Short Communications

Pakistan J. Zool., vol. 47(5), pp. 1487-1491, 2015.

Effects of Sub-lethal Doses of an Organophosphorus Insecticide Sumithion on Some Hematological Parameters in Common Carp, *Cyprinus carpio*

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Abstract.- Toxicological effect of an organophosphorous pesticide sumithion was studied in common carp, *Cyprinus carpio*. Acute toxicity of sumithion (96 h LC_{50} value 8.05 ppm) was first determined. Then the fish were exposed to three sub-lethal concentrations (0.8, 1.6 and 3.2 ppm) of sumithion for 24, 48, 72, and 96 h, at the end of which blood glucose levels and hematological parameters were analyzed. The blood glucose level increased significantly in higher concentrations (1.6 and 3.2 ppm) compared to control (0 ppm) during the toxicity of sumithion. On the other hand, the hemoglobin, RBC and hematocrit values declined significantly with the increasing concentration of sumithion. The MCV and MCH showed significant increases in higher concentrations. The present investigation revealed that sumithion has adverse effects on the biochemical and hematological parameters, which might alter physiological functions in the fish body.

Keywords: LC_{50}, blood glucose, hemoglobin, MCV, MCH, hematocrit.

Widespread use of pesticides in the agricultural field is currently alarming for their harmful effects on non-target animals like fish. Contamination of water by pesticides, either directly or indirectly can lead to fish kills, reduced fish productivity. In Bangladesh, more than 300 types of pesticides and insecticides are used for crop protection in agriculture. Among them, sumithion, the O, O Dimethyl O-(3-methyl-4-nitrophenyl) is important an organophosphate (OP). It is effective to control wide range of important insects and certain other arthropod pests, mainly used to control brittle in paddy field. It is also used in larval rearing fish pond to control tiger bug. Sumithion is considered somewhat toxic to fish (Thomson, 1989). Since sumithion is widely used for crop protection and for eradication of aquatic insects in aqua-ponds, it is very important to know the extent of damage done by this chemical to fish.

Different hematological parameters like hemoglobin, hematocrit, and blood cell counts can reflect physiological response of contaminated environment (Dethloff *et al.,* 2001; Venkataramana *et al.* 2006). Though the effects of different pollutants on the hematological parameters of fish have been documented in several fish species (Al-Attar, 2005; Ogueji and Auta, 2007; Abalaka *et al.,* 2011; Al-Kahem Al-Balawi *et al.,* 2011), the effects of sumithion on common carp *Cyprinus carpio* has not yet been reported.

In the present study, toxicity of sumithion has been studied on this fish. The changes in blood glucose levels and hematological parameters (hemoglobin, cell counts and hematocrit values) were monitored after sub-lethal exposure to this pesticide.

Materials and methods

Animals and chemicals

The fish was obtained from local fish farm and maintained in aquaria at 22±0.5°C under a controlled natural photo-regimen (14/10 h, light/dark) for a period of 21 days before the experiments. The length and weight of fishes ranged from 17 to 19 cm and 75 to 90 g, respectively. The fish were fed with artificial pellet feed twice a day at 8:00 am and 6:00 pm. The experimental procedures followed the guidance approved by the Animal Care and Use Committees of Bangladesh Agricultural University, Mymensingh, Bangladesh. To conduct

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0030-9923/2015/0005-1487 $ 8.00/0

the present experiment, sumithion (60E/C) was collected from retail pesticide shop from local market.

Determination of acute toxicity

Acute toxicity was determined according to the standard protocol to determine the 96 h lethal concentration values (LC_{50}) of sumithion. Six different concentrations (2, 4, 6, 8, 10, and 12 ppm) of sumithion with three replicates were used in the test series. Controls 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.

Determination of hematological parameters

The experiment was conducted with four treatments, each with three replications. Treatment one was used as control (0 ppm) and three sub-lethal concentrations, such as 0.8 ppm (10% of 96 h LC_{50}), 1.6 ppm (20% of 96 h LC_{50}) and 3.2 ppm (40% of 96 h LC_{50}). Ten fish were transferred in each aquarium. Fish were sacrificed 24, 48, 72, and 96 h after exposure. Blood was collected from the caudal peduncle into citrated tuberculin syringes. Blood glucose (mg/dL) and hemoglobin (Hb, g/dL) were measured by EasyMate® GHb, blood glucose/ haemoglobin dual-function monitoring system using glucose and hemoglobin strips. The red blood cell (RBC, $x10^6/mm^3$) count was done using Neubauer hemocytometer. Hematocrit (Hct, %) values were determined by using micro-hematocrit centrifuge. The mean corpuscular volume (MCV, μm^3), the mean corpuscular hemoglobin (MCH, pg) and the mean corpuscular hemoglobin concentration (MCHC, %) were calculated using the following formulas (Jain, 1993) :

MCV = (% of Hct/RBC in millions) x 10 μm^3
MCH = (Hb in g/RBC in millions) x 10 pg
MCHC = (Hb in g/ (% of Hct) x 100 g per 100 mL

Statistical analysis

Values are 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 different treatments. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using PASW Statistics 18.0 software (IBM SPSS Statistics, IBM, Chicago, USA).

Table I.- Number of dead specimens and their percentage of mortality (in parentheses) in different concentrations of sumithion at different time intervals.

Concentration (ppm)	Exposure time (h) 24	48	72	96
Control (0.0)	-	-	-	-
2.0	-	-	-	-
4.0	-	-	3 (10%)	6 (20%)
6.0	-	3 (10%)	6 (20%)	9 (30%)
8.0	-	3 (10%)	9 (30%)	15 (50%)
10.0	21 (70%)	30(100%)	-	-
12.0	27 (90%)	30(100%)	-	-

Results

LC_{50}

Table I summarizes the mortality patterns in relation to sumithion dosages. No mortality was observed in controls samples. The 96 h LC_{50} was calculated by probit analysis (Fig. 1). The LC_{50} value of sumithion for common carp during the 96 h of exposure was 8.05 ppm.

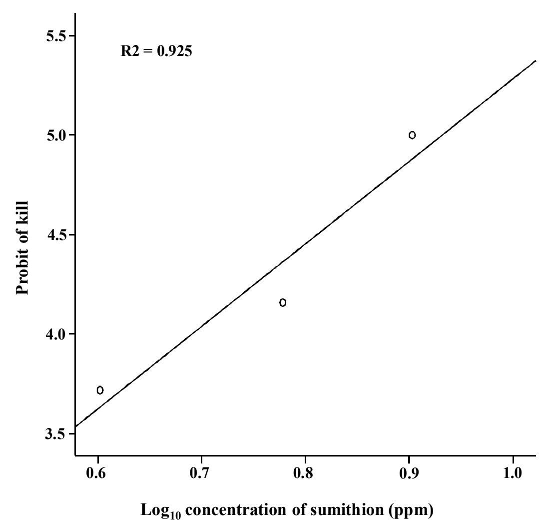

Fig. 1. Graph showing the relationship of probit of kill with log_{10} concentration of sumithion used to deduce the LC_{50}.

Blood glucose

We examined the blood glucose levels after exposure of fish to sumithion. The blood glucose levels were significantly (P<0.05) increased with the toxicity of sumithion at 24, 48, 72, and 96 h of exposure compared with control, though tended to increase in lower concentration. The glucose levels in control fishes were 110.50 to 111.50 mg/dL (Fig.2).

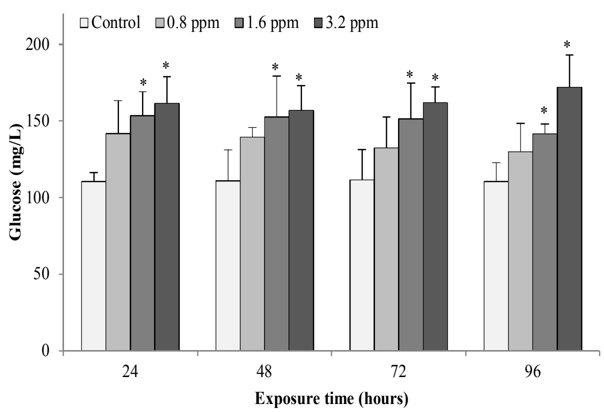

Fig. 2. Effects of different concentrations of sub-lethal doses of sumithion on blood glucose levels (Means ± SD) at different time intervals in common carp. Asterisk (*) indicates the statistically significantly different (P<0.05, n=6).

Hematological parameters

Table II shows the effect of sumithion on Hb, RBCs, Hct, MCV, MCH and MCHC. The Hb, RBCs and Hct decreased significantly (P<0.05) after exposure of fish in higher concentrations of sumithion for different periods compared to control. Conversely the MCV and MCH increased significantly (P<0.05) at higher concentrations, while MCHC showed no distinct change throughout the experimental periods.

Discussion

The 96 h LC_{50} value of common carp in the present study was 8.05 ppm which is less than the values of 11.8 ppm for *Heteropneustes fossilis* and 15.3 ppm for *Gila elegance* (Durkin, 2008; Faria *et al.,* 2010). In contrast, lower 96 h LC_{50} value (2.2 ppm) were recorded for *Oreochromis niloticus* (Pathiratne and George, 1998). The variation in the toxicity of the pesticide may be attributed mainly to the susceptibility of the test animals and factors like pH and hardness of water (Sial *et al.,* 2009).

Increased glucose levels in the present study can cause hypoglycemic condition, which may reflect stress induced hormone mediated response. Such elevation may be due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their new energy demands (Winkaler *et al.,* 2007; Abalaka *et al.,* 2011; Alkahem-Al-Balawi *et al.,* 2011). Alterations in blood glucose levels have been reported in *H. fossilis* exposed to rogor and aldrin (Singh and Singh, 1981; Borah and Yadav, 1995) and sub-lethal concentration of testosterone (Chowdhury, 2000). This is probably due to the rapid utilization of blood glucose during hyperexcitability, shocks and tremors, which are characteristic behavior of OP pesticide toxicity in fish (Singh and Singh, 1982).

In the present study, significant decrease (P<0.05) of RBC, Hb and Hct values compared to the control group were observed after sumithion treatment. A decrease in the number of RBC, Hb and Hct values were reported in common carp exposed to diazinon (Banaee *et al.,* 2011). Similarly, changes in erythrocyte profile induced by acute effect of OP pesticides, dichlorvos in *Clarias batrachus* (Benarji and Rajendranath, 1990), formothion in *H. fossilis* (Srivastava *et al.,* 1989), malathion in *Cyprinion watsoni* (Khattak and Hafeez, 1996), and trichlorphon in *Piaractus mesopotamicus* (Tavares *et al.,* 1999) provides evidence for decreased hematopoiesis followed by anemia induction in fish. Considering the previous findings, it is revealed that the decrease in RBCs count and Hb content observed in this study may be due to the disruptive action of the pesticides on the erythropoietic tissue as a result of which the viability of the cells might be affected. Changes in the hematological parameters were observed by diazinon as an anemic condition due to decreased synthesis of RBCs and erythrocyte in bone marrow (Morgan *et al.,* 1980). In addition, another type of hematological response to the effect of sumithion was a significant increase in MCV and MCH, which was also observed in this fish after acute effect of phenitrothion, imidan and dichlorvos (Svobodova, 1971, 1975; Rao, 2010).

Table II.- Changes in some hematological parameters (Means ± SD) of common carp exposed to different concentrations of sumithion.

Parameters	Treatments	Exposure time (hours)			
		24	48	72	96
Hb (g/dL)	Control	12.40 ± 0.59	12.23 ± 1.44	12.88 ± 1.81	12.65 ± 1.78
	0.8 ppm	10.75 ± 0.75	10.83 ± 1.63	10.88 ± 0.99	11.63 ± 0.68
	1.6 ppm	10.50 ± 0.93*	10.08 ± 1.04*	10.45 ± 1.03*	10.48 ± 1.63*
	3.2 ppm	10.05 ± 0.54*	10.15 ± 0.35*	10.35 ± 0.21*	10.25 ± 1.48*
RBCs ($\times 10^6/mm^3$)	Control	5.05 ± 0.11	5.20 ± 0.35	5.78 ± 0.68	5.83 ± 0.97
	0.8 ppm	3.98 ± 0.65	4.45 ± 0.07	4.85 ± 0.60	4.38 ± 0.75
	1.6 ppm	3.13 ± 0.47*	3.95 ± 0.92	3.78 ± 0.43*	2.18 ± 0.45*
	3.2 ppm	2.10 ± 0.30*	2.08 ± 0.24*	2.50 ± 0.85*	2.48 ± 0.32*
Hematocrit (%)	Control	43.19 ± 1.39	44.01 ± 3.08	41.90 ± 1.90	44.00 ± 4.48
	0.8 ppm	39.21 ± 3.72	38.20 ± 4.52	39.27 ± 5.46	37.75 ± 3.25
	1.6 ppm	33.58 ± 6.65*	34.70 ± 1.61*	36.37 ± 2.86	33.25 ± 2.47*
	3.2 ppm	30.50 ± 1.70*	29.47 ± 1.26*	30.43 ± 2.46*	31.13 ± 1.24*
MCV (μm^3)	Control	85.51 ± 1.39	84.63 ± 3.09	72.44 ± 1.90	75.43 ± 4.49
	0.8 ppm	98.44 ± 3.73	85.85 ± 4.52	80.96 ± 5.47	86.12 ± 6.25
	1.6 ppm	107.15 ± 6.66*	87.84 ± 1.61	96.12 ± 2.86	152.29 ± 2.47*
	3.2 ppm	145.47 ± 4.75*	142.00 ± 5.15*	121.72 ± 2.35*	125.76 ± 3.25*
MCH (pg)	Control	24.55 ± 0.59	23.51 ± 1.41	22.26 ± 1.81	21.69 ± 1.78
	0.8 ppm	26.99 ± 0.75	23.43 ± 1.63	22.42 ± 0.99	26.52 ± 0.68
	1.6 ppm	33.51 ± 0.93*	25.51 ± 1.04	27.62 ± 1.03	47.98 ± 1.63*
	3.2 ppm	47.93 ± 0.45*	48.92 ± 0.35*	41.40 ± 0.21*	41.41 ± 1.48*
MCHC (%)	Control	28.71 ± 1.09	27.78 ± 0.68	30.73 ± 1.02	28.75 ± 0.97
	0.8 ppm	27.41 ± 0.65	28.34 ± 0.60	27.69 ± 0.07	30.79 ± 0.75
	1.6 ppm	31.27 ± 0.47	29.04 ± 0.43	28.74 ± 0.92	31.50 ± 0.45
	3.2 ppm	32.95 ± 0.30	34.45 ± 1.24	34.01 ± 0.85	32.93 ± 0.32

Asterisk (*) indicate the statistically significantly different ($P<0.05$, n=6).

To conclude, sumithion enhanced the blood glucose level, which suggests that glycogen may have broken down to glucose due to sumithion. Conversely, reduction of Hb, RBCs and Hct might be because of failure of hematopoietic system. Therefore, the present study revealed that the sumithion has adverse effects on various blood parameters in common carp.

Acknowledgments

This work was supported by the grants from Aqua-drugs project of Bangladesh Fisheries Research Institute.

Conflict of interest statement

The authors have no conflict of interest to declare.

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(Received 3 January 2015, revised 14 February 2015)