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Histopathological alterations in the liver architecture of *Mystus tengara* exposed to sub-lethal concentrations of cypermethrin

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Abstract. The application of cypermethrin in agriculture may reach the aquatic environment through several routes including spray drift, surface runoff and ground water leaching and may contaminate the aquatic systems. The present study aimed at assessing the toxicity of cypermethrin in the liver histoarchitechture of *Mystus tengara*. Each of the 10 fish was stocked into 12 PVC tanks containing 100 L dechlorinated tap water. Fish were exposed to three sub-lethal concentrations of cypermethrin with one control (0, 0.026, 0.052 and 0.104 ppm) for 7, 14 and 28 days. Each of the control and treatment was maintained in three replicates. Results showed the histopathological alterations including cloudy swelling of hepatocytes, necrosis, vacuoles, hepatic cell rupture, karyolysis, nuclear pyknosis and degeneration of hepatic cells exposed to different sub-lethal concentrations at different sampling dates. The present study revealed that low levels (0.052 ppm) of cypermethrin exposure in the aquatic environment may cause histopathological alterations in the liver of *M. tengara*, however, studies should be carried out to understand the underlying mechanisms involved in long-term toxicity profile of cypermethrin.
Key words: Pyrethroid pesticides, *Mystus tengara*, Histopathology.

Introduction

In Bangladesh, the intensification of agriculture is needed for the ever-increasing population, decrease of available arable land and food security needs. Several agro-climatic conditions (e.g. sudden and flash floods, water scarcity, salinity intrusion in coastal belt, cyclones and storms) pose further difficulties in agricultural crop production and can cause the agricultural intensification (Sumon *et al.* 2017). While considering agricultural intensification, farmers produce high-yielding cultivars of different crops, which are mostly susceptible to diseases and pests (Ali *et al.* 2018). Hence, pesticides are being used indiscriminately to protect the crops (Shahjahan *et al.* 2017; Sumon *et al.* 2018). The use of pesticide in Bangladesh, however, was negligible until 1960, but it has increased rapidly from 7350 MT in 1992 to 45,172 MT in 2010 (Rahman 2013). Synthetic pyrethroids are less persistent in nature and less toxic to mammals and birds than other groups of pesticides like organochlorine, organophosphate, and carbamate. Hence, pesticides of pyrethroid groups are becoming increasingly popular in agricultural, veterinary and house-hold use over the decades (Yildirim *et al.* 2006; Shi *et al.* 2011). Cypermethrin and k-cyhalothrin are widely used as synthetic pyrethroids to control many pests of including cotton, fruits and vegetable crops (Crawford *et al.* 1981), and they are considered as the most effective pyrethroid preparations (Lewis *et al.* 2016; Ali *et al.* 2018). Cypermethrin contains cyano-3-phenoxy-benzyl groupsthat can act by blocking sodium channels and affect the function of GABA receptors of nerve filaments of fish (Bradbury and Coats 1989). Cypermethrin used in agriculture may reach the aquatic environment through different routes including spray drift, surface runoff and ground water leaching (Deka and Dutta 2012). After reaching into the aquatic systems, this insecticide might show high toxicity to a number of non-target aquatic organisms (fish, invertebrates, primary producers, etc.) at sub-lethal levels even at low concentrations (El-Sayed *et al.* 2007).

Histopathology provides a good tool to investigate the effects of toxicants in various organs of fish like liver, kidney, gill, gonad, etc. Fish liver is considered as a major site of storage, biotransformation and excretion of toxicants (Velmurugan *et al.* 2009). A number of studies have been conducted to assess the toxic effects of cypermethrin in the liver of various fish species (Sarkar *et al.* 2005; Dobsikova *et al.* 2006; Velisek *et al.* 2006; Joshi *et al.* 2007; Korkmaz *et al.* 2009; Velmurugan *et al.* 2009; Olufayo and Alade 2012). *Mystus tengara*, commonly known as 'tengra' is a freshwater catfish of the family Bagridae under the order Siluriformes. However, no study was undertaken to elucidate the toxic effects of cypermethrin in the liver of *M. tengara*. Therefore, the present study aimed at assessing the toxicity of cypermethrin in the liver histoarchitechture of *M. tengara*.

Materials and Methods

Collection of experimental fish and chemicals: Healthy adult of the *M. tengara* (n= 200; weight= 7.8±1.7 g, length= 8.2±0.8 cm) were collected from the local dealer of Mymensingh. They were reared in a cemented cistern for one month. During the rearing period, fishes were fed with artificial pellet feed. Cypermethrin (with 10% active ingredients) was purchased from a local pesticide trader.

Experimental design: Adult healthy fish (n= 120; weight= 8.2±1.6g, length= 8.7± 0.7 cm) were transferred to the PVC tanks for cypermethrin exposure. The experiment was carried out in the Wet Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Ten fish were stocked into each of the 12 previously prepared PVC tanks containing 100 L dechlorinated tap water. Aeration system was installed to provide sufficient oxygen during the experimental period of 28 days. The fish were exposed to three sub-lethal concentrations of cypermethrin with one control (0.00, 0.026, 0.052 and 0.104 ppm; as the 96-h LC_{50} of cypermethrin on *M. tengra* was 0.133 ppm described in Haque *et al.* (2018) for 7, 14 and 28 days. Each of the control and treatment was maintained in three replicates. Fish were fed with artificial pellet feed. Natural light and ambient temperature was maintained throughout the experimental period. Excess feed and excretions were removed everyday using a plastic pipe. Water was changed every alternate day and fresh cypermethrin concentrations were used. The water quality parameters measured (mean $\pm$ SD) every alternate day during the experiment were as follows: temperature: 26.8 $\pm$ 1.3 ^{o}C; dissolved oxygen: 6.2$\pm$0.9 mg/L; pH: 7.7$\pm$0.5; total alkalinity: 176.6$\pm$8.3 mg/L.

Collection of liver samples and histopathology: One fish was sacrificed from each of the replicate on 7, 14, 28 days for liver collection. Fish were killed by decapitation and liver samples were dissected out and transferred to 10% buffered formalin at ambient temperature for fixation. To observe histopathological alterations, liver samples were washed with running tap water, processed, dehydrated in graded alcohol, cleared in benzene, and embedded in paraffin. The paraffin blocks were sectioned with microtome at a thickness of 5 μm and were stained with hematoxylin and later counterstained with eosin. The histopathological alterations were observed under a compound binocular microscope and photographed using digital photomicroscope (Olympus CX 41).

Results

The liver of *M. tengara* collected from untreated fish showed normal histoarchitecture, which was characterized by polygonal shaped hepatocytes with granular cytoplasm and centrally placed round nuclei (Fig. 1A). Fig. 1B-F show histopathological alterations when exposed to different concentrations of cypermethrin. Histopathological alterations including cloudy swelling of hepatocytes, necrosis, vacuoles, hepatic cell rupture, karyolysis, nuclear pyknosis and degeneration of hepatic cells (Fig. B-F) were resulted when exposed to different sub-lethal concentrations of cypermethrin for different durations. No obvious histopathological alterations in the liver of *M. tengara* were evident when they were exposed to 0.026 ppm of cypermethrin during the whole experimental period. However, histopathological alterations including necrosis, vacuoles and hepatic cell rupture were observed after 14 days of cypermethrin exposure at 0.052 ppm while nuclear pyknosis was observed for the same concentrations after 28 days of exposure.

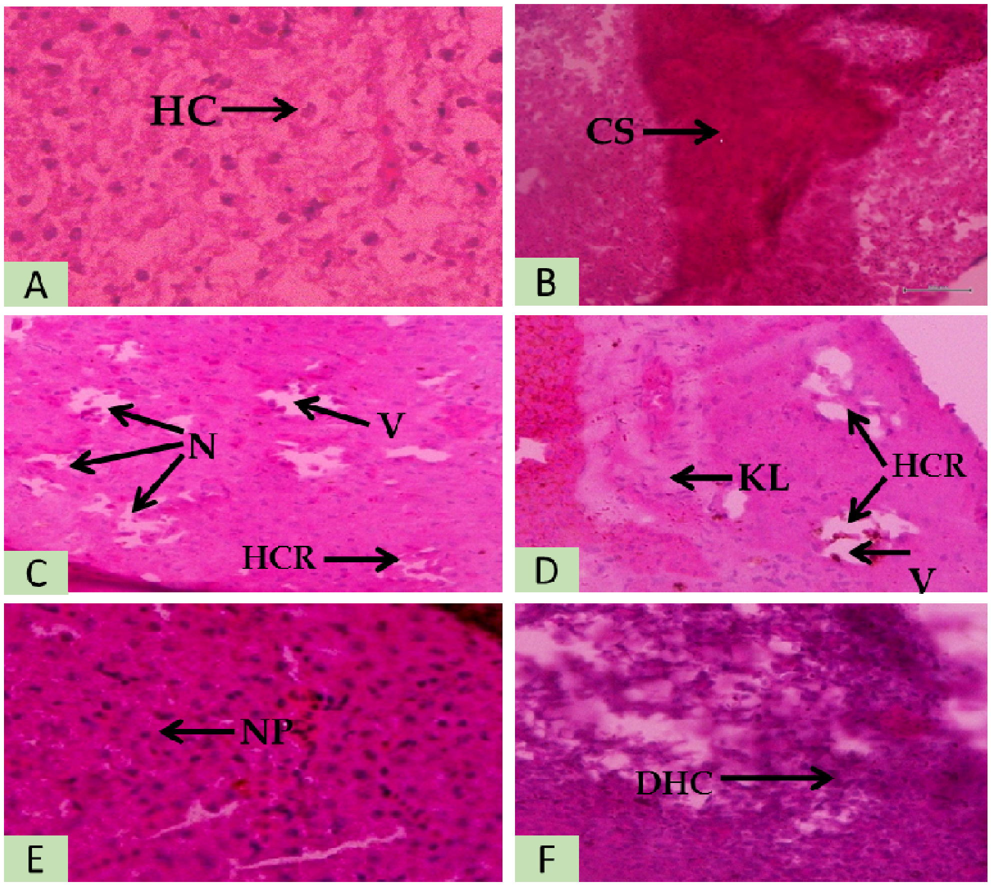

Fig. 1. Histopathological alterations in liver of *M. tengara* exposed to different cypermethrin concentrations. (A) Normal hepatic cell (HC) exposed to 0 ppm for 7 days (B) Cloudy swelling of hepatocytes exposed to 0.104 ppm for 7 days (C) Necrosis (N), vacuoles (V) and hepatic cell rupture exposed to 0.052 ppm for 14 days (D) Karyolysis (K), vacuoles (V) and hepatic cell rupture exposed to 0.104 ppm for 14 days (E) Nuclear pyknosis (NP) exposed to 0.052 ppm for 28 days and (F) Degeneration of hepatic cell exposed to 0.104 ppm for 28 days.

Discussion

Liver is the most important organ with processes of detoxification and biotransformation of toxic compounds and its regulating mechanisms can be affected by different concentrations of the compounds, which could result in histopathological alterations (Pal *et al.* 2012). In the present study, the liver of the treated fish compared to the control showed dose- and duration-dependent histopathological alterations e.g. cloudy swelling of hepatocytes, necrosis, vacuoles, hepatic cell rupture, karyolysis, nuclear pyknosis and degeneration of hepatic cells. Velmurugan *et al.* (2009) reported histopathological alterations in the liver of African catfish like cloudy swelling of hepatocytes, hepatocytes with some pycnotic nuclei, lipoid vacuoles and focal necrosis exposed to cypermethrin. Sarkar *et al.* (2005) observed histopathological alterations e.g. hyperplasia, disintegration of hepatic mass and focal coagulative necrosis in the liver of *Labeo rohita* when exposed to cypermethrin. Almost similar alterations were reported in the liver of *Heteropneustes fossilis* (Joshi *et al.* 2007). Similar alterations were reported in *Oreochromis niloticus* exposed to deltamethrin (Yildirim *et al.* 2006), *Cirrhinus mrigala* exposed to fenvalerate (Velmurugan *et al.* 2007), *Ctenopharyngodon idellus* exposed to fenvalerate (Tilak *et al.* 2001) and *Gambusia affinis* exposed to deltamethrin (Cengiz and Unlu 2006). In conclusion, the present study revealed several histopathological alterations in the liver of *M. tengara* could be easily detected at low levels (0.052 ppm) of cypermethrin exposure. Hence, the substantiation of histopathological alterations in various organs sequentially in contact with toxicants seems useful as a biomarker of pollutant exposure and effect. Further studies should be carried out to understand the underlying mechanisms involved in long-term toxicity profile of cypermethrin.

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