Evaluation of Hematological Response and Recovery Ability of Nile tilapia (Oreochromis niloticus) exposure to cypermethrin

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Abstract—***The present study was carried out to investigate the influences of sub-lethal toxicity of cypermethrin (25% EC) exposure to Oreochromis niloticus (fingerlings). The consequence was assessed on the basis of impact on short term exposures of its below safe concentrations (1/2 (0.05 µg/L) and 1/10 (0.01 µg/L) on some haematological (RBC, WBC and Hb) studies. The sub-lethal exposure studies were made on the same day and after 7 days of exposure. Haematological recovery was also studied by maintaining the cypermethrin exposed fish in a fresh water system for an additional 7 days. The blood parameters viz., total red blood cells (RBCs) and white blood cells (WBCs) count, Hb values were analyzed using standard methods. A decrease in RBCs count and hemoglobin (Hb) value were found in both concentrations of cypermethrin exposure while total WBCs count were increased in both concentrations of cypermethrin as compared to the control fish. Complete recovery was obtained in all the studies after a recovery period of 7 days and significant effects were observed for both concentrations in exposed fish.***

Keywords—Nile tilapia (Oreochromis niloticus), Cypermethrin, Haematology, Recovery

I. INTRODUCTION

Environmental pollution by toxicants becomes one of the most important problems in the world [1]. Fish are more frequently exposed to these pollutants because it is believed that regardless of where the pollution occurs, it will eventually end up in the aquatic environment. Pesticides are extensively used in agriculture, but a residue reaches aquatic ecosystems. They are ultimately transferred from phytoplankton to fish and human [2]. Earlier reports indicated that these compounds might be toxic to the wide range of other aquatic organisms in general and in particular in fish [3], [4], [5], and other aquatic organisms and mammals, too [6].

Synthetic pyrethroids are insecticides that have been introduced over the past two decades for agricultural and domestic use [7]. Owing to the excessive use of synthetic pyrethroids, the environment and water resources are being polluted, thus endangering aquatic life directly and human life indirectly [8]. Due to their lipophilicity, pyrethroids have a high rate of gill absorption even when present at very low concentrations in the water. This in turn is a contributory factor to the sensitivity of the fish to aqueous pyrethroid exposures, because fish seem unable to metabolize the pyrethroids efficiently [9].

Cypermethrin is an insecticide, having quite effective and vast spectrum, generally used to control cotton boll worm [10], insects in agriculture, home and garden [11], and also toxic for many aquatic organisms and fish [12]. The estimation of the ecotoxicological risks in non-target organisms of the ecosystem is based on data of the toxicity and effects of pesticide preparations. Fish are among of non-target aquatic organisms [13]. The metabolism and evacuation of cypermethrin in fish is slower than in mammals and birds [14].

Contamination of water by the pesticides can lead to fish kills, reduced fish production or elevated concentrations of undesirable chemicals in edible fish tissue, which can affect the health of human eating those fish [15]. Haematological analysis is crucial in many fields of ichthyological research, fish farming, toxicology and environmental pollution monitoring as an indicator of physiological or pathological changes in fishery management and disease investigation [15]. Blood is a pathophysiological reflective of the whole body, and therefore, blood parameters are important in diagnosing the structural and functional status of fish exposed to toxicants [12].

Among the aquatic species, the fish are the major targets of toxicants contamination. Fish are largely being used for the assessment of the quality of the aquatic environment and as such can serve as bioindicators of environmental pollution. Nile

tilapia, *Oreochromis niloticus*, is a teleost widely distributed around the world with economic importance for fisheries and aquaculture. It is a good biological model for toxicological studies due to diverse characteristics, namely their high growth rates, efficiency in adapting to diverse diets, greater resistance to diseases and to handling practices, easy reproduction in captivity and prolific rate, and finally, good tolerance to a wide variety of environmental conditions [16]. The exposure of aquatic organisms to very low levels or sub-lethal concentration of pesticides in their environment may result in various haematological alterations [17], and in the field of environmental mutagenesis [18]. Haematological characteristics have been widely used in clinical diagnosis at disease, pathologies of humans, monitoring of pollutants [19]. The evaluation of haematological characteristics of blood in fish has become an important means to understanding normal and pathological processes and toxicological impacts [20].

A number of studies have been reported on the effects of pesticides on haematology of various fish species in different parts of the world [21], [22], [23], [24], [25]. But, the studies demonstrating the changes in haematological indices of freshwater fishes due to specific pesticide pollution are scare in India. Therefore, the present study was conducted to investigate the effects of cypermethrin on haematology of Nile tilapia (*Oreochromis niloticus*) as a widely consumed fish species in India.

II. MATERIALS AND METHODS

A. Maintenance of experimental fish

Healthy fingerlings of *Oreochromis niloticus* (14.75 ± 3.2g of weight, 10.80 ± 1.5 (cm of total length, as mean ± S.E.), were collected from Department of Fisheries, Anantapur, Andhra Pradesh, India, and were acclimatized in 1500 L capacity cemented tank for one month before they were transferred to the test aquaria. During acclimatization, the fishes were fed with conventional fish feed (rice bran and soya cake in1:1 ratio) at the rate of 10% body weight. Water quality characteristics in the experimental units were recorded according to APHA [26].

B. Experimental design

Cypermethrin, trade name Challenger 25 (25% EC) was used. Forty five fingerlings of *Oreochromis niloticus* divided into three groups of 15 fish in each were kept in circular fiber tanks of 500 L capacity. Two groups were exposed to 1/2 (0.05 µg/L) and 1/10 (0.01 µg/L) part of safe concentrations of cypermethrin for seven days, while the third group served as control, without the addition of cypermethrin. During the experimentation, feeding and adding of fresh dose of cypermethrin was done and the water was changed on alternate days. After exposure for seven days, the treated fish were kept in fresh water (without cypermethrin) for another seven days with daily renewal of water and feeding. Fish were observed twice daily for mortality and for behavioural changes.

C. Blood sample preparation

Five fishes from each group were sampled initially at the beginning (on the same day) of the experiment while five fishes from each group were sacrificed after 7 days of exposure. Remaining five fishes in each group were kept in continuously flowing fresh water for recovery response for another one week and were sacrificed at the end of the experiment. The blood samples were obtained for analysis by severing of the caudal peduncle and collected in appendorf tubes containing EDTA anticoagulant for determination of RBC, WBC and haemoglobin content. Physico-chemical characteristics like dissolved oxygen (DO), pH and temperature were recorded twice in a week [26].

D. Analysis of haematological parameters

The haematological parameters such as the total count of red blood cells (RBCs), white blood cells (WBCs) and hemoglobin level (Hb) were analyzed. The total RBC counts were enumerated in an improved Neubaeur haemocytometer using Hendricks diluting fluid. Blood was diluted in 1:200 with Hayem's fluid [27]. The total RBC was counted in the loaded haemocytometer chamber and total numbers were measured in10^6 x mm^3 [28]. The total WBC counts were also enumerated using Shaw's diluting fluid. Blood was diluted 1:20 with WBC diluting fluid and placed in haemocytometer. The 4 large (1sq mm) corner squares of the haemocytometer were counted under the microscope. The total number of WBC was calculated in 10^3 x mm^3 [28]. Hb was determined by cyanmethemoglobin method [29]. The reading was taken on hemoglobin tube showing percentage of hemoglobin.

E. Statistical analysis

The results obtained were subjected to analysis for mean and standard deviation. The mean values were subjected to statistical analysis using one-way analysis of variance (ANOVA) to test for the level of significance between two concentrations of cypermethrin through software SPSS (version 14.0). The significance of difference between the mean values was determined by Duncan's multiple range test at the 5 % level ($P<0.05$).

III. RESULTS AND DISCUSSION

The fishes exposed to safe concentrations of cypermethrin significantly decrease in RBC content. Treatment with cypermethrin was found to cause a drastic reduction in the total count of RBC's. The reduction was dose dependent; as the concentration of cypermethrin increased the RBC level declined. The values mentioned (Table 1) showed a significant decrease in RBC as compared to the control ($P<0.05$). Also, a significant reduction was recorded in hemoglobin of cypermethrin treated group. However, significant increases in hemoglobin was recorded in fish kept in cypermethrin free fresh water for 7 days (Table 1). The values for treatments showed a significant decrease when compared with the control ($P<0.05$). Exposure of *Oreochromis niloticus* to cypermethrin results in disturbances in haematology. Earlier report also showed the survival of fish at sub-lethal concentration, even after prolonged periods of exposure [30].

TABLE 1
RBC, WBC AND HEMOGLOBIN IN BLOOD OF *OREOCHROMIS NILOTICUS* DURING EXPOSURE TO 1/2 (0.05 MG/L) AND 1/10 (0.01 MG/L) PART OF SAFE CONCENTRATIONS OF CYPERMETHRIN AND RECOVERY PATTERN AFTER ONE WEEK

Concentrations (µg/L)	**Exposure response**		**Recovery response**
	0 days	**One week**	**One week**
Total RBCs count (x 10^6 / mm^3)			
Control	2.91 ± 0.02	2.92 ± 0.017	2.91 ± 0 .010
0.01	2.59 ± 0.04	2.42 ± 0.018	2.82 ± 0.016
0.05	2.40± 0.027	2.28 ± 0.022	2.51 ± 0 .022
Total WBCs count (x 10^3 / mm^3)			
Control	18.38±0.027*	18.42±0.019*	18.42±0.017*
0.01	18.69±0.022*	22.05±0.061*	18.65±0.055*
0.05	21.19±0.035*	23.24±0.025*	19.67±0.053*
Hemoglobin (g %)			
Control	8.34± 0.038*	8.42± 0.038*	8.39± 0.013*
0.01	7.68± 0.032*	7.31± 0.033*	8.11± 0.026*
0.05	7.37± 0.069*	6.81± 0.056*	8.07± 0.083*

N = 5 fish in each group, * Significance level tested at 5% level.

Haematology parameters are important in the health status of any organism [31]. In fishes, they are used for clinical diagnosis of fish physiology which is determined by the effect of the internal and external physical environment [32]. Changes in haematological parameters might have been brought about by cypermethrin as an anemic condition due to decreased synthesis of Hb and RBC number in hemopoietic organs. The reduction of RBC is mainly due to development of hypoxic condition during the treatment which intern leads to increase in destruction of RBC or decrease in the rate of formation of RBC due to unavailability of Hb content in cellular medium [33].

Exposure of *C. Gariepinus* to sub-lethal concentrations of cypermethrin caused a significant decrease in the values of eosinophils, Hb, PCV, RBC, leucocratic, lymphocytes, monocytes and thrombocytes of the fish. Similar reductions were reported by earlier researchers, Sampath et al., [34] in *Oreochromis mossambicus* when exposed to Organophosphorus, Omoregie et al., [35] in *Oreochromis niloticus* when exposed to formalin, Svoboda et al., [36] in *Cyprinus carpio* when exposed to diazinon, and Gabriel et al., [37] in *C. Gariepinus* when exposed to refined crude oil products kerosene.

The total WBC count was significantly increased in cypermethrin exposed group. The fishes exposed to safe concentrations of cypermethrin showed the mean values (Table 1) on the same day and after 7 days of exposure of cypermethrin. However, treated fish recovered from ill-fated effect of cypermethrin within 7 days after withdrawal of exposure to the mean values (Table 1). The values mentioned above showed a significant change as compared to the control ($P<0.05$).

WBC is important in the immune system, because of their main defensive function. The WBCs respond immediately to the change in the medium due to xenobiotic transformation. During toxic exposure period of cypermethrin, the WBC counts were enhanced. It indicates that fish can develop a defensive mechanism to overcome the toxic stress. Increase in WBCs count occurred as a pathological response since these WBCs play a great role during infestation in stimulating the haemopoietic tissues and the immune system by producing antibodies and chemical substances working as a defense against infection [38]. WBC haematological response to the effect of cypermethrin causes a significant increase. This response was equally observed in common carp *Cyprinus carpio* after acute effect of phenitrothion, imidan and dichlorvos [39]. This may be due to release of white blood cells from the spleen into the blood stream to combat the toxicant.

The present study suggested that the perturbations in these blood indices attributed to a defense reaction against toxicity of cypermethrin through the stimulation of erythropoesis or may be due to the disturbances that occurred in both metabolic and haemopoetic activities of fish exposed to below safe concentrations of cypermethrin. The toxicant caused haematological disturbance which could lead to impairment of the fish ability to combat diseases, reduce its chances for survival and reduce the potential for growth and reproduction. The improvement in blood parameters of the test fish in response to transfer to cypermethrin-free fresh water for 7 days after acute exposure suggested that cypermethrin entering the system did not accumulate in the body and was slowly eliminated, resulting in recovery from the pesticide toxicity. Similar observations were recorded in the studies carried out earlier by

Adhikari et al., [12] on the toxicity of cypermethrin and carbofuran on haematological parameters and recovery in *Labeo rohita*.

IV. CONCLUSION

The above observations clearly demonstrate that a short term exposure of *Oreochromis niloticus* to cypermethrin at even very low concentrations is sufficiently effective in disrupting physiological processes of fish and it may be recovered from ill effects of the pesticide by providing a healthy environment.

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