EFFECTS ON HEAMATOLOGICAL PARAMETERS IN INDIAN MAJOR CARP, *LABEO ROHITA* EXPOSED TO CYPERMETHRIN

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Abstract: Heamatology is defined as the branch of biology, which deals with the morphology of blood and blood forming organs. Heamatological profiles of blood can provide important information about the internal environment of an organism and also an indicator of physiological condition of an animal. Thus, the study was conducted to investigate the heamatological parameters of fish, *Labeo rohita.* The effect of the pyrethroid pesticide, on *Labeo rohita* was assessed based on heamatological examination of fish exposed to 10 % EC Cypermethrin at sublethal concentration and lethal concentration respectively 0.03 ppm and 0.06 ppm. Haematologycally, fish was showed a significant decrease ($P < 0.05$) in Hb, RBC, WBC, PCV, MCV, MCH, MCHC compared to the control group. Thus the study reflects the extent of the toxic effects of Cypermethrin induced cumulative deleterious effects at various functional levels in the widely consumed freshwater fish, Labeo rohita.

Key Words: Effects, Cypermethrin, Heamatological Parameters, *Labeo rohita*

Introduction: With the growth of the modern civilization, our life is threatened due to pollution of water both from surface and underground. In our country, the scarcity of pure drinking water is so much felt that 50% of urban people and 80% of rural people are affected by water pollution. With the advent of Green Revolution in the second half of the 20th century when farmers began to use technological advances to boost yields, synthetic fertilizers, pesticides and herbicides became common place around the world not only on farms but in backyard gardens and on front lawns as well. A number of hazardous chemicals are being introduced directly or indirectly into the aquatic environment and pyrethroid as insecticide has been reported as one of the most alarming chemicals [1]. Cypermethrin a common synthetic pyrethroid, an important environmental contaminant, is present in the aquatic environment as a result of anthropogenic processes, through agricultural run offs, industrial effluent and other sources [2]-[3] studies have shown that contamination of water bodies and aquaculture facilities had great consequences for the fish species in these environment [4]-[5]. Cypermethrin is highly toxic to fish and 96 hr – low lethal concentration (96hr LC_{50}) of $8\mu g l^{-1}$ and $10\mu g l^{-1}$ has been reported for rainbow trout and common carp respectively [6]-[7].

Fish heamatology is a possible means of estimating the health status of fish stock [8]. Blood is a major circulating medium. It transports different substances from one organ to another [9]. Being very essential for assessing the effects, RBC count, Hb, WBC count, Hct, MCV, MCH, MCHC and change in erythrocytes were incorporated in the present study. In assessing the toxic effects of chemicals in aquatic organisms the use of heamatological techniques has become more useful in recent times, as a result of the intimate relationship between fish and its aqueous environment [10]-[11]-[12]. Sampath et al. [13] observed that heamatological studies in fish, lies in the possibility that the blood will reveal anomaly within the body of the fish long before there is any outward manifestation of symptoms of disease or effects of unfavorable environmental factors. Despite a number of studies on the effects of toxicants on hematology of *Labeo rohita* little is known on the heamatological changes that *Labeo rohita* may suffer under exposure to cypermethrin, a pesticide commonly applied in agriculture operations. The present study was carried out to assess the effect of exposure of *Labeo rohita* to cypermethrin on its heamatological parameters under laboratory conditions.

Methodology: Healthy and active fresh water fish *L. rohita* having weight of 8 ± 2 gm and size of 9 ± 2 cm were procured from the Krishna Fisheries, Sayan village of Surat district, India. Before investigation fish were maintained atleast for 10 days in large glass aquaria containing chlorine free tap water. All aquaria were kept in the laboratory in cool place and covered with nets to prevent the escape of animals and the foreign particles falling in. Dead specimens were removed immediately. Water was renewed every day and fish were fed daily with commercial dried feed pellets and fine powder of rice bran and oil cake.

Cypermethrin (10% EC) was obtained from Heranba Industries Limited, Vapi, Gujarat, India and used to check its impact on fish.

The median tolerance limit (TLm-96 hrs) of cypermethrin for the freshwater fish, *L. rohita* was determined in the laboratory through static renewal bioassay test using standard method as described in APHA, [14].

First, the animals were exposed to different concentrations for range finding test where the

lowest concentration at which 0 % and the highest concentration for 100 % mortality occurred. Based on these, the concentrations of 0.02, 0.04, 0.06, 0.08 and 0.1 ppm were selected for TLm-96 hrs exposing ten fishes of same size in each aquarium.

No feed was given before 24 hrs and during the bioassay test. Test medium was renewed for every 24 hrs with their respective test concentrations of the toxicant without aeration. Dead specimens were removed immediately as and when found and mortality rate was recorded at the end of 24, 48, 72 and 96 hrs. The median tolerance limit (TLm-96 hrs) was obtained by plotting graph with concentration series against the mortality rate for 96 hrs.

Based on lethal concentration (0.06 ppm) three different concentrations viz, sublethal, lethal and acute lethal of Cypermethrin were selected to study the damage caused in tissues through different parameters. About 15–20 fishes were exposed each time in aquaria of 100 liters capacity. One set of normal fishes was maintained under the same condition except the addition of pollutant for control study.

Experimental fishes were removed from aquaria, blotted in paper to avoid contamination with mucous. Blood was collected by puncturing heart using sterilized disposable plastic syringe and was transferred immediately into pretreated vials (anticoagulated with 0.02 ml of 10% EDTA) and was mixed gently.

The haematological variables viz; red blood cell (RBC) counts, Haemoglobin (Hb), white blood cell (WBC) counts, Heamatocrit value (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated according to the methods of Wedemeyer and Yusutake, [15] and Sahoo, [16].

Results: Table 1.1 depicts the mortality and survival of *Labeo rohita* exposed to different concentrations of Cypermethrin. Concentration at which minimum mortality (30 %) of *Labeo rohita* recorded was 0.04 ppm and the maximum concentration where 100 % mortality noted was 0.1 ppm. No mortality occurred with 0.02 ppm of Cypermethrin exposure. Low rate death of fishes was noted during the initial period upto 48 hrs. The exposure of *L. rohita* to Cypermethrin treatment was most critical at 96 hrs (Table 1.1). Lethal concentration (0.06 ppm) with 50 % mortality of fishes was observed and calculated (Table 1.1).

Haematological parameters like Hb (Hemoglobin), RBC (Red Blood Cell), WBC (White Blood Cell), PCV (Packed Cell Volume), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration) were observed from normal as well as treated fishes with Cypermethrin. Results after exposing fish *Labeo rohita* to sublethal (1st day, 2nd day, 4th day and 8th day) and lethal (1st day, 2nd day, 4th day and 6th day) concentrations of Cypermethrin as described here

Results of haemoglobin showed significant reduction at sublethal and lethal concentration. At sublethal concentration, 1st day recorded decrease 5.75 (11.54 %), 2nd day 5.05 (22.31 %), 4th day 4.85 (25.38 %) and 8th day 4.70 (27.69 %) are shown in table 1.2, the gradual decrease was recorded and the P value was found 0.067 where the significance level was > 0.05. The lethal concentration at day 1st 5.85 (10 %) day 2nd 5.20 (20 %) 4th day 4.75 (26.92 %) and 6th day 4.00 (38.46 %) showed higher reduction as depicted in table 1.2 and the P value was found 0.067 where the significance level was > 0.05. Results of total erythrocyte count indicated alteration at sublethal and lethal concentration. Reduction with sublethal concentration at day 1st 1.18 m/cu mm (2.48 %), day 2nd 1.06 m/cu mm (12.40 %), day 4th 0.96 m/cu mm (20.47 %), day 8th 0.88 m/cu mm (27.27 %) were recorded and are shown in table 1.2. The lethal concentration showed the reduction to 1.11 m/cu mm (8.26 %), 0.99 m/cu mm (18.18 %), 0.84 m/cu mm (30.58 %) and 0.79 m/cu mm (34.72 %) at the end of first, second, fourth and sixth day respectively. Findings are shown in table 1.2. The P value was > 0.05. Number of WBC showed slight gradual reduction. Table 1.2 represents the results where sublethal concentration showed maximum reduction in WBC by 7 % at the end of 8th day. The exposure of fish to lethal concentration of Cypermethrin represented 13 % at the end of 6th day. The WBC count was found to be altered during the different exposure period but the changes were statistically insignificant (P > 0.05).

Like RBC, values of PCV were also gradually decreased. The sublethal concentration showed maximum reduction as 9.36 % at the end of eighth day. Lethal concentration brought the reduction as 15.71 % at the end of the experiment. Gradual significant increase were noted in MCV value. Treatments of sublethal and lethal concentrations registered 25 % of increase at the end of experiments (Table 1.2).

MCH of haematological parameters did not show the consistency throughout the experiments with all the treatments. Results are shown through table 1.2.

MCHC showed the gradual decrease upto 23 % with sublethal exposure, 21.43 % at the end of 6th day with lethal concentration of Cypermethrin.

Discussion & Conclusion: Pyrethroids are preferred above organophosphates, carbamates and organochlorines as these have high efficiency, low toxicity and easy biodegradability [17]. For more than 30 years, pyrethroids are in use for home formulations and agricultural purposes and these

insecticides cover nearly one-fourth of the worldwide market [18]. In the last decade, their use has been increased [19]. Cypermethrin, synthetic pyrethroid, lipophilic in nature, is considered to be less toxic due to its speedy insect killing properties and having low toxicity to mammalian tissues [20]. Haematological parameters are important for the assessment of various systemic functions and health of animals under various environmental conditions importantly, for diagnosis of drugs and chemicals [21]. In the present experiments with *Labeo rohita*, significant differences were observed in the levels of RBC, WBC, Hb, PCV, MCV, MCHC and MCH. On the other hand, Atamanalp *et al.,* [22] and Atamanalp and Yanik [21] also found a significant decrease in the levels of RBC, Hb, MCH, MCHC, thrombocyte count and erythrocyte sedimentation rate in rainbow trout (*Oncorhynchus mykiss*) following Cypermethrin and Mancozeb acute exposure. The MCV, MCH, MCHC, Values are completely depend upon the factors of PCV, RBC count and hemoglobin concentration. In the present study, the PCV, RBC and hemoglobin concentration was completely altered affective the values of MCV, MCH and MCHC. Altered MCV, MCH and MCHC values were observed in *Channa punctatus* under Cypermethrin toxicity. The decrease in hematological indices could be due to macrocytic hypochromia, iron deficiency and increased haemolysis [23]. The quantitative haematological evaluations of present work show the similar trend and supportive decrease in RBC and Hb content in the present study are also comparable to those reported by Chen, [24] for catfish *Clarias leather* exposed to phenol. The decrease in RBC and Hb concentration indicate acute anemia. The fall in haematological parameters might be due to decreased rate of production and to an increased loss of destruction of RBC [25]. Another reason for RBC suppression could also be the damage to the haemopoietic tissue. PCV appeared to be positively correlated with RBC counts, hence, a decrease in PCV was observed. Similar results have been reported for several freshwater fishes exposed to pesticides [26]-[27].

Table 1.1: Mortality of *L. rohita* exposed to Cypermethrin

S.N.	Concentration of Cypermethrin (ppm)	No. of fishes exposed	Mortality in Numbers				Mortality in %	Survival rate (%)
			24 hrs	48 hrs	72 hrs	96 hrs		
1.	0.02	10	00	00	00	00	00	100
2.	0.04	10	00	00	01	02	30	70
3.	0.06	10	00	00	02	03	50	50
4.	0.08	10	01	01	01	03	60	40
5.	0.10	10	01	01	03	05	100	00

Table 1.2: Haematological parameters of *L. rohita* exposed to Cypermethrin

S.N.	Parameter	Normal Value	Exposed to								P Value
			Sublethal				Lethal				
			1st day	2nd day	4th day	8th day	1st day	2nd day	4th day	6th day	
1.	Hb %	06.50	05.75	05.05	04.85	04.70	05.85	05.20	04.75	04.00	>0.05
	Reduction in percentage		11.54	22.31	25.38	27.69	10.00	20.00	26.92	38.46	
2.	RBC m/cu mm	01.21	01.18	01.06	00.96	00.88	01.11	00.99	00.84	00.79	>0.05
	Reduction in percentage		02.48	12.40	20.67	27.27	08.26	18.18	30.58	34.72	
3.	WBC$x10^3$/cu mm	25.15	24.70	24.15	23.77	23.36	24.22	23.27	22.45	21.84	>0.05
	Change in percentage		01.79	03.98	05.49	07.12	03.70	07.48	10.74	13.16	
4.	PCV %	22.65	22.30	21.91	21.53	20.53	22.17	21.35	19.65	18.67	>0.05
	Change in percentage		01.55	03.27	04.94	09.36	02.12	05.74	13.25	15.71	
5.	MCV μ^3	187.97	189.79	207.57	221.00	234.65	199.75	216.76	233.89	236.32	>0.05
	Changes in percentage		00.96	10.43	17.57	24.83	06.27	15.32	24.43	25.72	
6.	MCH pg	53.94	48.95	47.88	50.50	53.82	52.72	52.79	56.55	50.63	>0.05

	Change in percentage		09.25	09.38	06.38	00.22	02.26	02.13	04.84	06.14	
7.	**MCHC %**	28.70	25.79	23.06	22.54	22.93	26.39	24.35	24.18	21.43	>0.05
	Change in percentage		10.14	19.65	21.46	20.10	08.05	15.16	15.75	25.33	

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