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IMPACT OF CYPERMETHRIN ON SOME HAEMATOLOGICAL PARAMETERS IN A FRESH WATER FISH, *CYPRINUS CARPIO* L.

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ABSTRACT

The present investigation was concluded to evaluate the lethality of cypermetrhin on common carp, *Cyprinus carpio.* The effect was assessed on the basis of 10,20 and 30 days exposure of its sublethal concentrations (1/5th (0.02 mg/L) and 1/10th (0.01mg/L) on some hematological parameters. Fish were sampled at 10, 20 and 30 days to test for blood analysis. There was most of the blood parameters measured such as hemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV) and MCHC was significantly decreased ($p < 0.05$), but the WBC levels were significantly increased ($p < 0.05$). Differential leukocyte counts such as lymphocyte, neutrophill and monocyte were significantly decreased ($p < 0.05$). Resulted changes in erythrocyte and leukocytes after exposing to cypermethrin are due to malfunction in haemopoiesis and in hypersensitivity of leukocytes to toxicants.

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 mechanism of toxicity (Burr and Ray, 2004). Pyrethroids generally affect central and peripheral nervous system. Cypermethrin is a class II -moderately toxic, highly active and broad spectrum, non accumulative pyrethroid insecticide, which is effective in public health and animal husbandry, and targets a wide range of pests in agriculture. 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 organisms. Fish are among the group of non-target aquatic organisms. Blood parameters are considered pathological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari *et al*., 2004). It was reported that the blood parameters of diagnostic importance are erythrocyte and leucocytes counts, haemoglobin, haematocrit and leucocyte differential counts would readily respond to incidental factor such as physical stress and environment stress due to water contaminant (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 information suggests that hematological parameters could be used as potential biomarkers of pyrethroid insecticides. Hematology of *Cyprinus carpio* has not been much documented, so this paper would provide an important contribution 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 long term exposure of 10, 20, and 30 days to 1/5 (0.02 µl/L) and 1/10 (0.01 µl/L) concentrations of cypermethrin.

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MATERIALS AND METHODS

The live healthy *Cyprius carpio* were obtained from a commercial fish farm. The mean length of the fish was

6.78 cm (range 5.0 to 8.5) and weight was 5.73 gm (range 3.8 to 7.3). The fish (n=150) were acclimated for 4 weeks. The fishes were maintained at a constant water temperature of 23 ± 1^0 C and a pH of 7-8. The fish were fed two percent total body mass twice daily, with conventional fish feed (rice bran and soya cake in 1:1 ratio) at the rate of 10 % body weight. The fish were divided into batches control and experimental. The effect of pesticide on fish becomes consistent within 96 hours exposures, LC_{50} 96 hours (0.101 mg/ L) of cypermethrin was taken as lethal concentration for *Cyprius carpio*. To study the haematological responses $1/5^{th}$ and $1/10^{th}$ (0.02 mg/L and 0.01 mg/L) of LC_{50} 96 hours was taken as sub lethal concentrations for further studies. Blood was collected by puncturing heart in vials coated with 2% EDTA, as an anticoagulant.

Estimation of Haemoglobin (Hb)

Haemoglobin content was analysed and measured to 0.0200 of blood 5.000 of the Drabkin's reagents (200mg potassium ferric cyanide, 50mg potassium cyanide and 1.0g sodium carbonate dissolved in one liter distilled water) was added, mixed well thoroughly and allowed to stand for ten minutes to ensure the completion of the reaction. The solution was read at 540nm together with standard solution of Cyanmethaemoglobin against a blank containing 5.000 of the Drabkin's reagent. The haemoglobin content was expressed as gm/dl.

Total erythrocytes count (RBC)

Erythrocytes were counted by the method of Rusia and Sood, (1992) using haemocytometer. The Neubaeur's counting chamber of the haemocytometer using counts, the total number of erythrocytes in millions per cubic millimeter of blood was calculated.

Total leucocytes count (WBC)

White blood cells were counted by the method of Rusia and Sood, (1992) using haemocytometer. The Neubaeur's counting chamber using counts, the total number of leucocytes in thousands per cubic millimeter of blood was calculated.

Estimation of HaematoCrit (Ht) or PCV

Haematocrit in percentage was estimated by microhaematocrit method described by Nelson and Morris, (1989) using micro-centrifuge and a microhaematocrit reader and the concentration of the red cells was taken as the haematocrit value which was expressed in percentage.

$$Ht\ (\%) = \frac{L_1}{L_2} \times 100$$

Where,
L_1 = is the height of the RBC column
L_2 = is the total length of the column (RBC + Plasma + buffer coat) in millimeter and expressed in percent.

Differential leucocytes count (Leucogram)

The group of granulocytes comprise leucocytes and may be basophil, eosinophil or neutrophil. The differential counts such as lymphocytes, monocytes and neutrophil were determined by blood film stained with May-Grunwald-Giesma stain.

Other blood indices

Heamatological indices such as Mean corpuscular haemoglobin content (MCHC), Mean corpuscular volume (MCV), Mean corpuscular heamoglobin (MCH), were calculated from the Hb content(%) and Hcrt (%) using the following formula proposed by Johansson – Sjobeck and Larsson (1978).

Mean Corpuscular Volume

To determine the average volume of a single red blood cell in cubic microns.

$$MCV\ (\mu m^3) = \frac{\text{Haematocrit (\%)}}{\text{Erythrocyte } (x10^6/mm^3)} \times 10$$

Mean Corpuscular Heamoglobin

To determine average haemoglobin content of a single red cell in micro- micrograms.

$$MCH\ (pg) = \frac{\text{Haemoglobin}}{\text{Erythrocyte } (x10^6/mm^3)} \times 10$$

Mean Corpuscular Heamoglobin Concentration

To determine the haemoglobin content of 100 ml of packed cells as a percentage as opposed to the percentage of the haemoglobin of whole blood.

$$MCHC\ (\%) = \frac{\text{Haemoglobin}}{\text{Haematocrit (\%)}} \times 100$$

Statistical study

The results of static bioassay were analyzed using linear regression probit analysis (Finney, 1971) using the statistical package (POLO- PC- LEORA software 1987 Haematological results were tested by using one way ANOVA (analysis of variance). Post hoc test were carried out using Tukey HSD procedure. Significance was tested at $p< 0.05$.

RESULTS

Acute toxicity

The LC_{50} values range from 0.091 (120 h) to 0.131 (12h) (Table 1). The 96h LC_{50} value (0. 101mg/l) obtained using probit analysis (Table 2) is used for fixing the two incipient lethal level exposure concentrations of 0.0202 mg/l ($1/5^{th}$ 96h LC_{50}) and 0.0101 mg/l ($1/10^{th}$ 96h LC_{50}).

Haematological Studies

The data in Table- 3 and 4 indicates that the fish exposed to two sub lethal concentrations (0.0202 and 0.0101 mg/l) of cypermethrin for 10, 20 and 30 days showed considerable variation over control.

Table 1. LC_{50} values (mg/L) of cypermethrin with their 95% confidential limits, Regression equation and Chi- square values of *Cyprinus carpio* exposed to pesticides for different durations

Hrs. of exposure	LCL (mg/L)	LC_{50} (mg/L)	UCL (mg/L)	Regression equation	Chi-square value
24	0.135	0.139	0.142	y =53.284 –55.88 x	2.42
48	0.127	0.130	0.133	y= 50.895 –51.76 x	2.08
72	0.110	0.118	0.126	y=15.278 –11.38 x	5.49
96	0.093	0.101	0.108	y =14.281 –9.35 x	2.88
120	0.082	0.091	0.099	y = 9.336 –3.94 x	1.52

LCL= Lower Confident Limit, UCL= Upper Confident Limit, LC_{50} = Lethal Concentration for 50 percent of the exposed fish.

Table 2. Log- dose/ probit regression line analysis of the response of *Cyprinus carpio* exposed to cypermethrin for 96 hrs

Dose (mg/Lit)	No.	Mor. %	Log Dose	Emp. Pro	Exp. Pro	Work Pro	Wt. Coef	Weight w	Wx	Wy	Y
0.80	10	10	0.90	3.72	3.65	3.72	0.34	3.36	3.04	12.51	3.55
0.90	10	30	0.95	4.48	4.38	4.48	0.56	5.58	5.33	25.00	4.28
0.100	10	50	1.00	5.00	5.04	5.01	0.64	6.37	6.37	31.88	4.93
0.120	10	70	1.08	5.52	6.17	5.25	0.37	3.70	3.99	19.41	6.07
0.125	10	90	1.10	6.28	6.42	6.27	0.30	3.02	3.31	18.94	6.32
0.130	10	100	1.11	7.33	6.66	7.06	0.21	2.08	2.32	14.69	6.56

STATISTICS:
SW = 24.110 SWX = 24. 356 X Bar = 1.010 SWY = 122.433 Y Bar = 5.078
SWX * X= 24.724 SWY *Y= 642.530 SWXY = 1225.145
b Value = 14.281
Regression Equation y = 14.281 x -9.35
If y = 5.0 then x= 1.005 This corresponds to dose of 0.101
Variance 0.0003 Chi- Square 2.88 (with 4 Deg. of freedom p)
Lower Limit 0.9721 Log Dose 1.0047 Upper Limit 1.0374
LCL= 0.093 UCL= 0.108

Table 3. Haematological parameters under the influence of sub lethal concentrations of cypermethrin

Parameters		Sub lethal (1/5)			Sub lethal (1/10)		
	Days	10	20	30	10	20	30
	Control	4.14±0.03[b]	4.16±0.02[b]	4.13±0.02[b]	4.14±0.03[b]	4.16±0.02[b]	4.13±0.02[b]
Hb	Exposed	3.48±0.02[b]	3.01±0.05[b]	2.41±0.03[b]	3.86±0.03[b]	3.59±0.03[b]	3.10±0.02[b]
	% change	-15.94	-27.64	-41.65	-6.76	-13.70	-24.94
	Control	1.36±0.2[a]	1.4±0.2[a]	1.41±0.2[b]	1.36±0.2	1.4±0.2	1.41±0.2[a]
RBC	Exposed	1.12±0.17[a]	1.08±0.06[a]	0.8±0.1[b]	1.20±0.01[b]	1.14±0.02	1.0±0.02[a]
	% change	-17.65	-22.86	-43.26	-11.76	-18.57	-29.08
	Control	15.22±0.40[b]	15.47±0.50[b]	15.57±0.57[**]	15.22±0.40[b]	15.47±0.50[b]	15.57±0.57[b]
WBC	Exposed	21.42±0.3[b]	28.78±0.82[b]	35.70±0.95[**]	17.2±0.72[b]	22.3±0.7[b]	30.46±1.60[b]
	% change	40.74	86.04	129.29	13.01	44.15	95.63
	Control	17.06±0.15[a]	17.2±0.2[b]	17.3±0.2[b]	17.06±0.15	17.2±0.2[b]	17.3±0.2[b]
PCV	Exposed	15.6±0.46[a]	14.18±0.33[b]	13.12±0.19[b]	16.6±0.6	15.12±0.19[b]	14.22±0.4[b]
	% change	-8.56	-17.56	-24.16	-2.7	-12.1	-17.80
	Control	127.2±17.75	124.43±16.5	124.23±16.3[a]	127.2±17.75	124.43±16.5	124.23±16.3
MCV	Exposed	141.33±19.78	131.48±4.44	165.57±18.5[a]	138.33±3.82	131.65±1.28	143.44±1.45
	% change	11.11	5.67	33.28	3.5	5.80	15.46
	Control	30.86±4.36	29.97±4.23	29.68±4.11	30.86±4.36	29.97±4.23	29.68±4.11
MCH	Exposed	31.61±5.19	27.91±0.77	30.41±3.41	32.17±0.16	31.50±0.3	31.01±0.42
	% change	2.43	-6.87	2.46	4.24	5.11	4.48
	Control	24.27±0.05[b]	24.18±0.17[b]	23.87±0.17[b]	24.27±0.05	24.18±0.17[a]	23.87±0.17[b]
MCHC	Exposed	22.34±0.52[b]	21.24±0.12[ab]	18.37±0.19[b]	23.28±0.66	23.76±0.15[ab]	21.82±0.49[b]
	% change	-7.95	-12.16	-23.04	-4.08	-1.74	-8.59

Values are means of three replicates ± SD. Column Values with different superscripts are significantly different ($p< 0.05$)

Table 4. Differential leucocytes count in common carp affected by sub lethal concentrations of cypermethrin

Parameter		Sub lethal (1/5[th])			Sub lethal (1/10[th])		
	Days	10	20	30	10	20	30
Lymphoc -ytes	Control	74.6±3.51[a]	74.6±3.51[b]	74.73±3.52[b]	74.6±3.51	74.6±3.51[a]	74.73±3.52[a]
	Exposed	69.48±1.58[a]	65.4±1.42[b]	60.52±0.99[ab]	70.84±1.21	68.04±1.7[a]	67.1±0.98[a]
	% change	-6.86	-12.39	-19.02	-5.04	-8.79	-10.21
Neutrophi-ll	Control	3.26±0.15[a]	3.33±0.15[a]	3.25±0.14	3.26±0.15	3.33±0.15	3.25±0.14[a]
	Exposed	2.72±0.11[a]	2.48±0.30[a]	2.24±0.37	2.96±0.14	2.58±0.47	2.32±0.62[a]
	% change	-16.56	-25.53	-31.08	-9.20	-22.52	-28.62
Monocyte	Control	2.54±0.13	2.57±0.15	2.56±0.15	2.54±0.13	2.57±0.15	2.56±0.15
	Exposed	2.22±0.4	2.12±0.41	1.76±0.32	2.44±0.6	2.36±0.44	2.02±0.1
	% change	-12.59	-17.51	-31.25	-3.94	-8.17	-21.09

Values are means of three replicates ± SD. Column Values with different superscripts are significantly different ($p< 0.05$).

Hemoglobin was found to be decreased in both concentrations throughout the exposure period. Maximum (43.26 %) decrease was recorded on 30 th day of 1/5th exposure while all the remaining values were (17.65%) decreased on 10 th day, and (22.86%) decreased on 20th day. 1/10th exposure showed maximum (24.94%) decrease on 30th day, (13.70%) decreased on 20thday and (6.76%) decrease on 10th day than the control. RBC count showed significant variation over control in both the concentrations. At 1/5th concentration maximum (43.26%) decrease was recorded on 30th day and minimum (17.65%) decrease was recorded on 10th day treatment. At 1/10th exposure showed maximum (29.08%) decrease on 30th day and minimum decrease (11.76%) was observed on 10th day exposure over the control values. WBC was found to be increased in both concentrations throughout the exposure period. Maximum (129.29%) increase was recorded on 30th day of 1/5th exposure while all the remaining values were (40.74%) increased on 10 th day, and (86.04%) increased on 20th day. 1/10th exposure showed maximum (95.63%) increase on 30th day, (44.15%) increased on 20thday and (13.01%) increase on 10th day than the control.

PCV values decreased in both concentrations on all exposure days with variable reduction. At 1/10th concentration (2.7%) decrease recorded on 10th day, (12.1%) decrease on 20th day and (17.80%) decrease on 30th day. At 1/5th exposure (8.56%) decrease on 10th day, (17.56%) on 20thday and (24.16%) decrease on 30th day than the control values. MCV values were above the control values. Positive at both the sub lethal exposure. MCH found (6.87%) decreased on 20th day of 1/5th exposure while all the values were above the control values. MCHC values decreased in both concentrations on all exposure days with variable reduction. At 1/10th concentration (4.08%) decrease recorded on 10th day, (1.74%) decrease on 20th day and (8.59%) decrease on 30th day. At 1/5th exposure (7.95%) decrease on 10th day, (12.16%) on 20thday and (23.04%) decrease on 30th day than the control values.

Differential Leucocytes

Lymphocyte was found to be decreased in both concentrations throughout the exposure period. Maximum (19.02 %) decrease was recorded on 30 th day of 1/5th exposure while all the remaining values were (6.86%) decreased on 10th day, and (12.39%) decreased on 20th day. 1/10th exposure showed maximum (10.21%) decrease on 30th day, (8.79%) decreased on 20thday and (5.04%) decrease on 10th day than the control. Neutrophill count showed significant variation over control in both the concentrations. At 1/5th concentration maximum (31.08%) decrease was recorded on 30th day and minimum (16.56%) decrease was recorded on 10th day treatment. At 1/10th exposure showed maximum (28.62%) decrease on 30th day and minimum decrease (9.20%) was observed on 10th day exposure over the control values. Monocyte values decreased in both concentrations on all exposure days with variable reduction. At 1/10th concentration (3.94%) decrease recorded on 10th day, (8.17%) decrease on 20th day and (21.09%) decrease on 30th day. At 1/5th exposure (12.59%) decrease on 10th day, (17.51%) on 20th day and (31.25%) decrease on 30th day than the control values.

DISCUSSION

Specific differences in haematological indices were evident. As far as values of RBC, Hb and PCV are concerned *Cyprinus carpio* showed a significant decrease in proportion to concentration of the pesticide exposure compared to control. In the present study the exposure of fish to sublethal concentrations (1/5th and 1/10th 96 hrs LC_{50}) of cypermethrin for 10, 20 and 30 days caused significant (<0.05) alterations in haematological parameters of Indian freshwater fish *Cyprinus carpio.*

In the light of the present study shows that the mean haemoglobin in the control and a decrease in the concentration of haemoglobin in blood is usually caused by the effect of toxicant like cypermethrin indicated anaemic conditions in fish due to stress-caused hemolysis. This result corroborates the findings of Ramesh and Saravana, (2010) reported in *Cyprinus carpio* exposed to chlorpyrifos under laboratory conditions inhibition of haemosynthesis. In the present study, when the fish *Cyprinus carpio* exposed to toxicant have shown a decrease in the RBC count. The decrease in the number of circulating RBCs probably reflects the physiological functioning of haemopoietic system, which is considered to be the most sensitive indicator towards environment pollutants. Akinrotimi *et al.* (2012) reported the similar findings in *Clarius gariepinus* exposed to cypemethrin, the reduction in RBCs, Hb, and PCV which are related to oxygen carrying of the blood may be due to the inhibition of erythropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in hemopoietic organs.

A significant increases (<0.05) in WBC count in the present study indicate a hypersensitivity of leucocytes to toxicants and these changes may be due to immunological reactions to produce antibodies to cope with stress induced by toxicants. Higher WBC count could be related to the inflammation of the stomach. Similar results have been reported by Venkatramanan and Santhya Rani, (2013) Significant decrease (<0.05) of monocytes count of *Cyprinus carpio* after the sublethal exposure of pyrethroid based pesticides leads to monocytopenia. The reduction in the values of monocytes reported in this study is in with the finding of Benariji and Kajendranath (1990) in *Clarius batrachus* exposed to dichlorvos. In the differential leucocyte cells count a sharp decrease was observed in the percentage neutrophills lead to neutropenia.

The erythrocyte indices like mean corpuscular volume (MCV), mean corpuscularhemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) seems to be changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with values of RBC count, hemoglobin concentration and packed cell volume. The values of erythrocyte indices were altered in *Cyprinus carpio* fishes after the exposure of sub-lethal concentrations of cypermethrin. Similar response was noted in common carp and other freshwater fish exposed to acute toxic level of pesticides (Rao, 2010).

Thus from the present study it can be concluded that the exposure of fish to cypermethrin pesticides resulted in significant alterations in haematological parameters. These alterations may negatively suppress normal growth, reproduction, immunity and even survival of fish in natural environment. And furthermore, the haematological studies provide a rapid and sensitive method for predicting the effects of sub-lethal exposure on general health and well being of fish.

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