tripathi *et al*., 2002).

Fisheries have always played a pivotal role in providing food to people in India as well as the world and fish water aquaculture constitutes more than 90% of the total aquaculture production. Since the mechanisms of pesticide excretion, deposition and detoxification in fish are not capable of handling pesticide in short time frames, pesticides tend to accumulate specifically in metabolically active tissues and organs. They also depend upon the age, size, feeding status and sex of the organism. Now a days a variety of toxicity testing programs have been launched throughout the world to assess the toxicity of these pesticides because there is a great need for global awareness towards the adverse effects of pesticides including organophosphorus to the aquatic ecosystem. Blood is the most assessable body fluid for checking the toxicity of any chemicals, (Hickey, 1979) thus selected in the present study. The selected pesticide dipterex in the present study has been aimed to assess the biochemical alteration in the blood of fresh water fish *Trichogaster fasciatus* (Bloch.). This fish has been selected in the present study due to its wide availability and easy handling under the laboratory conditions. The present study has thus been aimed to find put the haematotoxic potential of dipterex in *Trichogaster fasciatus* (Bloch).

* Email : babupratap@rediffmail.com

Materials and Methods

Trichogaster fasciatus (Bloch.) was selected for the present study. It is also known as snake headed fish. *Trichogaster fasciatus*, representing both sexes were collected alive from the local fish market of Agra. They were carefully examined for injury and were kept in 1% solution of potassium permangnate for a few minutes before transferring them into large aquaria measuring 75cmx37.5cmx37.5cm. Dechlorinated water was used in the aquaria, which was changed every alternate day. The fishes were fed daily two times with flour pellets and small pieces of boiled eggs albumin.

The Trichogaster fasciatus were divided into four groups (A, B, C, D). Each group consisted of four individuals with standard solution of experimental test compound. Dipterex, was given to each set of fish kept in 25 litre of water in aquaria by weighing. Then the survival number of fishes was recovered for each concentration after 24, 48, 72 and 96 hours. The data was analyzed statistically by log-dose/probit analysis method (Finney, 1971). Regression line was drawn on the basis of two variables, log dose and empirical probit, on a simple graph paper and was used to determine the expected probit necessary for LC_{50} determination. The concentration of Dipterex at which 50% of the fishes died is taken as the lethal concentration. The mortality percentage was calculated and the graph was plotted between the mortality percentage and the concentration of Dipterex. LC_{50} value is obtained by the straight line graphical interpolation.

Blood was collected after severing the caudal penducle of the living fish by a sharp scissors. Double oxalate vials were used for storing the blood samples for various haematological studies. Potassium oxalate 800 mg and 1200 mg ammonium oxalate was dissolved in 100 ml distilled water. One drop of this solution was added to each empty sterilized vial, shaken and dried in the oven at $80^{o}C$.

Total erythrocytes were counted with the help of improved Standard Neubauer Haemocytometer (Dacie and Lewis, 1968). Total leucocytes were counted by using improved Standard Neubauer Haemocytometer (Dacie and Lewis, 1968). The haemoglobin concentration of blood was estimated by the Standard Sahli's method outlined by Wintrobe *et al*. (1981). The packed cell volume or haematocrit value expresses the percentage sample occupied by the erythrocytes and was determined by the method of Wintrobe *et al*. (1981). The rate at which the red blood cells settle down is known as erythrocyte sedimentation rate. It is determined by Wintrobe *et al*. (1981) method. The experimental data was analysed by student's 't' test for determining the significance of the changes from control (Fischer and Yates, 1963).

Results and Discussion

It is well known that industrial wastes in aquatic environment have a toxic effect on organisms, especially on fish. The stress caused by environmental pollution changes the structure of red and white blood cells. Haematological techniques are the most common method to determine the sub-lethal effects of the pollutants. Thus, blood parameters such as TEC, TLC, Hb. Conc., ESR and PCV are the most common criteria used in the toxicity studies on fish. As an indicator of pollution, blood parameters are used in order to diagnose and describe the general health condition of some fish. Besides, this type of index reflects certain ecological changes in the environment.

Considering the significance of hematological parameters as indicator of fish health, the effect of organophosphate dipterex has been observed on different cell types in peripheral blood of *Trichogaster fasciatus* as a part of environmental studies on the use of these parameters for assessing nutritional status of fishes. The LC_{50} value of dipterex to *Trichogaster fasciatus* fish has been found to be 0.3 mg/L and four sub-lethal concentrations viz. 0.03, 0.04, 0.05 and 0.06 mg/L has been selected for present study. The effect of sub-lethal concentrations has been assessed for toxicity testing in *Trichogaster fasciatus* for 1, 7,

Table 1. TEC, TLC, Hb. conc., ESR and PCV in *Trichogaster fasciatus* (Bloch.) in control and after dipterex treatment

Experimental sets	1day	7days	15days	30days
	Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.
Total erythrocyte count (TEC) x10^6/mm^3				
Control	3.09±0.01	3.09±0.01	3.09±0.01	3.09±0.01
0.03mg/L	3.08±0.03*	3.05±0.09*	3.01±0.01*	2.99±0.08*
0.04mg/L	3.00±0.10*	2.98±0.15*	2.97±0.11*	2.95±0.03**
0.05mg/L	2.80±0.10**	2.78±0.03**	2.72±0.12**	2.70±0.06***
0.06mg/L	2.75±0.09**	2.70±0.05***	2.68±0.05***	2.65±0.09***
Total Leucocyte Count (TLC) 10^3/mm^3				
Control	62.5±0.07	62.5±0.07	62.5±0.07	62.5±0.07
0.03mg/L	63.8±0.70*	63.9±0.10*	64.5±0.21*	65.0±0.33*
0.04mg/L	65.4±0.10*	65.9±0.33*	66.1±0.09*	66.8±0.10*
0.05mg/L	68.0±0.10*	68.1±0.10*	69.8±0.33**	70.2±0.66**
0.06mg/L	70.4±0.55**	70.9±0.33**	71.8±0 .33**	72.0±0.67***
Haemoglobin Concentration (Hb. conc.) g/dl				
Control	12.1±0.20	12.1±0.20	12.1±0.20	12.1±0.20
0.03mg/L	11.4±0.22*	11.3±0.07*	11.1±0.07*	10.9±0.08*
0.04mg/L	11.0±0.10*	11.0±0.33*	10.8±0.09*	10.7±0.05*
0.05mg/L	10.9±0.10*	10.5±0.20*	10.3±0.20**	10.0±0.15**
0.06mg/L	10.5±0.13**	10.2±0.10**	10.0±0.12**	9.90±0.12***
Erythrocyte Sedimentation Rate (ESR) mm/hr				
Control	2.54±0.08	2.54±0.08	2.54±0.08	2.54±0.08
0.03mg/L	2.76±0.09*	2.77±0.07*	2.94±0.04*	3.00±0.10*
0.04mg/L	2.88±0.09*	2.90±0.12 *	2.99±0.12*	3.16±0.05**
0.05mg/L	3.10±0.08*	3.15±0.01*	3.56±0.05**	3.78±0.58**
0.06mg/L	3.41±0.05**	3.45±0.13**	3.68±0.67***	3.87±0.08***
Packed Cell Volume (PCV) %				
Control	51.4±0.90	51.4±0.90	51.4±0.90	51.4±0.90
0.03mg/L	50.2±0.87*	50.0±0.33*	49.0±0 .33*	48.7±0.66*
0.04mg/L	48.8±0.70*	48.5±0.50*	48.1±0.67*	47.6±0.67*
0.05mg/L	45.0±0.30*	44.7±0.22*	44.2±0.70*	43.9±0.09**
0.06mg/L	42.3±0.30*	42.0±0.08**	41.7±0.20**	40.2±0.09***

*Non-significant (P>0.05)

**Significant (P<0.05)

***Highly-significant (P<0.01)

****Very highly significant (P<0.001)

15 and 30 days in blood for TEC, TLC, Hb. Conc., ESR and PCV (Table1).

The Total Erythrocyte Count (TEC) in *Trichogaster fasciatus* has been found to be decreased after 0.03, 0.04, 0.05 and 0.06mg/L sub-lethal concentration treatment of dipterex for 1, 7, 15 and 30 days. The decrease is in systematic order which follows the time duration and concentration. The similar findings regarding decreasing trend in TEC was also observed by Rai and Qayyam (1984) in *Catla catla* due to Hg and Pb intoxication respectively, while Goswami and Dutta (1991) observed the same in fish *Heteropneustes fossilis* after treatment with thallium nitrate. Thakur and Bais (2000) were also in agreement with the findings as showed in *Heteropneustes fossilis* due to aldrin and fenvalerate treatment respectively. The decrease in TEC is because of the toxic effect of dipterex which is responsible for acute anaemia and further leads to haemorrhage condition and in turn induces erythrocytopaenic condition due to the adverse effect of dipterex on the erythropoietic tissue (bone marrow) of *Trichogaster fasciatus*. This may also be the effect of haemolytic anaemia which is characterized by abnormal destruction of erythrocytes in blood.

The Total Leucocyte Count (TLC) in *Trichogaster fasciatus* has been found to be increased after 0.03, 0.04, 0.05 and 0.06 mg/L sub-lethal concentration treatment of dipterex for 1, 7, 15 and 30 days. The increase is in systematic order which follows the time duration and concentration. This increase in the TLC in the present study was also supported by the findings of Garg and Tyagi (1989) observed in *Heteropneustes fossilis* due to manganese poisoning and Goswami and Dutta (1991) who observed similar findings in *Heteropneustes fossilis* due to aldrin and fenvalerate treatment respectively. Further, Saxena and Chauhan (1994) reported similar trend in *Heteropneustes fossilis* after copper sulphate intoxication, while Nath and Banerjee (1995) revealed similar findings in *Heteropneustes fossilis* after treatment with devithion pesticide. Ananad kumar *et al*. (2001) reported increase in TLC of *Heteropneustes fossilis* and Kumar and Malik (2006) observed in *Clarias batrachus*. This increase in TLC may be due to intoxication of dipterex and as a result of leukocytosis which is a protective reaction and here leukocyte protects the body when foreign substances invade the body.

The Haemoglobin Concentration (Hb. Conc.) in *Trichogaster fasciatus* has been found to be decreased after 0.03, 0.04, 0.05 and 0.06 mg/L sub-lethal concentration treatment of dipterex for 1, 7, 15 and 30 days. The decrease is in systematic order which follows the time duration and concentration. Similar decreasing trend in the haemoglobin concentration of *Trichogaster fasciatus* after treatment of dipterex has been confirmed by Garg and Tyagi (1989) in fish *Heteropneustes fossilis* following manganese poisoning and Goswami and Dutta (1991) in *Heteropneustes fossilis* receiving vitamin A deficient diet, while Singh (1995) observed in *Trichogaster fasciatus* due to copper sulphate and potassium dichromate poisoning and Raizada and Rana (1998) worked out in *Clarias batrachus* fish. Gupta and Gupta (2000) reported decreasing trend in Hb. Conc. in *Heteropneustes fossilis* after treatment and Saxena and Seth (2002) in *Trichogaster fasciatus* after cypermethrin treatment. Further, Das *et al*. (2004) reported that nitrate toxicity in *Labeo rohita* was followed by Masud *et al*. (2005) in *Cyprinus carpio* after mercuric chloride intoxication and Kumar *et al*. (2003) in *Clarias batrachus* fish after treatment. Red Blood Corpuscles (RBC) contains haemoglobin for oxygen transport. Hence it is clear that the decrease in RBC count is correlated with haemoglobin concentration as revealed in the present study. Dipterex disturbs the physiological state of fish *Trichogaster fasciatus* which induced uncontrolled entry of lactic acid into the tissues and interferes with internal mechanisms, which maintain the acid-base balance. Lactic acid may also reduce the affinity of hemoglobin for both oxygen and carbon dioxide, diminishing the oxygen-carrying

capacity of blood.

The Erythrocyte Sedimentation Rate (ESR) in *Trichogaster fasciatus* has been found to be increased after 0.03, 0.04, 0.05 and 0.06 mg/L sub-lethal concentration treatment of dipterex for 1, 7, 15 and 30 days. The increase is in systematic order which follows the time duration and concentration. This increase in ESR is also in conformity with Mishra and Srivastava (1979) who observed the same in *Colisa fasciatus fish*. Trivedi and Rajbanshi (1999) also reported the same in *Labeo rohita*, while Nath and Banerjee (1999) reported in *Anabas testudineus*. Hardikar and Gokhle (2000) and Thakur and Bais (2000) observed similar increase in *Sarotherodon mossambicus* following sewage treatment. Again Saxena *et al*. (2003) worked out this in *Gambusia affinis* and Das *et al*. (2004) in *Labeo rohita* following nitrate toxicity. Further, Tyagi and Srivastava (2005) reported increase in ESR in *Trichogaster fasciatus* and Oliveiia *et al*. (2006) reported in *Hoplias malabaricus* exposed to methyl mercury, inorganic lead and tributyltin chloride. Cruz and Miranda (2006) were also of similar view in *Oreochromis mossambicus* with Tilak *et al*. (2007) in *Catla catla*. The increase in ESR may be due to reduction in TEC as stated above. ESR depends on rouleaux formation and when rouleaux are formed in turn the density of its mass increases due to which RBC settle down.

The Packed Cell Volume (PCV) in *Trichogaster fasciatus* has been found to be decreased after 0.03, 0.04, 0.05 and 0.06 mg/L sub-lethal concentration treatment of dipterex for 1, 7, 15 and 30 days. The decrease is in systematic order which follows the time duration and concentration. Decreased PCV in present study is in conformity with Mishra and Shrivastava (1979) reported in *Colisa fasciatus* after zinc sulphate. Again, Singh (1995) worked out the same in *Trichogaster fasciatus* after treatment with copper sulphate and potassium dichromate and by Thakur and Bais (2000) in *Heteropneustes fossilis* due to aldrin and fenvalerate treatment. Gupta *et al*. (2001) reported similar trend in *Heteropneustes fossilis* fish after exposure to washing effluents from houses, while Nussey *et al*. (2002) disclosed that *Oreochromis mossambicus* exposed to copper and zinc mixture causes synergistic effect. Further, Acharya *et al*. (2005) observed similar findings in *Labeo rohita*. The decreased PCV may be due to reduced blood cell counts alongwith haemoglobin concentration. Packed Cell Volume is correlated with RBC count and as RBC count decreased after treatment of dipterex, it automatically reduced the PCV.

The macrocytosis as a result of chronic deficiency is probably an adaptive response through an influx of immature erythrocytes from the hemopoietic tissues to the peripheral blood to make up for the reduced RBC number and decreased hemoglobin concentration. The erythropoietic studies during present investigation revealed a decrease in the earliest stages of erythropoietic development, indicating thereby inhibition of cell proliferation at the stem cell stage. On the other hand, there was an increase in the relative population of young and mature reticulocytes in the hemopoietic tissues indicating failure of hemoglobin incorporation at this stage and consequent blocking of further development. Analysis of results recorded above shows that leukocytosis is basically due to an increase in the thrombocyte population such that it masks the actual relative decrease of all other cell types particularly those of neutrophils and small lymphocytes. Thrombocytosis may be an adaptive response to the hemorrhages and lesions accompanying the deficiency in view of their role in the clotting process. Aquatic ecosystems should be protected against all kinds of adverse activities, which may lead to dramatic changes. Studies aimed at determining the effects of various pollutants on the organisms take a long time and require expensive analytic operations. More practical biological tests have many advantages such as saving time and money, and obtaining reliable results and conclusions. The other aquatic organisms such as fish is a very suitable experimental organism for bio tests and toxicity assays.

It is believed that measuring the haematological parameters, which are used in this study, has

provided valuable information. It may show clearly that how fresh water fish responds to some changes in water quality parameters in its environment. It is also believed that further researches are needed to protect the aquatic ecosystem.

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