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Toxicity effects of sumithion on the breeding performance and viability of eggs, embryos and subsequent growth indices of *Heteropneustes fossilis* larvae

T. Das, M. M. Rahman*, M. S. Hossen and M. F. A. Mollah
Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
*E-mail: rahman.matiur@bau.edu.bd

Abstract

Sumithion, an organophosphate pesticide, has been used extensively in agriculture as well as in broodfish and nursery ponds in Bangladesh. Although it is being applied for beneficial purposes but eventually it exhibits some short and long term toxic effects to the aquatic ecosystem. Therefore, the present study investigated the toxic effects of sumithion on the breeding performance and subsequent viability of embryos and larvae of *Heteropneustes fossilis.* A total of 400 adult *H. fossilis* were equally stocked in two ponds. Sumithion at the dose of 0.24 ppm was applied for four months every 7 days interval in one pond while the rest one was considered as control. No significant (p>0.05) difference was found in growth between the treated and control *H. fossilis*; however, fertilization and hatching rates of the eggs obtained from the treated group were significantly (p>0.05) lower than that of control. Subsequently, the embryos and larvae produced from the broods of the control pond were exposed to 5 different concentrations of sumithion (0.5, 1.0, 2.0, 4.0, and 8.0 ppm) against a control (0 ppm). Each concentration was assessed in five replicates having 200 eggs. Data obtained from the sumithion acute toxicity tests were evaluated using the Probit Analysis. The mortality of embryos increased with the increasing sumithion concentrations from 0 to 8.0 ppm and the significant (p>0.05) differences were found only among the treated concentrations above 2.0 ppm. The 24 h LC_{50} value for embryos was estimated to be 7.803 ppm. Dose-response decreased in hatching success and recorded as 90.8, 85.0, 76.2, 73.0, 65.0, and 51.2 % for control and 5 different concentrations of sumithion, respectively (p<0.05). The numbers of dead larvae also increased significantly with the increasing sumithion concentrations exposed for 24-96 h. The 24, 48, 72, and 96 h LC_{50} values were estimated to be 8.677, 8.245, 7.664, and 6.782 ppm, respectively and these values were significantly (p<0.05) different to each other. Besides, the larvae obtained from 0.5, 2.0, and 8.0 ppm sumithion were reared against a control for 21 days to assess the residual effect. Significant negative effects on both growth and survival of the larvae were found among the sumithion concentrations used. The study therefore, suggested that sumithion has adverse and residual effects on the breeding performance along with early embryonic and larval development of *H. fossilis* even in lower concentration.

Keywords: Toxicity, Sumithion 50 EC, *Heteropneustes fossilis*, LC_{50}, Fertilized eggs, Larvae, Growth indices, Viability

Introduction

Pesticides are substances used to control pests, including insects, aquatic weeds, plant diseases, aquatic snails etc. These pesticides have been found to be highly toxic not only to fish but also to the other organisms, which constitute the food chain (Sabra and Mehana, 2015). Use of pesticides have been increased very extensively in agriculture, forestry, public health and in veterinary practices during the past two decades coinciding with changes in intensive farming practices. Although the pesticides are often very effective, many of them represent a potential hazard and some of them persist for a very long time in the environment and in most cases their impacts on the local fauna are unknown. Therefore, the uses of these pesticides worldwide give rise to concern on health and environmental effects.

Sumithion 50 EC, the *O*,*O*-Dimethyl *O*-(3-methyl-4-nitrophenyl) is one of the most frequently used pesticides in the agricultural region all over the world. Its principal use is to control a wide spectrum of chewing and sucking insects on rice, orchard fruits, vegetables, cereals, cotton, and forest (Minister *et al*., 1984). While such pesticides are used significantly for enhancing agricultural yield a certain portion are eventually transmitted and accumulated into the water body ensuing short and long term effects on the aquatic lives. Sumithion that reach into the aquatic habitats have been found to impair various physiological processes of fish (Bhuiyan *et al*., 2001). Accumulation of this toxic chemical is known to adversely affect many organs and systems of fish body such as the liver, kidney, thyroid gland, nervous

system, immune system as well as reproductive system of fishes. Sumithion exposure decreases the hemoglobin content of tilapia and 30 ppm sumithion has been found sufficient to kill all test fish within an hour (Koundinya and Ramamurthy, 1978; Haque and Barua, 1988). Ovarian recrudescence was also observed in *Channa punctatus* when treated with LC_{50} dose of sumithion (Saxena and Sehgal, 1986). Even human body can be exposed to such hazardous chemicals down the food chain for instance through eating contaminated fish.

Stinging catfish, *Heteropneustes fossilis* is one of the important catfishes of Bangladesh with good market demand due to its palatability and less spine (Tripathi *et al*., 1997). It breeds in shallow inland waters during the onset of monsoon and is capable of breeding in ponds when sufficient rainwater accumulates (Talwar and Jhingran, 1992). Their eggs, embryos and larvae are often exposed to the toxicity of pesticides in nature when pesticides run off into natural water body from agricultural field. Moreover, in all *H. fossilis* nursery pond, sumithion is usually applied to control the Backswimmer, *Notonecta glauca* where it can render its toxicity to the water. Although a large number of experiments have been carried out to investigate the effects of sumithion on the various morphological and physiological aspects of fishes (Kabir *et al*., 2013; Sancho *et al*., 1998) and other aquatic organisms (Pawar and Katdare, 1982) little attempts have been undertaken to examine its effect on the developing fish embryo and larvae; the most sensitive stage in the life cycle of a teleost fish. Therefore, the present study was carried out to determine the toxicity effects of sumithion to *H. fossilis* on their breeding performance as well as incubation period, survivability, hatching rate of fertilized eggs and subsequent larval growth. The stinging catfish was selected for the bioassay experiment because of its wide distribution and currently cultured everywhere in Bangladesh.

Materials and Methods

Brood fish and sumithion

The study was carried out at the Mini Hatchery cum Breeding Complex, adjacent to the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh during the period on 1st February to 30th May 2015. A total of 400 adult *H. fossilis* (average length: 20.77 cm, average weight: 30.25 g) were collected from the *haor* of Mohanganj, Netrakona, Bangladesh without any injury and transported to the study area through oxygenated polythene bags. Upon arrival at the hatchery the fish were acclimatized in the cistern (1.23×2.44×0.46m^3) for one week and then stocked in two brood rearing ponds of 2 decimal sizes each for a period of 4 months to monitor growth and breeding performances. Length and weight of 8 fish from each pond were recorded monthly. One of the two ponds was considered as experimental pond and the other as control. In experimental pond sumithion at the dose of 0.24 ppm was applied every 7 days interval while the control pond did not receive any pesticide dose. Before stocking the fish, ponds were prepared properly to produce sufficient natural food. Fish were fed with Quality premium; crumble-2 feed at 5% of their body weight. Both the ponds were under same management practices. Sumithion 50 EC (50% Fenitrothion), the product of Sumitomo Chemical Company Limited (Japan), was purchased from the Setu Agro Industries Limited, Bangladesh

Induced breeding and collection of fertilized eggs

Ten pairs of healthy and sexually mature *H. fossilis* were selected from both of the sumithion treated and control ponds on the basis of some external features. The gravid females were identified by the presence of soft and swollen abdomen as well as round and bulged urogenital papillae. The males were recognized by their flat abdomens and long protruding genital papillae (Fig. 1). In addition, the maturity of the ripe female was confirmed by a slight pressure on the ventral side of the fish for oozing of eggs. The selected broods were kept in cistern for about 6 h for conditioning to the new environment and constant water supply was maintained to ensure proper aeration. The female and male fish were artificially induced by intra-muscular injection with 50 mg carpPG/kg bw and 25 mg carpPG/kg bw, respectively. The induced breeding technique was same for both the sumithion treated and control group. Hormone injected fishes were then kept in separate *hapas* set within the cistern providing continuous water flow through porous PVC pipe (Fig. 2). After 12 h of hormone administration, females released eggs naturally and males also released their sperm to fertilize the eggs. Similar tendency was seen in both sumithion treated and control groups. The fertilized eggs were collected separately from the *hapas.* The fertilized eggs from the control

group were immediately placed in experimental units for embryo toxicity assay. Simultaneously, fertilization rate, incubation period and hatching rate of sumithion exposed fish were recorded. A portion of fertilized eggs were released into the glass aquarium to obtain hatchlings for the larval bioassay studies.

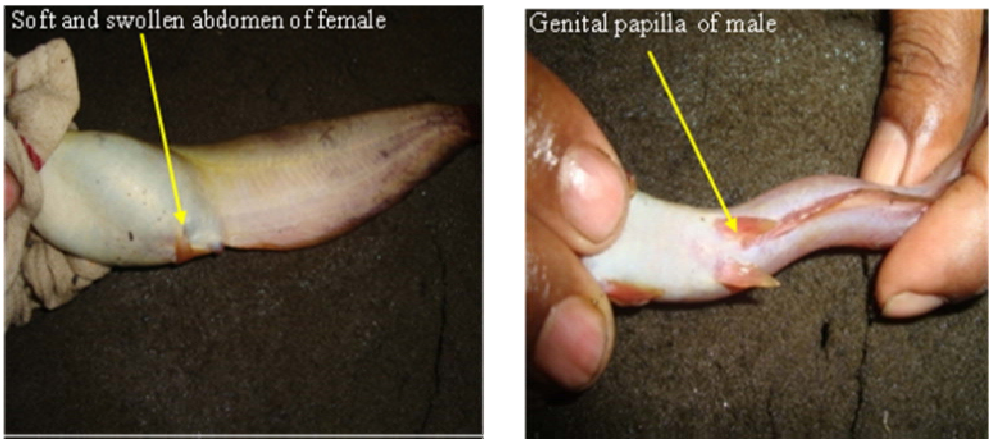

Fig 1. Brood female and male of *Heteropneustes fossilis* showing (a) soft and swollen abdomen of female and (b) long protruding genital papilla of male

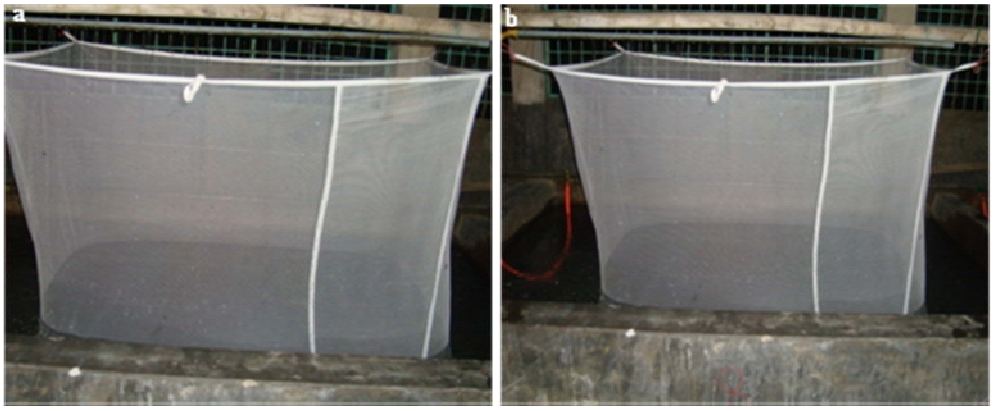

Fig 2. Hapa set in two adjacent cisterns for breeding of *Heteropneustes fossilis* (a) sumithion treated and (b) untreated

Effects of sumithion on the viability of embryo

Stock solution (1000 ppm) was prepared by dissolving a weighed amount of sumithion in distilled water. Then the appropriate amount of stock solution was taken into a glass jar and required amount of tap water was added in order to prepare the desired concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 ppm for embryonic and larval bioassay. The jar was then gently stirred to ensure complete mixing. The control (0 ppm) group was exposed to tap water. Approximately 6000 fertilized eggs of *H. fossilis* were randomly selected and exposed to previously prepared sumithion concentrations in plastic bowls as treatments with a control for assessing the effects of sumithion on the fertilized eggs. Each bowl was 6 cm deep having an internal diameter of 16 cm with effective water holding capacity of 1 L (Fig. 3). All the treatments were replicated five times. Therefore, a total of 30 plastic bowls were used having 200 eggs in each and the experiment was allotted following randomized complete block design (RCBD). Continuous oxygenation was ensured with the help of aerator connected with plastic tubes. Water quality characteristics of the experimental units were determined according to APHA (1985)[12]. The mean values for test water qualities were as follows: temperature 25.0±1.0°C, pH 7.3 ± 0.3, dissolved oxygen 7±0.2 mg/L and total

hardness 34.0±4.7 mg/L. Every after 6 h of incubation, the numbers of dead and mouldy eggs afterward dead larvae were counted and removed. Upon completion of hatching, the survival rate, hatching rate and incubation periods were determined using the following formulae:

$$\text{Survival rate (\%)} = \frac{\text{Number of eggs alive}}{\text{Total number of eggs stocked}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs}} \times 100$$

Incubation period (h) = Hatching time - Fertilization time

To study the larval toxicity, around 200 hatchlings were set into each aquarium and every test concentrations and control groups were executed with five replicates from the stock aquarium. After 24, 48, 72, and 96 h exposure periods to six different concentrations of sumithion, dead larvae in experimental and control groups were counted.

Growth indices of larvae produced from sumithion treated eggs

The larvae produced from three different sumithion concentrations (0.5, 2.0, and 8.0 ppm) were reared for 21 days to inspect subsequent toxicity effects of sumithion on growth indices. Around 1600 larvae of 5 days old having initial weight of 7.6±1.52mg and length of 5.0±0.71mm were collected and kept into separate plastic bowls. Each group was again replicated four times along with control bearing 100 eggs in each. The experimental bowl was 21 cm deep having an internal diameter of 32 cm with an effective water holding capacity of 10 L and facilitated for continuous flow of water from the porous PVC pipes as inlet along with outlet facilities (Fig. 4). The larvae were fed with live chopped Tubificid worms up to satiation level twice daily, once in the morning (8:00 am BDT) and next in the afternoon (5:00 pm BDT). The larvae were considered satiated when they stopped searching the food. The leftover food was removed from the bowls after 1 h of food application. Each bowl was cleaned once daily before providing food in the morning. All the experimental bowls were under same management protocol. Broken earthen pots locally known as "*chara*" was used as a shelter in the bowls as the larvae have a tendency to hide under the shelter. The experiment was continued for 21 days. During the study, water temperature by using a celcius thermometer, pH by a portable digital pH meter (MICRO-TEMP, pH 500), and dissolved oxygen (DO) by a digital DO meter (multi 340 i/set, DO-5509; Germany) were recorded as 26.4±0.9 °C, 7.5±0.3, and 6.7±0.5 ppm, respectively.

Fig. 3. Hatching devices for fertilized eggs of *Heteropneustes fossilis* under different sumithion concentrations

Fig 4. *Heteropneustes fossilis* larvae rearing unit

Performance evaluation of *H. fossilis* larvae

Periodic sampling was done every 7 days interval to assess the health condition of larvae. Ten larvae were randomly collected from each of the bowl by a small scoop net for measuring their length and weight. Weight was taken by a sensitive portable digital electric balance (METTLER TOLEDO, Switzerland) and the length by placing the larvae on a petridish placed on a graph paper. Furthermore,

the final mean length, weight, survival and specific growth rate (SGR) of larvae were recorded at the time of final harvesting. Sampling was done before the application of food to avoid the biasness of weight due to presence of excessive food. The following parameters were considered:

$$\text{Percent length gain} = \frac{\text{Average final length} - \text{Average initial length}}{\text{Average initial length}} \times 100$$

$$\text{Percent weight gain} = \frac{\text{Average final weight} - \text{Average initial weight}}{\text{Average initial weight}} \times 100$$

Specific growth rate:

$$\text{SGR (\%day)} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100 \text{ (Brown, 1957)}$$

Where, W_2=Final live body weight at time T_2

W_1= Initial live body weight at time T_1

$$\text{Percent survival (\% survival)} = \frac{\text{Number of larvae alive}}{\text{Total number of larvae stocked}} \times 100$$

Statistical analysis

The evaluations of the treatment mean were made by using one-way analysis of variance (ANOVA). Significant results were tested by using Duncan Multiple Range Test (DMRT) to identify the specific differences among the means. Data obtained from the sumithion acute toxicity tests were evaluated using the Probit Analysis Statistical Method. The LC_{50} values (with 95% confidence limits) were calculated and the significance level between the LC_{50} values and the different exposure times was analyzed using a χ^2test. The statistical data analysis was carried out with the help of the computer software SPSS (SPSS, Chicago, IL, USA) version 16.0.

Results and Discussion

The total length and weight were observed for sumithion treated and untreated *H. fossilis* from February to May and the values are presented in Table 1. The initial average lengths and weights of sumithion treated and control fish were 17.34±2.62 cm and 32.37±15.32 g, respectively. The final average lengths of sumithion treated and untreated fish were 25±2.33 and 27.21±4.25 cm, respectively and the final average weights were found to be 42.98±10.13 and 46.89±14.21 g, respectively. Although the treated fish apparently showed lower average length and weight than the control group but, statistical analysis showed no significant (p>0.05) difference in growth performance of sumithion treated and untreated fish.

Table 1. Growth performances of sumithion treated and untreated fish in terms of average length and weight during 4 months experimental period

Months	Sumithion Treated		Untreated (Control)	
	Length (cm) Mean±SD	**Weight (g)** Mean±SD	**Length (cm)** Mean±SD	**Weight (g)** Mean±SD
Initial	17.34±2.62	32.27±15.33	17.34±2.62	32.27±15.33
February	18.84±1.91	32.33±10.42	20.60±1.59	33.93±16.49
March	20.25±1.55	34.09±12.21	22.66±3.30	37.19±16.98
April	23.19±1.38	40.16±12.23	25.08±3.25	42.04±13.30
May	25.00±2.33	42.98±10.12	27.21±4.25	46.89±14.21

In order to determine the effects of sumithion on the breeding performances of *H. fossilis*, breeding trials were given at both sumithion treated and untreated fish in two separate cisterns. No significant difference was found in ovulation rate between sumithion treated and untreated group that is to say, ovulation rate was found to be 100% in both cases. The fertilization and hatching rates of sumithion treated fish were 72.67 and 64.33%, respectively whereas, such values for untreated fish were 85.33 and 79.33%, respectively. Statistical analysis showed that fertilization and hatching rates of treated group were

significantly (p<0.05) lower than the control. Bhuiyan *et al*. (2001) reported that exposure of sumithion at the dose of 100 ppm to sexually mature *Channa punctatus* showed fragmented ova with abnormal shape and arrangement while normal arrangement of ova was found in case of controlled fish. Sivarajah *et al*. (1978) observed fragmentation and karyolysis of ova when *Salmo gairdneri* and *Cyprinus carpio* were exposed to pesticide aroclor 1254. Jha and Jha (1994) mentioned the impact of urea (416 ppm) and ammonium sulphate (448ppm) on the ovary of *H. fossilis.* Urea induced initial stimulation of vitellogenesis followed by subsequent arrest of ovarian growth. Moreover, the cells of germinal epithelium developed hyperplasia leading to the complete fusion of the two follicles. Contrary to urea, ammonium sulphate produced severe adverse effects as evident from large number of early non-vitellogenic oocytes and traces of reovulatory degenerated oocytes. These may be reasons for getting lower fertilization and hatching ratesin pesticide treated group compared to that of control.

The acute toxicity of sumithion on the incubation, survival and hatching rates of embryo are presented in Table 2. It has been noticed that increasing sumithion concentrations has significant effects on incubation period, survival and hatching success of fertilized eggs of *H. fossilis.* The incubation period of *H. fossilis* embryo was estimated at 20.18 to 24.24 h for different sumithion doses and 19.17 h for control group. Statistical analysis revealed that incubation period increased significantly (p<0.05) with raising sumithion concentrations in all the treated groups. The present result is strongly supported by the findings of Konar (1969) who found incubation period increases with the increase of pesticide concentration of dichlorvos (2, 2-dichlorovinyl dimethyl phosphate). Marimuthu *et al*. (2013) found the similar result for incubation of African catfish, *Clarias gariepinus* when exposed to different buprofezin (an organophosphate insecticide) concentrations that is, the higher the pesticide concentration, the longer the time needed to hatch.

Table 2. Acute toxicity of sumithion on *Heteropneustes fossilis* embryos and larvae (n=200 for initial eggs and larvae in five replicates)

Concentration ppm	Embryonic stage			Larval stage Number of dead larvae			
	Number of dead embryos	Hatching success (%)	Incubation period (h)	24 h	48 h	72 h	96 h
0.5	39.2±4.2	85.0±2.7	20.2	29.2±6.6	39.6 ±4.3	46.0±4.2	54.2±5.1
1.0	49.8±5.5	76.2±2.4	21.3	38.6±7.9	48.2±5.6	55.4±3.2	65.6±4.0
2.0	60.0±10.1	73.0±4.9	22.2	48.2±6.3	55.0±3.8	60.0±4.3	70.0±2.4
4.0	70.8±8.0	65.0±4.5	23.2	56.4±6.8	62.4±5.9	68.8±4.3	75.6±4.4
8.0	85.6±6.8	51.2±6.6	24.3	80.4±4.5	84.0±6.0	89.8±6.5	97.8±7.4
Control	9.4±3.4	90.8±3.2	19.7	3.4±2.9	5.4±2.5	9.6±1.8	17.0±2.8
χ^2 value	46.370	-	-	68.093	68.835	57.658	50.101
P value	<0.05			<0.05	<0.05	<0.05	<0.05
LC_{50} value (ppm) with 95% confidence limits	7.803 (6.997-8.927)	-	-	8.677^{a} (7.612-10.289)	8.245^{ab} (7.205-9.832)	7.664^{c} (6.778-8.961)	6.782^{d} (6.064-7.791)

LC_{50} values with different superscripts are significantly different (p< 0.05).

The 24 h LC_{50} value (95% confidence limits) of sumithion for embryos was found to be 7.803 (6.997-8.927) ppm. The survival and hatching rates of fertilized eggs decreased with the increased sumithion concentration from 0.5 to 8 ppm. The survival rates of embryo at 0.5, 1.0, 2.0, 4.0, and 8.0 ppm were 80.40, 78.00, 74.00, 71.00, and 68.20%, respectively whereas; in control the survival rate was found to be 83.20%. The hatching rates of fertilized eggs when exposed to 0.5, 1.0, 2.0, 4.0, and 8.0 ppm sumithion were found to be 85.0, 76.2, 73.0, 65.0, and 51.2%, respectively while 90.8% for control group (Table 2). Statistical analysis showed that both survival and hatching rates of *H. fossilis* embryo at 1.0, 2.0, 4.0, and 8.0 ppm were significantly (p<0.05) lower than that of control group. Among the five treated groups, the survival and hatching successes of eggs at 0.5 ppm was found significantly higher (p<0.05) than those of others. Takimoto *et al*. (1984) stated that exposure time of *Oryzias latipes* embryos to increasing concentrations of the organophosphate fenitrothion (sumithion) resulted in significantly different degrees

of mortality and hatching success. Marimuthu *et al*. (2013) described that mortality of embryo of African catfish, *C. gariepinus* increased significantly with increasing buprofezin concentrations from 5 to 100 ppm. They also described that increasing buprofezin concentrations has significant effects on hatching success of fertilized eggs. The number of dead embryos has also been found to increase significantly with the increasing concentration of an organophosphate pesticide diazinon for 0.25, 0.5, 1, and 2, 4 to 8 ppm (Aydin and Koprucu, 2005). The present findings strongly support the results of the Marimuthu *et al*. (2013), Takimoto *et al*. (1984), and Aydin and Koprucu (2005). These observations clearly indicated that, sumithion has great adverse effects on the embryonic development of *H. fossilis*. However, more study is required to find out how sumithion interfere specifically the normal development and hatching process of *H. fossilis* embryo.

In the larval stage, the number of dead larvae at certain sumithion doses was examined in relation to the duration (24, 48, 72, and 96 h) of exposure. The number of dead larvae significantly increased with increasing concentrations exposed for 24-96 h. There were significant differences in number of dead larvae between the exposure times 24-96 h and in each concentration (p<0.05). The highest concentration of 8 ppm showed the highest larval mortality. The 24, 48, 72, and 96 h LC_{50} values (with 95% confidence limits) of sumithion for *H. fossilis* larvae were estimated to be 8.677 (7.612-10.289), 8.245 (7.205-9.832), 7.664 (6.778-8.961), and 6.782 (6.064-7.791) ppm, respectively (Table 2). There were significant differences in the LC_{50} values obtained at different exposure times (p<0.05) and we found here sumithion to be highly toxic to *H. fossilis* larvae. During development sensitivity may change with some compounds showing higher sensitivity in embryos whereas others are more toxic to larvae (Fent and Meier, 1994; Gaikowski, 1996). There are differences in the acute toxicity of sumithion for various fish species. In European eel (*Anguilla anguilla*), the 96 h LC_{50} values range even in hundredths ppm (Sancho *et al*., 1993). The 96 h LC_{50} values of an organophosphate diazinon was reported as 0.1-0.5 ppm for fry bluegill (*Lepomis macrochirus*), 0.88 ppm for American oyster (*Crassostrea virginica*), 1.47 ppm for sheepshead minnow (*Cyprinodon variegates*), 1.65 ppm for fry rainbow trout (*O. mykiss*), and 7.80 ppm for fathead minnow, *Pimephales minnow* (2000; Available at http://www.epa.govt.nz/; accessed July 2016). Again, the 96 h LC_{50} values of diazinon for guppy (*Poecilia reticulate*) was found to be 0.8 ppm but for zebra fish (*Brachydanio rerio*) it was found to be 8 ppm (Keizer *et al*., 1991). Oh *et al*. (1991) presented three factors causing the selective toxicity of organophosphate for various fish species: different inhibition of acety lcholinesterase, different detoxification and absorption.

Larvae produced from the pesticide exposed groups of three concentrations such as, 0.5, 2.0, and 8.0 ppm of sumithion were further reared in underground water to observe the effects of remaining sumithion on the subsequent growth and survival of the *H. fossilis* larvae. The result of the growth parameters for instance, percent length gain and percent weight gain, SGR and survival rate of larvae are summarized in Table 3. The percent length gains of the larvae produced from the control, 0.5, 2.0, and 8.0 ppm were 640±12.65, 599±29.46, 415.675±22.14, and 373.50±26.84 mm, respectively and percent weight gains were 2318.94±58.79, 1701.98±18.28, 1324.87±14.83, and 1109.21±75.25 mg, respectively. Significant differences (p<0.05) were found among the treated groups at both percent length gains and percent weight gains of the larvae. Amid the treated groups, larvae produced from 0.5 ppm sumithion showed the highest percent length gain and percent weight gain compared to 2.0 and 8.0 ppm.

The final SGR and survival rate of the larvae of *H. fossilis* were investigated and both the parameters were found significantly (p<0.05) different within the control and treated groups. The SGR and survival rate decreased significantly with increase of sumithion concentrations. The final SGR values in control, 0.5, 2.0, and 8.0 ppm were determined as 15.17±0.12, 13.77±0.05, 12.65±0.05, and 11.85±0.12 mm, respectively, and the survival rates were found to be 80.5±2.08, 71.25±2.63, 61.75±3.30, and 54±4.76mg, respectively (Table 3). These results suggested that the larvae produced from the fertilized eggs treated with 0.5, 2.0, and 8.0 ppm of sumithion had significantly (p<0.05) lower SGR and survival rate compared to those of control group after 21 days of experimental period. The SGR and survival rate of the larvae among the treated groups were also significant and the larvae produced from the eggs treated with 0.5 ppm sumithion showed significantly (p<0.05) higher SGR and lower mortality than those of 2.0 and 8.0 ppm, respectively. The results of this study revealed that the increase of sumithion concentrations to the

fertilized eggs of *H. fossilis* decreased growth and survival indicating toxic nature of sumithion to the *H. fossilis* larvae. Kabir *et al*. (2013), found similar mortality trend when *H. fossilis* larvae were treated with different sumithion doses that is, the mortality rates of the larvae gradually increased with the increasing concentrations of sumithion even in lower dose (from 145 µg/L to 314 µg/L). Our results are similar to those of many previous studies, decreased growth performance and increased mortality of the larvae in accordance with the increased pesticide concentrations (Rashid *et al*., 2012; Machova, 2010). The results of the current study denote that exposure of sumithion exerts developmental toxicity, creates deformities, and enhances mortality of larvae; and thus the toxic effect of sumithion to *H. fossilis* eggs, embryos and larvae are irreversible.

Table 3. Growth and survival rates of *H. fossilis* larvae produced from three different sumithion concentrations during 21 days experimental period

Sumithion concentraions (ppm)	Replication	Percent length gain (mm)	Percent weight gain (mg)	SGR (% day)	Survival rate (%)
0.5	R1	620.00	1697.37	13.76	75
	R2	580.00	1692.12	13.74	70
	R3	628.00	1728.95	13.84	69
	R4	568.00	1689.47	13.74	71
	Mean ± SD	**599.00^{b}±29.46**	**1701.98^{b}±18.28**	**13.77^{b}±0.05**	**71.25^{b}±2.63**
2.0	R1	391.50	1310.00	12.60	66
	R2	405.20	1318.42	12.63	62
	R3	423.60	1344.74	12.72	61
	R4	442.40	1326.32	12.65	58
Mean ± SD		**415.68^{c}±22.14**	**1324.87^{c}±14.83**	**12.65^{c}±0.05**	**61.75^{c}±3.30**
8.0	R1	344.80	1150.00	12.02	57
	R2	356.80	1005.26	11.44	59
	R3	400.00	1176.32	12.12	51
	R4	392.40	1105.26	11.85	49
Mean ± SD		**373.50^{d}±26.85**	**1109.21^{d}±26.85**	**11.86^{d}±0.30**	**54.00^{d}±4.76**
control	R1	624.00	2231.00	14.99	81
	R2	652.00	2352.63	15.23	80
	R3	648.00	2350.00	15.23	83
	R4	636.00	2342.12	15.23	78
Mean ± SD		**640.00^{a}±12.65**	**2318.94^{a}±58.78**	**15.17^{a}±0.12**	**80.50^{a}±2.08**

Mean values in the column with different superscripts are significantly different at $P<0.05$.

Conclusion

Long term exposure to pesticides causes a continuous health hazard of fish population. So, human population is at high risk by consuming these toxicated fishes. Therefore, attention must be given to the adverse effects of sumithion as well as other similar pesticides on non-targeted aquatic species. It could be said that sumithion contamination is dangerous to the aquatic ecosystems, and the issue should be taken into consideration when this pesticide is used in agriculture or in the control of insect populations. The findings of current research highlights that, exposure of sumithion to *H. fossilis* at an earlier stage of the life cycle significantly reduces the number of returning adults. However, for a safe use of this pesticide to the aquatic ecosystem, more research work should be carried out to determine the appropriate concentration and duration that will not induce remarkable sub-lethal effects to fish.

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Conflict of interest

We, the authors, declare that we have no conflict of interest.

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