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Effect of Organophosphorus Pesticide (Chlorpyrifos) on the Haematology of *Heteropnetues fossilis* (Bloch)

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Abstract
The present investigation has been designed to study the effect of sublethal concentrations of Chlorpyrifos, on the haematological parametes of *Heteropnetues fossilis* (Bloch) after exposure to 96 hours. The present study shows the alternation in haematological parameter such as significant decrease in RBC counts, Hb %, thrombocytes, monocytes %, eosinophils % and basophils % where as significant increase in WBC count, Lymphocytes % and NeutrophilS % in chlorpyrifos exposed fish. The response of the fish towards toxicity of chlorpyrifos was grossly dependent on concentration and length of exposure. Thus, this paper gives an overview of the manipulation of fish, *Heteropnetues fossilis* as a biomarker of pesticides through alternation in haematological parameters.

Keywords: Chlorpyrifos, Haematological parameters, *Heteropnetues fossilis.*

Introduction
Pesticides are the biological toxicants, which are being used by the man to kill the pests for increasing the yield of many crops and insect vectors to control the spread of disease. The use of pesticides has caused severe environmental and health hazards to organisms including human beings (Srivastava *et al*., 2012) [15]. The widespread use of pesticides not only brought adverse influence on agro ecosystems and also caused alteration in the ecological balance of many non-target organisms like fishes. These pesticides through surface runoff reach into the aquatic ecosystems and become a global environmental problem. These pesticides enter the food chain and their subsequent bioaccumulation and biotransformation at different trophic levels have catastrophic effect to the ecosystem (Grande *et al*., 1994) [7].

During the last two decade a tremendous progress has been made in the development of new compounds with better toxicity, therefore, a lot of work has been carried out on impact of pesticides on non-target aquatic organisms. The most of synthetic organic pesticides are extremely toxic to non-target species of freshwater fauna, which damage the population dynamics, complex food-web and food-web energetic (Chandra *et al.*, 2001) [2].

Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridil) phosphoro-thioate], a broad spectrum organophosphrous insecticide used against pod borers, fruit borers, stem borers, leaf miners, defoliating caterpillars, sucking pests, termites etc and in other settings, to kill a number of pests, including insects and worms. Organophosphates are highly toxic to fish and non-target aquatic organisms and are powerful nerve poisons, since they inhibit AChE activity (Klaverkamp and Hobden, 1980) [10]. Chlorpyrifos is considered moderately hazardous to humans by the World Health Organization. It enters into the aquatic ecosystem and affects aquatic organism (Chernyak *et al*., 1996; Livingstone, 2001) [3, 11]. Poisoning from chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system as well as a skin and eye irritant of fish (Cox, 1994 & 1995) [4, 5]. In many countries, large scale mortality of fishes has been recorded due to pesticides in water bodies as pollutants (Nikam, 2011) [12].

Blood is one of the most sensitive indicators of stress condition of an animal. It is highly susceptible to internal and external environmental fluctuations. In all aquatic animals, fishes are the most sensitive and best indicator of water pollution. The main route of entry for any toxicant in fishes is through gills. From the gill it is transported to other parts of the body through the blood stream, hence blood provides an ideal medium for toxicity studies. The change in the total number of blood cells both erythrocytes and leucocytes and haemoglobin factors assume considerable significance as a measure of response of the body to the adverse

environment. Fish is an important source of protein and easily digestible food for human being. It plays a vital role to fulfill our nutritional requirement.

Several studies have been conducted in assessing the toxicity of organophosphorous insecticides on different fish species (Verma *et al.*, 1980; Hoque *et al.*, 2000; Vasit and Patil, 2005; Thenmozhi *et al.*, 2011) [21, 8, 20, 19]. Perusal of literature reveals paucity of information on acute toxicity of Chlorpyrifos on freshwater fishes such as *Heteropneutes fossilis* which is one of the most preferred edible fish species of Uttar Pradesh. Hence the present study was undertaken to examine the toxic effects of chlorpyrifos on the haematological parameters of freshwater catfish, *H. fossilis.*

Materials and Methods

The healthy *Heteropneutes fossilis* ranging from 8.5-9.5 cm in length and 9.0-10.0 g in weight were collected from local fish ponds and washed with 1% solution of $KMnO_4$ for five minute and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. Based on 96 LC_{50}, fishes were exposed to sublethal concentrations (5, 10 and 15 ppm) for the period of 48, 72 and 96 hours. The fishes were regularly fed with commercial food and the medium was changed daily to remove faeces and food remnants. Five fishes from each set were sacrificed for the collection of blood. The blood sample were collected from caudal region after piercing the caudal peduncle of fish from both experimental and control groups on 24th, 48th, 72nd and 96th hours of exposure periods and subjected to analysis for haematological changes. Blood parameters like TEC, Hb, TLC, Thrombocytes and DLC were calculated following the methods of Dacie and Lewis (1977) [6].

Results and Discussion

Table 1: Effects of sublethal concentration of Chlorpyrifos on haematological parameters of *Heteropneustes fossilis* at different exposure period (N=5)

Haematological Parameters	Group	Exposure periods in Hours		
		48	72	96
RBC(TEC) ($x10^6/mm^3$)	Control	3.75±0.012	3.71±0.011	3.72±0.014
	5ppm	3.65±0.014	3.42±0.017	3.28±0.021*
	10ppm	3.52±0.015	3.35±0.016	3.20±0.013*
	15ppm	3.40±0.012	3.29±0.012*	3.12±0.021**
Hb (g/100ml)	Control	13.8±0.11	13.9±0.12	13.8±0.14
	5ppm	12.9±0.14	12.8±0.13	12.0±0.11
	10ppm	12.0±0.13	11.5±0.11*	10.8±0.17*
	15ppm	10.7±0.14*	10.2±0.14*	9.8±0.15**
WBC(TLC) ($x10^3/mm^3$)	Control	14.75±0.12	14.78±0.11	14.77±0.12
	5ppm	15.05±0.13	16.12±0.18	16.92±0.14
	10ppm	17.51±0.14	17.81±0.17*	18.10±0.13*
	15ppm	18.35±0.15*	19.11±0.21**	19.11±0.14**
Thrombocytes ($x10^3/mm^3$)	Control	40.25±5.11	41.11±4.12	41.09±3.19
	5ppm	31.12±1.12	28.12±1.32	24.14±3.14*
	10ppm	23.28±1.14*	21.41±5.12*	19.32±4.16*
	15ppm	18.14±1.85*	16.50±3.06**	12.82±3.08**
Lymphocyte %	Control	75.7±1.21	75.4±1.41	75.6±1.29
	5ppm	76.7±1.11	77.5±1.31	77.9±1.27*
	10ppm	78.0±1.14	78.9±1.24	79.5±1.26*
	15ppm	79.1±1.33*	79.8±1.31*	80.4±1.14**
Monocytes %	Control	8.7±0.77	8.8±0.14	8.6±0.28
	5ppm	7.9±0.82	7.2±0.19	6.7±0.21*
	10ppm	7.2±0.77	6.2±0.49*	5.4±0.31**
	15ppm	6.2±0.59*	5.4±0.44*	5.0±0.24**
Neutrophil %	Control	11.1±0.91	11.2±1.12	11.2±1.13
	5ppm	11.9±0.76	12.3±0.68	12.9±0.25*
	10ppm	12.3±0.74	12.8±0.81*	13.4±0.71*
	15ppm	12.8±0.66*	13.6±0.71*	13.8±0.57**
Eosinophil %	Control	3.5±0.24	3.6±0.26	3.6±0.31
	5ppm	3.2±0.33	2.9±0.34*	2.5±0.29*
	10ppm	2.5±0.44	2.1±0.47	1.7±0.34**
	15ppm	1.9±0.39*	1.2±0.45**	0.8±0.54**
Basophil %	Control	1.0±0.29	1.0±.39	1.0±0.34
	5ppm	0.3±0.21	0.1±0.31	0.0*
	10ppm	0.0±.0*	0.0*	0.0*
	15ppm	0.0*	0.0*	0.0*

*Significant at P< 0.05; ** significant at P< 0.01.

In the present study significant alterations was found in haematological parameters of Chlorpyrifos exposed catfish, *Heteropneustes fossils.* The significant decreases in RBC count, Hb%, Thrombocytes, monocytes, eosinophils and basophils of chlorpyrifos exposured *Heteropneustes fossilis* were found after 96 hours exposure to sublethal concentration of chlorpyrifos. The results obtained in this study showed significant reduction in RBC counts and Hb levels in chlorpyrifos exposed *Heteropneustes fossilis*. Similar reductions in RBC counts and Hb contents in different fish

species had been also reported after exposure to organophosphate pesticides (Svoboda *et al*., 2001; Ramesh and Saravanan, 2008) [13, 17]. The significant decrease in the Hb contents may also be due to either an increase in the rate at which the Hb is destroyed or to a decrease in the rate of Hb synthesis (Reddy and Bashamohideen, 1989) [14]. Thus in the present study significant decrease in haemoglobin content in chlorpyrifos exposed fish was due increased rate of destruction of haemoglobin or due to decrease rate of synthesis of haemoglobin or due to dysfunction / suppression of haemopoitic organ.

In the present study, WBC count increased significantly in *Heteropneustes fossilis* after exposure to sub lethal concentration of chlorpyrifos. The increases in WBC counts (leucocytosis) were reported in *Cyprinus carpio* exposed to acute toxicity of chlorpyrifos (Ramesh and Saravanan, 2008) [17]. However, the decrases in WBC counts (leucopenia) were also described in the *Cyprinus carpio* exposed to diazinon (Svoboda *et al*., 2001) [13]. The increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to sublethal concentrations of pesticide (Joshi *et al*., 2002) [9].

In the present study was significant reduction in thrombocyte counts with the exposure of the fish to chlorpyrifos. However, Adedeji *et al*. (2009) [1] reported a significant increase in thombocyte count in *Clarias gariepinus* exposed to diazinon. The decrease in thrombocytes may be related to decreased thrombocyte production or increased destruction of thrombocytes.

In the present study lymphocytes and Neutrophil percentage has significantly been increased causing lymphocytosis in fishes exposed to sub lethal concentration of chlorpyrifos. Percentage of eosinophils, monocytes and basiphils dropped in chlorpyrifos exposed fishes. Thakur and Pandey (1990) [18] and Srivastava *et al*. (2007) [16] also reported increase in percentage of lymphocytes and decrease in neutrophils and eosinophils in *Clarias batrachus* after exposure to BHC and distillery effluent, respectively. Thus lymphocytosis as evidences in the present investigation might be due to immunological reaction to produce more antibodies to cope with the stress induced by the toxicant.

Conclusions

The exposure of *Heteropneustes fossilis* to sublethal concentration of clorpyrifos for 96 hours showed significant alternation in hematological parameters which could lead to impairment of fish ability to combat against diseases and reduce its chances for growth and survival. This study clearly indicates that the presence of chlorpyrifos in fresh water bodies, even in small concentration, could cause deleterious effects on fish physiology and may potentially disturb their survivability in the natural environment. Therefore, controlling measures should be taken to prevent the possible contamination of the aquatic environment by such toxic pesticides.

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