Biospectra : ISSN : 0973-7057, Vol. 15(1), March, 2020, pp. 317-324 **34(AS).**
An International Biannual Refereed Journal of Life Sciences ***Animal Science***

ISSN : 0973-7057

Int. Database Index: 663 www.mjl.clarivate.com

Hematological changes in *Heteropneustes fossilis* exposed to organophosphate pesticides

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Received : 10th February, 2020 ; Revised : 20h March, 2020

Abstract: Defilement of anthropogenic xenobiotic chemicals Viz., pesticides as organophosphate–the dichlorvos alter the circulatory fluid, blood in the heterotrophic, cold blooded nektonic culturableexotic fish, Hetropneustes fossilis The fish were exposed to both lethal and sublethal concentrations of technical grade and 76% EC (Nuvan). In both the concentrations the branchial single circuit blood pumped through venous blood, right from the early point showed imbalance of homeostasis with a dire consequence of haemostasis. The alterations studied were RBC, WBC, Hb, Haematocrit, PCV, MCV and MCHC. A decrement and increment of the parameters studied were reported herewith.

Key words: ***Heteropneustes fossilis*, Dichlorvos, 76% EC (Nuvan) RBC, WBC, Hb, Haematocrit, PCV, MCH and MCHC, lethal**

INTRODUCTION

All pollutants are not toxicants but all toxicants are pollutants. The toxicants cause the death if it is in lethal where as in sublethal concentration; render the animal not suitable to maintain its life, the active buoyant swimmers especially the fish, heterotrophic cold blooded animals. Due to the presence even in low concentrations bring changes and in aquatic toxicology any variations in the blood serve as biomarker. Such rapid changes in the characteristics of the fish blood measured are used as indices of pollution by pesticides. The provide assessment of pollution and will be warning sign of the inhabiting organism. Such alterations either increase or decreases in the blood were studied and reported and even mentioned in the review articles.[1-4]

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Different chemicals of the four classes of pesticides organochlorines, organophosphates, carbamates and synthetic pyrethroids are reported and such changes in the fish are varied being species specific.[5-8] How ever paucity of information is available for the grass carp *Heteropneustes fossilis,* for the pesticide dichlorvos. Hence the present study is contemplated to assess the effects of the toxicant dichlorvos both in lethal and sublethal concentration using technical grade as well as 76% EC as Nuvan.[9-11]

MATERIAL & METHODS

Collection and maintenance

The freshwater fish *Heteropneustes fossilis* 3 to 5cm in length 4 to 5 gms in weight irrespective of their sex, have been chosen as the test organisms for the present investigation. Healthy and active fish were obtained from Madhepura fish hatcheries, Bihar, India in June 2015. The

fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28±1°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 on toxicity tests to aquatic organism were followed and such acclimatized fish only were used for experimentation.[12,13]

The technical grade which was 95-98% pure which was supplied by the Syngenta India Ltd, Mumbai-400 020. The pesticide 76%EC Nuvan is locally purchased manufactured by Hikal Limited 629/630 GIDC Industrial estate Panoli Bharuch Gujarat marked by Syngenta India., 14, J. Tata Road, Mumbai. The organophosphate pesticide was introduced into water from where the pesticide entered into the fish through gill. A total of 50 fish were taken each in sublethal and lethal concentrations for both technical grade and 76% EC Nuvan. The 96hrs LC_{50} values are determined by Finneysprobit analysis as mentioned in APHA.[14,15]

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.

The haematological variables analyzed were red blood cells count (RBC), hemoglobin (Hb), white blood cells count (WBC), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

RBC count was determined with an improved Neubauer crystalline counting chamber.[16] The blood was sucked up to 0.5mark on the RBC pipette and immediately, Hayem's solution as a diluent stain was drawn up to 101 mark and the pipette was rotated between the thumb and the forefinger 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 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 cu 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

WBC count was determined.[17] The blood was drawn up to 0.5 mark of WBC pipette and immediately the diluting fluid was drawn up to the 101 mark 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^o 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.

Hb concentration in the blood was estimated by cyanmethaemoglobin method.[18] Hb is converted into cyanmethaemoglobin by the addition of potassium ferricyanide (KCN) and the colour was read in a spectrophotometer at 540 nm against a reagent blank.

Packed cell volume was determined by micro haematocrit method.[19] 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 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.

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.

Mean corpuscular haemoglobin 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 reflects the average concentration of the haemoglobin in the red blood cells in a given 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 (%)

RESULT & DISCUSSION

The blood parameters in both lethal and sublethal concentration of technical grade as well as 76% EC Nuvan are presented in the Figure-1 and represented as graph in Figure-1. Marked changes are observed in exposed fish in the commercial formulation experimented fish resulted more percentage 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 and more percentage of decrement in 76% EC. The WBC count increased in both lethal and sublethal more in 76% EC. The haemoglobin content decreased in both lethal and sublethal concentration more in 76% EC. The haematocrit value altered accordingly to the RBC count. Accordingly the calculated values of MCV, MCH and MCHC also showed changes.

The review article mentioned about organochlorines organophosphates, carbamates and synthetic pyrethroids alter the haematological changes as effects in fish. The work of organophosphates was reviewed and gave a thought that the pesticides do effect the biochemical changes in the blood and also parameters of the blood change.[20-22] In the opinion of the review articles on toxicity of pesticides in fish documented the work where pesticide induce alteration in blood parameters and stressed the impact of such changes.[23,24] Haematobiochemical changes induced by pyrethroid insecticides in avian, fish and mammalian species.[25] The effects of pesticides in the blood of fish serve as indices of toxicity. The review on dichlorvos toxicity in fish indirectly opined that the toxic action result in fish alteration are likely and that is mentioned. The dichlorvos toxicity on fish, a review published and commented on the toxic effect of biochemical nature of the circulating fluid.[26] The review mentioned that the haematological parameters are important in toxicological research and indicative of toxic stress. After exposure to two sublethal concentration 203 µg/L and 68.0 µg/L of chlorpyrifos for 1, 3, 7, 14 and 21 days a decreased erythrocyte count, leucocyte count, haemoglobin and haematocrit mean values in *Channa punctatus* (Bloch)

Toxication altered the haematological parameters in *Cyprinus carpio* particularly ESR count due to chlorpyrifos toxicity which is another organophosphate.[27-29] The same was opined, for the same chlorpyrifos to *Cyprinus carpio*.[30] In bony fish *Tilapia guineensis* exposed to common pesticides produced characteristic alteration in the blood .[31]The review on the toxicity and other effects of dichlorvos an organophosphates pesticides to freshwater fish, documented exposure of *Orieochromis niloticus* to dichlorvos for 96 hours duration marked changes of packed cell volume (PCV), haemoglobin (Hb), Red Blood cell (RBC), neutrophil, monocyte and lymphocytes count. The review also mentioned the work done on the fish *Cyprinus carpio* exposed to dichlorvos.[32] The other works also emphasized the haematological changes are no doubt serve indices of toxicity.[33-36]

Haematological changes of silver carp *Hypoph thalmichthys molitrix* in response to diazonin pesticide after the exposed to half of the lethal concentration was reported.[37] The results showed that leucocytes (WBC), haematocrit (Ht) haemoglobin (Hb), MCHC and lymphocyte cortisol and glucose increased where MCV and MCH were significantly decreased. They also reported to significant differences in RBC Monocyte and Eosinophils among the fish exposed to the toxicant which are derivative changes. Haematological parameters of *Cyprinus carpio* exposed to Monocrotophosan organophosphate exposed to 4.5 ppm, 6.7 ppm and 13.5 ppm was studied and found that a significant decrease in all the sublethal concentrations and maximum decrease at 12.5 ppm RBC content, ESR PCV and a significant increase in RBC and WBC count was reported.[38] The effect of Glyphosite (Roundup 41%) on the haematology of the freshwater fish *Catla catla* was studied and the RBC, WBC, haemoglobin and haematocrit value showed marked decline while MCV, MCH and MCHC showed fluctuating pattern.[39] The present work can be

correlated with this work, but both of them belong to same group of pesticides i.e., organophosphate.

Changes in haematology of the freshwater fish *Channa punctatus* (Bloch) exposed to delta methrin for 45 days was reported, where there was a significant decrease.[40]

In haemoglobin content, total erythrocyte count, PCV, MCV and MCHC. On the other hand a significant increase in total leucocyte count and MCH which coincides the present work. Toxic impact of pesticide trichlorofon on the morphological characteristics of blood cells of fish *Channa punctatus* (Bloch) and opined not only the quantitative aspects of the blood parameters are altered but also the morphological aspects of the fish where compared with control are altered. Anisocytosis deformed erythrocytes and large bulged nucleus, deformed cells are clumped and finally cell lysis, an increase in the size of MCV has been associated in response to stress.[41] Haematological changes induced by pyrethroid insecticide fenvalerate in cat fish *Clarias gariepinus* was reported a significant decrease in the haemoglobin content haematocrit value and erythrocytes whereas the leukocyte was increased.[42] The work is similar of the present report even though both of them belong to two different classes of pesticides. Haematological response of grass carp *Ctenopharyngodon idella* after exposed to endosulphan was studied.[43] The fish were exposed to two sublethal concentrations for 15, 30 and 45 days. The RBC, haemoglobin content and hematocrit values showed declined trend and MCH, MCHC also showed declined trend while MCV showed an increasing trend. She opined variation in erythrocyte count is the indices of polluted aquatic environment. The present study also showed similar results but both of them belong to different class of pesticides.

The effects of Deltamethrin on haematological indices of Indian major carp *Cirrhinus mrigala* (Hamilton) was studied by exposing the fish to lethal and sublethal concentrations and the results showed that the RBC, HB and Haematocrit values decreased in both lethal and sublethal concentration. WBC, MCV and MCH were increased consequently.[44] An increase in WBC revealed haematological toxicity coincide the present work. Haematological changes in African catfish *Clarias gariepinus* exposure to mixture of Atrazine and metolachlor in the laboratory was studied.[45] Because of mixture, the two chemicals behaved differently when compared with the present result. A significant reduction in Hb, RBC, PCV and MCV were observed. The MCHC of result showed an increase. The haematological parameters are used in monitoring health status of the fish in the wild and culture medium. Even the present study fish is a culturable one and stringent steps have to be taken not to use the pesticides in disease management of culture practices. Haematological changes in the fish *Channa punctatus* (Bloch) exposed to fenvalerate. The percentage of haemoglobin PCV, RBC showed a gradual increase while WBC showed are increase.[46] The result showed similar trend of the present study. Haematological effects of commonly used fungicide on fish *Clarias batrachus* was studied where they exposed the fish to sublethal concentration and blood was assayed for selected haematological parameters.[47] A decrease in the Hb, MCV, MCHC, RBC levels and an increase in WBC level was observed. The report in strictly different owing to chemical nature of the two toxicants i.e., Chloropyrifos and Mancozeb.

Haematological changes in the fish *Cyprinus carpio* exposed to permethrin, a synthetic pyrethroid of class I type was reported.[48] Where in a decrease in RBC, Hb, PCV was found but an increase in WBC, MCV, MCH, MCHC was observed when the fish is exposed to technical grade and 25% ECin both lethal and sublethal concentrations. Commercial formulation concentration was causing more alteration because of the ingredients present which shows similar result of the present study even though both belong to different classes. A similar report was observed in sublethal effects of dichlorvos on the freshwater fish *Cyprinus carpio* var. Communis.[49] The fish were exposed to three sublethal concentrations 50% 60% and 70% and the results showed changes of the RBC and WBC count, Hb and haematocrit and PCV. Since osmoregulatory dysfunction was the cause for increase in the rate of destructions in hematopoietic organs. The same is reiterated. Studies on freshwater fish *Channa punctatus* (Bloch) in sublethal toxicity of deltamethrin in relation to sex was reported. The fish exposed to different concentrations of deltamethrin ranging from 0.02ppm and 0.2ppm of 5 exposure periods 24, 7, 15,20 and 30 days in sub lethal concentrations and due to long duration exposure, due to change in haemostatic mechanisms the fish showing leucocytosis initially and fish might have evoked a changed and neutralized the toxicant effects of deltamethrin.

However the present study cannot be compared with this result because of a different methodology. Haematological alterations in *Cyprinus carpio* as biomarkers of cypermethrin toxicity exposed to lethal and 1/10th of 96h LC_{50} values as sublethal for 5, 10, 15 days. RBC count, Hb content, PCV showed decrement at both lethal sublethal concentrations. WBC count and MCHC exhibited increasing trend at sublethal and decreasing trend at lethal concentrations whereas MCH increased in both concentrations. The presence of the toxicant disturbs the balance in the echo systems and the fish is definitely not comfortable.

CONCLUSION

Hence it may be concluded that, even the *Heteropneustes fossilis* a grass carp cultured along with the other major carps, when pesticides contaminate the culture medium, alter the blood parameters and such alterations are more severe in EC due to the ingredients mixed. Hence stringent measures have to be taken for quality control before giving pesticide representativeness for environmental usage.

REFERENCES

1. **Sahan A, Altun T, Cevik F, Cengizler I, Nevsat E, Genc E. 2007.** Comparative study of some haematological parameters in Europeaneel (*Anguilla anguilla* L., 1758) caught from different regions of Ceyhan River (Adana,Turkey). E.U. *Journal of Fisheries and Aquatic Sciences*. **24 (1-2):** 167–171.
2. **Di Giulio RT, Hinton DE: The toxicology of fishes. Taylor & Francis Group. 2008.** Hepatic antioxidant status and hematological parameters in rainbow trout, *Oncorhynchus mykiss*, after chronic exposure to carbamazepine. *Chemico-Biological Interactions*. **183:** 98–104.
3. **Velisek J, Stara A, Svobodova. Z.2011.** The effects of pyrethroid and trazine pesticides on fish physiology. In: Pesticides in the Modern World–Pests Control and Pesticides Exposure and Toxicity Assessment, Stoytcheva, M., (Ed.). *InTech Open Access Publisher*. 377–402.
4. **Shinde BR, Borane VR, Zambare SP 2014.** Ascorbate effect on endosulfan and cypermethrin induced alterations in the RBC of the fresh water fish, *Channa orientalis* (scheinder*). Journal of Environmental Research and Development*. **8(3A):** 673-675.
5. **Ullah S, Zorriehzahra MJ: Ecotoxicology2015:** A review of pesticides induced toxicity in fish.Advances in Animal and Veterinary Sciences**. 3(1):** 40-57.
6. **Shankar Murthy K, Kiran BR, Venkateshwarlu M. 2013.** A review on toxicity of pesticides in fish. *International journal of open scientific research*. **1(1):**15-36.
7. **Chandra SekharaRao J, Neelima P, GovindaRao K2017:** A review on the toxicity and other effects of Dichlorvos, an organophosphate pesticide to the freshwater fish. *Bioscience Discovery*. **8(3):**402- 415.
8. **Das S 2013:** A review of dichlorvos toxicity in fish. Current world environment.**8 (1):** 143-149.
9. **Neelima P, Sunitha K, Gopala Rao N, Chandra S, Rao J.2015.** Haematological alterations in *Cyprinus carpio* as biomarkers of cypermethrin toxicity. *International journal of current research*. **7(8):** 18864-18870.
10. **HomairaAfreen, Toufiq-Ul Amin, Siddiqul Islam. Md, Salma Parvin, Rayhanus Salam. 2016.** In vitro phytochemical analysis and cytotoxic assay of leaves of *Solanum lycopersicum* Linn by brine shrimp bioassay. *Journal of Innovations in Pharmaceuticals and Biological Sciences*. **3 (3):**81-85.
11. **Finney DJ.** Probit analysis 3rd Ed., *Cambridge University Press, London, New York*, 333p.
12. **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 Work Association, Water Environment Federation, Washington DC. USA.
13. **Shaperclaus. W. 1979.** Fish Diseases, vol. 1. AkademieVerlag, Berlin.570.
14. **Donald H and Bonford RR. 1963.** Hutchinson Clinical Methods. 14th Edtion. London.; 145.

15. **Blaxhall PC, Daisley KW. 1973.** Routine haematological methods for use with fish blood. *Journal of fish biology*; **5:** 771- 781.

16. **Schalm OW, Jain NC, Carroll EJ. 1975.** Veterinary Haematology, 3rd Edition.; Lea and Febiger, Philadelphia.

17. **Saeedi FM, Roodsari HV, Zamini A, Mirrasooli E, Kazemi R. 2012.** The Effects of Diazinon on Behavior and Diazinon on Behaviour and Some Haematological Parameters of Fry Rainbow. Trout (*Oncorhynchus mykiss*). *World Journal of Fisheries and Marine Sciences.* **4(4):** 369- 375.

18. **Sampath K, James R, Velammal S. 2003.** Effect of methyl parathion on blood parameters and its recovery in a catfish, *Mystus keletius*. *Indian Journal of Fisheries.* **50(2):** 191-197.

19. **Svoboda M, Luskova V, Drastichova J, Zlabek V. 2001.** The effect of diazinon on haematological indices of common carp (*Cyprinus carpio* L.). *Acta Vetrinary Bruno*. **70:** 457-465.

20. **Satyanarayan S, Bejankiwar RS, Chaudhari PR, Kotangale JP, Satyanarayan A 2004:** Impact of some chlorinated pesticides on the haematology of the fish *Cyprinus carpio* and *Puntius ticto*. *Journal of Environmental Science.* **16(4):** 631-634.

21. **Geyer HJ, Steinberg CE, Scheunert I, Brüggemann R, Schütz W, Kettrup A, Rozman K. 1993.**A review of the relationship between acute toxicity (LC50) of gamma-hexachlorocyclohexane. (gamma-HCH, Lindane) and total lipid content of different fish species. *Toxicology*. **83 (1-3):**169-179.

22. **Ahrar khan, Latif Ahmad and Muhammad Zargham Khan. 2012.** Review article, Hemoto -Biochemical changes induced by pyrethroid insecticides in Avian, fish and mammalian species. *International Journal of agriculture & biology*. **14(5):**834-842.

23. **Deka S, Mahanta R. 2015.** Dichlorvos toxicity on fish –A review. *European Journal of Biological Research.* **5 (3):**78-85.

24. **Sunanda M, Chandra SekharaRao J, Neelima P, GovindaRao K, Simhachalam G. 2016.** Effects of Chlorpyrifos (an Organophosphate Pesticide) in Fish. *International Journal of Pharmaceutical Sciences Review and Research*; **39(1):** 299-305.

25. **Ali D, Kumar S. 2012.** Study on the effect of chlorpyrifos on acetylcholinesterase and hematological response in *Channa punctatus* (Bloch*), IIOAB Journal*. **3(5):** 12-18.

26. **Malla FA, Sharma G, Singh S. 2009.** Chlorpyrifos pesticide toxicity on erythrocyte sedimentation rate in fish, *Channa punctatus* (Bloch.) *Biology and Medicine*; **1(2):** 54-55.

27. **Ramesh M, Saravanan M 2008.** Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. *International Journal of Integrative Biology*; **3(1):** 80-83.

28. **Chindah AC, Sikoki FD, Vincent-Akpu I. 2004.** Changes in haematological characteristics of a bony fish, *Tilapia guineensis* (Bleeker, 1862), exposed to common pesticides in the Niger Delta Wetland, Nigeria, *Ghana Journal of Agricultural Science*; **37:** 59

29. **Tak AM, Bhat FA, Jan U, Shah GH 2014.** Sublethal haematological effects of dichlorvos on the freshwater fish, *Cyprinus carpio* var. communis. *International Journal of Recent Scientific Research;* **5(7):** 1334- 1337.

30. **Lakshmanan SA, Rajendran C, Sivasubramaniyan 2013.** Studies on impact of Dichlorvos on selected haematological parameters of freshwater fish, *Orechromis mossambicus* (Peters*). International Research Journal of Biological Science*s. **3(1):** 28-33.

31. **Ashade OO, Ashiru AW, Obiri CM 2011.** The comparative study of the toxic effects of 2-3 diclorovinyl dimethyl phosphate (DDVP) and chlorpyrifos on the behaviour and haematology of african- (*Clarias gariepinus). International Journal of Science and Society Yabatech*. **1(1):** 38-47.

32. **Sobhana KS, George KC, Sanil NK, Surendran KK, Paul Raj R. 2006.** Immunosuppressive action of nuvan (dichlorvos) in the banded pearl spot, *Etroplus suratensis* - evaluation of effect on haematology and humoral immune response. *Journal of the Marine Biological Association of India*; **48(1):** 29-34

33. **Das BK, Mukherjee SC 2000.** Sublethal effect of quinalphos on selected blood parameters of *Labeo rohita* (Ham.) fingerlings. *Asian Fisheries Science*; **13**: 225-233.

34. **Hedayati A, Hassan NatajNiazie E 2015.** Hematological changes of silver carp in response to Diazinon pesticide. *Journal of Environmental Health Science*

35. **Vaiyanan V, Sridharan G, Raveendran S, Chairman K 2015.** Impact of pesticide on haematological parameters of *Cyprinus carpio*. *World Journal of pharmacy and pharmaceutical sciences*; **4(8)**: 1424-1430*and Engineering*;**13 (52):** 1-5

36. **Jerald Felix F, Saradhamani N. 2015.** Impact of the herbicide glyphosate roundup (41%) on the haematology of the freshwater fish, *Catla catla* (Hamilton). *IOSR Journal of Environmental Science, and Food Technology*; **9 (4) 3**: 56-60.

37. **Jayaprakash C, Shettu N. 2013.** Changes in the hematology of the freshwater fish, Channa punctatus (Bloch) exposed to the toxicity of deltamethrin. *Journal of Chemical & Pharmaceutical Research*;**5(6)**:178-183.

38. **Mishra A. 2017.** Toxic impact of pesticides on the morphological characteristics of blood cells of fish *Channa punctatus* (Bloch*) Indian Journal of scientific Research*. **12 (2):** 068-072.

39. **Sheikh Jamal AL. 2006.** Haematological changes induced by a pyrethroid insecticide fenvalerate in catfish *Clarias gariepinus*. *Sci. J. Fac. Sci. Minufa Univ*.; **20**: 89 -100.

40. **Renu Bala Singla. 2016.** Morphometric alterations in exotic freshwater fish, *Ctenopharyngodon idella* (Cuv. and Val.) upon exposure to endosulfan *International Journal of Fisheries and Aquatic Studies*. **4(3):** 590- 593.

41. **David M, Sangeetha J, Shrinivas J, Harish ER, Naik VR. 2015.** Effects of deltamethrin on haematological indices of indian major carp, *Cirrhinus mrigala* (Hamilton) *International Journal of Pure and Applied Zoology*. **3(1):** 37-43.

42. **George ADI, Akinrotimi OA, Nwokoma UK. 2017.** Haematological changes in African Catfish (*Clarias gariepinus*) exposed to mixture of Atrazine and Metolachlor in the laboratory. *Journal of Fisheries Sciences*. **11(3):**048-054.

43. **Tilak K S, Satyavardhan K. 2002.** Effect of feneveralate on oxygen consumption and haematological parameters in the fish, *Channa punctatus* (Bloch). *Journal of Aquatic Biology*; **17**:81-86.

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

45. **GopalaRao N, Bala Krishna Naik R, SrinivasaRao G 2017:** Haematological changes in the fish *Cyprinus carpio* exposed to a synthetic pyrethroid [Class I], Permethrin and its 25% EC. *Current Trends in Technology and Science*. **6 (5):** 759-763.

46. **Abdul MajidTak, Bhat FA, UlfatJan, Mustafa Shah G. 2014.** Sublethal haematological effects of dichlorvos on the fresh water fish, *Cyprinus carpio* var. Communis. *International Journal of Recent Scientific Research Research*. **5 (7):**1334-1337.

47. **Jenkins F, SmithJ, Rajanna B, Shameem U, Umadevi K, Sandhya V, Mahadevi R. 2003.** Effect of sublethal concentration of endosulfan on haematological and serum biochemical parameters in the carp *Cyprinus carpio*. *Bulletin of Environmental Contamination and Toxicology.* **70:**993-997.

48. **Seth N, Saxena KK. 2003**. Hematological responses in a freshwater fish, *Channa punctatus* due to fenvalerate. *Bulletin of Environmental Conta mination and Toxicology*. **71:** 1192-1199.

49. **Venkata Ramudu M, Nagabhushan Reddy M, Chennaiah K, Indira P. 2008.** Carbohydrate metabolism in freshwater fish *Channa punctatus* (Bloch) during sublethal toxicity of deltamethrin in relation to sex. *Bulletin of Pure & Applied Sciences Section A*. **27(1):** 7-16.

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