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HAEMOTOLOGICAL CHANGES INDUCED BY THE DELTAMETHRIN A SYNTHETIC PYRETHROID TECHNICAL GRADE AND 11% EC IN THE FISH *CYPRINUS CARPIO* LINNAEUS

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Abstract - Haematological changes determined in the fish *Cyprinus carpio* exposed to Deltamethrin technical grade and 11% EC both in lethal as well as in sublethal concentrations are reported. The blood parameters of Erythrocytes (RBC), Leucocytes (WBC), Haemoglobin (Hb), Hematocrit values (Ht), mean corpuscular volume (MCV), Mean corpuscular Haemoglobin (MCH), Mean corpuscular concentrations (MCMC) that determined by standard protocols are reported. The changes as effects when control are reported as: (a) RBC decreased (b) WBC increased (c) HB, Hb/MCV decreased, (except for lethal technical grade), (d) MCH and MCHC increased and that are going to be discussed with the earlier reports to elicit the information of the possible ways of explanation for such changes.

INTRODUCTION

Pyrethroid chemicals used to control insect pests, are definitely not recommended things to overcome the problems caused by the other groups of pesticides because they are causing the problem, globally and also cautioned to have more research on the effects of them on the living organisms as of the opinion of the world wild life fund (WWF) which was mentioned by Hasibur Rahman *et al*., 2014).

As and when these chemicals are transported into the aquatic environment, the non-target organisms, fish whose number is highest in the vertebrate, class of animals being present in the largest subdivision of earth the hydrosphere. Any warning signs of change in the ambient waters of thesuch sub-division resulted by an act of defilement, transported from the entry point and such causative factors, known as thepollutants, out of which few are the toxicants. To monitor such things of early warnings of the water quality and as such the studies of the blood as they are only affected serve as indices of toxicity.

Witeska *et al*. (2016) reported on the various blood cells of the fish *Cyprinus carpio*, viz., RBC and WBC, etc., and the present studied one is within the range of the quantitative aspects that were mentioned, including other aspects also. In the report of Vijayakumar (2016) where a comparative account of the three fish studies too reported on the shape and amount of cells of the blood. We know that the erythrocytes are nucleated, heart is a single circulated blood, popularly known as venous heart. The study of Svodbora *et al.* (2008) too mentioned the blood components of the *Cyprinus carpio.* Hence, in the present study is attempted to know the effects of the blood in the fish *Cyprinus carpio* exposed to both technical grade and 11% EC of deltamethrin while in toxic stress.

MATERIALS AND METHODS

Collection and maintenance of test organism

The freshwater fish *Cyprinus carpio* 3 to 5 cm in length 4 to 5 g in weight irrespective of their sex, have been chosen as the test organisms for the present investigation. Healthy and active fish were

obtained from local fish farms, Nandivelugu, 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±1°C. Water was renewed every day with 12-12h dark and light cycle and the one used for acclimatization and conducting experiments was clear unchlorinated ground water and the hydrographical conditions as physical and chemical properties of water were: Turbidity-8 silica units. Electrical conductivity at 28°C – 816 Micro ohms/cm, pH at 28°C-8.1, Alkalinity Phenolphthalein-Nil mg/l, Alkalinity; Methylorange-172 mg/l, Total Hardness (as $CaCO_3$-232 mg/l, Carbonate Hardness (as $CaCO_3$)-232 mg/l, Non Carbonate Hardness (as $CaCO_3$)-Nil mg/l, Calcium Hardness (as $CaCO_3$)-52 mg/l, Magnesium Hardness-40 mg/l, Nitrite Nitrogen (as N)-Nil mg/l, Sulphate (as SO_{42})- Trace mg/l, Chloride (as Cl-)-40 mg/l, Fluoride (as F-)-1.8 mg/l Iron (as Fe)-Nil mg/l, Dissolved Oxygen-8-10 ppm, Temperature-28+2 °C. 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 acute toxicity test. All the precautions laid by committee on toxicity tests to aquatic organism APHA (1998, 2005 and 2012) methods are 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 technical grade which was 95-98% pure which was supplied by the M/s. Tagroos Chemical India Ltd., (101/102, Kanchanganga factory lane, Boravil (West), Mumbai. The pesticide 11% EC (Decis) is locally purchased, manufactured and marked by the share company. The toxicant pesticides were introduced into water from where the pesticide entered into the fish through gills. A total of 50 fish were taken each in sublethal and lethal concentrations for both technical grade and 11% EC (Decis). The 96 hrs LC_{50} values are determined by Finneysprobit analysis (1971). Haematological changes were determined at the end period of exposure to 96 hrs LC_{50} value for Technical grade (2.0 µg/l) and for 11% EC(0.8 µg/l) and also $1/10^{th}$ of LC_{50} of 96 hrs exposed for 10 days for both technical grade and 11% EC (Decis) and the concentrations are 0.2µg/L, 0.08 µg/l respectively. 50% of the organisms were dead in lethal concentrations and 1% in sub lethal, while the remaining live organisms were sacrificed during the experimentation in both lethal and sub lethal concentrations for assays of blood parameters.

Sampling of blood

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

Haematological 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 mean corpuscular haemoglobin concentration (MCHC).

Determination of red blood corpuscles (RBC) count

RBC count was determined with an improved Neubauer crystalline counting chamber as was by the method of Shaperclaus (1979). The blood was sucked up to 0.5 mark on the RBC pipette and immediately Hayem's solution as a diluents stain was drawn upto 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° with the tip of the pipette at the junction of the edge of cover glass and the counting chamber. A pint of blood was placed from the tip of the pipette on the central platform near the edge of the cover slip, so that it was sucked up between the central platform and cover slip by the capillary force. The cells were allowed to settle for 2 to 3 min. The ruled area of the counting chamber was focused under the microscope and the numbers of RBC were counted in 80 small squares (4 squares of 16 at the four corners and one of 16 at the centre). The cells touching the upper and left hand lines were counted. 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: 1/400 sq mm

The depth of the counting chamber: 1/10 mm

Therefore the volume of a small square is: 1/400 x

1/10= 1/4000 cumm

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 using method described by 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 101mark 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 cover slip 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 millimetres and the number of WBC per cubic-millimetre was calculated.

Estimation of haemoglobin (Hb)

Hb concentration in the blood was estimated by cyanmethaemoglobin method of Blaxhall and Daisely (1973). 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.

Determination of packed cell volume (PCV) or Haematocrit value

Packed cell volume was determined by micro haematocrit method by Sclam*et al.* (1975). The heparinised blood was filled up to the mark 100 of the haematocrit tube with the help of Pasteur pipette and centrifuged at 3000 rpm 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.

Determination 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 femtoliter (fL).

MCV = Haematocrit (%) x10/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 pictogram (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 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 blood parameters alterations as well as present changes in both lethal and sub-lethal concentrations of technical and 11% EC (Decis) of deltamethrin are presented as graphical images of Figure 1 & 2.

DISCUSSION

Sana Ulla *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. Ullah *et al*. (2019b) reported the variations in the blood constituents of the fish. *Hypophthalmichthys moltrix*, RBC, WBC, Haemoglobin, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations were determined. 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 haemogynthesis and as well destruction was due to haemopoietic tissues. Hypoxid conditions that resulted in changes in oxygen consumption had impact on RBC, which might be the possible reason even in the present study.

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

Saumya Biswas *et al*. (2019 and Prusty *et al*. (2015)

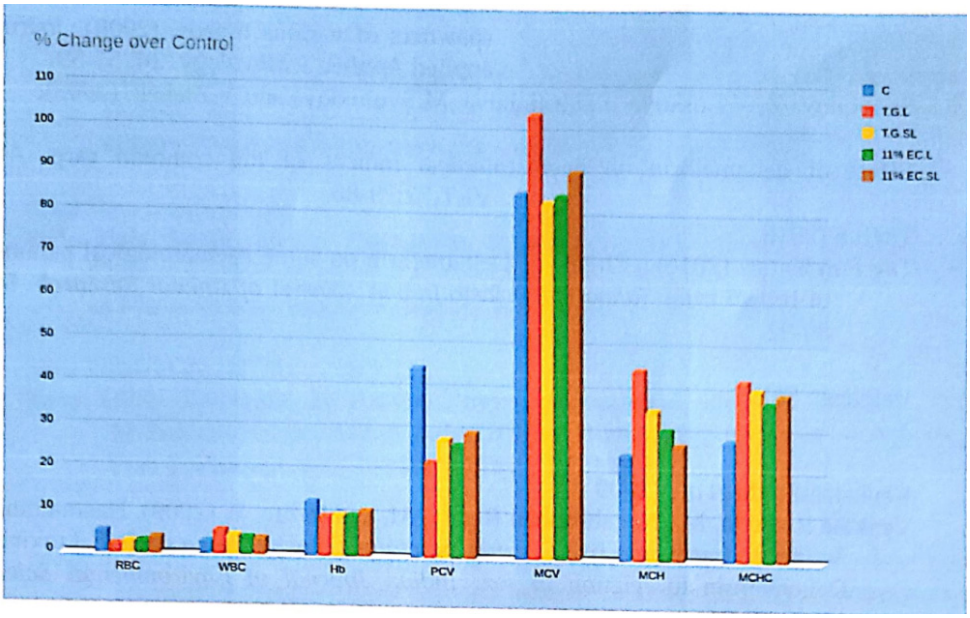

Fig. 1. Heamatological changes in the parameters of blood of *Cyprinus carpio* exposed to lethal(K) and sub lethal (SL 1/10th of LC50 value) concentrations both technical grade(TG) and 11% EC of delta methrin

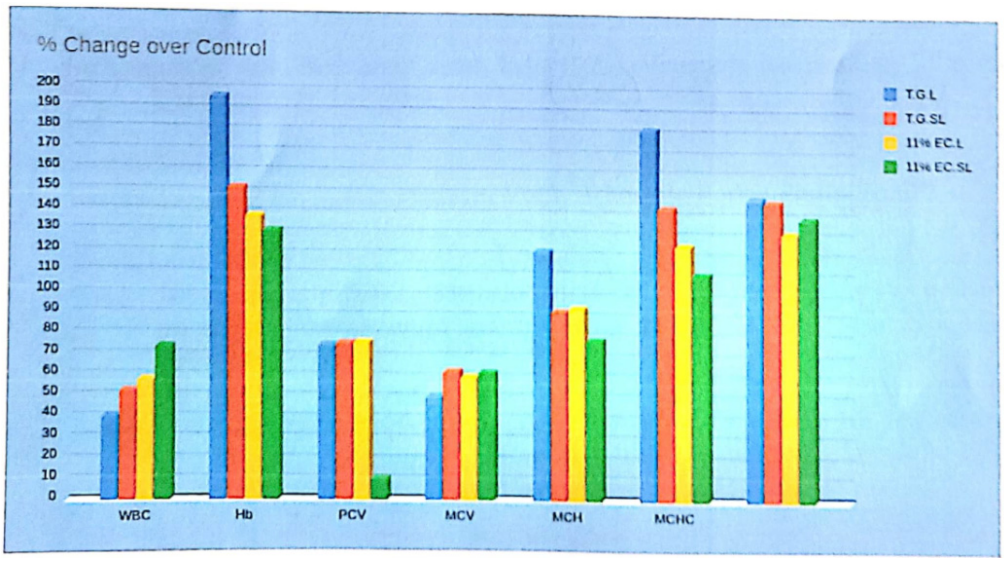

Fig. 1. Heamatological changes in the parameters of blood of *Cyprinus carpio* exposed to lethal(L) and sub lethal (SL 1/10th of LC50 value) concentrations both technical grade(TG) and 11% EC of delta methrin agains control (C, taken as 100%)

for synthetic pyrethroids and Sana Ullah, Zorrizahare (2015) and Krishna Murthy (2013) for other pesticides too in their review articles mentioned, all, about the Haematological alterations which serve as indices of toxicity.

Tayfun (2016) reported the effect of deltamethrin on some of the haematological parameters of brown trout (*Salmo trutta* fario), after exposure for four days, in two different concentrations of Deltamethrin i.e. 0.91 mg/l^{-1} and 188µg/l^{-1} and the results that reported were the WBC, Hb, PCV, MCV, MCHC decreased however RBC cells increased. The results of their study are quite contradicting of the present study may be due to the nutritional status of the fishes they studied and the present experimented one and the toxic stress as such that

behaved differently.

David *et al*., (2015) reported effect of Deltamethrin, the Haematological indices of Indian major carp *Cirrhinusmrigala* (Hamilton). The fish are exposed to both lethal and sublethalDeltamethrin 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 reported were RBC, Hb and haematocrit values decreased, whereas WBC, MCV and MCH were increased MCHC remain unchanged. The results are in agreement 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).

Vani *et al*. (2014) in the fish *Catla catla* exposed to deltamethrin exposed to 1/3rd of LC_{50} value i.e., 1.61 micro gms, had a decrement in total erythrocytes as well as haemoglobin which might be due to the condition of erythropenia that resulted anaemic condition either by destruction or inhibition of synthesis. Similar such report was also observed in the fish *Cyprinu scarpio*, by Svobdova*et al.* (2007).

Jayaprakash and Shettu (2013) reported changes in the haematology of the fresh water fish *Channa punctatus* exposed to Deltamethrinboth in lower concentration of 0.075 mg/l and higher one of 0.15 mg/l for 15, 30 and 40 days as sub-lethal and lethal exposures. 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. They had given the above new mechanism of action for changes in the blood of the fish.

According to Ahrar Khan *et al.,* (2012) report on the study of haemato– biochemical changes induced by pyrethroid insecticide, who also compared the toxicity and of Cypermethrin and Deltamethrin. Total erythrocyte count, Haemoglobin,Haemotocrit and Leucocytes at 2 ppm concentration being low along with MCV, MCH and MCHC and similar result for Deltamethrin at 1.61 mg/L exposure both ofthem belong to Type II type synthetic pyrethroids, with cyanogroup but Deltamethrin is more toxic to fish than Cypermethrin. Leukocytosis has been resulted for Cypermethrin exposure but for deltamethrin, it was leucopenia. But the present study, of *Cyprinu scarpio,* RBC values decreased sufficiently, more so in 11% EC whereas WBC values are increased more so in 11% EC, whereas WBC values are increased more so in 11% EC. Consequently MCH increased whereas Hb, PCV, MCHC, decreased and such measurement of the toxicity effect in haematological alterations serve as indices of toxicity. They opined that haemotological and biochemical disturbances result ultimately the damage to the tissues of the excretory organ kidney and metabolic organ the liver also. That is why they recommended that the doses of pesticides application must be strictly followed and also cautioned,their use, related to other insecticides for this group of insecticides as well, along with other groups.

Velisek *et al*. (2009) using bifenthrin as toxicant in the fish *Cyprinu scarpio* and also in the in different fishes as brown trout and common carp using triazone and deltamethrin (Velisek *et al*. (2011) again reported that the effects of pyrethroids and triazine pesticides on the fish physiology and mentioned about blood changes. The present study revealed the 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.

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 sub-lethal 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 that due to 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 *Cyprinus carpio* 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.

Gopalarao *et al.,* (2017) reported 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.

Neelima *et al*., (2015) reported on haematological alteration in *Cyprinus carpio* and considered as a biomarker of toxicity due to cypermethrin wherein RBC 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 at both lethal and sub lethal. 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 the status of healthy nature and sustenance is in jeopardy.

Mohan and Muthuswamy (2014) reported haematological alterations of pyrethroidcypermethrin 10% EC, exposure to the fish *Catlacatla* resulted RBC, MCV, MCHC being decreased whereas haematocrit, WBC, MCH values increased. Deltamethrin, Cypermethrin and Fenvalerate all the three belong to the synthetic pyrethroid of group II with cyanogroup.In the fish exposed to lower concentration Hb 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 destroyed or decrease in the rate of its synthesis. The MCV, MCH and MCHC values are completely dependent upon the RBC count.

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.

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

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

Julia Jasmin *et al*., (2018) reported haematological changes induced by Thiamethoxam a synthetic pyrethroid to the fish *Labeorohita*. When exposed to 0.002 and 0.004 ml/l of the toxicant, RBC and WBC decreased significantly in the fish exposed to 0.002 ml/l whereas decreased slightly in the fish exposed to 0.004 ml/l.

Similarly, when the fish *Labeorohita* exposed to Difenoconazole, a fungicide by Alkamisra *et al*. (2018) WBC, RBC, Hb, PCV and MCV decreased significantly in 0.002 ml/l whereas MCH and MCHC values increased. The results of the above due to the two toxicants exposure are contracting to the WBC count of the present study of Deltamethrin toxicant to the fish *Cyprinus carpio*. According to Alka Mishra (2017), who reported the toxic impact of pesticides on the morphological characteristics of blood cells of the fish *Channa punctatus* after the exposure to trichlorofan - an organophosphate and he opined that expansion of membrane 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.

Haematological alterations of the different toxicants such as Monocrotophos to the fish *Cyprinuscarpio* by Vaiyanan (2015), Diazonin by

Pourgholam*et al*., (2017) to the fish *Ctenopharyngodonidella*, RenuBala Singh (2016) testing endosulfan as toxicant to *Ctenopharyngodonidella*, Nile tilapia *Oreochromisniloticus* exposed to sublethal concentration of Mercury by Nilton *et al*., (2007), Jaya and Ajay (2014) to the fish *Clariasbatracus* by the toxicant Manocozeb, Abdul *et al*., (2014) to the fish *Cyprinuscarpio* by the toxicant Dichlorvos; Mallum *et al*., (2016) to the fish *Oreochromisniloticus* to the toxicant Dichlorvos. Aliakbar and Niazie (2015) to the fish sliver carp by the toxicant Diazion, George *et al*., 2017 Jerald felix (2015) and Anusiya Devi *et al*., (2017) are noteworthy.

The haematological parameters of the fish altered due to exposure to the different types of toxicant synthetic pyrethroids, Type I Permethrin, Type II Cypermethrin. Deltamethrin and Fenvalerate wherein the stress after swap resulted in the blood of the fish certain parameters even in low concentration termed as sub-lethal. Such sublethalas toxicants slowly is alienation of the time factor to succumb instead of not being in lethal concentration. The ambient waters defilement definitely poses a threat, as a result, alterations the blood of the fish and such changes of which when studied serve as a biomarker as opined by Kaviraj and Gupta (2014). They mentioned higher erythrocyte count haemoglobin, haematocrit, MCV, MHC and MCHC fail to harmonize resulting a disturbance of the equilibrium in homeostasis, a failure ultimately reflected on haemostasis is rendering to be unsuitable to lead a normal mode of life of the fish really, one can view that sublethal is really lethal wherein later there is a happy death but in the former it is slow suffocative death.

CONCLUSION

Hence, it may be concluded that even in the fish *Cyprinus carpio* common carp cultured along with the other carps when pesticide 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 cellular respiration is impaired there by growth is curtailed finally, it leads to a loss in the business venure of aquaculture. Hence, stringent measures have to be taken for quality control before giving pesticide representativeness for environmental usage.

REFERENCES

Abdul Majid Tak, F.A. Bhat Ulfat Jan and Mustafa Shah, G. 2014. Sublethal Haematological effects of Dichlorvos on the fresh water fish *Cyprinuscarpio* Var. Cummunis. *International Journal of Recent Scientific Research*. 5 (7): 1334-1337.

Ahrar Khan, Lahf Ahmmad and Muhammad Zargham Khan, 2012. Haemato biochemical changes induced by pyrethroid insecticides in avian, fish and Mammalian species. *International Journal of Agriculture and Biology*. 14: 834-842.

Aliakar Hedayati and Elahah Hassan Nataf Niazie, 2015. Haematological changes in silver carp *Hypothalmichthrysmoltrix* in response to Diazonin pesticide. *Journal of Environmental and Engineering*. 13: 52-59.

Alka Mishra, Md. Mahabubbur Rahaman and Md. Rahaman, 2018. Haematological changes in *Labeorohita* (H.) due to the exposure of the pesticides. Difenoconozale and Thiamehoxam.

Alka Mishra, 2017. Toxic impact of pesticides on the Morphological characteristics of blood cells of fish *Channa punctatus* (Bloch). *Indian J. Science Research.* 12 (2): 068-072.

Anusiya Devi, K., Dinukumari, S. and Vasanthi, J. 2015. Effects of Monocrotophos on Haematological Analysis of Blood in an Indian freshwater fish *Labeorohita. International Journal of Recent Advances in Multidisciplinary Research.* 2 (10) : 0870-0874.

APHA, AWWA and WEF 1998. *Standard Methods for the Examination of Water and Waste water,* 20th Edition, Clesceri L.S. Greenberg A.E. and Eaton A.D. (Eds). American Public Health Association, American Water Works Association Water Environment Federation, Washington, DC, USA.

APHA, AWWA and WEF 2005. *Standard Methods for the Examination of Water and Waste Water,* 21st Edition, Clesceri L.S. Greenberg A.E. and Eaton A.D. (Eds). American Public Health Association, American Water Works Association Water Environment Federation, Washington, DC, USA.

APHA, AWWA and WEF 2012. *Standard Methods for the Examination of Water and Waste water,* 22nd Edition, Clesceri L.S. Greenberg A.E. and Eaton A.D. (Eds). American Public Health Association, American Water Works Association Water Environment Federation, Washington, DC, USA.

Blaxhall, P.C and Daisley, K.W. 1973. Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5: 771-781.

David, M., Sangetha, J., Srinivas J. Harish, E.R., Naik, V.R. 2015. Effects of Deltamethrin on haematological indices of Indian major carp *Cirrhinusmrigala* (Hamilton). *International Journal of Pure and Applied Zoology*. 3 (1): 37-43.

Donald, H. and Bonford, 1963. *Hutchinson Clinical Methods* 14thEdn. London; pp: 145.

George, A.D.I., Akinrotimi, O.A. and Nwokoma, U.K. 2017. Haematological changes in African catfish *Clariusgariepinus* exposed to mixture of Atrazine and Metolachlor in the laboratory. *Journal of Fisheries Sciences.com*, 11 (3): 048-054.

Gopala Rao, N., Balakrishna Naik, R. and Srinivasa Rao, G. 2017. Haematological changes in the fish *Cyprinuscarpio* exposed to a synthetic pyrethroid (Class I) Permethrin and its 25% EC. *Current trends is Technology and Science*. VI (V): 759-763.

Hasibur Rehman, A.I. 2014. Thbiani, Azia, ShaliniSaggu, Zahid Khorshid Abbas Anad Mohan, Abid A. Ansari. Systematic Review on Pyrethroid toxicity with special reference to deltamethrin. *Journal of Entomology and Zoology*. 2 (6): 60-70.

Jaya Sahi and Ajay Singh 2014. Genotoxic and haematological effect of commonly used fungicide on fish *Clariasbatracus*. *Journal of Biology and Earth Sciences*, 4 (2): B137-B143.

Jayaprakash, C. and Shettu, N. 2013. Changes in the haematology of the fresh water fish *Channa punctatus* (Bloch) exposed to the toxicity of Deltamethrin. *Journal of Chemical and Pharmaceutical Research*. 5 (6): 178-183.

Jerad, F. Felik and Saradhamani, N. 2015. Impact of the Herbicide Glyphosate Roundup (41%) on the Haematology of the freshwater fish *Catlacatla* (Hamilton). *Journal of Environmental Science, Toxicology and Food Technology.* 9 (4) : 56-60.

Julia Jasnmin, Md. Mahabubur Rahman and Md. Motiur Rahman 2018. Haematological changes in *Labeorohita*due to exposure of pesticides, Difenoconazole and Thiamethoxam. *International Journal of Contemporary Research and Review*. 9 (1) : 20199-20205.

Kartas, T. 2016. Effects of deltamethrin on some haematological paramters of Brown trout (*Salmo truttafario*). *Indian Journal of Animal Research*. 50: 89-92.

Kaviraj Anilava and Abhik Gupta, 2014. Biomarkers of type II Synthetic pyrethroids, pesticides in freshwater fish. *Biochemical Research International*. 7 pages article ID 928063, http://dx-org.10.115/2014/92806.

Krishna Murthy, S. Kiran, B.R. and Venkateswarlu, M. 2013. A review on toxicity of pesticide in fish. *International Journal of Open Scientific Research*. 1 (1): 15-36.

Mallum, S.S., Sogbesan, O.A. and Haruna, A.B. 2016. Acute toxicity of Dichlorovos to *Oreochromisniloticus* and its Haematological effects. *International Journal of Novel Research in Life Sciences*. 3 (6): 53-60.

Mohan Kannan Padmanabhan and Muthu Swamy Venkatachalam 2014. Response of Synthetic pyrethroidcypermethrin of 10% EC induced stress in Biochemical and Haematological parameters of Indian major carp *Catlacatla* (Hamilton) 1822. *World Journal of Pharmaceutical Research.* 3 (4): 1976-1995.

Neelima, Cyrill, C. Arun Kumar, J. Chandra Sekhara Rao and N. Gopala Rao 2015. Histopathological alterations in gill, liver and kidney of *Cyprinus carpio* (Linn) exposed to cypermethrin 25% EC. *J. Adv. Research Biological Sciences* 2 (2) : 34-40.

NiltonMassuo Ishikawa Maria Josi Tavares, RanzaniPaiva Julio Vicente Lombardi and Claudia Maris Ferreira, 2017. Hematological Parameters in nileTalapia, *Oreochromisniloticus*. Exposed to sublethal concentrations of Mercury. *Brazilian Archives of Biology and Technology*. 50 (4) : 619-626.

Nuri Cakmak and Girgin, 2003. Toxic effects of synthetic pyrethroid, cypermethrin on blood cells of Rainbow trout *(Oncorhychinus mykiss:* Wabaum). *Online Journal of Biological Sciences*. 3 (8): 694-698.

Patole, S.S., Patil, M.U. and Bhoi, S.S. 2016. Effect of Fenvalerate Synthetic Pyrethroid on a certain Haematological parameters of Freshwater fish *Channamarulius* (Hamilton-Bach). *International Journal of Life Sciences Scientific Research*. 2(3): 269-272.

Pourgholan, R., Soltani, M., D.M. Hassan, F., Esmelli, H., Farhourm and P. Usefi, 2017. Evaluation of Blood parameters of grass carp *Ctenopharyngodonidella* after exposure to organophosphate diazonin. *Indian Journal of Fisheries Science.* 3 (2): 1-16.

Prusty, A.K., Meena, D.K., Mohapatra, S., Panikkar, P., Das, P., Gupta, Sk. and Behera, B.K. 2015. Synthetic pyrethroids Type II and freshwater fish culture: Perils and Mitigations. *Int. Aquatic Research*. 7: 163-191.

Renu Bala Singh, 2016. Evaluation of haematological response of grass carp *Ctenopharyngodonidella*after exposure to Endosulphan. *International Journal of Fisheries and Aquatic Research*. 1 (1): 01-04.

Rodriguez, M.R., F.J. Ordonez, Rasety, J.M. and M. Rasety, 2005. Erythrocyte antioxidant enzymes of gill head as early warning indicators of oxidative stress induced by Malathion haematology. 8 (2) : 237-240.

Sana Ullah and Mohammad Jallil Zorrieha Zahra, 2015. Ecotoxicology: A Review of pesticides induced toxicity in fish. *Advances in Animal and Veterinary Sciences.* 3 (1): 41-57.

Sana UllahZhongglu hi Amina Zuberi, Muhammad Zain UlArifeen Mirza Muhammad Faran Ashraf Baig, 2019a. Biomarkers of Pyrethroid toxicity in Fish. *Environmental Chemistry Letters.* 17 : 945-973.

Sana Ullah, Li Z, UI arifeen, MZ Khan, Fahal, 2019b. Multiple biomarkers based appraisal of deltamethrin induced toxicity in silver carp *Hypophthalmichthys molitrix*. *Chemosphere*. 214 : 19-523.

Saumya Biswas, KausikMondal, Salma Hague, 2019. Review on effect of type II synthetic pyrethroid pesticides in fresh water fishes. *Environmental Ecology.* 37 (1): 80-82.

Schalm, O.W. Jain, N.C. and Carrol, E.J. 1975. Veterinary haematology 3rd edition, Lea Febiger Philadelphia.

Shaperclaus, W. 1979. *Fish Diseases*. Vol. I, Academic Verlag Berlin, pp.570.

Sheik Jamal, A.L. 2006. Haematological changes induced by a pyrethroid insecticide Fenvalerate in catfish *Clariasgariepinus. Sci. J. Fac. Sci. Minufa Univ*. 20 : 89-

100.

Svobodava, Z., V. Luskova, J. Drastichova, M. Svobodava, and V. Ziabek, 2003. Effect of Deltamethrin on haematological indices of the common corp. *ACTA VET*. 72 : 79-85.

Svobodova, Z., H. Kronpora, H.Monda, M. Flajasham, T. Randak, L.V. Suvina and D. Gela, 2007. Haematological profile of common carp speniceus of various breeds. *J. Applied Ichthyology*. 24: 55-59.

Tay Fun Kartas, 2016. Effects of Deltamethrin on some haematological parameters of brown trout *Salmo truttafario*. *Indian Journal of Animal Research*. 50 (1): 89-92.

Vaiyanan, V., Sridaran, G., Raveendran, S. and Chairman, U. 2015. Impact of pesticide on Haematological parameters of *Cyprinus carpio*. *World Journal of Pharmacy and Pharmaceutical Sciences.* 4(8): 1424-1430.

Vani, T.,N. Saharan, S.C. Mukherjee, Ritesh Rajani, Rajesh Kumor, R. and K. Brahmachari, 2014. Deltamethrin induced alterations of haematological and Biochemical parameters in fingerlings of *Catlacatla* (Hamilton). *Pesticide Biochemistry and Physiology*. 101: 16-20.

Velisek, J., Stare, A. and Svobodova, Z. 2011. The effects of pyrethroid and Triazine pesticides on fish Physiology Chapter (17) Pesticides in the modern World Pests Control and Pesticides exposure and Toxicity Assessment. 377-402.

Velisek, J., Z. Svobdova and J. Machova, 2009. Effects of bifenthrinon some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). *Fish. Physiol. Biochem.* 35 : 583-590.

Venkata Ramudu, M., Nagabhushan Reddy, M. and Indira, P. 2009. Haematological Studies in freshwater fish *Channa punctatus* (Bloch) during sublethal toxicity of Deltamethrin in relation to sex. *Indian Journal of Environmental Sciences.* 13 (1): 79-84.

Vijayakumar, M. 2016. Morphometric studies of blood cells in *Cyprinuscarpio*, *Ctenopharyngodanidella* and *Hypophthalmichthys molitrix* cultured fish in west Godavari region of Andhra Pradesh. *International Journal of Fisheries and Aquatic Studies*. 4 (5): 489-493.

Witeska, M.K., Lugowska and Kondara, E. 2016. Reference values of haematological parameters for juvenile *Cyprinuscarpio*. *Bull. Env. Ass. Fish Pathology*. 36 (4).