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Changes in hematological parameters and gill morphology in common carp exposed to sub-lethal concentrations of Malathion

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Abstract: Malathion is an organophosphorous insecticide widely used in the agricultural field in Bangladesh and reach in the aquatic environment through rain wash. In the present study, we examined the effects of Malathion on hematological parameters and gills morphology in common carp exposed to two sub-lethal concentrations (1.5 and 3.0 mg/L) for a period of 192h (8 days). For hematological parameters fish were sacrificed at 24, 48, 72, and 96h after start of exposure. Gills were collected at 192h after start of exposure. The blood glucose level was significantly elevated with increasing the concentration of Malathion. Red blood cells (RBCs), hemoglobin (Hb) and hematocrit (Hct) values were significantly decreased after the exposure of Malathion. Significant increments were observed in case of the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), while no distinct changes were noted for mean corpuscular hemoglobin (MCH). Interestingly, white blood cells (WBCs) count was significantly increased with toxicity of Malathion. Several morphological changes, such as telangiectasia, blood lamellar congestion, hypertrophy of filaments, lamellar fusion were observed in the gills of fish exposed to Malathion. The present study revealed that the insecticide had adverse effects on various blood parameters and gills morphology in common carp. Thus, the use of insecticide in the agriculture field may be a threat to fauna and flora of the aquatic environment.

Keywords: fish; environment; pesticide; toxicology; histopathology

1. Introduction

In the last few decades, indiscriminate use of insecticides has raised the risk of contamination of terrestrial environment as well as aquatic habitat. Though they have been used for the safety of humans, their harmful effects are alarming on other non-target animals like fish. Contamination of water by insecticides, either directly or indirectly can lead to fish kills and reduced productivity of waterbodies (Rahman *et al.*, 2012; Siddika *et al.*, 2012; Talukdar *et al.*, 2012; Nupur *et al.*, 2013). In Bangladesh, more than 300 types of pesticides and insecticides are used for crop protection in agriculture (Uddin *et al.*, 2016). Malathion (O, O-dimethyl dithiophosphate of diethyl mercaptosuccinate) is an insecticide in the chemical family known as organophosphates. Products containing Malathion are used outdoors to control a wide variety of insects in agricultural settings and around people's homes. It has been used in public health mosquito control and fruit fly eradication programs. Malathion may also be found in some special shampoos for treating lice. Malathion is toxic via skin contact, ingestion, and inhalation exposure (Tomlin, 2006). The organophosphate compounds altered metabolic activities to death of fishes (Anam and Mitra, 1995). It is important to examine the toxic effects of pesticides on fish as the fish is an important part of the food chain of human.

Similar to mammalian species fish appear to possess the same biochemical pathways to deal with the toxic effects of endogenous and exogenous agents (Lackner, 1998). Hematological parameters like Hb, Hct, and blood cell counts can be used to find physiological responses of contaminated environment (Dethloff *et al.*, 2001; Salam *et al.*, 2015; Sharmin *et al.*, 2015; Sharker *et al.*, 2015; Shahjahan *et al.*, 2016). Therefore, the blood parameters are often measured when a clinical diagnosis of fish physiology is applied to determine the sub-lethal effects of pollutants (Venkataramana *et al.*, 2006). The effects of different pollutants on the hematological parameters have been documented in several fish species (Abalaka *et al.*, 2011 and Al-Kahem Al-Balawi *et al.*, 2011). However, the reports on the effects of sub-lethal dosages of Malathion in common carp, *Cyprinus carpio* are still scanty. Moreover, so far there is no study yet conducted in Bangladesh which warrants the present study.

The common carp 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 species. The changes in hematological parameters (glucose, Hb, cell counts and Hct) were monitored after sub-lethal exposure of this insecticide. In addition, effects of Malathion on histoarchitecture of gills were examined after sub-lethal exposure of this insecticide.

2. Materials and Methods

2.1. Animals and insecticide

Healthy and active specimens of common carp (*Cyprinus 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 fishes were maintained in aquaria at $25 \pm 0.5^\circ\text{C}$ under a controlled natural photo-regimen (14/10 h, light/dark) for a period of 21 days before the experiments. The fish were fed twice a day. The experimental procedures followed the guidance approved by the Animal Care and Use Committees of Bangladesh Agricultural University, Mymensingh, Bangladesh. To conduct the present study, the Malathion with 57% active ingredient was collected from local retail pesticide shop.

2.2. Experimental design and sample collection

To conduct the experiment nine aquaria (75 cm \times 45 cm \times 45 cm) were first cleaned and washed thoroughly by tap water. Then the aquaria were filled with 100 L of tap water. 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 (15.24 mg/L) value (Sharmin *et al.*, 2015). A control set was also run for the same time and with the same number of fish but without Malathion. Ten fish were transferred in each aquarium. For hematological parameters fish were sacrificed at 24, 48, 72, and 96 h after start. Gills were collected at 192 h after start and preserved in 10% formalin until use for sectioning.

2.3. Blood sampling and measurement of hematological parameters

After each exposure periods of Malathion, blood was collected from the caudal peduncle into citrated tuberculin syringes. Blood glucose (mg/dL) and hemoglobin (Hb; g/dL) were measured using glucose and hemoglobin strips in a digital EasyMate® GHb, blood glucose/haemoglobin dual-function monitoring system. Hematocrit (Hct; %) values were determined by using a micro-hematocrit centrifuge. The red blood cell (RBC, $\times 10^6/\text{mm}^3$) and white blood cell (WBC, $\times 10^4/\text{mm}^3$) count was made using Neubauer hemocytometer. 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 (Jain, 1993) :

$$\text{MCV} = (\% \text{ of Hct/RBC in millions}) \times 10 \mu\text{m}^3$$

$$\text{MCH} = (\text{Hb in g/RBC in millions}) \times 10 \text{ pg}$$

$$\text{MCHC} = (\text{Hb in g/} (\% \text{ of Hct}) \times 100 \text{ g per } 100 \text{ mL}$$

2.4. Histology of gills

The fixed gills were passed through graded alcohol series to dehydrate them. The dehydrated gill samples were embedded into paraffin. The embedded gill samples were sectioned at 5-10 μm thickness using microtome machine (KD- 3358, Kede, USA). The gill sections were then stained with hematoxyline-eosin stain. Finally the gill sections were observed under microscope and taken photograph at $10 \times$ magnifications.

2.5. Statistical analysis

Values are expressed as means \pm standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess statistically significant differences among different treatments. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using PASW Statistics 18.0 software (IBM SPSS Statistics, IBM, Chicago, USA).

3. Results

3.1. Effects of Malathion on blood glucose

We examined the blood glucose levels after exposure of fish to Malathion. The blood glucose levels were significantly ($P < 0.05$) increased with increasing the concentrations (1.5 and 3.0 mg/L) of Malathion compared to control (0 mg/L) at 24, 48, 72, and 96h of exposure periods (Figure 1). The glucose levels in control fishes were ranged from 104.67 to 115.00 mg/dL.

3.2. Effects of Malathion on hemoglobin (Hb)

The Hb of blood was measured after exposure of fish to Malathion. The Hb values in control fishes were ranged from 13.27 to 14.27 g/dL (Figure 2). The values of Hb (g/dL) was significantly ($P < 0.05$) decreased due to the toxicity of Malathion at 24, 48, 72 and 96h of exposure periods in both concentrations (1.5 and 3.0 mg/L) compared to control (0 mg/L).

3.3. Effects of Malathion on red blood cells (RBCs)

Similar to Hb, RBCs ($\times 10^6/\text{mm}^3$) values were significantly ($P < 0.05$) decreased during the toxicity of Malathion at 24, 48, 72 and 96h of exposure periods in both concentrations (1.5 and 3.0 mg/L) compared to control group (0 mg/L). The RBCs ($\times 10^6/\text{mm}^3$) values varied from 5.583 to 7.700 ($\times 10^6$) cells/ mm^3 in the fishes of all groups (Figure 3).

3.4. Effects of Malathion on hematocrit/pet cell volume (Hct/PCV)

The Hct (%) values in control fishes were ranged from 36.61% to 37.53% (Figure 4). Similar to Hb and RBCs, Hct/PCV (%) was significantly ($P < 0.05$) decreased with increasing the toxicity of Malathion at 24, 48, 72 and 96h of exposure periods in both concentrations (1.5 and 3.0 mg/L) compared to control (0 mg/L).

3.5. Effects of Malathion on MCV, MCH and MCHC

We calculated the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). The MCV showed significant ($P < 0.05$) decreasing tendency with the increasing of toxicity of Malathion at 24, 48, 72, and 96h, while MCH showed no noticeable changes (Table 1). On the other hand, significant ($P < 0.05$) increments were observed in case of MCHC.

3.6. Effects of Malathion on white blood cells (WBCs)

The values of WBCs ($\times 10^4/\text{mm}^3$) are presented in Figure 5. There was a significantly ($P < 0.05$) increase of WBCs in experimental groups (1.5 and 3.0 mg/L) compared to control group (0 mg/L) at 24, 48, 72, and 96h of exposure periods.

3.7. Effects of Malathion on gills morphology

In the present study, morphological changes in gills exposed to Malathion were examined by histological analysis (Figure 6). The gills of fish exposed to low dose (1.5 mg/L) showed curling and clubbing of secondary lamellae called talangiectasis (T) and barrier for blood movement called blood lamellar congestion (C), while talangiectasis (T), blood lamellar congestion (C), hypertrophy of filaments (H), edema with lifting of lamellar epithelium and hyperplasia of lamellar epithelium called lamellar fusion (LF) were observed in the gills of fish exposed to high concentration (3.0 mg/L).

Table 1. Changes in MCV, MCH and MCHC of common carp exposed to different concentrations of Malathion.

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

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

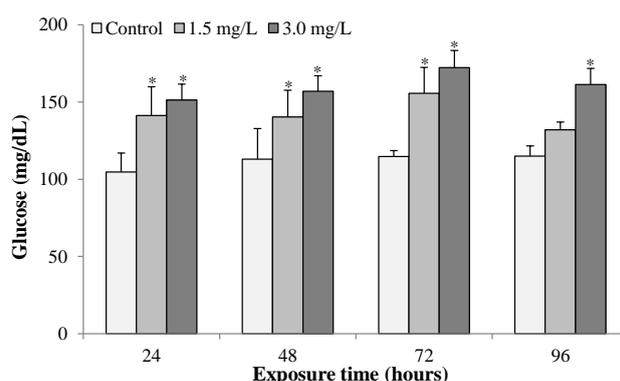


Figure 1. Changes in blood glucose levels (Means \pm SEM) of common carp exposed to sub-lethal dosage of Malathion at different time intervals. The blood glucose levels of fish exposed to 1.5 mg/L and 3.0 mg/L Malathion were compared to the fish of control group (0 mg/L). Asterisk (*) indicate the statistically significantly different ($P < 0.05$, $n = 6$).

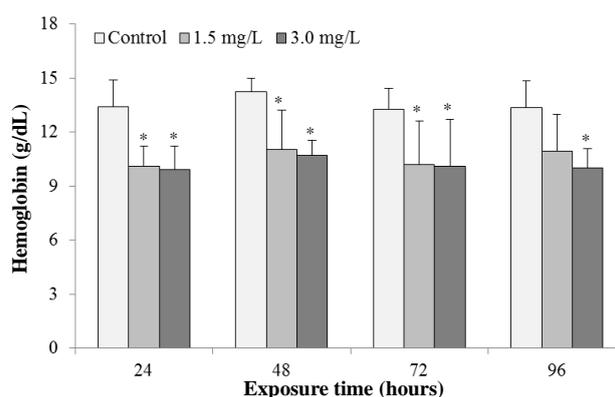


Figure 2. Changes in hemoglobin (Means \pm SEM) of common carp exposed to sub-lethal dosage of Malathion at different time intervals. The haemoglobin of fish exposed to 1.5 mg/L and 3.0 mg/L Malathion were compared to the fish of control group (0 mg/L). Asterisk (*) indicate the statistically significantly different ($P < 0.05$, $n = 6$).

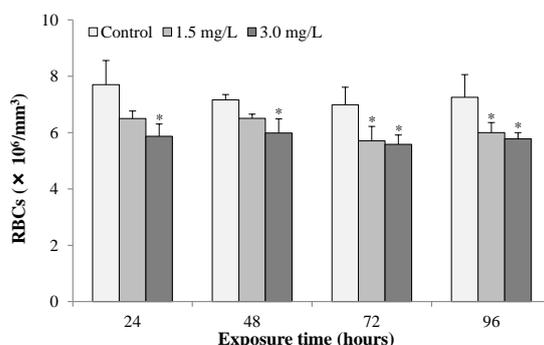


Figure 3. Changes in RBCs (Means ± SEM) of common carp exposed to sub-lethal dosage of Malathion at different time intervals. The RBCs of fish exposed to 1.5 mg/L and 3.0 mg/L Malathion were compared to the fish of control group (0 mg/L). Asterisk (*) indicate the statistically significantly different (P<0.05, n=6).

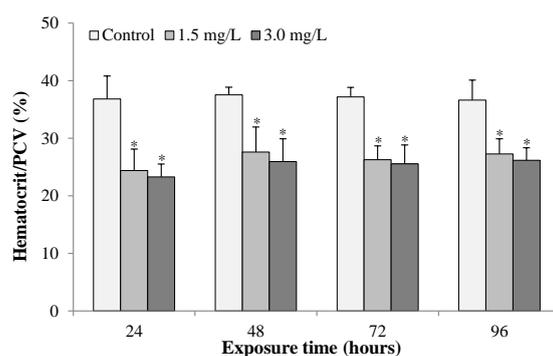


Figure 4. Changes in hematocrit/PCV (Means ± SEM) of common carp exposed to sub-lethal dosage of Malathion at different time intervals. The haematocrit/PCV of fish exposed to 1.5 mg/L and 3.0 mg/L Malathion were compared to the fish of control group (0 mg/L). Asterisk (*) indicate the statistically significantly different (P<0.05, n=6).

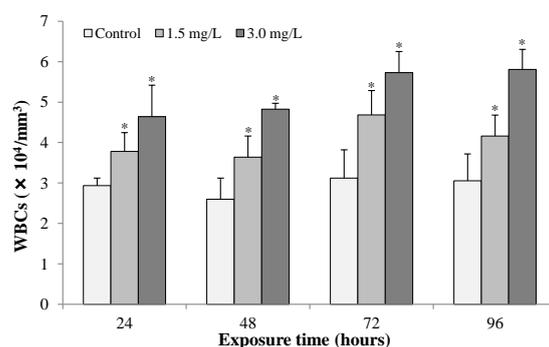


Figure 5. Changes in WBCs (Means ± SEM) of common carp exposed to sub-lethal dosage of Malathion at different time intervals. The WBC of fish exposed to 1.5 mg/L and 3.0 mg/L Malathion were compared to the fish of control group (0 mg/L). Asterisk (*) indicate the statistically significantly different (P<0.05, n=6).

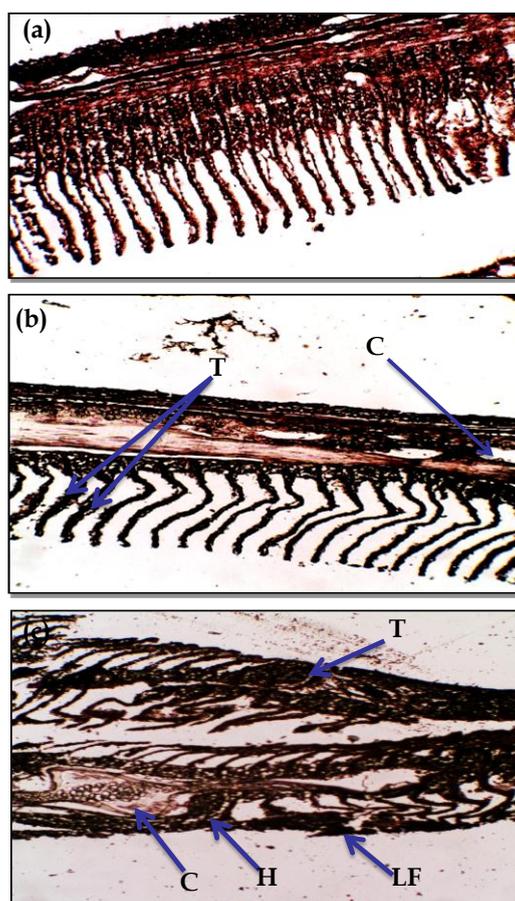


Figure 6. Morphological changes in gills exposed to Malathion; (a) Control (0 mg/L), (b) 1.5 mg/L and 3.0 mg/L. Arrowheads are indicating Talangiectasis (T), Blood Lamellar congestion (C), Hypertrophy of filaments (H), Lamellar fusion (LF).

4. Discussion

To understand the toxic effects of an organophosphorous insecticide Malathion on fish, we examined the effects of Malathion on hematological parameters and gill morphology of common carp. Differential changes were observed in hematological parameters and gills morphology indicating the toxicological impacts of Malathion on fish body.

The blood glucose level is the amount of glucose (sugar) present in the blood of an animal which is the primary source of energy. In the present study, increased glucose levels in the fish exposed to Malathion might be due to the mobilization of glycogen into glucose to meet the increased demand for energy. Glucocorticoids and catecholamine hormones are known to produce hyperglycemia in animals and stress stimuli elicit rapid secretion of these hormones from adrenal tissue of the fish (Pickering, 1981). Such increase may be due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their new energy demands (Winkaler *et al.*, 2007). The hyperglycemic condition in the present study may also be attributed to increased secretion of these hormones which causes glycolysis in the fish exposed to Malathion (Abalaka *et al.*, 2011; Alkahem-Al-Balawi *et al.*, 2011). The pesticide may change the functions of vital organs like liver and kidney, disrupting the homeostatic condition of the body. Similar observations have been reported in the carp, *Cyprinus carpio*, after the exposure of dietary copper and support to the present study (Al-Akel *et al.*, 2010).

Significant decreases in various blood parameters (Hb, RBC and Hct) might be because of failing of hematopoietic system in common carp exposed to Malathion. Similar to the present results, a decrease in the number of RBC, Hb and Hct values were reported in common carp exposed to diazinon (Banaee *et al.*, 2011) and in *Clarius gariepinus* exposed to lead nitrate (Adeyemo, 2007). Generally, toxicants exposure exerts an adverse effect on the hematopoietic organs which in turn alters blood parameters. Decline in RBCs, Hb and Hct values apparently reproduced erythrocyte hemolysis, damage of gill morphology and function in Korean rockfish (*Sebastes schlegeli*) exposed to cypermethrin (Jee *et al.*, 2005). The blood cell indices like MCV, MCH and MCHC seem to be changes in the present study that can cause reversible changes in the homeostatic system

of fish. Fluctuations in these indices correspond with values of RBCs, Hb and Hct. The values of blood cell indices were enhanced in common carp and other freshwater fish after the exposure of acute toxic level of pesticides (Rao, 2010; Hossain *et al.*, 2015; Salam *et al.*, 2015).

In the present study, WBC significantly increased with increasing the toxicity of Malathion at 24, 48, 72 and 96h of exposure periods in both concentrations compared to control (Figure 8). Increased WBC count established leucocytosis, which is considered to be of an adaptive value for the tissue under chemical stress. This also helps in the removal of cellular debris of necrosed tissue at a faster rate (Marti *et al.*, 1996). In the presence of foreign substances or under pathological conditions leucocytosis in fish may be the consequence of direct stimulation of immunological defense (Marti *et al.*, 1996). The increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to Malathion (Joshi *et al.*, 2002).

In fish, the gills participate in many important functions, such as respiration, osmoregulation and excretion, remain in close contact with external environment and particularly sensitive to changes in the quality of the water are considered the primary target on contaminants (Camargo and Martinez, 2007; Fernandes and Mazon, 2003). Toxic substances can injure gills, thus reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms (Saravana *et al.*, 2000). In the present study, exposure of common carp to Malathion resulted in structural alterations of the gill including talangiectasis (curling and clubbing of secondary lamellae), blood lamellar congestion (barrier for blood movement), hypertrophy of filaments, lamellar fusion (edema with lifting of lamellar epithelium and hyperplasia of lamellar epithelium). These pathological changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants thorough the gill surface and probably due to increased capillary permeability (Olurin *et al.*, 2006). The present results are in agreement with those observed in other fish species under the influence of different pollutants (Olurin *et al.*, 2006; Parikh *et al.*, 2010; Ahmed *et al.*, 2015).

5. Conclusions

The effects of Malathion on hematological parameters and gills morphology were examined in common carp. Malathion enhanced the blood glucose level suggests that glycogen might be breakdown to glucose due to toxicant of Malathion. On the other hand, reduction of Hb, RBCs and Hct might be because of failing of hematopoietic system. Increased WBCs count established leucocytosis, which is considered to be of an adaptive value for the tissue under chemical stress. Several morphological changes in gills were observed which indicates a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants thorough the gill surface and probably due to increased capillary permeability. Taken all together, the present study revealed that the Malathion has adverse effects on various blood parameters and gills morphology in common carp.

Conflict of interest

None to declare.

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