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Toxicity of Chlorpyrifos and Dimethoate to Fingerlings of the Nile Tilapia, *Oreochromis niloticus*: Cholinesterase Inhibition

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Abstract

Toxicity of chlorpyrifos and dimethoate, two organophosphorus insecticides commonly used in agricultural pest management in Sri Lanka, to the fingerlings of *Oreochromis niloticus* was evaluated. The 96 hour LC50 values for chlorpyrifos and dimethoate were determined to be 0.117 mg l^{-1} and 14.84 mg l^{-1} respectively. Exposure to sublethal concentrations of the insecticides for 15 days caused marked inhibition (51-96%) of the acetylcholinesterase activity of brain, skeletal muscle, liver and spleen tissues in the fish. Upon transfer to insecticide free water, acetylcholinesterase activity increased but complete recovery was not attained even after 20 days. The results indicate that chronic exposure to even sublethal concentrations of these insecticides affect the fingerlings of *O. niloticus*. Of the two insecticides tested, chlorpyrifos was more toxic than dimethoate.

Introduction

Organophosphorus (OP) insecticides act as neurotoxicants by blocking synaptic transmission in cholinergic parts of the nervous system. The major group of enzyme which is inhibited by OP insecticides is Acetylcholinesterase (AchE) (Coppage & Braidech 1976; Fest & Schmidt 1982). AchE measurements have been recognized as a potential biochemical indicator of OP insecticide poisoning of fish in the water bodies (Murthy 1986; Vander Wel & Welling 1989; Bruijn & Hermens 1993).

OP insecticides are widely used in Sri Lanka to control insect pests of agricultural crops especially in rice cultivation. In Sri Lanka, water for cultivation is provided mainly from reservoirs. Water from rice fields sometimes repeatedly drains into irrigation canals and other reservoirs thereby distributing the insecticides over a wide area posing risks to aquatic organisms especially fish. Recently it has been suggested that rice fields in Sri Lanka could be utilized for secondary food in the form of fish (Fernando 1996). Some activities on rice - fish culture are being carried out in Sri Lanka on experimental basis (Edirisinghe & Perera 1995; Edirisinghe & Bodhiwansa 1996). However, contamination of irrigation canals and rice fields with insecticides used to control pests of rice plants may affect the fish yield. To date, there is little information on toxicity of OP insecticides on inland food fish species in Sri Lanka.

Nile tilapia, *Oreochromis niloticus* is one of the commercially most important inland food fish species introduced to reservoirs in Sri Lanka. In the present investigation, acute toxicity and sublethal effects of chlorpyrifos and dimethoate, two OP insecticides

commonly used in Sri Lanka on fingerlings of *O. niloticus* were evaluated. The acute toxicities were quantified through median lethal concentration (LC50). Sublethal toxicities were quantified through *in vivo* inhibition of AchE. Recovery patterns of the insecticides inhibited AchE in the fish were also studied.

Materials and Methods

Fish

Fingerlings of *O. niloticus* (1.6-2.2 g in body weight) were obtained from the Udawalawe Fisheries Station, Ministry of Fisheries and Aquatic Resources Development, Sri Lanka and allowed to acclimate to laboratory conditions in rectangular aquaria with aged tap water under natural photoperiod for two weeks. The fish were fed daily with commercial fish meal.

Insecticides:

Commercial formulations of two insecticides, chlorpyrifos, available in 40% emulsifiable concentration (EC), and dimethoate available in 50% EC were used.

Acute toxicity tests

Static acute toxicity tests were conducted in accordance with the standard procedure outlined by FAO (Reish & Oshida 1986). Solutions of the insecticides were made by diluting commercial formulations of the insecticides with aged tap water to obtain desired concentrations. Range finding tests were conducted to find the range of concentrations of the insecticides to be used for the definitive toxicity tests. Based on the results of the range finding tests, groups of 10 fish (1.8-2.3 g in body weight) were placed in rectangular glass aquaria with 36 l of series of concentrations of chlorpyrifos (0.05-0.40 mg l^{-1}) or dimethoate (5-20 mg l^{-1}). In all tests, triplicates were used and control sets were maintained simultaneously with only the aged tap water. Temperature, pH and dissolved oxygen concentration of the test water were measured daily and found to be within the acceptable limits for fish. The mortalities of fish exposed to different concentrations of the insecticides were recorded every 24 hours for 96 hours, dead fish being removed every 3-12 hours. LC50 values were determined using the computer programme, "Toxicologist" (Euro-Med centre on Marine contamination Hazards 1990). The concentration - mortality data were also analyzed statistically following Finney (1971).

Sublethal toxicity tests

Based on the 96 hour LC50 values obtained from acute toxicity tests, two concentrations of each insecticide (10% LC50 and 20% LC50): chlorpyrifos 0.012 mg l^{-1} and 0.024 mg l^{-1} ; dimethoate 1.5 mg l^{-1} and 3.0 mg l^{-1} were used as sublethal concentrations. Fish (1.9-2.6 g in body weight) were exposed to the sublethal concentrations in groups of 20-25 fish for 15 days. Control fish were maintained simultaneously with the aged tap water and the experiments were conducted in triplicates. The test solutions were renewed regularly at every 96 hour interval. As in the acute toxicity tests, the water quality parameters were measured daily and found to be within the acceptable limits. After 15 days of insecticide exposure, a sample of fish in each concentration was sacrificed for AchE assay. The remaining fish were transferred to clean aged tap water for recovery. These fish were sacrificed at 10 day intervals for AchE assay.

AchE assay:

Fish were sacrificed and brain, skeletal muscle, liver and spleen were removed. The enzyme sources were prepared by homogenizing the tissues in 0.1M phosphate buffer, pH 8.0. AchE activities of the homogenates were assayed using acetylthiocholine iodide as the substrate following the spectrophotometric method of Ellman et al. (1961). Enzyme activities are presented as mean±SD. The differences between the mean values were compared by analysis of variance (ANOVA). Where differences were significant ($P<0.05$), mean values were compared by Scheffe's test (Kleinbaum et al. 1988).

Results

The results of the toxicity tests are presented in Table 1. The acute toxicity varied depending on the insecticide tested and the duration of the exposure. The LC50 for 24 hour exposure was 0.135 mg l^{-1} for chlorpyrifos and 17.94 mg l^{-1} for dimethoate. After 96 hours, the toxicity was greater with LC50 values of 0.117 mg l^{-1} for chlorpyrifos and 14.84 mg l^{-1} for dimethoate.

Table 1. LC50 values (24,48,72 and 96 hours) of chlorpyrifos and dimethoate for fingerlings of *Oreochromis niloticus*.

Insecticide	Exposure period (hours)	LC50 (mg l^{-1})	95% confidence limits(mg l^{-1})	slope
chlorpyrifos	24	0.135	0.100 - 0.186	1.72
	48	0.124	0.091 - 0.170	1.73
	72	0.120	0.087 - 0.165	1.75
	96	0.117	0.083 - 0.161	1.77
dimethoate	24	17.94	15.43 - 28.44	1.45
	48	15.95	13.68 - 21.92	1.48
	72	14.98	13.18 - 17.72	1.36
	96	14.84	13.04 - 17.49	1.36

During the 96 hour acute exposure, fish exposed to higher concentrations of the insecticides (>0.10 mg l^{-1} chlorpyrifos and >12 mg l^{-1} dimethoate) showed pronounced behavioural changes; they were incapable of normal swimming and made erratic movements. Changes in integumental pigmentation were also observed. Behavioural changes were also observed in the fish exposed to sublethal concentrations of the insecticides (0.012 and 0.024 mg l^{-1} chlorpyrifos; 1.5 and 3.0 mg l^{-1} dimethoate) for 15 days. These fish showed erratic swimming and later became lethargic.

Acetylcholinesterase activities of various tissues of control fish and the fish exposed to sublethal concentrations of the insecticides for 15 days are presented in Table 2. The *in vivo* sublethal toxicity of the insecticides was reflected by marked inhibition of the AchE activities in the brain, skeletal muscle, brain and spleen tissues of insecticide exposed fish in comparison with the controls. AchE activities of the brain tissues of chlorpyrifos exposed fish were more strongly inhibited (92-96%) than that of fish exposed to dimethoate (51-86%). Inhibition of the AchE activity in brain tissues was dependent on the concentration of the insecticide. Percentage inhibition of the AchE activity of the other

tissues namely skeletal muscle and liver of fish exposed to chlorpyrifos was also greater than that of the dimethoate exposed fish.

AchE activity of the brain tissues during recovery phase are shown in Fig. 1. At the end of 20 days of post exposure, the AchE activity of the brain tissues of fish exposed to dimethoate recovered up to 78% of the normal value. However, the enzyme activity of the chlorpyrifos exposed fish recovered only up to 49% of the normal value at the end of the test period. Results show that after termination of the insecticide exposure, the AchE activities slowly increased in the fish but complete recovery was not attained even by the end of 20 days.

Table 2. Acetylcholinesterase activities of various tissues of *Oreochromis niloticus* following 15 days exposure to sublethal concentrations of insecticides. In each column, means not followed by the same superscript are significantly different from each other($P<0.05$). Numbers in parentheses indicate percentage inhibition in enzyme activity in comparison to the controls (n=10-12).

Exposure	Enzyme activity (n moles min^{-1} mg^{-1})			
	Brain	Skeletal muscle	Liver	Spleen
control	23.04 ± 1.67^{a}	4.60 ± 1.20^{a}	9.89 ± 0.12^{a}	4.18 ± 0.45^{a}
chlorpyrifos(mg l^{-1})				
0.012	1.87 ± 0.45^{d} (92%)	0.22 ± 0.07^{c} (95%)	1.38 ± 0.08^{c} (86%)	0.66 ± 0.05^{b} (84%)
0.024	0.82 ± 0.24^{e} (96%)	0.21 ± 0.02^{c} (95%)	1.04 ± 0.35^{c} (89%)	0.62 ± 0.05^{b} (85%)
dimethoate (mg l^{-1})				
1.5	11.20 ± 3.09^{b} (51%)	0.53 ± 0.18^{b} (88%)	1.94 ± 0.38^{b} (80%)	0.83 ± 0.23^{b} (80%)
3.0	3.34 ± 0.65^{c} (86%)	0.42 ± 0.01^{b} (91%)	1.62 ± 0.41^{bc} (84%)	0.63 ± 0.17^{b} (85%)

Discussion

The 96 hour LC50 values of chlorpyrifos and dimethoate for fingerlings of *O. niloticus* were found to be 0.117 mg l^{-1} and 14.84 mg l^{-1} respectively. Results showed that acute toxicity of chlorpyrifos is more than 100 times greater than the toxicity of dimethoate for fingerlings of *O. niloticus*. Chlorpyrifos was found to be very toxic to guppies (Vander wel and Welling, 1989) and even to freshwater shrimps (Abdullah et al. 1993).

Using the pesticide safety level for OP insecticides, suggested by FAO (1969), the estimated safety ranges for chlorpyrifos and dimethoate from this study correspond to 0.00117 mg l^{-1} - 0.0117 mg l^{-1} and 0.148 mg l^{-1}-1.48 mg l^{-1} (1% 96 hour LC50-10% 96 hour LC50) respectively for *O. niloticus* fingerlings. Recommended application rates of chlorpyrifos and dimethoate are 1.008 kg ha^{-1} and 0.728 kg ha^{-1} respectively which

correspond to concentrations of 1.008 mg l^{-1} and 0.728 mg l^{-1} in irrigated rice fields with 10 cm of water. When chlorpyrifos is applied at the recommended rate, its concentration in irrigated rice fields would not be within the safety range estimated from this study indicating that the recommended application rates of chlorpyrifos for controlling rice pests could affect the fingerlings of *O. niloticus*. However, at the recommended application rates, the concentration of dimethoate in irrigated rice fields would be within the safety range estimated from this study.

AchE enzyme is vital for the functioning of the sensory, integrative and neuromuscular systems of fish (Murthy 1986). In the present study, AchE activities in the brain, skeletal muscle, liver and spleen in the fingerlings of *O. niloticus* were significantly inhibited following 15 days of exposure to sublethal concentrations of chlorpyrifos and dimethoate clearly demonstrating the high anticholinesterase effect of these insecticides. Verma et al. (1979) also observed high anticholinesterase effect in two freshwater fishes, *Channa gachua* and *Cirrhinus mrigala* after sublethal exposure to three OP insecticides, Zolone, Roger and Malathion. It has also been reported that exposure to sublethal concentrations of malathion significantly affected the behaviour and AchE activity in red tilapia (*Oreochormis mossambicus* X *Oreochromis niloticus*) (Sulaiman et al. 1989) and in bluegill sun fish, *Lepomis macrochirus* (Richmonds & Dutta 1992).

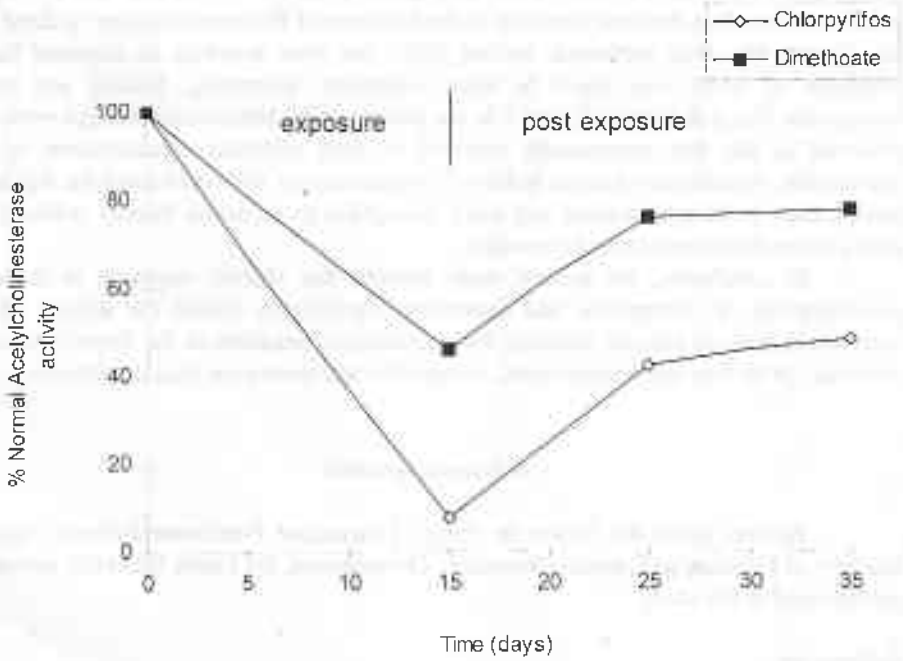

Fig. 1. Brain acetylcholinesterase activity of *Oreochromis niloticus* fingerlings at the end of the insecticide exposure and its recovery during postexposure period.

In the present study, percentage inhibition of AchE in brain and other tissues caused by the exposure to sublethal concentrations of chlorpyrifos was significantly higher than the inhibition caused by the sublethal concentrations of dimethoate. Results indicate that even chronic toxicity of chlorpyrifos is greater than that of dimethoate for *O. niloticus* fingerlings. When the insecticide exposed fish were transferred to insecticide free water, slow recovery of AchE activity was observed. By the end of the 20th day, AchE activity in the fish exposed to dimethoate had mostly recovered (78%) while AchE activity in chlorpyrifos exposed fish had recovered the least gaining only 49% the activity. The results indicate that AchE activity in these fish had not recovered fully within 20 days and a longer time may be needed before the normal activity of AchE could be attained. OP insecticides bind irreversibly to cholinesterase enzymes (Fest & Schmidt 1982). Hence, fish exposed to OP insecticides must synthesize new enzymes in order to return activity to normal, a process that appears to take time (Rand & Petrocelli 1985). The recovery time was found to vary with the species of fish (Rand & Petrocelli, 1985; Zinkle et al. 1987; Sulaiman et al. 1989).

Although OP insecticides generally do not persist for very long, with repeated inputs to the aquatic environment, the fish may be exposed to low sublethal concentrations of the insecticides for long period of time (Edwards 1977). Hence, inhibition of AchE activity in populations of *O. niloticus* may occur upon repeated sublethal exposure to these insecticides affecting the functioning of the nervous system in the fish. In the long run, these repeated sublethal concentrations may prove more deleterious than the short term lethal concentrations because alteration in the functions of the nervous system in these fish may in turn alter their behaviour, feeding habits and other activities. In salmonid fishes, inhibition of AchE was found to alter respiration, swimming, feeding and social interactions (Rand & Petrocelli 1985). In the present study, behavioural changes were also observed in the fish continuously exposed to even sublethal concentrations of the insecticides. Behavioural changes in the wild populations of fish could make the fish more conspicuous in the environment and more susceptible to predation thereby reducing the ability of the fish to survive and reproduce.

In conclusion, the present study showed that chronic exposure to sublethal concentrations of chlorpyrifos and dimethoate significantly inhibit the activity of the acetylcholinesterase enzyme affecting the neurological functions in the fingerlings of *O. niloticus*. Of the two insecticides tested, chlopyrifos was more toxic than dimethoate.

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