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HAEMATOLOGICAL ALTERATIONS INDUCED BY BIFENTHRIN (A SYNTHETIC PYRETHROID) TECHNICAL GRADE AND 10% EC IN THE FRESH WATER FISH *LABEO ROHITA* (HAMILTON, 1822)

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Abstract – Alterations in the blood components of the freshwater fish *Labeo rohita* are studied after exposing them to both technical grade and10% EC of bifenthrin a synthetic pyrethroid as toxicant for 10 days in 1/ 10^{th} of LC_{50} values 96 hrs LC_{50} values as sublethal and lethal of 4 days. The parameters that are studied include erythrocytes (RBC), Leucocytes (WBC), Haemoglobin (Hb) Haemotocrit (Ht), Mean Corpscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpscular Haemoglobin Concentration (MCHC). The first four are determined and the last three are calculated values. Except WBC, all others are decreased as quantitative alterations and will be the impediments for the normal functioning of all physiological processes and are the one of the biomarker study of the toxicity and will be the indices of toxicity and pollution load. They are the one of the reasons for causing the death of the fish. The results are going to be discussed with earlier studies and objective is to not to allow the pollution of such caused by pesticides and in the agriculture practices and such contaminated waters should not be used as it is going to be detrimental for the impairment of growth for the fish which are cultured.

INTRODUCTION

Blood, is the nutritive, excretory, immunity media apart from the respiratory gases to carry for the living organisms especially more so in vertebrate group with a closed circulatory system. Metabolism of such heterotrophic organism including the fish depends on the quantitative components of its constituents of the mesoderm derivative which is differentiated into plasma and formed elements (Erythrocytes, Leucocytes and Platelets). In normal condition, fish health is dependant mainly on the circulatory fluid as it helps in proper metabolism carrying oxygen to different tissues/cells and also only defense mechanism which it provides with attacking by parasites or antigens or even in toxic stress (Saddar Faheem *et al.* (2021).

The highly developed, vertebrates due to blood of its ecological adaptation are divided into exto and endotherms and also poikilotherms and homeotherms. The fish candidate species for testing in the laboratory to offer any indices of the pollution due to the toxicant, pollutants, the pesticides as of one major category Fish are poikilothermic with a single circulation of blood of the various heart perceive any subtle changes in the waters with a sensory system. Any such situation of the, which external environment will be reflected as an effect in the blood, there by any components of its parameters quantitatively change had an impact on the overall survival of the organism/fish.

Bifenthrin, the toxicant selected for the study is resistant in freshwaters because it can have the process of break down neither can undergo the process of hydrolysis or photolysis or it can have metabolic nor in quick time (North West Center for alternatives to pesticides, 2017). It was a persistent one and also its bioconcentration is also high because it is lipophilic and magnify in the food chain as per the same report as above. Hence, the best management practices to protect water and fish is 0.1 microgram/l which is the concentration that is

permissible as a protector to the aquatic life and is considered as the 'benchmark'. Hence, there is a justification in selecting the synthetic pyrethroid of type I, non-cyanogroup member the bifenthrin against a candidate species *Labeo rohita* one of the major carp and also a capture and culture fishery component as a part is selected as test species.

The studies of the haematological nature of the effects caused by the synthetic pyrethroids, the biomarker studies were extensively discussed and promoted by the review article by Sana Ullah *et al.* (2019, 2021 and 2022). They mentioned some of earlier reports exclusively for the pyrethroids including the present tested toxicant for the different species and also even in the review articles by Prusty *et al.* (2015); Murthy *et al.* (2013) and also by Ahrar Khan *et al.* (2012) which had a mention of the haematological alterations of the quantitative compounds of studies that can be the indices of pollution of the pesticides.

Hence, an attempt is made to study the variations of the quantitative nature in the blood components in the fish *Labeo rohita* both in lethal and sublethal concentration of the bifenthrin a synthetic pyrethroid of type I, of its technical grade as well as its commercial formulation 10% EC, in the laboratory by following standard protocols.

MATERIALS AND METHODS

Collection and Maintenance of Test Organism

The freshwater fish *Labeo rohita* size and weight selected as per the toxicity experiments (3-5 cm length and 3-5 gms weight). Healthy and active fish were obtained from local fish farm of Nandivelugu, 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±1°C. Water was renewed every day with 12-12h dark and light cycle. During the period of acclimatization, the fish were fed *(ad libitum)* with groundnut oil cake and rice bran. The feeding was stopped one day prior to the acute toxicity tests and also the haematological studies. As per the recommendations of the precautions laid by the committee on toxicity tests to aquatic organism (APHA, 1998, 2005 & 2012 and OECD, 2019) were followed. The acclimated fish were selected for haematological evaluation. If the mortality, if any 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°C-816 Micro ohms/cm, pH at 28 °C-8.1. Alkalinity Phenolphthalein=Nil, Alkalinity; Methyl orange-472, Total Hardness (as $CACO_3$)-232, Carbonate Hardness (as $CaCO_3$)-232, Non-Carbonate Hardness (as $CaCO_3$)-Nil. Calcium Hardness (as $CaCO_3$)-52, 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)-Ni, Dissolved Oxygen-8-10 ppm, Temperature-28±2 °C.

The Bifenthrin technical grade is supplied by M/s. Kalyani Industries (Agrochemical suppliers 1202/1204, 12th floor, B-wing, Kailash Business Park, Ghotpur Power, India) and 10% EC is purchased in the local market.

The fish, fifty numbers each were exposed to technical grade and 10% EC in lethal and sublethal concentrations for 4 days and the days respectively of 96 hrs concentration and the values are 0.22 µg/l and 0.022 µg/l (1/10th of LC_{50} value) and 0.11 µg/l and 0.011 µg/l respectively for technical grade and 10% EC, as per recommendations of APHA (1998, 2005 and 2012 and OECD, 2019). A control of the fish without an exposure to the toxicants is also maintained a control. At the end of the exposure to fish are subjected to analysis of the blood constituents for observation of any haematological changes as alterations.

The 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.

Haematological examination

The haematological variables analyzed by the standard procedures are RBC count, haemoglobin (Hb), white blood cells count (WBC), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

The RBC was determined by with a 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). 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./dL0 x 10/ RBC count.

The mean corpuscular Haemoglobin concentration (MCHC) was obtained by the following formula and expressed in terms of gram percent (8%).

RESULTS

The results after exposing the fish *Labeo rohita* (96 h) and sublethal (1/10 of LC_{50} value for 10 days) concentrations bifenthrin is appreciable. The blood parameters were estimated are presented graphically as Figure 1 and 2 for technical grade and 10% EC in lethal concentrations and sub-lethals and percent changes of haematological alterations in *Labeo rohita* on exposure to both sublethal and 10% EC. Except in WBC all other parameters decreased both in lethal and sublethal concentrations of technical grade as well as 10%. The impact of the commercial formulation in the alteration of parameters is significant and increases in WBC also.

A decrease of RBC 19% (18.9%) in technical sublethal and 23% (23.24%) concentration of technical grade when compared with 10% EC of sublethal 7% and 11% in lethal showed, the decrement of the oxygen carrying capacity and fish

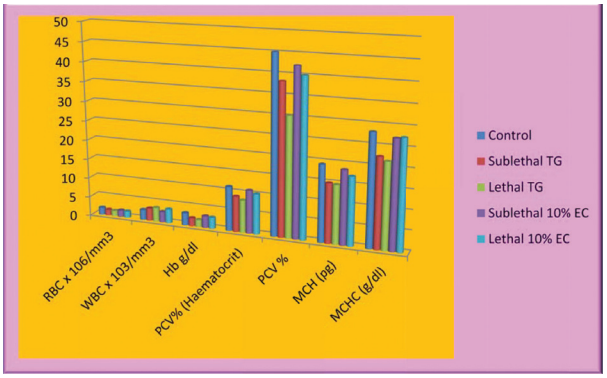

Fig. 1. Haematological changes in the parameters of blood of the fish *Labeo rohita* exposed to sublethal and lethal concentrations of both Technical grade Bifenthrin as well as 10% EC.

is deprived of it.

Similarly the WBC count showed for technical grade sublethal as 30.7% and 37.12% in lethal whereas for 10% EC of sublethal as 3.4% and 8.71% in lethal as increase which can be presumed of that much increase an unspecific immunity due to the toxic stress to the technical grade toxic stress.

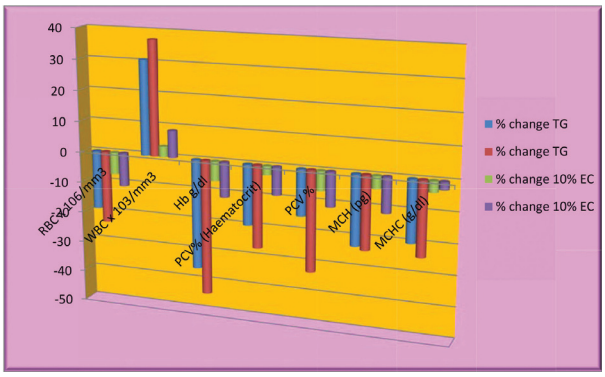

Fig. 2. Haematological changes of percentages in the parameters of blood of the fish *Labeo rohita* exposed in to sublethal and lethal concentrations of both Technical grade Bifenthrin as well as 10% EC.

Similarly, the haemoglobing decreased in sublethal by 35.62% and in lethal 43.75% whereas for 10% it is 6.25% and 11.25% and that much decrease of haemoglobin on oxygen carrying capacity and ultimately it has a bearing on Haematocrit values that too are changed by 26.17% and 19.61% for technical grade in lethal and sublethal respectively, whereas for 10% EC it was 2.82% and 9.01% in sublethal and lethal respectively.

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

Such appreciable changes in the blood parameters of the fish due to the toxic stress made a point clear that the fish in lethal concentration (acute), suffer the death whereas in sublethal concentration (acute) suffer the death whereas in sublethal concentration (chronic) suffer in the long run too.

DISCUSSION

With group I and II synthetic pyrethroids

Sana Ullah *et al.* (2022) in the fish *Ctenopharyngodon idella* using the bifenthrin as toxicant reported the changes in the blood components. Erythrolysis or

inhibition of RBC production that was responsible for decrease in RBC count reflected on haemoglobin and also on haematocrit values. For the increase of WBC, lymphopoiesis was responsible due to the toxic stress which is similar even to the present study.

Suchiang (2021) in his review article, specifically mentioned about the haematological aspects of changes of the pesticides in catfishes only. The reasons for decrement of RBC was due to exposure of the pesticides, in cat fishes *Clarias gariepinus* was due to direct 'feedback' mechanism which was also due to structural damage to RBC membrane, haemolysis or might be due to the impairment of its synthesis. The kidney is the haemopoietic tissue and the blood cells reservoir. Any decrement of the cells of it was due to dysfunction of the entire haemopoietic system of the fish. The increase of WBC due to an increased antibody production to cope the stress, which in a nutshell put it as disruption of the haematopoiesis and a decrease in the immunity of non-specific nature.

Saha and Saha (2021) in the fish *Clarias batracus* Saha *et al.*(2021) in the fish *Heteropneustes fossils* (Bloch). Saha *et al.* (2020) in the fish *Oreochromis mossambicus* too reported that the toxic stress manifested alterations as effects via the behavioural changes and the said parameters that showed variations. Even in the fish *Oncorhynchus mykiss* (rainbow trout) the bifenthrin the present tested toxicant had an impact on the fish.

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

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 were 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.

Faraq *et al.* (2021), in the fish *Oreochromis niloticus* due to bifenthrin intoxication resulted some neuronal behavioural and physiological alterations in which haematological changes are one of them.

Bano *et al.* (2021) due to the effect of bifenthrin and chlorpyrifos in the fish *Labeo rohita* due to the toxic action reported that the erythrocytes were effected which resulted a decrement of them quantitatively which ultimately lead to impairment of the whole physiological respiratory process due 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 and of such act the blood of the fish showed variations which in the environment at the sublethal levels in which such changes are quite possible.

Satyanarayana *et al.* (2020) while reporting on the permethrin intoxification in the fish *Ctenopharyngodon idella*, the blood of the grass carp had changes/alterations. The results of the toxicant which also belongs to the same group of the present toxicant bifenthrin were similar (All parameters decreased except WBC), in both 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. The present tested fish *Labeo rohita* 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 who visualized the contaminated waters should not be used as a source for such aquacultures ventures.

Sana Ullah *et al.* (2019a) in their review article gave a vivid aspects of the makers due to the pesticides toxic action to some of the different types of fish. The alterations of the blood such study Ullah Sana (2019b) reported on the changes in the fish *Hypophthalmichus maltrix* (Silver carp) of due to the Deltamethrin type II with cyanogroup. Even Kartas (2016) in the fish *Salmo trutta fario*, RBC increased and decrement in other cells due to the same toxicant.

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

Virinalis (red swamp cray fish) and also to other cray fishes and all of them had an impact on the

haematology. Except the oxygen carrying pigment (copper) and having closed system of blood circulations the shrimps that belong to the arthropods are similar to the fishes (poikilothermic/ exothermic).

Pradeep Kumar *et al.* (2018) in the fish *Heteropneustes fossils* when exposed to waters contaminated with pesticides in Muzaffnagar (M.P.) had an impact on the blood parameters, which serve as indices of the pollution load especially with pesticides. Such contaminated waters should not be used as source for the culture due to the synergestic effects the variations that were reported are different for the different pesticides individually. The concentrations were at both lethal and sublethal and was field/environmental study.

Dhanya and Sushma (2018) reported on 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 and 10, 20 and 40 days respectively. Only three parameters were studied RBC, WBC and Hg and the results that were obtained as of the present study. The toxicant they tested and the present studied one both belong to the same group of the synthetic pyrethroid (type/ group I non-cyano one). 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 agreed upon as the explanation for the present study also.

Julia Jasmin *et al.* (2018), reported on the fish *Labeo rohita* due to the exposure difenoconazole (asynthetic 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 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. The toxicant had several effects including the blood changes in its internal components.

Yadav rao (2017) due to the exposure of the synthetic pyrethroid polo (difenthurion – a formulated product of Bifenthrin of the present toxicant) in the fish *Labeo rohita* (the present tested fish) had an impact on the haemetological variations due to the toxic stress. All the parameters decreased including WBC, that are different of the present study. The structural damage of the RBC, of haemolysis and hypoxia conditions were the aspects they mentioned as the valid reasons for decrement of RBC and immune response made an impact on WBC. The same might be the same even in the present study.

Huma Naz *et al.* (2017), due to the pesticide mixture exposure in the fish, *Catla catla*, *Cirrhinus mrigala, Labeo rohita*, in which the bifenthrin, (the present studied toxicant) chlorpyrifos (organo-phosphate) and Endosulfan (organochlorine) had an impact on the enzyme superoxide dismulase in the liver which had a bearing on the metabolism and haematological variations.

Taufan (2016) due to deltamethrin in the fish *Salmo trutta*, David *et al.* (2015) in the fish *Cirrhinus mrigala* Venkataramudu *et al.* (2009) in the fish *Channa punctatus* had the similar results of the present study. But their study pertains only with EC, the commercial formulation whereas the present study pertains to both technical grade as well as 10% EC.

Kartas *et al.* (2016) in the fish *Salmo trutta fario* reported that RBC increased and WBC decreased due to deltamethrin a contrary result of the present study.

Neelima *et al.* (2016) in the fish *Cyprinus carpio* (Common carp) due to cypermethrin toxicity. Considering it as a biomarker study, erythrocytes count, Haemoglobin and the haematocrit values decreased and WBC increased apart from MCHC. Except the MCHC result, the present studied toxicant showed similar results. The stress factor that resulted the health status of the fish disturbed and the very cause of the organism the sustainance which is in jeopardy. Mohan *et al.* (2014) reported the same result in the fish *Catla catla* due to 10% EC of cypermethrin. The same stress factor was the causative effect that finally led to the death of the fish.

Nasir Khan *et al.* (2016), in the fish *Labeo rohita* (the present studied one)exposed to cypermethrin reported that RBC, Hb and haematocrit, MCH/ MCHC also decreased whereas WBC increased which is similar of the present study of the toxicant bifenthrin. The reasons of the decrement of the erythrocytes include inhibition of synthesis, erythropoiesis and also haem synthesis. The osmoregulatory dysfunction (plasma-formed

elements-RBC-WBC-platelets) and well as the destruction of the hametopoietic organs are the reasons. The increase of WBC was due to general immune response and defense response and above reasons reiterate the present studied fish for the variations in the blood.

Renu Chaudhari and Kamal Kumar Saxena (2016) reported on the bioallethrin another synthetic pyrethroid of type I exposed to the fish *Channa punctata* that due to sublethal concentrations of the toxic stress the fish blood erythrocytes got damaged in their DNA (Fish erythrocytes are nucleated not enucleated as of mammals) as a result the erythrocytes count decreased.

Thirumavalavan and Ganesan (2016) in the fish *Catla catla* exposed to cypermethrin (10 and 30% of LC_{50} values of 0.22 ppm) had an impact on haematological changes. The erythrocytes were damaged by the toxicant which in their view secondary but primarily the tissues of erythropoietic nature were damaged as a result decrement of blood components. The same may be true even in the present study.

Patole *et al.* (2016) reported in the fish *Channa marulus* exposed to ¼ of the LC_{50} value (not $1/10^{th}$ of LC_{50} value) of sublethal concentration the results as of the present study are reported except MCHC.

Neelima *et al.* (2015) in the fish *Cyprinus carpio*, due to cypermethrin toxicity the similar results of the present study are reported.

David *et al.* (2015) the effect of Deltamethrin in the blood of the fish *Cirrhinus mrigala* exposing both in lethal and sublethal concentrations and the same results as of above except MCHC of the present study. For the decrease of the RBC, erythrolysis and erythrocytopania were the main reasons of the for it which might be the same even in the present study.

Prusty *et al.* (2015) in their review article of the synthetic pyrethroids mentioned some of the different studies and reasons of the alterations were the same which later was mentioned by Sana Ullah *et al.* (2022), Saha *et al.* (2020) and Velisek *et al.* 2009 (earlier).

Jaya Sahi *et al.* (2013), when the fish *Channa punctatus* exposed to synthetic pyrethroids plant origin (RUTIN, TARAXEROL APIGENIN and FURADON (Carbamate) that resulted haematological changes similar to the present study. They opined that the stressors (toxicants) evoke non-specific responses in fish and as a result to cope with all the prevailing disturbances to maintain homeostatic state the changes occurred as effects. The same is true even in the present study.

According to Ahrar Khan *et al.* (2012) due to cypermethrin and deltamethrin where in the study they reported that later was more toxic than former. Leucocytosis and leucopenia were the resultant process and erythropoises were the process that are involved in the toxic stress.

Venkataramudu *et al.* (2009) reported in the fish *Channa punctatus* due to deltamethrin toxicity wherein they studied only two parameters RBC and WBC. The fish reacted in quick time of the stress in order to eliminate the toxicant which in detoxification that had an impact on the blood. Erythropoises was inhibited and to have a defense mechanism of homeostasis an aspect of it the WBC increased.

Velisek *et al.* (2009a&b) in the same above fish due to the same toxicant of the present studied one reported that the same result of the present study. Haematopoieses was the reason they explained for the reduction of the RBC and leucocytothermia as for increase of WBC and the same may be true even in the present study.

CONCLUSIONS

The poikilothermic blood of the fish if it is altered due to toxic stress had a bearing on the survival even though the concentrations are not lethal. In such sublethal concentration acting as slow poison, making the fish to suffer as of haematological changes if so, RBC when decreased, oxygen carrying capacity is reduced, metabolism is impaired and growth will be curtailed. WBC changes of increase definitely are immunological response. Both erythrocytopenia and leucocytosis are dangerous for the organisms well being and that is not good for the species to be cultured. The commercial formulations too significantly altered the blood parameters which has to be noted.

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