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PROOF
RESEARCH PAPER

# Histopathological Alterations in Liver and Kidney Tissues of Banded Gourami (*Trichogaster fasciata*) Exposed to Thiamethoxam

**Md. Mohibul Hasan[1,#], Md. Helal Uddin[1,#], Md. Jakiul Islam[2], Shema Biswas[1], Kizar Ahmed Sumon[1], Mohammad Dalower Hossain Prodhan[3], Harunur Rashid[1,*]**

[1]Bangladesh Agricultural University, Department of Fisheries Management, Mymensingh-2202, Bangladesh.
[2]Sylhet Agricultural University, Faculty of Fisheries, Sylhet-3100, Bangladesh.
[3]Bangladesh Agricultural Research Institute (BARI), Entomology Division, Pesticide Research & Environmental Toxicology Section, Gazipur-1701, Bangladesh.
[#]These authors share the first authorship.

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**Corresponding Author**
Tel.: +8801924429971
E-mail: rashid@bau.edu.bd

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## Abstract

Thiamethoxam is a broad-spectrum pesticide used in Bangladesh to protect crops from pests. In several ways, this pesticide ends up in aquatic ecosystems. Researchers have detected its existence in waterbodies. However, the harmful effects on non-target aquatic residents are not well studied and reported. As a result, the study sought to investigate the effect of thiamethoxam on the liver and kidney tissues of banded gourami (*Trichogaster fasciata*) using histopathological observations. Fish were exposed to five sub-lethal concentrations (in triplicates) of thiamethoxam (9.37, 18.75, 37.5, 75, and 150 mg/L) and 0 mg/L (control) for 90 days. Fish livers and kidneys were sampled on days 30, 60, and 90 of thiamethoxam exposure. The histopathological changes observed in thiamethoxam exposed fish were autolysis, acute cellular swelling, vacuolation, necrosis, and fatty change in the liver, whereas irregular renal corpuscle and cellular degradation of tissue were also observed in the kidney. The present study reveals that thiamethoxam can affect the livers and kidneys of Banded Gourami; therefore, the usage of this insecticide in agriculture should be applied carefully and monitored to avoid entry into water bodies.

## Introduction

Agriculture is an important sector in Bangladesh, contributing to supplying the country's ever-expanding food requirements. Bangladesh's government has advocated the use of pesticides to promote agricultural production to meet the country's increasing food demand since the 1980s (Dasgupta et al., 2007). Pesticide usage in Bangladesh was fairly low until the 1960s. However, it abruptly increased by about 7 times between 1992 and 2010 (Rahman, 2013; Ali et al., 2018).

These agro-pesticides can enter aquatic habitats in a variety of ways, including spray drift, runoff, and leaching (Shahjahan et al., 2017). The discharge of these compounds into the aquatic environment might have an impact on non-target aquatic invertebrates (Rubach et al., 2011; 2012; Sumon et al., 2018), and vertebrates (Mishra and Mohanty, 2008; Mohammed, 2009; Saeedi et al., 2012; Satyavardhan, 2013; Manjunatha and Philip, 2015; Sumon et al., 2017; 2018; 2019). The widespread use of systemic insecticides in agriculture results firstly in contamination of the soil of the treated crops, and secondly in the transfer of residues to the aquatic environment (Bayo et al., 2016). More generally, pesticides reduce species diversity in the animal kingdom and contribute to population decline in animals and plants by destroying habitats, reducing food supplies, and impairing reproduction (Shefali et al., 2020; Zahid et al., 2019).

Thiamethoxam (THM) is one of the most widely used pesticides in Bangladesh to control whiteflies, aphids, and lepidoptera (Al-Emran et al., 2020; Lewis et al., 2016). This insecticide is considered a second-generation neonicotinoid insecticide, having a half-life of 385-408 days in water and 6-3001 days in soil (Borsuah et al., 2020). THM has been reported to be hazardous to many aquatic creatures, including fish and invertebrates, due to its poor soil retention, high leaching capacity, high solubility in water, and resistance to biological treatment (Cohen et al., 1995; Satyavardhan, 2013; Ullah et al., 2014; Rani and Kumaraguru, 2014; Morrissey et al., 2015; Uğurlu et al., 2015; Saraiva et al., 2017).

Among vertebrates, fish are regarded as an excellent bio-indicator (Gupta et al., 2009; Narra et al., 2011; Correia et al., 2017). Banded gourami, *Trichogaster fasciata* has been used as a model organism for ecotoxicological research (Sumon et al., 2017; 2019). This benthopelagic fish favors weedy settings like estuaries, ponds, major rivers, ditches, and lakes; and is endemic to Bangladesh, India, Myanmar, Nepal, and Pakistan (Mitra et al., 2007). This fish has become a prominent model for aquatic stress studies due to its short generation, ease of acclimation, and captive breeding characteristics under laboratory conditions (Akter et al., 2016; Islam et al., 2017; Mitra et al., 2007).

In fish, the kidney is responsible for electrolyte and water balance, as well as the preservation of stable internal homeostasis (Hyodo et al., 2014). The liver is thought to be a significant location for xenobiotic storage, biotransformation, and excretion (Dutta et al., 1993). Histopathological investigation of the liver and kidney has been acknowledged as an excellent tool for studying the acute toxic effects of agro-pesticides on fish, as well as essential indicators of physiological stress caused by any anthropogenic stressor (Hossain et al., 2016; Islam et al., 2019; Sumon et al., 2019; Reza et al,. 2020). There was plenty of fish and rice that make Bengali in Bangladesh. In order to boost crop production to feed the rising human population, the indiscriminate applications of agro-pesticides are increasing tremendously. The bioavailability of these pesticides is increased at an alarming rate in the open water environment and caused harm to fish. Therefore, the objectives of the present research work were to determine the effects of THM on vital internal organs like the liver and kidney of an important freshwater fish banded gourami (*T. fasciata*) and to observe the abnormalities and deformities in these organs exposed to different sub-lethal concentrations of THM.

## Materials and Methods

### Procurement of Tested Fish

Sexually matured, healthy, and disease-free *T. fasciata* (n=250, body weight: 8.12±0.34 g and length: 9.2±0.8 cm) were obtained from the Bangladesh Fisheries Research Institute (BFRI), Mymensingh, Bangladesh. Fish were transferred in well-oxygenated polythene bags to the Wet Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, where they were housed in a cement cistern with a constant water supply. For acclimation, fish were reared under natural photoperiod for 21 days. Fish were fed twice a day with commercial floating feed (32% protein; Mega Fish Feed Ltd. Bangladesh) up to the satiation level. The experiment was approved by the Animal Care and Use Committee of Bangladesh Agricultural University, Bangladesh.

### Pesticide

Commercial grade THM (CAS number: 153719-23-4; trade name: spike 25WG; manufacturer: Syngenta) was purchased from an authorized dealer at Shambhugonj Market, Mymensingh-2202, Bangladesh.

### Experimental Design

After 21 days of acclimatization, based on a previously estimated 96-h LC50 (161.06mg/L) for Banded Gourami (Hasan et al., 2021), fish were exposed to five sub-lethal concentrations of THM (9.37, 18.75, 37.5, 75 and 150mg/L) and control (0 mg/L) in triplicates. A total of 180 individuals were allocated to eighteen 100 L glass aquaria (10 fish per aquarium). Aquaria was filled with dechlorinated tap water and equipped with aerators. Fish were fed twice a day with commercial feed Mega Fish Feed, Bangladesh) up to satiation. Every day, unutilized feedstuffs and fecal wastes were cleansed by siphoning. Around 80% of water was exchanged every 15 days with previously prepared water with appropriate pesticide dosages. On days 30, 60, and 90 of THM exposure, three fish were sampled from each aquarium to collect liver and kidney samples. Samples were immediately fixed in Bouin's fluid for histopathological observation later. Water quality parameters (viz. DO, temperature, pH, and total alkalinity) were measured during the experimental period and found within permissible limits as per the recommended value of APHA and American Public Health (APHA, 1998).

### Histological Procedures

The fixed liver and kidney tissues were dehydrated in a graded alcohol series, cleaned with xylene, and embedded in paraffin. Embedded tissues were sectioned at a thickness of 5 µm with a microtome machine (HM 430; Thermo-Scientific). The tissue slices were arranged on slides like ribbons and stained with standard Haematoxylene-Eosin (H-E) protocol before being mounted with DPX and a coverslip. Finally, stained slides were examined under a microscope to identify histological abnormalities in liver and kidney tissues (Uddin et al., 2022; Bell et al., 2000).

**Semi-quantitative Analysis**

Following Mishra and Mohanty (2008), semi-quantitative histopathological alterations were observed. The histopathological changes in the tissues were investigated as the mean of ten randomly selected slides from a total of fifty specimens for each organ. The mean prevalence of each histopathological parameter was classified as follows: no abnormalities (–, 0% of sections), mild abnormalities (+, 10% of sections), moderate abnormalities (++, 10% to 50% of sections), and severe abnormalities (+++, > 50% of sections).

## Results

**Changes in Liver Tissue**

Livers of THM-exposed fish displayed a range of histopathological changes that were dosage and exposure dependent. The structural features of the liver of a Banded Gourami subjected to 0 mg/l of THM (control) are illustrated in Figure 1A. The control fish liver had normal hepatocytes hexagonal, hepatic portal vein branch with erythrocytes, capillaries, Kupffer cells, and well-spaced sinusoids (Figure 1A). Whereas several histopathological changes were observed in the liver of THM exposed groups like autolysis (A), acute cellular swelling (ACS), fatty change (FC), vacuolation (V), and necrosis (N) during the experiment (Figure 1B-F). The severity of observed abnormality frequencies of the THM-exposed fish increased with the increased concentrations and exposure duration (Table 1).

**Changes in Kidney Tissue**

The kidney tissues of the control group showed normal glomerulus and tubules with well-defined Bowman's space, blood vessel (BV), well-defined renal tubules (RT), and well-defined renal corpuscle (RC) with nucleus (Figure 2A). Several abnormalities in kidney tissues were observed after THM exposure [e.g., cellular degradation (CD), necrosis (N), acute cellular swelling (ACS), and irregular renal tubule (IRT)]. (Figure 2B-F). The frequency of histological changes in the Banded Gourami kidney increased with increasing THM concentrations and exposure duration (Table 2).

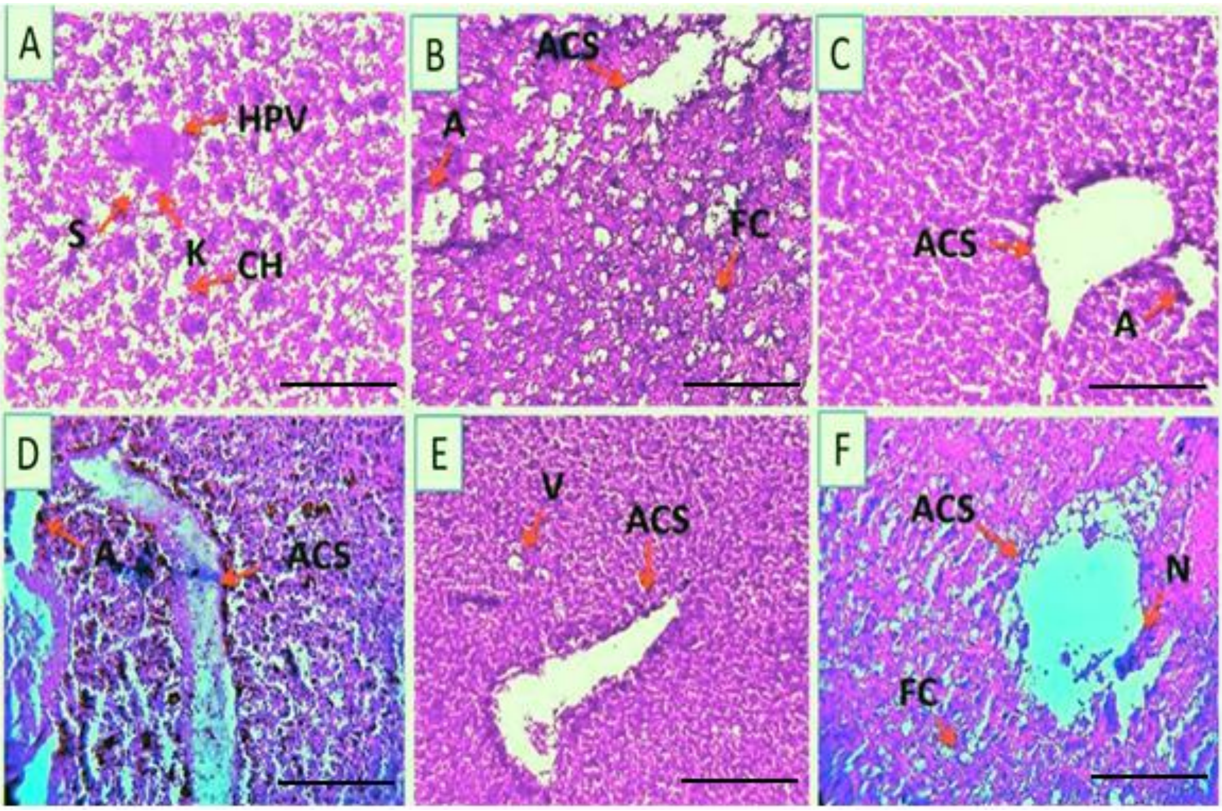


**Figure 1.** Histopathological changes in the liver of Banded Gourami, *T. fasciata* subjected to thiamethoxam exposure for 90 days. A. After 90 days of exposure to 0 mg/L (control), hepatocytes in typical hexagonal form, hepatic portal vein branch containing erythrocytes, capillaries, Kupffer cells, and well-spaced sinusoids; B. autolysis (A), acute cellular swelling (ACS), and fatty change (FC) after 60 days exposure to 37.5 mg/L; C. Acute cellular swelling (ACS), and autolysis (A) after 90 days exposure to 18.75 mg/L; D. Vacuolation (V) and acute cellular swelling (ACS) after 30 days exposure to 75 mg/L; E. Necrosis (N), fatty change (FC) and F. acute cellular swelling (ACS) after 90 days exposure to 150 mg/L. magnification (40x); scale bars (100µm).

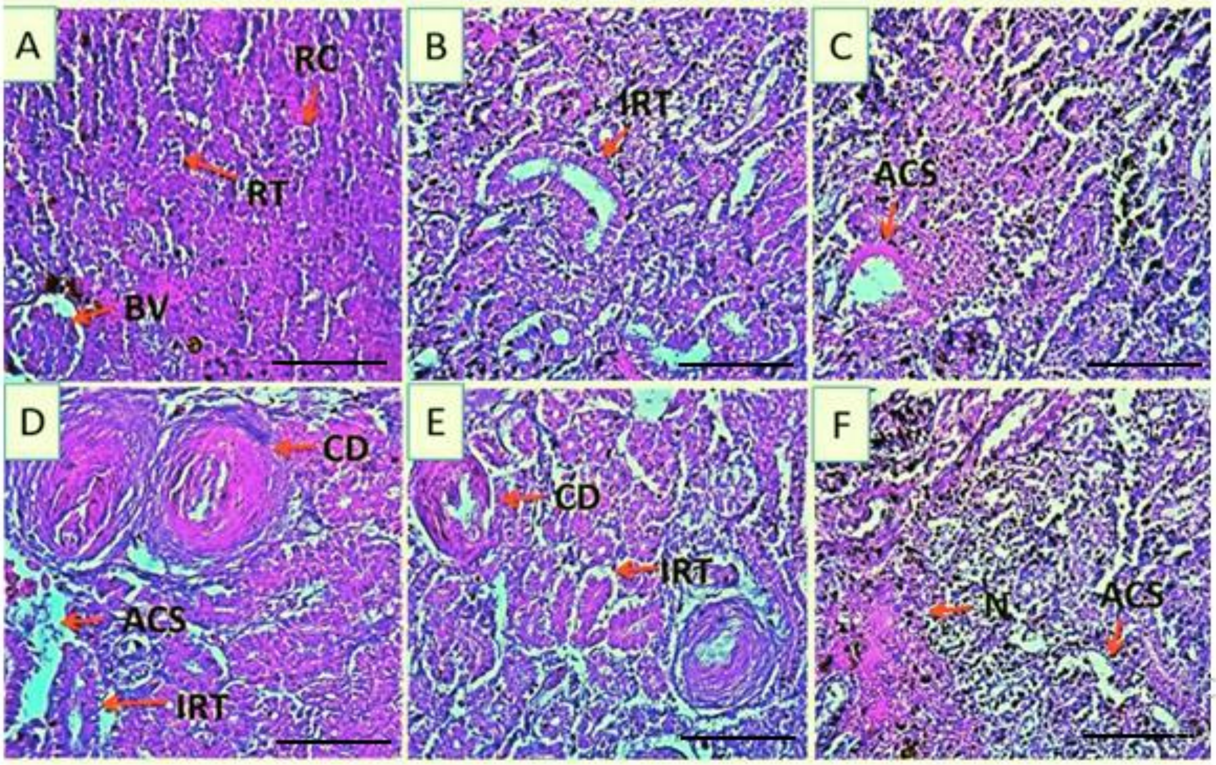


**Figure 2.** Histopathological changes in the kidney of Banded Gourami, *T. fasciata* subjected to thiamethoxam exposure for 90 days. A. normal structure of renal corpuscle (RC), renal tubule (RT), and blood vessel (BV) after 90 days exposure to 0 mg/L (control); B. irregular renal corpuscle (IRT) after 30 days exposure to 37.5 mg/L; C. Acute cellular swelling (ACS) after 60 days exposure to 18.75 mg/L; D. Cellular degradation of tissue (CD), acute cellular swelling (ACS), and irregular renal corpuscle (IRT) after 60 days exposure to 75 mg/L; E. Cellular degradation of tissue (CD), and irregular renal corpuscle (IRT) after 60 days exposure to 37.5 mg/L; F. Necrosis (N), acute cellular swelling (ACS) after 90 days exposure to 150 mg/L. magnification (40x); scale bars (100µm).

**Table 1.** Histopathological alterations in the liver of Banded Gourami, *Trichogaster fasciata* subjected to exposure at various thiamethoxam concentrations

| Alterations | Thiamethoxam concentrations (mg/L) | Exposure time (days) | | |
|---|---|---|---|---|
| | | 30 | 60 | 90 |
| Fatty Change (FC) | 0 | – | – | – |
| | 9.37 | – | – | + |
| | 18.75 | – | – | ++ |
| | 37.5 | – | ++ | ++ |
| | 75 | + | + | ++ |
| | 150 | + | + | +++ |
| Vacuolation (V) | 0 | – | – | – |
| | 9.37 | – | – | – |
| | 18.75 | – | – | ++ |
| | 37.5 | – | + | ++ |
| | 75 | ++ | ++ | ++ |
| | 150 | ++ | ++ | ++ |
| Necrosis (N) | 0 | – | – | – |
| | 9.37 | - | - | + |
| | 18.75 | - | + | ++ |
| | 37.5 | + | + | ++ |
| | 75 | ++ | ++ | +++ |
| | 150 | +++ | +++ | +++ |
| Acute Cellular Swelling (ACS) | 0 | – | – | – |
| | 9.37 | + | + | ++ |
| | 18.75 | ++ | +++ | +++ |
| | 37.5 | +++ | +++ | +++ |
| | 75 | +++ | +++ | +++ |
| | 150 | +++ | +++ | +++ |
| Autolysis (A) | 0 | – | – | – |
| | 9.37 | + | + | + |
| | 18.75 | + | + | ++ |
| | 37.5 | ++ | ++ | +++ |
| | 75 | ++ | +++ | +++ |
| | 150 | +++ | +++ | +++ |

(–, none (0%); +, mild (< 10%); ++, moderate (10 to 50%); +++, severe (>50%).q

**Table 2.** Histopathological alterations in the kidney of Banded Gourami, *Trichogaster fasciata* subjected to exposure at various thiamethoxam concentrations

| Alterations | Thiamethoxam concentrations (mg/L) | Exposure time (days) | | |
|---|---|---|---|---|
| | | 30 | 60 | 90 |
| Cellular Degradation (CD) | 0 | – | – | – |
| | 9.37 | – | + | + |
| | 18.75 | + | ++ | ++ |
| | 37.5 | ++ | ++ | +++ |
| | 75 | + | +++ | +++ |
| | 150 | ++ | +++ | +++ |
| Irregular Renal Tubule (IRT) | 0 | – | – | – |
| | 9.37 | – | – | + |
| | 18.75 | – | – | ++ |
| | 37.5 | + | + | ++ |
| | 75 | ++ | ++ | +++ |
| | 150 | ++ | ++ | +++ |
| Necrosis (N) | 0 | – | – | – |
| | 9.37 | – | + | + |
| | 18.75 | + | + | ++ |
| | 37.5 | ++ | ++ | ++ |
| | 75 | ++ | ++ | ++ |
| | 150 | ++ | ++ | +++ |
| Acute Cellular Swelling (ACS) | 0 | – | – | – |
| | 9.37 | + | + | ++ |
| | 18.75 | ++ | +++ | +++ |
| | 37.5 | +++ | +++ | +++ |
| | 75 | +++ | +++ | +++ |
| | 150 | +++ | +++ | +++ |

(–, none (0%); +, mild (< 10%); ++, moderate (10 to 50%); +++, severe (>50%).

## Discussion

Histopathological examination is a sensitive bio-monitoring method for determining the impact of toxicants on fish (Marchand et al., 2009; Panseri et al., 2019; Vieira et al., 2019). Primarily, pesticides accumulate in metabolically active tissues (e.g., gills, liver, kidney, and muscle) of fish residing in polluted aquatic ecosystems (Oruce and Usta, 2007). The liver is the primary organ for xenobiotic detoxification (Dutta et al., 1993), and in pesticide-exposed fish, it undergoes significant structural changes (Özaslan et al., 2018; Kaur and Mishra, 2019). Because of its massive blood supply and involvement in metabolism, the liver is especially vulnerable to these harmful substances (Roganovic-Zafirova and Jordanova, 1998). The current study found that after THM exposure, the liver of Banded Gourami exhibits degenerative changes such as autolysis, acute cellular swelling, fatty change, vacuolation, and necrosis, which are comparable to those shown in previous studies on other fish subjected to different toxicants. When exposed to malathion, Magar and Shaikh (2013) observed comparable abnormalities (cytoplasm degradation, clumping, and vacuolization of hepatocytes) in the liver of snakehead, *Channa punctatus*.

In *Clarias gariepinus* subjected to chlorpyrifos-ethyl, Ogueji et al. (2013) noticed degenerative alterations such as sinusoidal spaces, extensive infiltration of sinusoidal spaces, and central vein, pyknotic nuclei, necrosis, and coagulation. Vijayakumar (2013) observed degeneration in hepatopancreatic tissue, blood cells among hepatocytes, the appearance of blood streaks in the parenchyma, the formation of vacuoles along with atrophy, severe necrotic patches, and the disappearance of the cell membrane and hepatic cords in the liver of snakehead *C. punctatus*. Sarkar et al. (2005) found vacuolation, hyperplasia, disruption of hepatocytes, localized necrosis, and disorganization of hepatic canaliculi in Rohu, *Labeo rohita* after cypermethrin exposure. Anomalies such as hepatocyte enlargement and blood vessel infiltration were seen in the liver of *L. rohita* after exposure to hexachlorocyclohexane (Das and Mukherjee, 2000) and fenvalerate (Susan et al., 2012). Similar histological alterations were seen in the liver tissue of tilapia, *Oreochromis niloticus,* and European common carp, *Cyprinus carpio* subjected to sub-lethal carbaryl and cyfluthrin concentrations, respectively (Matos et al., 2007; Sepici-Dinc¸el et al., 2009).

In the current study, these changes in the morphological structure of the liver might be coupled with a disruption in tissue function, which could potentially be related to the lower antioxidant capability in THM-treated fish. Cellular swelling can occur for one of two reasons: it can occur directly owing to the denaturation of volume-regulating ATPases, or it can occur indirectly due to disruption of the cellular energy transfer pathways necessary for ionic control (Hinton and Lauren, 1990). Oxidative stress caused by lipid peroxidation is an obvious source of membrane bilayer vulnerability, which leads to cell demise (Li et al., 2000;

Avci et al., 2005). Several authors have previously documented the appearance of vacuoles in the hepatocytes of fish subjected to chemical pollutants such as insecticides (Khan et al., 2021; Dong et al., 2017; Magar and Afsar, 2013). These effects were thought to be caused by tissue hypoxia (Matton and LaHam, 1969).

Similarly, in the present study, necrosis, melanomacrophage center, and hepatocyte enlargement were seen with increasing THM concentration, indicating the acute toxicity of pesticides on hepatocytes. Because the liver is the primary organ in all xenobiotic detoxification routes, the observed alterations in hematopoietic tissues might be attributed to the direct lethal effects of the toxin on hepatocytes. Sublethal stages of the xenobiotic methyl parathion (folidol 600) were shown to be responsible for localized necrosis and hazy edema of the liver in *Corydoras paleatus* (Fanta et al., 2003). In reaction to atrazine poisoning, lipid droplets and vacuolization were seen in the enlarged liver of *Liza ramada*. (Biagianti-Risbourg and Bastide, 1995).

THM-exposed fish's kidneys exhibited cellular deterioration, necrosis, acute cellular swelling, and abnormal renal tubules when compared to controls. Moreover, similar findings have been reported for *Heterobranchus bidorsalis* exposed to various doses of cypermethrin (Olufayo and Alade, 2012), *L. rohita* exposed to organochlorine pesticide endosulfan (Indirabai et al., 2010), *Cirrhinus mrigala* exposed to pyrethroid derivative cypermethrin (Prashanth, 2011). According to Gupta et al., (2016) glomerulosclerosis in the kidney is caused by renal tubule degeneration, necrosis of hematopoietic tissue, vacuolation, the presence of sinusoidal space, tubular necrosis, damage to the epithelial cells of the renal tubules, and a rise in the Bowman's space. Sharmin et al (2021) reported that the toxicological effects of sumithion on Nile tilapia (*Oreochromis niloticus*) resulted in patch degeneration, vacuolation, and intense form of pyknotic nuclei in the kidney. Haque et al. (2017) observed vacuolation, necrosis, cellular degeneration, karyolysis, and rupturing of renal tubules as histopathological changes within the kidney tissues of *Mystus tengara* due to cypermethrin assault. *Labeo rohita* exposed to pyriproxyfen (PPF), a well-known synthetic substance, resulted in necrosis, edema, widening of Bowman's space, and necrosis of tubules in the kidney (Naseem et al. 2022). All of the above-mentioned findings positively supported the current research output.

The kidney's renal tubules are the first to be harmed by pesticides, which cause hazy swelling of the tubules (Singh, 2012; Das and Mukherjee, 2000). As a result, greater dosages of THM exposure resulted in increased diameter of renal tubes, glomerular enlargement, and patch degradation. Nuclear alterations are caused by damage and necrosis caused by pollutant(s) accumulation; resulting in the condensation of nuclear material to generate darkly colored pyknotic nuclei (Velmurugan et al., 2007). The degradation of the glomerulus and glomerular enlargement in acute exposure was seen in the current investigation and can be interpreted as signs of intoxication (Cengiz, 2006). THM at lethal concentrations induced significant histological damage to the organs investigated in this study.

## Conclusions

The freshwater Gourami, *Trichogaster fasciata*, was exposed to various concentrations of THM (a highly toxic synthetic pesticide with a fatal dosage (LC50) of 161.06 mg/L) that resulted in different histopathological alterations in the liver and kidney tissues which hampered the growth and reproductive performance which ultimately reduced the biodiversity of the test fish in the open waters. As a result, THM, which is utilized in aquaculture as a treatment and disinfection, should be handled with extreme caution. Thus present study helps to improve the understanding of local people and communities about the impacts of different pesticides on aquatic ecosystems and fish health and their use should be closely monitored.

## Ethical Statement

The experimental protocol was approved by the Ethics Committee of Bangladesh Agricultural University, Mymensingh, Bangladesh (2018/696/MOE/BAURES).

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## Author Contribution

Md Helal Uddin & Md. Mohibul Hasan: Conceptualization, Methodology, Writing-Original Draft Preparation; Shema Biswas: Experimentation, Methodology and Data Curation; Md Jakiul Islam & Kizar Ahmed Sumon: Resources and Analysis; Mohammad Dalower Hossain Prodhan: Investigation and Visualization; Harunur Rashid: Supervision, Review and Editing.

## Conflict of Interest

The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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