



Histopathological alterations in the gills of banded gourami (*Trichogaster fasciata*) exposed to thiamethoxam

MD. MOHIBUL HASAN^a, MD. MAHMUDUL HASAN^a, MD. HELAL UDDIN^a,
KIZAR AHMED SUMON, AL-AMIN AND HARUNUR RASHID*

Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

^aEqually contribution first authors; *Corresponding Author: rashid@bau.edu.bd

Abstract. Thiamethoxam (THM) is a widely used agro-pesticide in Bangladesh, though European Union has banned this neonicotinoid insecticide due to its toxic effects on non-target organisms. A 60-day experiment was conducted to study the effect of sub-lethal concentrations of THM on gills of Banded Gourami *Trichogaster fasciata*. The experiment was conducted in 15 PVC tanks each containing 100L water. The 96-h LC50 value of THM was estimated as 161.06mg/L for Banded Gourami. Based on estimated 96-h LC50 value, the experiment was carried out using a control (0 mg/L of THM) and five sub-lethal concentrations (9.37, 18.75, 37.5, 75 and 150 mg/L of THM in water) with three replications. For collection of gills, fishes were sampled on day-30 and day-60 of exposure to THM. Several histopathological changes were observed in the gill tissues of treated fishes viz. clubbing and reduction of gill filaments, telangiectasis of gill lamellae, haemorrhage and damage of gill raker. Water quality parameters (e.g. DO, pH and temperature) were recorded every 15 days interval. The DO levels were found to decrease significantly with increasing THM concentrations and time of exposure, whereas no noticeable changes were observed for pH and temperature. Therefore, neonicotinoid crop insecticide like THM is capable of damaging gill tissues of a small indigenous fish Banded Gourami.

Keywords: Banded Gourami, *Trichogaster fasciata*, thiamethoxam, histopathology, gill

Introduction

To meet the food demand for the increasing population, the government of Bangladesh has promoted the use of pesticides for producing more agricultural crops (Dasgupta *et al.* 2007). There was very minimum used of pesticides in Bangladesh before 1960s but abruptly increased about 7 times in amount from 1992 to 2010 (Ali *et al.* 2018). High Yielding Varieties (HYVs) are very much susceptible to pest for that reason the uses of pesticides are in increasing trends that also makes the environment polluted (Sumon *et al.* 2017). Pesticides eventually find their way into aquatic environments in many ways like spray drift, run off and leaching (Van den Brink 2013, Shahjahan *et al.* 2017). The release of this insecticide into the aquatic environment may have potential toxic effects on non-target aquatic organisms like invertebrates (Rubach *et al.* 2011; 2012), and vertebrates (Manjunatha and Philip 2015, Sumon *et al.* 2016). Considering the importance of fish as food as well as having long life span and ability to respond against environmental xenobiotics treated as a good bio-indicator among vertebrates (Gupta *et al.* 2009; Narra *et al.* 2011, Correia *et al.* 2017). Fish may be directly affected by pesticides in many ways like changes in normal behavior e.g. feeding or swimming (Satyavardhan 2013, Ullah *et al.* 2014, Rani and Kumaraguru 2014), alteration of physiological functions such as changes in blood parameters (Blood glucose, WBCs, RBCs etc) as well as histo-architectural changes in some important internal organs like gills, liver, kidney, intestine etc. (Saeedi *et al.* 2012, Salam *et al.* 2015, Sharmin *et al.* 2015). THM is one of the mostly used neonicotinoid insecticide, which is commonly used for controlling insect in Bangladesh and causes severe

detrimental effects on the cellular level of respiration on aquatic organisms particularly fish (Betoulle *et al.* 2000).

Trichogaster fasciata, the Banded Gourami, has been used as a fish model for ecotoxicological studies (Sumon *et al.* 2017, 2019). This benthopelagic species of fish prefers weedy environments such as estuaries, ponds, large rivers, ditches, lakes etc. and native to Bangladesh, India, Myanmar, Nepal and Pakistan (Mitra *et al.* 2007). Short generation time and easy acclimatization and captive breeding in laboratory conditions have made this fish a popular model for aquatic stress studies (Sumon *et al.* 2017, Reza *et al.* 2020). Therefore, the present study was conducted to examine the effects of agro-pesticide THM on the gill tissues of Banded Gourami *T. fasciata* as a model animal.

Materials and Methods

Experimental fish and pesticide collection: Healthy and adult *T. fasciata* (body weight: 8.12 ± 0.34 gm and length: 9.2 ± 0.8 cm) were collected from natural waters in Mymensingh. Live *T. fasciata* (n=250) were transported in well oxygenated polythene bags to the Wet Laboratory, Faculty of Fisheries, Bangladesh Agricultural University and were kept in cemented cistern with continuous water supply. They were reared under natural photoperiod for conditioning. Fish were fed with commercial fish feed at the rate of 3% body weight and twice daily. The fishes were conditioned for a period of 21 days. The pesticide THM 25WG (trade name: spike) was used for this experiment which was purchased from local retailer at Mymensingh town.

Experimental design: After 21 days, the acclimatized fish were exposed to five sub-lethal concentrations of THM (9.37, 18.75, 37.5, 75 and 150mg/L) in three replicates based on previously estimated 96-h LC50 value, 161.06mg/L for Banded Gourami. Fifteen 100-L plastic drums were used for fish holding, filled with dechlorinated water, equipped with aerators, released with 10 fish per drum, feeding daily at 3% of body weight and exposed for a period of 60 days. Unutilized feedstuffs and fecal wastes were cleaned daily by siphoning. Water was exchanged fortnightly and the pesticide with respective doses was added accordingly. Fishes were sampled (n=3) on day 30 and day 60 to collect gills and fixed in Bouin's fluid for histopathological studies. Water quality parameters (DO, pH, and temperature) were recorded weekly.

Histological procedures: Fixed gill tissues were dehydrated by passing through graded alcohol series, cleared by chloroform and embedded into molten paraffin. Embedded tissues were then sectioned at 5 μ m thickness using a microtome. The sections were placed on slides like ribbons, then stained with routine Haematoxyline-Eosin (H-E) protocols, followed by mounting with DPX and cover slip. The stained sections were observed under a microscope to detect the histopathological changes in gill tissues.

Statistical analysis: Values are expressed as means \pm standard deviation (SD). Data were analyzed by a 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).

Results

Histopathological results indicated that gill tissue was the primary target affected by pesticide THM. The gills are key organs involved in nutrient uptake, ingestion and respiration. Various dose and duration-dependent histopathological alterations in Banded Gourami gills were observed exposed to THM (Figs. 1 and 2). The gills which extracted from the control fish showed almost no histopathological alterations during the experimental period. The control gills showed regular-shaped of gill arches (GA), gill rakers (GR), gill lamellae (GL) and gill filaments (GF) (Figs. 1A and 2A). The gills of exposed fishes were revealed different histopathological alterations like necrosis (N), telangiectasis (T), clubbing (C) and reduction of gill filaments (RF) (Figs. 1B-E and 2B-E). A significant increase in histopathological alterations in gills was observed at all the pesticide treated groups on day-60 compared to day-30, respectively. There was a significant increase of histopathological alterations in the gills of Banded Gourami when exposed to 150 mg/L after both day-30 and day- 60 (Figs. 1F and 2F).

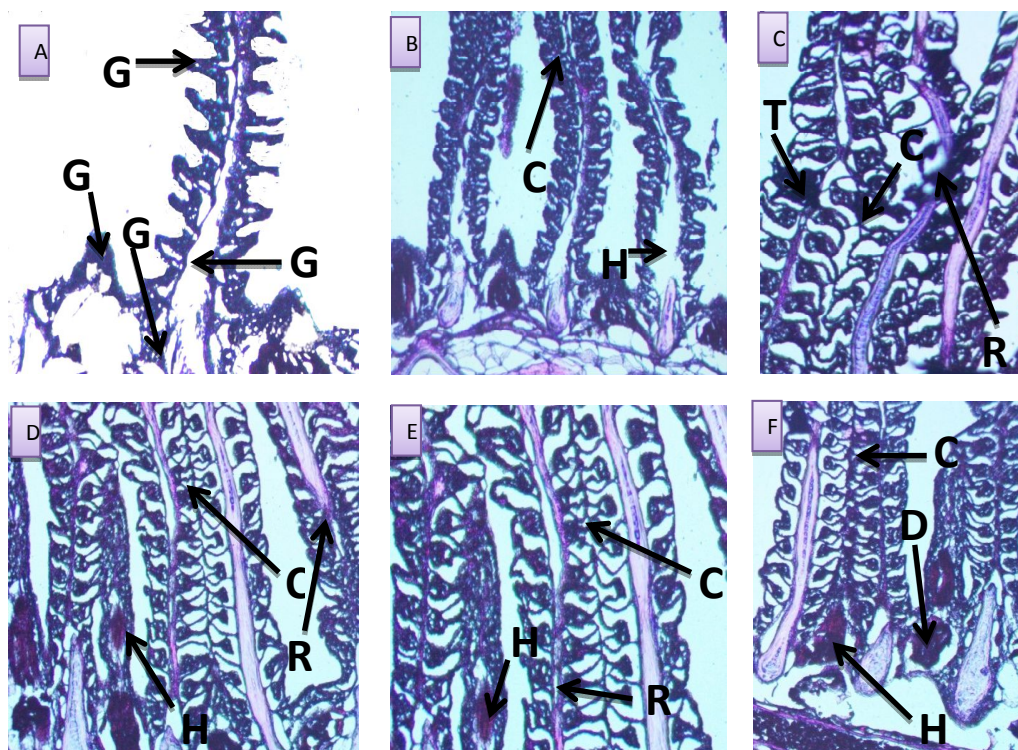


Fig. 1. Histopathological alterations observed in Banded Gourami *T. fasciata* exposed to thiamethoxam pesticide on 30 day, during the experimental period. (A) Almost normal structure of structure of gill arches (GA), gill rakers (GR), gill lamellae (GL) and gill filaments (GF) after 30 days exposure to 0 mg/L (control gill); (B) clubbing (C) and hypertrophy (HY) after 30 days exposure to 9.37 mg/L ; (C) clubbing (C), telangiectasis (T) and reduction of gill filaments (RF) after 30 days exposure to 18.75 mg/L ; (D) clubbing (C), haemorrhage (H) and reduction of gill filaments (RF) after 30 days exposure to 37.5 mg/L; (E) clubbing (C), haemorrhage (H) and reduction of gill lamellae (RF) after 30 days exposure to 75 mg/L; (F) clubbing (C), haemorrhage (H) and damage of gill raker (DR) after 30 days exposure to 150 mg/L; H and E stain $\times 400$.

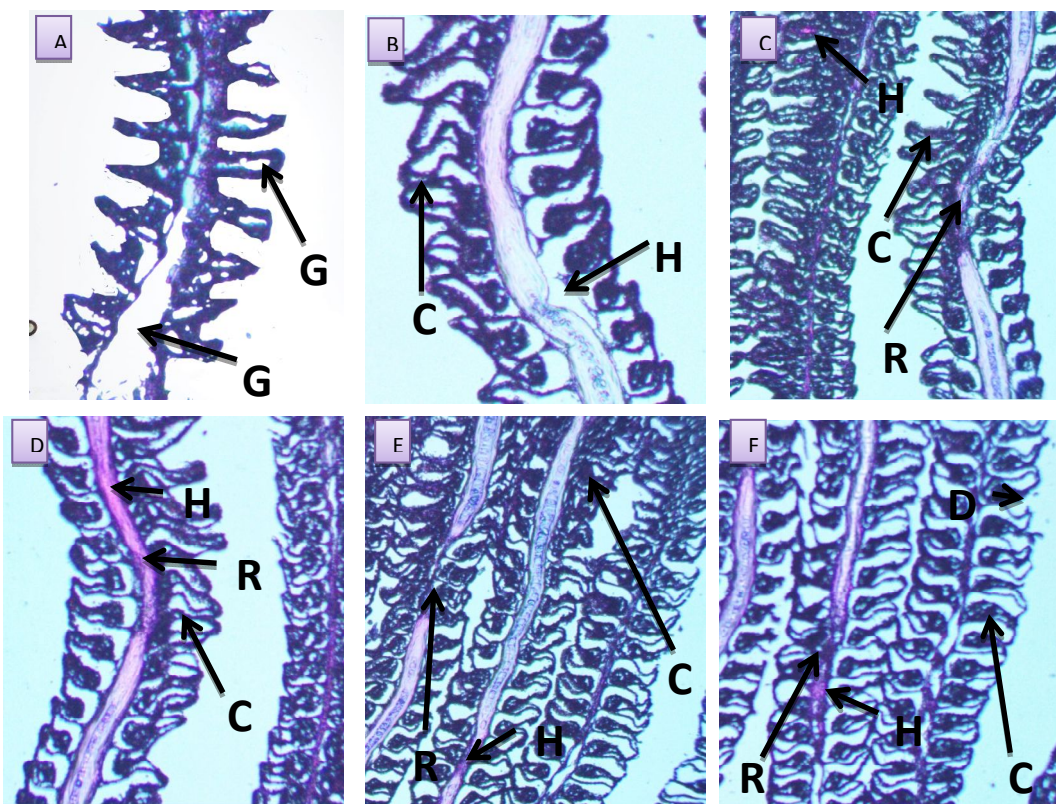


Fig. 2. Histopathological alterations observed in Banded Gourami *T. fasciata* exposed to thiamethoxam pesticide on 60 day, during the experimental period. (A) Almost normal structure of structure of gill lamellae (GL) and gill filaments (GF) after 60 days exposure to 0 mg/L (control gill); (B) clubbing (C) and hypertrophy (HY) after 60 days exposure to 9.375 mg/L ; (C) clubbing (C), haemorrhage (H) and reduction of gill filaments (RF) after 60 days exposure to 18.75 mg/L; (D) clubbing (C), reduction of gill filaments (RF) and haemorrhage (H) after 60 days exposure to 37.5 mg/L; (E) clubbing (C), reduction of gill filaments (RF) and haemorrhage (H) after 60 days exposure to 75 mg/L; (F) clubbing (C), damage of gill raker (DR), reduction of gill filaments (RF) and haemorrhage (H) after 60 days exposure to 150 mg/L; H and E stain $\times 400$.

The value of dissolved oxygen (mg/L), pH, and temperature were presented in Table 1. Dissolved oxygen (mg/L) values were found to be significantly reduced in all the pesticide treated groups compared the control group. The reduction of DO level at two higher pesticide exposed groups (75 and 150 mg/L) were significantly significant. Whereas, the values of pH and temperature were almost unchanged throughout the study period (Table I).

Table I. Water quality parameters (mean \pm SD) during the experimental period*

Parameters	Treatments (mg/L)	Sampling Days			
		Day-15	Day-30	Day-45	Day-60
DO (mg/L)	0	6.26 \pm 0.61 ^b	7.19 \pm 0.5 ^c	6.19 \pm 0.5 ^b	5.85 \pm 0.5 ^a
	9.37	7.19 \pm 0.5 ^c	6.35 \pm 0.35 ^b	5.33 \pm 0.65 ^b	4.68 \pm 0.41 ^a
	18.75	7.85 \pm 0.84 ^c	6.19 \pm 0.84 ^b	5.12 \pm 0.31 ^b	4.26 \pm 0.44 ^a
	37.5	5.97 \pm 0.55 ^b	5.33 \pm 0.31 ^b	5.47 \pm 0.36 ^b	4.67 \pm 0.65 ^a
	75	5.12 \pm 0.61 ^b	4.40 \pm 0.45 ^a	4.55 \pm 0.69 ^a	4.76 \pm 0.40 ^a
	150	5.10 \pm 0.61 ^c	4.15 \pm 0.61 ^b	4.02 \pm 0.61 ^b	3.68 \pm 0.61 ^a
pH	0	8.15 \pm 0.40	7.5 \pm 0.40	7.11 \pm 0.40	7.29 \pm 0.40
	9.37	7.64 \pm 0.20	8.09 \pm 0.26	7.44 \pm 0.42	7.81 \pm 0.32
	18.75	7.59 \pm 0.39	7.66 \pm 0.21	7.71 \pm 8.1	7.77 \pm 0.17
	37.5	7.57 \pm 0.32	7.63 \pm 0.54	7.69 \pm 7.63	7.77 \pm 0.32
	75	7.67 \pm 0.39	7.75 \pm 0.22	7.82 \pm 0.45	7.88 \pm 0.22
	150	7.17 \pm 0.39	7.37 \pm 0.39	7.43 \pm 0.39	7.50 \pm 0.39
Temperature (°C)	0	28.18 \pm 7.20	27.58 \pm 7.20	28.38 \pm 7.20	28.50 \pm 7.20
	9.37	29.35 \pm 8.2	29.22 \pm 8.2	29.36 \pm 8.7	29.82 \pm 6.59
	18.75	29.12 \pm 7.31	29.16 \pm 7.36	29.21 \pm 0.62	29.63 \pm 8.30
	37.5	28.22 \pm 7.51	29.36 \pm 7.15	29.55 \pm 0.63	29.87 \pm 7.57
	75	29.36 \pm 7.65	28.13 \pm 7.36	29.54 \pm 8.20	28.33 \pm 7.39
	150	29.16 \pm 7.65	29.56 \pm 7.65	29.06 \pm 7.65	29.09 \pm 7.65

*Values with different alphabetical superscripts in a row differ significantly ($p < 0.05$) among different treatments. All values were expressed as mean \pm SD.

Discussion

Histopathological observation is a sensitive bio-monitoring tool to indicate the effect of toxicants on fish in pesticides polluted aquatic ecosystems (Marchand *et al.* 2009). Pesticides in polluted aquatic ecosystem are accumulated in fish mainly through metabolically active tissues such as gill, liver, kidney so on (Oruce and Usta 2007). Several histopathological changes were observed in the gill tissues of Banded Gourami, *T. fasciata* exposed to different sub-lethal concentrations of THM agro-pesticide for a period of 60 days. Histopathological alterations include clubbing, reduction of gill filaments, telangiectasis of gill lamellae, haemorrhage and damage of gill raker. Several investigators have reported that the histopathological changes were recorded in gills of different fish species exposed to various pesticides (Cengiz and Unlu 2002, 2003; Cengiz 2006). Mucus extrusion, lamellar swelling, fused and reduced microridges, were observed in bluegill sunfish, *Lepomis macrochirus* when exposed to different sub lethal concentrations of diazinon (Dutta *et al.* 1997). Histopathological effects of sub lethal concentrations of monocrotophos on the gills of *Anabas testudineus* was reported by Santhakumar *et al.* (2001). The observed histopathological changes were hemorrhage in the primary and secondary gill lamellae, degeneration and necrosis of epithelial cells, distortion of the secondary lamellae, and disruption of epithelial cells from pillar cells. Shorter gill lamellae, fusion, complete destruction of lamella, increased vacuolation, irregular appearance of gill lamellae were observed in guppy *Poecilia reticulata* exposed to chlorpyrifos (De Silva and Samayawardhena 2002). Degenerative changes in gill, such as intraepithelial edema in the secondary lamellae, thick coating of mucus covering the entire gill filaments and lamellae, erosion of secondary lamellae, thickening of lamellae, inflammation of epithelial cells,

breakages in primary lamellae, degeneration of secondary lamellae, necrosis, rupture of epithelium were noticed during exposure of sub lethal concentrations of monocrotophos by Rao *et al.* (2005). Rao *et al.* (2005) observed uncontrolled regeneration of the primary lamellae and secondary lamellae, hypertrophy, hyperplasia, necrosis of the epithelial cells, epithelial lifting, and dilation of the blood sinuses of the secondary lamellae, lamellar aneurism, and hemorrhages in the gill of fish exposed to profenofos. Mukti *et al.* (2018) reported that female Banded Gourami exposed to different sub-lethal concentrations of chlorpyrifos resulted in hemorrhage, hypertrophy, necrosis, pyknosis, vacuums, splitted gill lamellae and missing of gill lamellae. All of the findings cited above are supportive to the present research. Among the water quality parameters, DO levels were found to be significantly low in all the treatments compared to control. Reduced DO levels in the higher pesticide concentrations made fishes become stressed and might have hampered the respiratory system resulting in the damaged of gills lamellae. On the other hand, no noticeable changes were observed between control and treatments in case of pH and temperature.

In conclusion, the results of the present study revealed that a neonicotinoid crop insecticide like thiamethoxam can cause histopathological alterations in gills of an indigenous freshwater fish Banded Gourami. Therefore, indiscriminate use of thiamethoxam to agriculture should be restricted.

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