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RESEARCH ARTICLE

COMBINED EFFECT OF CADMIUM AND CHLORPYRIFOS ON HAEMATOLOGICAL CHANGES IN TILAPIA (*OREOCHROMIS MOSSAMBICUS*)

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ABSTRACT

Laboratory bioassay studies were carried out to evaluate the toxic effect of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos on the haematological changes in Tilapia, *Oreochromis mossambicus.* The lethal toxicity of commercial grade organophosphate insecticide, Chlorpyrifos (20% Emulsified concentration), heavy metal, Cadmium and Cadmium + Chlorpyrifos on *Oreochromis mossambicus* was tested. The lethal toxicity of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos to the tilafia fingerlings exposed for 96 hr was found to be 169.80ppm, 0.022ppm and 92.04ppm respectively. The fingelings were exposed to lethal concentrations (LC_{50}) of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos to study the alterations of different haematological parameters at 24hrs and 48 hrs of exposure. Reduction in the number of red blood cells, Haematocrit value, Haemoglobin conten and increase in Mean corpuscular haemoglobin, Mean carpuscular volume, Mean carpuscular haemoglobin concentration were evident.

INTRODUCTION

The presence of pollutants such as pesticides, heavy metals, etc. in an aquatic environment cause several structural and functional changes in the biota including fishes. Chlorpyrifos (O, O-diethyl-O-3, 5,6-trichlor-2-pyridyl phosphorothioate; CPF) is a broad spectrum organophosphate insecticide widely used to control foliar insects in agricultural crops (Rusyniak and Nanagas, 2004) and subterranean termites (Venkateswara Rao *et al.*, 2005). It is the second highest selling organophosphate insecticide and is more toxic to fish than organochlorine compounds (Tilak *et al.*, 2001). Cadmium is a non-essential, non-biodegradable element with no known biological function and is a major contaminant causing adverse effects on aquatic organisms. Fishes have greater sensitivity to changes in the aquatic environment (Vinodhini and Narayanan, 2008). Capacity to accumulate large quantity of pollutants and important link in the food chain, fishes are often used as indicator organisms to monitor quality of aquatic systems the world over (Rajkowska and Protasowicki, 2011).

Fish blood is highly susceptible both to internal and external environment fluctuations because pollutants mainly transfer in the body through this. Hematocrit values, haemoglobin content, number of red blood cells, white blood cells and haematological indices such as MCV, MCH and MCHC are the indicators of toxicity with wide potential for use in environmental monitoring and toxicity studies (Sancho *et al.*, 2000; Barcellos *et al.*, 2003).

The toxic effect of pesticides to the blood of fishes has been studied by many workers. Consequences of pesticides on hematological factors of a number of fish species have been investigated: in *Cyprinus carpio* (Salvo *et al.*, 2008); *Clarias batrachus* (Kharat and Kothavade, 2012; Summarwar and Verma, 2012); *Oreochromis mossambicus* (Desai and Parikh, 2012); *Heteropneustes fossilis* (Deka and Dutta, 2012); *Cyprinion wabsoni* (Khattak and Hafeez, 1996) and in *Piaractus mesopotamicus* (Carraschi *et al.*, 2012).

Haematological abnormalities have also been studied in heavy metal exposed fish: *Chana punctatus* to Cadmium (Karuppasamy *et al.*, 2005); *Cyprinus carpio* to Carbofuran (Chandra *et al.*, 2001). Similarly, the changes in the haematological profile of fish exposed to mercury have been observed in *Hoplias malabaricus* (Oliveira-Ribeiro *et al.*, 2006), *Oreochromis aureus* (Allen, 1994), *Ctenopharyngodon idella* (Shakoori *et al.*, 1994).

With this background, it was planned to study the haematological changes of fish exposed to Cadmium, Chlorpyrifos and mixer of Cadmium and Chlorpyrifos to know

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the extent of effect that it causes. Hence in the present work, the toxic effects of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos on Haematological changes in fish *Oreochromis mossambicus* was studied.

MATERIALS AND METHODS

Oreochromis mossambicus fry (2-3 cm) acquired from Chintamani fish farm, Chickaballapur district, Karnataka were transported to the FRIC Hebbal, Bengaluru in well oxygenated polythene bags containing clean pond water. The fish were reared to fingerling size (9-10 cm) with artificial feeding. Later, the fishes were released into the freshwater aquariums of 50 liter capacity (10 No's each) for proper acclimation in the laboratory and were fed every 24 hr with commercial feed. The walls of the tank were periodically cleaned to avoid algal growth. The excreta was siphoned off on a daily basis to prevent the buildup of ammonia in the medium. Fishes were conditioned for 10 days prior to use them for the experiments. The water temperature, dissolved oxygen level and pH are monitored regularly. Individual fishes measuring 9±0.5 cm in total length and weighing 13±0.5 g were selected for the present study.

Toxicity study was carried out by following the standard guidelines (APHA, 2005) to determine the lethal (LC_{50}) level of toxicants using static system for Cadmium, static renewal method for Chlorpyrifos and Cadmium + Chlorpyrifos (Varying concentration of cadmium + Fixed concentration of Chlorpyrifos i.e 1/5th of its LC^{50} value). Ten fish each were accommodated in 45 liters of test solution in the aquarium. The experiment was conducted in triplicate. Dead fishes were removed immediately from the test medium to avoid disintegration.

Three set of replicates were performed for each concentration. The 96 h LC_{50} value of the mortality in each exposure concentration of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos were recorded and tested by probit analysis program as described by (Finney, 1971).

Haematological Estimation

The haematological changes of those fishes treated with lethal concentration (LC_{50}) of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos were analysed after 24 hr and 48 hr of exposure, compared with that of healthy fishes (control). For this purpose, the blood samples were taken by puncturing the caudal vessel using 1ml insulin syringe. The blood was taken in a vial containing 1% EDTA as anticoagulant. RBC counting was done with Neubauer Chamber (Davidson and Henry, 1969). The haemoglobin content was estimated by acid – Haematin method using Sahli's haemocytometer. Hematocrit value was analysed by microhematocrit (capillary) method. Erythrocyte indices MCV, MCH and MCHC were calculated using the values of haemoglobin content, haematocrit value and total erythrocyte count using formula of Dacie and Lewis (1975). The results were processed stastically using analysis of variance (ANOVA) and are presented as mean±SD.

RESULTS

The concentration at which 50% survival/mortality occurred was taken as a lethal concentration (LC_{50}) for 96 h, which was 169.80 ppm for Cadmium (Table 1), 0.022ppm for Chlorpyrifos (Table 2) and 92.04ppm for Cadmium + Chlorpyrifos (Table 3).

Table 1 Determination 96hr LC_{50} of Cadmium on *Oreochromis mossambicus*

Conc. (ppm)	No. of fishes used	Mean % Mortality
160	10	16
164	10	30
168	10	43
172	10	56
176	10	80
180	10	96
184	10	100

Table 2 Determination 96hr LC_{50} of Chlorpyrifos 0n *Oreochromis mossambicus*

Conc.(ppm)	No. of fishes used	Mean % Mortality
0.015	10	16
0.018	10	26
0.021	10	40
0.024	10	63
0.027	10	83
0.03	10	100
0.033	10	100

Table 3 Determination 96 hr LC_{50} of Cadmium (Varying conc.) + Chlorpyrifos (Fixed conc. i.e 1/5th of LC_{50}) 0n *Oreochromis mossambicus*

Conc. Of Cadmium (ppm)	No. of fishes used	Mean % Mortality
80	10	16
85	10	26
90	10	43
95	10	56
100	10	80
105	10	96
110	10	100

Haematological Estimation

R.B.C. count (× 10^6 /mm^3)

The R.B.C. count of control fish was found to be 2.79± 0.006. In fishes exposed to lethal concentration (LC_{50}) of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos, the R.B.C. count was recorded to be 1.84± 0.005, 1.08± 0.018 and 1.75 ±0.021 respectively after 24hr exposure and 1.72 ± 0.012, 0.92 ± 0.027 and 1.62 ±0.011 respectively at the time period of 48hr exposure (Table 4). In all the cases, it is observed that R.B.C. count significantly decreased compared with the control.

Haemoglobin (g/dL)

The haemoglobin content of control fish was found to be 5.2 ± 0.081. In fishes exposed Cadmium, the haemoglobin content was recorded to be 3.4 ± 0.036 in 24hr and 3.2 ± 0.070 at the time period of 48hr (Table 4). In fishes exposed to Chlorpyrifos, the haemoglobin content was recorded to be 3.7 ± 0.054 in 24hr and 3.6 ± 0.027 at the time period of 48 hr (Table 4). In fishes exposed to Cadmium+Chlorpyrifos, the haemoglobin content was recorded to be 4.7 ± 0.097 in 24hr and 4.4 ± 0.100 at the time period of 48 hr (Table 4). In all the cases, it is observed that haemoglobin content significantly decreased compared with the control.

Mean Corpuscular Haemoglobin Concentration (in g/100ml)

The Mean Corpuscular Haemoglobin Concentration of control fish was found to be 22.6±1.455. In

Table 4 Change in certain haematological parameters in fish *tilapia* after exposure to Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos. Values are mean± standard deviation of four replicates

Exposure/Time(hrs)	Control	Cadmium (169.80 ppm)	Chlorpyrifos (0.022ppm)	Cadmium + Chlorpyrifos (92.08 ppm)
		R.B.C Count (x $10^6/mm^3$)		
24 hr	2.79 ± 0.005	1.84± 0.005	1.08± 0.018	1.75 ±0.021
48hr	2.79± 0.007	1.72 ± 0.012	0.92 ± 0.027	1.62 ±0.011
		Haemoglobin (g/dL)		
24 hr	5.2±0.074	3.4±0.036	3.7±0.054	4.7±0.097
48hr	5.2±0.086	3.2±0.070	3.6±0.027	4.4±0.100
		Haematocrit (%)		
24 hr	23±0.054	19±0.038	16±0.075	22±0.092
48hr	23±0.063	17±0.042	15±0.052	20±0.033
		Mean cell volume (μ^3m)		
24 hr	82.43±1.303	103.26±1.086	148.14±1.934	125.71±1.967
48hr	82.43±1.657	98.83±2.435	163±2.543	123.5±0.987
		Mean Corpuscular Haemoglobin (Pg/cell)		
24 hr	18.63±1.987	18.47±2.345	34.25±1.342	26.85±1.212
48hr	18.63±1.564	18.60±1.876	39.13±2.230	27.16±1.232
		Mean Corpuscular Haemoglobin Concentration(in g/100ml)		
24 hr	22.6±0.113	17.8±0.217	23.1±0.119	21.3±0.342
48hr	22.6±0.178	18.8±0.314	24±0.210	22±0.439

P<0.05 Significant difference

Haematocrit (%)

The Haematocrit value of control fish was found to be 23±0.058. In fishes exposed to lethal concentration (LC_{50}) of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos, the Haematocrit was recorded to be 19±0.038, 16±0.075 and 22±0.092 respectively in 24hr and 17±0.042, 15±0.052 and 20±0.033 respectively at the time period of 48hr (Table 4). In all the cases, it is observed that haematocrit value significantly decreasedcompared with the control.

Mean cell volume (μ^3m)

The Mean cell volume of control fish was found to be 82.43±1.480. In fishes exposed to lethal concentration (LC_{50}) of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos, the Mean cell volume was recorded to be 103.26±1.086, 148.14±1.934 and 125.71±1.967respectively in 24hr and 98.83±2.435, 163.00 ±2.543and 123.50 ±0.987respectively at the time period of 48 hr (Table 4). In all the cases, it is observed that Mean cell volume significantly increased compared with the control.

Mean Corpuscular Haemoglobin (Pg/cell)

The Mean Corpuscular Haemoglobin of control fish was found to be 18.63±1.770. In fishes exposed to lethal concentration (LC_{50}) of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos, the Mean Corpuscular Haemoglobin was recorded to be18.47±2.345, 34.25±1.342 and 26.85 ±1.212 respectively in 24hr and 18.60 ±1.876, 39.13±2.230, and 27.16±1.232 respectively at the time period of 48hr (Table 4). In all the cases, it is observed that Mean Corpuscular Haemoglobin significantly increased compared with the control except in Cadmium.

fishes exposed to lethal concentration (LC_{50}) of Cadmium, Chlorpyrifos and Cadmium + Chlorpyrifos, the Mean Corpuscular Haemoglobin Concentration was recorded to 17.8±0.217, 23.1±0.119 and 21.3 ±0.342 respectively in 24hr and 18.8±0.314, 24 ±0.210and 22 ±0.439 respectively at the time period of 48hr (Table 4). In all the cases, it is observed that Mean Corpuscular Haemoglobin Concentration significantly increased compared with the control except in Cadmium.

DISCUSSION

In the present investigation, the significant decrease in the various parameters of blood was observed in *Oreochromis mossambicus* due to the treatment of different toxicants for short durations. The haematological parameters in fish can significantly change in response to chemical stressors; however, these alterations are nonspecific to a wide range of substances (Modesto and Martinez, 2010).

In this study, the RBC count decreased significantly in the Chlorpyrifos treated fish. This result agrees with the previous observation made by Yonar *et al*. (2012), who demonstrated that Chlorpyrifos caused a significant decrease in the RBC count of carp. Similarly, Ramesh and Saravanan (2008) reported that significantly lower values of red blood cells in *Cyprinus carpio* that were exposed to Chlorpyrifos. The decrease in the RBC count levels may be due to the inhibition of erythropoiesis, haemosynthesis or osmoregulatory dysfunction or due to an increased rate of erythrocyte destruction in the hematopoietic organ (Vani *et al*., 2011). In this study, the haemoglobin content decreased significantly in the Chlorpyrifos treated fish. The decrease in the haemoglobin content in the present study result from rapid oxidation of haemoglobin to methaemoglobin or release of O_2 radical brought about by the toxic stress of Chlorpyrifos. Similar observation made by Matkovics *et al*. (1981) in *Cyprinus carpio,* noted a quick decrease in haemoglobin content in response to paraquat toxicity and the authors suggested that it might presumably through methaemoglobin formation and a direct response of O_2-radical. Haematocrit values of *Oreochromis mossambicus* exposed to lethal concentrations of Chlorpyrifos for two exposure periods i.e. 24 hr and 48hr followed the same pattern as for hemoglobin content. The hematocrit values decrease when a fish loses its appetite, is diseased or poisoned by pesticides (Gill and Pant, 1985). In addition an alteration in the fish metabolism would have also led to decreased values of haematocrit in *O. mossambicus.* MCV gives an indication of the status or size of RBCs (Alwan *et al*, 2009). MCV value was significantly higher in fish treated with Chlorpyrifos. The MCHC is a good indicator of red blood cell swelling or shrinkage (Wepener *et al*., 1992). The increase in the MCHC values in the exposed fish is thus probably an indication of shrinking of the red blood cells and/or an increase in haemoglobin synthesis.

In the present study, the RBC count decreased significantly in the Cadmium treated fish. The decrease in RBC count during lethal exposure to Cadmium is due to exaggerated disturbances that occurred in both metabolic and haemopoietic activities of fish exposed to the pollutant (Kori-Siakpere and Ikomi, 2011). The significant reduction of haemoglobin and Hct (%) in the present study exposed to Cadmium was probably due to internal bleeding and haemolysis from the damaged tissues of different vital organs like kidney, gills and liver preceded by bio-concentration and bioaccumulation of Cadmium during exposure (Kori-Siakpere *et al*., 2006; Jayakumar and Paul, 2006; Mauskar, 2007; Omer *et al*., 2012). The reduction in Haematocrit of *O. mossambicus* may also be due to decreased rate of erythropoiesis as well as hemolysis as observed in the *Flounder pleuronectus flesus* when subjected to Cadmium toxication (Larson, 1975). The reduction in MCH and MCHC values was attributed probably as a defence mechanism against the toxic effect of Cadmium through the stimulation of erythropoiesis.

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