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Hematological and Biochemical Changes in the Freshwater Fish, *Pseudetroplus Maculatus* Exposed to Sublethal Concentrations of Chlorpyrifos

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Abstract
Sublethal effects of chlorpyrifos on hematological and biochemical parameters were assessed in the freshwater fish, Pseudetroplus maculatus. Fish were exposed to chlorpyrifos at two sublethal concentrations, 0.661 µg/L (one-tenth of LC_{50}—96 h) and 1.32 µg/L (one-fifth of LC_{50}—96 h), for 15 and 30 days maintaining the control group. Blood collected from the control and treated groups were used for analyzing the hematological and biochemical parameters. Chlorpyrifos exposure significantly (P<.05) decreased red blood cell count, hemoglobin concentration and packed cell volume with significant (P<.05) increase in white blood cell count at both sublethal concentrations when compared with the control group. Erythrocyte indices like mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration showed significant (P<.05) increase after chlorpyrifos exposure. Differential leukocyte counts such as lymphocyte, monocyte, and eosinophil were significantly (P<.05) increased meanwhile the number of neutrophil and basophil showed significant (P<.05) reduction. Serum protein and globulin level were significantly (P<.05) increased with reduction in the level of albumin at both concentrations. The activities of alanine aminotransferase and aspartate aminotransferase increased significantly (P<.05) after chlorpyrifos exposure. The study suggests that chlorpyrifos induced sublethal toxic effect in Pseudetroplus maculatus which is evident by the alteration in hematological and biochemical parameters. Thus, the widespread application of chlorpyrifos in the natural environment could cause potential adverse effects to aquatic organisms.

Keywords: *Chlorpyrifos, biochemical parameters, hematology, Pseudetroplus maculatus, sublethal toxicity*

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INTRODUCTION

There has been a growing concern that the natural aquatic ecosystems are extensively facing adverse ecological impacts due to the widespread use and disposal of contaminants such as pesticides, which may contribute to short-term behavioral imbalance to long-term genetic abnormalities in the inhabitants. Presently, various categories of pesticides such as organochlorines, organophosphates, carbamates, synthetic pyrethroids, and other natural products are widely used to control agricultural pests [1]. Chemicals exposed due to increased agricultural activity enter into the aquatic environment through atmospheric deposition, surface water run-off or leaching, and also frequently known to accumulate in sediments which harms the aquatic organisms [2]. Therefore, monitoring the effects of insecticides is essential as the toxicity of chemical depends on several factors such as duration, concentration and the number of target receptors in the organism. In the present study, toxic effect of one of the organophosphate insecticides, chlorpyrifos to nontarget aquatic organism was studied in detail.

Chlorpyrifos (O, O-diethyl-O-3, 5, 6-trichlor-2-pyridylphosphorothioate; CPF) is the second highest selling organophosphate insecticide widely used to control foliar insects in agricultural crops and is branded under the names as Dursban and Lorsban. The foremost use of chlorpyrifos in farming is to protect corn, cotton, and fruit trees against insects and also used in termite control, mosquito control, and pet collars. The toxicity of chlorpyrifos is determined by the biotransformation of chlorpyrifos into chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol (TCP), which are potent acetylcholinesterase inhibitor than the parent compound [3]. Chlorpyrifos has been shown to stimulate oxidative stress and inhibit antioxidative and physiological activities in fish [4–6].

Fish are considered as one of the very sensitive and significant bio-indicators to detect contamination in the freshwater ecosystems as it respond instantly by altering certain physiological and biochemical processes. Both commercial and edible species of fish have been widely investigated in ecotoxicological studies in order to confirm the adverse effects of toxicants on human health through the food chain [7]. Blood of fish gill is in direct contact with the water medium thus any adverse change in the aquatic environment could be revealed in the circulatory system. In toxicological studies, the use of hematological parameters are considered as an ideal tool since it provides valuable information to detect physiological and biochemical functions including the health status, nutrition, diseases, and stress in response to the change in the environmental conditions [8].Thus, blood is an important component for studying the effects of toxicants as it is highly susceptible to environmental fluctuations [9]. Blood physiology is also used to analyze the stress or disease conditions in fish associated to internal and external environmental fluctuations. The analysis provide systematic relationship and physiological adaptations including the assessment of respiratory activity that fluctuate with ecophysiological factors such as pH of water, temperature, salinity, nativity, oxygen tension, respiratory metabolism, blood constituents, age, sex, body length, weight, and seasonal variations [10].

Most of the literatures have focused on the effect of chlorpyrifos on hematological and biochemical parameters in several fishes as *Cyprinus carpio* [11, 12], *Heteropneustes fossilis* [13], *Oreochromis mossambicus* [14]. However, the hematological parameters such as erythrocyte and leukocyte counts, hemoglobin concentration, hematocrit value along with serum protein and blood glucose level vary among different species based on the properties of toxicants exposed. Hematological parameters reflect the early detection of physiological abnormalities and promptly describe the health status of fish in toxicant-stress condition. The aim of the present study was to assess the toxic effect of chlorpyrifos at sublethal concentration on the hematological and biochemical parameters in the erythrocytes of the fish, *Pseudetroplus maculatus* in order to predict the physiological adaptations of fish under stress condition and also provide future understanding of ecological impacts.

MATERIALS AND METHODS

Animals and Maintenance

Healthy freshwater cichlid fish, *Pseudetroplus maculatus* weighing 3.5±0.5 g and length 6±0.3 cm collected from the local fish farm near Parappanangadi, Malappuram district, Kerala, India were acclimatized to the laboratory conditions for 2 weeks prior to the experiment. Fish were fed with standard fish pellets during and at the time of experiment, and are maintained in large cement tank containing dechlorinated and well-aerated water. The physiochemical features of the tap water were analyzed maintaining the water temperature at 28 ±2°C, dissolved oxygen level at 70% to 100% and pH at 7.4 to 7.6 as described in APHA guidelines (1998) [15].

Chemicals

Chlorpyrifos (O,O-diethyl O-[3,5,6-trichloro-2-pyridyl]phosphorothioate) of technical grade (97%) was obtained commercially from Hikal Chemical Industries, Gujarat, India. Drabkin reagent, bromocresol green, 2,4-dinitrophenol were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and obtained from local commercial sources.

Experimental Exposure

The median lethal concentration of chlorpyrifos (LC_{50}—96 h) in *Pseudetroplus maculatus* determined by Probit analysis was 6.61μg/L [16]. After acclimatization, two sublethal concentrations of chlorpyrifos, that is, one-tenth (0.661 μg/L) and one-fifth (1.32

μg/L) of LC_{50}—96 h were selected and exposed for 15 and 30 days. Ten fish each were accommodated in 40 L capacity of test solutions containing different concentrations of chlorpyrifos and the experiment was conducted in triplicate. One group of fishes was maintained as control besides experimental group which were provided with dechlorinated, well-aerated tap water without test solution. At the end of every experiment, fishes from the control and experimental groups were caught very gently using a small dip net, one at a time with least disturbance in order to avoid stress to the animal.

Collection of Blood Sample

After each exposure period, blood from the control and treatment groups was collected by cardiac puncture using 2 ml glass syringe and transferred into a vial containing 1% ethylenediaminetetraacetic acid as anticoagulant. The whole blood was then immediately used for hematological studies. Remaining blood samples were centrifuged at 800 *g* for 15 min at 4°C to obtain serum for biochemical estimation.

Hematological Studies

The hematological parameters include the analysis of erythrocyte count (red blood cell [RBC]) and leukocytes count (white blood cell [WBC]) by the method of Blaxhall and Daisley [17]. Hemoglobin content (Hb) of the blood was determined using Drabkin's reagent [18]. Packed cell volume (PCV) [19] and the differential counts such as neutrophils, monocytes, lymphocytes, basophils, and eosinophils were determined on blood film stained with Grumwald Giemsa stain [20]. Erythrocyte indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulas [21] as mentioned below:

MCV (pg) = Hct (%)/RBC count in millions/$mm^3 \times 10$

MCH (pg) = Hb (g/dl)/RBC count in millions/$mm^3 \times 10$

MCHC (g/dL) = Hb (g/dL) Hct (%) × 100

Biochemical Estimations

Biochemical studies include the analysis of total protein by using the method of Lowry et al. [22]. The level of albumin was determined by bromocresol purple method [23] and serum globulin level was calculated by subtracting the albumin content from the total serum protein. Activities of asparate aminotransferase, and alanine aminotransferase (ALT) were estimated as described by Reitmenand Franckel (1957) [24].

Data Analysis

Statistical analysis was performed using one-way analysis of variance followed by Duncan's Multiple Range test using statistical package SPSS 19.0. Differences were considered to be significant at $P<.05$ against control group and the data are presented as $M \pm SD$ for 10 animals per group.

RESULTS

Hematological Studies

Exposure of chlorpyrifos at sublethal concentrations (0.661 μg/L and 1.32 μg/L) showed a significant ($P<.05$) decrease in erythrocyte count, hemoglobin, and PCV in time-dependent manner when compared with the control fish group (Table 1). However, the leukocyte count was found significantly ($P<.05$) increased after 30 days of one-tenth of LC_{50} concentration and after 15 and 30 days of one-fifth of LC_{50} concentration (Table 1). Chlorpyrifos treatment significantly ($P<.05$) increased the values of blood indices like MCV, MCH, and MCHC when compared with control group (Table 1). Percentage of differential counts such as lymphocyte, monocyte, eosinophil showed significant ($P<.05$) increase after exposure to both sublethal concentrations whereas the percentage of neutrophil and basophil counts decreased significantly ($P<.05$) after chlorpyrifos exposure (Table 1).

Biochemical Estimations

Chlorpyrifos exposure at sublethal concentrations showed significant ($P<.05$) increase in serum total protein and globulin level and significant ($P<.05$) reduction in the level of serum albumin than that of control group (Figures 1-3). Activities of ALT and aspartate aminotransferase showed significant ($P<.05$) increase at both sublethal concentrations of chlorpyrifos exposure when compared with the control group (Figures 4 and 5).

Table 1: *Effect of Chlorpyrifos on the Hematological Parameters in the Freshwater Fish, Pseudetroplus maculatus (M ± SD; *denotes P<.05 Against the Control Group).*

Parameters	Control	One-tenth of LC_{50}—96 h (0.661 µg/L)		Control	One-fifth of LC_{50}—96 h (1.32 µg/L)	
		15 Days	30 Days		15 Days	30 Days
RBC ($10^6/mm^3$)	0.397±0.017	0.185±0.036*	0.113±0.015*	0.397±0.017	0.107±0.012*	0.038±0.004*
WBC ($10^4/mm^3$)	19.19±0.65	22.55±0.54	30.51±3.18*	19.19±0.65	58.03±1.09*	75.68±2.12*
Hb (g/dL)	5.49±0.22	3.89±0.17*	2.93±0.44*	5.49±0.22	2.94±0.12*	1.78±0.33*
PCV (%)	16.15±0.66	11.46±0.50*	8.63±1.28*	16.15±0.66	8.64±0.31*	5.25±1.00*
MCV (pg)	407.69±31.76	636.16±97.9*	788.35±74.91*	407.69±31.76	815.64±97.6*	1373.57±105.1*
MCH (pg)	138.51±10.8	216.066±33.2*	274.603±22.4*	138.51±10.8	277.65±33.1*	502.73±31.2*
MCHC (g/dL)	33.98±0.02	33.964±0.03	33.98±0.08	33.98±0.02	34.14±0.22	34.97±0.17*
Lymphocyte (%)	43.6±2.59	47.7±1.88*	51.5±0.52*	43.6±2.59	50.3±0.82*	55.4±1.43*
Neutrophil (%)	31.5±1.50	23.8±1.03*	19.5±0.53*	31.5±1.50	19.8±1.47*	15.3±0.82*
Monocytes (%)	14.5±0.90	15.9±0.73*	16.8±1.31*	14.5±0.90	16.4±0.84*	17.8±0.91*
Eosinophil (%)	7.3±1.09	8.8±0.91*	9.8±1.03*	7.3±1.09	9.5±1.08*	9.7±0.48*
Basophil (%)	3.1±0.10	2.8±0.03	2.4±0.11*	3.1±0.10	2.9±0.13	1.3±0.18*

Note: RBC = red blood cell; WBC = white blood cell; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

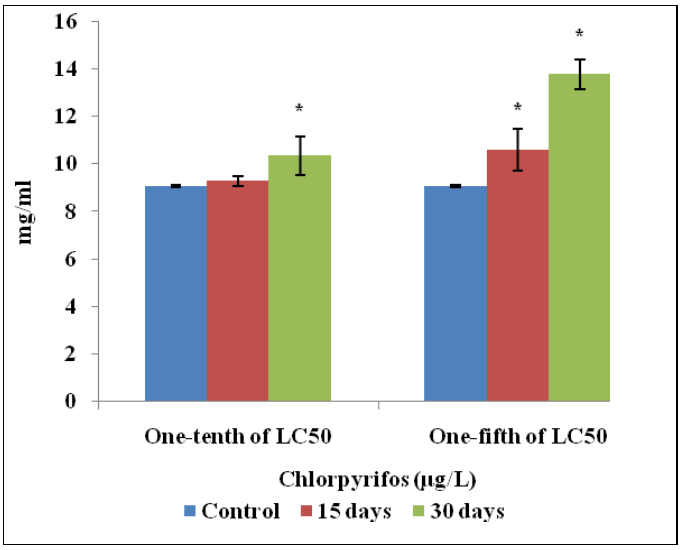

Fig.1: *Effect of Chlorpyrifos on the Serum Total Protein in the Fish, Pseudetroplus maculatus.*

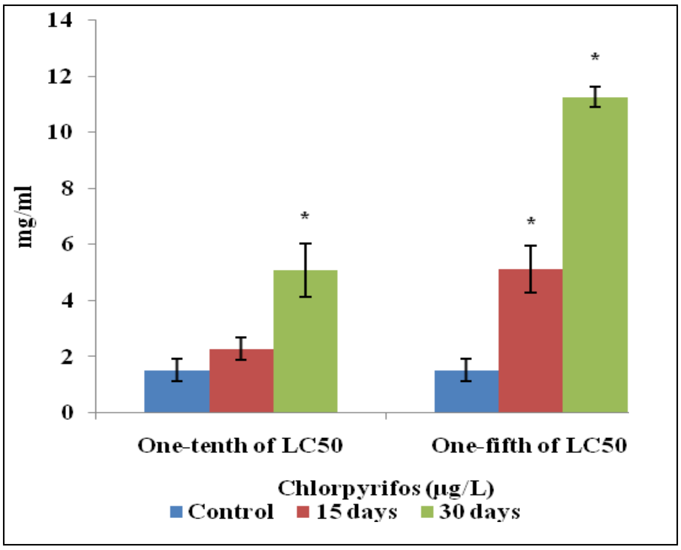

Fig. 2: *Effect of Chlorpyrifos on the Level of Serum Globulin in the fish, Pseudetroplus maculatus.*

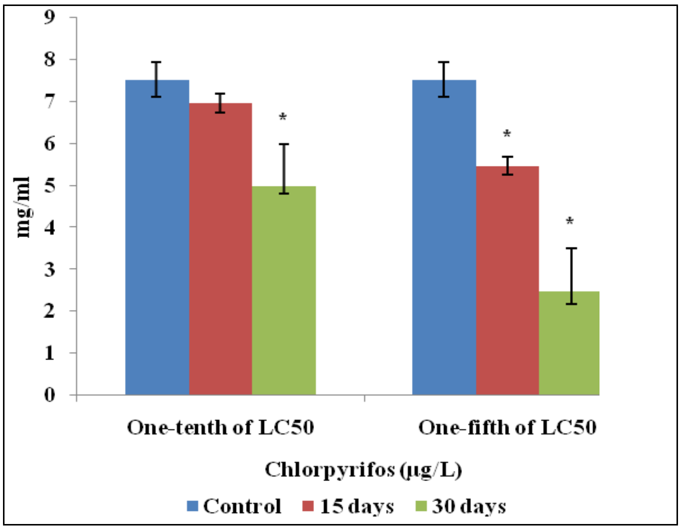

Fig. 3: *Effect of Chlorpyrifos on the Level of Serum Albumin in the Fish, Pseudetroplus maculatus.*

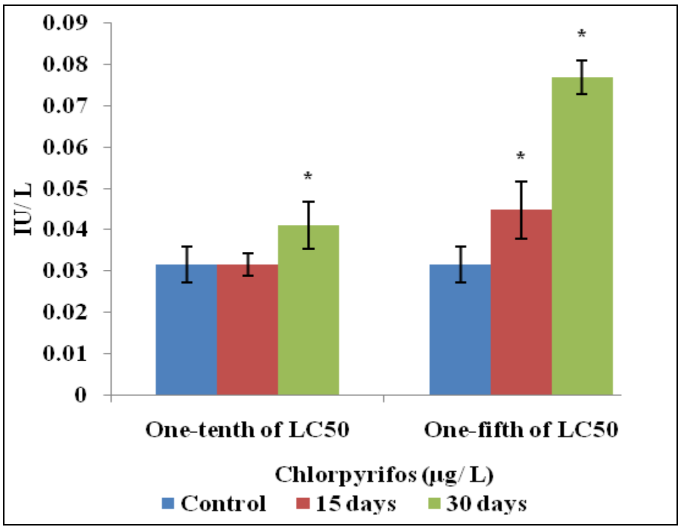

Fig. 4: *Effect of Chlorpyrifos on the Activity of Alanine Aminotransferase in the Serum of the Fish, Pseudetroplus maculatus.*

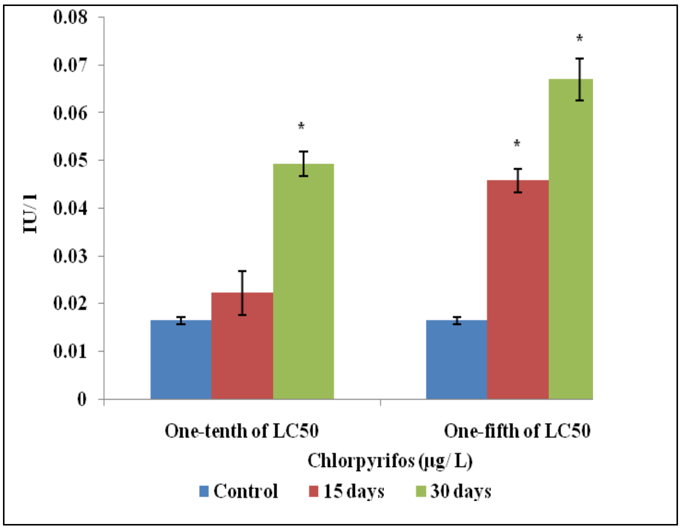

Fig. 5: *Effect of Chlorpyrifos on the Activity of Aspartate Aminotransferase in the Serum of Fish, Pseudetroplus maculatus.*

DISCUSSION

In India, more than 70% of the chemical formulations used in agricultural practices find their way to freshwater bodies that ultimately affect nontarget organisms including fish [25]. Widespread use of chlorpyrifos as pesticide in both agricultural and domestic purposes can adversely affect the health status of aquatic animals. Fish occupy a major role in aquatic communities as it considered as an indicator of water quality. Moreover, the variety and diversity of fish species and its position in the uppermost trophic level of aquatic food chain makes fish more vulnerable victim of pesticides. Hematological parameters are widely accepted diagnostic tool to assess the health status of fish and to monitor the stress response in relation to pollutant exposure [26]. It is well established that various environmental factors and stress condition grounds for the alterations in different blood parameters. The hypothesis of the present study was to assess the toxic effect of one of the widely used organophosphate pesticides, chlorpyrifos on hematological and biochemical parameters in the freshwater fish, *Pseudetroplus maculatus*.

Fish exposed to chlorpyrifos at both sublethal concentrations showed a significant decrease in number of erythrocytes (RBC), hemoglobin concentration (Hb) and percentage of PCV. The decrease in erythrocyte count and PCV could be due to the inhibition of erythropoiesis, haemosynthesis, and osmoregulatory dysfunction or due to the increased rate of erythrocyte destruction in the hematopoietic organ [27]. Thus, decrease in the production of erythrocytes finally lead to altered physiological activities and severe anemic condition in the fish exposed to chlorpyrifos. Lyzing or shrinkage of erythrocytes due to chlorpyrifos exposure on the erythropoietic tissue is also correlated with the reduction in the concentration of hemoglobin and PCV, which was evident after chlorpyrifos exposure. Increased leukocyte (WBC) count is considered as an adaptive and protective mechanism of fish against chlorpyrifos toxicity. It also indicates the activation of immune system to increase in antibody production which helps to manage the fish against the stress caused by the exposure of chlorpyrifos. Our findings are in agreement with the prior studies in which chlorpyrifos induced similar changes in the fishes as *Labeo rohita* and *Cirrhinus mrigala* [28, 29].

The erythrocyte indices like MCV, MCH, and MCHC significantly increased in concentration and time-dependent manner after chlorpyrifos exposure. The observed increase in MCV and MCH might be due to the release of large RBCs into the circulation [30]. MCHC is a good indicator of RBC swelling or shrinkage and the increase in MCHC values after chlorpyrifos exposure may be an indication of shrinking of the RBCs or decrease in hemoglobin synthesis due to the toxic effect of chlorpyrifos or may be due to sphaerocytosis [31]. Similar observations have been reported when malathion was exposed to the fish, *Clarias batrachus* [32].

Leukocytes, which play a major role in the defense mechanism of fish, consist of lymphocytes, monocytes, neutrophils, basophils, and eosinophils. Sublethal exposure to chlorpyrifos increased the percentage of lymphocytes, monocytes, and eosinophils, whereas the number of neutrophils, and basophils showed significant reduction. Lymphocytes, the major cells of leukocytes are more responsible for immune response and the observed increase in the lymphocyte count possibly indicates increased antibody production under chlorpyrifos intoxication. In addition, elevated monocyte and eosinophil counts also indicate increased phagocytic activity in order to provide protection against toxicants there by to overcome the toxicant-related stress [33]. Reduction in neutrophil and basophil counts after chlorpyrifos exposure could be due to toxic effect of chlorpyrifos. Similar changes have been observed in the fish, *Channa punctatus* exposed to dimethoate [34] and in *Labeo rohita* exposed to difenoconazole and thiamethoxam [35].

General health condition of aquatic organisms and the alteration in physiological activities after pollutant exposure are evaluated by several biochemical parameters. Determination of serum protein is usually considered as the link of biochemical and physiological architecture of animal cell or tissue. Albumin and globulin constitute major part of the total protein and are used to monitor disorders of the immune system, renal and hepatic dysfunctions [36]. The present findings showed an elevated serum protein and globulin level with reduction in the level of albumin after sublethal exposure to chlorpyrifos. The increase in serum protein could be due to the degradation of tissue protein and its release into the blood for the need of more alternative source of energy to detoxify and overcome the toxicant related stress condition [37]. Increased serum protein level was also observed in fish, *Channa punctatus* treated with the pesticide phorate [38] and in Caspian brown trout treated with chlorpyrifos [39]. Increase in the level of globulin in fish after chlorpyrifos exposure may be due to hyperglycemia to manage the energy demand [40]. The reduction in the level of serum albumin after chlorpyrifos exposure may be due to reduced blood viscosity as well as impaired liver function or kidney damage [41]. Similar results have been observed in *Oreochromis niloticus* exposed to malathion and copper sulfate [42].

ALT and aspartate aminotransferase are the biomarker enzymes for liver functions. Increase in the activities of both enzymes in the serum of fish exposed to chlorpyrifos indicates hepatocellular damage or liver necrosis [43]. The altered activities of the enzymes also possibly indicate muscular dystrophy, brain injury or myocardial damage due to cellular degradation after the exposure to chlorpyrifos. The findings coincide when carbaryl and parathion were exposed to *Clarias batrachus* [44] and chlorpyrifos exposure to *Cyprinus carpio* [45]. Thus the increase in the activities of aminotransferase enzymes in serum of *Pseudetroplus maculatus* could be due to the leakage of enzymes from the liver cytosol into the blood stream as a result of liver damage by chlorpyrifos intoxication.

CONCLUSIONS

Results of the present investigation indicate that exposure to sublethal concentration of chlorpyrifos is toxic to fish that altered hematological and biochemical parameters of fish. The study provide early warning signal to limit the usage of pesticide nearby the natural freshwater ecosystem. Chlorpyrifos even at sublethal concentration is harmful to the aquatic inhabitants and this could seriously affect the fish population as a whole.

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