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EFFECTS OF CYPERMETHRIN AND DIAZINON ON HAEMATOLOGY OF *LABEO ROHITA* (H.)

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ABSTRACT

The study was conducted to investigate the effect of cypermethrin and diazinon on haematology of *Labeo rohita* (H.) in glass aquaria. Twelve batches of fish were exposed to two concentrations of cypermethrin (0.15 and 0.30 µl/L) and diazinon (0.002 and 0.004 ml/L) for 96 hours and three batches of fish were treated as control. The blood parameters viz., total WBC and RBC count, Hb, PCV, MCV, MCH and MCHC values were analyzed using standard methods. A decrease in WBC and RBC count, Hb and PCV values were found in both concentrations of cypermethrin exposure ($P<0.05$). MCV value was decreased in low concentration and increased in high concentration whereas MCH and MCHC values were increased in low concentration and decreased in high concentration of cypermethrin exposure as compared to the control fish ($P<0.05$). On the other hand, WBC count and MCHC value were increased with increasing concentration of diazinon exposure whereas RBC count, Hb, PCV, MCV and MCH values were increased in both concentrations of diazinon as compared to the control fish ($P<0.05$). This study concluded that short-term exposure of cypermethrin and diazinon (even in low concentration) make substantial changes in haematology of *L. rohita*.

INTRODUCTION

The use of pesticides has increased dramatically in worldwide contemporary agriculture. Cypermethrin and diazinon are two of widely used pesticides which are beneficial by providing reliable, persistent and relatively complete control against harmful pests with low cost (Lawrence and Isioma, 2010). Due to injudicious and indiscriminate use of these pesticides, water bodies like ponds, lakes, river and low lying water areas are continuously getting polluted through surface run off, sediment transportation from treated soil and direct application as spray for controlling pests (Kumari and Subisha, 2010) which lead some serious problems to the non-target organisms such as fishes, mammals and birds (Riebeiro *et al*., 2003). Cypermethrin is a highly potent synthetic pyrethroid insecticide that virtually used to control insects in agriculture, home and garden (Jee *et al*., 2005). Due to its lipophilicity, cypermethrin has a high rate of absorption in the gill of fish even at very low concentrations in the water. This in turn is a contributory factor to the sensitivity of the fish to aqueous pyrethroids exposures because fish seem unable to metabolize the pyrethroids efficiently (Viran *et al*., 2003). On the other hand, diazinon is an organophosphate pesticide extensively used to control insects. Due to its chemical properties and widespread application, diazinon is frequently found in waste water treatment plant effluent and in storm water runoff in urban and agricultural areas. Contamination of water by the pesticides can lead to fish kills, reduced fish production or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of human eating those fish (Adedeji *et al.,* 2009). Haematological analysis is crucial in many fields of ichthyological research, fish farming, toxicology and environmental pollution monitoring as an indicator of physiological or pathological changes in fishery management and disease investigation (Adedeji *et al*., 2009). However, a number of studies have been reported on the effects of pesticides on haematology of various fish species in different parts of the world (Singh and Srivastava, 1994; Nath and Banerjee, 1996; Khattak and Hafeez, 1996; Tavares *et al*., 1999; Parma *et al*., 2007). But, the studies demonstrating the changes in haematological indices of freshwater fishes due to specific pesticide pollution are scare in Bangladesh. Therefore, the present study was conducted to investigate the effects of cypermethrin and diazinon on haematology of *Labeo rohita* (H.) as a widely consumed fish species in Bangladesh.

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MATERIALS AND METHODS

Experimental site and unit

The experiment was conducted in fifteen glass aquaria at the wet laboratory in the Department of Fisheries, University of

Rajshahi, Bangladesh. The aquaria were neat and clean with well aeration and water exchange facilities.

Experimental design

The fishes were exposed to two concentrations of cypermethrin (0.15 as low and 0.30 µl/L as high concentration) and diazinon (0.002 as low and 0.004 ml/Las high concentration) for 96 hours (from 11^{th} to 15^{th} March, 2013) in glass aquaria. Three batches of fish (10 fishes in each batch) were exposed to each concentration of cypermethrin and diazinon. At the same time, three batches of fish were kept in 3 aquaria with clean water treated as control fish.

Maintenance of experimental fish

Healthy juvenile of *L. rohita* (body weight 123±39.58 g and length 22.5±2.67 cm) were collected from the fish ponds at the Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh. The collected fishes were kept in aquaria containing 60L water for acclimatization. During acclimation, the fishes were fed with artificial feed twice daily. The water in aquarium was aerated continuously with aerators. Constant amount of the test chemical and test water were renewed every 24 hours to remove faecal metabolites and waste matter and also to maintain the constant concentration of the chemicals.

Monitoring of water quality

Water quality was monitored with water temperature, dissolved oxygen (DO) and pH by using Celsius thermometer, DO meter (HI-9142) and pH meter (Jenway, 3020), respectively.

Blood collection

At the end of exposure, blood samples were collected by serving off the caudal region of fish with a sharp scalpel blade in a haeamatocrit tube containing EDTA solution as an anticoagulant agent.

Analysis of blood parameters

The blood parameters such as total count of white blood cells (WBC) and red blood cells (RBC), haemoglobin level (Hb), haematocrit or pack cell volume (Ht or PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were analyzed. The total WBC counts were enumerated in an improved Neubaeur haemocytometer using Shaw's diluting fluid. The total RBC counts were similarly enumerated in an improved Neubaeur haemocytometer using Hendricks diluting fluid. Hb was determined by cyanmethemoglobin method (Lee *et al*., 1998). Ht was determined by the microhaematocrit method (Snieszko, 1960). MCV was calculated according to Feldman *et al*. (2000). MCH and MCHC were calculated according to Stoskopf (1993).

Statistical analysis

The results obtained were subjected to analysis for mean and standard deviation. The mean values were subjected to statistical analysis using one-way analysis of variance (ANOVA) to test for the level of significance between two concentrations of cypermethrin and diazinon through software SPSS (version 16). The significance of difference among the mean values was determined by Duncan's multiple range test at 5% level ($P<0.05$).

RESULTS AND DISCUSSION

Water quality

Water quality is one of the important factors responsible for variations in fish haematology since they are sensitive to slight fluctuation that may occur within their internal milieu (Fernades and Mazon, 2003). During the study period, water temperature, DO and pH of water in the experimental aquaria did not show any significance difference and the mean values were recorded as 24.4 to 24.6 °C, 5.94 to 6.50 mg/l and 7.21 to 7.33, respectively. The values of these parameters were within the acceptable ranges for fish according to the previous reports that water temperature 25.5°C to 30.0°C (Rahman *et al*., 1982), DO value 5.0 mg/l or more (Rahman *et al*., 1982) and pH 6.5-8.5 (Boyd 1990) are suitable for fish growth.

Behavioural changes in the fish due to cypermethrin and diazinon exposure

During this study, there was no death of fish occurred due to cypermethrin and diazinon exposure. By changing behavior, fish try to reduce the entrance of pesticides present in the medium or to minimize the damage of their body tissues. The fish exposed to the cypermethrin and diazinon primarily decreased their food intake and exhibited frantic swimming, and sluggish movement. Moreover, paleness was also observed over the body surface of the fish. Similar behavioural changes have been reported in *Poecilia reticulata* due to cypermethrin exposure (Li *et al*., 2005), and in *C. carpio* (Svoboda *et al*., 2001) and in *Huso huso* due to diazinon exposure (Khoshbavr-Rostami *et al*., 2005). The behavioural changes observed in the fish might be due to the effect of pesticides on the central nervous system or the disturbances in physiological mechanism (Marler and Hamilton, 1966).

Changes in blood parameters due to cypermethrin exposure

The changes in blood parameters of *L. rohita* due to cypermethrin exposure are shown in Table 1. The total WBC, RBC, Hb level and PCV values were decreased in both concentrations of cypermethrin as compared to the control fish. Decrease in total WBC counts reflects a state of stress in fish and points to the role of cypermethrin as a potential environmental stressor. Stress-induced lowering of WBC counts have been reported by Iwama *et al.* (1976) in *Oncorhynchus kisutsh* exposed to dehydroabietic acid and by Mcleay and Gordon (1977) in salmonid fish exposed to kraft pulpmill wastes. In the present study, the total RBC count was decreased significantly in high concentrations of cypermethrin exposure. Adhikari *et al*. (2004) and Parma *et al*. (2007) found significant decrease in RBC in *Prochilous lineatus* exposed to cypermethrin. This finding is supportive to the present study. The decrease found in total RBC might be due to the disruptive action of pesticides on the erythropoietic tissue (Larsson, 1985). The level of Hb was insignificantly decreased in low concentration whereas significantly decreased in high concentration of cypermethrin exposure. Similar results have been reported by previous studies (Nuri Cakmak and Girin, 2003; Vani *et al*., 2012; Deka and Dutta, 2012). The decrease found in Hb level might be due to either an increase in the rate of Hb destroying or a decrease in the rate of Hb synthesis (Matkovies and Witas, 1981). In this study, a significant decrease in PCV value was recorded in cypermethrin exposure which is supported by Khoshbavar-Rostami *et al.* (2004). Gill and Pant (1985) reported that PCV value decreased when a fish loses its appetite or poisoned by pesticides. This report is also supportive to the present study.

During this study, MCV value was decreased significantly in low concentration of cypermethrin exposure which is supported by Atamanalp and Yanik (2003) whereas MCV value was increased significantly in high concentration which is supported by Adhikari *et al*. (2004). As compared to the control fish, MCH value was increased insignificantly in low concentration of cypermethrin exposure which is supported by Parma *et al*. (2007) and Desai and Parikh (2012) whereas MCH value was decreased in high concentration which is identical to the findings of Koprucu *et al*. (2006). MCHC value was significantly decreased in high concentration of cypermethrin exposure than the control fish which is similar to the finding of Parma *et al*. (2007) who observed significant decrease in MCHC value in *Prochilous lineatus* exposed to sub-lethal concentration of cypermethrin but the value of MCHC in low concentration was differed from their result. The changes found in MCV, MCH and MCHC values might be due to stress induced by cypermethrin which exaggerates further disturbances in haemopoietic activities of fish (Rao, 2010).

Table 1. Blood parameters of the control fish and the fish exposed to cypermethrin

Parameters	Concentration of cypermethrin		
	Control	0.15 µl/L	0.30 µl/L
RBCs (10^6×mm^{-3})	2.26±0.13^a	2.19±0.10^a	1.85±0.09^b
WBC (10^4× mm^{-3})	12.74±0.36^a	11.52±0.29^b	9.59±0.24^c
Hb (g/dl)	8.20±0.30^a	8.10±0.41^a	6.40±0.23^b
PCV (%)	36.33±3.57^a	32.01±2.03^b	31.01±2.27^b
MCV (fl)	160.0±5.00^b	146.0±7.00^c	167.0±9.00^a
MCH (pg)	36.25±1.45^a	36.98±1.92^a	34.54±2.04^b
MCHC (g/dl)	22.55±1.24^b	25.29±1.58^a	20.63±1.03^c

* Values of blood parameters are mean of triplicate determination. Values on the same row with different superscripts are significantly different (P<0.05).

Changes in blood parameters due to diazinon exposure

The changes in blood parameters of *L. rohita* due to diazinon exposure are shown in Table 2. During this study, it was observed that total WBC count was increased significantly (P<0.05) in the fish exposed to diazinon in both concentrations as compared to the control fish. An increase in lymphocytes number may be the compensatory response of lymphoid tissues to the destruction of circulating lymphocytes (Shah and Altindag, 2005). Such lymphocytes response might be due to the presence of toxic substances or pollutant induced tissue damage (Haniffa, 1990). Shah and Altindag (2005) reported that total WBC count is increased in *Tinca tinca* exposed to lethal and sub-lethal treatments with mercury. This report is supportive to the present study. The haematological response to the diazinon exposure caused significant decrease in RBC counts. Similar result has been reported by previous studies in *Cyprinion watsoni* due to malathion exposure (Khattak and Hafeez, 1996) and in *Piaractus mesopotamicus* due to trichlorphon exposure (Tavares *et al.,* 1999).

Table 2. Blood parameters of the control fish and the fish exposed to diazinon

Parameters	Concentration of diazinon		
	Control	0.002 ml/L	0.004 ml/L
WBC (10^4× mm^{-3})	12.74±0.18^c	13.02±0.12^b	14.10±0.21^a
RBC (10^6×mm^{-3})	2.27±0.09^a	2.19±0.05^a	2.10±0.08^b
Hb (g/dl)	8.22±0.27^a	7.20±0.31^b	6.32±0.25^c
PCV (%)	36.67±2.57^a	31.17±1.04^b	25.00±2.00^c
MCV (fl)	160.00±9.00^a	142.00±7.67^b	119.00±4.33^c
MCH (pg)	36.25±2.02^a	30.02±1.72^b	30.09±1.41^b
MCHC (g/dl)	22.55±0.48^c	23.09±0.37^b	25.26±1.17^a

* Values of blood parameters are mean of triplicate determination. Values on the same row with different superscripts are significantly different (P<0.05).

A lower count of RBC found in the fish might be due to anemia resulted from the conditions that decreased the number or size of red blood cells, reduced synthesis of RBC (Morgan, 1980) or abnormality formed in haemoglobin (Hisa and Connie, 1998). In the present study, Hb level in the fish was decreased significantly with increasing concentration of diazinon. Similar findings have been reported by Adedeji *et al*. (2009) in *Clarias gariepinus* and by Far *et al*. (2012) in *Oncorhynchus mykiss* due to diazinon exposure. The decreased in haemoglobin found in the present study might be due to either an increase in the rate of haemoglobin destroying or a decrease in the rate of haemoglobin synthesis (Matkovies and Witas, 1981).

During this study, PCV value was decreased significantly in the fish exposed to diazinon. This finding is supported by Svoboda *et al*. (2001) who found decreased PCV value in common carp due to diazinon exposure. The reduction in PCV value might be due to the fish suffers from anemia or haemodilution (Wedemeyer *et al.,* 1976). During this study, diazinon exposure caused a decrease in MCV and MCH values as compared to the control fish which might be due to the disruptive action of diazinon on the erythropoietic tissue. On the contrary, the MCHC value was increased with increasing concentration of diazinon as compared to the control fish. Similar result has been reported by Chandrasekar and Jayabalan (1993) in *Cyprinus carpio* exposed to the pesticide, endosulfan. The change found in MCHC value might be due to the drastic decrease in the levels of Ht caused by haemolysis.

The overall study revealed that short-term cypermethrin and diazinon exposure (even in low concentration) resulted considerable haematological changes in *L. rohita*. These changes may seriously disruptive to the normal behaviour and growth of this fish in its natural environment as well as in culture conditions. Thus the present study calls for careful application of cypermethrin and diazinon in insect/pest controlling operation in agricultural fields surrounding their natural freshwater reservoirs.

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