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Haematological changes in the fish Cyprinus carpio exposed to a synthetic pyrethroid [Class I], Permethrin and its 25% EC

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ABSTRACT - *The fish Cyprinus carpio exposed to Permethrin technical grade and 25% EC resulted alterations in RBC, WBC, HB, PCV, MCV, MCH and MCHC parameters in both lethal and sub lethal concentrations. The percent changes observed are 44%, 61%, 36%, 44%, 130%, 126% and 99% for technical grade and 75%, 104%, 98%, 89%, 107%, 115% and 107% for 25%EC respectively in lethal concentrations of the parameters studied. Similarly the changes are 65%, 120%, 85%, 80%, 115%, 122% and 106% for technical grade and 52%, 127%, 88%, 76%, 105%, 118% and 111% for 25%EC in sub lethal exposure. These changes are the indices of toxicant action in the fish which is linked to human beings in energy circuit.*

Keywords: - *Blood, Hb, PCV, Permethrin, MCV, MCH, MCHC, RBC, and WBC.*

I. INTRODUCTION

Fish, poikilothermic, stenothermal and heterotrophic animals inhabiting aqueous medium which is very close when compared with other animals living in other environment. The teleosteon fish, actively swim in the water and is subjected to any change whenever the quality of medium changes.

Pollutants entering the environment by any mean change the physical and chemical characters of ambient water and such changes are immediately perceived by the organism's blood and pave the way to have changes in this circulating fluid. The two chambered heart popularly known as venous heart in fish, alterations in the blood parameters reflect on blood circulation. The changed blood constituents due to contaminants and their count really serve as indices of the toxic stress due to toxicant as pollutant.

Death may not be resulted in the sub lethal concentrations but such latent concentrations do reflect in the blood parameters. According to the report by [1] in the fish Siamese fighting fish, *Betta splendens*, both in quality and quantity mentioned about the cell sizes as Thrombolytic: 4.7-5.6 microns, Lymphocyte: 7.4-8.4 microns, Monocyte: 7.0 -17 microns, Eosinophil: 7.4-8.4 microns and Neutrophils: 100-200 microns.

Reference [2], reported that the morph metric studies of blood cells in *Cyprinus carpio* along with two others, the sizes showed differences. The size differences in the other commonly cultured fish and this numerical objection of the most suitable modifications that can help in research application in cytological and histopathological studies.

Among the aquatic organisms fish occupy an important position in the field of aquatic toxicology [3]. Fish in close association with their environment would be reflected in alterations in their haematological studies [4].

Various stressors particularly the toxicant pollutants generally cause rapid changes in the blood characteristics of fish [5]. Among such, the pesticides xenobiotics compounds, even in low concentration, i.e., sub lethal may bring about changes in haematological parameters [6]. Such alterations serve as important toxicological tool and have been widely used as indices for environmental monitoring and disease diagnosis and environmental stress [7], [8]. Reference [9] considered that evaluation of haematological characteristics of blood in fish has become an important means to understand normal and pathological processes and toxicological impacts.

Reference [10] opined that the blood parameters are considered as pathological indicators of the whole body and therefore are important in diagnosing the structural and functional status of the fish exposed to the toxicants. Even reference [11] reported that Haematocrit values are the indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in animals.

The blood parameters are important for maintenance of any physiological activity [12]. Studies of the effects of pesticides on aquatic organisms enable the establishment of maximum acceptable concentrations, as permissible limits in the environment without causing significant damage to the resident biota, including fish. Such studies are therefore indispensable for assessing risks and outlining guidelines for the use of pesticides [13]. Any such things as changes in blood are considered potential biomarkers of exposure to

chemical agents, since the later can induce changes; as an increase or decrease in the various haematological components [14]. Reference [15] stated that the haematology of any animal is widely used as potent bioindicator in aquatic toxicology.

Hence the present study an attempt is made to study the changes in the blood parameters of the fish *Cyprinus carpio* exposed to widely used Permethrin a synthetic Pyrethroid which belongs to the class I type.

II. MATERIALS AND METHODS

The fresh water common carp, *Cyprinus carpio* is an edible and economically important fish was selected with a range of size about 3 to 5 cm and 3 to 6 grams of weight, irrespective of their sex, have been chosen as the test organisms for present investigation. Healthy and active fish were obtained from Ratna Singh Hatcheries, Kuchipudi, Guntur (A. P), India. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28 ± 1º C. Water was renewed every day with 12-12 h dark and light cycle. During the period of acclimatization, the fish were fed (*ad libitum*) with groundnut oil cake and rice bran. Feeding was stopped one day prior to the actual toxicity test. All the precautions laid by committee on toxicity tests to aquatic organisms [16] were followed and such acclimatized fish only were used for experimentation. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded. The fish are exposed to 1/10th (96 h) of LC50 value for 10 days both in technical and 25% EC of Permethrin. From such exposed fish the survived fish blood sample were taken for analysis.

2.1 SAMPLING OF BLOOD

Fish were euthanized by an overdose of MS-222 and then weighed and measured. Blood was sampled by caudal severance from the disease free test fish during the early hours of the day and stabilized with 50 IU sodium heparin (anticoagulant)/ml blood.

2.2 EMATOLOGICAL EXAMINATION

The haematological variables analyzed were red blood cells count (RBC), haemoglobin (Hb), white blood cells count (WBC), haematocrit (Ht), mean corpuscular volume (MCV) mean corpuscular haemoglobin concentration (MCHC). RBC count was determined with an improved Neubauer crystalline counting chamber as described by [17]. The blood was sucked up to 0.5 marks on the RBC pipette and immediately, Hayem's solution as a diluent stain was drawn up to 101 mark and the pipette was rotated between the thumb and the forefinger. WBC count was determined by following the method described [18]. The blood was drawn up to 0.5 mark of WBC pipette and immediately the diluting fluid was drawn up to 101 marks above the bulb. The solution was mixed thoroughly by shaking gently and allowed to stand for 3 min. The WBC was counted in the four corner square millimetres and the number of WBC per cubic millimetre was calculated.

Hb concentration in the blood was estimated by cyanmethaemoglobin method as described by [19]. Hb is converted into cyanmethaemoglobin by the addition of potassium ferricyanide (KCN) and the colour was read in a spectrophotometer at 540 nm against a reagent blank. Packed cell volume was determined by micro haematocrit method of reference [20]. The heparinised blood was filled up to the mark 100 of the haematocrit tube with the help of Pasteur pipette and centrifuged at 3000rpm for 30 min. The relative volume of the height of the RBC'S packed at the bottom of the haematocrit tube was recorded as packed cell volume in terms of percentage of total blood column taken in the haematocrit tube. The Mean Corpuscular volume (MCV); Mean Corpuscular Haemoglobin (MCH); Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated from Haematocrit, RBC count, Haemoglobin values.

III. RESULTS

1. The results of the haematological parameters are found to be time dependent. In the present study the effect of exposing the fish *Cyprinus carpio* to lethal (96 h) and sub lethal (1/10 of LC_{50} value for 10 days) concentrations of Permethrin are shown as follows. The blood parameters changes associated were presented in table 1 and figure 1 for technical grade Permethrin and 25% EC along with standard deviation and percent change over control.

The total erythrocyte count showed alterations at both lethal and sub lethal concentrations of Permethrin in both technical grade and 25% EC. The RBC count was 5.96 million /cu mm in the blood of control fish and this count was decreased to 2.64 millions/ cu mm at lethal concentration at 96 h of exposure and 3.86 millions/ cu mm sub lethal concentration in technical grade at 10 days exposure period. The decreasing trend was the function of exposure period and time. When the control RBC count was compared to the lethal and sub lethal concentrations in 25% EC a slight decrease are observed as 72.1% and 51.6%

The percentage change in the RBC count was also calculated at lethal and sub lethal concentration in technical grade, maximum reduction was of 44.2% & 64.7% observed at 96 h exposure period over control. In 25% EC of Permethrin, the percentage change in the RBC count was also calculated at lethal and sub lethal concentrations over control were 72.1% and 51.63 %. The WBC count altered specifically and the WBC count exhibited a different trend when compared to the RBC count. It exhibited a slight increasing trend at sub lethal concentrations. At lethal concentration in technical grade for 96 h exposure period slight decrease was observed as 14.24 cells/ cu.mm and 61.1%. In sub lethal concentration of Permethrin in technical grade, WBC count increased as 28.02 cells/

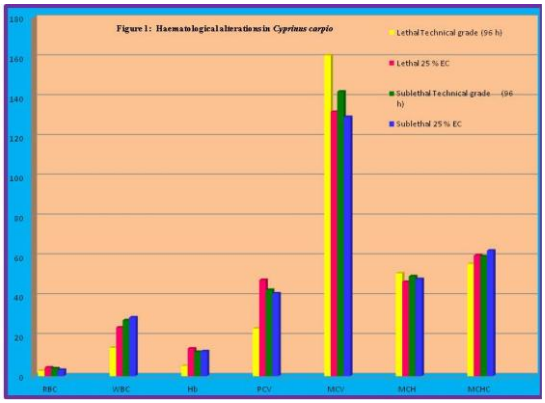
cu.mm and 120.7%. When the control WBC count was compared to the lethal and sub lethal concentrations in 25% EC an increment was observed as 104% and 126.5%.The haemoglobin content in blood sample of *Cyprinus carpio* was 14.12% in control fish. The Hb content decreased in test fish when exposed to both lethal and sub lethal concentrations in technical grade were 35.97% and 85.69%. When the control Hb content was compared to the lethal and sub lethal concentrations in 25% EC a decrement was shown as 97.87% and 88.3% but an increment was observed than technical grade as The decreasing content is comparable to that or RBC count in the blood of fish exposed. The trend of PCV value was like that of RBC and Hb at lethal and sub lethal concentration decreased in both technical grade and 25% EC when compared with the control fish PCV concentrations were observed as 43.9%, 88.7% and 79.55%, 76.3% over control concentration. MCV value calculated on the basis of PCV and RBC values. Elevated values of MCV were recorded over control 124.38 at both lethal and sub lethal concentrations. At lethal concentration in technical and 25% EC, 29.52 % and 6.6%elevation was observed respectively and on the other hand at sub lethal concentration in technical and 25% EC, the elevation was seen as 14.8% and 4.5% respectively High MCV indicates macrocytic (large average RBC size). MCH exhibited increment in all exposure periods over control as 41.08. At lethal concentration in both technical grade and 25% EC an elevation of 25.7% and 15.1% were observed. In sub lethal concentration in both technical grade and 25% EC too increase was observed over control as 21.9% and18.4%. The MCHC of blood of Cyprinus carpio was 58.64 in control fish. The MCHC value decreased in lethal and increased at sub lethal concentrations. The decrement was observed in lethal and sub lethal concentrations both in technical grade and 25%EC as 0.6%, 7.02% and 6%, 11.2% respectively.

Table 1 Haematological alterations in Cyprinus carpio on exposure to lethal and sublethal concentrations of Permethrin

Parameters	Control	Lethal		Sublethal	
		Technical grade (96 h)	25 % EC	Technical grade (96 h)	25 % EC
RBC (Millions/cu.mm)	5.96 ± 0.36	2.64 ± 0.28 (44.2)	4.3 ±0.56 (72.1)	3.86 ± 0.42 (64.7)	3.08 ± 0.62 (51.6)
WBC (Cells/ cu.mm)	23.28 ± 0.3	14.24 ± 0.26 (61.1)	24.36 ± 0.4 (104)	28.02 ± 0.38 (120.7)	29.46 ± 0.86 (126.5)
Hb (g/dL)	14.12 ± 1.28	5.08 ± 0.54 (35.97)	13.82 ± 1.18 (97.87)	12.1 ± 1.22 (85.69)	12.48 ± 0.76 (88.3)
PCV (%)	54.36 ± 0.68	23.88 ± 1.04 (43.9)	48.24 ± 0.96 (88.7)	43.22 ± 0.5 (79.5)	41.5 ± 0.98 (76.3)
MCV (cu µm)	124.38 ± 0.4	161.1 ± 0.74 (129.5)	132.6 ± 0.34 (106.6)	142.86 ± 0.3 (114.8)	130.1 ± 0.54 (104.5)
MCH (pg)	41.08 ± 0.28	51.64 ± 1.14 (125.7)	47.3 ± 0.56 (115.1)	50.1 ± 0.82 (121.9)	48.66 ±0.24 (118.4)
MCHC (%)	56.64 ± 0.92	56.32 ± 0.46 (99.4)	60.62 ± 0.32 (107.02)	60.04 ± 0.50 (106)	63.0 ± 0.64 (111.2)

Values are the mean of 5 observations, Standard Deviation is indicated as (±),
Values are significant at $p < 0.05$., Percent change over control are given in Parenthesis

MCV along with mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), is a part of RBC indices (erythrocyte indices), which are measurements and/or calculations for determining the size, content, and haemoglobin concentration. The results indicate profound impact of toxicants both technical and 25%EC where the changes are more significant even in just 25% EC concentrations. Considering the fact that the farmers spray the commercial formulations rather than technical grade 100% where the ingredients are imparting the cumulative toxicity which of concern that the sub lethal are really lethal.



IV. DISCUSSION

Reference [21] reported that the impact of pesticide on haematological parameters of *Cyprinus carpio*, studied the monocrotophos of pesticide in sub lethal concentrations. The toxicant resulted in a significant decrease in all the sub lethal concentration and maximum at 12.5 ppm, RBC (-42), haemoglobin content (-71), ESR (-68) and PCV (-59) and a significant increase in WBC Count (+52). They opined to determine the normal values of haematological parameters carp are intensive rearing, it is necessary the concentration of studies that could offer standards for physiological condition and a pathological inquest. In the present work on *Cyprinus carpio*, similar study resulted that EC had more pronounced impact. The monocrotophos resulted in significant alterations in different haematological parameters and this kind of physiological changes may directly affect the survivability of these fishes in their natural habitat. Reference [22] reported effects of deltamethrin on haematological indices of Indian major carp *Cirrhinus mrigula* (Hamilton). The type II synthetic Pyrethroid, deltamethrin which had different *exposure* to the toxicant contrary to the present study had similar alterations but only percent variation is different. The present study related to technical grade (96 h LC_{50}) in Continuous flow through system and $1/10^{th}$ of 25 % EC which is more similar to natural conditions for 10 days. The report gave the information, that the erythrocytes, haemoglobin and Haematocrit values decreased, WBC, MCH and MCV increased whereas MCHC remain unchanged. The values in the present study are almost

similar except that for 25 % EC, MCHC also increased for technical grade Permethrin unchanged. In aquaculture systems, the carp is surface inhabited Labeo is middle watered and Cyprinus is a bottom dweller.

In the present study, *Cyprinus carpio* behaved differently in MCHC. The haematological effects of this chemical pollutant and they may be a helpful tool in monitor the health status of this and other related fish species. The evaluation of haematological parameters will help in early detection of clinical pathology as well as the presence of disturbance in the environment.

Reference [23] reported genotoxic and haematological effect of commonly used fungicide on fish *Clarias batracus*. After exposure to 80 % of LC_{50} values of 24 h for one week, it was reported that the pesticide caused a decrease in haemoglobin (Hb), MCH, MCHC and red blood cell levels and increase in WBC level. The indices observed differently using different chemical pollutant on barbelled fish. In the present study Permethrin on *Cyprinus carpio* showed difference in alterations of blood constituents. The conclusion that can be drawn that the damage of the toxicant to aquatic organism and its use in domestic and agriculture pests and one must show severe concern. The fish *Cyprinus carpio* is an omnivorous where as *Clarias batracus* is carnivorous, according to the haematological variations as well as in the toxicant stress.

Reference [24] reported sub lethal effects of Diazonin on haematological indices in Indian major carp *Cirrhinus mrigala* (Hamilton). The observations were a significant decrease in erythrocyte count, haemoglobin concentration, MCH and MCHC. It is also omnivorous but bottom dweller more detritus feeder. The *Cyprinus carpio* as already mentioned is omnivorous showed variations in the alterations of haematological parameters as the method of exposure is different and toxicant is organophosphate pesticide. The toxicant could cause deleterious effects on fish physiology and may potentially disturb their survivability in the natural environment. Therefore controlling measures should be taken to prevent the possible contamination of the aquatic environment by such toxic pesticides. Similar conclusion can be drawn for *Cyprinus carpio* for using synthetic Pyrethroids.

The fish site, (Aquanac) have given haematology and blood chemistry for fish species after understanding the blood cell morphology it is essential for each species of fish in order to establish reference ranges. It was mentioned that the fish *Cyprinus carpio* require a more elaborate study for drawing better conclusions. The present toxicity on the reference fish needs to be studied still extensively on haematology.

Reference [25] reported on the toxicity of the chloropyriphos based pesticide Termifos® effects haematological parameters of African *Clarias gariapinus*. When fish exposed to 96 h LC_{50} value and sub lethal concentration ($1/5^{th}$ of the value) for different duration of 5, 10 and 15 days significantly lowered the red blood cell count and haematocrit values and both sub lethal concentrations. The haemoglobin level did not change significantly; MCH and MCHC were significantly elevated compared to the control specimens. The different toxicants and method of exposure showed variations and in the present study, continuous flow system LC_{50} value and its 96 h as lethal and at sub lethal concentration cannot be compared with result.

Reference [26] made a comparative on the fish *Channa puncta* using synthetic and plant origin pesticides and reported more toxic pesticide fenvadan; an organophosphate altered the haematological values. But here *Channa* is not more sensitive fish when compared to present study fish *Cyprinus carpio* which is more sensitive.

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