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HAEMATOLOGICAL VARIATIONS OF THE QUANTITIES OF THE PARAMETERS IN THE FISH *CATLA CATLA* EXPOSED TO DICHLOROVOS TECHNICAL GRADE AND 76% EC (NUVAN) AS EFFECTS

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Abstract

Hamatological changes as impediments in the fish *Catla catla* (Hamilton) exposed in the laboratory conditions (*in vivo*) due to the Dichlorvos an organo phosphate, both technical grade and 76% EC (Nuvan) are studied. The fish blood parameters studied includes erythrocytes (RBC), White blood corpscules (WBC), Haemoglobin (Hb), Haematocrit value (Hct & PCV), Mean corpuscular volume (MCV), Mean corpsuclar Haemoglobin (MCH) and Mean corpsuclar haemoglobin concentration (MCHC) of which the first four are analysed to determine them quantitatively and the last three are determined as by calculation. The RBC, Hb and HCt decreased whereas WBC increased and accordingly the calculated and MCV, MCH and MCHC are too varied. Due to the toxic stress the blood regeneration of components failed to function normally and to cope the toxic action leucocytes increased. The effects of the pesticide of technical grade and 76% EC (Nuvan) both in lethal and sublethal concentration had a significant impact and in total the metabolism is impaired hence the growth will be limited as the fish is for culture candidate species.

Keywords: *Catla catla*, Dichlorvos, Technical Grade, 76% EC Nuvan, Sublethal, lethal, blood, RBC, WBC, Hb, Hct or PCV, MCV, MCH and MCHC..

INTRODUCTION

Blood the circulating mesodermal derivative of the fluid component performs so many functions in the living organisms more so in vertebrates. The environmental variations of the physico-chemical parameters as pollutants cause changes perceived through the sensory system of the organisms. Accordingly the living creatures more so fishes and other highly developed vertebrates ecologically divided into poikilothermic and homeothermic and also eurythermal and stenothermal are effected.

Fish, the first group of the vertebrates with the closed system of the circulation (phylum Annelida and vertebrates also) are poikilothermic and stenothermal. Being heterotrophic, the blood takes all the nutrients, hormones and carry oxygen to different parts of the body apart from collecting the waste materials. Multi-functional aspect of the blood of fish if, by any means, perceived through lateral line sensory system (scales), if it is varied all the components of it, the repercussions that will result ultimately none other than the survival. Such changes as alterations of the constituents of it, are the indices of toxicity and such biomarker studies earlier reported for the fish by several authors.

In fact, Sana Ullah *et al.* (2019), Anilava and Gupta (2014) for the synthetic pyrethroids and for all the pesticides recognized the study as a biomarker in the fishes. The same view of it was reflected in the review articles of Ullah and Zorriezahara (2015) and Murthy *et al.* (2013) for all the pesticides, Sunanda *et al.* (2016) for chlorpyrifos and for the toxicant Dichlorvos (Chandrasekhara Rao *et al.* (2017) for the present studied toxicant) which had a mention of such studies earlier.

Somayyeh *et al.* (2017) reported the impact of the organophosphates, a survey study as a review of the reports in the last 40 years emphasized that because of hepato-toxicity via the blood the fish are subjected to death. Pallavi Srivastava *et al.* (2016) even in their review article considering the waters as the ultimate end they reach, and the ambient organisms are of great risk. Even such situations were possible as per reports of Anamika Srivastava *et al.* (2018) and Indira Devi *et al.* (2017) with reference to Indian conditions, the scenario of the pesticidal pollution.

In fact, the fundamental aspect of the blood components of *Catla catla* by Kandeepan (2014) and also by Deshmukh *et al.* (2020) are worth mentioning to know the different constituents of the blood of the present studied test fish. If these quantitative parameters are altered, the very metabolism and growth both are impaired and will be more important for the study, if so, the test species is a candidate one for culturing aspect.

Nnamdi *et al.* (2020) reported on the comparative assessment of ten different chemicals of pesticides in which Dichlorvos was also one and the reason to have the effect of toxic action was due to haematological quantitative alternations which act as impediments for the normal and routine life.

Hence, in the present study an attempt is made to know the alterations in the blood constituents of the fish viz., White Blood Corpuscles (WBC), Haemoglobin (Hb), Haematocrit (Hct), Erythrocytes (RBC), Mean Corpscular Volume (MCV), Mean Corpscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCMC) in the fish *Catla catla* a cultivable species by using both technical grade as well as 76% EC (Nuvan). It is the indices of the toxic action of both the chemicals as toxicants.

MATERIALS AND METHODS

Collection and maintenance of the test organism

The freshwater fish *Catla catla* size and weight selected as per the toxicity experiments (2-3 cm length and 3-4 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±1ºC. 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) 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.

A total of 50 fish were taken each in sublethal and lethal concentrations for both technical grade and 76% EC (Nuvan) exposure to study the effects of blood parameters. The 96 hrs LC_{50} concentrations that is determined by Finneys probit analysis (1971) as mentioned above. Haematological changes were determined at the end period of exposure to 96 hrs LC_{50} values of technical grade and for 76% EC (Nuvan) Dichlorvos as lethal exposure as per the table 2.8 of second chapter and also $1/10^{th}$ of LC_{50} of 96 hrs as sublethal exposure of duration of 10 days in both the toxicants and the concentrations are 1.77, 0.177, 1.4 & 0.14 µg/L for technical grade and 76% EC respectively. The organisms that were dead in lethal concentrations only 50% and in sublethal only 1%, while the remaining live organisms were sacrificed during the present experimentation in both lethal and sublethal concentrations for assays of different blood parameters.

The Sampling of the blood

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

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).

Determination of red blood corpuscles (RBC) count

RBC count was determined with an improved Neubauer crystalline counting chamber as described by Shaperclaus, 1979. The blood was sucked up 0.5 of the pipette and immediately, Hayems solution as a diluent stain was drawn up to 101 mark and the pipette was rotated between the thumb and the forefinger to facilitate adequate mixing of the solution (dilution: 1:200). The counting chamber and the cover glass were cleaned thoroughly and cover glass was placed in position over the ruled area. The fluid from the stem of the pipette was expelled as it contains only the diluting fluid. The pipette was then held at an angle of 45^0 with the tip of the pipette at the junction of the edge of cover glass and the counting chamber. A drop of blood was placed from the tip of the pipette on the central platform near the edge of the cover slip, so that the drop was sucked up between the central platform and cover slip by the capillary force. The cells were allowed to settle for a period just for 2 to 3 minutes. The ruled area of the counting chamber was focused under the microscope and the number of RBC were counted in 80 small squares (4 squares of 16 at the four corners and one of 16 at the center). The cells touching the upper and left-hand lines were only counted and the cells touching the lower and the right-hand lines were omitted.

The numbers of RBC per sq mm were calculated as follows:

The area of small square: 1/400sq mm
The depth of the counting chamber: 1/10mm
Therefore, the volume of a small square is: 1/400x 1/10 = 1/4000cu mm
The dilution of blood is 1:200
Total number of RBC = n x 4000 x 200/80
n = Number of cells counted in 80 small squares

Determination of white blood corpuscles (WBC) count

WBC count was determined by the method described Donald and Bonford (1963). The blood was drawn up to 0.5 mark of WBC pipette and immediately the diluting fluid was drawn up to the 101 marks above the bulb (the dilution fluid consists of 1.5ml of glacial acetic acid and 1 ml of aqueous gentian violet solution and made up to 100 ml with distilled water). The solution was mixed thoroughly by shaking gently and allowed to stand for 3 min. Cleaned Neubauer counting chamber and cover glass were placed over the ruled area. Excess solution was expelled, and a drop of fluid was allowed to flow under the coverslip by holding the pipette at an angle of 40° and allowed to stand for 2 to 3 min. The WBC was counted in the four-corner square millimeters and the number of WBC per cubic millimeter was calculated.

Estimation of haemoglobin (Hb)

Hb concentration in the blood was estimated by cyan methaemoglobin method as described by Blaxhall and Daisley (1973). It is converted into cyanmet-haemoglobin by the addition of potassium ferricyanide (KCN) and the colour was read in a spectrophotometer at 540 nm against a reagent blank.

Determination of packed cell volume (PCV) or haematocrit value

Packed cell volume was determined by micro haematocrit method of Schalm *et al.* (1975). The blood that is hyperinised was filled up to the mark 100 of the haematocrit tube with the help of Pasteur pipette and centrifuged at 3000rpm for 30min. 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 Determination as calculation of mean corpuscular volume (MCV)

MCV indicates the average size of the blood cell in a given sample of blood. MCV was calculated by the following formula and expressed as femto liter (fL).

MCV = Haematocrit (%) x 10/RBC count

Determination of mean corpuscular haemoglobin (MCH)

MCH represents the average content of the HB in each red blood cell. MCH is influenced by the HB concentration and the number of RBC. MCH was calculated by the following formula and expressed in picogram (pg).

MCH = Haemoglobin (g/dl.) x 10/RBC count

Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC reflects the average concentration of the haemoglobin in the red blood cells in a given sample of the blood. MCHC was obtained by the following formula and expressed in terms of gram percent (g%).

MCHC = Haemoglobin (g/dL) x 100 / Haemoglobin (%)

RESULTS

The constituents of the blood in both lethal and sublethal concentration of technical grade as well as 76% EC Nuvan exposure that altered are presented as graph in Fig. 1, and the percent changes are also represented in Fig. 2.

Changes that are observed in the exposed fish of the two toxicants whereas in 76% EC i.e. the commercial formulation of the fish that experimented resulted more % of changes rather than the technical grade, because of the ingredients mixed imparting additive toxicity. The RBC count decreased in both lethal and sublethal concentration exposure and more % of decrement in 76% EC. The WBC count increased in both lethal and sublethal and more in 76% EC. The haematocrit value altered, proportionately to the RBC count and also the values of MCV, MCH and MCHC are altered accordingly.

DISCUSSION

Studies on Dichlorvos

Nwamba *et al.* (2020), in the African cat fish *Clarias gariepinus* with a modulator reported the alterations in the blood by exposing them to 21.391 mg/L and (96h LC_{50} value of $^{1}/_{5}{}^{th}$) and in two concentrations of 2.14 mg/L and 4.28 mg/L as 96 hrs 1/10 value for 1^{st}, 5^{th}, 10 days and 15 days of exposure. The results which they reported were concentration and duration dependent. The RBC, PCV, Hb, MCH, MCV and MCHC decreased while WBC increased. The present work differs with the following aspects.

- The 96h LC_{50} value of CFTS is taken and $1/10^{th}$ of the value is taken as sublethal and lethal concentration is the actual value of said duration LC_{50} value and the total exposure is for 10 days as per APHA (1998, 2005 and 2012) guidelines.
- The tests are for both technical grade as well as 76% EC (Nuvan) and are not of static bioassay type.
- The reported fish is a cat fish (Hard fish), whereas the present studied one is a carp (sensitive).

The inhibition of the erythropoiesis and leucocytosis to cope the demand of toxic stress were the valid reasons they mentioned which are of similar lines even in the present study.

Nanda and Siba Prasad (2020) even on the same genus fish, *Clarias batracus* too reported of haematological changes due to Dichlorvos toxic effect. The study period of experiment was limited for 72 days (3 days) exposure both RBC and WBC decreased contrary to the present study and accordingly other parameters too resulted alterations as per the formed elements RBC and WBC of the blood. The authors did not offer any explanations for WBC decrease which might be due to short time exposure of just 3 days.

Serpil Mise Yonar *et al.* (2020) reported in the fish *Cyprinus carpio*, the changes in the blood due to exposure of trichlorfon (Active ingredient Dichlorovos, Turkey brand name and market and one as for the authors place of work). The exposure was for the days of two different concentrations (11 mg/L & 22 mg/L $1/6^{th}$ and 1/3 rd LC_{50} value of 96h determined by static brassay method). RBC and WBC both decreased contrary to the present study the increased value for WBC and other parameters were made to have quantitative differences. The non-specific immune activity made to have a decrease of WBC and the decrease of RBC were due to inhibition of erythropoiesis, haemosynthesis and might be due to osmoregulatory dysfunction or due to increased erythrolysis which might be true even in the present study.

Akinortini *et al.* (2018) in both juveniles and adults of the fish *Tilapia guinensis* exposed to four different concentration of Dichlorvos for about 12 days (The value was as per the static bioassay of 96h LC_{50} value). They mainly focused their attention with WBC and found increase of them as in the present study resulted by an increase of leucocytosis and the same can be considered even in the present study.

Swarna Kumari *et al.* (2018) in the fish *Ctenopharyngodon idella* exposed to Dichlorvos in technical grade as well as in 75% EC (Nuvan). The decrease and increase of the RBC and WBC was reported as in the present study to the fish *Catla catla*. The effect of the commercial formulation was significant due to the ingredients mixed in the commercial formulation as in the present study.

Chandrasekhar Rao *et al.* (2017) in their review articleon Dichlorvos mentioned about the earlier reports by Mallum *et al.* (2016); Tak *et al.* (2014); Lakshmanan *et al.* (2013); Ashade *et al.* (2011); Sobhana *et al.* (2006), Das and Mukherjee (2003); Medda *et al.* (1993), Benerji and Rejendranath (1990) and Verma *et al.* (1982), in different fish. Both the RBC and WBC values decreased or only RBC values decreased whereas WBC increased in all the reports of study. Hence, some of the results are in agreement whereas in others (few) contradictory results of WBC. The reasons of the variations were not specific but overall aspect of the changes in the blood components which serve as the indices of toxicity.

Sadia Sarma *et al.* (2016) in the fish *Labeo rohita* the haematological alterations in the blood due to exposure of Dichlorvos in the sublethal concentration for 15 days (The concentrations were based on 96h LC_{50} value that was determined by static bioassay). A decrease of RBC and increase of WBC and Haematocrit values. The reasons for variations were not mentioned in the report of the study.

Studies on other organophosphates

Styanova *et al.* (2020) in the fish liver of the common carp *Cyprinus carpio*, due to exposure to chlorpyrifos effect of toxic action the hepatotyoxicity was noticed due to mainly circulating fluid, the blood only which had a great impact. The changes in the blood, made to have biochemical changes in the liver which was the study of indices of toxicity.

Nnamdi *et al.* (2020) report on the comparative toxicity of the African catfish *Clarias gariepinus* emphasized in their report that the pesticides were toxicants that made alterations in the blood which had an ultimate effect of mortality. They reported contradictory result of the present study of WBC count and not offered a favourable explanation for the cause of the same.

Akter *et al.* (2020) due to exposure of the pesticide, Envoy 50% an organophosphate reported in the fish *Heteroneusteus fossils*, where in only one parameter that RBC was aimed of the study in two different concentrations and result that was decrement and also morphological changes in them. They firmly opined that any chemical condition in the ambient waters, if it is unfavourable perceived through sensory system reflected in blood only. Medium of water and the respiring organs of the fish gills separated by a thin of layer as a 'film' through the toxicant penetrated ultimately the circulating poikilothermic fluid is effected. Now actually the stress started at that point leading to reduction of oxygen supply to the different parts of the body through capillaries as a squeezing mechanism got effected as a result low RBC count. The present study is no exception to that only, the fish in the opinion of the authors require more oxygen to evert the situation and finally this, when the concentration reaches lethal the fish succumb to death. Overall the whole study was the indices of toxicity according to authors of course no exception even in the present case.

Sarma and Pooja (2021) reported using Biphenol an organic compound used for making plastic items, as a toxicant, in the fish *Channa punctata*, at three different concentrations (0.1, 0.2 and 0.4 ppm) had an profound impact of reduction in RBC. They mentioned that the teleostean bony fish where the erythropoises was from the bone only and due to damage of cells resulted a reductions of the RBC bone count. The reason apart from this they mentioned that haemolysis enhancement and the endocrine disruption system too had an impact which all cumulatively had reduced the RBC quantitatively. The decrement resulted the anarmic nature of the blood and fish had suffered the hypoxic condition which resulted CO_2 toxicity. The reduction in RBC made the haemoglobin which got catabolised into bilerubin which was excreted and all the above explanation offered by the authors holds good even in the present study. The study of the result had also a decrement of WBC (a contradictory result of the present study) either by activation a stimulation of inhibitory and synthesizing factors respectively. The Channa fish is carnivore and *Catla catla* is a omnivore.

Yanchova (2019), using chlorpyrifos as a toxicant exposing it for 72 hrs in the fish *Cyprinus carpio*, commented that due to architectural damage of the gill only that had reduced the oxygen carrying capacity apart from alterations in the blood finally lead to the death of the fish.

Islam *et al.* (2019) in the fish *Paugasianodon hypophithalmas* reported on the haemotological changes that underwent due to the exposure of the pesticide Sumithion an organophosphate. The result of the present study coincide as of a decrease of RBC and Haemoglobin and increase of WBC in the respective fishes studied. The valid reasons they offered for were disruption of haem protein as well as action at the origin point of the RBC cells. All had resulted a failure of blood regeneration system. The two aspects of inhibition of the erythrocytes regeneration or due to lysis (erythropoiesis and erythrolysis) and the histopathological damage of the excretory organ of aminotellic nature of the kidney that paved the way for the ultimate death of the fish. The increase of the WBC cells was due to leucocytosis which due to the chemical stress the immune system to produce more antibodies that to more in number and the explanation holds good in the present study too.

Edwin *et al.* (2019), in two fishes *Oreochromis niloticus* and *Cyprinus carpio* due to chlorpyrifos toxicity which due to the profound action had changes in the blood, internal components. Mainly in the RBC reduction the oxygen carrying capacity reduced substantially, as a result, the death was caused which sounds good even in the present study as explanation and same explanations were too offered by Arumugan Stalin *et al.* (2019) in the fish *Channa punctata*, using the same toxicant.

In the fish *Cyprinus carpio*, using monocrotophos as toxicant of 30 days both at as lethal and sublethal concentrations reported by Sharmila and Kavitha (2019) the same outcome of the present one. The distruction of haemopoietic tissue resulting anaemic condition too was the valid reason of explanation for decrement of RBC and for increase of WBC to cope the stress demand proliferation of them was a must and all the explanation given by them are true for the present study.

In the fish, *Labeo rohita*, Julia Jasmin *et al.* (2018) observed in the blood exposed to Difenoconzale and thiamethoxan exposed to different concentration and reported that after tested with the two chemicals and the results obtained by them was that of contradictory nature when compared with the present study. In fact for difenoconazole RBC and WBC decreased whereas for thiamethoxan increased and opined that the results were due to the stress condition that induced haematological variations.

Satish *et al.* (2018) in the fish of *Channa punctata*, by using Acephate an organic phosphate as testing chemical after 5 and 10 days of exposure at a sublethal concentration. The WBC count decreased a contradictory result of the present study and concluded that the bioconcentration of the toxicant in the kidney had made haemopoietic tissue improper in (zn metal) resulting the tissue not to have normal function.

Bheem Rao *et al.* (2018) in the fish *Heteroneusteus fossils* after methyl parathion exposure, Mohammad Ismail *et al.* (2018) in the fish *Labeo rohita*, Somayyah *et al.* (2017) in the review article, Antiha Bhatnagar *et al.* (2016) in the fish *Cirrhinus mrigala* due to chlorpyrifos toxicity, Pallavi Srivastava *et al.* (2016) in the review article had a mention of haematological changes in the fish blood.

Sadia *et al.* (2016) in the fish, *Cyprinus carpio* reported haematological alterations due to the toxic action of the Malathion another, organo phosphate, in two sub-lethal concentrations of 1.5 and 3.0 mg/L for 8 days exposure (A different methodology of the present study) but the same result of the present study. Haemolysis of RBC and damage of the gill were reasons they mentioned for the decrement of erythrocytes, to adopt the toxic stress the increase of WBC was justified. The same by using carbofuran as toxicant in the fish *Channa punctatus* was reported by Ashaduzzaman *et al.* (2016), also and even such thing might be possible in the present study.

Golam *et al.* (2015) reported in the fish, *Barbony musgonionotus*, (Silver barb) exposed to an organo-phosphate quinalphos, 25% EC, at 0.47 ppm and 0.94 ppm of sub-lethal concentrations. The same result of the present study was also mentioned and haemolysis and shrinkage of RBC or decrease in production of them (haematopoiesis). The same will apply even in the present study even though the toxicant is different but both are organophosphates.

Md. Jamal Haider and Abdul Rauf (2014) in the fish Rohu when exposed to Diazinon, an organophosphate, exposed to two sub-lethal concentrations of 0.815 mg/L and 1.63 mg/L for 30 days had a report of the contradictory result of decrement of WBC that resulted and the possible reason they mentioned was the failure or suppression of haemopoietic system' due to the toxic action. The review articles of Deb and Das (2013) mentioned certain haematological alterations including the genotoxicity of the fish.

Jaya *et al.* (2013) reported on the haematological of the fish *Channa punctata*, using both synthetic as well as plant origin chemicals and pesticides, furadan, rutin, taraxerol and apigenin in sub-lethal concentration for 7 days. The results of the present study are similar and the reasons of decrement of the RBC, they opined as of haemolysis and for WBC increment the leucocycotis due to the toxic stress. The same applies true even in the present study.

Zubair Ahmad (2011) reported in the fish *Cyprinus carpio*, due to diazinon exposure that caused the blood parameters variations, an increase of RBC values and a decrease of WBC values, a contradictory result of the present study. Biochemical changes in the blood of the fish 'hyperactiveness' of the fish to the stress conditions, to have more oxygen intake promoted more synthesis of RBC and decrement of WBC too the failure of blood system functioning, leukocytosis, heterophilia and lymphonemia were the terms used in their report, to substantiate their findings.

Ramesh and Saravanan (2008), Chlorpyrifos in the fish *Cyprinus carpio*, (size of 7.5 cm length and weight of 6.0 gms) exposure, at 24 h LC_{50} value of 5.28 ppm determined by the static method and the blood was tested, after 24 hrs only. The methodology was entirely different for comparison yet the result was the same. Anemia, erythropoiesis, haemosynthesis and osmoregulatory dysfunction for the decrement of erythrocytes, heterophilia and lymphopenia for WBC increase were the valid reasons they mentioned to justify the result. All the terminology used, that can be a favourable explanation even of the present study results also.

With synthetic pyrethroids

Sana Ullah *et al*. (2019a) in their review article giving an vivid aspects of biomarkers due to the pyrethroid toxic action in fish. One such indicator as biomarker is none other than haemotological toxicity Sana Ullah *et al*. (2019b) reported the variations in the blood constituents of the fish. *Hypophthalmichthys moltrix*, viz., RBC, WBC, Haemoglobin, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations. When the fish was exposed at 96 h toxicity value [1/10th of it] for 10 days showed the manifestations in the blood cells and reflected finally on its toxic action. Reduction in RBC, Hb, MCV and MCHC was due to the inhibition of Erythropoieses or haemo-synthesis and as well destruction was due to haemopoietic tissues. Hypoxic conditions that resulted in changes in oxygen consumption had impact on RBC, which might be the possible reason even in the present study.

According to Alka Mishra (2018), who reported the impact of pesticides toxicity on the morphological characteristics of blood cells of the fish *Channa punctatus* after the exposure to trichlorofan - an organophosphate and he opined that membrane expansion increases the area/volume proportion and could allow swelling of the blood cell thus reaching the largest volume before any lysis. The swelling of RBC increases the activity of MCV and is generally considered as stress factor.

Gopalarao *et al*., (2017) reported also the haematological changes in the fish *Cyprinus carpio* exposed to synthetic pyrethroid permethrin (class I) and its 25% EC. In the study when exposed to technical grade and its 25% EC, of lethal and sublethal concentrations RBC, Hb, PCV decreased whereas WBC, MCV and MCHC increased. The % change is more in EC due to more toxic effect of the ingredients mixed. The result is in agreement of the present study even though both the toxicants belong to different class of synthetic pyrethroids.

Tayfun (2016) reported the effect of deltamethrin on some haematological parameters of brown trout (*Salmo trutta* fario), after exposure for four days, in two different concentrations of Deltamethrin i.e. 0.91 $\mu g/L^{-1}$ and 188 $\mu g/L^{-1}$and the results are that the WBC, Hb, PCV, MCV, MCHC decreased however RBC cells increased. The results are quite contradicting of the present study may be due to herbivorous nature of the fish *Ctenopharyngodon idella* in toxic stress that behaved differently.

Kartas (2016) in the fish *Salmo trutta fario* report was also mentioned wherein red blood cells increased and there was a decrement in other cells that reflected on the calculated values also, due to deltamethrin toxicity.

Patole *et al*., (2016) reported effect of fenvalerate another synthetic pyrethroid, to the fish *Channa marulius* exposed to ¼ of LC_{50} value at sublethal concentration (0.086 ppm). Hb percentage as TEC, PCV and MCHC counts significantly decreased whereas TLC, MCV and MCH increased slightly. The Fenvalerate and Deltamethrin, the two belong to type II synthetic pyrethroid and the study had similar results of the present study fish.

David *et al.*, (2015) reported effect of Deltamethrin on Haematological indices of Indian major carp *Cirrhinus mrigala* (Hamilton). The fish are exposed to both lethal and sublethal Deltamethrin of 8 mg/L and 0.8 mg/L respectively, for 1, 2, 3 and 4 days and 1st, 5th, 10th and 15th day respectively. The result that are reported are RBC, Hb and haematocrit values decreased, whereas WBC, MCV and MCH were increased MCHC remain unchanged. The results are in agreed of the present study except MCHC. The increase of MCV and MCH values after exposure to deltamethrin indicates that a reduced RBC count which may be due to destruction of erythrocytes or then decreased synthesis (Erythrolysis and Erythropenia).

Prusty *et al.* (2015) for synthetic pyrethroids and Saumya Biswas *et al.* (2019) for synthetic pyrethroid too in their review articles mentioned about haematological alterations which serve as indices of toxicity.

Neelima *et al.*, (2015) reported on haematological alteration in *Cyprinus carpio* and considered as a biomarker of toxicity due to cypermethrin wherein erythrocytes count, Hb content and PCV showed decrement at both lethal and sublethal concentrations. WBC and MCHC values increased at sublethal and decreased at lethal concentrations. The study concluded that elevated values of MCV in exposed concentrations. The stress factor and the presence of the toxicant in the ecosystem by any means of transportation may cause a lot of disturbances resulting not to have a status of healthy nature and sustenance is in jeopardy. Cypermethrin at 2µg/L and opposite and Deltamethrin at 1.61µg/L concentrations exposure reported the similar and opposite results of the present study, being type II synthetic pyrethroids with cyanogroup. Leukocytosis was for Cypermethrin and for deltamethrin it was leucopenia.

Jayaprakash and Shettu (2013) reported changes in the haematology of the fresh water fish *Channa punctatus* exposed to Deltamethrin in the lower concentration of 0.075 mg/l and higher one of 0.15 mg/l for 15, 30 and 40 days. The study reported that: MCV and WBC are increased significantly after the exposure period whereas RBC, Hb, PCV, MCH and MCHC values decreased. The results support the present study of the fish of deltamethrin exposure. They opined that decrease in Hb, TEC and PCV values leading to anemia is due to impaired absorption of iron. The stress factor leads to changes in physiology that resulted alterations in the blood parameters.

Mohan *et al.*, (2014) reported haematological alterations of pyrethroid cypermethrin 10% EC, exposure to the fish *Catla catla* resulted RBC, MCV, MCHC being decreased whereas haematocrit, WBC, MCH values increased. Deltamethrin, Cypermethrin and Fenvalerate all the three synthetic pyrethroids of group II are with cyanogroup. In the fish exposed to lower concentration, Hb showed a minute decrease, when compared to higher concentration which significantly increased. The values of PCV, MCH and MCHC significantly increased in both the concentrations but MCV value showed significant decrease in both the concentrations. Stress is the factor, where the pesticide had a negative impact on Hb level, wherein it is made to be destroyed or lysed to had decrease of synthesis. The MCV, MCH and MCHC values are completely dependent upon the RBC count.

Velisek *et al.* (2012) reported that the effects of pyrethroids and triazine pesticides on the fish physiology and mentioned about blood changes. The result of present study showed that Deltamethrin exposure to the fish, common carp resulted significantly lower values of RBC, HB and PCV, whereas in the rainbow trout significantly higher values of erythrocyte count, haemoglobin content and haematocrit than the control group. The results drastically are different in the two fish with the same toxicant.

According to Ahrar Khan *et al.*, (2012) report on the study of haemato - biochemical changes induced by pyrethroid insecticides Cypermethrin and Deltamethrin compared the toxicity.

Venkataramudu *et al.*, (2009) reported haematological studies in freshwater fish *Channa punctatus* during sublethal toxicity of Deltamethrin in relation to sex. They studied only the two parameters RBC and WBC in both males and females of carnivorous fish *Channa punctatus* (Bloch) exposing the fish to sublethal concentration for periods of 24 h, 7 day, 15 day, 20 day and 30 day. A decreasing trend in RBC except in 24 h period and increasing trend of WBC in all the exposure periods in both sexes was revealed in their study. The fish reacted quickly to the stress conditions tried to eliminate the pesticide which can be correlated a detoxification process. They opined the presence of pesticide that might have induced hypoxia which in turn accelerate the haemopoietic tissue. They referred Rodriguez *et al.*, 2005 in their discussion that the decline in RBC count obviously is due to the entry of the toxicant into the body of the fish and in turn entitled erythropoiesis. They also opined the increase in WBC may be attributed as a work of defensive mechanism against the pesticide that has entered and as a homeostatic mechanism of such a change resulted an increase in the WBC. The report is also in the agreement of the present study of the fish, wherein a decrease and increasing trend of RBC and WBC count respectively, which ultimately have profound bearing on Hb, Ht, MCV, MCH and MCHC values.

Sheik Jamal (2006) reported haemotological alterations induced by pyrethroid insecticide fenvalerate in catfish *Clarias gariepinus* exposed to 1/10 of LC_{50} of fenvalerate for one, 5 and 10 days. The toxicant induced decrease that was significant in the haemoglobin due to haemolysis content haematocrit and erythrocytes. The leukocytes count was increased, the results are in similarity to the present study of the fish, but both the toxicants belong to the same class.

Nuri Cakmak and Girgin (2003) reported cypermethrin induced in the fish Rainbow trout (*Oncorhychinus mykiss*: Wabaum) as haematocrit, haemoglobin leucocyte RBC, MCHC decreased with increasing concentration but MCV value increased and MCH values are not affected. This is another contradicting result of the present study.

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 EC 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 its in the environmental usage.

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Figure 1: Haematological changes in the parameters of blood of the fish *Catla catla* exposed in both sublethal and lethal concentrations for both Technical grade Dichlorvos as well as 76% EC as (Nuvan)

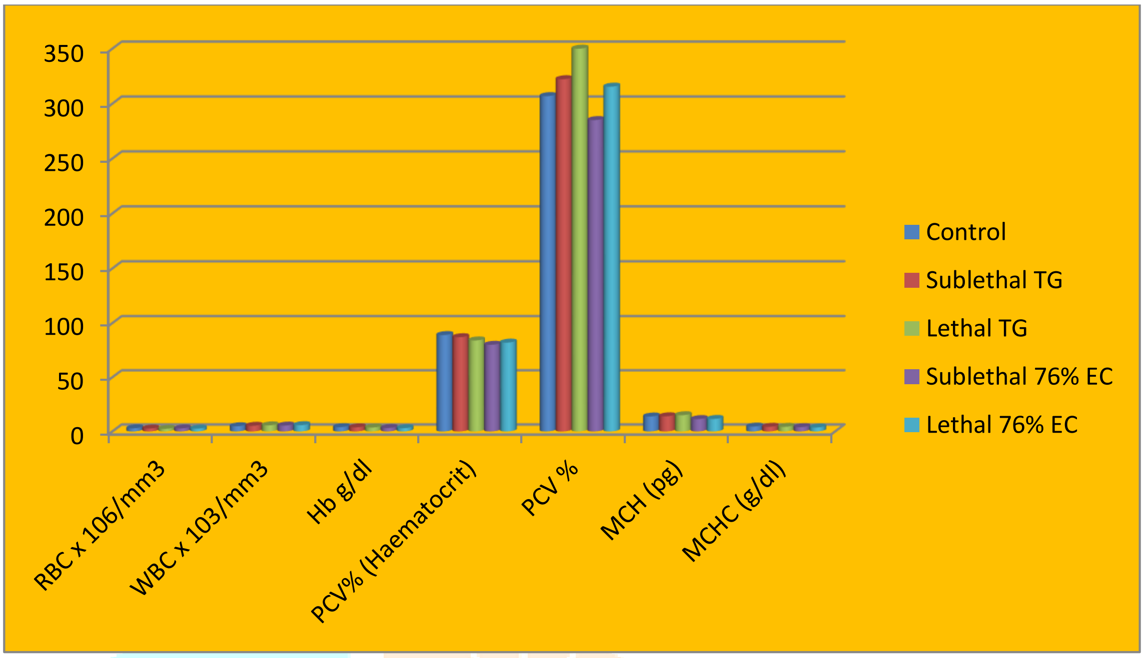

Figure 2.: Haematological changes of percentages in the parameters of blood of the fish *Catla catla* exposed in both sublethal and lethal concentrations for both Technical grade Dichlorvos as well as 76% EC as (Nuvan)