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HISTOPATHOLOGICAL AND ENVIRONMENTAL EFFECTS OF THE INSECTICIDE, SUMITHION ON THE FISH, TILAPIA (*Oreochromis niloticus*) IN POND CONDITION

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Abstract

The present research work was conducted to evaluate the effects of organophosphate insecticide, sumithion on water quality parameters, density of plankton population and histological changes of kidney and liver of the fish, tilapia (*Oreochromis niloticus*) in aquaculture ponds during July to December 2016. The experiment was conducted with four treatments, each with two replications. Treatment T_0 was used as control (no sumithion) and other three treatments with 0.025 ppm (T_1), 0.050 ppm (T_2) and 0.100 ppm sumithion (T_3). The water quality parameters such as dissolved oxygen, free carbon dioxide, pH, total alkalinity, NO_3-N and PO_4-P fluctuated significantly under four treatments during the experimental period but they were not affected by sumithion application. The phytoplankton densities ($\times 10^5$ cells L^{-1}) was not affected by sumithion. Six genera of phytoplankton populations were found in the experimental ponds. On the other hand, zooplankton population densities ($\times 10^3$ cells L^{-1}) were significantly reduced with increasing doses of sumithion (T_2 and T_3) in comparison with that of control (T_0). Histological changes of kidney were observed after application of sumithion. The renal corpuscle, collecting duct, hematopoietic cells and other cells of the kidney in control (T_0) were normal and systematically arranged. Abnormal collecting duct, Intra-cellular space, degenerated renal corpuscle, irregular shaped blood vessel, ruptured membrane large vacuole and necrosis were found in T_1, T_2 and T_3.Normal structure of liver cells such as hepato-pancreas, hepatic cell and blood vessel were observed in T_0 (control). Sumithion exposed liver sections showed rupturedhepato-pancreas, necrosis, hemorrhage, intra-cellular space, degenerated hepato-pancreas and large vacuole were found in T_1, T_2 and T_3. Therefore, it reveals that sumithion has adverse effects on kidney and liver of the test fish. So, sumithion should not be used indiscriminately in agriculture and aquaculture practices. It may be concluded from the research finding that dissolved oxygen, free carbon dioxide, pH, total alkalinity, PO_4-P, NO_3-N, phytoplankton and zooplankton values under treatment, T_0, are significantly different from treatments T_1, T_2, and T_3 in most cases.

Keywords: Histopathological, Environmental, Sumithion, Tilapia, Pond, Effects of Insecticide.

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Introduction

Bangladesh has diversified freshwater fishery resources comprising of about 260 indigenous species and as many as 13 exotic species. Bangladesh is one of the world's leading fish producing countries with a total production of 3.68 million MT, where aquaculture production contributes about 56 percent of the total production. The significance of fisheries can be indicated through its contribution to the national GDP. Fisheries contribute about 4.43% to the national GDP along with its contribution of about 23.37% of total agricultural GDP (DoF, 2016). It is no longer a surprise that Fisheries sector is now

contributing to more than 2.27% of the country's total export earnings (DoF, 2016).

Constant use of pesticides in crop fields has led to decreased biodiversity of fauna. Fishes live in the aquatic column; they are facing challenge for surviving from pollutants, particularly from various chemical fertilizers and pesticides used in or adjacent paddy fields or flood lands. Pesticides in aquatic environment have become a matter of concern because of their toxicity and tendency to accumulate in food chain. Pesticides reach aquatic environment by direct application, spray drift, aerial spraying, and erosion and run off from agriculture land. These huge amounts of pesticides may be deposited in the flood lands and other open water wetlands and rivers of the country (Plimer, 1988, Miskiewicz and Gibbs, 1994). The hazardous effect of pollutants has its impact on all living organisms. The indiscriminate use of pesticides in agriculture, animal husbandry and post-harvest technology is a threat to the natural water system, public health and welfare of mankind (Tilak *et al.*, 2007). Aquatic environment contaminated through pesticides that show some altered behavioral patterns, which may include avoidance, locomotive activity and aggression (Morgan *et al.*, 1991). Xenobiotics usually contaminate water bodies, a number of researchers have found the effects of different pesticides on aquatic organisms. Water quality parameters such as temperature, dissolved oxygen, pH, turbidity, alkalinity as well as conductivity are influenced by the rate of pollutants entering the water or lethal effects on the aquatic organisms (Fagbenro, 2002; Olufayo, 2009). Fish are often used as indicators of such biological impacts of pollutants as they respond to low concentrations of toxic substances (Ayas *et al.*, 2007). Alteration in the histology of the tissue such as gill, liver, kidney or intestine that are directly related to the contaminants which serve as important bio-monitoring tools or bio-markers to assess the toxicity.

Sumithion, the O, O Dimethyl O-(3-methyl-4~nitrophenyl) is an organophosphate insecticide, which is widely used in aquaculture ponds for eradication of aquatic insect (mainly tiger bug) prior to release of larvae. It is effective against a wide range of pests, i.e. penetrating, chewing and sucking insect pests on cereals, cotton, orchard fruits, rice, vegetables, and forests. Molecular formula of Sumithion: $C_9H_{12}NO_5PS$; Molecular weight of Sumithion: 277.231 g mol^{-1}; Solubility in water: 38.0 mg L^{-1}.

Pesticides are used on large scale to improve the production of food grains. Pesticides are used to kill and keep away the unwanted and harmful insects and pests. However, pesticides are used for better production of crop but at the same time, these pesticides also enter into aquatic environment through rain water. It affects the life of living things of water mainly fishes.

Materials and Methods

Experimental design: The experiment was set up with four treatments each with two replications, i.e., ponds without sumithion and with sumithion at the dose of 0.025 mg L^{-1}, 0.050 mg L^{-1} and 0.100 mg L^{-1}. The ponds receive water from a water-supply system of a deep-tube well and from rain. The experimental layout has been given in the Table 1 below.

Table 1.Layout of the experiment.

Treatment	Replication	Concentration of the sumithion	Fish species for test, it's length & weight and no. per decimal of pond
T_0	2 ponds	No sumithion	*Oreochromis niloticus*, L=4-5 cm, wt.= 10-12 g, 40 fishes/decimal
T_1	2 ponds	0.025 mg L^{-1}	-do-
T_2	2 ponds	0.050 mg L^{-1}	-do-
T_3	2 ponds	0.100 mg L^{-1}	-do-

Water Quality Monitoring

Throughout the experimental period, the water quality parameters were studied. Water quality measurements and sample collections were done on each sampling day following the standard methods.

Methods for analysis of physical and chemical parameters

All data of air temperature (°C) and water temperature (°C) of the sampling dates during the experimental period were collected from the 'Weather Yard', Bangladesh Agricultural University, Mymensingh.

Dissolve oxygen of the water samples were measured by a portable D.O meter (Model DO5509, Lutron, made in Taiwan). pH of the water samples were measured by a portable pH-meter (Model number-RI 02895, HANNA instruments Co.). The concentration of nitrate-nitrogen (NO_3-N) was determined by a nitrate meter (Model number-MI3874, HANNA instruments Co.). Concentration of Phosphate

phosphorus (mg L^{-1}) of the water samples were determined by a phosphate meter (Model number-MI3833, HANNA instruments Co.).

Methods for study of biological parameters

Collection and preservation of plankton sample

Water samples were randomly collected in a 500 ml bottle for quantitative and qualitative analysis of phytoplankton and zooplankton of water from different locations of each of the ponds and passed through a plankton net (mesh-size 55µ) and finally concentrated to 100 ml. Then concentrated samples were preserved in small plastic bottles in 5% formalin for further study under a compound microscope.

Counting of plankton

For counting both phytoplankton and zooplankton Sedgwick-Rafter Counting Cell (S-R cell) was used. The S-R cell is 500 mm long, 20 mm wide and 1 mm deep. The volume of the chamber is equally divided into 1000 fields of which each is 0.001 ml. From the concentrated plankton samples, 1 ml was taken by a dropper and put in the S-R cell. Before starting to count the plankton sample was left to stand for about 10 minutes to allow the plankton settle down and then it was studied under a compound microscope. Planktons were counted in 10 squares of the S-R cell chosen randomly.

Calculation of plankton

The plankton population was determined by Sedgwick Rafter Counting Cell (S-R Cell) using the following formula (Rahman, 1992):

$$N = \frac{A \times 1000 \times C}{V \times F \times L}$$

Where, N = No. of plankton cells per liter of original water, A = Total no. of plankton counted, C = Volume of final concentrate of the sample in ml, V = Volume of a field = 1 mm^3, F = No. of the fields counted and L = Volume of original water in liter.

The number of phytoplankton and zooplankton were expressed as cells L^{-1}.

The qualitative study of phytoplankton and zooplankton were done according to Pennak (1953), Ward and Whipple (1954), Needham and Needham (1962) and APHA (1992). Planktons were identified up to genus level.

Histological study (kidney and liver) of test species

Test species collection: Test species Tilapia (*Oreochromis niloticus*) were collected from experimental ponds by using push net. Total 132 fishes were collected. Fish samples were collected from the ponds under different treatments on different sampling days fixed before starting the experiment.

Selection and collection of chemicals: An organophosphorus compound, Sumithion was collected from the authorized dealer of the pesticide in original sealed container from a shop in city market, Mymensingh. The expiry date of the test substance checked prior to initiation of the treatment was found to be suitable for the application.

Determination of water volume of the ponds: At first, length and width of water area of the ponds were measured by using measuring tape. Then about 12 nos. depths were measured with a depth meter at regular positions of water area at regular 1 meter distances from one to another position. After that, water volume (in m^3) of the ponds was calculated by using the following formula:

Pond Water Volume (m^3)

= Water Area (m^2) × Average water depth (m)
= Water volume (m^3) = (m^3× 1000) Liter

Application of sumithion in the ponds

Sumition was applied in pond water according to water volume and calculated amount of sumithion have been shown in the table below.

Table 2. Water volume of ponds, amount of sumithion used, and concentration of sumithion in pond water.

Treatment	Pond No.	Total amount of Sumithion applied in pond	Volume of water of pond (m^3 or liter)	Concentration of Sumithion(mg L^{-1}) in pond water
T_1	P_3	About 1.00 ml	37.37 or 37370	0.025
	P_5	1.00 ml	41.23 or 41230	0.025
T_2	P_2	2.32 ml	46.45 or 46450	0.050
	P_6	2.40 ml	47.98 or 47980	0.050
T_3	P_1	4.04 ml	40.39 or 40390	0.100
	P_4	4.90 ml	49.01 or 49010	0.100

Materials used in the experiment

Plastic bowl, plastic bucket, Dissection box, Gloves and masks, Tube, Glass slide, Glass cover slip, OLYMPUS microscope (Model: CX21, Japan), Microscope digital camera: Optika OPTIKAM B3 4083.B3 (BG- Italy); 3.14 mega pixel, Slide warmer, Oven, Water bath, Electronic balance, Counter Machine, Incubator, Microtome machine and Thermometer.

Examination of the effect of sumithion on organs and tissues

Collection of organs: The animals from both control and treated ponds were collected on prefixed sampling days and organs like liver and kidney were carefully collected by dissection and immediately kept in vials filled with 10% formalin and kept at room temperature for preservation.

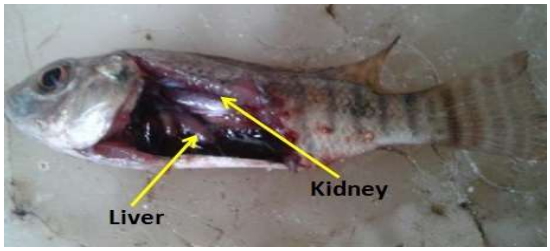

Figure 1. Collection of kidney and liver of *O. niloticus*

Histological study of organs: The slices of the kidney and liver were fixed in 10% neutral buffered formaldehyde. Fixing prevent autolysis and purification of tissues. The preserved samples were taken out from vials and put into cassettes separately (Figure 1).

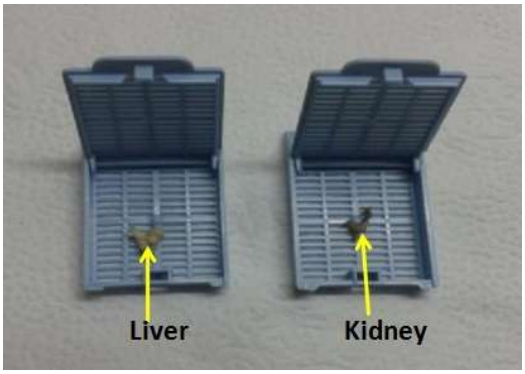

Figure 2. Samples in the cassette

Then dehydration process was carried out manually followed by clearing, infiltration, embedding, sectioning, staining, mounting and finally microscopic observation. These steps are mentioned below:

Dehydration: The fixed samples were kept into the cassette and passed through a graded alcohol series. The samples that kept in 10% formalin fixative were started dehydration from 70% ethyl alcohol and proceeded on up to 100% ethyl alcohol (Table 3).

Table 3. The Dehydration schedule.

Sl. No.	Solution	Time
1	80% Ethyl alcohol	12 hours
2	90% Ethyl alcohol	12 hours
3	100% Ethyl alcohol	12 hours
4	70% Ethyl alcohol	4 hours
5	100% Ethyl alcohol	4 hours

Clearing: Dehydrated samples were cleared by using chloroform twice successively for 2-3 hours to remove traces of alcohol in order to have consistent paraffin blocks. The process is mentioned in Table 4.

Table 4. The Clearing schedule.

Sl. No.	Solution	Time
1	Chloroform	1 hour
2	Chloroform	1 hour

Infiltration: The cassettes holding the samples were marked previously in accordance with the number of samples. From chloroform, the samples were taken out from the cassette and placed in paraffin sequentially in incubator (EL - 450B) following the steps below (Table 5).

Table 5. The Infiltration schedule.

Sl. No.	Solution	Time
1	Paraffin	40 minutes
2	Paraffin	40 minutes

Embedding: After infiltration, the cassettes were taken out one by one, being opened, settling the sample in the middle of a small paper-box

previously marked as in the cassette and filled with melted paraffin from wax dispenser. Then the paper-boxes were allowed to cool in room temperature. Thus the embedded blocks containing the sample were formed which allowed smooth sectioning.

Trimming: Trimming is a process in which the undesirable wax layers of the embedded blocks are trimmed by knife to obtain suitable blocks. Trimming allowed easy sectioning. Trimming was done by using microtome blades. In this step both side trimming and surface trimming were conducted

Sectioning: Paraffin embedded blocks were cut by microtome knife in microtome machine (KEDEE KD-3358, China) at 5 µm size. The sections were placed on lower part of a glass slide previously tagged and filled with water drops. The sections were kept overnight at room temperature for proper drying.

Staining: Staining was done following the schedule in Table 6 below.

Table 6. The staining schedule.

Sl. No.	Solution	Time	Process
1	Xylene	30 minutes	Clearing
2	Xylene	30 minutes	
3	100% Alcohol	5 minutes	Rehydration
4	100% Alcohol	5 minutes	
5	90% Alcohol	3 minutes	
6	80% Alcohol	3 minutes	
7	70% Alcohol	3 minutes	
8	50% Alcohol	2 minutes	
9	Distilled water	15 dips	Washing
10	Haematoxylene	2-3 seconds	Staining
11	Washed in tap water	15 minutes	Washing
12	50% Alcohol	10-15 dips	Dehydration
13	95% Alcohol	30 seconds	
14	Eosin	3-4 seconds	Counter staining
15	95% Alcohol	2 minutes	Dehydration
16	100% Alcohol	1 minute	
17	100% Alcohol	3 minutes	
18	100% Alcohol	1 minute	
19	Xylene	30 minutes	Clearing
20	Xylene	30 minutes	
21	Drying at room temperature	Over night	Drying

Mounting: DPX was used for mounting as a mounting agent. A drop of DPX was put on each slide followed by attachment of cover slip (22 mm × 22 mm). After mounting the slides were put for several hours in room temperature.

Microscopic view of the tissue sections: The mounted slides were observed under a microscope (OPTICA 4083.B3, Italy) which was connected to computer with OPTIKA MB3 Digital camera (3.14 Mega pixel).

Statistical analysis

Data were analyzed by using Microsoft Excel software and values are expressed as means ± standard deviation (SD). Statistical analyses were done by IBM SPSS Statistics 20.0 software (IBM SPSS Statistics, IBM, Chicago, USA).

Results

An attempt has been made in the present investigation to demonstrate the effects of organophosphate insecticide, sumithion, on plankton populations i.e. phytoplankton and zooplankton populations along with water quality parameters as well as to observe the histological changes of liver and kidney of the test fish, tilapia (*Oreochromis niloticus*).

Parameters of Water quality

In the present study, several physicochemical parameters of water such as temperature (^{0}C), dissolved oxygen (mg L^{-1}), free carbon dioxide (mg L^{-1}), pH, total alkalinity (mg L^{-1}), nitrate-nitrogen (mg L^{-1}), phosphate-phosphorus (mg L^{-1}) were recorded during the exposure period of sumithion at various concentrations along with control.

Water and air temperature

Data of water and air temperature (^{0}C) collected from the records of 'Weather Yard' of the Dept. of Irrigation and Water Management, BAU, Mymensingh.

Dissolved oxygen (mg L^{-1}): The ranges and mean values of DO during the experimental period in T_0 (control), T_1, T_2 and T_3 were 6.05-8.55 (6.40±2.60), 7.90-8.65 (7.36±2.80), 7.45-8.60 (7.35±2.80) and 7.80-8.65 (7.30±2.78) mg L^{-1}, respectively. The determined values have no

significant variations due to application of the pesticide. The highest value was found 8.65 mg L^{-1} in case of T_1 and T_3 and the lowest value was 6.05 mg L^{-1} in T_0.

Free carbon dioxide (mg L^{-1}): The ranges and mean values of free CO_2 during the experimental period in T_0, T_1, T_2 and T_3 were 2.0-7.0 (3.5±1.8), 0.0-5.0 (2.0±1.8), 1.0-4.0 (1.75±1.30), 0.0-7.0 (2.38±2.20) mg L^{-1}, respectively. The lowest value was found 0.0 mg L^{-1} in case of T_1, T_2, T_3 and the highest value was 7.0 mg L^{-1} in T_0 and T_3.

pH: The ranges and mean values of pH during the experimental period in T_0, T_1, T_2 and T_3 were 6.95-7.90 (7.40±0.40), 7.25-8.30 (7.86±0.33), 7.30-8.20 (7.91±0.30), and 7.25-8.40 (7.83±0.36), respectively.

Total alkalinity (mgL^{-1}): Total alkalinity was determined on different sampling dates and days during the study period. The ranges and mean values of total alkalinity during the experimental period in T_0, T_1, T_2 and T_3 were 111-146 (129.62±12.18), 114-177 (136.75±23.15), 129-169 (145.87±13.65), and 126-160 (147.37±10.92), respectively.

Phosphate-phosphorus PO_4-P (mg L^{-1}): The ranges and mean values of PO_4-P during the experimental period in T_0, T_1, T_2 and T_3 were 3.50-7.95 (5.21 ±1.44), 4.25-10.25 (6.41±2.37), 4.25-6.55 (5.81±0.80), and 4.35-9.30 (5.53±1.80) mg L^{-1}, respectively. The highest value was found 10.25 mg L^{-1} in case of T_1 and the lowest value was 3.50 mg L^{-1} in T_0, respectively.

Nitrate–nitrogen (NO_3-N) (mg L^{-1}): The ranges and mean values of nitrate- nitrogen during the experimental period in T_0, T_1, T_2 and T_3 were 2.35-6.60 (3.60±2.00), 1.15-5.85 (3.30±1.72), 2.35-9.55 (4.8±3.0), and 1.60-10.0 (5.06±3.12) mg L^{-1}, respectively. The highest value was found as 10.00 mg L^{-1} in case of T_3 and the lowest value was 1.15 mg L^{-1} in T_1.

Study of plankton

Quantitative study of phytoplankton

The total number of phytoplankton ranged from 1.2 to 2.3 (×10^5) cells L^{-1}, 0.4 to 3.7 (×10^5) cells L^{-1}, 0.2 to 4.1 (×10^5) cells L^{-1} and 0.4 to 3.3 (×10^5) cells L^{-1} in the ponds of T_0, T_1, T_2 and T_3, respectively. The phytoplankton concentration in T_0 remained more or less constant during the study period. But, when the ponds were treated with sumithion it intended to fluctuate in volume. Based on mean values, it was found that phytoplankton concentrations showed its highest density in T_0 (1.81±0.41 (×10^5) cells L^{-1}) followed by T_2 (1.75±1.15 (×10^5) cells L^{-1}), T_1 (1.64±1.07 (×10^5) cells L^{-1}) and T_3 (1.56±0.91 (×105) cellsL^{-1}). However, no prominent changes were observed in phytoplankton concentrations due to application of sumithion.

Quantitative study of zooplankton

The total number of zooplankton ranged from 5.0 to 7.9 (×10^3) cells L^{-1}, 2.3 to 9.6 (×10^3) cells L^{-1}, 2.9 to 9.9 (×10^3) cells L^{-1} and 2.1 to 6.3 (×10^3) cells L^{-1} under the treatments of T_0, T_1, T_2 and T_3, respectively. The zooplankton density in T_0 remained more or less same during the study period. But in sumithion treated ponds, it was found to decrease in volume. With the advancement of the study period, we found that zooplankton densities were found distinctly reduced.

On the basis of mean values, zooplankton densities showed its highest density in T_0 (6.39±1.00 (×10^3) cells L^{-1}) followed by T_2 (5.05±2.31 (×10^3) cells L^{-1}), T_3 (4.64±1.44 (×10^3) cells L^{-1}) and T_1 (4.62±2.42 (×10^3) cells L^{-1}). At the end of the experiment, we found a distinct change of reduction in zooplankton density.

Histological alteration of fish organs

Kidney

Renal corpuscle, hematopoietic tissue, collecting duct, distal tubule and proximal tubule were normal and systematically arranged in the control treatment (Figure 3.1 A, 3.2 A and 3.3 A). Sumithion exposed kidney sections showed disintegration of convoluted tubules with large intra-cellular space and vacuoles in the epithelial cells and lumen, necrosis, hemorrhage, degenerated renal corpuscle and irregular shaped blood vessel. Kidney tissue from tilapia exposed to 0.025 ppm showed abnormal collecting duct at 7 days of exposure (Figure 3.1 B). Intra-cellular space was found at the dosage of 0.05 ppm (Figure 3.1 C) and necrosis and intra-cellular space were observed at the dosage of 0.10 ppm (Figure 3.1 D) at 7 days of exposure. Blood vessel and vacuoles were found at the dosage of 0.025 ppm at 15 days of exposure (Figure 3.2 B). Irregular shaped blood vessel and degenerated renal corpuscle were found at the dosage of 0.05 ppm (Figure 3.2 C) and large vacuole was also recorded at the dosage of 0.10 ppm (Figure 3.2 D) at 15 days of exposure. Ruptured membrane and intra-cellular space were observed at the dosage of 0.025 ppm at 30 days of exposure (Figure 3.3 B). Vacuole and ruptured renal corpuscle were found at the dosage of 0.05 ppm at 30 days of exposure (Figure 3.3 C). Large vacuole and ruptured collecting duct were also found at the dosage of 0.10 ppm at 30 days of exposure (Figure 3.3 D).

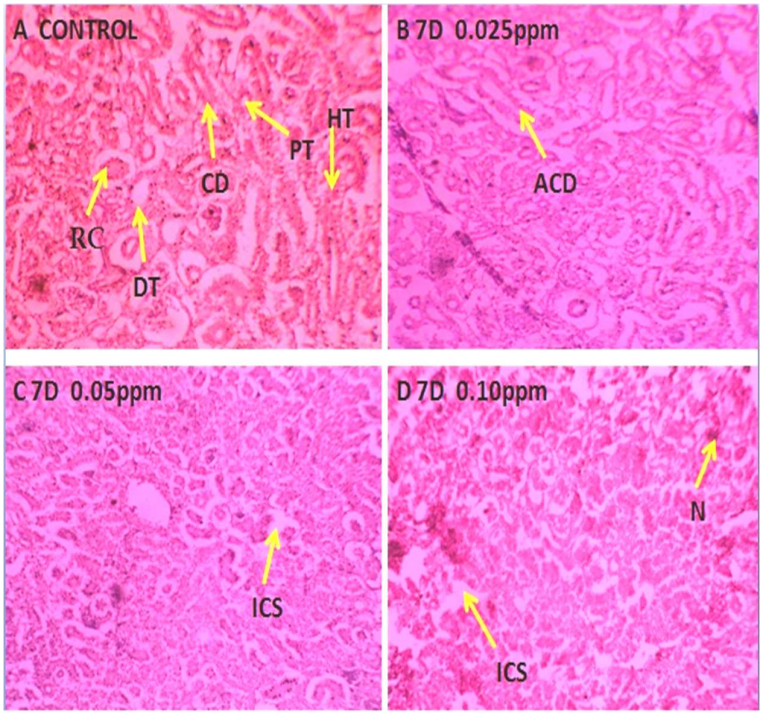

Figure 3.1. Histological changes in kidney (H and E stained, X100) exposed to sumithion (A) Control (T_0); (B) 0.025ppm (T_1); (C) 0.05ppm (T_2) and (D) 0.10ppm (T_3) at 7 days of exposure. Arrowheads are indicating- RC (renal corpuscle), PT (proximal tubule), DT (distal tubule), CD (collecting duct), HT (hematopoietic tissue), ACD (abnormal collecting duct), ICS (intra-cellular space) and N (necrosis).

Liver

Hepato-pancreas, hepatic cell and other cells were normal and systematically arranged in the control treatment (Figure 3.4 A, 3.5 A and 3.6 A). Sumithion exposed liver sections showed ruptured hepato-pancreas, necrosis, vacuole, degenerated hepatic cell, intra-cellular space and hemorrhage. Liver tissue from tilapia exposed to 0.025 ppm showed ruptured hepato-pancreas at 7 days of exposure (Figure 3.4 B). Necrosis and ruptured hepato-pancreas were found at the dosage of 0.05 ppm (Figure 3.4 C) and vacuole and hemorrhage were observed at the dosage of 0.10 ppm (Figure 3.4 D) at 7 days of exposure. Ruptured hepato-pancreas, necrosis and vacuoles were found at the dosage of 0.025 ppm at 15 days of exposure (Figure 3.5 B). Vacuole and ruptured hepato-pancreas were found at the dosage of 0.05 ppm (Figure 3.5 C) and blood vessel and vacuole were also recorded at the dosage of 0.10 ppm (Figure 3.5 D) at 15 days of exposure. Necrosis and vacuole were observed at the dosage of 0.025 ppm at 30 days of exposure (Figure 3.6 B). Intra-cellular space was found at the dosage of 0.05 ppm at 30 days of exposure (Figure 3.6 C). Degenerated hepato-pancreas, vacuole and hemorrhage were also found at the dosage of 0.10 ppm at 30 days of exposure (Figure 3.6 D).

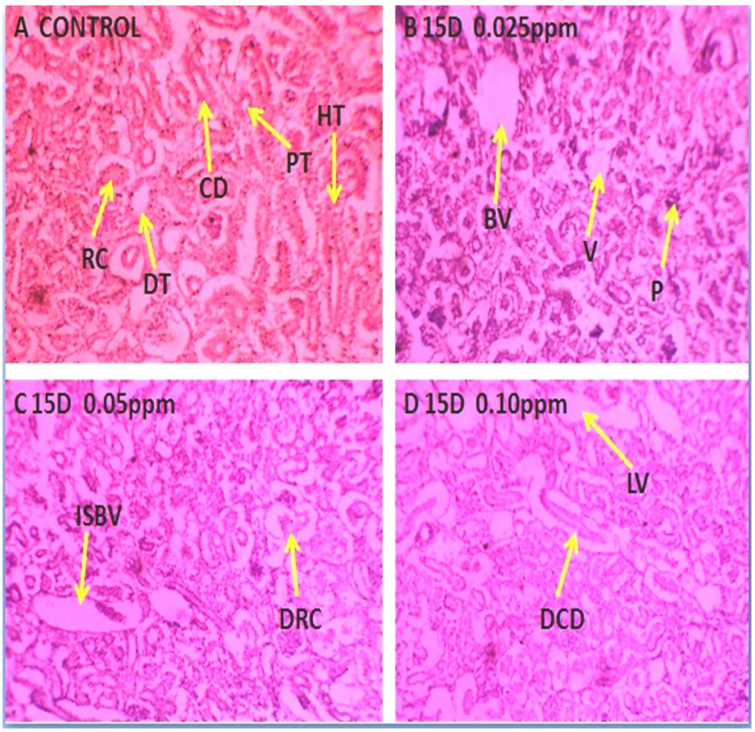

Figure 3.2. Histological changes in kidney (H and E stained, X100) exposed to sumithion (A) Control (T_0); (B) 0.025ppm (T_1); (C) 0.05ppm (T_2) and (D) 0.10ppm (T_3) at 15 days of exposure. Arrowheads are indicating- RC (renal corpuscle), PT (proximal tubule), DT (distal tubule), CD (collecting duct), HT (hematopoietic Tissue), BV (blood vessel), V (vacuole), P (pyknosis), ISBV (irregular shaped blood vessel), DRC (degenerated renal corpuscle), DCD (degenerated collecting duct) and LV (large vacuole).

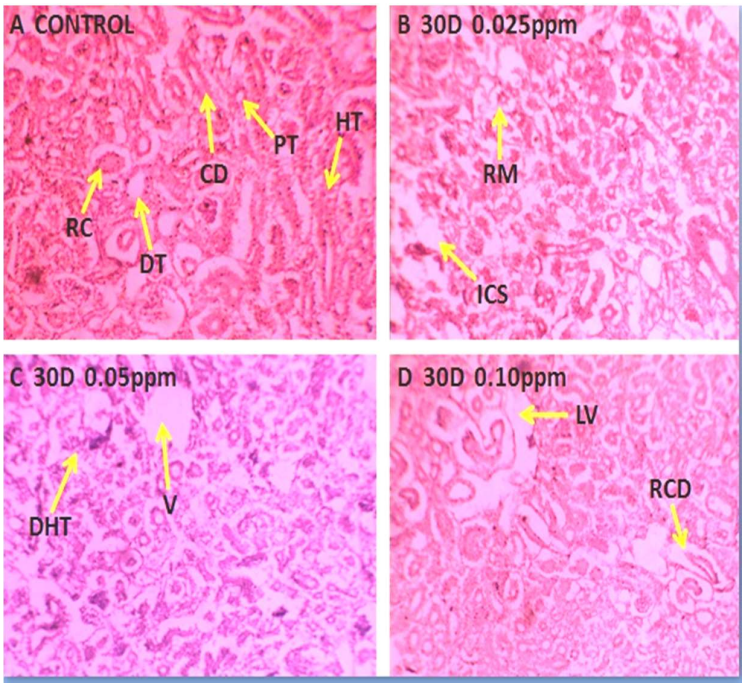

Figure 3.3. Histological changes in kidney (H and E stained, X100) exposed to sumithion (A) Control (T_0); (B) 0.025ppm (T_1); (C) 0.05ppm (T_2) and (D) 0.10ppm (T_3) at 30 days of exposure. Arrowheads are indicating- RC (renal corpuscle), PT (proximal tubule), DT (distal tubule), CD (collecting duct), HT (hematopoietic tissue), RM (ruptured membrane), ICS (intra-cellular space), DHT (degenerated hematopoietic tissue), RCD (ruptured collecting duct), V (vacuole) and LV (large vacuole).

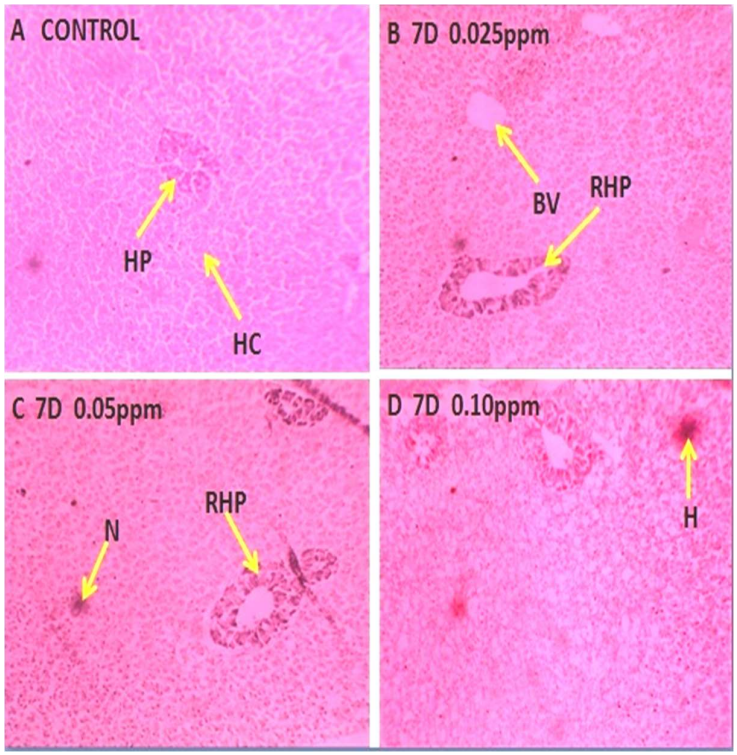

Figure 3.4. Histological changes in Liver (H and E stained, X100) exposed to sumithion (A) Control (T_0); (B) 0.025ppm (T_1); (C) 0.05ppm (T_2) and (D) 0.10ppm (T_3) at 7 days of exposure. Arrowheads are indicating- HP (hepato-pancreas), HC (hepatic cell), BV (blood vessel), RHP (ruptured hepato-pancreas), N (necrosis) and H (hemorrhage).

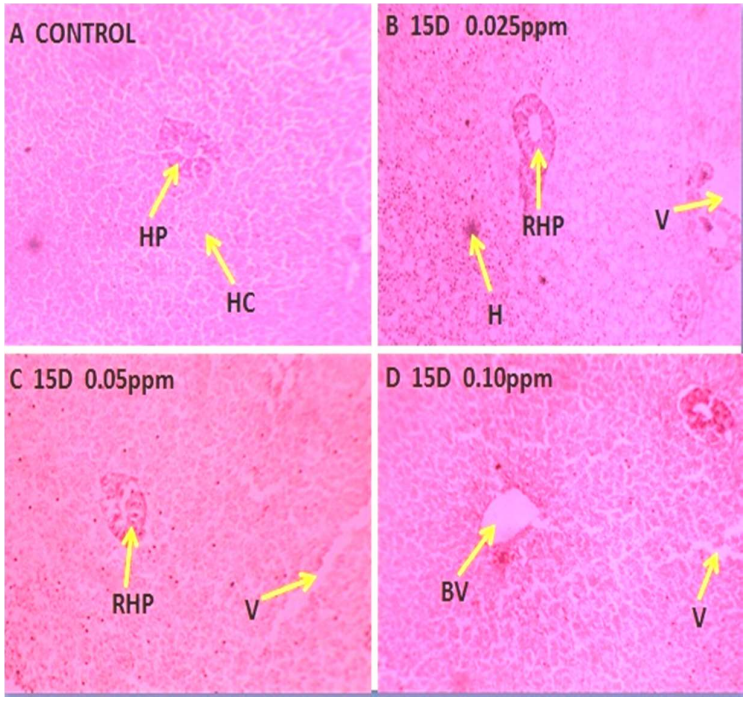

Figure 3.5. Histological changes in Liver (H and E stained, X100) exposed to sumithion (A) Control (T_0); (B) 0.025ppm (T_1); (C) 0.05ppm (T_2) and (D) 0.10ppm (T_3) at 15 days of exposure. Arrowheads are indicating- HP (hepato-pancreas), HC (hepatic cell), BV (blood vessel), RHP (ruptured hepato-pancreas), V (vacuole) and H (hemorrhage).

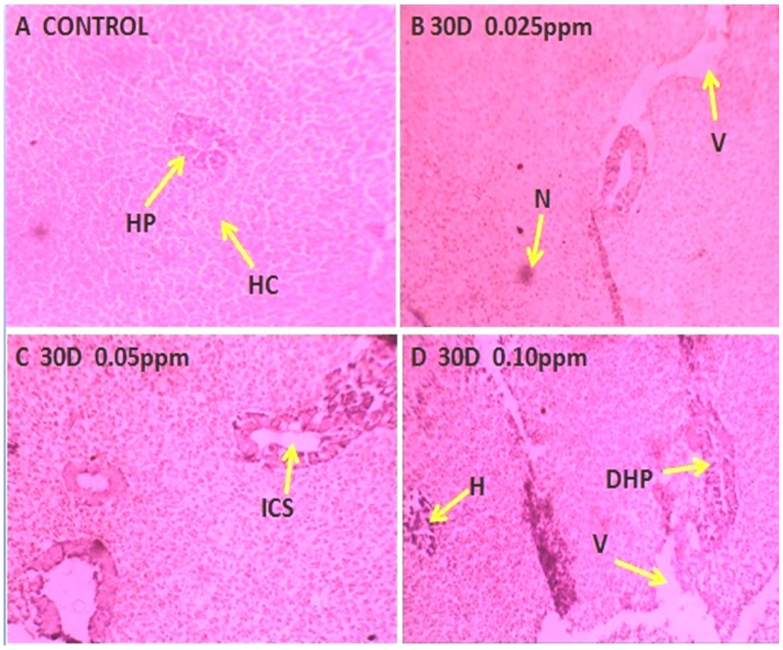

Figure 3.6. Histological changes in Liver (H and E stained, X100) exposed to sumithion (A) Control (T_0); (B) 0.025ppm (T_1); (C) 0.05ppm (T_2) and (D) 0.10ppm (T_3) at 30 days of exposure. Arrowheads are indicating- HP (hepato-pancreas), HC (hepatic cell), N (necrosis), V (vacuole), ICS (intra-cellular space), DHP (degenerated hepato-pancreas), and H (hemorrhage).

Discussion

The present study was conducted to determine the effects of organophosphate insecticide, sumithion on water quality parameters, plankton population in aquaculture ponds and effects on histological changes in kidney and liver of Tilapia (*O. niloticus*). Changes in various water quality parameters, abundance of phytoplankton and zooplankton changes and different changes in kidney and liver histology have been documented in the present study.

Water quality parameters

Water quality parameters have a great role in causing the toxicity of different pesticides that ultimately have harmful effect on diversity, abundance and dynamics of aquatic flora and fauna. It has been reported that mass mortalities of grass carp attributed to a multi-factorial disease primarily caused by bacterial agents and might be triggered by unsuitable environmental factors, such as poor water quality, limited oxygen supply, poor feed bases and chronic and acute exposure to pesticides dissolved in water or included in feeds (Pucher *et al.*, 2012). In the present study, several physicochemical parameters of water such as dissolved oxygen (mg L^{-1}), free carbon dioxide (mg L^{-1}), pH, total alkalinity (mg L^{-1}), nitrate-nitrogen (mg L^{-1}), phosphate-phosphorus (mg L^{-1}) were determined during the exposure period of sumithion at various concentrations along with control. Such water quality parameters have also been observed by a number of authors (Talukdar *et al.*, 2012) in the aquaculture ponds of the present experimental area. The water quality parameters such as dissolved oxygen, free carbon dioxide, pH, total alkalinity, NO_3-N & PO_4-P values of pond water under different treatment, T_0, T_1, T_2, and T_3 were significantly different in most cases. Uddin *et al.* (2016) found that NO_3–N and PO_4–P concentrations were significantly ($p < 0.05$) decreased in sumithion on high dose and low dose, compared to control pond (no sumithion).

Various physicochemical properties of pesticides like hydrolysis, volatilization and in balancing the dissociated and undissociated forms are influenced by pH (Weber, 1972). With few exceptions, pH generally does not influence the toxicity of organophosphate (OP) compounds. The toxic effects of 2, 4-D were reduced when the pH was raised by the addition of sodium chloride. Only few works have been carried out on the influence of low oxygen on the toxicity of pesticide to fish. Davies (1975) tried 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. Such water parameters have also been observed by a number of authors (Siddika *et al.*, 2012; Nupur *et al.*, 2013) in the aquaculture ponds of the experimental area.

Plankton Population

In the present study, phytoplankton and zooplankton population densities were studied after exposure to sumithion, which are discussed below:

Phytoplankton

The phytoplankton concentration in T_0 remained more or less same during the study period. But, in the sumithion treated ponds it was found to fluctuate in density. With the progress of the study period, we found that phytoplankton densities were slightly reduced. On the basis of mean values, it was found that phytoplankton concentration showed its highest density in T_0 (1.81±0.41 (×10^5) cells L^{-1}) followed by T_2 (1.75±1.15 (×10^5) cells L^{-1}), T_1 (1.64±1.07 (×10^5) cells L^{-1}) and T_3 (1.56±0.91 (×10^5) cells L^{-1}). However, no prominent changes were observed in phytoplankton concentration due to sumithion application. Pathania *et al.*, (2010) found phytoplankton density ranged from 1.98 × 10^5 cells L^{-1} to 2.37 × 10^5 cells L^{-1}, which is similar to those of the present study. Interestingly, the quantity of phytoplankton was not affected by sumithion but qualitatively affected as fewer genera were found in T_2 (4 genera) and showing that sumithion may have some adverse qualitative effects on some phytoplankton.

Zooplankton

The total number of zooplankton ranged from 5.0 to 7.9 (×10^3) cells L^{-1}, 2.3 to 9.6 (×10^3) cells L^{-1}, 2.9 to 9.9 (×10^3) cells L^{-1} and 2.1 to 6.3 (×10^3) cells L^{-1} in the ponds of T_0, T_1, T_2 and T_3, respectively. The zooplankton concentration in T_0 remained more or less same during the study period. But in the sumithion treated ponds it was found to decrease in density. With the advancement of the study period, it was found that zooplankton densities were distinctly reduced. Hossain (2015) also observed the zooplankton population densities (cells L^{-1}) significantly decreased with toxicity of sumithion during 30 days of treatment.

Histological study

Pesticides in polluted aquatic ecosystem are accumulated mainly in the metabolically active tissues of fish such as liver, kidney, gonads and gill and cause histopathological damage of those organs. *O. niloticus* was exposed to different sub-lethal concentrations such as 0.025 (T_1), 0.050 (T_2) and 0.100 mg L^{-1} (T_3) of sumithion with a control treatment (T_0).

Kidney

In the present study, the kidney of the test fish *O. niloticus* compared to the control showed vacuole, necrosis, abnormal collecting duct, degenerated renal corpuscle, intra-cellular space and ruptured membrane. More or less similar findings have been reported in Silver barb (*Barbonymus gonionotus)* exposed to an organophosphorous insecticide Quinalphos 25 EC in *Cirrhinus mrigala* expose to lethal (5.13 µg L^{-1}) and sub-lethal (1.026 µg L^{-1}) dosage of pyrethroid derivative cypermethrin (Choudhuri *et al.*, 1984).

Liver

Hepato-pancreas, hepatic cell and other cells were normal and systematically arranged in the control treatment. Tissue sample from fishes treated with 0.025 ppm of sumithion showed vacuole, hemorrhage, ruptured hepato-pancreas and necrosis as also observed by Hossain *et al.*, (2012). However, at the higher tested concentrations of sumithion *viz.*, 0.05 and 0.10 ppm, marked severe necrosis, hemorrhage, ruptured and degenerated hepato-pancreas, intra-cellular space and large vacuole were observed in all the test species which agreed with the finding of Rahman *et al.*, (2012). Kabir and Begum (1978) reported cytoplasmic degeneration, pyknotic nuclei in liver tissues; vacuolation in hepatic cells and rupture of blood vessels; degenerative hepatic cells and necrotic nuclei when *H. fossilis* was exposed for 25 days to 5, 10 and 20 ppm Diazinon, respectively. This finding is similar to the present study.

From the above discussion, it can be deduced that sumithion is significantly toxic to Nile tilapia *(O. niloticus*). The exposure of sumithion in low concentrations produced significant changes in some hematological parameters of *O. niloticus* and caused stress to the fish. The important aspects of this research are, besides the description of the histopathological effects of sumithion on *O. niloticus*, the detection that some of them are significant and appear very shortly after exposure. One wants to point out that the observed effects are serious if considered that the concentration of the product used in the tests is considered safe according to a perspective of environmental safety. Ecological consequences may be significant if the action of the pesticide persists. Therefore, not only one can recommend that the allowed levels for pesticides should be lower to make them really more secure, that the exposure shall be the shortest as possible and that different species should be tested as their sensitivity may vary.

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