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Haematological Parameters Effected By The Toxicant Cyfluthrin And 10% Wp: The Impediments As Effects – A Biomarker Study

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Abstract

Alterations in the blood parameters of the freshwater fish *Catla catla* are studied in the laboratory after exposing the fish to both technical grade and 10% WP of Cyfluthrin, a synthetic pyrethroid for four days and 10 days in 1 /10^{th} of LC_{50} values of 96 hours respectively in lethal and sublethal concentration. The parameters studied are Erythrocytes (RBC), White Blood Cells (WBC), Haemoglobin (Hg), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and also Mean Corpuscular Haemoglobin Concentrations (MCHC). Except White Blood Cells (WBC) all other parameters resulted or decrement for both technical grade and 10% WP of Cyfluthrin exposure in lethal and sub-lethal concentrations of the toxicants in the fish. The WP of commercial formulation had a significant impact on the blood parameters which will have impact on the physiology of the fish particularly of the metabolism and if it impaired render the fish not to have a proper growth which is important for the cultivable species like the one studied in the present experimentation.

Key Words: *Catla catla*, Cyfluthrin, technical grade, 10% WP, Synthetic pyrethroid, RBC, Hb, WBC, HCT, MCV, MCH and MCHC.

INTRODUCTION

Blood, the circulating fluid proforms many functions in the closed system of it as, in vertebrates. It is the nutritive, immunity, and respiratory media and carry gases to all the different parts. It has the oxygen carrying pigment haemoglobin and the health of the fish depends on the mesodermal derivative with erythrocytes, leucocytes and platelets. According to Saddar Faheem *et al* (2021), metabolism of the fish depends on oxygen carrying capacity to different tissues/cells providing a definite defense mechanism fighting against any pathogen or cope any toxic stress.

Fish easy to maintain, poikilothermic, nektonic, ectothermic which offer many indices of the pollution including the toxicant pesticids. Any variation of the external environment can be easily perceived by the sensory system of the fish (scales & lateral line system). Malgorzata *et al* (2022) and Sinha *et al* (2022) reviewed the haematological aspects due to the pesticide toxic stress, The studies of the haematological nature of the effects caused by the synthetic pyrethroids, as the biomarker studies were extensively discussed and promoted in the review articles of Sana Ullah *et al* (2022 and 2019) and also by Anvila Kaviraju and Gupta (2014). They mentioned of some of the earlier reports exclusively for the pyrethroids including the present tested toxicant for the different species and according to Prusty *et al* (2015) Ullah and Zoriezahara (2015), Murthy *et al* (2013) and Ahrar Khan *et al* (2012) also which had a mention of the haematological alterations of the quantitative components of the studies that can be the indices of the pollution of the pesticides.

Hence, an attempt is made to study the variations of the quantitative nature in the blood components of *Catla catla* fish both in acute and chronic levels of Cyfluthrin of technical grade as well as its commercial formulation 10% WP, in the laboratory by following the standard protocols.

MATERIALS AND METHODS

Collection and Maintenance of Test Organism

The freshwater fish *Catla catla* length and weight 3-5 cm in length, 3-5 g in weight respectively, irrespective of their sex, have been chosen as the test organisms for the present investigation. Healthy and active fish are obtained from Nandivelugu hatchery (Guntur district, Andhra Pradesh, India). The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28 ± 1ºC. 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 (APHA, 1998 2005 and 2012 and OECD 2019) were followed and such acclimatized fish only are used for experimentation, If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded.

The water that was used for experimentation is similar to the one used for toxicity determination and the physical and chemical characteristics are:

Turbidity-8 silica units; Electrical conductivity at 28 Micro ohms/cm, pH at 28^{o} C-8.1, Aklalinity: Phenolphthalein Nil, Alkalinity: Methyl orange-472, Total Hardness (as $CaCO_3$)-232; Carbonate Hardness (as CaCO Carbonate Hardness (as $CaCO_3$)-232; Non Hardness (as $CaCO_3$)-Nil, Calcium Hardness (as CaCO Magnesium Hardness-40; Nitrite Nitrogen (as N)-Nil, Sulphate (as SO_4^{2})-Trace; Chloride (as Cl^{-})-40; Fluoride (as F) - 1.8; Iron (as Fe)-Nil; Dissolved Oxygen 8-10 ppm; Temperature -28^{o}±2C.

The pure as technical grade (obtained from a reputed company, the manufacturers of the Cyfluthrin, from a Agro Chemical multinational Company by name M/s. Bayer India Ltd., (Bombay, Maharashtra-400 079, India) and the formulation was purchased from the available pesticides shops, local of Guntur, Andhra Pradesh, India.

The fish, fifty numbers each were exposed to technical grade to 96 hrs LC_{50} value lethal 2.2 µg/L, sublethal LC_{50} i.e., 0.22 µg/L and also 10% WP lethal 1.4 mg/L and 10% WP sublethal 1/10th of LC_{50} value (0.14 µg/L) as per recommendations of APHA (1998, 2005 & 2012 and OECD 2019). A batch of the fish without an exposure to the toxicants is also maintained as a contorl. At the end of the exposure to

fish are subjected to analysis of the blood constituents for the observation of any haematological changes as alterations.

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.

Hematological 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 (MCH) and also its concentration as mean corpuscular concentration (MCHC).

The RBC was determined by with an Neubauer Crystalline Country Chouscher as described by Sheperclaus (1979). The white blood cells count was determined by the method described by Donald and Bonford (1963). The Haemoglobin was estimated by cyanomethanoglobin method as described by Blaxhall and Daisley (1973),and the heamatocrit by the method of Scahalm *et al* (1975). The mean corpuscular volume (MCV) was calculated by the following formula and expressed as fembliter.

MCV = Haematocrit % x 10/RBC count. The mean corpuscular Haemoglobin (MCH) was calculated by the following formula and expressed in pictogram (Pg) MCH = Haemoglobin (8/dL) x 10/RBC count. The mean corpuscular Haemoglobin concentration (MCHC) was obtained by the following formula and expressed in terms of gram percent (g%).

RESULTS

The quantitative data, determined is represented as graph 1 and the percent variations of changes compared to control are also appended as graph 2.

Even the diagrammatic representation as graphs depicts the same (Refer Fig. 1 & 2). The variations that are observed even in 10% WP are also significant when compared to the technical grade. The parameters viz., erythrocytes, Haemoglobin, Haematocrit, Mean Corpuscular volume, Mean Corpuscular haemoglobin and mean corpuscular Haemoglobin concentrates all of the results showed decrement whereas Leucocytes on the contrary increased.

A decrease of 93.10 percent in technical sublethal and 82.75 percent concentration of technical grade when compared with 10% WP of sublethal 96.55 percent and 89.65 percent in lethal showed, the decrement of the oxygen carrying capacity and fish is deprived of it.

Similarly the WBC count showed for technical grade sublethal as -8.3 and -16.67 in lethal whereas for 10% WP of sublethal as -12.5 and -20.83 in lethal as increase which can be presumed of that much increase is unspecific immunity due to the toxic stress to the technical grade toxic stress.

Similarly, the haemoglobin decreased in sublethal by 95% and in lethal 75% whereas for 10% WP it is 80% and 75% and that much decrease of haemoglobin had an impact on oxygen carrying capacity and ultimately it has a bearing on haematocrit values that too are changed by 97.75% and 5.6% for the technical grade in sublethal and lethal respectively,whereas for 10% WP it was 89.88% and 92.43% in sublethal and lethal respectively.

The calculated values of MCV decreased as of series lethal technical > sublethal technical > lethal 10% WP > sublethal > 10% WP. Similarly, MCH values decreased as of the similar of the above series and MCHC values too followed the same pattern.

Such appreciable blood parameters as alterations in the tested organism due to toxic action made a point clear that the fish in lethal concentration (acute), suffer the death whereas in sublethal concentration (chronic) suffer for the long run too.

DISCUSSION

With synthetic pyrethroids of type I and II

Sana Ullah *et al* (2022) reported on the haematological changes in the fish *Ctenopharyngodon idella* due to exposure of Bifenthrin, a synthetic pyrethroid of group I. Similarly, Sambasiva Rao *et al* (2022) in the fish *Labeo rohita*, using the same toxicant reported the same. Erythrolysis or inhibition of RBC production both are responsible for decrease and that result a reduction in haematocrit value too. The increase of leucocytes was due to toxic stress and immunity of non-specific nature were the valid reasons for the results of the similar as in the present study.

Ghosh *et al* (2022) reported alterations in the blood of the fish *Channa punctatus*, due to exposure of Cypermethrin, which was also reported in the fish *Cyprinus carpio* by Srinivasa Rao *et al* (2018) due to the exposure of the deltamethrin to the fish *Ctenopharyngodon idella*. Inhibition of erythropoieses and non-specific immunity all are responsible for variations.

Malgorzata *et al* (2022) and also Sinha *et al* (2022) provided all the relevant information of haematological variations in fish due to exposure of the pesticides including the synthetic pyrethroids.

In cat fishes, Suchiang (2021) reported that due to direct 'feedback' mechanism responsible for decrease of RBC, haemolysis and also impaired synthesis all cumulatively made such variations which is similar even in the present study kidney, the haemopoietic tissue damage the lead to decrease of RBC cells and the entire dysfunction of the whole haemopoietic system resulted alterations in blood parameters including the RBC production of antibiotics to cope the toxic stress all combinedly made to have changes.

Saha and Saha (2021) in the fish *Clarias batracus* and also in the fish *Heteropneustes fossils* (Bloch), Saha *et al* (2020) in *Oreochromis mossambicus* too opined that due to toxic action only, that manifested alterations as effects via the behavioural changes and the said parameters that showed variations in the blood. Even in *Oncorhynchus mykiss* (rainbow trout) the present tested chemical had an impact on the fish (Velisek *et al*. 2007).

Aysel (2021) opined that the pesticides at the concentration of the chronic level due to the oxidative stress resulted several biochemical alterations and of the haematological variations in the different fishes.

According to Siebel (2021), the fish blood gives much information about its physiological state of it. The contamination of the external medium is inevitable at any time and was perceived through the blood examination. Any such deviation of its quantitative internal components speaks about its ill-health or due to the effect of chemical stress.

Farag *et al* (2021), in *Oreochromis niloticus*, due to the bifenthrin toxic action which resulted some neuronal behavioural and physiological alterations in which haematological changes also one among them.

Bano *et al* (2021) due to the effect of the bifenthrin and chlorpyrifos in *Labeo rohita* reported that erythrocytes were affected because of its effect which resulted a decrement of them quantitatively that ultimately lead to impairment of the whole physiological respiratory process, finally caused to the reduction in the oxygen carrying capacity.

Deshmukh *et al* (2020) while investigating the polluted waters of river Godavari (near Paithan, Maharashtra state) in the fishes, *Catla catla* and *Labeo rohita* reported on the haematological changes due to contamination of the waters including the pesticides. Because such act the blood of the fish showed variations while in the environment even in the sublethal levels as a result such changes are quite possible.

The permethrin intoxification in the fish *Ctenopharyngodon idella* resulted that the blood of the grass carp had changes/alterations which was a study by Satyanarayana *et al* (2020). The results of the toxicant which also belongs to the same group of the present, were of similar (All parameters decreased except WBC), for technical grade as well as 25% EC formulation exposures. Kidney damage by which blood regeneration is effected and due to decrement of the RBC and histopathological lesions of the gills had an

impact on the oxygen carrying capacity and there was a decrement of the haemoglobin too. The present tested fish *Catla catla* along with other two major carps and also the grass carp are cultured in polyculture practices and when such situation of the Deshmukh *et al* (2020) report, one can visualize the contaminated waters should not be used as a source for such aquacultures ventures, as practices.

Biomarker studies Sana Ullah *et al* (2019a) as a review paper article gave the vivid aspects as the makers due to the pesticides toxic action to some of the different types of fish. The alterations of the blood, the study reported on the changes of the blood in the fish *Hypophthalmichus maltrix* (Silver carp) of due to the Deltamethrin in particular (type II) with cyanogroup. Even Kartas (2016) in the fish *Salmo trutta fario*, reported that RBC increased and of decrement in other cells of the blood due to the same toxicant. Gopala Rao *et al* (2017) in the fish *Cyprinus carpio*, Ullah *et al* (2015) in the fish *Tor putitova* due to cypermethrin reported same result.

Rahman and Hasan (2019), exposed the pesticides Lambda cyhalothrin a synthetic pyrethroid of group I and Dimethoate an organophosphate in the fish *Barbonymus gonionotus* (Silver barb). They reported that all the parameters decreased except MCHC which increased in the blood of the fish exposed at two different concentrations of both toxicants and exposure time was only 96 hrs. The results of contradictory nature of the present study and also the reasons for that not mentioned in their report except of comparing the results of the earlier studies.

Dhanya and Sushma (2018) reported on the lambda cyhalothrin on the haematological alterations in the same fish which was selected for the present study, both in lethal as well as sublethal concentrations of 24, 48, 72 and 96 hrs for 10, 20 and 40 days respectively. Only three parameters were studied RBC, WBC and Hb and the results that were obtained similar as of the present study. The toxicant they tested and the present studied one both belong to the same groups of pyrethroids (type/group I non-cyano one). The stress factor had an impact and the reduction in Hb that resulted a decrement in oxygen carrying capacity. The stimulation of the immune system was responsible for enhancement of WBC. The same explanations can also be concur upon as the explanation even for the study also.

Julia Jasmin *et al* (2018), reported in *Labeo rohita* while exposing of the difenoconazole (a synthetic pyrethroid) and thiamethoxam (neonicotinoids / new class) of pesticides. The results that are reported in the study are quite contradictory because they had a decrement even in WBC. Even though the reasons were not mentioned in the study however just compared to the earlier reports of the study.

Dhruv Kumar and Mamta Kumari (2018) in the fish *Heteropneustes fossils* and *Channa punctata* while assessing the toxicity due to the exposure of the lambda cyhalothrin (the group/type I) synthetic pyrethroid similar to the present tested toxicant reported several effects including the blood changes in its internal components.

Ahrar Khan *et al* (2012) reported haemato biochemical aspects in fishes, Avian and Mammalian species and the variations are due to changes in kidney and liver tissues that got damaged.

Aylin *et al* (2009) reported in the fish *Cyprinus carpio* due to exposure of sublethal exposure of Cyfluthrin, the present studied toxicant, the alterations that occurred in the blood of the fish. Because of the toxic stress, structural damage of kidney and liver made to have changes in the void. The decrement of the RBC need not be the cases wherein they mentioned contrary results too (Velisek *et al* 2007) in the fish *Onchorynchus mykiss* due to deltamethrin exposure.

CONCLUSIONS

Hence, it may be concluded that even in the fish *Catla catla* one of the major carp cultured along with the other carps when pesticides contaminate the culture medium alter the constituents of blood and such alterations are more severe in WP due to the ingredients mixed. If RBC is decreased the oxygen carrying capacity is reduced and the cellular respiration is impaired there by the growth is curtailed. Hence, stringent measures have to be taken for the quality control before giving pesticide representativeness, for, of in the environmental usage.

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Figure 1: Haematological alterations as impediments of the fish *Catla catla* exposed to both sublethal (SL) and lethal (L) concentrations of Cyfulthrin technical grade and 10% wettable powder (WP)

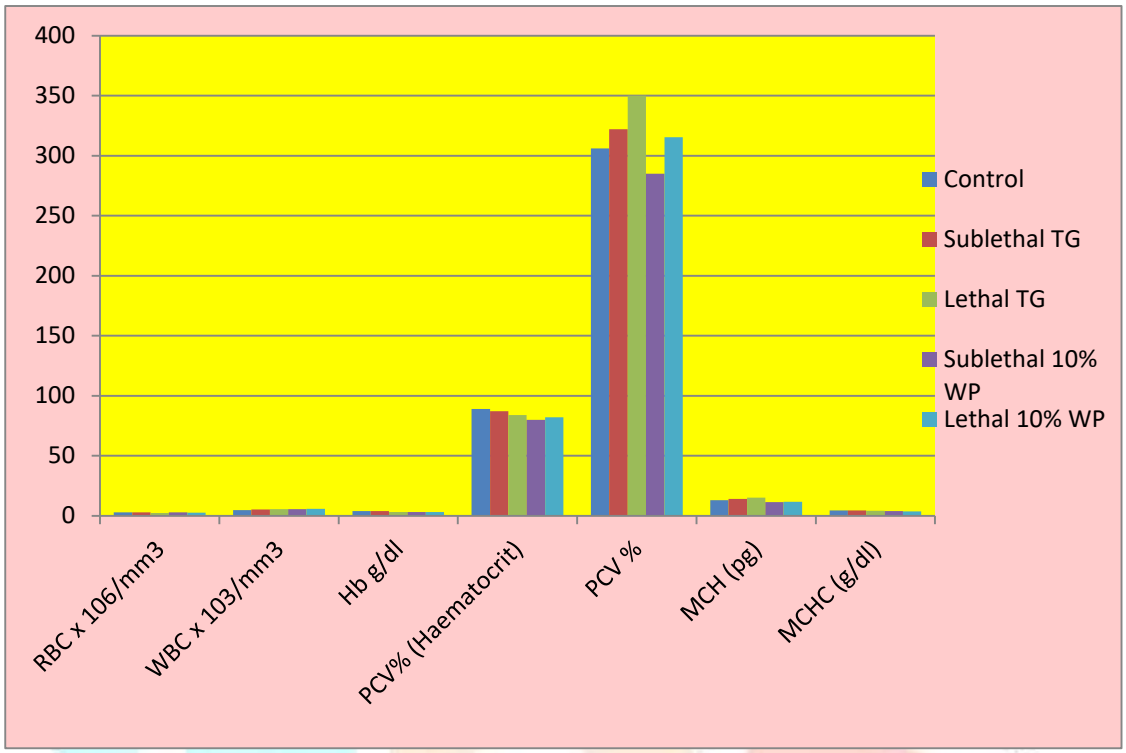

Figure 2.:Haematological alterations of percentages in the parameters of blood of the fish *Catla catla* exposed to both sublethal (SL) and lethal (L) concentrations of Cyfulthrin technical grade and 10% wettable powder (WP)