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RESEARCH PAPER

Effects of Pyrethroid Pesticide Cypermethrin on the Gonad and Hemato-biochemical Parameters of Female Gangetic Mystus (*Mystus cavasius*)

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Abstract

A 90-day experiment was conducted to study the effect of sub-lethal concentration of cypermethrin exposure on mortality, Gonadosomatic index (GSI), ovarian cell development and hemato-biochemical parameters of Gangetic mystus (*Mystus cavasius*). According to previous findings of LC50 value (30µg/L) of cypermethrin the experiment was carried out using four sub-lethal concentrations as treatment (0.0, 4.0, 8.0, and 16.0µg/L). The whole water was exchanged in every 4th day and pesticide was mixed accordingly. For collection of gonad and blood, fish were sampled at 30, 60 and 90 days after starting of pesticide exposure. There were no significant changes on GSI. Several histopathological changes were observed in ovary. Wrinkle oocyte, cytoplasmic clumping, atretic follicle, degenerated granulose layer, degenerated oocyte wall, increased inter follicular space, adhesion, cyst, necrosis were found in the ovary of *M. cavasius* with increasing cypermethrin concentration and exposure time. Blood glucose level and WBCs were significantly increased whereas blood hemoglobin and RBCs were significantly decreased in exposure groups compared to control groups over the three sampling days. The current findings revealed that cypermethrin had negative impact on the hemato-biochemical parameters and female gonad of *M. cavasius*. So the use of cypermethrin in agriculture should be done with a great caution.

Introduction

Bangladesh is a densely populated country with high population growth rate. 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 use 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 our environment polluted (Sumon et al., 2016). 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). 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, 2016; Sumon et al., 2017). Considering the importance of fish as food as well as having long life span and ability to response against environmental xenobiotics treated as a good bio-indicator among vertebrates (Gupta et al., 2009; Narra et al., 2011; Correia et al., 2017). There are four pesticide groups among them cypermethrin belongs to pyrethroid having a good commercial agricultural applications as it

considered a fast acting neurotoxin in insects. According to the National Pesticides Telecommunications Network (NPTN) cypermethrin is very much toxic to fish, bees and other aquatic insects. It is easily degraded on soil and plant and can be effective on water up to 30 days. Decomposition of this pesticide will stimulate when exposure to sun light, water and oxygen.

Pesticides are found in different types of water bodies from lower to higher concentrations and resulted to be toxic for aquatic organisms especially for fish (Banaee et al., 2008). The impacts of this type of pesticide are the occurrences of muscle convulsion along with loss of balance of Na and K in neuron and finally stopped the activities of central nervous system (Sachsen and Sultana, 1999). 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, Hb, WBCs, RBCs etc) as well as histo- architectural changes in some important internal organs like liver, kidney, intestine etc. (Saeedi et al., 2012; Salam et al., 2015; Sharmin et al., 2015). Cypermethrin may also affect the reproductive system of fish which is an important consideration for evaluating the impacts of pesticide chemicals (De Silva and Samayawardhena, 2005).

The effects of pesticide pollution on non-target organisms (e.g. fish) can be measured by detecting changes in organisms at the physiological, biochemical or molecular levels, which can provide 'early warning' tools in monitoring environment quality (Crane and Maltby, 1991; Miren et al., 2000). These sensitive early warning biomarkers can measure interaction between environmental xenobiotics and biological effects. Hematological parameters are commonly used as a significant index for measuring the health condition of the fish (Ruane et al., 2000; Okomoda et al., 2013). The physical and chemical changes of water may be expressed in the blood components of fish (Wilson and Taylor, 1993). Blood parameters can provide a lot of information about physiological response of fish to environmental changes that affect homeostasis (Lohner et al., 2001; Cazenave et al., 2005; Elahee and Bhagwant, 2007; Sharmin et al., 2015; Salam et al., 2015). The analysis of haematological and biochemical parameters in fish can contribute to the assessment of the animal's health and also the habitat conditions (Thrall, 2004).

There are about 260 freshwater fish species in Bangladesh among them 143 is considered as small indigenous fish species (SIS) having maximum size in length less than 25cm. Gangetic mystus is a popular SIS in Bangladesh due to its taste and nutritional value and also found in Nepal, India, Pakistan, Sri Lanka and West Africa (Yadav, 1997). Rivers, lakes, canals, floodplains, swamps, ponds and ditches are considered as natural habitat for feeding and breeding of this fish species (Talwar and Jhingran, 1991). Gangetic mystus is locally known as gulsa. There are a number of researches conducted all over the world assessing the toxicity of cypermethrin on the reproduction and haematological parameters of different fishes but not yet on Gangetic mystus. Therefore, the present study aimed at assessing the toxicity of cypermethrin on the reproductive organs along with hemato-biochemical parameters of *M. cavasius*.

Materials and Methods

Experimental Fishes

Healthy and living female Gangetic mystus was procured from a local reputed fish farm and disinfected in 0.1% solution of potassium permanganate for 5 minutes to avoid dermal infection. They were reared in an indoor cemented rectangular cistern with continuous aeration for three months at wet laboratory, Bangladesh Agricultural University. The experiment was conducted under natural light and ambient temperature condition. Due to nocturnal behavior fish were fed at evening at a rate of 4%/kg body weight. The experiment was approved by the Animal Care and Use Committee of Bangladesh Agricultural University, Mymensingh, Bangladesh.

Pesticide

Technical grade synthetic pyrethroid (cypermethrin) 10% EC active ingredient manufactured by Hayleys PLC, Sri Lanka was purchased from a local registered pesticide seller (Mymensingh, Bangladesh).

Experimental Design

Total 240 mature female *Mystus cavasius* (length=13.2±0.72 cm; weight=15.71±1.09 g) were selected for the experiment. Previously prepared 12 PVC tanks having 100L de- chlorinated tapwater with aeration facilities were used to ensure sufficient oxygen supply during the experimental period of 90 days. Twenty female fish were stocked in each of the PVC tank and acclimatized at laboratory condition for a period of 21 days prior to pesticide exposure. Fish were exposed to four different concentrations of cypermethrin (0, 4.0, 8.0 and 16.0 µg/L; the 96-h LC50 in this study was 30 µg/L) for 30, 60 and 90 days. The control and treatments were set up in three replicates. Water (about 80%) in the experimental units was changed every three days and fresh cypermethrin concentrations were used.

Gonads Collection and GSI Estimation

To prevent suffering during gonad collection test fish were anesthetized using clove oil (5mg/l) on days-30, 60 and 90 after the first cypermethrin exposure. Three fish were sacrificed from each replication at each sampling day. Immediately after collection of ovary electric balance was used to weight and rinsed with

physiological solution (0.9% sodium chloride) and transferred to 10% buffered formalin solution at ambient temperature for appropriate fixation. The GSI was measured using following formula: GSI = [(gonad weight/body weight) × 100].

Histopathology of Gonads

In order to histological observation, the collected ovarian tissues were washed with running tap water and passed through a graded series of alcohol for dehydration then in benzene for clearing and embedded with using paraffin. The paraffin blocks were sectioned with a thickness of 5 µm using microtome and stained with hematoxylin and counterstained with eosin. Finally, the histopathological alterations were photographed using digital photomicroscope (Olympus CX 41).

Blood Sampling

For collecting blood, fish were anesthetized with clove oil (5 mg/l) and blood was collected by cutting the caudal peduncle and pushed into a sterilized centrifuge tube containing anticoagulant (20mM EDTA). The blood collection process for each fish took less than one minute to minimize stress impacts on normal blood values.

Measurement of Hemato-biochemical Parameters

Blood glucose level (mg/dl) was measured using glucose strips in adigital EasyMate® GHb, blood glucose/hemoglobin dual-function monitoring system (Model: ET- 232, Bioptik Technology Inc. Taiwan 35057). SAHLI's hemometer (Model-3243000, MARIENFELD, Germany) was used to calculate the Hb (%) following the routine procedure. Total count of RBCs and WBCs were done by established Neubaur haemocytometer counting method.

Semi-quantitative Analysis

Histological evaluation like mean assessment value (MAV), degree of tissue changes (DTC) and observed frequency of the pathological lesions were evaluated after observing six sections per fish tissue by light microscope (Olympus CX 41). Semi-quantitative observations on histopathological alterations were done according to Schwaiger et al. (1997) with some modifications. A numerical value was given for each alteration according to their frequency of occurrences

Statistical Analysis

Values were presented as, means ± standard deviation (SD). We used one-way analysis of variance (One-way ANOVA) followed by Tukey's post hoc test. Statistical significance was set at $p<0.05$. Data were analyzed using SPSS (version 14.0).

Results

Effects of Cypermethrin on Mortality

The calculated 96-h LC10, LC50 and LC90 (with 95% confidence limits) of cypermethrin for Gangetic mystus were 13.6, 25.5 and 46.6 µg/L respectively. During the static bioassay, mortality was not observed in the control groups. The short term 24h LC50 for Gangetic mystus was around 30 µg/L with the LC10 and LC90 being 1.5 times lower and higher, respectively (Table 1). Similarly, at 48h the LC50 value (29.5 µg/L) is two times higher and lower than LC10 and LC90 values sequentially. Whereas the LC90 at 72h was maximum around 60 µg/L with near about two times and three times higher than LC50 and LC10 values individually.

Effects of Cypermethrin on GSI

We measured female GSI at day-30, -60 and -90 after exposure to four different pesticide concentrations. At the day-30 and -60, the values of female GSI were decreased with increasing pesticide concentration compared to control group. While in the day- 90 female GSI were more or less similar among all pesticide treated groups along with control group (Figure 1).

Table 1. Number of dead individuals of Gangetic mystus exposed to different concentrations of cypermethrin during the experimental period.

Cypermethrin concentrations (µg/L)	Exposure time (h)			
	24h	48h	72h	96h
0	0	0	0	0
5	0	0	0	0
10	0	0	0	0
15	0	10±0	20±0	20±0
20	15±7	25±7	30±14	30±14
25	35±7	35±7	40±14	50±14
30	45±7	50±0	50±0	60±0
LC10 value with 95% confidence limits	19.3(13.9-21.9)	15.7(10.3-18.7)	13.5(8.2-16.6)	13.6(9.0-16.4)
LC50 value with 95% confidence limits	29.9(26.5-39.9)	29.5(25.2-41.9)	28.4(23.9-40.9)	25.5(22.1-31.9)
LC90 value with 95% confidence limits	46.2(36.3-101.0)	55.2(39.8-145.5)	59.8(41.4-169.8)	47.6(36.3-92.9)

Data were expressed as mean ± SD (n=3)

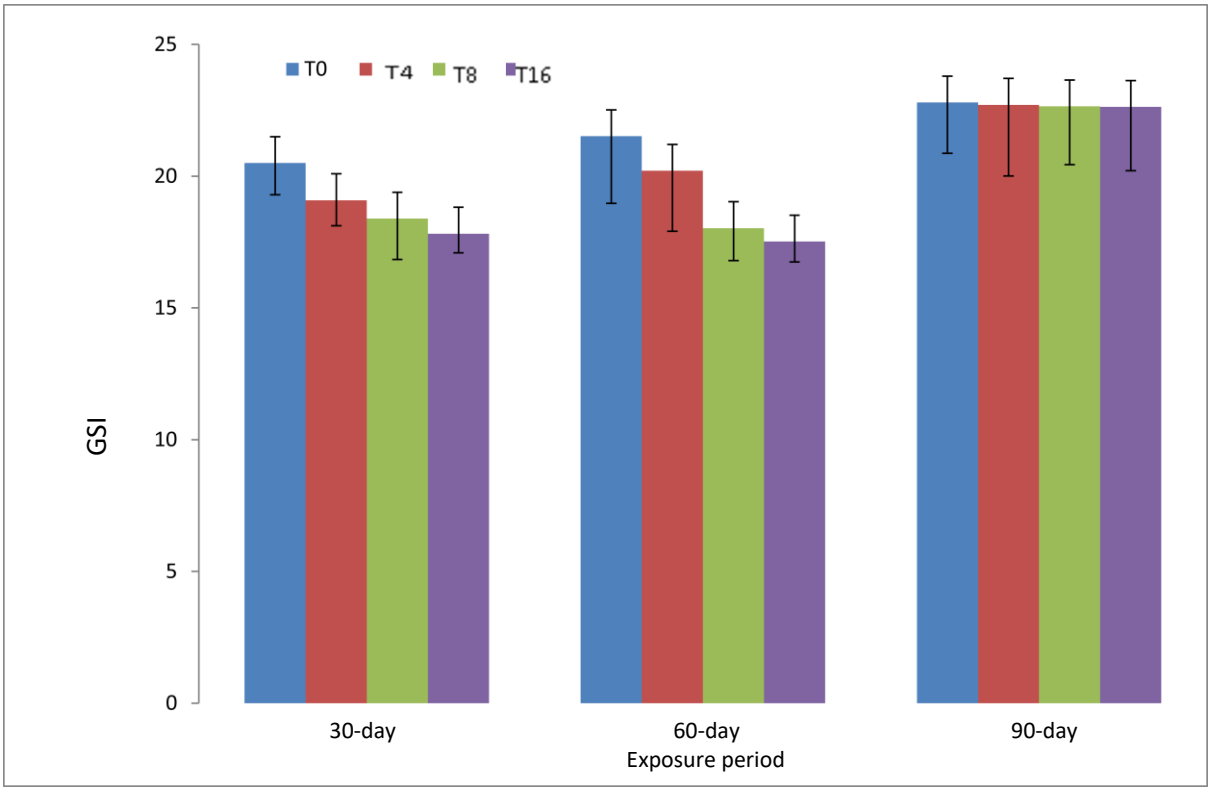

Figure 1. Gonadosomatic index of female *Mystus cavasius* exposed to cypermethrin for 90 days (TO= 0 µg/L; T4= 4 µg/L; T8=8 µg/L & T16=16 µg/L).

Effects of Cypermethrin on Ovary

The ovaries extracted from the control fish did not show any histopathological alterations at day-30 associate with regular shape of nucleus (Figure 2A). After 30 days of pesticide exposure several abnormalities in female gonad were observed like WO (wrinkle oocyte), AF (atretic follicle), DGL (degenerated granulose layer) at pesticide concentration 4µg/L (Figure 2B); IFS (inter follicular space), AD (adhesion) CC (cytoplasmic clumping) at 8µg/L pesticide treated group (Figure 2C) and CT (cyst), DGL (degenerated granulose layer), CC (cytoplasmic clumping), DO (disrupted oocyte) at 16µg/L pesticide exposure group (Figure 2D). Similarly, at day-60 and day-90 ovaries collected from control groups showed regular pattern of nucleus and granulose layer (Figure 3A & 4A). The treated ovary showed DGL (degeneration of granulose layer), IFS (inter follicular space), AF (atretic follicle), CT (cyst), CC (cytolasmic clumping), AD (adhesion) were evident after 60 and 90 days of pesticide exposure (Figure 3B-3D and 4B-4D). Histopathological alterations of *M. cavasius* ovary exposed to different cypermenthrin concentration were found to be increased linearly with exposure time and concentrations (Table 2).

Effects of Cypermethrin on Hemato-biochemical Parameters

The blood glucose level is the amount of glucose (sugar) present in the blood of a human or animal which is the primary source of energy. Present study showed that blood glucose level increased significantly with increasing pesticide concentration and time of exposure. Blood glucose level was consecutively higher in the higher pesticide treated groups than the control group (Table 3). On the other hand, blood hemoglobin level showed opposite scenario i.e. the Hb level decreased significantly with increasing pesticide concentrations along with exposure time (Table 3). Whereas the numbers of RBCs and WBCs are significantly decreased and increased respectively with increasing pesticide exposure periods and concentrations (Table 3).

Discussion

Effect of Cypermethrin on Mortality

The 96-h LC50 of cypermethrin for Gangetic mystus was about 30µg/L in the static bioassay. Ayoola et al. (2008) reported the 96-h lethal concentration (LC50) value of cypermethrin was 0.063mg/l for Juvenile African Catfish (*Clarias gariepinus*) which is about two times higher than what we reported for Gangetic mystus. Marigoudar et al. (2009) found the 96-h LC50 value for Freshwater Teleost, *Labeo rohita* as 4.0 µg /L about seven times lower than our findings. Deka and Dutta (2012) revealed that the 96-h LC50 for *Heteropneustes fossilis* was about forty-five times lower than the present experiment as 0.67µg/L. Rabia Sarıkaya (2009) found 96-h the LC50 of cypermethrin for Nile

tilapia was estimated as 5.99 µg/L. Saha and Kaviraj (2007) estimated the 96-h LC50 value of aqueous cypermethrin ranged from 0.03 µg/L for the crustacean (*Diaptomus Forbesi*) to 9.0 µg/ L for the tadpole larva (*Bufo melanostictus*). The higher LC50 value reported in the present study indicates that Gangetic mystus is less toxic to cypermethrin than some fish species which are tested in previous studies. The variation of sensitivity of fish to different chemicals depends on prevailing environmental conditions (Odukkathil and Vasudevan, 2013), physico-chemical properties of pesticides (Al-Emran et al., 2021), size and age group of fish tested, and detoxification and absorption potential of fish (Oh et al., 2009).

Effect of Cypermethrin on GSI

The GSI value did not show the significant change ($p>0.05$) from the first sampling (day-30) to the end of the experiment (day-90) among the control and treatments. Compared to control the GSI values was in decreasing trend with increasing pesticide concentration at day-30 and day-60 of pesticide exposure on the other hand it remains almost similar at day-90. This reduction in GSI value maybe due to delayed maturity, decrease in the percentage of different stages of oocytes as well as reduction in reproductive efficiency. An investigation was conducted to know the effects of organophosphate pesticide dimethoate (rogor) on the gonadosomatic index of the fish, *Cyprinus carpio var. communis* was. The GSI values was found to be increased in all control groups compared to the sub- lethal pesticide treated groups (0.85, 1.20 and 1.53 mg/L) (Maqbool and Ahmed, 2013). However, the decreased in GSI was found directly proportional to the pesticide concentration and the period of the exposure (Mir et al., 2011).

Effect of Cypermethrin on Ovary

The current research found several dose and time dependent alterations in the ovarian histopathology of Gangetic mystus exposed to different concentrations of cypermethrin. The most common histopathological alterations in Gangetic mystus ovary when exposed to different concentrations of cypermethrin was degenerated granulose layer and oocyte wall. These two alterations induced by cypermethrin toxicity result in the loss of follicles or empty follicles, indicating that the genetic material within the follicles has been lost. The loss of genetic material in Gangetic mystus ovarian follicles may impede estrogen synthesis. The findings of this investigation are consistent with previous studies of Dutta and Maxwell (2003) and Maxwell and Dutta (2005) in Bluegill ovary exposed to diazinon; and Marutirao (2013) in *Puntius ticto* ovary exposed to dimethoate.

Inter follicular space can be caused by oocytes adhesion, as shown in our experiment. In the present study, fusion of oocytes accompanied with inter follicular space was noticed after 30 days and onwards of cypermethrin exposure to 8.0 and 16.0 µg/L. Oocytes that adhered to one another are unable to progress to the next stages of maturation resulted the inhibition of

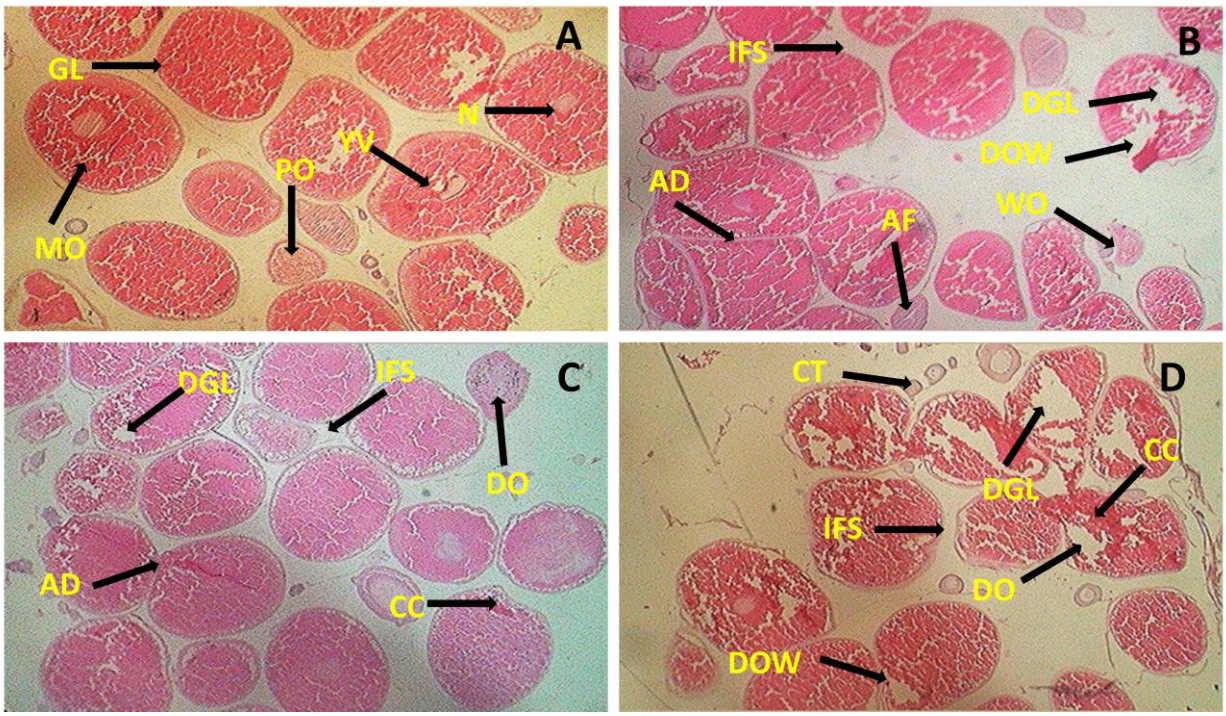

Figure 2. Histo-architectural changes in ovary exposed to cypermethrin at day-30; (A) Control (0 µg/L), (B) 4 µg/L, (C) 8 µg/L and (D) 16 µg/L. Arrow heads indicate mature oocyte (MO), pre-mature oocyte (PO), yolk vesicle (YV), wrinkle oocyte (WO), atretic follicle (AF), degenerated granulose layer (DGL), inter follicular space (IFS), adhesion (AD), cytoplasmic clumping (CC) cyst (CT), cytoplasmic clumping (CC), disrupted oocyte (DO). Magnification 4×; scale bars = 100 µm.

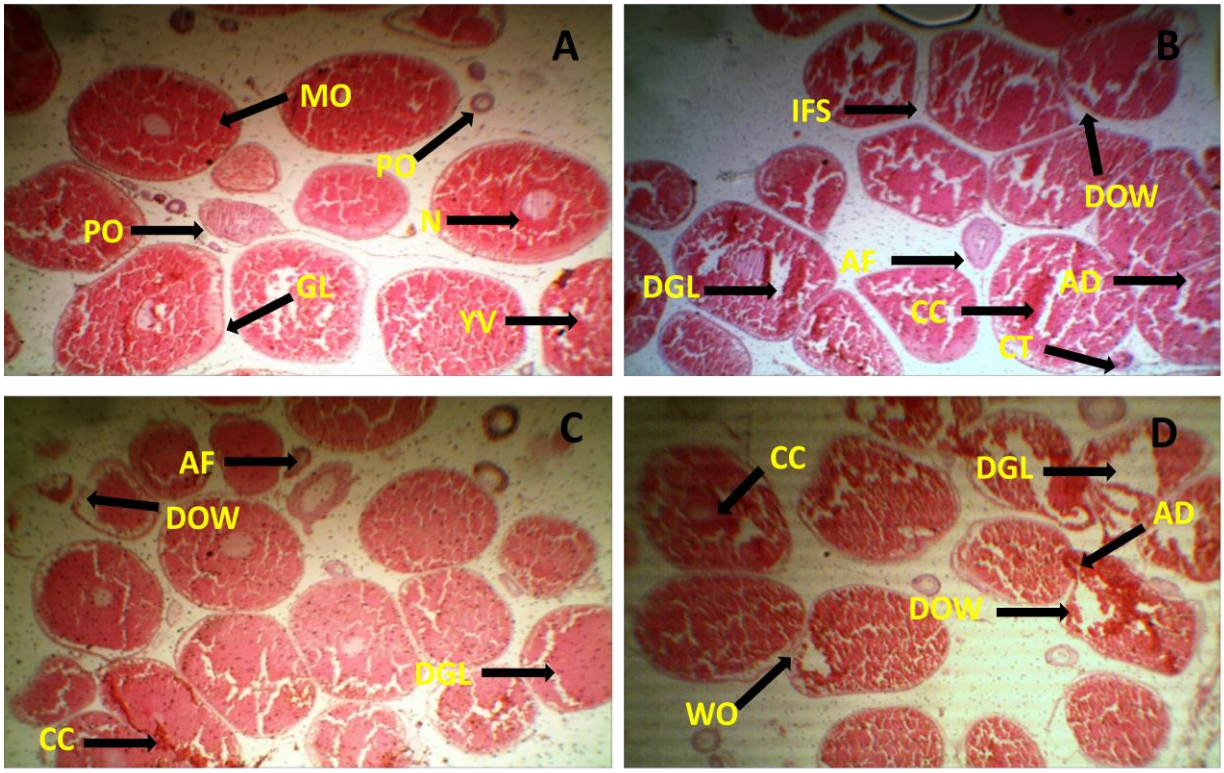

Figure 3. Histo-architectural changes in ovary exposed to cypermethrin at day-60; (A) Control (0 µg/L), (B) 4 µg/L, (C) 8 µg/L and (D) 16 µg/L. Arrow heads indicate mature oocyte (MO), Nucleus (N), atretic follicle (AF), wrinkle oocyte (WO), inter follicular space (IFS), degenerated granulose layer (DGL), adhesion (AD), cytoplasmic clumping (CC) cyst (CT), cytoplasmic clumping (CC), disrupted oocyte (DO). Magnification 4×; scale bars = 100 µm

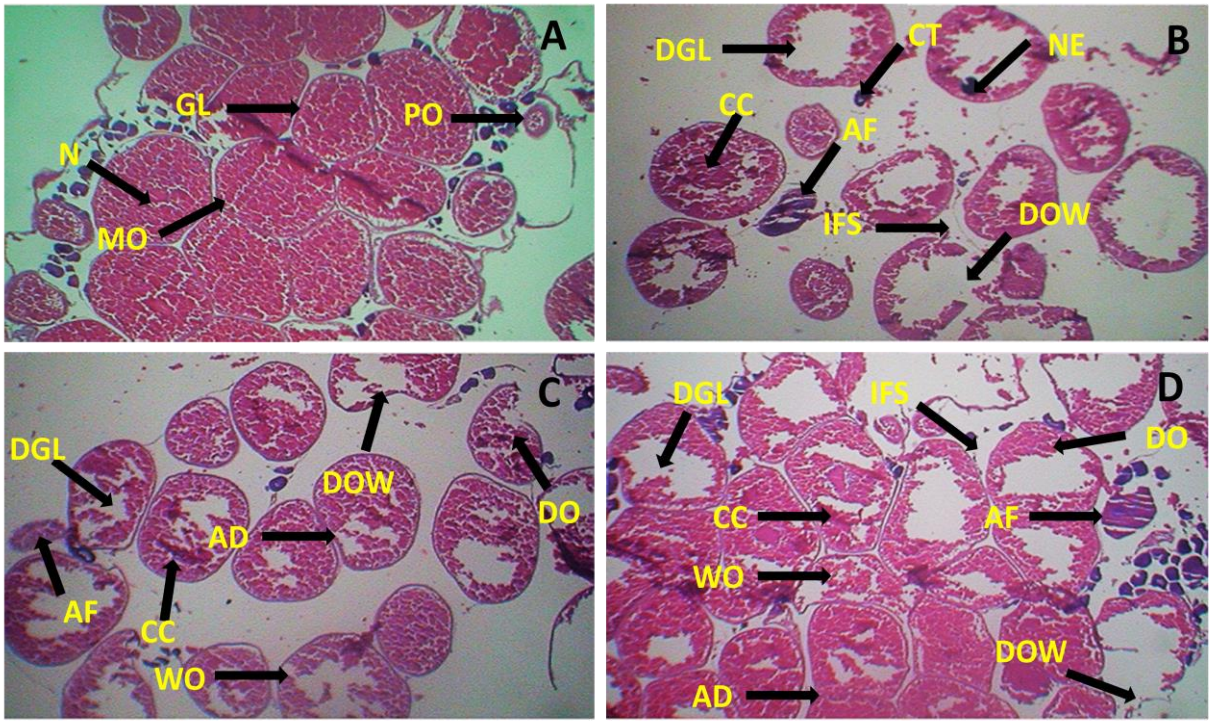

Figure 4. Histo-architectural changes in ovary exposed to cypermethrin at day-90; (A) Control (0 µg/L), (B) 4 µg/L, (C) 8 µg/L and (D) 16 µg/L. Arrow heads indicate mature oocyte (MO), granulose layer (GL), degenerated oocyte wall(DOW), atretic follicle (AF), wrinkle oocyte (WO), inter follicular space (IFS), degenerated granulose layer (DGL), adhesion (AD), cytoplasmic clumping (CC) cyst (CT), cytoplasmic clumping (CC), disrupted oocyte (DO). Magnification 4×; scale bars = 100 µm.

Table 2. Summary of histopathological alterations of *M. cavasius* ovary exposed to different concentrations of cypermethrin during the experimental period.

Alterations	Treatments (µg/L)	Sampling days		
		Day- 30	Day- 60	Day- 90
Degenerated granulose layer (DGL)	0	–	–	–
	4	–	–	+
	8	+	++	++
	16	+++	+++	+++
Atretic follicle (AF)	0	–	–	–
	4	–	–	–
	8	–	++	++
	16	++	+++	+++
Inter follicular space (IFS)	0	–	–	–
	4	–	+	+
	8	++	++	++
	16	+++	+++	+++
Wrinkle oocyte (WO)	0	–	–	–
	4	–	–	–
	8	+	+	+++
	16	++	++	+++
Adhesion (AD)	0	–	–	–
	4	–	+	+
	8	+	+	+
	16	++	++	++
Disrupted oocyte (DO)	0	–	–	–
	4	–	–	–
	8	–	+	+
	16	++	++	++
Cytoplasmic clumping (CC)	0	–	–	–
	4	–	–	–
	8	+	++	++
	16	++	++	++
Cyst (CT)	0	–	–	–
	4	–	+	+
	8	++	+	++
	16	+++	+++	+++
Degenerated oocyte wall (DOW)	0	–	–	–
	4	–	+	++
	8	++	++	+++
	16	+++	+++	+++
Necrosis (NE)	0	–	–	–
	4	–	+	+
	8	+	+	+
	16	++	++	++

(–) indicates absent, (+) low frequent, (++) moderately frequent, (+++) highly frequent

Table 3. Summary of hemato-biochemical parameters of *M. cavasius* after exposed to different concentrations of cypermethrin concentrations during the experimental period

Parameters	Treatments (µg/L)	Sampling Days		
		Day-30	Day-60	Day-90
Blood glucose (mg/dL)	0	5.9±0.01^{a}	5.6±0.03^{a}	5.7±0.01^{a}
	4	6.5±0.07^{a}	5.8±0.05^{a}	7.2±0.03^{a}
	8	8.1±0.11^{b}	6.8±0.19^{b}	7.8±0.05^{a}
	16	10.4±0.18^{c}	9.9±0.28^{c}	10.1±0.17^{c}
Hb (%)	0	9.8±0.01^{c}	9.6±0.01^{c}	9.9±0.01^{c}
	4	7.7±0.04^{b}	7.3±0.05^{b}	7.8±0.07^{b}
	8	6.1±0.04^{b}	6.5±0.04^{b}	6.7±0.04^{b}
	16	4.6±0.11^{a}	4.3±0.17^{a}	4.8±0.09^{a}
RBC ($\times 10^{6}/mm^{3}$)	0	3.4±0.03^{c}	3.2±0.03^{c}	1.8±0.05^{c}
	4	1.8±0.05^{b}	1.9±0.04^{b}	1.5±0.03^{b}
	8	0.4±0.04^{a}	1.7±0.05^{b}	1.4±0.04^{b}
	16	0.5± 0.13^{a}	1.3±0.17^{a}	1.1±0.04^{a}
WBC ($\times 10^{3}/mm^{3}$)	0	38.3±0.03^{a}	35.3±0.03^{a}	26.0± 0.05^{a}
	4	60.8±0.04^{c}	47.0±0.07^{c}	34.3±0.07^{b}
	8	44.6±0.06^{b}	43.3±0.09^{b}	32.5±0.11^{b}
	16	48.0± 0.07^{b}	50.3±0.11^{c}	53.8±0.01^{c}

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

steroids production in the ovary. The results of our study confirm with those of earlier studies in Bluegill ovary after 72 h of diazinon exposure to 60 mg/L (Maxwell and Dutta, 2005; Dutta and Maxwell, 2003).

Cytoplasmic degeneration like cytoplasmic clumping (CC) was observed among the three sampling days exposure to both 8.0 and 16.0 µg/L of cypermethrin. Earlier studies demonstrated a substantial link between the damaged ovarian structures and levels of estrogen production in fish exposed to different pesticides (Manjunatha and Philip, 2016; Maxwell and Dutta, 2005). Deka and Mahanta (2012) found cytoplasmic degenerations in Stinging Catfish (*Heteropneustes fossilis*) ovary after exposed to 200 mg/L malathion for 10 days. The cytoplasmic alterations were also observed in previous studies in Bluegill (*Lepomis macrochirus*) ovary exposed to diazinon (Maxwell and Dutta, 2005) and in *Puntius ticto* ovary exposed to dimethoate (Marutirao, 2013).

Follicular atresia in Gangetic mystus ovary was detected after 60 and 90 days of cypermethrin exposure to 8.0µg/L, as well as after all three sampling days of cypermethrin exposure to 16.0µg/L. The presence of follicular atresia in cypermethrin-exposed ovaries of Gangetic mystus at oocyte stages could reflect an interruption in the normal processes of final maturation of oocytes as well as disturbances of ovulation and oviposition, finally reduction of fertility. The atretic follicles were observed by Manjunatha and Philip (2016) in Zebrafish (*Danio rerio*) ovaries after an acute exposure (96 h) to 200 mg/L of chlorpyrifos; by Dutta and Maxwell (2003) in Bluegill ovary exposed to diazinon and by Narayanaswamy and Mohan (2014) in *Glossogobius giuris* ovary exposed to malathion. The follicular atresia was also noticed in *Puntius ticto* ovary after a chronic exposure to dimethoate (Marutirao, 2013) and in *Channa punctatus* ovary after a sub-chronic exposure to monocrotophos (Maqbool and Ahmed, 2013).

Necrosis was evident in Gangetic mystus ovary after exposure to 4.0, 8.0 and 16.0 µg/L at Day-30, Day-60 and Day-90. This indicated the lack or absence of genetic material after a longterm exposure, which may reduce the hormone production. Maqbool and Ahmed (2013) observed similar alterations in *Channa punctatus* ovary after 45 days of 2,000 mg/L monocrotophos exposure. Necrosis or loss of genetic materials was also observed in Stinging Catfish ovary after an acute exposure to malathion (Dutta et al., 1994) and in Bluegill ovary exposed to diazinon (Maxwell and Dutta, 2005).

Alteration of Hemato-biochemical Parameters Exposed to Cypermethrin

Blood glucose levels are general stress indicators in fish (Pacheco and Santos 2001). In the present study, increased glucose levels recorded in the fish exposed to cypermethrin might be due to the mobilization of glycogen into glucose to meet the increased demand for energy under stressed condition due to pesticide exposure. Glucocorticoids and catecholamine hormones are known to produce hyperglycemia in animals and stress stimuli elicit rapid secretion of these hormones from adrenal tissues of the fish (Pickering 1981). Fresh water catfish *H. fossilis* exposed to different pesticide concentrations such as rogor and aldrin reported hypoglycemia (Borah et al., 1995). Such elevation may be due to the increased reaction of stressed fish to gluconeogenesis to meet their additional energy requirements (Winkaler et al., 2007). Chowdhury et al. (2000) reported that *H. fossilis* subjected to testosterone sub-lethal concentrations resulted to change of blood glucose level. This is likely owing to the fast use of blood glucose during hyper excitability, shocks and tremors, characteristic behavior of fish toxicity to pesticides (Singh et al., 1982).

In the current research, Hb content in the blood of the fish exposed to the different concentrations of cypermethrin decreased significantly. The observed decrease in hemoglobin levels might be because of failing of hematopoietic system under stressed condition in Gangetic mystus. A similar reduced value of Hb was also reported in common carp exposed to sumithion (Salam et al., 2015) and malathion (Sharmin et al., 2015). Similar to Hb, the number of RBCs was found to be decreased in fishes subjected to different concentrations of cypermethrin, also might be because of failing of hematopoietic system. Similar to the present results, a decrease in the number of RBC was reported in rainbow trout exposed to diazinon (Banaee et al., 2011) and in *Clarias gariepinus* exposed to lead nitrates (Adeyemo et al., 2008). Inhibition of erythropoiesis and increase in the rate of erythrocyte destruction in hematopoietic organs is the cause of decrease in RBC count (Joshi et al., 2002). In the present study, the significant decrease of RBC content might have resulted from the oxygen deficiency in the body or from the lowering content of the water due to the presence of cypermethrin.

Remarkably, there was a significant increase in WBC count in the blood of fishes exposed to different concentrations of cypermethrin, which can be correlated with an increase in antibody production that helps in survival of the fish in adverse environmental condition. The immediate stimulation of immunological defense may result in leucocytosis in fish due to the presence of foreign particles or stressed conditions (Marti et al., 1996). The rise in the number of WBCs can be linked with rise in the manufacturing of antibodies, which helps in the survival and regeneration of stressors exposed fish (Joshi et al., 2002)

Ethical Statement

The experimental protocol was approved by the Ethics Committee of Bangladesh Agricultural University, Mymensingh, Bangladesh.

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Author Contribution

Md Helal Uddin: Conceptualization, Methodology, Writing -review and editing; Md Haider Ali: Experimentation, Methodology and Data Curation; Kizar Ahmed Sumon: Resources and Analysis; Md Shahjahan: Investigation and Visualization; Harunur Rashid: Supervision, review and editing.

Conflict of Interest

The authors declare no competing interests.

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