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Original Article

THE TOXICITY EFFECT OF MONOCROTOPHOS 36% E. C ON THE HAEMATOLOGY, *LABEO ROHITA* (HAMILTON, 1882)

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ABSTRACT

Objective: Pesticides are stable compounds and they enter the aquatic ecosystem through the agriculture run off. The evaluation of nature and degree of harmful effects produced by the toxic substance in the aquatic organisms are evaluated by toxic tests. The 96 hour LC_{50} values have generally been found to be satisfactory for the measurement of acute toxicity. The differences in 96 h LC_{50} of the same toxicant in different fishes may be attributed to individual traits including those of behavior and additional structure such as accessory respiratory organs. The individual characters such as size and weight, sex and biological behavior are important determination for variation in LC_{50} values.

Methods: Therefore, in this present study is an attempt to study the toxicity of the pesticide with respect to the hematology, biochemical and histology of fish *Labeo rohita (Ham).* The Monocrotophos affects not only fish but also organisms in the food chain through the process of consumption of one by the other. The pesticide, which enters the body tissues of the fish, affects the physiological activities.

Results: The cytometric measurements of erythrocytes of sublethal exposure showed that there is not much differences from the control. In the control fish, the erythrocytes were oval in shape with elongated nucleus. Fish, exposed to sublethal concentration of Monocrotophos showed abnormal size Reduction in the volume of the cytoplasm of cells and swelling of nuclei were observed in fish exposed to concentration.

In the hematology, the total Red Blood Corpuscle and Haemoglobin content were decreased with the increasing hours of exposure of the monocrotophos 36% EC. The amount of the Mean Corpuscular Haemoglobin (MCH) also was increased. The haemoglobin content directly relationship for RBC content it indicate count leading to anemia as a result of inhibition of erthropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in haemopoietic organs.

However, the total was total White Blood Corpuscle was increased with the increasing hours of exposure of the Monocrotophos 36% E. C. The constant increasing in the differential count clearly indicates that the pesticide stress certainly stimulate the white blood cells to produce more at all time of exposure. A linear relationship was established with respect to pesticide monocrotophos and total White Blood Corpuscle. The constant increase in the differential count clearly indicates that the pesticide stress certainly stimulate the white blood cells to produce more at all times of exposure.

Conclusion: It has been suggested that the enumeration of differential cell ratio counts provide of useful diagnostic procedure to assess the physiological stress in the fish.

Keywords: Labeo rohita, Monocrotophos, Heamatology.

INTRODUCTION

Adversely human activities are directly or indirectly affect the environment. Due to development activities such as construction, transportation and manufacturing not only deplete the nature resources but also produce large amount of wastes that leads to pollution of air, water and soil. Today environmental pollution has become not only a national but also an international problem.

The major sources of water pollution are domestic, agricultural and industrial wastes which are discharged into natural water bodies [1]. Water pollution is usually caused by various human sources, typically (point and non point) industrial facilities and agrochemicals especially in aquatic ecosystem, has become a serious environmental problem nowadays. These agrochemicals and industrial discharges may carried away effectively by rains, winds, rivers and floods into the large water bodies and change their physico-chemical properties with high toxicity. The water contamination cause damages to aquatic life especially to fishes which are very sensitive to wide range of toxicant in the water [2].

Pesticide is widely used in modern agriculture to aid in the production of high quality food. However, some pesticides have the potential to cause serious health and environment damage. Repeated exposure to sub-lethal doses of some pesticides can cause physiological and behavioral changes in fish that reduce populations, such as abandonment of nests and broods, decreased immunity to disease and increased failure to avoid predators [3]. The increasing use of pesticides causes chemical pollution results potential health hazards to live stock, especially to fish, birds, frogs, and mammals [4].

The most common cause of water pollution in developing countries is domestic and industrial waste that is directly released into streams or ponds without treatment. These wastes mostly contain various types of pollutants such as heavy metals, radioactive elements, pesticides, herbicides and corrosive substances like acids and bases [5].

Aquatic water bodies are frequently polluted with a multiple of potentially hazardous substance [6]. Pesticide poisoning is an important cause of the morbidity and mortality in developing countries. Now a day's formers are using the verity of pesticide, insecticide, herbicide using agricultural field. The pesticide mainly two types organochlorine and organophosphate in recent year monocrotophos is organophasphate using their field of controlling the insect pest.

Fishes play an important role in human nutrition. Fish proteins are well balanced with essential amino acids and are comparable to other proteins of animal's origin [7]. Further fishes contains lipids especially omega fatty acids from the human nutrious point of view.

Among organophosphate pesticides, monocrotophos is one of the important to controlling insect pesticide, indiscriminately using by India. The formers residue reaches to the environment by direct application, spray drift, aerial spraying, and washing from the atmospheric precipitation and runoff from agricultural lands where they ravage the biotic life [8].

In assessing the safety level of any poisonous chemical for higher animals, the first task is to determine the acute toxic LC_{50} value, a simple expression of the degree of toxicity that can be understood by toxicologists [9]. The increasing awareness of aquatic pollution demands toxicity tests to assess the efficacy of the contamination and to extrapolate their safe levels permissible in the environment. The median tolerance limit of any pollutant is meant as an elementary guide in the field of toxicology [10].

Indiscriminate use of different pesticides in agriculture to prevent crop damage from pests has been increasing over two decades especially in developing countries [11]. When pesticides come in contact with internal organs, irreversible changes in metabolic activities, many pesticides have been reported to produce a number of biochemical changes in fish at sub-lethal levels [12].

Toxicity data for a variety of pesticides such as organophosphate, organ chlorine, carbamide and pyrethroid pesticides have been reported for the number of fish species by various author's [13-21].

Blood is a pathophysiological reflector of the whole body and therefore, blood parameters are important in diagnosing the structural and functional status of fish exposed to toxicants [22]. Hematological parameters can provide satisfactory information on the physiological response of fish to environmental stressors for two major reasons, namely, the close association of the circulatory system with the external environment and the ease of availability of fish blood [23-24].

In general, the end points used in toxicity studies are mortality, survival and growth with acute toxicity tests, the parameters are quite appropriate, but for long-term sublethal concentration's these relavant parameters are difficult to ascertain [25].

In the present study is an haematology studies of cytometric measurement, red blood corpuscle estimation, total white blood corpuscle estimation, haemoglobin content and mean of corpuscle heamoglobin in the fresh water fish in *Labeo rohita (Hamilton 1882).* exposed in monocrotophos.

MATERIALS AND METHODS

Healthy freshwater fish, Labeo rohita of the weight (15±1g) and length (8.0±0.5 cm) were selected for the experiment and were collected from the local commercially culture farm near Kumbakonam. Fish was screened for any pathogenic infections. Glass contamination aquaria were washed with 1% KMnO4 to avoid fungal contamination and then dried in the sun light. Healthy fishes were then transferred to glass aquaria (35´20´20 cm) containing dechlorinated tap water.

Fish was acclimatized to laboratory conditions for 10 to 15 d prior to experimentation. The rate of mortality during acclimatization was less than 10%. They were regularly fed with commercial food. Chlorinated tap water was changed daily to remove faces and food remnants.

Toxicity tests were conducted in accordance with standard methods [26]. Stock solution of monocrotophos 36 % EC with a concentration of 0.1 ml per liter (equivalent to 1 ppm) was prepared in distilled water. Based on the progressive bisection of interval on a logarithmic scale, log concentrations were fixed after conducting the range finding test. After the addition of the toxicant into the test tank with 10 liters of water having twenty fish, mortality was recorded after 24, 48, 72 and 96 h. Five replicates were maintained simultaneously. Percent mortality was calculated and the values were transferred into probit scale probit analysis was carried out as suggested [27].

As already indicated *labeo rohita* was used in this investigation. The fish was maintained in the aquaria at room temperature 28±2 °C. The fish was introduced into ten litre capacity containers of water containing specific Organophosphat Monocrotophos. Caudal peduncle was cut with a sharp blade and the blood was collected in a watch glass containing EDTA, anticoagulant (6% Ethylene diamine tetra acetic acid).

The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining the Red Blood Corpuscle Count (RBC), Total Leucocyte Count (TLC) and Haemoglobin content (Hb). For RBC count, a method devised by Yokayama and later modified by Christensen *et al.,*was followed. For counting the total number of WBC, the pipette with white bead was used. The number of cells present in the four large corner squares marked by capital letter *'L'* was counted and multiplied by 10^3which give the total number of WBC per cubic millimeter of blood. Haemoglobin determination is the quickest means for detecting anaemia. However, many factors are known to influence the haemoglobin level. The Sahli Hellige method was followed for haemoglobin determination. Sahlis pipette was filled slightly above the 20 mm mark, the pipette was wiped with a filter paper or cotton to remove excess blood and the volume was adjusted to exactly 20 $mm^3$by blotting the tip. The blood was expelled into a calibrated (transmission) test tube containing 2 ml of 0.1 N HCl. The pipette was rinsed several times in the acid solution. The sample was allowed to stand for 15 min.

The principle behind the method is the conversion of haemoglobin to acid haematin. The acid haematin was then diluted with distilled water till colour matched with the colour of the standard in the haemoglobinometer. The height of the column at which the colour match obtained gives the value of haemoglobin in g%. Oxygen carrying of blood was calculated by multiplying the haemoglobin content with 1.25, oxygen combining power of Hb/g [28].

RESULTS

Haematological studies

The diameter of erythrocytes cell and their nucleus of the control and treated animal were observed. In the control fish the mean diameter of cell and nucleus were 6.06±0.56µ and 2.35±0.43 µ respectively. In the treated fish the diameters of corpuscle and its nucleus were found to be decreased and the values were 5.78±0.62 µ (cell), 2.05±0.34 µ (nucleus) in sublethal concentration respectively. The cytometric measurements of erythrocytes of sublethal exposure showed that there is not much differences from the control.

In the control fish, the erythrocytes were oval in shape with elongated nucleus Fish, exposed to sublethal concentration of Monocrotophos showed abnormal size. Reduction in the volume of the cytoplasm of cells and swelling of nuclei were observed in fish exposed to concentration.

Table 1: Cytometric measurement of Blood Cells of *L. rohita* control and treated at sublethal concentration(0.40 ppm) of Monocrotophos 36 % EC at 24 and 96 H. (Values are expressed in µm)

Parameter (µm)	Control	Treated
Cell	6.06±0.56	5.78±0.43
Nucleus	2.35±0.43	2,05±0.34

Table 2: Total Haemoglobin contents in the Blood of *L. rohita control and 0.40 ppm sublethal concentration of Monocrotophos 36%. EC.* at various time intervals (values are expressed in mgs.)

Hours of Exposure	Control	Treated	% of Changes
24	8.8±0.12	8.2±0.72	6.8
48	8.6±0.12	8.1±0.30	5.8
72	8.2±0.72	7.9±0.26	3.6
96	7.9±0.30	7.7±0.06	2.5

Value are mean±SD of six observation–or+indicate percentage decrease or increase over control.

The haemoglobin content in the blood of *Labeo rohita* are showing in the table: 2 in this study showing, fish treated with sublethal concentration (0.40 ppm) of Monocrotophos, the heamoglobin content (mg) and hours of exposure were 8.2, 8.1, 7.9, 7.7 and 24, 48, 72, 96 respectively. The total amount of heamoglobin content was gradually reduced by hours to hours.

Table 3: Total RBC contents in the Blood of *L. rohita control and 0.40 ppm sublethal concentration of Monocrotophos 36%. EC.* at various time intervals (values are expressed in mgs)

Hours of exposure	Control	Treated	% of Changes
24	2.95±0.52	2.84±0.23	3.7
48	2.85±0.75	2.70±0.35	5.5
72	2.75±0.75	2.60±0.47	5.5
96	2.70±0.59	2.50±0.75	7.4

Value are mean±SD of six observation–or+indicate percentage decrease or increase over control.

Table 4: Total WBC contents in the Blood of *L. rohita control and 0.40 ppm sublethal concentration of Monocrotophos 36%. EC.* at various time intervals (values are expressed in mgs)

Hours of exposure	Control	Treated	% of changes
24	9740±0.12	9780±0.25	-0.41
48	9765±0.15	9820±0.87	-0.56
72	9770±0.85	9980±0.90	-2.14
96	9780±0.13	10110±0.88	-3.37

Value are mean±SD of six observation–or+indicate percentage decrease or increase over control.

The RBC count in the blood of *Labeo rohita* are shown in the table: 3 In this study fish treated with sublethal concentration (0.40 ppm) of Monocrotophos, the RBC level and Hours of exposure were 2.84, 2.70, 2.60, 2.50 and 24, 48, 72, 96 respectively amount of RBC count increasing by hours to hours.

The WBC count in the blood of *Labeo rohita* are shown in the table: 4 in this study fish treated with sublethal concentration (0.40 ppm) of Monocrotophos, the WBC count and hours of exposure were 9780, 9820, 9980, 10110 and 24, 48, 72, 96 respectively amount of WBC count gradually reduced by hours to hours.

Table 5: Total MCH contents in the Blood of *L. rohita control and 0.40 ppm sublethal concentration of monocrotophos 36%. EC.* at various time intervals (values are expressed in mgs)

Hours of exposure	Control	Treated	% of changes
24	29.83±0.1	28.87±0.1	3.3
48	30.17±0.1	29.12±0.3	3.4
72	29.81±0.7	30.38±0.2	1.9
96	29.25±0.7	30.82±0.0	5.3

Value are mean±SD of six observation–or+indicate percentage decrease or increase over control.

The total MHC count in the blood of *Labeo rohita* are shown in the table: 5 in this study fish treated with sublethal concentration (0.40 ppm) of Monocrotophos, the MHC count and hours of exposure were 28.87, 29.12, 30.82, 30.38 and 24, 48, 72, 96 respectively amount of MHC count gradually reduced by hours to hours.

DISCUSSION

The hematology of fishes has gained recognition as an applied science. Hematology tests have become important diagnostic tools in medicine. Recent studies have shown that the haematological parameters may be equally valuable, in indicating the disease of the stress in the fish. The composition of blood of fishes varies with the changing conditions of the environment and response immediately to any changes in water quality because of intimate contect. Out of varied haematological parameters differential red blood cells counts are of immense physiopathological importance. In the present investigation, an attempt has been made to elucidate the effect of pesticide with different sublethal hours on certain physiological properties of the blood of fish *Labeo rohita.*

The measurement of the red blood corpuscle (RBC), cell and their nucleus was not much changes in the treated fish,due to the pesticide effects of the cell organals. Fish,exposed to sublethal concentration of monocrotophos showed abnormal size reduction in the volume of cytoplasm of cell and swelling of nuclei was observed in fish exposed concentration. Fukushima Were noticed normal cell and treated nucleus was indicated with anemia and destruction of heamopoietic organs and erthropoiesis [29].

The *Labeo rohita* exposed to sublethal concentration of Monocrotophos resulted in a significant decrease in RBC's count leading to anemia as a result of inhibition of erthropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in haemopoietic organs. Similar report has been made by Venkataramana and Sandhya [30]. The long term exposure to pesticides reduced the red blood cell count and haemoglobin value. This indicates the high doses of pesticides produced anaerobic condition and limit the oxygen carrying capacity and there by decrease the mobility.

Totel leucocytes count showed an increased in their number in the higher concentration. But at low concentration, there is the gradual increase in their number. In the present investigation at lower concentration and hour of exposure period an increase total WBC count an *labeo rohita* suggested by Venkataramana Sandhya [30] and Sudha summarwar [31]. Haematological investigation on *Labeo rohita* following chronic exposure to zn & cu. Then Patil, V. K. and M. David, 2007 [32]. Hepatotoxic potential of malathion in the freshwater teleost, *Labeo rohita.*

A linear relationship was established with respect to pesticide Monocrotophos and total leucocytes. The constant increasing in the differential count clearly indicates that the pesticide stress certainly stimulate the white blood cells to produce more at all time of exposure. It has been suggested that the enumeration of differential cell ratio counts provides of useful diagnostic procedure to assess the physiological stress in the fish.

The amount of mean corpuscle heamoglobin (MCH) in the blood of *labeo rohita* exposed to monocrotophos found to increased at different study periods. Same finding given by Binukumari,S and J. Vasanthi [33],on the effect of pesticide dimethoate 30 % EC,in the blood of fresh water fish, *labeo rohita*.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. De AK. Environmental Chemistry. 3rd edition. New Age International Pvt. Ltd. New Delhi; 1996.
2. Herger W, Jung SJ, Peter H. Acute and prolonged toxicity to aquatic organisms of new and existing chemicals and pesticides. Chemosphere 1995;31:2707-26.
3. Veeraiah. Effect of pesticide on non-target organisms. Residue Rev 2012;76:173-301.
4. Nagaraju, B Venkata, Rathnamma V. Effect of profenofos an organophosphate on protein level in some tissues of fresh water fish labeo rohita (Hamilton). Int J Pharm Sci 2013;5(Suppl 1):276-9.
5. Mhadhbi L, Palanca A, Gharred T, Boumaiza M. Bioaccumulation of metals in tissues of *Solea vulgaris* from the outer coast and Ria de Vigo, NE Atlantic (Spain). Int J Environ Res Publ Health 2012;6:19-24.
6. Cattaglin WA. Fairchild JF. Potential toxicity of pesticides measured in mid-western streams to aquatic organisms. Water Sci Technol 2002;45:95-103.
7. Tont S. Food source of the oceans: an outline status of potential. Environ Conserv 1977;4:243.
8. Thangnipon W, P Luangpaiboon, W Thangnipon, S Chinobul. Effects of the organophospahate insecticide, monocrotophos. On acetylcholine esterase activity in the tilapia fish (*Orecheomosis niloticus*) brain. J Neurochem Res 1995;20:515-9.
9. Dubois KP, Geiling EMK. Text book of Toxicology. Oxford University Press. Oxford; 1959. p. 302.
10. Ward GS, Parrish PR. Manual of methods in aquatic environment research. Part 6 Toxicity test FAO. fish tech. pub; 1982. p. 185.
11. Muthukumaravel K, Rajarajan P, Nathiya N, Govindarajan M, Raveendran S. Studies on the histopathology of selected organ of fresh water water fish *Labeo rohita* exposed to pesticide monocrotophos; 2013.
12. Nisar Shaikh, Yeragi SG. Effect of rogor 30E (organophosphate) on muscle protein in the fresh water fish *Lepidocephalecthyes thermalis.* J Ecotoxicol Environ Morit 2004;14:233-5.
13. Anees MA. Acute toxicity of four organophosphours insecticides to fresh water teleost *Channa punctatus.* Park J Zoo 1975;7:135.
14. Arunachalam S, Palanichamy S. Sublethal effects of carboryl on surfacing behavior and food utilization in the air breathing fish *Macropods cupanus*. Physiol Behav 1982;29:23-7.
15. Baskaran P, Palanichamy S, Balasubramanian MP. Effect of pesticides on protein metabolism in *Mystus vittatus.* J Ecobiol 1989;1:90-7.
16. Singh NN, VK Das, S Singh. Effect of aldrin on carbohydrate, protein and ionic metabolism of a fresh water catfish Heteropneustes fossilis. J Bulletin of Environ Contam Toxicol 1996;57:204-10.
17. Malla Reddy P, Bashamohideen MD. Toxic impact of fenvalarate on protein metabolism in the branchial tissue of a fish *Cyprinus carpio.* Curr Sci 1989;57:211-2.
18. Gurusamy K, Ramadoss V. Impact of DDT on oxygen consumption and opercular activity of *Lepidocephalichthys thermalis*. J Ecotoxicol Environ Monit 2000;10:239-48.
19. Sapna shrivasatava, Sudha Singh, Keerty Shrivastava. Effect of carbaryl on glucose content in the brain of Heteropneustes fossilis J Ecotoxicol Environ Monit 2002;12:205-8.
20. Nisar Shaikh, Yeragi SG. Effect of Rogor 30E (organophosphate) on muscle protein in the fresh water fish *Lepidocephalecthyes thermalis.* J Ecotoxicol Environ Monit 2004;14:233-5.
21. Visvanathan P, C Maruthanayagam, M Govindaraju. Effect of malathion and endosulfan on biochemical changes in *Channa punctatus.* J Ecotoxicol Environ Monit 2009;19:251-7.
22. Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT, Ayyappan S. Effects of cypermethrin and carbofuran on certain haematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxicol Environ Saf 2004;58:220–6.
23. Cazenave J, Wunderlin DA, Hued AC, de los Angeles-Bistoni M. Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae), captured from pristine and polluted water. Hydrobiology 2005;537:25–33.
24. Houston AH. Are the classical hematological variables acceptable indicators of fish health *Trans.* Am Fish Soc 1997;126:879-93.
25. Sulekha BT. Certain biological aspects on the effect of pesticides on fishes. Ph. D. Thesis. Mahatma Gandhi University, Kerala; 2002.
26. American Public Health Assosciation. Standard Methods of water and wastewater. 18th ed. American water works Association, Water Environment Federation Publication. APHA, Washongton D.C; 1992.
27. Finney DJ. Probit Analysis. Cambridge Univ. Press: London. Folch J, M Lees, SH Sloane-Stanley. A simple method for isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497-507.
28. Johansen. Effects of aldiocarbon the blood and tissues of a fresh water fish. Bull Environ Contam Toxicol 1970;38:36-41.
29. Fukusima H, a Bailone RL, Weiss LA. Triploidy in the hematology of jundia juvaniles (Siluriformes: Hetapterida) *Braz.* J Biol 2012;72:147-55.
30. Venkataramana GV, PN Sandhya, PS Murthy. Impact of malathion on the biochemical parameter of gobid fish, Glossogobius giuris (Ham). J Environ Biol 2006;27:119-22.
31. Sudha summarvar K. Haematological investigation on *Labeo rohita* following chronic exposure to zn and cu; 2012.
32. Patil VK, M David. Hepatotoxic potential of malathion in the freshwater teleost, *Labeo rohita (Hamilton).* Veteinarski Arch 2007;73:179-88.
33. Binukumari S, J Vasanthi. Studies on the effect of pesticide Dimethoate 30% EC on the lipid content of the fresh water fish *labeo rohita*; 2013.