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Toxicity bioassay of organophosphorous pesticide malathion in common carp, *Cyprinus carpio*

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Abstract

The present study was conducted to evaluate the effects of an organophosphorous pesticide Malathion on hematological parameters of common carp, *Cyprinus carpio*. Acute toxicity of Malathion (96 h LC50 value) was determined. The experiment was conducted with three treatments, each with three replications. Two sub-lethal concentrations (1.5 and 3.0 mg/L) were selected considering the 96 h LC50 value representing treatment two (T2) and treatment three (T3). A control set was also run for the same time with the same number of fish without Malathion representing treatment one (T1). For hematological parameters fish were sacrificed at 24, 48, 72, and 96 h after exposure. The LC50 value of common carp during the 96 h of exposure was 15.24 mg/L. The values of Hb, RBC and hematocrit were significantly decreased with the increasing concentration of Malathion. On the other hand, WBC was significantly increased with toxicity of Malathion. The present study revealed that Malathion has unpleasant effects on the hematology and metabolism of macromolecule of fish. Therefore, the use of Malathion in the agricultural field may be a hazard to fauna and flora of the environment.

INTRODUCTION

Contamination of water by pesticides, either directly or indirectly can lead to fish kills, reduced fish productivity. In Bangladesh, more than 300 types of pesticides and insecticides are used for crop protection in agriculture (1). Malathion (O, O-dimethyl dithiophosphate of diethyl mercaptosuccinate) is an insecticide in the chemical family known as organophosphates. Malathion is toxic via skin contact, ingestion, and inhalation exposure (2). Hematological parameters like hemoglobin, hematocrit, and blood cell counts can be used to find physiological response of contaminated environment (3).

The common carp, *Cyprinus carpio* is an economically important freshwater fish, native to China and has been introduced all over the world to form a significant part of freshwater fishery. It is a highly palatable and preferred for culture due to its high growth rate and prolific breeding in confined water. In the present study, an effort was made to examine the toxicity of Malathion to this fish.

MATERIALS AND METHODS

A. Selection of test fish species

Healthy and active specimens of common carp, C. carpio were collected from local fish farm. The length and weight of fishes ranged from 14 to 16 cm and 50 to 70 g, respectively. The experiment was conducted in the wet laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh during January to June 2014.

B. Pesticide collection

To conduct the present study, the Malathion (50 E/C) was collected from a retailer shop in Mymensingh town.

C. Experimental procedure for acute and behavioral toxicity

Five different concentrations (5, 10, 15, 20, and 25 mg/L) of Malathion with three replicates were used. Then 10 fishes were transferred into each aquarium. Mortality was assessed at 24, 48, 72, and 96 h after exposure.

D. Experimental design for measurement of haematological parameters

The experiment was conducted with three treatments, each with three replications. Control set (0 mg/L) was used as Treatment one (T1) and two concentrations, such as 1.5 mg/L and 3.0 mg/L were used as Treatment two (T2) and Treatment three (T3.) For hematological parameters fish were sacrificed at 24, 48, 72, and 96 h after start of exposure.

E. Blood sampling and measurement of hematological parameters

Blood was collected from the caudal peduncle into citrated tuberculin syringes and immediately analyzed for the estimation of hematological parameters. Hemoglobin (Hb; g/dL) were measured using EasyMate® GHb, blood glucose/hemoglobin dual-function monitoring system using hemoglobin strips. Hematocrit (Hct; %) values were determined by using a micro-hematocrit centrifuge. The Red Blood Cell (RBCs; $x10^6/mm^3$) and White Blood Cell (WBCs; $x10^3/mm3$) count was made using Neubauer haemocytometer. The mean corpuscular volume (MCV; μm^3), the mean corpuscular hemoglobin (MCH; pg) and the mean corpuscular hemoglobin concentration (MCHC; %) were calculated using the following formulas (4) :

MCV = (% of Hct/RBC in millions) x 10 μm^3
MCH = (Hb in g/RBC in millions) x 10 pg
MCHC = (Hb in g/ (% of Hct) x 100 g per 100 mL

RESULTS

A. Acute and behavioral toxicity of Malathion in common carp

The mortality patterns in relation to Malathion dosage are presented in Table 1. No mortality was taken place in control. The LC50 was calculated by probit analysis (Fig. 1). The LC50 value of common carp during the 96 hrs of exposure was 15.24 mg/L. Several behaviors, such as restlessness, sudden quick movement, rolling movements, swimming on the back etc. were observed during the study period. The affected fish became very weak, settled at the bottom and died in increasing numbers at the higher dosages.

Table 1. Mortality of fish species exposed to different concentration of Malathion at different time intervals.

Concentration (mg/L)	Initial No. of fish	No. of dead fish after different exposure time (hrs)			
		24	48	72	96
Control	30	-	-	-	-
5.0	30	-	-	-	3
10.0	30	-	-	3	6
15.0	30	-	6	9	12
25.0	30	21	9	-	-

B. Effects of Malathion on hematological parameters in common carp

The values of Hb, RBCs and Hct decreased with the exposure period of Malathion concentration where's WBCs increased with the increased concentration of Malathion (Table 2).

Another type of hematological response such as Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated in the present study. MCV showed significant decreasing tendency with the increasing of toxicity of Malathion, while MCH showed no noticeable changes and significant increments were observed in case of the MCHC (Table 3).

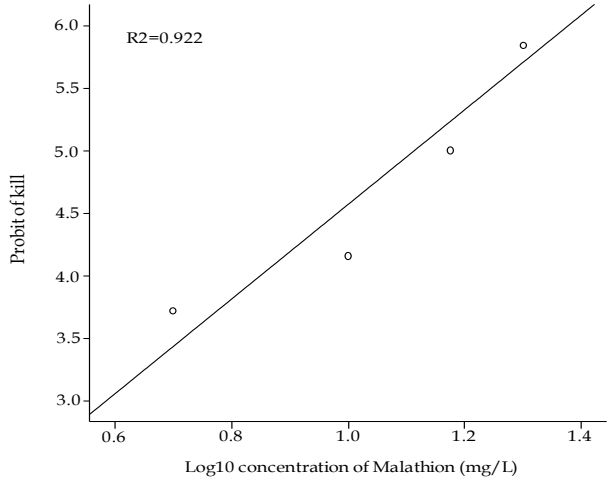

Fig. 1. Graph showing the relationship of probit of kill with $\log_{10}$ concentration of Malathion used to deduce the LC50.

Table 2. Alteration of different hematological parameters of fishes to different concentration of Malathion at different time intervals.

Parameters	Trea-ments	Exposure time (weeks)			
		24	48	72	96
Hb (mg/dL)	T1	13.40 ±1.50	14.27 ±0.75	12.93 ±0.58	12.70 ±1.63
	T2	11.43 ±1.37	12.40 ±2.00	11.20 ±1.58	11.93 ±1.90
	T3	10.93 ±1.32*	12.03 ±1.92*	10.80 ±1.42*	11.03 ±2.00*
RBC ($x10^6/mm^3$)	T1	7.70 ±1.92	7.16 ±0.33	6.99 ±1.07	7.25 ±1.39
	T2	6.50 ±0.46	6.51 ±0.26	5.71 ±0.88*	6.00 ±0.63*
	T3	6.53 ±0.64	6.65 ±0.58	5.58 ±0.58*	6.11± 0.30*
Hct (%)	T1	36.81 ±4.00	37.53 ±10.12	35.18 ±1.66	36.61 ±7.99
	T2	21.40 ±1.04*	28.92 ±2.76*	29.61 ±5.23*	29.60±1. 51*
	T3	23.29±2. 24*	27.2± 4.18*	23.55± 0.25*	27.18± 0.89*
WBC ($x10^3/mm^3$)	T1	2.93± 0.32	2.60± 0.90	3.12± 1.56	3.05± 0.12
	T2	3.78± 0.81	3.64± 0.90*	3.68± 1.57*	4.16± 0.90*
	T3	4.31± 1.05*	4.16± 0.90*	5.73± 0.91*	6.81± 0.86*

Asterisk (*) indicate the statistically significantly different (P<0.05, n=6).

Table 3. Changes in MCV, MCH and MCHC of common carp exposed to different concentrations of Malathion.

Parameters	Treat-ments	Exposure time (weeks)			
		24	48	72	96
MCV (μm^3)	T1	47.81 ±1.50	52.42 ±0.75	50.32 ±0.58	50.48 ± 1.63
	T2	32.92 ±1.37*	44.44 ±2.00	51.86 ±1.58	49.37 ± 1.90
	T3	35.65 ±1.32*	41.00 ±1.9*	42.19 ±1.42*	44.46 ±2.00*
MCH (pg)	T1	17.40 ± 1.92	19.93 ±0.33	18.50 ±1.07	17.51 ± 1.39
	T2	17.59 ± 0.46	19.06 ±0.26	19.61 ±0.88	19.90 ± 0.63
	T3	16.73 ± 0.64	18.10 ±.58	19.34 ±0.30	18.05 ± 0.58
MCHC (%)	T1	36.40 ± 4.00	38.01 ±6.14	36.77 ±1.66	34.69 ± 3.99
	T2	53.44 ±1.04*	42.88 ±2.76	37.82 ± 5.23	40.31 ±1.51*
	T3	46.94 ±2.24*	44.13 ±4.18*	45.85 ±0.25*	40.59 ± 0.89*

Asterisk (*) indicate the statistically significantly different (P<0.05, n=6).

DISCUSSION

In the present study, the LC50 value of common carp during the 96 hrs of exposure was 15.24 mg/L. The LC50 value of malathion for common carp in the present investigation was more or less within ranges reported by Verma *et al*., (5) where they examined the LC50 for twenty three pesticides for freshwater fish species. The fish showed characteristic changes in behavior when exposed to various concentrations of Malathion. Difficulty in respiration, convulsions and short erratic jerky body actions were observed. The fish settled at the bottom before death. In low concentration of Malathion, the responses were of a lesser degree. Control fish behaved normally. Similar observations have been made in different fish species following exposure to various biocides (6-7).

The values of Hb, RBCs and Hct were significantly decreased indicated the failing of hematopoietic system. Significant decrease of RBCs, Hb and Hct were recorded in ATR exposed fish species (8). Interestingly, WBCs count was increased in the present study. The increase in WBCs count can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to lindane and Malathion (9).

MCV showed decreasing tendency with the increasing of toxicity of while MCH showed no noticeable changes (Table 3). On the other hand, significant increments were observed in case of the MCHC. This response was registered in common carp after acute effect of phenitrothion, imidan and dichlorvos (10).

CONCLUSION

It can be concluded that Malathion seems to be toxic to common carp. The present study provides the information that hematological alteration in fish due to sub-lethal exposure of Malathion. The data obtained in the present investigation amply emphasized that Malathion has adverse effects on the metabolism of macromolecule and hematopoietic organs of fish.

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