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RESEARCH ARTICLE

EFFECTS OF MONOCROTOPHOS ON HAEMATOLOGICAL ANALYSIS OF BLOOD IN AN INDIAN FRESHWATER FISH, LABEO ROHITA

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ABSTRACT

Pesticides are an integral part of present day agricultural technology. They are greatly contributing towards increasing world food supply by protecting the crop yield. Due to intensive development of agriculture in recent years and rapid growth of industrialization in our country, there has been a great increase in manufacture and utilization of fertilizers, pesticides, petrochemical products, detergent and other synthetic chemicals and agricultural wastes pose a serious threat to the water ecosystem and aquatic life. Monocrotophos is one of the organophosphorous pesticide used in this study. The median lethal concentration(LC_{50}) of MC to fish *L. rohita* for 96 hour was found to be 45.1 ppm.In sublethal concentration($1/10^{th}$ of LC_{50} 96 hour value, 4.51ppm) fishes were exposed for 24, 48, 72 and 96 hrs, 10 days, 20 days and 30 days.A significant decrease in erythrocyte (RBC), Haemoglobin (Hb),mean cellular volume (MCV), mean cellular haemoglobin (MCH) and packed cellular volume were observed throughout the study period when compared to their controls. In contrast, leucocyte (WBC) were increased in this study period.It is essential for assessing the ecological risk of these pesticides.

INTRODUCTION

The environmental pollution due to extensive usage of the pesticides, herbicides and ammonical fertilizers without proper management has far reaching effects on the survival potential of aquatic animals, for some of these toxic chemicals may persist in the environment for longer periods and show damage at histological level (Roy *et al*., 2006).Fish as a bioindicator species can play an important role in the monitoring of water pollution, as they respond with great sensitivity to changes in the aquatic environment (Lindstrom Seppa *et al*., 1981; Smolowitz *et al.,* 1991).Blood is the most important and abundant body fluid. Its composition often reflects the total physiological condition.

The main route of entry for any pesticide is through the gills. From the gills, it is transported to various parts of the body via the blood stream. Blood provides an ideal medium for toxicity studies. The haematological parameters have been considered as diagnostic indices of pathological conditions in animals. Fish blood can serve as a valuable tool in detecting physiological changes taking place in animal (Tilak *et al*., 2005).Haematological parameters are used as an index to detect physiological changes and to assess structural and functional status of health during stress conditions in a number of fish species (Adhikari *et al*.,2004; Suvetha *et al*., 2010).In addition, hematological studies are proceeded frequently and routinely applied in the diagnosis of diseases in aquaculture (Ranzani -Paiva *et al*., 2000).

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MATERIALS AND METHODS

Collection and Maintanence of Fish

Fingerlings of the fresh water fish, *Labeo rohita* ranging in weight from 4g to 8g and measuring (4cm to 6cm in length) were procured from Aliyar Fish farm, Tamilnadu, India. The procured bulk samples of *Labeo rohita* were transported to the laboratory in well aerated polythene bag and acclimatized to the laboratory conditions under natural photo period for one week in large plastic containers at (26 ± 5°C). The tank was previously washed with potassium permanganate to prevent any fungal infection. The fishes were maintained in dechlorinated tap water of the quality used in the test and water was renewed every day to provide freshwater rich in oxygen.During the periods of acclimatization they were fed everyday with oil cake mixed with rice flour.Unhealthy fish and those with infections were removed. Feeding was stopped two days prior to the experiment to maintain same state of metabolic requirements. Fish belonging to both sexes were selected for the present investigation.All the precautions, laid down on recommendations of the toxicity tests to aquatic organisms are followed Anon,(1975). The tap water free from contaminants was used as dilution water for the present study. The physico-chemical analysis of water used in the experiments was carried out using the method of APHA, (2005); temperature 27.2 ± 0.9 (°C), pH 7.1 ± 0.1, dissolved oxygen 5.4 ± 0.4 (mg /l), total hardness 180 ±1.9 (mg /l), salinity 0.3 ± 0.1 (ppt). Continuous artificial aeration was maintained throughout the acclimation and exposure periods.

Toxicant

Monocrotophos is one of the organophosphorus insecticides extensively used in agriculture and animal husbandry (Rao,

2004). Monocrotophos is a brownish yellow liquid with a sharp smell that irritates the eyes and skin. The IUPAC name is dimethyl (E)-1-methyl-2-(methyl-carbamoyl) vinylphosphate. Molecular formula is C7H14NO5P and molecular weight is 223.2.

Determination of 96 h LC_{50} value of MC

The concentrations of the pollutant at which 50 percent of the test animals die during a specific test period of time is referred to as median lethal concentration (LC_{50}) (or) median tolerant limit. In aquatic toxicology the traditional LC_{50} test is often used to measure the potential risk of a chemical (Jack de Bruijin *et al*., 1991).Batches of 10 healthy fishes were exposed to different concentrations of pesticide, Monocrotophos to calculate the LC_{50} value. One more set of fishes are maintained as control in tap water. To find the wide range of concentration 100-600 ml were choosen and the number of dead or affected fishes was counted at regular intervals upto 48 hrs. The level of the dissolved oxygen, P^H, alkalinity and hardness were monitored and maintained constant.Appropriate narrow range of concentration was used to find the median lethal concentration using a minimum of 6 fishes for each concentration and the mortality was recorded for every 24hrs upto 96hrs.It was found as for 48hrs, using probit analysis method (Finney, 1971).From the stock solution various sublethal concentrations were prepared for bioassay studies.

Sublethal toxicity

Seven groups of fishes were exposed to $1/10^{th}$ of the pesticide 'Monocrotophos' for 24, 48, 72 and 96 hrs, 10 days, 20 days and 30 days.Another group was maintained as control. All the groups received the same type of food and other conditions were maintained similarly. At the end of each exposure period, fishes were sacrificed for further analysis.

Preparation of blood samples

After each exposure periods blood samples were taken from fish by gill puncture using plastic disposable syringes.The syringe and needle were prechilled and coated with heparin(Beparin R heparin sodium,IP 1000 IU/ml derived from beef intestinal mucosa containing 0.15% (w/v) chlorocresol IP preservative), an anticoagulant manufactured by Biological E Limited, Hyderabad,India.The collected blood sample was transferred into small vials,which is previously rinsed with heparin.The whole blood sample was used for the estimation of haematological parameters like Hb,RBC,WBC,MCV,PCV and MCH.

Haematological analysis

Hb was estimated by acid haematin method (Sahil, 1962).Total Red blood cells (RBC) were counted using the improved Neubaur haemocytometer (Shah and Altindag,2004).Blood was diluted (1:200) with Hayem's fluid (Mishra and Pandey,1977).Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as 10^6 mm^3 (Wintrobe,1967).Total White blood cells (WBC) were counted using an improved Neubaur haemocytometer (Shah and Altindag,2005;Mgbenka and Oluah,2003).Blood was diluted (1:20) with Turk's diluting fluid and placed in haemocytometer.4 large (1Sq.mm) corner squares of the haemocytometer were counted under the microscope (Olympus) at 640x.The total number of WBC was calculated in mm^3x10^3 (Wintrobe,1967).The mean corpuscular volume was calculated by using values of PCV % and the red blood cell counts and expressed in µm Anderson and Klontz,(1965).Blood was sucked into the heparinized haematocrit capillary tube (7.5cm length, 0.1 cm width). After sealing both the sides of the tube it was centrifuged in the microhaematocrit centrifuge at 6000 rpm for 2 min. From the volume of blood taken and cell volume after centrifugation, the PCV percentage was calculated employing standard method and formula (Sandhu, 1990).

$$\text{MCH (in pico gms)} = \frac{\text{Hb in gm per100 ml}}{\text{RBC'S in millions/mm3}} \text{ x10}$$

Statistical analysis

The data were analysed statistically at $p< 0.05$.To test their significance the t values were calculated by Student's t-test.

RESULTS

LC 50 value-96 hour

In the present study the 96 h LC 50 value of MC to *L. rohita* was determined to be 45.1ppm.

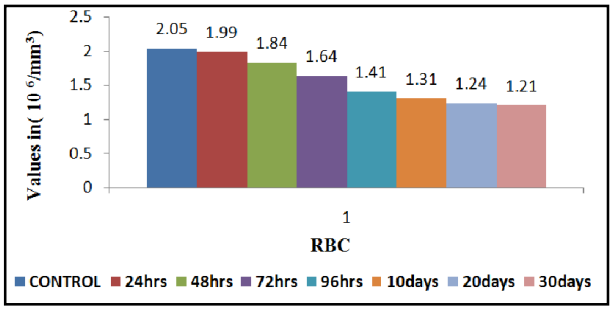

Fig. 1. Changes in the RBC count in a fresh water fish, *L.rohita* treated with sublethal concentration of MC(4.51 ppm;30 days).Bars represent means and standard deviation of six individual observations.Significant at p<0.01(based on t-test) of short and long term exposures

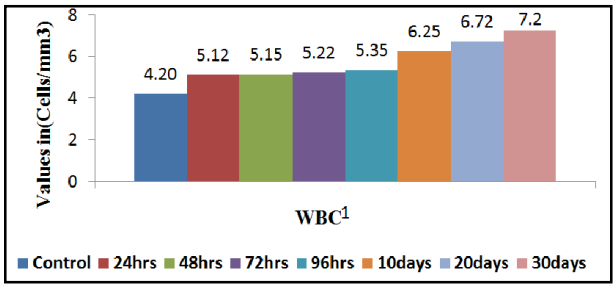

Fig. 2. Changes in the WBC count in a fresh water fish,*L.rohita* treated with sublethal concentration of MC(4.51 ppm;30 days).Bars represent means and standard deviation of six individual observations.Significant at p<0.01(based on t-test) of short and long term exposures

Haematological profiles

The haematological profiles such as RBC (Fig. 1), Hb (Fig. 3), MCV (Fig. 4), MCH (Fig. 5) and PCV (Fig. 6) levels were significantly decreased ($p<0.05$) whereas WBC (Fig. 2)

contents were significantly increased ($p<0.05$) in MC treated fish throughout the exposure period when compared to that of their control groups.

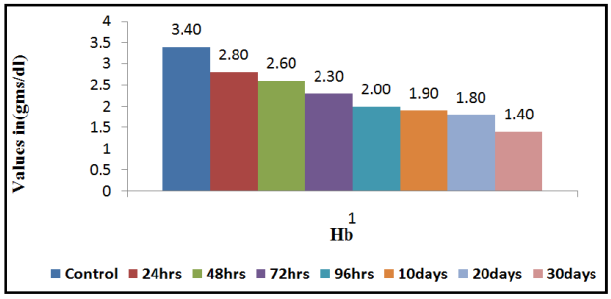

Fig. 3. Changes in the Hb content in a fresh water fish,L. rohita treated with sublethal concentration of MC(4.51ppm;30days).Bars represent means and standard deviation of six individual observation.Significant at p<0.01(based on t-test) of short and long term exposures

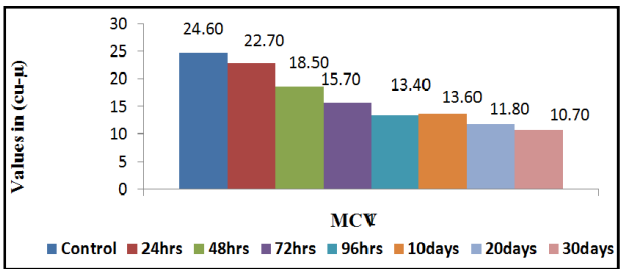

Fig. 4. Changes in the MCV value in a fresh water fish *L. rohita* treated with sublethal concentration of MC(4.51 ppm;30 days).Bars represent means and standard deviation of six individual observations.Significant at p<0.01(based on t-test) of short and long term exposures

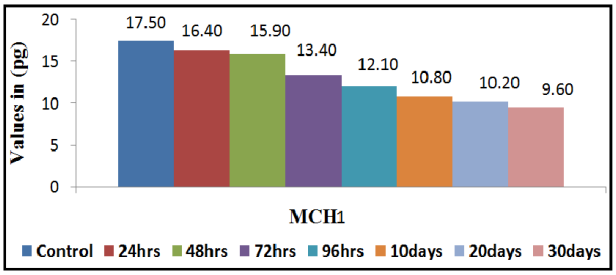

Fig. 5. Changes in the MCH value in a fresh water fish, *L.rohita* treated with sublethal concentration of MC(4.51 ppm;30 days).Bars represent means and standard deviation of six individual observations. Significant at p<0.01(based on t-test) of short and long term exposure

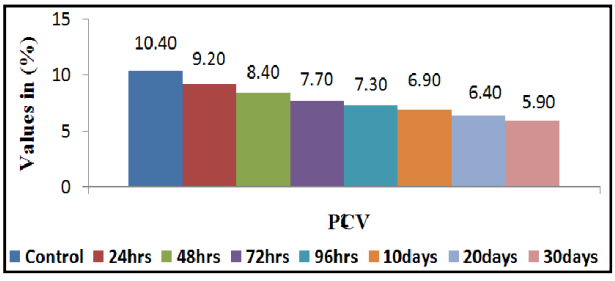

Fig. 6. Changes in the PCV value in a fresh water fish L. rohita treated with sublethal concentration of MC(4.51 ppm;30 days).Bars represent means and standard deviation of six individual observations.Significant at p<0.01(based on t-test) of short and long term exposures

DISCUSSION

Blood often exhibit pathological changes before the appearance of any external symptom of toxicity. Therefore, the haematological studies in animal form a promising tool for the investigation of physiological alteration caused by environmental pollution.Toxicants might cause an adverse effect on the haematopoietic organs which reduces the supply of RBC either due to less production and/or increased rate of removal from circulation. Fall in the level of haemoglobin may be the consequence of toxic effects of Monocrotophos on the synthesis of this molecule. The insecticide may disrupt the synthetic pathway by affecting the activity of enzymes involved in the synthesis of haemoglobin. Consequently, reduces the level of haemoglobin of exposed fish.

The results of the present study showed significant decrease in RBC, Hb, MCV, MCH and PCV in the tissues studied (Fig. 1,3,4,5and 6). Per cent decrease in 24hrs, 48hrs, 72hrs and 96hrs was found to be more in MCV -45.52 during 96 hours and less in RBC -2.92 during 24 hours. The results of the present study showed significant increase 27.38% in WBC during 96 hours (Fig.2). Per cent decrease in 10 days, 20 days and 30 days was found to be more in Hb -58.82 during 30 days and less in MCH -32.57 during 10 days. The results of the present study showed significant increase 71.42% in WBC during 30 days (Fig.2).Decrease in RBC and haemoglobin values in *Labeo rohita* exposed to pesticide in this study is similar to the observations conducted by Gill and Pant, (1981b) in *Clarias batrachus* exposed to different toxicants. Decrease in RBC may be due to oxygen carrying capacity of the blood which may be due to the inhibition of erythropoiesis, haemosynthesis and increase in therate of erythrocyte destruction in haemopietic organs.Low haemoglobin level according to Joshi *et al*. (2000c) might decrease the ability of fish to enhance its activity in order to meet occasional demands.Adeyemo, (2007) reported that decreased haemoglobin, RBC count and haematocrit values in *Clarias gariepinus* exposed to Lead nitrate.

Increase in the TLCs in the fish, *Oreochromis mossambicus* could be due to stimulated lymphopoiesis and enhanced release of lymphocytes from lymphomycloid tissues. Such a lymphocyte response might be due to the presence of toxic substances induced tissue damage as was observed by Haniffa,(1990).The increase of WBC in T-guineensis exposed to toxicant may be caused by migration of White blood cell from the spleen to the blood circulation or mobilization of neutrophils and monocytes from the bone marrow reserves into the blood stream (Barcellos *et al.*, 2004).The significant increase in total leucocyte count might be due to immunological reaction to produce more antibodies to cope with the stress induced by these toxicants (Shanthi *et al.*, 2009). Decrease in the Hb levels may impair oxygen supply to various tissues resulting in slow metabolic rate and low energy production (Ahmad *et al.*, 1995).The significant reduction of Hb could be indication of severe anaemia caused by destruction of erythrocytes (Kori – Siakpere *et al.*, 2009).A decrease in Hb has been reported in *Common carp* after poisoning with cypermethrin was reported by Dorucu and Girgin, (2001). In the present study MCV, MCH and PCV was found to decrease in all exposure periods. Verma *et al.* (1979) studied that the decrease in MCV indicates microcytic

normochromic anaemia. Shakoori *et al*. (1996) stated a significant decline in MCH and MCV levels are indicative of hypochromic microcytic anemia.This is domino effect of fenvalerate treated with *Cenopharyngodon idella*. The decrease MCV and MCH clearly indicate the hypochronic microcytic anaemia. The above findings are supported by Ramalingam *et al*. (2000). Ahmad *et al*. (1995) reported that the decrease in PCV may show the extent of shrinking cell size and decrease in the number of cells due to Danitol intoxication in the fish, *Ctenopharyngodon idella*. Svoboda *et al*. (2001) showed that diazinon exposure in carp led to a decrease in PCV and their results are similar to the present study. The low value of PCV in fish exposed to stress was attributed to a reduction in red blood cell volume caused by osmotic changes (Aiwan *et al*., 2009).The low value of PCV in fish exposed to stress was attributed to a reduction in red blood cell volume caused by osmotic changes (Aiwan *et al*., 2009).Benarjee *et al.* (2010) recorded decrease in RBC, Hb, PCV and MCV values in the fish, *channa punctatus* due to the effect of Rayon industry effluents. All these observations confirm the findings of the present study.

Conclusion

In the present study,it is concluded that MC has a profound influence on the haematological analysis of an Indian major carp *L.rohita* and MC is toxic to aquatic organisms.The results imply a better understanding on the toxicological endpoint of this specific pesticide and provide significant information on safe levels in the aquatic environment.

Conflict of Interest: None declared.

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