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Toxicity of cypermethrin on the embryo and larvae of Gangetic mystus, *Mystus cavasius*

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Abstract The objective of the present study was to elucidate the effects of cypermethrin on the embryo and the larvae of Gangetic mystus, *Mystus cavasius*. Therefore, fertilized eggs (n = 100) and 1-day-old larvae (n = 100) were exposed to six different concentrations of cypermethrin (0, 2, 4, 8, 16 and 32 μg L^{-1}) in each of the 18 plastic bowls. Each of the treatment and control was maintained in three replicates. The LC_{10} and LC_{50} values for Gangetic mystus embryos and larvae were calculated using probit analysis. Results showed the mortality of embryos significantly increased with increasing cypermethrin concentrations. The 24-h LC_{10} and LC_{50} (with 95% confidence interval) values of cypermethrin for embryo were 0.42 (0.14–0.81) and 5.60 (4.16–7.19) μg L^{-1}, respectively. Hatching success decreased and mortality of larvae increased significantly with increasing cypermethrin concentrations. The 24-h LC_{10} and LC_{50} values (with 95% confidence limits) of cypermethrin for larvae were 1.72 (1.24–2.20) and 11.57 (10.09–13.42) μg L^{-1}, respectively; the 48-h LC_{10} and LC_{50} for larvae were 1.34 (0.83–1.89) and 8.25 (6.87–9.91) μg L^{-1}, respectively; the 72-h LC_{10} and LC_{50} for larvae were 1.13 (0.63–1.66) and 6.12 (4.91–7.47) μg L^{-1}, respectively. Furthermore, results showed several malformations in embryos and larvae when exposed to the two highest concentrations of cypermethrin. The findings of the study suggest that 2 μg L^{-1} cypermethrin concentration in the aquatic environment may have deleterious effects on the development and the reproduction of Gangetic mystus.

Keywords Pyrethroid insecticide · Early-stage toxicity · Gangetic mystus · Malformation · Aquatic ecosystem

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Introduction

Cultivation of high-yielding varieties of crops is a common practice in Bangladesh to meet the ever-growing demand of food. The important phenomenon of these high-yielding varieties is that most of them are susceptible to pests and diseases (Bagchi et al. 2009; Sumon et al. 2016a, b). Farmers use pesticides indiscriminately to protect the crops and to increase the quantity of agricultural yields (Rahman 2013). The use of pesticide in Bangladesh was negligible until 1960 (Rahman 2013), but it has increased rapidly from 7350 MT in 1992 to 45,172 MT in 2010 (Hasan et al. 2014).

Pyrethroids, a class of broad-spectrum and high-efficiency pesticides, are becoming increasingly popular in agricultural, veterinary and home use over the decades; accounting for about one-quarter of the world pesticides market (Oros and Werner 2005; Shi et al. 2011). In recent years, residues of pyrethroids have been extensively detected in soil, urban and agricultural streams, as well as indoor dust, which poses a potential risk to aquatic organisms and humans (Hladik and Kuivila 2009; Kuivila et al. 2012). However, great attention has been paid to pyrethroids residues in the runoff and stream water because of their high toxicity towards aquatic organisms, like fish and invertebrates (Werner and Moran 2008). Pyrethroids have been shown to be up to 1000 times more

toxic to fish than to mammals and birds (Edwards et al. 1986; Bradbury and Coats 1989).

Cypermethrin [RS-a-cyano-3-phenoxybenzyl (1RS)-cis-, trans-3-(2,2,-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] and *k*-cyhalothrin [a-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3,-trifluoropropenyl)- 2,2-dime-thyl-cyclopropane carboxylate; CAS number: 52315-07-8] are widely used as synthetic pesticides of pyrethroids, and they are among the most effective pyrethroid preparations (Bradbury and Coats 1989; Lewis et al. 2016). They are the type II pyrethroid insecticides, used to control many pests including moth pests of cotton, fruits and vegetable crops (Crawford et al. 1981). These insecticides have emerged as a major agricultural pesticide in both developing and developed countries owing to their superior insecticidal activity and broad insecticidal range. The mechanism of their effectiveness in the case of fish is the same as that of other pyrethroids containing cyano-3-phenoxy-benzyl groups. Cypermethrin acts by blocking sodium channels and affecting the function of GABA receptors of nerve filaments (Bradbury and Coats 1989; Dobsikova et al. 2006).

The pesticides used in the crop fields, animal husbandry, post-harvest technology, public health and industry ultimately reach the aquatic systems through different pathways like direct deposition or spray drift, runoff, precipitation, soil conditions and slope of the catchment area (Liess et al. 1999; Deka and Dutta 2012). Application of these synthetic derivatives of pyrethroids is highly toxic to a number of non-target aquatic organisms even at low concentrations (Oudou et al. 2004; Begum 2005; El-Sayed et al. 2007). Cypermethrin has potentially deleterious effects on fish at sub-lethal levels (Saha and Kaviraj 2009). Like other groups of vertebrates, however, fish embryos and larvae are also considered to be the most sensitive stages in the life cycle and sensitive to low levels of environmental pollutants (Sumon et al. 2016b). A number of studies have been conducted to assess the toxicity of cypermethrin to various stages of different fishes (Das and Mukherjee 2003; Adhikari et al. 2004; Gonzalez-Doncel et al. 2004; Aydin et al. 2005; Kumar et al. 2007; Saha and Kaviraj 2009; Suvetha et al. 2010; Shi et al. 2011; Jin et al. 2011).

Gangetic mystus (*Mystus cavasius*; order: Siluriformes; family: Bagaridae), locally known as 'gulsha', is one of the important small indigenous fish species (SIS) in Bangladesh. The species is also abundant in India, Nepal, Pakistan, Sri Lanka, Myanmar, Thailand, Indo-China, Malaysia, East Indies, Syria and West Africa (Yadav 1997). *M. cavasius* is commonly found in rivers, lakes, canals, floodplains, swamps, ponds and ditches (Talwar and Jhingran 1991). The fish is very popular to consumers because of its taste and nutritional values. As per our literature survey, no study has been conducted to investigate the toxicity of cypermethrin on the developmental stages of *M. cavasius* to date. Therefore, the present study aimed at assessing the toxicity of cypermethrin on the embryo and the larvae of *M. cavasius*. The findings of this study could serve as a baseline for other researchers in using Gangetic mystus as a model fish for assessing the embryonic and larval toxicity of environmental contaminants.

Materials and methods

Collection of experimental fish and pesticide

Mature male and female of *M. cavasius* were collected from a local commercial fish breeding farm (Sharnalata Agro-Fisheries Limited, Fulbaria, Mymensingh). Fish were transported in oxygenated plastic bags to the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, and were stocked in an earthen pond (length: 12 m; width: 6 m; water height: 1.5 m). Fish were fed daily in the evening with a commercial floating catfish feed 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.

Cypermethrin (10 EC, manufactured by Hayleys PLC, Sri Lanka) was purchased from a local registered pesticide seller (Mymensingh, Bangladesh).

Hormone administration, collection of gametes, artificial fertilization and incubation of eggs

Healthy and fully matured male (n = 5; length: 12.97 ± 0.76 cm; weight: 15.64 ± 2.22 g) and female (n = 10; length: 14.56 ± 0.70 cm; weight: 21.40 ± 2.80 g) fishes were selected for spawning. Matured males were identified by a slightly pointed genital papilla and females by a swollen abdomen and a reddish swollen vent. The maturity of each female was confirmed by slightly pressing the ventral side of the fish for oozing of eggs. Fish were artificially induced by intramuscular injection of carp pituitary powder suspended in a 0.9% NaCl solution. The powder was administered at a rate of 10%/kg body weight of both male and female fish. Hormone-injected fish were transferred in previously prepared aerated glass aquaria (45 cm × 30 cm × 32 cm) containing 50 L dechlorinated tap water.

Fish were taken out from aquarium after 10 h of pituitary administration and placed in an aluminium bowl for stripping. Female fishes were stripped by gentle pressure on the abdomen to collect the eggs in the bowl, and simultaneously, testes were collected from the male fishes by using a scalpel and cut into small pieces to collect milt. Collected eggs and milt were mixed properly by using a clean and soft poultry feather. Few drops of water were added for proper mixing of eggs and milt to ensure effective fertilization. A portion of the fertilized eggs were placed into previously prepared experimental units for

studying embryonic toxicity, and the rest of the fertilized eggs were released into a glass aquarium. Continuous water supply was ensured by providing shower in aquarium until hatching of the eggs, and 1-day-old larvae were used to study larval toxicity.

Study of embryonic and larval toxicity

In this study, 18 plastic bowls containing 2 L of dechlorinated tap water were used for embryonic and larval bioassay. Six different concentrations of cypermethrin (0, 2, 4, 8, 16 and 32 μg L^{-1}) were used by adding cypermethrin stock solution to study the embryonic and larval toxicity. Each of the control and treatment was executed in three replicates. The stock solution was prepared by dissolving the weighed amount of cypermethrin in distilled water containing 100 g L^{-1} cypermethrin. Water quality parameters in the experimental units were determined according to APHA (APHA 1985). The values (mean ± SD) for water quality were as follows: temperature, 27.41 ± 0.09 °C; dissolved oxygen, 8.21 ± 0.11 mg L^{-1}; pH, 8.80 ± 0.02 and total alkalinity, 186 ± 2.42 mg L^{-1}.

To study the embryonic toxicity, each of the 100 fertilized eggs was released randomly in the 18 plastic bowls. The hatching rate and incubation period were recorded for all the treatment and control groups. The number of dead embryos was counted at 24 h of cypermethrin exposure. The live embryos were kept in the bowl until hatching. For the larval bioassay, we selected 18 sets of 100 1-day-old larvae that were released in 18 plastic bowls. The number of dead larvae was counted at 24 h, 48 h and 72 h of cypermethrin exposure. Dead embryos and larvae were identified as white opaque colour and not responding to agitation of water by plastic spoon.

Malformations were observed for embryos at every 6-h interval and for larvae at every 12-h interval from each of the 18 plastic bowls under a digital microscope (Olympus CX 41). Images were snapped by using a camera (Magnus analytics, Model-MIPS) connected between the microscope and a computer.

Statistical analysis

The data on hatching success and mortality of embryo and larvae were presented in this study as the average of three replicates ± standard deviation (SD). Data were analysed by one-way analysis of variance (one-way ANOVA) followed by Tukey's post-hoc test to assess statistically significant differences among the different treatments. Statistical significance was set at $p < 0.05$. The one-way ANOVA assumptions of normality and homoscedasticity were evaluated using the Shapiro-Wilks test and Levene's test, respectively, before analyses were performed. The LC_{10} and LC_{50} values were calculated by using probit analysis. Statistical analysis was performed using SPSS Version 23.0 for Windows (SPSS Inc., Chicago, IL).

Results

A prolonged incubation period was observed with increasing concentrations of cypermethrin (Table 1). The mortality of embryos significantly increased (one-way ANOVA; $F_{5,12} = 92.43$; $p = 0.000$) with increasing cypermethrin concentrations (Table 1). The 24-h LC_{10} and LC_{50} values (with 95% confidence interval) of cypermethrin for Gangetic mystus embryos were presented in Table 1. Hatching success significantly decreased with increasing cypermethrin concentrations (one-way ANOVA; $F_{5,12} = 147.54$; $p = 0.000$). Mortality of larvae at 24 h (one-way ANOVA; $F_{5,12} = 142.28$; $p = 0.000$), at 48 h (one-way ANOVA; $F_{5,12} = 81.64$; $p = 0.000$) and at 72 h (one-way ANOVA; $F_{5,12} = 82.46$; $p = 0.000$) significantly increased with increasing concentrations of cypermethrin (Table 1). Table 1 presents the 24-h, 48-h and 72-h LC_{10} and LC_{50} values (with 95% confidence interval) of cypermethrin for Gangetic mystus larvae.

In embryos, several malformations were observed including dark and dark-brown yolk sac, broken eggshell and notochord, unhatched eggs exposed to different cypermethrin concentrations (Fig. 1). In the present study, malformations were also evident in Gangetic mystus larvae, like deformed and broken notochord, yolk-sac edema, body arcuation, lordosis and irregular caudal region when exposed to different cypermethrin concentrations (Fig. 2). All malformations in Gangetic mystus embryos and larvae were found when exposed to the two highest concentrations of cypermethrin (i.e. 16 and 32 μg L^{-1}). No obvious malformation was observed in embryos and larvae when they were exposed to <16 μg L^{-1} of cypermethrin concentrations (Figs. 1 and 2).

Discussion

The present study showed several effects of cypermethrin on the hatching success, incubation period and mortality of embryos and larvae of Gangetic mystus. Hatching success significantly decreased with increasing concentrations of cypermethrin. For example, eggs exposed to the lowest cypermethrin concentration (2 μg L^{-1}) had 65% hatching success, while those with the highest concentration (32 μg L^{-1}) had only 8% hatching success. Our study is in line with the hatching success described by Aydin et al. (2005) for common carp embryos exposed to cypermethrin. They found a hatching success of 87% for common carp eggs exposed to 0.0001 μg L^{-1}; a hatching success of 23% for the same species when exposed to 8 μg L^{-1} of cypermethrin concentration.

Table 1 Toxicity of cypermethrin on the embryo and the larvae of Gangetic mystus (n = 100 embryos and 1-day-old larvae)

Concentration (μg L^{-1})	Incubation period	Number of dead embryos at 24 h	Hatching success (%)	Number of dead larvae at 24 h	Number of dead larvae at 48 h	Number of dead larvae at 72 h
0	19 h	8 ± 1	81.3 ± 2.5	3.3 ± 1.5	5 ± 0.58	8 ± 0.5
2	19 h 30 min	28 ± 3*	64.6 ± 5.0*	10 ± 2	13.6 ± 1	17 ± 1
4	20 h 30 min	51 ± 5*	45.3 ± 3.2*	22 ± 2*	28.3 ± 3*	37 ± 3*
8	22 h	55 ± 6.2*	38.3 ± 4.1*	47 ± 5.5*	56 ± 6.6*	65.6 ± 6.6*
16	25 h	62 ± 3.2*	28.6 ± 3.2*	59 ± 3.7*	70 ± 4.3*	75 ± 4.3*
32	29 h	86 ± 7.6*	8.3 ± 3.5*	72 ± 6.5*	79 ± 12.5*	87.6 ± 12.5*
LC_{10} value with 95% confidence limits		0.42 (0.14–0.81)		1.72 (1.24–2.20)	1.34 (0.83–1.89)	1.13 (0.63–1.66)
LC_{50} value with 95% confidence limits		5.60 (4.16–7.19)		11.57 (10.09–13.42)	8.25 (6.87–9.91)	6.12 (4.91–7.47)

*Significance level ($p < 0.05$)

Koprucu and Aydin (2004) reported a significant decrease in hatching success of common carp embryos exposed to different concentrations of pyrethroid deltamethrin. A similar finding was reported by Ansari and Ansari (2012) for zebrafish embryos exposed to alphamethrin. Richterva et al. (2014) also observed reduced and delayed hatching success for common carp embryos exposed to cyhalothrin. In another study, Richterva et al. (2015) reported a significant decrease of hatching success for common carp embryos when exposed to cyperkill (a cypermethrin-based pesticide). Earlier reports showed that other groups of pesticides than pyrethroids may also have negative effects on the hatchability of different fishes. For instance, Aydin and Koprucu (2005) reported a significant decrease in hatching success of common carp embryos due to different diazinon concentrations. A similar finding was also reported by Mhadhbi and Beiras (2012) for turbot eggs when exposed to diazinon concentrations. Another study by Ansari and Ansari (2011) found a significant decrease of hatching success for zebrafish embryos exposed to dimethoate concentrations. A reduced hatching success was also observed for African catfish embryos when exposed to different buprofezin (Marimuthu et al. 2013) and endosulfan concentrations (Agbohessi et al. 2013) and for banded gourami embryos when exposed to chlorpyrifos (Sumon et al. 2016b).

The present study observes a prolonged incubation period of Gangetic mystus embryos due to the toxicity of cypermethrin. This might be due to hypoxia or disturbances of the hatching enzyme. During the normal hatching process of fish embryos, the chorion is digested by the hatching enzyme, which is a proteolytic enzyme secreted from hatching gland cells of the embryo. The structure and function of the protease might be destroyed by toxicants that block the pore canals of the chorions; thus, resulting in shortage of oxygen supply for the development of embryos (Fan and Shi 2002). The physiological

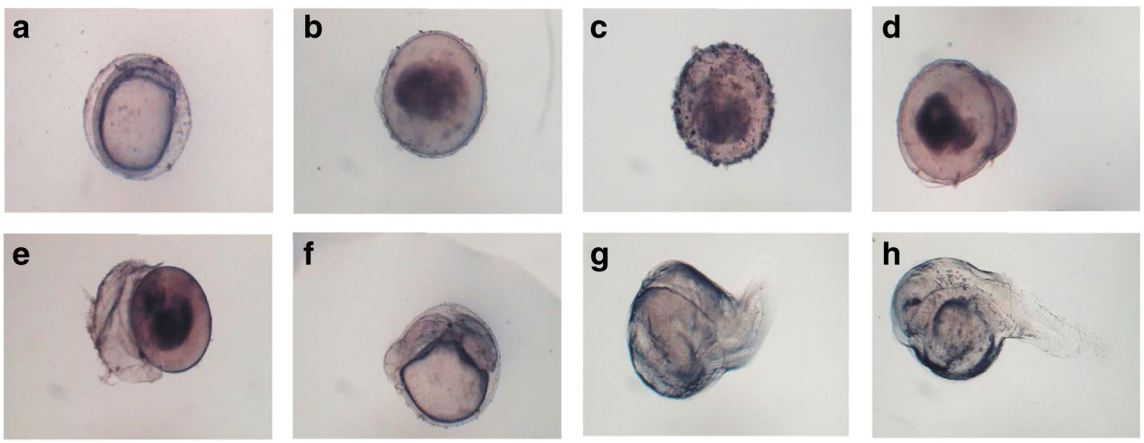

Fig. 1 Malformation observed in Gangetic mystus embryos due to cypermethrin toxicity. **a** Normal fertilized embryo after 12 h of exposure to 0 μg L^{-1} of cypermethrin. **b** Dark yolk sac after 12 h of exposure to 16 μg L^{-1} of cypermethrin. **c** Unhatched egg after 30 h of exposure to 32 μg L^{-1} of cypermethrin. **d** Dark-brown yolk sac after 18 h of exposure to 16 μg L^{-1} of cypermethrin. **e** Eggshell broken after 18 h of exposure to 32 μg L^{-1} of cypermethrin. **f** Notochord broken after 24 h of exposure to 32 μg L^{-1} of cypermethrin. **g** Abnormal embryo after 24 h of exposure to 32 μg L^{-1} of cypermethrin. **h** Notochord broken after 24 h of exposure to 32 μg L^{-1} of cypermethrin

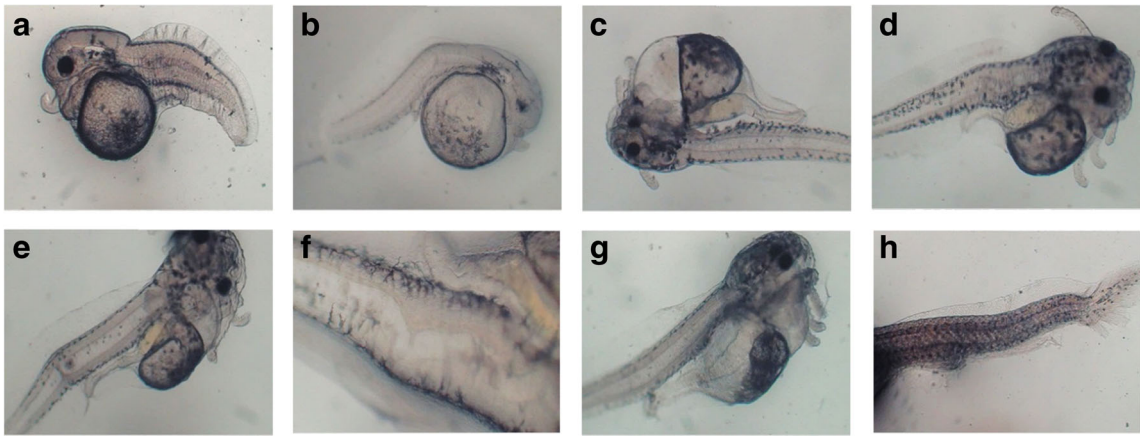

Fig. 2 Malformation observed in Gangetic mystus larvae due to cypermethrin toxicity. **a** Notochordal abnormality after 12 h of exposure to 32 μg L^{-1} of cypermethrin. **b** Broken notochord after 12 h of exposure to 16 μg L^{-1} of cypermethrin. **c** Yolk-sac edema after 36 h of exposure to 32 μg L^{-1} of cypermethrin. **d** Deformed notochord after 48 h of exposure to 16 μg L^{-1} of cypermethrin. **e** Notochordal abnormality and yolk-sac edema after 48 h of exposure to 32 μg L^{-1} of cypermethrin. **f** Deformed notochord after 72 h of exposure to 32 μg L^{-1} of cypermethrin. **g** Body arcuation and yolk-sac edema after 72 h of exposure to 32 μg L^{-1} of cypermethrin. **h** Lordosis and irregular caudal region after 72 h of exposure to 32 μg L^{-1} of cypermethrin

processes involved, as well as the mechanism underlying neural control in hatching of fish embryos are still unclear. Therefore, it is important to know the normal biology of the hatching process and how cypermethrin interferes with the development of the hatching gland of Gangetic mystus.

The number of dead embryos of Gangetic mystus increased significantly with increasing concentrations of cypermethrin. The 24-h LC_{50} value of cypermethrin for Gangetic mystus embryos was found to be 5.60 μg L^{-1}, which is about five times higher than those of the 24-h LC_{50} for common carp embryos (Aydin et al. 2005). Koprucu and Aydin (2004) estimated the 48-h LC_{50} of 0.213 μg L^{-1} of deltamethrin for common carp embryos, which is several times lower than we reported for Gangetic mystus embryo. The 72-h LC_{50} value of alphamethrin for zebrafish embryo was calculated to be 0.024 μg L^{-1} (Ansari and Ansari 2012). Sumon et al. (2016b) reported the 24-h LC_{50} of 11.8 μg L^{-1} for banded gourami embryos exposed to chlorpyrifos which is two times higher than the present study reported for Gangetic mystus embryos. The mortality of Gangetic mystus larvae significantly increased with increasing cypermethrin concentrations. In the present study, we observed embryos of Gangetic mystus which are more toxic to cypermethrin than 1-day-old larvae. However, during the early development, fish show variable sensitivity to some compounds displaying higher sensitivity in embryos whereas others are more toxic to larvae (Arufe et al. 2010; Ansari and Ansari 2012). The LC_{50} values of cypermethrin for Gangetic mystus larvae at 24, 48 and 72 h were 11.57, 8.25 and 6.12 μg L^{-1}, respectively. Aydin et al. (2005) estimated the 72-h LC_{50} of cypermethrin to be 1.304 μg L^{-1} for common carp larvae, which is about six times lower than we reported for Gangetic mystus. The 72-h LC_{50} of cypermethrin for Caspian roach (*Rutilus rutilus* caspicus) and silver carp (*Hypophthalmichthys molitrix*) larvae were estimated as 0.73 μg L^{-1} and 1.03 μg L^{-1}, respectively (Shaluei et al. 2012); which were again several times lower for both species than our study. Earlier studies showed almost similar toxicity of deltamethrin for common carp larvae (Koprucu and Aydin 2004), rainbow trout fry (Ural and Saglam 2005), European catfish fingerling (Koprucu et al. 2006) and spirlin larvae and fingerling (Vajargah et al. 2013).

In the present study, malformations were evident in the embryos and larvae of Gangetic mystus exposed to different cypermethrin concentrations (Figs. 1 and 2). All deformities were observed when the embryos and larvae were exposed to concentrations higher than 8 μg L^{-1}. Shi et al. (2011) observed malformation in zebrafish embryo and larvae when exposed to different concentrations of cypemethrin. Almost similar malformation was found by Shahjahan et al. (2017) in stinging catfish when exposed to sumithion and Marimuthu et al. (2013) in African catfish when exposed to buprofezin and Sumon et al. (2016b) in banded gourami when exposed to chlorpyrifos. In this study, the most observed notable malformation of banded gourami embryo and larvae was notochordal deformity, when they were mostly exposed to the two highest concentrations (16 and 32 μg L^{-1}) of cypermethrin. Our study is supported by earlier findings on zebrafish exposed to chlorpyrifos (Sreedevi et al. 2014; Yu et al. 2015), cartap (Zhou et al. 2009), malathion (Fraysse et al. 2006), bifenthrin (Jin et al. 2010), fipronil (Stehr et al. 2006), acetofenate (Xu et al. 2008) and endosulfan (Moon et al. 2016).

Conclusions

We report a first study assessing the developmental toxicity of cypermethrin by using Gangetic mystus as a model. Cypermethrin significantly affects the hatching, survival of

embryo and larvae and induces malformations. The results of the study suggest that 2 μg L^{-1} of cypermethrin in the aquatic environment may have an adverse effect on the development and the reproduction of Gangetic mystus. Our study also suggests that Gangetic mystus fish could serve as an ideal model species for evaluating the developmental toxicity of environmental contaminants. This study, however, addresses only the exposure of Gangetic mystus fish during their early developmental stages. Therefore, for potential persistence of the toxic effects in the long-term, we recommend future studies to evaluate the same endpoints in juvenile or adult of Gangetic mystus to determine whether the effects of cypermethrin are transitory or permanent.

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