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Full Length Research Paper

Hematological Responses of Cyprinus carpio to Cypermethrin: An Analysis of Toxicity and Recovery Potential

Sahar Masud[1]* and I. J. Singh[2]

[1]Division of Livestock Production and Management, F.V. Sc & AH, SKUAST-Jammu, India.
[2]Department of Fishery Biology, C.F.Sc., G.B.P.U.A. & T. Pantnagar, Uttarakhand, India.

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The present investigation was conducted to evaluate the lethality of cypermethrin "pyrethroid" (25% EC with 100% purity) on common carp, *Cyprinus carpio* (fingerlings). The effect was assessed on the basis of impact of short term exposures of its below safe concentrations (1/2 (0.05 µl/L) and 1/10 (0.01 µl/L) parts of safe concentration) on some hematological parameters. The sub-lethal exposure studies were done on the same day and after 7 days of exposure. Hematological recovery was also studied by maintaining the pesticide-exposed fish in a fresh water system for an additional 7 days. The hematological analysis showed significant reduction in red blood cells (RBCs) count and hemoglobin (Hb) value while total white blood cells (WBCs) count were significantly increased in the fish *C. carpio.* Complete recovery was obtained in all the parameters after a recovery period of 7 days.

Key words: Cypermethrin, *Cyprinus carpio,* hematology, recovery**.**

INTRODUCTION

Cypermethrin, a synthetic pyretheroid has become one of the most important insecticides in wide-scale use. In 1988, pyrethroids amounted to 40% of the sales for in-secticides for cotton treatment in the world (cypermethrin 8%). Pyrethroids are synthetic analogs of pyrethrins belonging to non-systemic chemical group of insecticides. This group can be classified into two categories- Type I and II, depending on their structure, properties and mec-hanism of toxicity (Burr and Ray, 2004). Pyrethroids generally affect central and peripheral nervous system. The primary site of action of these pesticides is the sodium channel in the nerve membrane. They cause sudden and prolonged progressive increase in Na^+ per-meability of the nerve membrane resulting in long lasting chain of impulses in the sense organs and frequency dependent depression of nerve impulses in nerve fibers (Roberts and Hudson, 1998). The pyrethroid insecticides are extremely toxic to fish with 96-h LC_{50} values generally below 10 µg/l. Corresponding LD_{50} values in mammals and birds are higher (hundred to several thousand mg/kg). Sensitivity of fish to the pyrethroids is dependent on their relatively slow metabolism and delayed elimina-tion (Bradbury and Coats, 1989).

Cypermethrin [CAS: 52315-07-8, Chemical Name: (R,S)-alpha-Cyano-3- phenoxybenzyl - 2,2-dimethyl (1R,1S) - cis, trans - 3 - (2,2 - dichlorovinyl)cyclopropane carboxylate] is a class II - moderately toxic, highly active and broad spectrum, non accumulative pyrethroid insecti-cide, which is effective in public health and animal hus-bandry, and targets a wide range of pests in agriculture. The products containing cypermethrin are classified as Restricted Use Pesticides (RUP) by the US Environ-

*Corresponding author. E-mail: sahar_masud@rediffmail.com. Tel: 09419195303. Fax: 01923250639

mental Protection Agency (US EPA, 1989) because of their very high toxicity to fish. The LC50values for small fish and other aquatic organisms typically lie below 1 µg/l. This is mainly because it is metabolized and eliminated significantly more slowly by fish than mammals or birds. Their mean life in water is two weeks but they get rapidly absorbed by aquatic organisms with a significant toxicity (Rand and Petrocelli, 1985; Phillip and Rajasree, 1996).

The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on toxicity data and the effects of pesticide preparations on non-target orga-nisms. Fish are among the group of non-target aquatic organisms. Blood parameters are considered patholo-gical indicators of the whole body and therefore are important in diagnosing the structural and functional sta-tus of fish exposed to toxicants (Adhikari et al., 2004). It was reported that the blood parameters of diagnostic importance are erythrocyte and leucocytes counts, hae-moglobin, haematocrit and leucocyte differential counts would readily respond to incidental factor such as physical stress and environment stress due to water contaminants (Bhatnagar and Bana, 1992; Ralio and Nikinmaa, 1985). Some authors (Reddy and Bashamohideen, 1989; Chauhan et al., 1994; Agarwal and Chaturvedi, 1995) have reported a decrease in hematocrit, hemoglobin and red blood cells values of some fish after their exposure to insecticides. The infor-mation suggests that hematological parameters could be used as potential biomarkers of pyrethroid insecticides. Hematology of *Cyprinus carpio* has not been much docu-mented, so this paper would provide an important contri-bution to the knowledge of the specimen constitution variation. The aim of the present investigation was to assess and contribute to knowledge on the hematological changes in *C. carpio* following the short term exposure of 7 days to 1/2 (0.05 µl/L) and 1/10 (0.01 µl/L) part of safe concentrations of cypermethrin and recovery response after withdrawal of exposure.

MATERIALS AND METHODS

Collection and maintenance of test organism

Live specimens (fingerlings) of *C. carpio*, average length 10.78 (range 9.0 to 12.5 cm) and average weight 14.73 g (range from 11.5 to 19.5g), were obtained from the Instructional Fish Farm, College of Fisheries, Pantnagar and were acclimatized in 1500 L capacity cemented tank for one month before they were transferred to the test aquaria. During acclimatization, the fish 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 experi-mental units were recorded according to APHA (1980).

Test pesticide specification used

Agriculture grade cypermethrin, trade name Challenger 25 (25% EC with 100% purity) was used for the experiment. Micropipette was used to measure the doses of cypermethrin for test exposure.

Experimental design for static bioassay

Forty five fingerlings of *C. carpio*, divided into three groups of 15 fish in each, were used for the experiment in circular fiber tanks of 500 L capacity.

Exposure of fish to cypermethrin

Two groups were exposed to 1/2 (0.05 µl/L) and 1/10 (0.01 µl/L) part of safe concentrations of cypermethrin for seven days, while third group served as control. During the experimentation, feeding and adding of fresh dose of cypermethrin were done fresh after changing water on alternate day. After exposure for seven days, the treated fish were kept in fresh water (without cypermethrin) for ano-ther seven days with daily renewal of water and feeding. Fish were observed twice daily for mortality and other behavioral changes.

Hematological study

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 clean water for recovery response for another one week and were sacrificed at the end of the experiment. During each sampling blood samples were obtained by severance of caudal peduncle and collected in Appendorf tubes containing EDTA anticoagulant (Mgbenka et al., 2003) for determination of RBC, WBC and hemoglobin content. Physico-chemical characteristics like dissolved oxygen (DO), pH and temperature were recorded twice weekly (APHA, 1980).

Total count of RBC

Total red blood cells (tRBCs) were counted using Neubaur haemo-cytometer. Blood was diluted 1:200 with Hayem's fluid (Mishra et al., 1077). Erythrocytes were counted in the loaded haemocyto-meter chamber and total numbers were reported as 10^6 mm^{-3} (Wintrobe, 1967).

Total count of WBC

Total white blood cells (WBC) were counted using Neubaur haemo-cytometer (Mgbenka et al., 2003). Blood was diluted 1: 20 with WBC diluting fluid and placed in haemocytometer. 4 large (1sq mm) corner squares of the haemocytometer were counted under the microscope. The total number of WBC was calculated in mm^3 $x10^3$ (wintrobe, 1967).

Estimation of hemoglobin (Hb)

It was done by using Sahli's (1962) method. Graduated hemoglobin tube was filled with N/10 HCl upto mark 10 then blood was sucked in hemoglobin pipette upto mark 20 cu.mm. The blood was poured into hemoglobin tube already containing N/10 HCl. After leaving for 10 min, the mixture was diluted with N/10 HCl with stirring conti-nuously with a glass rod. The addition of N/10 HCl was continued drop by drop into the hemoglobin tube till the color matches with that of the standard brown glass rod. The reading was taken on hemoglobin tube showing percentage of hemoglobin.

Statistical analysis

Hematological changes were tested by using one way ANOVA (analysis of variance). Post hoc test were carried out using Duncan's multiple comparison procedure. Significance was tested at 5% level. All the statistical analysis were performed via SPSS 14.0 for windows.

Table 1. RBC, WBC and hemoglobin in blood of *C. carpio* during exposure to 1/2 (0.05 µg/L) and 1/10 (0.01 µg/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 erythrocyte count (x 10^6 /mm^3)			
Control	2.95 ± 0.02	2.94 ± 0.019	2.95 ± 0 .011
0.01	2.63 ± 0.05	2.46 ± 0.019	2.86 ± 0.017
0.05	2.44 ± 0.029	2.32 ± 0.023	2.55 ± 0 .023
Total Leukocyte count (x 10^3 mm^3)			
Control	18.44±0.029*	18.48±0.021*	18.48±0.019*
0.01	18.73±0.024*	22.08±0.063*	18.69±0.059*
0.05	21.24±0.038*	23.29±0.027*	19.71±0.056*
Hemoglobin (g %)			
Control	8.38±.040*	8.46±.040*	8.43±.015*
0.01	7.73±.034*	7.35±.035*	8.15±.029*
0.05	7.42±.072*	6.85±.058*	8.11±.085*

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

RESULTS AND DISCUSSION

Exposure of common carp to cypermethrin results in disturbances in hematology. Below safe concentrations of cypermethrin significantly decrease the RBC content. The mean values of RBC's were 2.63 (0.01 µl/L), 2.44 (0.05 µl/L), 10^6 mm^{-3} and 2.49 (0.01 µl/L), 2.32 (0.05 µl/L), 10^6 mm^{-3} within few hours and after 7 days of exposure, respectively. However, fishes showed significant recovery within 7 days after withdrawal of cypermethrin treatment with the mean values of RBC's being 2.86 (0.01 µl/L) and 2.55 (0.05 µl/L), 10^6 mm^{-3}, respectively (Table 1 and Figure 1). Treatment with cypermethrin was found to cause a drastic reduction in the total count of RBC's. The reduction was dosage depended; as concentration of cypermethrin increased the RBC level declined. The values mentioned above showed a significant decrease as compared to the control ($P<0.05$). Also, a significant reduction was recorded in hemoglobin of cypermethrin treated group. The control fishes showed mean value of 8.38 g % for hemoglobin. Fishes exposed to below safe concentrations of cypermethrin showed the hemoglobin mean value 7.73 (0.01 µl/L), 7.42 (0.05 µl/L), 7.35 (0.01 µl/L), 6.85 (0.05 µl/L), g % on the same day and after 7 days of exposure, respectively. However, significant increase in hemoglobin was recorded when treated fish were kept in cypermethrin free fresh water for 7 days and the mean values were 8.43 (0.01 µl/L) and 8.11 (0.05 µl/L), respectively (Table 1 and Figure 2). The values for treatment showed a significant decrease when compared with the control ($P<0.05$).

Changes in hematological 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 rate of formation of RBC due to non availability of Hb content in cellular medium (Chen et al., 2004). The damaging of toxicant on erythrocyte may be secondary, as resulting from a primary action of toxicant on erythropoietic tissues on which there exist a failure in red cell production and or due to increase in the erythrocyte destruction (Verma et al., 1982; Agarwal and Srivastava, 1980). Saxena and Seth (2002), Paul (2004), Bunn et al. (1996) and Kumari and Banergi (1986) also reported that the RBC count and Hb concentration decrease may depend on age of animal, stress condition, sex and availability of food in a particular medium

Total WBC count was significantly increased in cyper-methrin exposed group. The results of the total count of white blood cells revealed that the blood of the control fish showed a mean value of 18.44, mm^3 x 10^3. The fishes exposed to safe concentrations of cypermethrin showed the mean values of WBC to be 18.73 (0.01 µl/L), 21.24 (0.05 µl/L) and 22.08 (0.01 µl/L), 23.29 (0.05 µl/L), mm^3 x 10^3 on the same day and after 7 days of exposure of cypermethrin, respectively. However, treated fish reco-vered from ill-fated effect of cypermethrin within 7 days after withdrawal of exposure with the mean values of 18.48 (0.01 µl/L) and 19.71 (0.05 µl/L) respectively (Table 1 and Figure 3). The values mentioned above showed a significant change ascompared to the control ($p<0.05$).

Increase in WBCs count occurred as a pathological response since these WBCs play a great role during infestation by stimulating the haemopoietic tissues and the immune system by producing antibodies and chemi-cal substances working as defense against infection

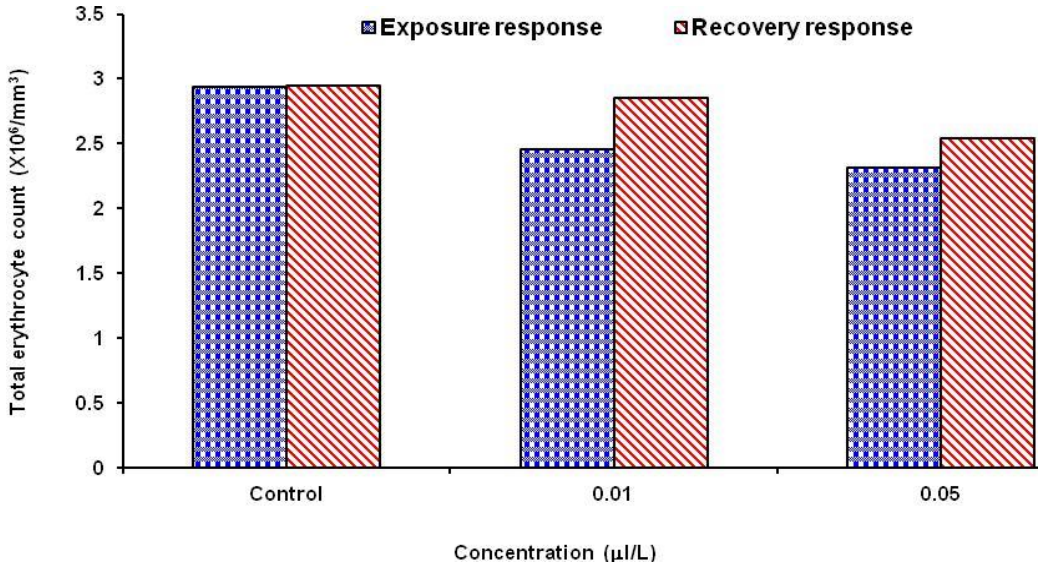

Figure 1. Total RBC during exposure to 1/2 (0.05 µg/L) and 1/10 (0.01 µg/L) part of safe concentrations of cypermethrin and recovery pattern after one week.

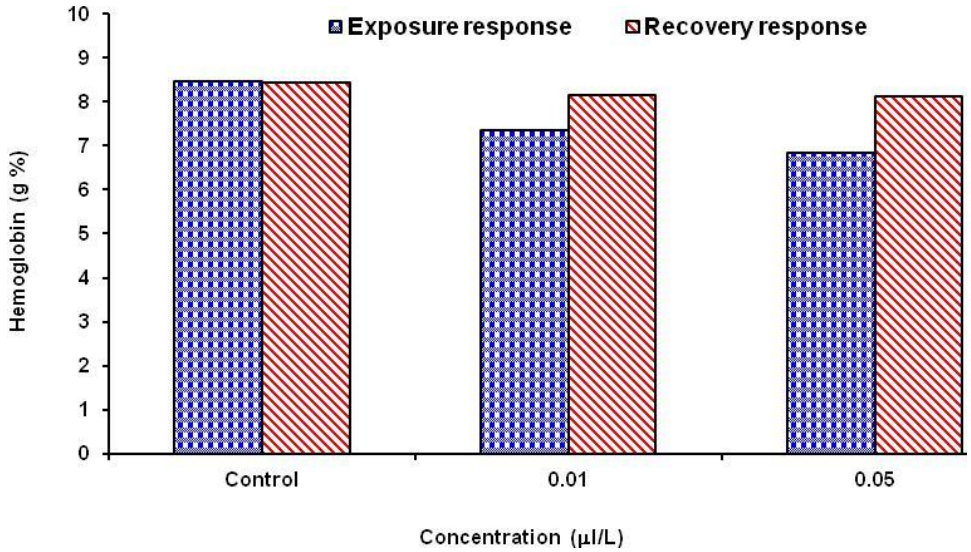

Figure 2. Hemoglobin during exposure to 1/2 (0.05 µg/L) and 1/10 (0.01 µg/L) part of safe concentrations of cypermethrin and recovery pattern after one week.

(Wedmeyer and Wood, 1974; Lebelo et al., 2001, Hassen, 2002). WBC is important cells in the immune system, because of their main defensive function. The WBCs respond immediately to the change in 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.

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 hematological disturbance which could lead to impairment of the fish ability to combat diseases, reduce its chances for survival and potential for growth and reproduction.

The improvement in blood parameters of the test fish in response to transfer to cypermethrin-free freshwater 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 pesticidal toxicity. Similar observations were recorded in

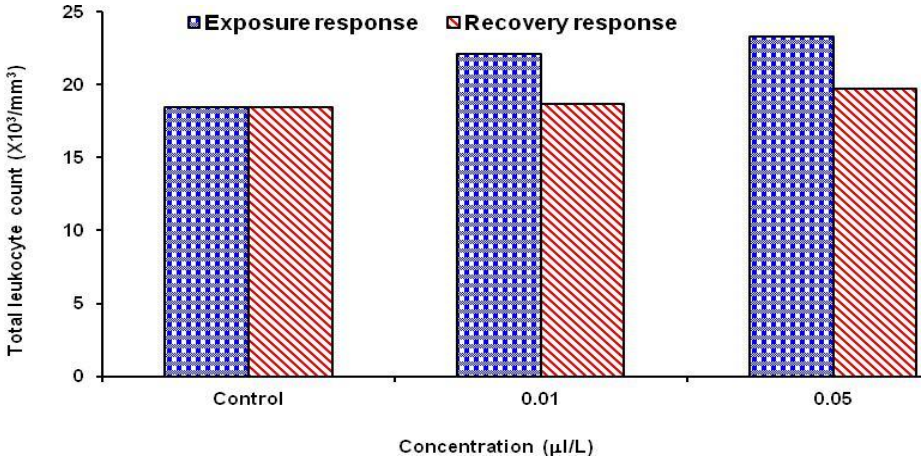

Figure 3. Total WBC during exposure to 1/2 (0.05 µg/L) and 1/10 (0.01 µg/L) part of safe concentrations of cypermethrin and recovery pattern after one week.

the studies carried out earlier by Adhikari et al. (2004) on the toxicity of cypermethrin and carbofuran on hemato-logical parameters and recovery in *Labeo rohita*.

Conclusion

The above observations clearly demonstrate that the short term exposure of *C. carpio* to cypermethrin at even very low concentrations was sufficiently effective in dis-rupting physiological processes of fish and it may be recovered from the ill effect of pesticide by providing healthy environment.

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