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Effect of Repeated Application of Fenthion as a Mosquito Larvicide on Nile Tilapia (*Oreochromis niloticus*) Inhabiting Selected Water Canals in Sri Lanka

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Abstract Health status of feral Nile tilapia following repeated applications of fenthion as a mosquito larvicide to selected water canals in Sri Lanka was assessed. With three spray applications of fenthion to the study sites at weekly intervals at the concentration recommended for mosquito control, condition factor and brain acetylcholinesterase activity of the fish were depressed in a time dependent manner. Prominent histopathological alterations displayed were gill hyperplasia and telangiectasis and vacoulation of hepatocytes. Observed ill health effects of fenthion on the fish demonstrate probable ecological risk to the fish populations inhabiting the water canals which receive repeated inputs of fenthion.

Keywords Fenthion · Tilapia · Acetylcholinesterase · Histology

Fenthion (*o*,*o*-dimethyl-*o*-4-methylthio-*m*-tolylphosphorothioate) is an organophosphate widely used throughout the world as a broad spectrum insecticide and avicide (Thomson 1976; http://www.pesticideinfo.org, retrieved on 19 July 2007). Fenthion is also used in controlling mosquito larvae. As an organophosphate insecticide, fenthion inhibits acetylcholinesterase enzyme causing persistent depolarization of post-synaptic membrane of cholinergic synapses which finally leads to paralysis and death of animals. Long term exposure to sub lethal concentrations of fenthion (0.1 mg L^{-1} and 0.2 mg L^{-1}) has decreased the survival and growth rate of *Oreochromis mossambicus* (Pathiratne 1999). Fenthion was developed as a safer pesticide, which is not easily converted to the oxon form (Roberts and Hutson 1999). However, Kitamura et al. (2000) found that fenthion was converted to fenthion oxon, which was likely to be highly toxic to gold fish. It was also reported that the main metabolites of fenthion were fenthion oxon and fenthion sulfoxide in gold fish (Kitamura et al. 2000). More recent study showed that cytochrome P 450 is involved in the sulfoxidation of fenthion as well as the formation of other metabolites of fenthion in three species of fish (Bawardi et al. 2007). Lacorte et al. (1997) demonstrated that fenthion was converted to fenthion oxon in the environment. Contamination with fenthion and its oxidation products has been reported in aquatic ecosystems (Fukushima 1991; Tsuda et al. 1996). Tsuda et al. (1996) reported that fenthion was accumulated at a higher level in killifish exposed to the insecticide. These reports indicate that even though applications of fenthion are targeted at destroying mosquito larvae, it may pose a risk to non target aquatic organisms.

Stagnation of water due to rapid urbanization and deforestation has converted some shallow water canals in Sri Lanka to mosquito breeding grounds posing a serious health hazard to people living in their vicinity. Repeated application of the organophosphate insecticide fenthion to shallow canals including Bolgoda canal and several of its tributaries running through terrain belonging to the Dehiwala Mount-Lavinia Municipal council in the Western province of Sri Lanka has been carried out by this local authority to control breeding of vector mosquitoes. Community based information gathered from the area indicate that these canals are being used by villagers for transportation of goods and passengers by a canoe service over several decades. In order to control the mosquito breeding, fenthion 50% E.C. has been sprayed by the local authority

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over at least the past 10 years. The routine application of fenthion at intervals of 7–10 days is mainly aimed at disrupting the life-cycle of vector mosquitoes by killing a large number of larvae. The routine application of fenthion to control mosquito breeding may be a long-term stressor for resident fish in these water canals. According to community based information, the most abundant fish found in catches at present is the food fish Nile tilapia, O*reochromis niloticus*. The main occupation of a few people in the low income group of the area is fishing in these canals with hook and line or cast net regardless of insecticide application. The purpose of the present study was to assess the effects of repeated application of fenthion for mosquito control in the Western province of Sri Lanka, on feral fish populations inhabiting these canals.

Materials and Methods

Three sites, A, B and C were chosen for this study from three different tributaries of the main Bolgoda canal. Insecticide application to these three tributaries was suspended from 3rd January 2004, several months prior to the commencement of experiment. During the month of May 2004, the three sampling sites were visited daily and the fish caught by a cast net (1.5 cm stretch size) were sorted according to the species and counted to determine the relative abundance of fish species in these canals. Fish species inhabiting the study sites were *Oreochromis niloticus*, *Anabas testudineus*, *Ophiocephalus punctatus* and *Ophiocephalus striatus. O. niloticus* was selected as the test species since it was the most abundant at all three sites (relative abundance 99.9%).

A commercial preparation of fenthion (Baytex® 50% EC) was used for this study. Fenthion was applied using a knapsack sprayer at the concentration recommended for mosquito control (i.e. 1,000 mg/100 m^2 surface area), on three occasions to each study sites at weekly intervals starting from 4th June 2004. The concentration of insecticide in the water corresponds to approximately 0.1 mg L^{-1} of fenthion in the water body up to 10 cm depth. Fenthion was applied during the study period on 4th June 2004, 10th June 2004 and 16th June 2004. The fish samples were collected from each of the three study sites on three occasions just before the application of the insecticide, using cast nets of 1.5 cm stretch size. They were taken to the laboratory in polythene bags filled with oxygenated water from their natural habitats. The temperature and pH of the water at the collection sites at the time of sampling ranged from 27 to 28°C and 6.7 to 6.9, respectively.

In the laboratory, fish were sacrificed by pithing and their individual wet weight and total length were measured. The size ranges of sampled fish including the fish exposed to the insecticide were 8.9–12.8 cm in total length and 8.9–21.8 g in body weight. There were no significant inter-site differences in the body size of the fish. The condition factor (CF) of the fish in each sample was determined using the equation $CF = (W/L^3) \times 100$ where W = body weight (g), L = total length (cm). The CF was expressed in g cm^{-3}.

Brain tissue of the fish was removed and enzyme source was prepared by homogenizing the tissue of individual fish in ice-cold 0.1 M Phosphate buffer, pH 8.0 (in a ratio of 20 mg tissue: 1 mL of the buffer) using the Ultra-Turrax T25 tissue homogenizer (IKA Labortechnik, Germany) at high speed. All preparation steps of the enzyme source were carried out on ice. ChE activities in the homogenates were determined following the method of Ellman et al. (1961) as a kinetic assay using a recording spectrophotometer (GBC Cintra 10e, Australia) fitted with a thermostated cuvette holder. The tissue homogenate (100 µL) was added to a cuvette containing 2.8 mL of 0.1 M phosphate buffer pH 8.0 and 100 µL of 0.01 M 5,5′-dithiobis-2-nitrobenzoic acid (Sigma-Aldrich Corporation, USA). The contents were then mixed and the absorbance was read at 412 nm at 10 s intervals for 1 min. Then 20 µL of 0.076 M acetylthiocholine iodide (Sigma-Aldrich Corporation, MO, USA) was added to the reaction mixture and the absorbance was read at 10 s intervals for another 1 min. The reaction was carried out at 25°C. At least two samples were assayed for each tissue and the absorbance values were corrected for non-enzymatic reaction. Brain tissues of 16 fish were assayed from each location at each sampling stage.

Liver and gill tissues of the three fishes in each site at every sampling stage were processed for histopathological examinations as described by Bucke (1989). Abnormalities in the tissues were identified under the light microscope. The data with respect to the fish prior to insecticide application and the fish after application of fenthion in each site were compared by analysis of variance (ANOVA). Where differences were significant ($p < 0.05$) mean values were compared by Tukey's test (Zar 1999).

Results and Discussion

O. niloticus is an introduced species to these water bodies and this species has become abundant probably due to their high tolerance to habitat alteration.

Table 1 presents the condition factor of the fish prior to and after fenthion exposure. The results showed that the condition factor has decreased significantly after each application of fenthion compared with the fish prior to insecticide application. In the three sites after first applications of fenthion, the condition factor of fish has

Table 1 Condition factor (in g cm^{-3}) of *Oreochromis niloticus*

Site	Prior to insecticide application	Condition factor (in g cm^{-3})		
		7 days after first application	7 days after second application	7 days after third application
A	1.76 ± 1.15[a]	1.43 ± 0.06[b] (19%)	1.28 ± 0.04[c] (27%)	1.06 ± 0.08[d] (40%)
B	1.78 ± 1.16[a]	1.40 ± 0.04[b] (21%)	1.22 ± 0.04[c] (31%)	1.03 ± 0.09[d] (42%)
C	1.73 ± 0.11[a]	1.38 ± 0.05[b] (20%)	1.24 ± 0.06[c] (28%)	1.03 ± 0.08[d] (40%)

Data are presented as mean ± standard deviation, n = 16 for each site at each sampling location. In each row, means followed by the same superscript are not significantly different from each other (ANOVA, Tukey's test, $p < 0.05$). Numbers in parentheses are the percent reduction in the condition factor

decreased by 19%–21%, respectively, compared with that of the fish prior to insecticide exposure. After the second and third applications of fenthion, the condition factor of fish has decreased by 27%–31% and 40%–42%, respectively, compared with that of the fish prior to insecticide exposure. This study revealed that the general well being of the fish has been affected due to repeated application of fenthion at the recommended concentration for mosquito control. Prolonged exposure to fenthion may be a stressor for *O. niloticus*. Fenthion has been found to induce gluconeogenesis and glycogenolysis in the fish, *Heteropneusteus fossilis* (Srivastava and Mishra 1983). Utilization of body protein and lipid reserves for gluconeogesis may lead to retardation of growth in fish. Long term exposure to sub lethal concentrations of fenthion (0.1 mg L^{-1} and 0.2 mg L^{-1}) has decreased the survival and growth rate of another species of tilapia, *Oreochromis mossambicus* (Pathiratne 1999).

The results of the present study showed a significant cumulative depression in the brain AChE activity of the feral fish by repeated application of fenthion in the field (Table 2). Hence, apart from inhibition of brain AChE activity by the amount of fenthion input in second and third applications separately, persisting amount of fenthion in the water due to previous applications also can affect the inhibition of brain AChE activity. Although it is generally assumed that organophosphate insecticides degrade sufficiently and quickly in the environment, the half-life of fenthion in water under field conditions has been reported to range from 2.9 to 21.1 days (Eto 1979). In a recent study, significant depression of cholinesterase activities in brain and liver tissues of *O. niloticus* following single and multiple exposure of chlorpyrifos (an organophosphate insecticide) and carbosulfan (a carbamate insecticide) in the laboratory was reported by Chandrasekera and Pathiratne (2005). They found that the history of prior exposure to anticholinesterase pesticides influences the recovery period of ChE activity in *O. niloticus* exposed subsequently to these pesticides if the enzyme had not returned to normal levels before subsequent exposure. Hence the return to normalcy of this enzyme in these fish may be prolonged and this would adversely affect the health of these fish. Inhibition of acetylcholinesterase (AChE) may also affect the physiological processes associated with body growth

Table 2 Brain acetylcholinesterase activity (in nmoles min^{-1} mg^{-1} brain) in brain tissue of *Oreochromis niloticus*

Site	Prior to fenthion application	AChE activity (nmoles min^{-1} mg^{-1} brain)		
		After first application	After second application	After third application
A	15.5 ± 9.2[a]	8.6 ± 1.2[b] (45%)	3.8 ± 1.7[c] (75%)	1.8 ± 0.6[d] (88%)
B	15.4 ± 4.9[a]	7.6 ± 2.1[b] (51%)	4.9 ± 1.3[c] (68%)	1.8 ± 0.3[d] (88%)
C	12.7 ± 3.7[a]	7.6 ± 2.9[b] (40%)	4.6 ± 2.9[c] (64%)	1.6 ± 0.4[d] (87%)

Data are presented as mean ± standard deviation. n = 16 for each site at each sampling stage. In each row, means followed by the different superscripts are significantly different from each other (ANOVA, Tukey's test, $p < 0.05$). Numbers in parentheses indicate percentage inhibition in comparison to pre-exposed fish such as food consumption and digestion to certain extent which could contribute to the depletion of condition factor of the fish evident in this study.

The gill and liver tissues of the fish exposed to fenthion in the field displayed some histological changes. Erythrocyte engorged club-shaped deformities in gill lamellae (telangiectasis), excessive hyperplasia of interlamellar epithelial cells and filling up of spaces between secondary lamellae were observed in the gills of the fish exposed to fenthion. Displacement of the nucleus in some hepatocytes and vacuolation of the cytoplasm of hepatocytes were observed in the liver tissues of the fish exposed to fenthion. Progress of pathological changes in gills and liver tissues to an advanced state may impair all the functions carried on by these vital organs.

The present study revealed that condition factor and brain acetylcholinesterase activity of the feral fish *O. niloticus* were depressed by 19%–42% and 40%–88%, respectively, in a time dependent manner, when subjected to three consecutive spray applications of fenthion to their habitat at weekly intervals at the concentration recommended for mosquito control. Prominent histopathological alterations displayed were hyperplasia and telangiectasis in the gills and vacoulation of hepatocytes in liver tissues. Observed ill health effects of fenthion on the food fish *O. niloticus* demonstrate probable ecological risk to the fish populations inhabiting the water canals which receive repeated inputs of fenthion.

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