INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 18, No. 5, p. 77-85, 2021

RESEARCH PAPER OPEN ACCESS

Chronic toxicity effects of cypermethrin on some hematological parameters of freshwater fish *Cyprinus carpio*

Puja Boidya, Sumaiya Afrin, Md. Sherazul Islam*

Department of Fisheries and Marine Bioscience, Faculty of Biological Science and Technology, Jashore University of Science & Technology, Jashore-7408, Bangladesh

Key words: Cypermethrin, Hematology, *Cyprinus carpio,* Growth performance.

http://dx.doi.org/10.12692/ijb/18.5.77-85 Article published on May 16, 2021

Abstract

The effects of an organophosphorus pesticide, cypermethrin, (10%EC) on hematological parameters of mirror carp (*Cyprinus carpio*) were investigated in the present experiment. Two sub-lethal concentrations of cypermethrin 0.00143 (T_1) and 0.00286 (T_2) mg/L were estimated and the experimental fish mirror carp, *Cyprinus carpio*, was exposed sublethally for 28 days. In this study, the hematological parameters like total red blood cell (RBC) counts, hemoglobin (Hb), white blood cells (WBCs), mean corpuscular hemoglobin (MCH), packed cell volume (PCV), mean corpuscular volume (MCV) were considered to investigate the chronic effect using cypermethrin. During the experimental period, fish showed a significant decrease ($P<0.05$) in RBC counts, Hb, PCV when compared with controlled fish. On the other hand, WBCs and MCH were significantly ($P<0.05$) increased with the toxicity of cypermethrin in the last experimental days of exposure. On the 14th day of exposure to cypermethrin, MCV exhibits a significant increasing tendency at the concentration of 0.00286 mg/L but slight degeneration observed at 0.00143 mg/L of cypermethrin. When fish were exposed to both sublethal concentrations of cypermethrin, the value of mean corpuscular hemoglobin concentration was not significantly different on the 7th and 21st days of exposure but on the 14th and 28th days of exposure the values were significantly reduced. Growth parameters after 28 days showed a significant decrease ($P<0.05$) and increase of feed conversion ratio in all treatments compared with the control group. The present study revealed that the sublethal exposure of cypermethrin changes the hematological parameters of *Cyprinus carpio* as well as reduced growth parameters with increasing toxicity.

* **Corresponding Author:** Md. Sherazul Islam ✉ tuhinkk@yahoo.com

Introduction

Water pollution affects aquatic organisms and the impact is harming not as it were to the individual species and their communities but also the natural ecosystem. Organophosphate pesticides are broadly being used in agribusiness and account for approximately 50% of the worldwide pesticide utilizes (John, 2007). One of the organ phosphorus pesticides named cypermethrin is a complex and synthesized pesticide, belongs to the pyrethroid family, discovered from chrysanthemum flowers.

The IUPAC name of cypermethrin is: (+/-) alpha-cyano-(3-phenoxyphenyl) methyl (+)-cis, Trans-3-(2, 2-dichloroethenyl)-2, 2-dimethyl cyclopropane carboxylate (WHO, 1989) and trade name is Relothrin 10 EC.

The degradation and elimination rate of cypermethrin is significantly slow for fish rather than by mammals or birds, which may indicate toxic effects of fish compared to other organisms (Stephenson, 1983). Hematological parameters play a vital role in the diagnosis of numerous infectious diseases (Kone *et al.,* 2017). Blood parameters are considered pathological indicators of the whole body (Adhikari *et al.,* 2004) as well as assessing the effects of contaminants because blood parameters respond to low doses of pollutants. Hematological parameters including red blood cells (RBC), white blood cell (WBC), hemoglobin (Hb) concentration, packed cell volume (PCV) or hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). For ecotoxicological studies, hematological parameters are considered as biomarkers with a wide range of potential applications (Fairbrother *et al.*, 1998; Sancho *et al.*, 2000) and it may vary depending on age, gender, race, environmental and genetic background (Lugada *et al.*, 2004). Hematological hazards happened in the aquatic systems whereas fish exposed to agrochemicals or any kind of toxic effluents and such phenomena has not only been seen in laboratory state but also nature.

Several studies have been performed on the diagnosis of blood parameters including RBC, WBC counts, hemoglobin, hematocrit and leukocyte differential counts would readily respond to irregular factors such as physical stress and environmental stress due to water contaminants (Ralio and Nikinmaa, 1985; Bhatnagar and Bana, 1992).

The decrease in hematocrit, hemoglobin and red blood cell values has also been studied in some fish after their exposure to insecticides (Reddy and Bashamohideen, 1989; Chauhan *et al.*, 1994; Agarwal and Chaturvedi, 1995). Arunachalam *et al.* (1980) investigated the toxic and sublethal effect of carbaryl on a freshwater catfish, *Mystus vittatus* and reported retarded growth and conversion efficiency as consequences of more energy expenses in stress conditions. The study on stress of fishes particularly to their hematological study in exposure to a pesticide in Bangladesh is very scarce. The mirror carp, *Cyprinus carpio* is a highly palatable economically important freshwater fish species; due to its accessibility throughout the year and easy acclimatization to laboratory conditions, the species is also an ideal model organism for toxicity tests. The present study aimed to assess and identify the effect of the pesticide Cypermethrin on *C. carpio* in regards to hematological aspects and evaluate the comparative growth rate of experimental fish species.

Methodology

Collection of species and conditioning

The fish (average weight for big fish is 50.7g and average weight for small fish is 19.98g) were collected from the nearby fish market Bablatola bazar, Chachra, Jashore and transported to the laboratory of Fisheries and Marine Bioscience department inappropriately aerated plastic bags. The fish were placed into the glass aquarium for about 24hrs for acclimatization and fed with commercial pelleted food (2% of the body weight) at least once a day. Live fishes were treated with a 75 ppm potassium permanganate ($KMnO_4$) solution to avoid any dermal infection. Then the fish were reared for conditioning for about 7 days.

Experimental design

The study was designed with two treatments with three replications, which indicated as T_1 and T_2, a control set was maintained where no pesticides were used. Total 54 species of *C. carpio,* randomly divided into three groups of 18 fish in each and each group was subdivided into a set of three subgroups. The nine subgroups were kept in nine different aquariums in 60 liters of water. The physicochemical properties of the test water, namely temperature, pH, dissolved oxygen were analyzed using standard methods (APHA, 1998).

Exposure of C. carpio to test pesticide

Cypermethrin pesticide (Trade name: Relothrin 10 EC) was selected as the test chemicals and collected from the local market. To find out the sublethal concentration of Cypermethrin for mirror carp, safe levels were estimated by multiplying the 96 h LC_{50} with different application factors and was based on the methods of Sprague (1971), CWQC (1973), IJC (1977) and CCREM (1991). Two sublethal concentrations were selected to fish exposure, where 0.00 mg/L will be termed as Control treatment as well as 0.00286 mg/L is treatment 1 (T_1) and 0.00286 mg/L is treatment 2 (T_2) respectively.

The test solution was changed every alternate two days to counter-balance the decreasing pesticide concentration. The exposure lasted for 28 days during which the fish were fed daily with a small quantity of food approximately 2 % of total body weight about an hour before the test solution was renewed to avoid catabolism and subsequent mortality.

Blood sampling and measurement of hematological parameters

During each sampling blood samples were collected from the caudal vein of each fish on the 7th, 14th, 21st and 28th days. Collected blood transferred into the EDTA containing hematocrit tubes for the analysis of red blood cells count (RBC), white blood cell counts (WBC), packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Diagnosis of the hematological parameter was carried out in a nearby medical diagnostic center, Jharna Clinic, Jashore. MCV, MCH, MCHC in a given sample of blood were calculated by following formulas.

$$MCV = \frac{PCV\ (\%) \times 10}{RBC}$$

$$MCH = \frac{Hemoglobin\ \left(\frac{g}{dL}\right) \times 10}{RBC}$$

$$MCHC = \frac{Hemoglobin\ \left(\frac{g}{dL}\right) \times 100}{PCV\ (\%)}$$

Determination of growth performance

To determine the changes in growth parameters of Cypermethrin for *C. carpio* exposed to Cypermethrin for 28 days, the difference between the initial and the final weights of fishes at selective time intervals 7th, 14th, 21st and 28th days from both control and pesticide-exposed treatment tanks were recorded. To evaluate the changes in growth parameters the following parameters were used as Weight gain (%); Feed conversion ratio (FCR) and Specific growth rate (SGR).

$$\text{Weight gain } (\%) = \frac{\text{Final wt}\,(g) - \text{Initial wt.}\,(g)}{\text{Initial wt}\,(g)} \times 100$$

$$\text{Feed Conversion Ratio} = \frac{\text{Food given}(g)}{\text{weight gain}(g)}$$

$$\text{Specific growth rate } \left(\frac{\%}{day}\right) = \frac{\ln W2 - \ln W1}{T} \times 100$$

Where,

Ln = the natural log,

W_1 = initial body weight (g),

W_2 = final body weight (g) and

T = days of exposure

Statistical analysis

Qualitative and quantitative analyses of all kinds of data were stored and arranged by using Microsoft excel software. Hematological changes were tested by using one-way ANOVA (analysis of variance). Significance was tested at a 5% level Chi-Square test to find out any significant relationship between cypermethrin concentration and mortality by using SPSS ver.16.0.

Result and discussion

Hematological parameters

In the present study, RBC counts, Hb and PCV were significantly ($P<0.05$) decreased with the toxicity of cypermethrin on the 7th, 14th, 21st and 28th days of exposure periods at higher concentrations of 0.00286 mg/L (T_2) compared to the control group. This is maybe due to impaired oxygen supply to various tissues, resulting in a slow metabolic rate and low energy production. On the other hand, in lower concentration 0.00143 mg/L (T_1) RBC, Hb and PCV tend to decrease but not significantly decrease compared to the control group. On the 14th day of exposure, the mean values of RBCs were 2.71(0.00143 mg/L), 1.94(0.00286 mg/L) and 2.12(0.00143 mg/L), 1.43(0.00286 mg/L) 10^6/mm^3 were on the 28th days of exposure. In the present study, cypermethrin in water may reduce the oxygen content which in turn responsible for a significant decrease of RBC count. Fishes showed the hemoglobin mean value 5.68 g/dL (0.00143 mg/L) and 5.48 g/dL (0.00286 mg/L), on the 14th days of exposure whereas 5.93 g/dL (0.00143 mg/L) and 5.43 g/dL (0.00286 mg/L), on the 28th days of exposure (Table 1).

Table 1. Effects of cypermethrin (10%EC) exposure on hematological parameters of *Cyprinus carpio* in the concentration of 0.00143mg/L (T_1) and 0.00286 mg/L (T_2).

Parametes	Treatments	7th day	14th day	21st day	28th day
RBC (10^6/mm^3)	Control	2.4 ± 0.05^a	2.51 ± 0.06^a	2.39 ± 0.05^a	2.27 ± 0.5^a
	T1	2.01 ± 0.12^a	2.71 ± 0.26^a	2.18 ± 0.11^b	2.12 ± 0.6^a
	T2	1.81 ± 0.32^b	1.94 ± 0.09^b	1.75 ± 0.25^b	1.43 ± 0.1^b
WBC (10^3/mm^3)	Control	32.52 ± 0.41^a	34.23 ± 0.6^a	35.21 ± 0.7^a	35.54 ± 0.65^a
	T1	32.28 ± 0.77^a	32.53 ± 0.52^b	33.61 ± 0.66^b	33.97 ± 0.67^b
	T2	30.47 ± 0.50^b	30.91 ± 0.6^c	30.51 ± 0.6^c	30.47 ± 0.62^c
Hb (g/dL)	Control	5.42 ± 0.5^a	6.25 ± 0.15^a	5.50 ± 0.59^a	6.1± 0.09^a
	T1	4.93 ± 0.25^a	5.68 ± 0.3^b	5.35 ± 0.1^a	5.93 ± 0.11^a
	T2	4.39 ± 0.72^a	5.48 ± 0.12^b	5.1± 0.25^a	5.43 ± 0.15^b
PCV (%)	Control	35.42 ± 1.18^a	36.35 ± 0.75^a	37.21 ± 0.5^a	36.62 ± 0.71^a
	T1	34.59 ± 0.5^a	35.18 ± 0.52^a	34.71 ± 0.8^b	35.18± 0.82^b
	T2	32.09 ± 0.72^b	33.68 ± 0.62^b	32.81 ± 0.5^c	33.49 ± 1.05^b
MCV (fl)	Control	147.56 ± 1.9^a	144.8 ± 0.48^a	155.71 ± 1.16^a	166.32±34.33^a
	T1	156.75 ± 6.4^a	130.63±13.69^a	161.39 ± 5.53^a	138.85±24.39^a
	T2	180.62±28.46^a	173.76 ±4.87^b	189.81±24.51^a	172.71 ±2.60^a
MCH (pg)	Control	22.56 ± 1.61^a	24.9 ± 0.08^a	23.52 ± 2.20^a	27.72 ± 5.84^a
	T1	18.65 ± 6.53^a	21.1 ± 2.25^b	24.56 ± 0.78^a	29.49 ± 8.14$_b$
	T2	24.31 ± 0.52^a	28.27 ± 0.69^c	29.41 ± 2.79^b	38.04 ± 1.61^b
MCHC (%)	Control	15.28 ± 0.911^a	17.19 ± 0.06^a	15.19 ± 1.41^a	16.66 ± 0.08^a
	T1	14.25 ± 0.51^a	16.14 ± 0.13^b	15.41 ± 0.06^a	16.86 ± 0.08^b
	T2	13.66 ± 1.94^a	16.27 ± 0.06^b	15.53 ± 0.52^a	16.21 ± 0.06^c

RBC=Red Blood Cell; Hb=hemoglobin concentration; PCV=Packed Cell Volume; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; WBC= White Blood Cell/leukocyte count. Significance level tested at 5% level ($P<0.05$).

The destruction of RBC content and formation of RBC principally depend on the Hb content in cellular medium and the development of hypoxic environment (Chen *et al.,* 2004). RBC count decreased due to the prohibition of erythropoiesis and an increase in the rate of erythrocyte destruction in hemopoietic organs (Joshi *et al.,* 2002). Svoboda *et al.* (2001) reported that acute exposure to diazinon, another variety of organophosphorus pesticide, was responsible for decreasing RBC count and Hb content in *Cyprinus carpio*. Adhikari *et al.* (2004) studied a time and concentration-dependent response in *L. rohita,* chronically treated with a sub-lethal concentration of cypermethrin and noticed erythropenia and a marked decrease in Hb content, PCV and oxygen-carrying capacity of the blood.

Moreover, the fishes exposed to sub-lethal concentrations of cypermethrin the mean value of PCVs were 35.18(0.00143 mg/L), 33.68(0.00286 mg/L) % on the 7th days and 35.16(0.00143 mg/L), 33.49(0.00286 mg/L) % on the 28th days of exposure (Table 1). Lower PCV values make reduced cell size as a result of intoxication. The present study indicates that cypermethrin causes hematological changes that can interfere with oxygen uptake, which may jeopardize the animal's overall health.

Table 2. Changes in growth parameters of mirror carp exposed to Cypermethrin for 28 days. Values are the mean of three replicates ± SD.

Parameters	Control (0 mg/L)	Treatment 1 (0.00143mg/l)	Treatment 2 (0.00286mg/l)
Initial weight (g)	70.98 ±1.61	75.70±3.02	68.02±0.81
Final weight (g)	91.54±4.71	93.83± 0.92	79.7± 52
Net weight gain (g)	20.56 ±3.84	18.12 ±3.89	11.68±4.37
Weight gain (%)	29.41±5.94[a]	24.11±6.25[a]	17.12±6.19[a]
SGR (%/day)	0.91 ± 1.4[a]	0.77 ±0.18[a]	0.56 ±0.18[a]
FCR	1.96 ±0.03[a]	2.42±0.55[a]	3.56±1.22[a]

The results were similar in accordance with earlier reports that stated a significant decrease in RBCs, Hb and PCV of freshwater fish exposed to cypermethrin (Velisek *et al*., 2011; Akinrotimi *et al*., 2012; Kannan *et al*., 2014). Velmurugan *et al.* (2016) have studied *Anabas testudineus* and observed that with the increase of cypermethrin concentration, RBC counts, Hb levels and PCV levels decreased. Acute, sub-acute and chronic exposure of *C. carpio*, *H. fossilis*, *Ctenopharyngodon idella*, *Sebastes schegeli* to sublethal concentrations of cypermethrin, deltamethrin, fenvalerate, respectively, showed significantly reduced erythrocyte counts, Hb content and PCV values (Reddy and Bashamohideen, 1989; Ghosh and Banerjee,1992; Dorucu and Girgin, 2001; Jee *et al*. 2005).

In the present study, the WBC was significantly increased with increasing the toxicity of cypermethrin on the 7th, 14th, 21st and 28th days of exposure periods in both concentrations (T_1 & T_2) compared to the control group. The maximum increase was found at concentration 0.00143 mg/L as 33.97 ± 0.67 x103 µ/L on the 28th day. The increase in WBC count can be correlated with an increase in antibody production which helps in the survival and recovery of the fish exposed to lindane and Malathion (Joshi *et al.,* 2002). Since the leucocyte cells are important cells in the immune system play a major role in the defense mechanism of the fish. Thus increasing or decreasing numbers of leucocyte cells are a normal reaction to a chemical such as cypermethrin as in the present investigation. Similar results were reported in *Catla catla* (Vani *et al*., 2012), *Labeo rohita* (Adhikari *et al*., 2004), *Channa orientalis* (Shinde *et al*., 2014), *Cyprinus carpio* (Masud and Singh, 2013).

Compared to control, MCV counts showed a significant ($P<0.05$) increasing tendency (173.76fl ±4.87) at concentration 0.00286 mg/L of cypermethrin on the 14th day of exposure periods. However, slight degeneration was found for MCV counts as 130.63 and 138.85 fl on 14th and 28th days respectively. The present study found that the highest mean value (38.04 ± 1.61) of MCH recorded when treated with 0.00286 mg/L after the 28th day of exposure, while the lowest mean (18.65 ± 6.53) was recorded in 0.00143mg/L after 7th of exposure and the values were significantly increased in the last experimental days of exposure compared to control. Adedeji *et al.* (2009) reported increased values of MCV and MCH after diazinon exposure in African catfish, *C. gariepinus*.

The MCHC count of controlled and exposed fish was not significantly different from each other after 7th

days and 21st days of exposure to cypermethrin, but after 14th days and 28th days of exposure, a significant decrease in MCHC count of both experimental fish groups was observed when compared to the controlled fish group. Elevated levels of MCH and MCHC recorded in the present investigation were similar in accordance with the findings of Parma *et al.* (2007) and Atamanalp *et al.* (2002). In fish, many chemical pollutants such as pesticides can initiate anemia and the results in the present experiment support a feasible state of anemia (Min and Kang, 2008). Unusual oxidation of hemoglobin and other cellular materials resulting ROS-induced oxidative injury that may lead to anemia (Bloom and Brandt, 2008).

Growth parameters

In the present study, the toxicity effect of cypermethrin, exposed to sub-lethal concentrations for 28 days, reduced the growth of *C. carpio* significantly ($P<0.05$) as compared to control. The Initial weight, final weight and weight gain of *C. carpio* were 70.98 ±1.61g, 91.54±4.71g and 29.41±5.94 % respectively in the control condition whereas the weight gain of *C. carpio* reduced to 24.11±6.25% at 0.00143 mg/L concentrations and 17.12±6.19% at concentration 0.00286mg/L. The highest reduction for weight gain and SGR were 17.12±6.19% and 0.56 ±0.18 %/day respectively recorded at concentration 0.00286mg/L (T_2). Fish exposed to cypermethrin showed more reduction in SGR both in T_1 and T_2 compared to control where SGR 0.91 ± 1.4 %/day was observed in control, 0.77 ±0.18 and 0.56 ±0.18 %/day in T_1 and T_2 respectively. FCR increased significantly ($P<0.05$) in T_2 than T_1 compared to control where the value was 1.96 ±0.03 on 28th days exposure period. The maximum increase was observed at concentration 0.00286mg/L (T_2) as 3.56±1.22 (Table 2). Fish exposed to cypermethrin showed more reduction in weight gain (%) but showed more increase in FCR in T_2 than those treated by cypermethrin in T_1. Similar results were reported by Majumder and Kaviraj (2017), where the growth of *O. niloticus* was reduced when exposed to sub-lethal concentrations (1.25, 2.5 µg/L) of cypermethrin for 90 days. Long-term exposure of fish to pesticides induces stress and it may adversely affect fish growth, formation of protein and lipid in the fish body. The decrease in body weight might be observed due to appetite depression for longer under toxic environmental conditions periods and can result in a unambiguous influence on growth and reproduction reported by Astm (2008). Another study was carried out by Sharmila and Abhik (2013) and investigated the Sublethal effects on growth of *Channa punctatus* where fish was exposed to 1/3 and 1/10 of the 96 h LC50 values of three pesticides endosulfan, malathion and carbaryl for 21 days and resulted in decreased bodyweight of fishes in both sublethal concentrations of the three pesticides when compared with those in the control.

Conclusion

The present study demonstrated that cypermethrin was highly toxic for fishes and aquatic animals as short-term exposure to such chronic sub-lethal concentrations of cypermethrin resulted in significant hematological alterations. These changes suggest that the treated fish are faced with a serious metabolic crisis. The elevated values of RBC count, hemoglobin concentration and hematocrit values in the exposed fish are indicative of stress-mediated production of RBC and hemoglobin by the fish.

The results indicate that the usage of cypermethrin in the fields of aquaculture may be a threat to *Cyprinus carpio*. Besides, for the safe use of this pesticide more judicious control should be implemented.

Acknowledgement

The authors would like to thank the medical diagnostic center, Jharna Clinic, Jashore for helping and providing all the facilities to estimate hematological parameters of fish blood.

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