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Acute Toxicity of Sumithion Insecticide on Freshwater Catfish, *Clarias batrachus* (Linnaeus, 1758)

Neetu Singh and Ravindra Kumar Singh
PG Department of Zoology, Jai Prakash University, Chapra (Bihar)
Corresponding author: nitu3146@gmail.com

ABSTRACT

Sumithion is widely used for crop safety and eradication of tiger bugs (*Cicindela* spp.) from larval rearing aquaculture ponds. The present experiment was conducted to evaluate the toxic effects of sumithion on catfish fingerlings. Fish were exposed for 96 h to six concentrations of sumithion (0, 3, 4, 5, 6 and 7 mg/l) each with three replications. The 96 h LC50 value was calculated using probit analysis. The fishes were sacrificed after 96 hour exposure to measure hemato-biochemical parameters. The 96 h LC50 value of sumithion for catfish was 5.876 mg/l. The values of RBCs and Hb significantly decreased in different dozes of the toxicant compared to control, while the values of WBC and blood glucose levels showed opposite condition. Consequently, the frequencies of formation of MN significantly increased in different concentrations of the toxicant compared to the control. The results of the current study denoted that sumithion exerts toxicity to catfishes. It is expected that the results of the present research will help in the development of awareness of the concerned people about the toxic effect of sumithion as well as other insecticides and pesticides in the environment.

Keywords: Insectisides, organophospates, acute toxicity, catfishes, freshwater

INTRODUCTION

The India is an agrarian country where economy mainly depends on agricultural crop production; however, this sector is frequently invaded by pests and parasites causing a severe economic loss. The farmers using insecticides, pesticides, herbicides and also fungicides which chemicals come in direct contact after spraying or by runoff into aquatic resources. The water contamination in this way causing severe affect on the physiology, biology and ontogeny of aquatic organisms that can lead to fish mortality or reduced fish productivity (Hossain et *al*, 2016).

The Sumithion, O, O Dimethyl O-(3-methyl-4-nitrophenyl) is widely used among pesticides in India. It is effective to control a wide range of important insects and certain other arthropod pests. It is mainly used to control beetles in paddy fields and also to control tiger bugs (*Cicindela* spp.) in nursery ponds. Thus wide use for crop protection and for eradication of aquatic insects in fish ponds, ultimately, the surface and ground water might be highly contaminated due to this agricultural runoff pesticide (Ray et *al*, 2012). This pesticide is toxic to fishes and affects negatively the aquatic ecosystem resulting in the loss/shift in abundance of natural invertebrate and vertebrate species in the aquatic environment (Ventura et *al*, 2008; Vargas and Ponce-Canchihuaman, 2017). It is essential to know the damage extent being done by this chemical to fish and other aquatic organisms.

Fish is very vulnerable to the changes in different water quality parameters which might be directly observed in their blood parameters (Sadiqul et *al*, 2016). Blood parameters are considered as essential indicators to physiological stress caused by any disturbances that affect fish homeostasis (Salam et *al*, 2015; Sharmin et *al*, 2015). Micronucleus (MN) is a small mass of chromatin present in cytoplasm which is made during the nuclear division of the acentric chromosome fragments (Winter et *al*, 2007). The formation of micronucleus in the erythrocyte assay has been used to examine the stress caused by different pollutants Sadiqul et *al*, 2016; Anbumani and Mohankumar, 2012). Similarly, assessment of nuclear and cellular abnormalities of erythrocytes is also a very important analytical technique to assess the stress caused by any environmental contaminants (Ghaffar et *al*, 2015).

The catfishes are great importance in culture due to its fast growth rate, tolerance of wide range of environmental conditions, grow well in high stocking density, easy rearing and seed production, high consumer demand, advantage of long distance transportation in live condition and farmers` opportunity to get higher economic gain than in culturing some other species (Sarker et *al*, 2007).This study may be useful to catfish culturists and policy makers to minimize pollutionl through insecticides essentially to sustainable production in future.

MATERIALS AND METHODS

The sumithion was procured from an authorized dealer in Chapra. It was in liquid form and white in color. A static acute toxicity bioassay was performed according to standard method to determine the LC50 of sumithion for catfish fingerlings. Ten fingerlings were stocked in each cleaned glass aquarium (75 cm × 45 cm × 45 cm) with 30 litre of tap water. Adequate aeration was maintained throughout the experimental period. The fishes were exposed to six (0 mg/l as control, 3 mg/l, 4 mg/l, 5 mg/l, 6 mg/l and 7 mg/l) concentrations of sumithion each with three replications. The application of the pesticide was repeated at every 24 h with a regular total exchange of water. Records of mortality were made at different time intervals. A fish was considered as dead when respiratory movement of the opercula stopped and there was no response to touch.

Hematological experimentation: After 96 h of exposure, blood was collected from the survived fishes. The fishes were carefully collected and immediately anesthetized with clove oil (5 mg/l). After cutting the caudal peduncle, blood samples were collected and pushed into a sterilized centrifuge tube containing anticoagulant (20 mM EDTA). It took less than one minute per fish to complete the blood withdrawal process, which was deemed important to prevent stress impacts to minimize any mistake in normal blood values.

Hemoglobin estimation: The Hb% was measured using a Sahil's hemometer. At first, 90 µl 0.1N HCl was taken in Ependorf tube using micropipette and then 10 µl of blood added and the tube was shake thoroughly for proper mixing. After 2-3 min the mixture was transferred to the tube of the hemometer. Then distilled water was added in drops until the color was adjusted with the colorimeter and when the color was adjusted then the reading was taken up to the level of the mixture specified on the body of the tube.

Statistical analysis: The values are expressed as means±standard deviation (SD). To test the statistically significant difference among the different concentrations of sumithion, one-way analysis of variance (ANOVA) was carried out followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using Minitab software.

OBSERVATION

The lethal concentration of sumithion for the catfish was determined at pesticide level ranged from 3 to 7 mg/l. There was no mortality at control (0 mg/l) during 96 h exposure period. Percentage mortality of fish in different concentrations of sumithion is shown in Table 1.

Table 1: Mortality percentage of fish exposed to sumithion (0-7 mg/l) in water

Sumithion (mg/l)	Fishes	Cumulative dead fish in exposure				Mortality (%)
		24 h	48 h	72 h	96 h	
0	30	0	0	0	0	0
3	30	0	0	0	3	10
4	30	0	0	0	6	20
5	30	0	0	0	12	40
6	30	3	3	15	18	60
7	30	15	15	30	30	100

The probit analysis on number of observed dead fishes was performed after 96 h exposure at different concentrations of sumithion. The Probit analysis showed that the median lethal concentration for 50% mortality of the fishes was 5.886 ppm. The linear transformation of the percentage mortality against the log concentration of sumithion is shown in Figure 1.

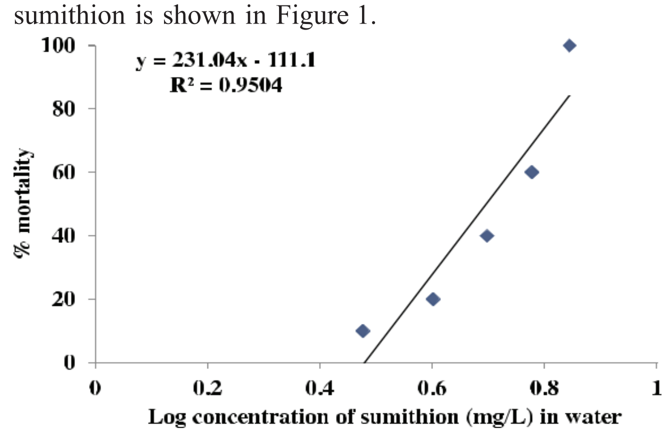

Figure 1: Linear Probit of sumithion concentrations used to determine LC_{50} value.

The values of the blood Hb level of the experimental fishes were examined after exposure of the fishes to different sumithion concentrations. The Hb level were significantly decreased ($p < 0.05$) with the increment of the sumithion toxicity at 96 h of exposure period in the concentrations of 3–6 mg/l compared to control (0 mg/l) fishes died within 72 hour exposure period (Figure 2).

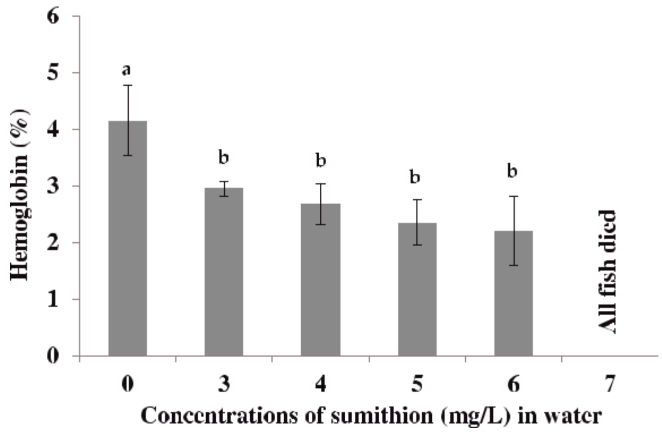

Figure 2: Alteration in the Hemoglobin (%) of C. batrachus exposed to 96 hour sumithion concentrations. Values represent the mean ± SD (n = 4).

The Erythrocytes (RBC) are such blood cells that supplying oxygen to the body tissues via the blood flow through the blood vascular system in vertebrate animals. The RBCs count ($\times 10^6/mm^3$) was found to be significantly decreased ($p < 0.05$) in higher concentrations of sumithion at 96 h of exposure (Figure 3).

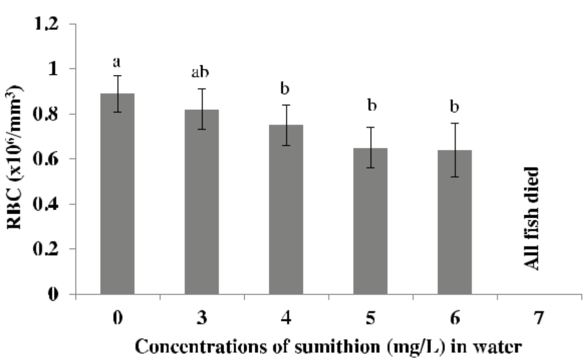

Figure 3: Alteration in the RBC (cells x $10^6/mm^3$) of C. batrachus exposed to 96 hour sumithion concentrations. Values represent the mean±SD (n = 4).

The white blood cells (WBCs) or leukocytes are the cells of the immune system those are involved in defending the body against both infectious diseases and foreign materials. The WBC was significantly ($p < 0.05$) increased in higher concentrations of sumithion at 96 h of exposure (Figure 4).

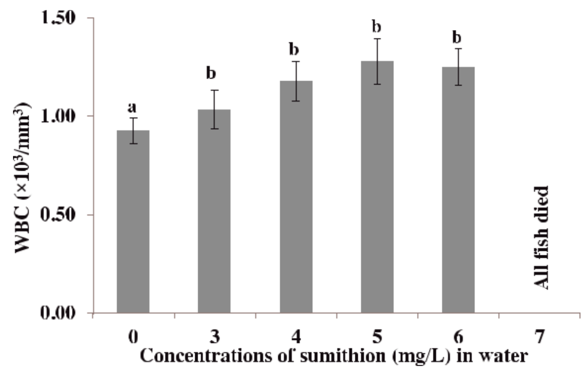

Figure 4: Alteration in the WBC (cells x $10^3/mm^3$) of C. batrachus exposed to 96 hour sumithion concentrations. Values represent the mean±SD (n = 4).

The blood glucose levels were also significantly ($p<0.05$) increased with the sumithion toxicity at 96 h of exposure period in concentrations of 3-6 mg/l compared to control (0 mg/l), whereas all the stocked fishes died at 7 mg/l (Fig. 5).

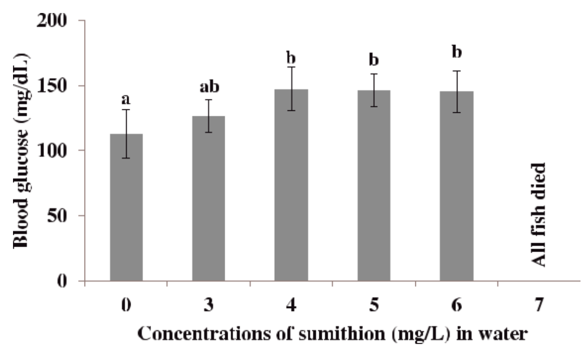

Figure 5: Alteration in blood glucose level (mg/dL) of C. batrachus exposed to 96 hour sumithion concentrations. Values represent the mean±SD (n = 4).

A statistically significant ($p < 0.05$) increase in the frequency of MN was noted in fishes exposed to sumithion concentrations of 3–6 mg/l compared to control (0 mg/l). All fishes died at 7 mg/l and about 3 to 4 folds increase in the frequency of MN noted at higher concentrations indicated the genotoxic effects of the pesticide (Figure 6).

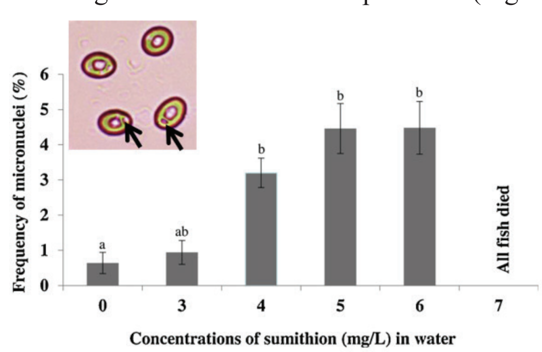

Figure 6: Micronuclei (shown by arrow) frequency in the catfish erythrocytes exposed to different concentrations of sumithion. All values are expressed as mean±SD (n=3).

DISCUSSION

The differential toxicity of sumithion for several fish species were reported in previous researches. Thr 96 hour LC50 (5.8 mg/l) of sumithion for Clarias batrachus in the present study is less than the values of 8.1 for common carp (Feneh et *al*, 2003) and 11.8 mg/l for *Heteropneustes fossilis* (Durkin, 2008). The variation of acute toxicity of any chemicals depends on the physiological conditions of the concerned species, their habitat and chemical purity of the used chemicals and some water quality factors (Sial et *al*, 2009).

The hemoglobin (Hb) is the iron-containing oxygen-transporter in the RBCs which carries oxygen from the respiratory organs to the body tissues where it releases the oxygen to provide energy harvest to be used in metabolism of animals. The present study revealed significant fall in Hemoglobin content after sumithion exposure in different concentration during the study period as similar decreased value of Hb also reported in common carp exposed to sumithion (Salam et *al*, 2015) and malathion (Sharmin et *al*, 2015). The observed hemoglobin fall in the present study may be due to the disruptive action of the pesticides on the erythropoietic tissue as a resulted in erythrocytic blood cells. There is also fall in RBC count observed in sumithion exposure might be caused by disturbances in hematopoietic system as also reported in *Clarias gariepinus* exposed to lead nitrate (Adeyemo, 2007). The significant decrease in RBC count might have resulted from hypoxic catfish or lowering of oxygen content of the water due to presence of pesticide in the research.

In this study, WBC increment with the sumithion toxicity at 96 hour exposure in comparison to control is may be due to the leukocytosis under chemical stress. The sudden rise in immunity may result with leucocytosis in fish during infection or under pathological conditions (Marti et *al*, 1996). The rise in WBCs count can be linked with enhanced antibodies production which helps in the survival and recovery of pesticide-exposed fishes (Joshi et *al*, 2002).

In the present investigation, blood glucose was found to be increased with gradual increase of sumithion exposure showed hormonal reaction of stressed fish to gluconeogenesis for their surplus energy requirements (Winkaler et *al*, 2007). There is previous research about hyperglycemic *H. Fossilis* subjected to testosterone (Chaudhary and Joy, 2000) or pesticide exposure (Borah and Yadav, 1995). This is due to the fast use of blood glucose during hyper excitability, shocks and tremors, characteristic behavior of fish toxicity to organophosphate pesticides (Singh and Singh, 1982).

The blood is considered as the pathological reflector of the body and so erythrocytes in toxicant exposure diagnosing about fish physiology. In the present study, there was a significant increase in the micronucleus (MN) formation as also observed in *Oreochromis mossambicus* (Ahmed et *al*, 2011) and *Channa punctatus* [(Patowary et *al*, 2012) due to arsenic exposure. The MN in the present study after exposure is identified as a good genotoxic biomarker for monitoring the impact of agricultural pesticide in the environment.

The findings of the present research will help the policy makers to make people conscious about the impact of excess use of insecticides in crop fields on normal physiological development of fish and other aquatic organisms. Moreover the research findings will help to find out a safety level of using this pesticide in crop lands through further research.

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Declaration: *We also declare that there is no conflict of interest among authors, and all ethical guidelines were followed during the present study.*

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