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Histopathological Changes in Liver and Kidney of Common Carp Exposed to Sub-lethal Doses of Malathion

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Abstract.- The present study was conducted to evaluate the effects of an organophosphorous pesticide malathion on histo-architecture of liver and kidney of common carp exposed to two sub-lethal concentrations (1.5 and 3.0 mg/L) for a period of 8 days. The 96 h LC_{50} value of malathion for common carp was 15.24 mg/L. Several morphological changes were observed in the liver after exposure to the malathion. Hypertrophy of hepatocytes, mild to severe necrosis and minor vacuolation was found in liver exposed to both concentrations. Similarly, a number of changes such as, pycknosis, necrosis, disintegrated renal tubule, vacuole, shrunk glomerulus etc were observed in the kidney exposed to both concentrations. The changes in these vital organs might have resulted in physiological and metabolic dysfunction. Therefore, the use of pesticide in the agricultural field may be a threat to fish health and their population in the aquatic environment.

Keywords: Organophosphorous, malathion, *Cyprius carpio.*

Over the decades, various types of pesticides are widely used in agriculture field in Bangladesh. Though the use of pesticides contributed to enhance crop production, but their harmful effects are alarming for non-target animals like fish. These pesticides have widely polluted the aquatic environment (Pandey *et al.,* 2000; Sial *et al.,* 2009). The major route of pesticides to water ecosystems is through rainfall runoff and atmospheric deposition. Another source of water contamination by pesticides is from municipal and industrial dischargers. Most insecticides ultimately find their way into rivers, lakes and ponds (Werimo *et al.,* 2009; Arjmandi *et al.,* 2010) and have been found to be highly toxic to non-target organisms that inhabit natural environments close to agricultural fields, leading to mortality of fish (Rahman *et al.,* 2002). Malathion (O, O-dimethyl dithiophosphate of diethyl mercaptosuccinate) is an organophosphate which is used to control a wide variety of insects in agricultural field and residential area. It is toxic via skin contact, ingestion and inhalation exposure (Tomlin, 2006).

Histopathological investigations on different tissues of fish are valuable tools for toxicology studies (Thophon *et al.,* 2003). In the present study, an effort has been made to examine the changes in liver and kidney morphology of a common carp, *Cyprinus carpio* after sub-lethal exposure of malathion.

Materials and methods

A total of 200 fish (14-16 cm, 50-70 g) were collected from local fish farm (Bangladesh Catfish Limited, Valuka, Mymensingh) and maintained in aquaria at 25±0.5°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 with artificial pellet feed twice a day at 8:00 am and 6:00 pm.

Malathion (Sithion 57 E/C, Siam Crop Care, Bangladesh) was purchased from local retail pesticide shop. Its 96 h LC50 value was determined against common carp. Five different concentrations (5, 10, 15, 20, and 25 mg/L) of malathion with three replicates were used. A control was also run without malathion treatment with each replicate. Malathion was directly added to the water of aquarium. Exceeding aeration was applied to the aquarium for 2 h in order to obtain a homogeneous concentration of the toxic compound, and then 10 fish were transferred into each aquarium. Mortality was assessed 24, 48, 72, and 96 h after start of exposure to malathion. Dead fishes were recorded and then immediately removed.

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To study effect of sub lethal doses of malathion on liver and kidney of common carp, following experiment was setup. Nine aquaria (75 cm × 45 cm × 45 cm) were cleaned, washed thoroughly with tap water and then filled with 100 L of tap water. The experiment was conducted with three treatments, each with three replications. Two sub-lethal concentrations *viz.,* 1.5 and 3.0 mg/L were added to the water of aquarium. A control aquarium was also run without malathion. Ten fish were transferred to each aquarium and kept for a period of 8 days. Water and malathion were changed every 24 h at 10:00 am. The fish were anesthetized with 0.03% tricaine methane sulfonate (MS222, Sigma-Aldrich, Japan) before sampling. Liver and kidney were removed immediately after decapitation and preserved in 10% formalin. The fixed liver and kidney samples were processed by a routine procedure, and embedded in paraffin wax, 6-8 µm thick sections were cut using microtome machine (KD-3358, Kedee, USA) and stained with hematoxylene and eosin.

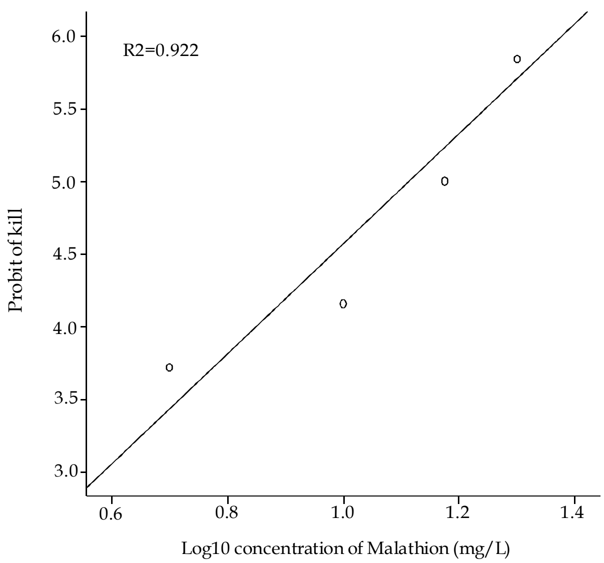

Fig. 1. Graph showing the relationship of probit of kill with $\log_{10}$ concentration of malathion used to deduce the LC_{50}.

Results and discussion

The LC_{50} value of malathion calculated by probit analysis against common carp during 96 h of exposure was 15.24 mg/L (Fig. 1). This value was similar to that of another study where it was reported 16.6 mg/L for common carp (Hassan *et al.,* 1993).

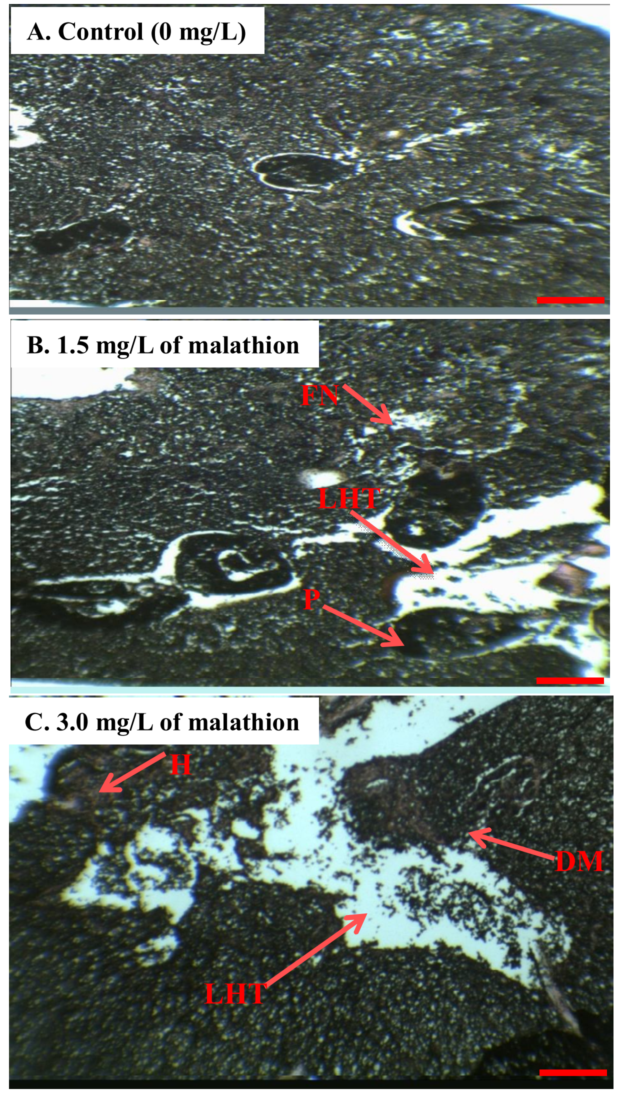

Fig. 2. Histo-architectural changes in liver exposed to malathion; (A) Control (0 mg/L), (B) 1.5 mg/L and (C) 3.0 mg/L. Arrow heads indicate focal necrosis (FN), loosening of hepatic tissue (LHT), pycknosis (P), haemorrhage (H) and degeneration of muscle (DM). Magnification 10×; scale bars = 100 µm.

Histological structure of liver of fish exposed to both concentrations (1.5 and 3.0 mg/L) of malathion showed several changes such as focal necrosis (FN), loosening of hepatic tissue (LHT), pycknosis (P), haemorrhage (H), and degeneration of muscle (DM) (Fig. 2). Since liver is the site of detoxification of all type of toxins and chemicals, these changes of vacuolization, swelling and necrosis may be attributed to the direct toxic effects of pollutants on hepatocytes. Similar observations have also been reported after exposure to other organophophorous pesticides in various fishes (de Rodrigues and Fanta, 1998; Camargo and Martinez, 2007; Mohamed, 2009).

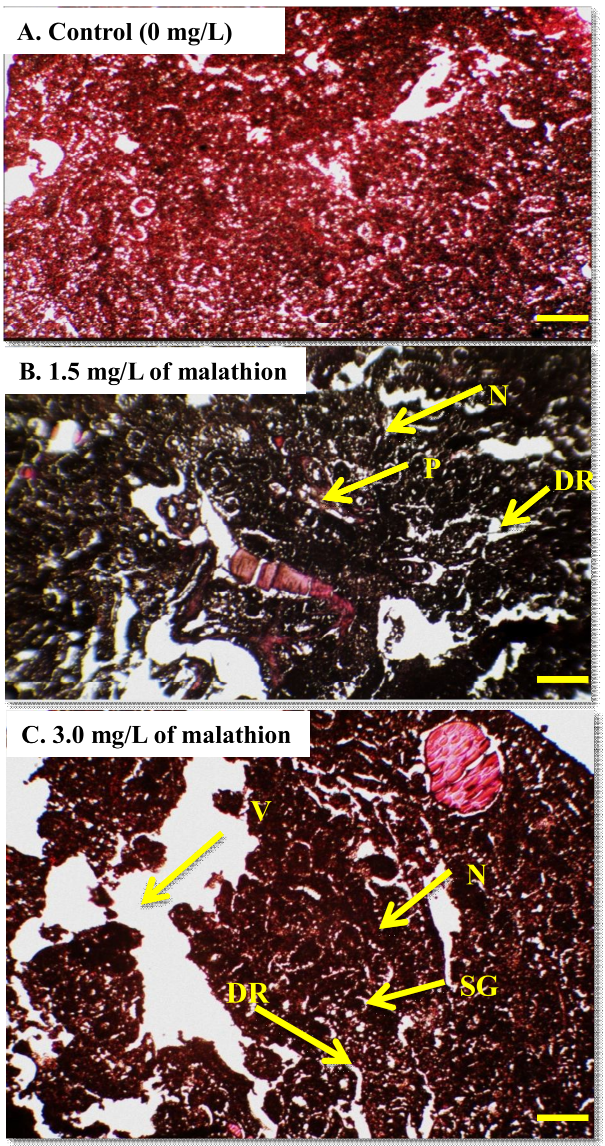

Fig. 3. Morphological changes in kidney exposed to malathion; (A) Control (0 mg/L), (B) 1.5 mg/L and (C) 3.0 mg/L. Arrow heads indicate pycknosis (P), necrosis (N), disintegrated renal tubule (DR), vacuole (V), shrunk glomerulus (SG). Magnification 10×; scale bars = 100 µm.

Kidney is another vital organ that maintains the homeostasis of a body and plays an important role in erythropoiesis (Iqbal *et al.,* 2004). The alterations found in the kidney of fish in the present study were pycknosis (p), necrosis (N), disintegrated renal tubule (DR), vacuole (V), shrunk glomerulus (SG) etc after exposure to both concentrations (1.5 and 3.0 mg/L) of malathion (Fig. 3). Severity in alterations increased in proportion to increased dose. Similar results have been reported by Cengiz (2006) in *Cyprinus carpio* after acute exposure to deltamethrin. With severe intoxicated conditions, the degenerative process leads to tissue necrosis (Yokote, 1982). The necrosis of the tubules will affect the metabolic activities and promotes metabolic abnormalities in fish. The present results are in agreement with those observed in *C. carpio* exposed to sewage (Veiga *et al.,* 2002; Thophon *et al.,* 2003). Necrosis of tubular and surrounding haematopoietic cells, pycknosis and karyorrhexis of kidney tissue were observed when coho salmon was exposed to Amitrole for 144 h (Rand and Petrocelli, 1985).

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