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HISTO-ARCHITECTURAL CHANGES OF INTESTINAL MORPHOLOGY IN ZEBRA FISH (*Danio rerio*) EXPOSED TO SUMITHION

Sk. Istiaque Ahmed[1,2], Mirja Kaizer Ahmmed[2], Subrata Kumar Ghosh[2], Md. Moudud Islam[2] and Md. Shahjahan[1*]

[1]Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; [2]Faculty of Fisheries, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong- 4225, Bangladesh

***Corresponding author:** Dr. Md. Shahjahan; E-mail: mdshahjahan@bau.edu.bd

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Organophosphorous pesticide sumithion, the O, O Dimethyl O- (3-methyl-4-nitrophenyl), is a widely used pesticide in agricultural land and in aquaculture to control some harmful pests. A study was conducted in laboratory condition with aquaria (36 inch × 10 inch × 12 inch) to evaluate the effects of sumithion on histo-architecture of intestine in zebrafish (*Danio rerio)*. The experiment was carried out with three treatments (T_1: 0.5 ppm, T_2: 1.0 ppm, T_3: 2.0 ppm) and a control (T_0: 0ppm), each having three replications. Zebra fishes (*Danio rerio*) (4±1cm and 0.9±0.2g) were stocked for the experiment and sacrificed after 7 days of exposure of sumithion. During the study period, the temperature was almost constant (21-22^{O}C) but dissolved oxygen, pH and total alkalinity values were tended to decrease with the increase in concentrations of test chemicals. The histo-architectural changes in intestine suggested that the intestinal epithelial cells, lumen, villi and intestinal folding were varied significantly ($P<0.05$) in treatment groups rather than the control groups (T_0). Disappeared mucosa (DM) along with abnormal lumen (AL) were found in case of T_1, while destructed intestinal villi (DV), sloughing of superficial epidermal cells (SEC) and uneven intestinal folding (UF) were found in T_2 and T_3. The obtained result supports the toxic potentiality of sumithion. Therefore, the use of sumithion must be evaluated carefully in agriculture and aquaculture.

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INTRODUCTION

Pesticides have been considered as a major threat of gradual degradation for the aquatic ecosystem (Salam et al., 2015; Sharmin et al., 2015). Thirty nine insecticides, under four major groups *viz.* organochlorine, organophosphate, carbamate and pyrethroid, are being used in agricultural and public health sector (Satter, 1985) of which over 98% of sprayed insecticides and 95% of herbicides have impacts not only upon their target species but also on non-target species, air, water, bottom sediments and food (Miller and Miller 2004; Rahman et al., 2012).

Sumithion, an organophosphate insecticide is considered somewhat toxic to fish (Thomson, 1989) but no clear evidence on the intestinal morphological alteration was found so far. Toxicology studies on different tissues (Thophon et al., 2003) can provide information about tissues injuries and damages of organs resulting in morphological dysfunction.

Being one of the most common, small and robust vertebrate model organisms, zebrafish got the highest priority to conduct this experiment (Shahjahan et al., 2013). Generally, every species possess an immense power of biotic potential in favorable condition by increasing the population to the explosive level. But, in adverse condition, different organs such as intestine, gonad etc. may be affected. Therefore, this study was carried out to determine the effect of sumithion on intestine of zebrafish.

MATERIALS AND METHODS

Proper and absolute analysis of impacts of pesticides and their contamination under field conditions is extremely difficult, but the need for field tests in this aspect can be reduced to a greater extent by the implementation of controlled laboratory tests. That is why, the present study was carried out from January to April, 2014 at the wet laboratory of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh.

Species selection

The Zebrafishes (*Danio rerio*) with a length of 4±1 cm and weight of 0.9±0.2g, were selected on the basis of the health condition from different ponds adjacent to academic building of Fisheries Faculty, BAU. The selected fishes were acclimatized in aquaria at 22 ± 0.5°C under a controlled natural photo-regimen (14/10 h, light/dark) condition for a period of 21 days before the experiments. During acclimatization process, the fish were fed twice a day with commercial grower feed (CP Bangladesh Co., Ltd.).

Experimental procedure for acute and behavioral toxicity

According to the standard method, a static acute toxicity bioassay was performed to determine the 24, 48, 72, and 96 h lethal concentration values (LC50) of sumithion for Zebra fish. Seven different concentrations (4, 5, 6, 7, 8, 9, and 10 ppm) of sumithion with three replicates were used in the test series. Control units with three replicates were also prepared. Exceeding aeration was applied to the aquarium for 2 h in order to obtain a homogeneous concentration of the toxic compound, and then 10 fish were transferred into each aquarium. Mortality was assessed at 24, 48, 72, and 96 h after the start and dead fishes were removed immediately. Several behavioral changes, such as reduced activity, equilibrium imbalance, abnormal swimming and motion inactivity of the fishes were observed during the exposure period.

Experimental design

Twelve aquaria (36 inch × 10 inch × 12 inches) were collected, cleaned, washed and sun-dried properly prior to set in the wet laboratory. The experiment was conducted with three treatments (T_1: 0.5 ppm, T_2: 1.0 ppm, T_3: 2.0 ppm) and a control (T_0: 0ppm), each having three replications. Ten fish were stocked in each aquarium containing 20L of tap water. Fish were sacrificed after the desired exposure of sumithion (7 days) to observe the effects on intestine. The fish sample was collected and fixed in 10% formalin for the use of histological analysis. The application of pesticide at desired concentration was reapplied at every 24 h with a regular exchange of water.

Feeding frequency

The feeding frequency was 2 times per day (9.00am and 9.00pm) at a rate of 70% of their body weight throughout the experimental period. Before introducing feed for the next feeding, previous uneaten feeds and feces in aquarium were removed by siphoning using a plastic pipe.

Monitoring of water quality parameters

During the experimental period, the water quality parameters such as temperature with a thermometer, pH with a pH meter, dissolved oxygen with a DO meter and total alkalinity with hachkit were recorded.

Histological study of intestine

For histological analysis of intestine, three fish species were selected from each treatment. Then cephalic and caudal portion of the selected fishes were cut off and the intestinal section was preserved at 10% formalin. The preserved samples were taken out from vials and put into cassettes separately. Then dehydration process was carried out manually followed by clearing, infiltration, embedding, sectioning, staining and mounting. Finally, intestinal sections were observed under microscope and photographs were taken at 10x magnification.

Statistical analysis

Values were expressed as means ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess statistically significant differences among the control and different treated values. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using PASW Statistics 18.0 software (IBM SPSS Statistics, IBM, Chicago, USA).

RESULTS AND DISCUSSIONS

Acute and behavioral toxicity

The mortality patterns of the test species exposed to different doses of sumithion are presented in Table 1. No mortality was observed in control treatment, whereas the mortality percentage increased as the concentration of sumithion increased. The LC50 value of zebrafish for sumithion during the 96 h of exposure was 7.89 ppm (≈8). A series of abnormal behavior such as restlessness, sudden quick movement, rolling movements, swimming on the back (at higher doses) etc. was observed during the continuation of experiment. Due to the application of higher doses, the affected fish became extremely weak and ultimately died. No such abnormalities were observed in terms of behavior in the control group.

In the present study, the LC50 value (7.89 ppm) recorded for *D. rerio* is less than the values (9.14 ppm for *Ptychocheiilus lucius*, 11700 µg/L for black bullhead, 11.8 ppm for *Heteropneustes fossilis*, 15.3 ppm for *Gila elegance* and 17.0 ppm for *Ictalurus furcatus*) determined by Durkin (2008) and Faria *et al.* (2010) for different fish species. In contrast to the above-mentioned values, Pathiratne and George (1998) reported a lower 96 h LC50 value (2.2 ppm) for *Oreochromis niloticus*. Newhart (2006) tabulated the LC50 values of malathion for different species of fish which ranges from 0.06 to 7620 µg/L. Malathion was found to be highly toxic to fry of *Labeo rohita* (LC50 value 9 µg/L), Patil and David, (2008); *Opheocephalus punctatus* (LC50 16 µg/L), Pugazhvendan *et al.,* (2009); walleye (LC50 64 ppb), brown trout (LC50 101 ppb) and cutthroat trout (LC50 280 ppb) and moderately toxic to minnows (LC50 8.6 ppm) and murrels (LC50 5.93 ppm) as summarized by Durkin (2008). The difference in the potentiality of pesticides toxicity may be attributed mainly to the susceptibility of the test animals and several factors like pH and hardness of water. The observation of a series of abnormal behaviors such as restlessness, loss of equilibrium, increased opercular activities, surface to bottom movement, sudden quick movement, resting at the bottom, etc. were similar to the observations of Haque *et al.* (1993) and Lovely (1998). Some aspects that are contrary to the findings of Kabir and Begum (1978) and Lovely (1998) such as swelling in the abdominal region and gas-filled stomach were not observed.

Effects of sumithion on water quality parameters

Recording of water quality parameters (Temperature, Dissolved oxygen, pH and Total Alkalinity) was a regular task during the exposure period of sumithion at various concentrations as well as with control. During the study period of 7 days the values of Temperature were almost constant regardless of the application of treatments. Dissolved oxygen, pH and total alkalinity values were tended to decrease with the increase in concentrations of test chemicals (sumithion) (Table 2), but the values were not significantly varied (P> 0.05).

Table 1. Mortality percentages of the selected fish samples exposed to different concentrations of sumithion at different time intervals.

SL No.	Concentration (mg/L)	Initial No. of fish	Count of dead fish after				% of mortality
			24 h	48 h	72 h	96h	
1	Control	10	-	-	-	-	00
2	4.0	10	-	-	-	1	10
3	5.0	10	-	-	-	2	20
4	6.0	10	-	-	2	3	30
5	7.0	10	1	2	3	4	40
6	8.0	10	-	2	4	5	50
7	9.0	10	1	3	7	9	90
8	10.0	10	2	7	9	10	100

Table 2. Water quality parameters (Means ± SD) during the study period.

Parameteres	Treatments			
	T_0 (0 ppm)	T_1 (0.5 ppm)	T_2 (1.0 ppm)	T_3 (2.0 ppm)
Temperature (^OC)	21.6± 0.71	21.48 ± 0.34	21.34 ± 0.15	22.20 ± 0.40
pH	7.21 ± 0.07	6.81 ± 0.07	6.58 ± 0.11	6.40 ± 0.13
Dissolved oxygen (mg/L)	4.62 ± 0.12	3.41 ± 0.15	3.18 ± 0.19	2.93 ± 0.12
Total Alkalinity (mg/L)	175.71 ± 1.38	163.57 ± 1.98	159.28 ± 6.36	149.28 ± 6.89

There have been profound significances on environmental parameters in affecting the toxicity of different pesticides. Considering the significance of temperature on different factors like enzyme activity, metabolic rate, oxygen uptake etc, it has been studied more widely than any other environmental parameters. Generally, toxicity is more or less proportionate to high temperature. Macek et al. (1969) studied the effects of 10 pesticides to the rainbow trout and 11 pesticides to the bluegills at different temperatures and found that the toxicity increased with increasing temperature. P^H has also been found significant in influencing various physicochemical properties of pesticides like hydrolysis, volatilization and in balancing the dissociated and undissociated forms (Weber, 1972). The toxicity of organophosphate (OP) compounds is not influenced by pH very commonly except in few cases. The toxic effects of 2, 4-D were reduced when the pH was raised by the addition of sodium chloride (Holcombe et al., 1980). Davies (1975) attempted to formulate the criteria for minimum dissolved oxygen requirement of fish. His approach was on examining the threshold levels of dissolved oxygen that cause changes in some physiological lesions. Channel Catfish exposed for 72h to an oxygen content of 1.5 ppm showed anomalies in gill, liver, kidney and spleen (Scott and Rogers, 1980). In the present investigation the variation of the different water quality parameters (Temperature, Dissolved oxygen, pH and Alkalinity) that were monitored during the exposure period within various concentrations of pesticides as well as with the control were not significant. The limited variations in these parameters among different treatments may be due to regular renewal of water and pesticide at every 24 hours.

Effects of sumithion on histo-architecture of intestine

In this experiment, the histo-architecture of zebrafish intestine was observed through histology. The intestinal epithelial cells, lumen, villi and intestinal folding were almost regular in control groups (T_0), while in treatments various abnormalities were found. In T_1, the intestinal mucosa was almost disappeared (DM) along with abnormal lumen (AL). On the other hand, destructed intestinal villi (DV), sloughing of superficial epidermal cells (SEC) and uneven intestinal folding (UF) were found in T_2 and T_3 (Figure 1). The result also indicated that the Zebra fish intestine was damaged by 20%, 50% and 70% in T_1, T_2 and T_3, respectively (Figure 2) and varied significantly ($P<0.05$) among treatments.

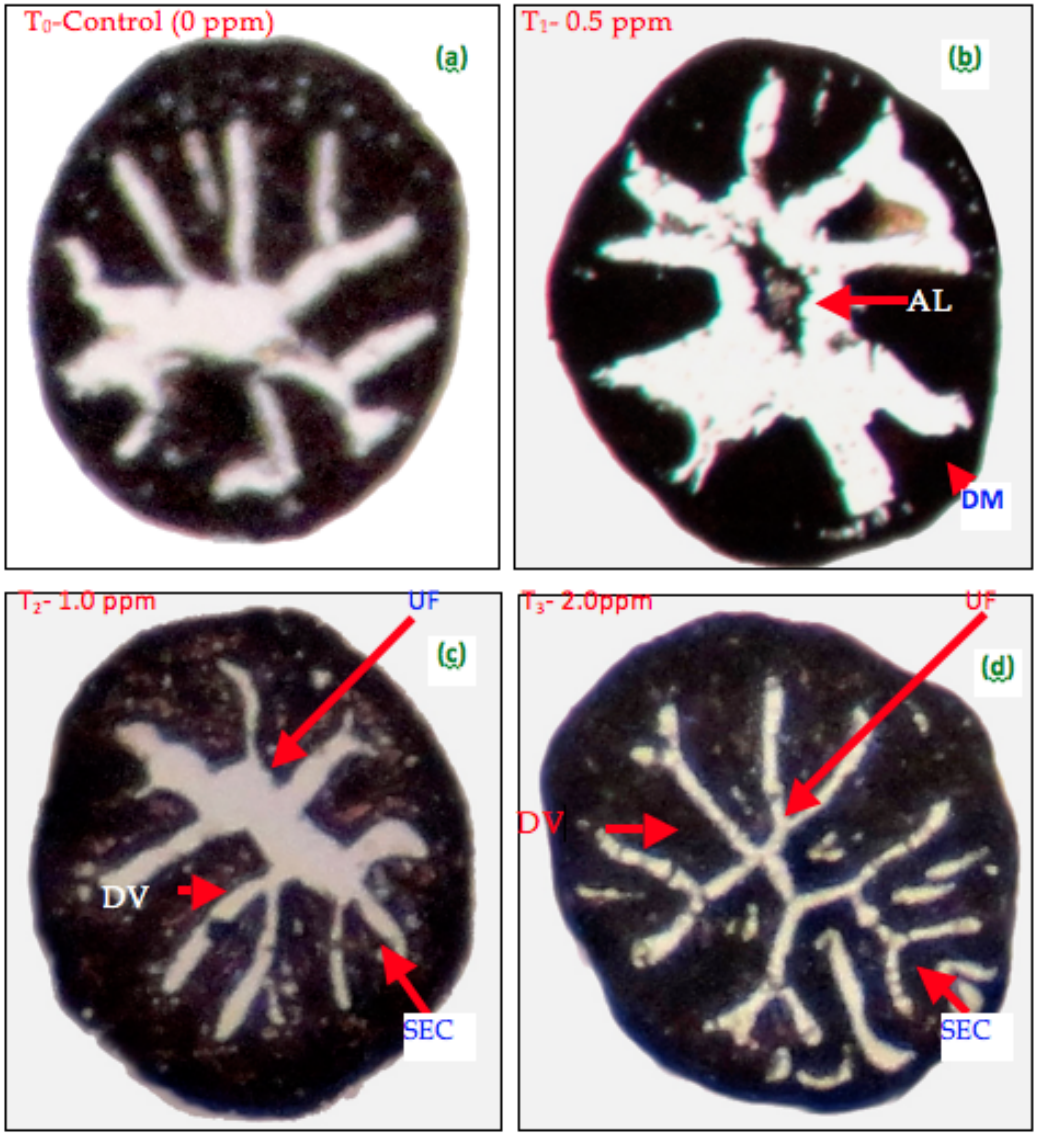

Figure 1. Histo-architectural changes in intestine exposed to sumithion; (a) Control (T_0), (b) 0.5 ppm (T_1), (c) 1.0 ppm (T_2) and (d) 2.0 ppm (T_3). Arrowheads are indicating Abnormal Lumen (AL), Disappeared Mucosa (DM), Destructed Villi (DV), Uneven intestinal Folding (UV) and Sloughing of Epithelial Cells (SEC).

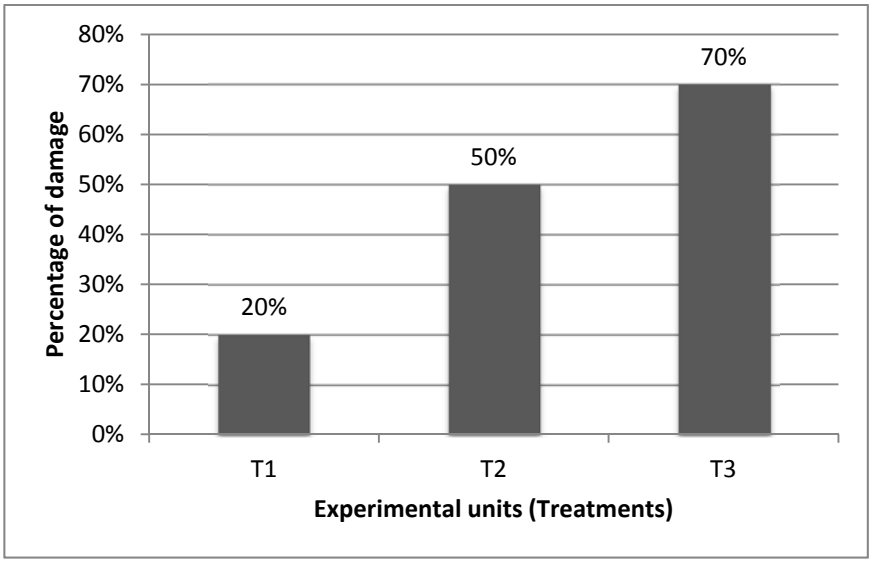

Figure 2. Percentage of damage in Zebra fish intestine due to sumithion exposure

Das and Gupta (2013) suggested a severe damage in intestinal mucosa, destructed epithelial walls, infiltration of lymphocytes and vacuolation in the intestine of Indian Flying Barb, *Esomus danricus* when it was exposed to malathion at 1.79, 0.179 and 0.00179 µg/l concentration for 28 days. Velmurugan *et al.* (2007) observed atrophy of epithelial cells, necrosis of epithelial cells and infiltration of lymphocytes into the lamina propia in the intestine of *Cirrhinus mrigala* exposed to fenvalerate. According to Desai *el al.* (1984), the degenerative and necrotic changes observed in the different intestinal layers of the studied fish may be due to a direct effect of the detected pesticides on the cells, to an accumulation of acetylcholine in the tissues or to a reduction in oxygen supply. According to Bhatnagar *et al.* (2007), the observed irritation and destruction of the mucosa membrane of the intestine hamper absorption. The pathological alterations in the intestine of the studied fish is in agreement with those observed by many investigators about the effects of different toxicants on fish intestine (Hanna *et al.*, 2005; Cengiz and Unlu, 2006). Walsh and Ribelin (1975) reported hyperemia, degenerative changes in the tips of villi, loss of structural integrity of mucosal folds, hypertrophy vacuolation and necrosis in the intestine of *Cyprinus carpio* exposed to the pesticide atrazine. The present study, thus, concludes that although organophosphate pesticides are found to be less toxic to mammals than organochlorines, yet, very low doses of such pesticides (sumithion) can cause apparent damage in the intestine of fish.

CONCLUSION

The current research activity was conducted on the basis of an aim to evaluate the effects of sumithion on histo-architecture of intestine in zebrafish. It is concluded that sumithion seems to be somewhat toxic to zebrafish. The data obtained in the present experimental protocol revealed significance that sumithion has adverse effects on the intestinal arrangement of fish. In addition, the indiscriminate use of pesticide in the field may be a threat to human, fauna and flora of the environment.

CONFLICT OF INTEREST

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

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