Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 1, 2020, Pages. 1507 - 1517
Received 18 April 2020; Accepted 23 June 2020.

Effects of Sub Lethal Toxicity of Cypermethrin on Haematological Parameters of *Labeo Rohita* (Hamilton)

Berjin Beaula N[1] and Christo Queensly C[*]

[1] Research Scholar (reg.No.19113092192014) Department of Zoology, Muslim Arts College, Thiruvithancode-629174, Affiliated to Manonmanium Sundaranar University, Tirunelveli.

[*]Assistant professor, Department of Zoology, Muslim Arts College, Thiruvithancode-629174, Affiliated to Manonmanium Sundaranar University, Tirunelveli.

ABSTRACT

Pesticide pollution is an effective environmental issue throughout the world. In some cases, acute amount of different pesticides affected haematological changes of fish. The present study was designed to understand the sub lethal effects of pyrethroid cypermethrin. In this study *Labeo rohita* was subjected to the treatment with two sub lethal concentrations [$1/5^{th}$,$1/10^{th}$] of 96 hr LC_{50} cypermethrin for a total exposure periods of 10,20 and 30 days. During the experiment haematological parameters including RBC, HB, PCV, WBC, Lymphocytes, Neutrophil, Monocytes were found to decrease and MCV, MCHC, MCH were increased while compared to control. Results obtained in this study explained that sub lethal concentrations of cypermethrin stimulated stress and altered the haematological parameters of *Labeo rohita*.

Key words : Haematological, Cypermethrin, *Labeo rohita*, Sub-lethal

INTRODUCTION

Aquatic ecosystems support a substantial source of the earth's biological diversity. Aquatic biodiversity is comprehensive term that comprises freshwater ecosystems with lakes, ponds, reservoirs, rivers, streams, groundwater and wet lands. The other part of aquatic biodiversity has marine ecosystems, which makes up an ocean, estuaries, salt marshes, coral reefs, mangroves and algal colonies. Aquatic ecosystem continuously damaged with variety of agricultural chemicals such as pesticides, fungicides and other biocides are used to control crop pests in agricultural production. Due to indiscriminate use of these pesticides, water bodies like ponds, lakes, river and low lying water areas are continuously getting polluted through surface run off, sediment transportation from treated soil and direct application as

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spray for controlling pests. Pesticides may be directly and indirectly toxic, detoriate the water quality and cause ecological imbalance leading to health hazards to aquatic biota (Mahabubur Rahman and Rakibul Hasan, 2019). Aquatic environment are continuously being polluted with a variety of pollutants including synthetic pesticides. Among synthetic pesticides, Pyrethroids are widely used insecticides in houses and agriculture. Wide spread use of pyrethroids results in exposure and toxicity to non-target organisms (Neelima e*t al*.,2016). Cypermethrin is a very effective pyrethroid insecticide against several pests which are attacking cotton, cabbagebrnjal sugarcane and wheat etc. Pyrethroid pesticides are very toxic even at low concentrations on non-target aquatic organism. Fish are very susceptible to pyrethroid contamination and the rate of toxicity to fishes in a range of micrograms per litre. (Neelima *et al.,* 2017).The purpose of this study was evaluate the toxicity of cypermethrin using a non- target aquatic organism , *Labeo rohita* to assess its changes in the haematological parameters.

MATERIALS AND METHODS

Based on the acute toxicity test (96 hr LC_{50}) selected two sub-lethal concentrations ($1/5^{th}$ 96 hr LC_{50}) and ($1/10^{th}$ 96 hr LC_{50}) were obtained from cypermethrin which were used as the experimental concentration of the pesticide to this present investigation. A group of *Labeorohita* with a body weight of 8.6 ± 1.6gm were collected from stock tanks and were exposed to sub-lethal concentrations in pesticide cypermethrin [0.284 mg/l ($1/5^{th}$ 96 hr LC_{50}) and 0.142 mg/l ($1/10^{th}$ 96 hr LC_{50}]. Ten fishes were exposed to each concentration for a period of 10, 20 and 30 days. Another set of 10 fishes were simultaneously maintained in water without pesticide and considered as control.

Blood collection for Haematological studies.

Samples from both the control and test fish were collected for haematological study by puncturing the heart with a sterile Syringe with 21-G needle. During blood collection the syringe rinsed with sufficient quantity of anticoagulant heparin solution. The whole blood Samples was used for the estimation of RBC, Hb, Hct, WBC, MCHC, MCV, MCH, lymphocyte, monocyte and neutrophil. Haemoglobin content was at analysed by cyano-haemoglobin method , RBC analaysed by Neubaeurshaemocytomer, Haematocrit was estimated by micro haematocrit method using microcentrifuge and a micro haematocrit reader, while differential counts such as neutrophils, lymphocytes, and monocytes were determined on blood film stained with May - GrunwaldGiemsa stain . Other blood indices

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Such as Mean Carpuscular haemoglobin Content (MCHC), Mean Corpuscular volume (MCV), Mean carpuscular haemoglobin (MCH) were calculated from the Hb Content (%) and Hct (%) using the following formula proposed by Johansson - Sjobeck and Lassen(1978).

MCV

$$MCV\ (Um^3) = \frac{\text{Haematocrit (\%)}}{\text{Erythrocyte}(x10^6/mm^3)} \quad X\ 10$$

MCH

$$MCH\ (pg) = \frac{\text{Haemoglobin}}{\text{Erythrocyte}\ (x\ 10^6 mm^3)} \quad X\ 10$$

MCHC

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

Statistical Analysis

Data obtained for haematological parameters of *Labeo rohita* were subjected to Analysis of variance (ANOVA) using statistical package for Social Sciences (SPSS) version 19 to generate the mean and standard deviation and $p<0.05$ was considered Statistically significant.

RESULT

Acute toxicity

The table 1 shows the LC_{50} values of cypermethrin on the exposure periods from 0.219(24hr) to 1.340 (120hr).The 96 hr LC_{50} value (1.420mg/l) calculated using Probit analysis (Table 2) used for two sub lethal level exposure concentrations of 0.284mg/l ($1/5^{th}$ 96 hr LC_{50}) and 0.142mg/l ($1/10^{th}$ 96 hr LC_{50}).

HAEMATOLOGICAL STUDIES

The results in Table 3 and 4 explained the haematological alterations of *Labeo rohita* exposed to two sub lethal concentrations. (0.284 and 0.142mg/l)of 96 hr LC_{50}cypermethrin for 10,20 and 30 days exposure.

Haemoglobin contents were decreased in both concentrations throughout the exposure period compared with control. Maximum (3.30±0.18mg/l) decrease was obtained on 30 th day of $1/5^{th}$ exposure while all remaining values were (3.96±0.01 mg/l) decreased on 10^{th} day, and(3.69±0.01mg/l) decreased on 20^{th}day.At $1/10^{th}$ exposure showed maximum (2.30±0.01

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mg/l) decrease on 30th day,(3.15±0.01 mg/l) decreased on 20th day, (3.66±0.01mg/l) decrease on 10th day compared with control. RBC count recorded significant changes over control in both concentrations. At 1/5th concentration maximum (1.06±0.02mg/l) was identified on 30th day and minimum (1.24±0.09mg/l) was recorded on 10th day treatment. At 1/10th exposure showed (0.8±0.04mg/l) decrease on 30 th day and decrease (1.20±0.05mg/l) on 10th day exposure over the control. PCV values decreased in both concentrations on all exposure days with reduction. At 1/5th concentration (17.07±1.06mg/l) decrease recorded on 10th day (16.3±1.02mg/l) decline on 20th day and (15.54±1.06mg/l) decline on 30th day. At 1/10th concentration (16.27±1.04mg/l) decline on 10th day,(15.90±1.05mg/l) decline on 20thday,and 14.64±1.06mg/l) was observed on 30th day when compare with control. The total leucocytes (WBC) were found to be decreased in both concentrations throughout the exposure period. At 1/5th concentration (18.01±1.79) was recorded on 10 th day, (15.90±1.34) was recorded on 20th day,(13.28±1.30) was observed on 30th day treatment. At 1/10 th concentration maximum (12.40±0.02) decreased on 30th day, remaining values was observed (16.15±1.19) on 20th day, (17.60±1.84) decrease on 10th day treatment compare with control. Lymphocytes were observed in decreased both concentrations throughout the (63.42±0.14) decrease on 20th day and (68.16±0.01) decrease on 10th day treatment. At 1/5th concentration (73.42±0.22) was recorded on 10thday,(71.36±3.15) was observed on 20th day and (65.60±3.05) was recorded on 30 th day compared with control values . Neutrophil maximum (2.82±0.02) decline on 30th day and minimum (3.08±0.19) on 10thday.At1/10th concentration maximum (2.29±0.02) decrease on 30th dayminimum (2.98±0.27) decrease on 10th day treatment with control values. Monocyte values decreased in both concentrations on all exposure days with reduction. At 1/10th exposure (1.96±0.09) decline on 30 th day ,(1.98±0.07) was observed on 20 th day and (2.04±0.06) was recorded on 10 th day. At 1/5thexposure (2.48±0.04)decrease on 10th day,(2.38±0.01) was recorded on 20th day ,(2.16±0.03) was observed on 30th day decreased compared with control. MCV, MCHC and MCH values were increased in both concentrations of cypermethrin.MCV values found (144.98±2.75) increased on 10th day ,(14.52±2.77) was recorded on 20thday and (140.48±5.20) increased on 30 th day at the concentration of 1/5.At 1/10 th concentration (119.68±2.26) on 30thday,(127.7±3.04) was observed on 20thday and (132.86±3.17) was recorded on 10thday increased with control values.MCH values increased with control values. At 1/5 th concentration (30.38±0.44) on 30 th day,(32±0.48) on 20thday,(31.28±0.58) on 10th was observed with treatment .At 1/10thconcentration (31.82±0.40) was recorded on 10thday,(27.56±0.65) was observed on 20thday and (28.94±4.91) was recorded on 30thday

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increased compared with control values. MCHC values also increased in both concentration of exposure compared with control. At 1/5thconcentration (23.86±0.78) was recorded on 10thday,(22.46±0.27) was observed on 20th day and (21.80±0.79) increase on 30thday.At 1/10thconcentration (20.46±0.51) was observed on 30 th day,(22.34±0.13) was observed on 20thday and (23.20±0.1) on 10 th day treatment.

TABLE 1

LC_{50} values (mg/l) of cypermethrin with their 95% confidential limits, Regression equation and chi-square values of *Labeo rohita* 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	0.219	2.568	0.299	Y=7.196X-5.14	0.69
48	0.174	1.879	0.201	Y=12.410X -10.81	4.15
72	0.159	1.720	0.185	Y= 16.297X -15.14	0.82
96	0.139	1.420	0.162	Y=18.946X -17.32	3.63
120	0.108	1.340	0.165	Y=3.980X -10.51	6.60

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 *Labeorohita* exposed to cypermethrin for 96 hrs.

Dose (mg/l)	No	Mor %	Log Dose	Emp Pro	Exp Pro	Work Pro	Wt Coef	Weight W	Wx	Wy	Y
1.20	10	10.00	1.08	3.72	3.39	3.81	0.24	2.38	2.57	9.07	3.13
1.40	10	30.00	1.15	4.48	4.66	4.49	0.62	6.16	7.06	27.63	4.39
1.60	10	50.00	1.20	5.00	5.76	4.81	0.50	5.03	6.06	24.17	5.49
1.80	10	100.00	1.26	7.33	6.73	7.06	0.21	2.08	2.61	14.69	6.46

STATISTICS

SW = 15.650 SWX = 18.296 X Bar = 1.69
SWY = 75.568 Y Bar = 4.829
SWX*X =21.433 SWY* Y =378.466 SWXY = 89.006

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b value = 18.946
Regression Equation Y = 18.946 X - 17.32
If Y = 5.0 then X =1.178 .This corresponds to dose of 1.420 Variance 0.0003 Chi-square 3.63 (with 2 Deg. Of freedom p)
Lower Limit 1.1447 Log Dose 1.1781
Upper Limit 1.2116
LCL = 0.139 UCL = 0.162

TABLE 3

Haematological parameters under influence of sub lethal concentrations of cypermethrin

PARAMETERS	DAYS	SUB LETHAL 1/5			SUB LETHAL 1/10		
		10	20	30	10	20	30
HB	CONTROL	4.23±0.03	4.18±0.02	4.14±0.01	4.23±0.03	4.18±0.02	4.14±0.01
	EXPOSED	3.96±0.01	3.69±0.01	3.30±0.18	3.66±0.01	3.15±0.01	2.30±0.01
	% CHANGE	(-6.81)	(-13.27)	(-25.45)	(-15.57)	(-32.69)	(-80)
RBC	CONTROL	1.30±0.02	1.42±0.02	1.47±0.02	1.30±0.02	1.42±0.02	1.47±0.02
	EXPOSED	1.24±0.09	1.18±0.04	1.06±0.02	1.20±0.05	1.12±0.06	0.8±0.04
	%CHANGE	(-4.83)	(-20.33)	(-38.67)	(-8.33)	(-26.78)	(-83.75)
WBC	CONTROL	19.22±0.03	19.23±0.23	19.28±0.22	19.22±0.03	19.23±0.02	19.28±0.22
	EXPOSED	18.01±1.79	15.90±1.34	13.28±1.30	17.60±1.84	16.15±1.19	12.40±0.02
	%CHANGE	(-88.70)	(-20.94)	(-45.18)	(-9.20)	(-19.07)	(-55.48)
PCV	CONTROL	17.10±0.2	17.8±0.2	17.4±0.02	17.10±0.2	17.8±0.2	17.4±0.02
	EXPOSED	17.07±1.06	16.3±1.02	15.54±1.06	16.27±1.04	15.9±1.05	14.64±1.06
	%CHANGE	(-0.17)	(-9.20)	(-11.96)	(-5.10)	(-11.94)	(-18.85)
MCV	CONTROL	127.74±1.03	126.26±2.98	125.4±2.29	127.74±1.03	126.26±2.98	125.4±2.29
	EXPOSED	144.98±2.75	141.52±2.77	140.48±5.20	132.86±3.17	127.7±3.04	119.68±2.26
	%CHANGE	(11.8)	(10.78)	(10.73)	(3.85)	(28.82)	(-4.770
MCH	CONTROL	30.38±0.44	28.48±0.43	27.2±0.65	30.38±0.44	28.48±0.43	27.2±0.65
	EXPOSE	31.28±0.5	32±0.48	30.38±0.4	31.82±0.4	27.56±0.6	28.94±4.9

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	D	8		4	0	5	1
	%CHANGE	(65.84)	(11)	(10.46)	(4.52)	(-3.33)	(6.01)
MCHC	CONTROL	22.52±0.13	21.54±0.11	21.44±1.14	22.52±0.13	21.54±0.11	21.44±1.14
	EXPOSED	23.86±0.78	22.46±0.27	21.80±0.79	23.20±0.13	22.34±0.13	20.46±0.51
	%CHANGE	(5.61)	(4.09)	(1.65)	(2.93)	(3.58)	(4.78)

TABLE 4

Differential Leucocytes Count in Rohu affected by Sub lethal concentrations of cypermethrin

PARAMETERS	DAYS	SUB LETHAL ($1/5^{th}$)			SUB LETHAL($1/10^{th}$)		
		10	20	30	10	20	30
Lymphocytes	CONTROL	75.80±3.51	75.70±3.51	75.72±3.52	75.80±3.51	75.70±3.51	75.72±3.52
	EXPOSED	73.42±0.22	71.36±3.15	65.6±3.05	68.16±0.01	63.42±0.14	61.14±0.02
	%CHANGE	(-3.24)	(-6.08)	(-15.42)	(-11.20)	(-19.36)	(-23.84)
Neutrophil	CONTROL	3.35±0.02	3.30±0.02	3.30±0.02	3.35±0.02	3.30±0.02	3.30±0.02
	EXPOSED	3.08±0.19	2.96±0.13	2.82±0.02	2.98±0.27	2.77±0.02	2.29±0.02
	%CHANGE	(-8.76)	(-11.48)	(-17.02)	(-12.41)	(-19.13)	(-44.10)
Monocyte	CONTROL	2.58±0.01	2.60±0.02	2.60±0.02	2.58±0.01	2.60±0.02	2.60±0.02
	EXPOSED	2.48±0.04	2.38±0.01	2.16±0.03	2.04±0.06	1.98±0.07	1.96±0.09
	%CHANGE	(-4.03)	(-9.24)	(-20.37)	(-26.47)	(-31.31)	(-32.65)

DISCUSSION

Behavioural effects of cypermethrin

In the present study, the fish showed some abnormal behaviours when they exposed to cypermethrin pesticide .The abnormal behaviours were decolourized, scales sheddening, protruding eyes, restlessness. Similar abnormalities investigated by Khatun *et al.*, (2015).They reported abnormal behaviours such as restlessness, asymmetrical swimming activities, motionlessness and loss of equilibrium due to exposure on cypermethrin. Nasir khan *et al*., (2018) explained above similar behaviours on *Labeo rohita* due to exposure cypermethrin.

Haematological effects of cypermethrin on *Labeorohita*

Acute and sub-lethal toxicity tests were commonly used to assess the toxicity of chemicals on non- target animals .The 96hr LC_{50} is one of the most important factors for

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evaluating the toxic effects of contaminants. The 96hr LC_{50} value of cypermethrin was found to be 1.420 mg/l which suggest that the pesticides are toxic to fish. In the present study the exposure of the fishes to sub-lethal concentration ($1/5^{th}$ and $1/10^{th}$ 96 hr LC_{50}) of cypermethrin for 10, 20 and 30 days exposure caused significant (0.05) alterations in haematological parameters of Indian fresh water *fish Labeo rohita*. There was a decrease in RBC, Hb content and PCV, WBC, lymphocytes, neutrophil monocytes Observed with dose dependent manners. Anita Bhatnagart (2017) reported RBC count, Hb level and Hct showed decreasing with concentration and exposure of cypermethrin which might be attributed to the decreased blood cell production rate.

Oruc (2019) explained kidney damages cause a decrease in blood cell production level which turn in RBC count, Hb Concentration, and haematocrit values by the action of cypermethrin. The present findings are similar to the reports (Saravanan *et al.*, 2017; Maurya *et al*., 2019; Akter *et al.*, 2020). Further changes in blood cell indices like MCH, MCV, MCHC were also observed in the present study. This may be due to the fact that these are very sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices directly concerned with the values of RBC count, Hb concentration and PCV reported by (Anita Bhatnagart *et al*., 2017). In the present investigation the total WBC exhibited decreased in sub-lethal concentration of cypermethrin.

Zubais Ahmad (2016) investigated the total leukocyte count was decreased which might be due to abnormal functioning of the haematopoietic system. Changes in the leukocyte system manifest in the form of leukocytosis with heterophilia and lymphopenia which are characteristics of leukocytic response in animals exhibiting stress. A decreased in WBC count were found two concentrations of cypermethrin exposed on *Labeorohita* studied by Khutan*et al.,* 2015). Similar observations by Dawood *et al.,* (2020) and KonDera *et al.,* (2018) reported decreased level of WBC for different toxicants. Al-kahem (2019) reported reduction in the number of lymphocytes in trichlorfun exposed *O. niloticus*. Sathyianarayana (2020) noticed that the decrease the count of neutrophils exposure of cypermethrin in*Cyprinuscarpio*. Akintromini *et al.,* (2019) reported reduction of monocytes in juveniles of C*lariasgariepinus* the exposure of cypermethrin.

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Conclusion

The haematological parameters of the fish is altered due to pesticide toxic stress. The RBC content of the blood is important to carry oxygen to the venous heart and in turn the increase of WBC is a good sign of the fish, an immunological response. But both erythrocytopenia and leucocytosis are threatning to reflecting changes in other blood parameters. The variation in these parameters may provide early warning signals for the determination of toxic levels of pesticides used in the field and its effects on the aquatic organisms. Hence, discharge of this pesticide in the aquatic ecosystems should be restricted in order to reduce its risk to fishes as well as humans.

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