J. Natn. Sci. Foundation Sri Lanka 1999 27(2): 79-91

TOXICITY OF FENTHION IN LEBAYCID® TO TILAPIA, *OREOCHROMIS MOSSAMBICUS* (PETERS): EFFECTS ON SURVIVAL, GROWTH AND BRAIN ACETYLCHOLINESTERASE ACTIVITY.

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(Received: 19 June 1997 ; accepted : 07 May 1999)

Abstract:Effects of long term exposure to sublethal concentrations of organophosphorous insecticide, fenthion (used as Lebaycid®) on survival, growth and brain acetylcholinesterase (AchE) activity of fry and fingerlings of *Oreochromis mossambicus*, a widely distributed food fish species in the inland waterbodies of Sri Lanka were investigated. The 96h LC_{50} values of fenthion for fry (0.19 - 0.26 g in body weight) and fingerlings (2.1 - 2.9 g in body weight) were 0. 83 mgl^{-1} and 2.07 mgl^{-1} respectively. Exposure to sublethal concentrations of fenthion (0.1 mgl^{-1} and 0.2 mgl^{-1}) for 10 weeks significantly decreased the survival and growth of fish compared to the control fish. Long term exposure to sublethal concentrations of fenthion also exerted significant inhibitory effects on brain AchE activity of fish, ranging from 26 - 29 % inhibition in the fingerlings and 38 - 40% inhibition in the fry. Upon transfer to insecticide free water for 10 weeks, AchE activity of the fry and fingerlings exposed to fenthion recovered completely but the fry stage exposed to high concentrations of fenthion continued to grow at a slower rate. The fry stage of *O. mossambicus* was more sensitive to fenthion exposure than fingerlings.

Key words: Acetylcholinesterase, fenthion, insecticides, *Oreochromis*, organophosphorous compounds, tilapia

INTRODUCTION

Organophosphorus (OP) insecticides are widely used in many countries to control agricultural pests. Insecticides when used in the vicinity of aquatic ecosystems may enter the water bodies as a result of spray drift and run off from agricultural land and leaching from the soil in concentrations which may exert lethal and sublethal effects on fish populations. OP insecticides generally do not persist for very long, but with repeated input to the water bodies, fish may be exposed to low sublethal concentrations of the insecticide for an extended period of time.[1,2] In a review on measurement of pollutant toxicity to fish, Sprague[3] reported that impaired growth was a good indicator of long term metabolic stress from sublethal levels of toxicants. Inhibition of enzymes involved in nerve function, especially acetylcholinesterase (AchE) in fish and shellfish has been suggested as a measure of impact of OP insecticides.[4-6]

To date, there is little information on chronic effects of pesticides on food fishes in Sri Lanka. Tilapia, *Oreochromis mossambicus* is a widely distributed food fish in the inland waterbodies in Sri Lanka. The present investigation was carried out to assess the effects of long term exposure to sublethal concentrations of fenthion

(Lebaycid®), a commonly used OP insecticide in Sri Lanka, on survival, growth and brain acetylcholinesterase activity of young *O. mossambicus*. Lebaycid® which is a commercial formulation of fenthion (50% w/v emulsifiable concentrate of fenthion) is widely used as an insecticide in Sri Lanka for agriculture. Wild fish are therefore exposed to fenthion along with the other additives in Lebaycid®

METHODS AND MATERIALS

Fish:

Young *O. mossambicus* (0.17 - 3.1 g in body weight) were brought to the laboratory from a freshwater hatchery of the Ministry of Fisheries & Aquatic Resources Development, Sri Lanka. Fish were allowed to acclimate to laboratory conditions for one week under natural photoperiod in glass aquaria with aged tap water. Fish were fed with commercially available fishmeal during the period of acclimation.

Acute toxicity tests:

Prior to the chronic toxicity experiments short term acute toxicity tests were conducted according to the standard procedure of Reish & Parrish[7] to evaluate the sensitivity of the species and to establish the sublethal concentrations to be used in chronic toxicity tests. Solutions with desired concentrations of the insecticide were made by diluting Lebaycid® (50% w/v emulsifiable concentrate) with aged tap water. The concentrations and nature of the other components of Lebaycid® are not available. Fingerlings (2.12 - 2.94 g in body weight and 48 - 54 mm in total length) were placed in groups of 10 individuals in rectangular glass aquaria containing 30 l of different concentrations of fenthion (0, 0.1, 0.5, 1, 2, 3, 4, 8 mg l^{-1}) and the mortality of the fish was observed for 96h. Each concentration of the insecticide had triplicates. The same procedure was followed with the fry stage of fish (0.193- 0.264 g body weight and 20 - 24 mm in total length) with different concentrations of the insecticide (0, 0.02, 0.1, 0.5, 1, 2 mg l^{-1}). Concentrations to be used in the definitive acute toxicity tests were determined by the preliminary range finding acute toxicity tests. The 96h LC_{50} values of fenthion with 95% confidence limits were determined using the computer programme, "Toxicologist" (Euro- Med Centre on Marine Contamination Hazards, 1990). The acute toxicity data were also analysed by Probit analysis[8].

Chronic toxicity tests:

Based on the 96h LC_{50} values of the acute toxicity tests, three sublethal concentrations of the insecticide, 0.02 mgl^{-1}, 0.1 mgl^{-1} and 0.2 mgl^{-1} were chosen for the chronic toxicity test which was designed with a static renewal procedure. Acclimated fish were divided into two groups according to their size: fry

(0.193 - 0.268 g body weight, 19 - 24 mm in total length) and fingerlings (2. 02 - 2.94 g body weight, 45 - 55 mm in total length). Fish in each size group were randomly divided into 4 groups of 60 individuals. First group of fish in each size group were placed in glass aquaria containing aged tap water and treated as controls. Twenty fish were included in each aquarium containing 50 l of test water. The other three groups were placed in glass aquaria containing sublethal concentrations of the insecticide (0.02 or 0.1 or 0.2 mgl^{-1}). Each treatment had triplicates.

The fish were fed with a commercially available fishmeal twice daily (6% of the body weight per day approximately at 0830 and 1530h. Unconsumed food was removed after 2h of feeding to reduce the contamination of the medium. Before presentation of the meal each day, dead fish were counted and removed and the faecal material was siphoned out. Every fourth day, each aquarium was cleaned and the test water was renewed with the same concentration of the insecticide and this procedure was followed for 10 weeks. The weights of the fish were measured every two weeks. In addition, the total number of fish surviving in each group was determined and the food ration was adjusted accordingly.

Subsequent to the chronic exposure, 3 - 4 fish from each experimental aquarium were sacrificed (n = 10-12 for each treatment) and their brains were removed for AchE assay. The remaining fish in each aquarium containing different concentrations of the insecticide were placed separately in aquaria with clean aged tap water for recovery. Fish were fed as stated previously and their body weight was measured every 2 weeks. After leaving them for 10 weeks in insecticide free water, their final body weight was recorded and their brains were removed for AchE assay. Overall growth responses of fish maintained in different concentrations of fenthion and the growth responses of fish over the post-exposure period were expressed in terms of mean fish body weight, % weight gain and specific growth rates.

AchE and protein assay:

AchE activity was assayed in brain homogenates in 0.1 M phosphate buffer (pH 8.0), following the spectrophotometric method of Ellman *et al*[9]. Protein concentration in the brain homogenates was estimated by the method of Lowry *et al*[10] with bovin serum albumin as the standard.

Water quality parameters:

Acute toxicity tests and chronic toxicity tests were conducted under natural photoperiod. Glass aquaria were provided with continuous aeration. Temperature of

the test water was monitored once daily and pH and dissolved oxygen concentration once in two days.

Statistical analysis:

In the chronic toxicity study, data from control and test concentrations were compared by analysis of variance (ANOVA). Where differences were significant ($p < 0.05$) mean values were compared by Schefee's test[11].

RESULTS

Acute toxicity data for fry and fingerlings of *O. mossambicus* exposed to fenthion are presented in Table 1. Acute toxicity varied depending on the size group of the fish. The 96h LC_{50} values of fenthion for the fry and fingerlings were determined to be 0.83 mgl^{-1} and 2.07 mgl^{-1} respectively. The confidence limits for the two LC_{50} values do not overlap with each other indicating that the LC_{50} value for fry is significantly different from that for the fingerlings. Three sublethal concentrations of fenthion used in the 10 weeks chronic toxicity tests (0.02 mgl^{-1}, 0.1 mgl^{-1}, and 0.2 mgl^{-1}) were 2.5%, 12.5% and 25% of the 96h LC_{50} value for fry which correspond to 1%, 5% and 10% of the LC_{50} value for the fingerlings.

Table 1 : Acute toxicity of fenthion for young *O. mossambicus*.

Fish	Body weight (g)	96 h LC_{50} (mgl^{-1})	Confidence limits (mgl^{-1})
Fry	0.193-0.264	0.83	0.28-1.35
Fingerlings	2.12-2.94	2.07	1.48-2.88

Survivor curves of fry and fingerlings exposed to different sublethal concentrations of the insecticide are shown in Figure 1. Survival of the control fry and fingerlings after 10 weeks period was 90% and 95% respectively. Percentage surival of fry and fingerlings exposed to the lowest concentration (0.02 mgl^{-1}) of fenthion was not significantly different from the respective control fish. However the survival of fry and fingerlings decreased considerably during 10 weeks exposure to 0.1 $mg.l^{-1}$ and 0.2 $mg.l^{-1}$ fenthion. Fry and fingerlings exposed to 0.2 $mg.l^{-1}$ of fenthion showed only 68% and 78% survival respectively at the end of the exposure period.

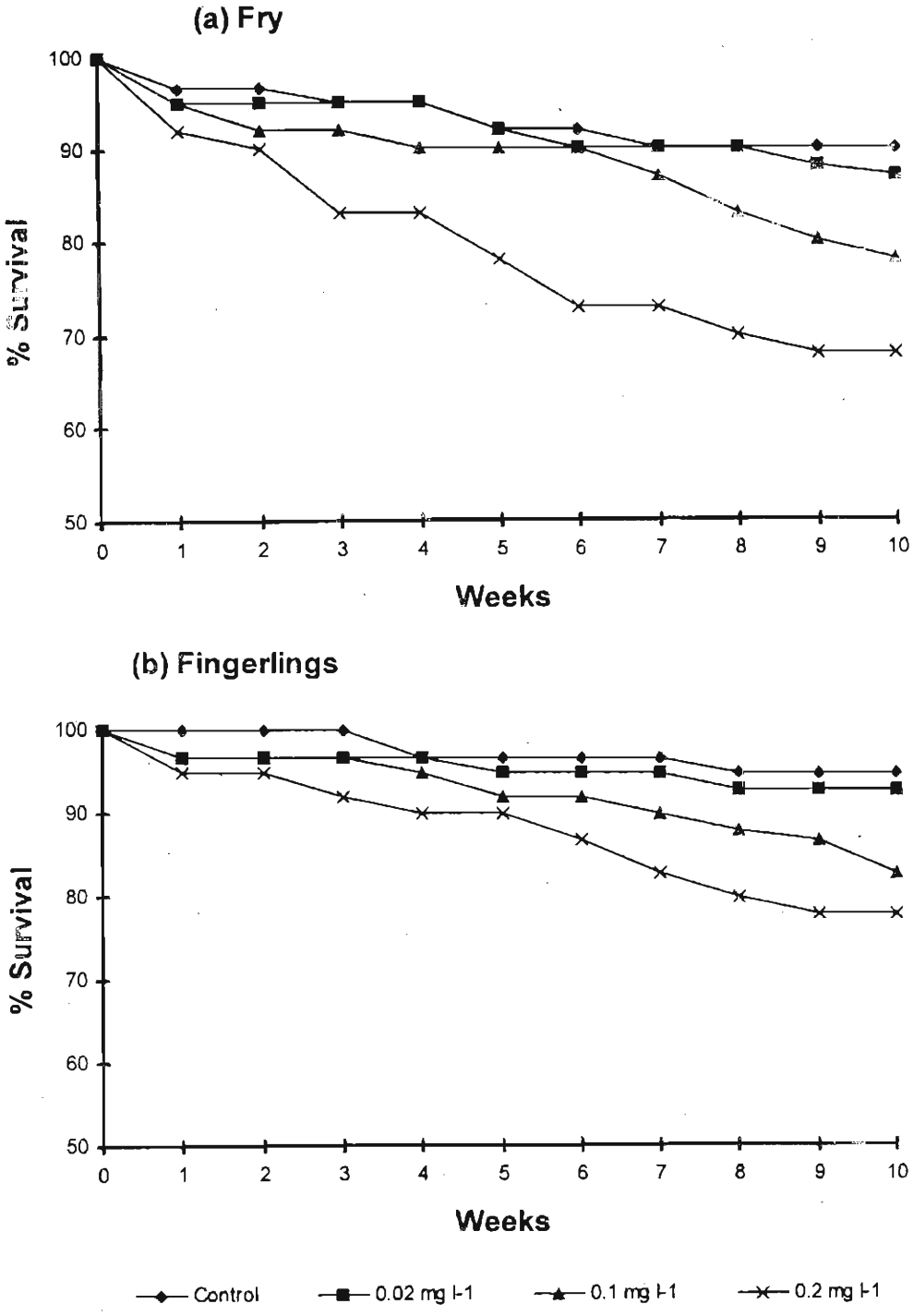

Figure 1: Percentage survival of *O. mossambicus* exposed to sublethal concentrations of fenthion. (a) Fry (b) Fingerlings.

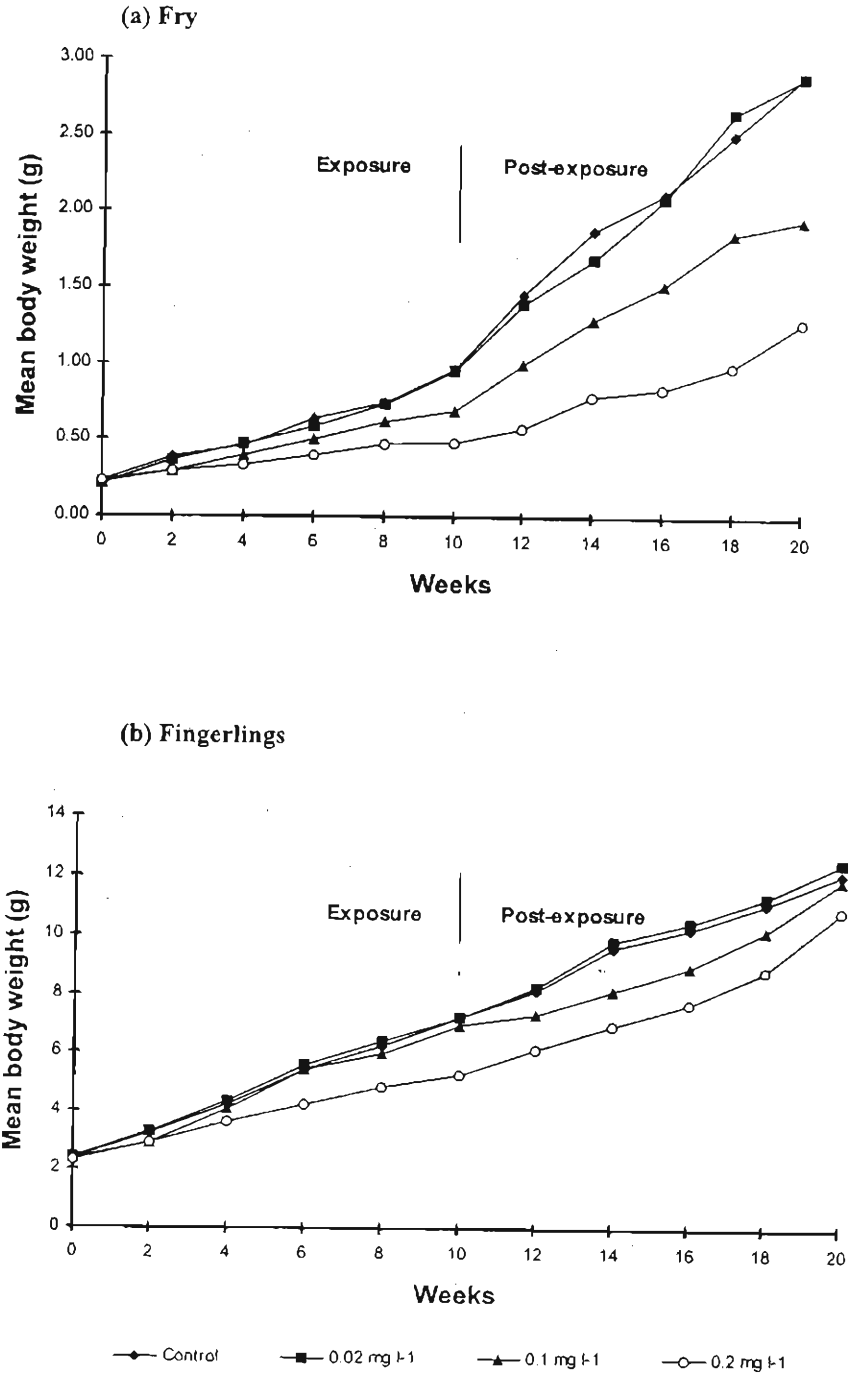

Figure 2 : Growth of *O. mossambicus* exposed to sublethal concentrations of fenthion during 10 weeks exposure and 10 weeks post exposure period. (a) Fry (b) Fingerlings.

Table 2 : **Growth responses of young *O. mossambicus* exposed to sublethal concentrations of fenthion for 10 weeks and after 10 weeks post-exposure to fenthion free water.**

Treatment	Initial body weight (g)	After 10 weeks exposure			After 10 weeks post-exposure		
		Body weight after exposure (g)	Weight gain %	Specific growth rate (% d^{-1})	Final body weight (g)	Weight gain %	Specific growth rate (% d^{-1})
Fry							
Control	0.226±0.032[a]	0.962±0.083[a]	326[a]	2.07[a]	2.91±0.41[a]	195[a]	1.58[a]
0.02 mgl^{-1}	0.214±0.032[a]	0.953±0.081[a]	345[a]	2.13[a]	2.89±0.39[a]	203[a]	1.58[a]
0.1 mg l^{-1}	0.223±0.031[a]	0.692±0.072[b]	210[b]	1.62[b]	1.91±0.28[b]	176[b]	1.45[b]
0.2 mg. l^{-1}	0.226±0.039[a]	0.486±0.089[c]	115[c]	1.09[c]	1.28±0.31[c]	163[b]	1.38[b]
Fingerlings							
Control	2.37±0.32[a]	7.23±0.79[a]	205[a]	1.59[a]	12.14±3.1[a]	68[a]	0.74[a]
0.02 mg l^{-1}	2.42±0.41[a]	7.19±.0.82[a]	197[a]	1.56[a]	12.53±3.3[a]	74[a]	0.79[a]
0.1 mg l^{-1}	2.39±0.42[a]	6.92±0.99[a]	190[a]	1.52[a]	11.92±3.4[a]	72[a]	0.78[a]
0.2 mg l^{-1}	2.33±0.23[a]	5.24±0.32[b]	125[b]	1.16[b]	10.88±4.9[a]	108[b]	1.04[b]

Data are presented as mean ± standard deviation of 32-60 fish for each treatment. In a column, for each size group of fish, means not followed by the same superscript are significantly different from each other (ANOVA, Schefee's test, $p<0.05$).

Biweekly changes in mean body weights of fish during fenthion exposure and subsequent recovery period are shown in Figure 2. The results show that body weight increased in all groups of fish during both exposure and recovery period. Growth parameters for the exposure and post-exposure periods are presented in Table 2. In both size groups of fish, exposure to 0.02 mgl^{-1} of fenthion had no significant effect on normal growth of fish. However, fry exposed to 0.1 mgl^{-1} fenthion exhibited a significantly slower growth rate compared with control fishes' whereas the fingerlings exposed to the same concentration had a growth rate comparable to the controls. In both size groups, fish exposed to 0.2 mgl^{-1} grew significantly slower than the respective controls. When fenthion exposed fish were transferred to insecticide free water for 10 weeks, the fry exposed to 0.1 mgl^{-1} and 0.2 mgl^{-1} continued to grow at a slower rate during the 10 weeks post exposure period. However, fingerlings were able to grow faster and attain body weights comparable to the controls. Final body weights, percentage body weight gain and specific growth rates of the fry and fingerlings reflect these differences (Table 2).

Table 3: Brain acetylcholinesterase activities in young *O. mossambicus* exposed to sublethal concentrations of fenthion.

	Enzyme activity (nmoles min^{-1} mg^{-1})	
Treatment	After 10 weeks exposure	After 10 weeks post exposure
Fry		
Control	183 ± 14^{a}	194 ± 24^{a}
0.02mgl^{-1}	179 ± 18^{a}	189 ± 25^{a}
0.1 mgl^{-1}	114 ± 11^{b}	181 ± 17^{a}
0.2 mgl^{-1}	110 ± 14^{b}	189 ± 24^{a}
Fingerlings		
Control	241 ± 23^{a}	248 ± 34^{a}
0.02 mgl^{-1}	234 ± 29^{a}	239 ± 39^{a}
0.1 mgl^{-1}	179 ± 11^{b}	218 ± 46^{a}
0.2 mgl^{-1}	172 ± 19^{b}	236 ± 44^{a}

Data are presented as mean ± standard deviation of 10-12 fish. In a column for each size group of fish, means not followed by the same superscript are significantly different from each other (ANOVA, Schefee's test, $p\leq0.05$)

The effects of long term exposure to sublethal concentrations of fenthion on AchE activity of brain tissues of fish are presented in Table 3. Long term exposure of fry and fingerlings to fenthion at 0.02 mgl^{-1} had no significant effect on brain AchE activity. However, exposure of fry to 0.1 - 0.2 mgl^{-1} fenthion resulted in 38 - 40% reduction in brain AchE activity. The same levels of exposure resulted in 26 - 29% reduction in brain AchE activity in the fingerlings. Upon transfer to fenthion free water for 10 weeks, complete recovery of AchE activity was attained in both size groups of fish.

Table 4 presents the data on water quality parameters in experimental aquaria containing different test solutions of fenthion. Statistical analysis showed that there were no significant differences ($p>0.05$) in the water quality parameters of different concentrations of insecticide solutions.

Table 4 : Water quality parameters of different aquaria during the experiment.

Treatment	Temperature (^{0}C)	pH	Dissolved oxygen (mgl^{-1})
Fry			
Control	28.3±2.1^a	6.72±0.21^a	5.4±0.7^a
0.02 mgl^{-1}	28.2±1.9^a	6.69±0.19^a	5.6±0.8^a
0.1 mgl^{-1}	27.9±2.1^a	6.73±0.13^a	5.3±0.5^a
0.2 mgl^{-1}	28.6±2.9^a	6.72±0.14^a	5.4±0.6^a
Fingerlings			
Control	28.4±2.3^a	6.71±0.14^a	5.2±0.5^a
0.02 mgl^{-1}	28.9±1.4^a	6.76±0.21^a	5.3±0.4^a
0.1 mgl^{-1}	27.7±2.3^a	6.73±0.19^a	5.4±0.6^a
0.2 mgl^{-1}	28.8±2.3^a	6.74±0.22^a	5.1±0.7^a

Data are presented as mean ± standard deviation of the parameters recorded in three aquaria per treatment. In each column, mean followed by the same superscript are not significantly different from each other (ANOVA, $p>0.05$)

DISCUSSION

The 96 h LC_{50} values of fenthion for fry and fingerlings of *O. mossambicus* were found to be 0.83 and 2.07 mgl^{-1} respectively. Using the pesticide safety levels for organophosphorous pesticides suggested by the FAO[12] the safety range (1% of 96 h LC_{50}-10% of 96 h LC_{50}) of fenthion estimated from this study corresponds to 0.008 - 0.083 mgl^{-1} for fry stage and to 0.021- 0.208 mgl^{-1} for fingerlings.

Present study showed that 10 weeks exposure to 0.02 mgl^{-1} of fenthion (which is within the safety range estimated from this for fry and fingerlings), had no adverse effects on the survival or normal growth rates of the fish. However, long term exposure to the other two sublethal concentrations of fenthion (0.1mgl^{-1} and 0.2 mgl^{-1}) significantly decreased the survival and growth rates of young *O. mossambicus*. Fry stage of fish was more sensitive to the level of exposure than fingerlings. When fenthion exposed fish were transferred to insecticide free water for 10 weeks, fingerlings were able to grow faster and attain body weights comparable to control fish. However longer time appears to be needed for recovery of fry exposed to higher concentrations of fenthion.

Impaired growth of *O. mossambicus* exposed to sublethal concentrations of fenthion corroborates the earlier findings with other insecticides for *Channa punctatus*[13], *Macropodus cupaneus*[14], *Puntius stigma*[15] and *Lepidocephalichthyes thermalis*[16]. Fenthion has been found to induce muscle and liver glycogenolysis in the fish *Heteropneustes fossilis*[17]. Reduction in growth following fenthion exposure may result from enhanced metabolism to compensate for high maintenance energy demands of fish under insecticide stress[17]. Reduction in growth of fish following insecticide exposure could also result from decrease in food consumption and food conversion efficiency[14-16]. Sublethal concentrations of two OP insecticides, phosphamidon and methylparathion significantly depressed the feeding rate and food conversion efficiency in *O. mossambicus*[18].

AchE is vital for the functioning for the sensory, integrative and neuromuscular systems of fish[19]. AchE inhibition causes an accumulation of acetylcholine at nerve synapses and disruption of nerve impulse transmission.[19] Chronic exposure to sublethal concentrations of two OP insecticides, dimethoate and chlorpyrifos has resulted in marked inhibition (51% - 96%) of AchE activity in several tissues of *Oreochromis niloticus*.[20] The present study also showed that brain AchE activities in *O. mossambicus* were significantly depressed following long term exposure to sublethal concentrations (0.1 and 0.2 mgl^{-1}) of fenthion. Cholinergic system in fish brain contributes the mechanism controlling feeding behaviour and inhibition of AchE has been found to alter feeding in fish.[21] Decrease in growth rates of the fish exposed to OP insecticides may be attributed to impairment of nerve functions leading to depressed feeding capacity.

In conclusion, the present study showed that long term exposure to sublethal concentrations ($\geq 0.1 mgl^{-1}$) of fenthion reduce the survival, growth and brain acetylcholinesterase activity of young *O. mossambicus.* Fry stage was more sensitive to fenthion exposure than fingerlings. Results indicate that continuous presence of even low levels of fenthion in aquatic environment due to repeated inputs, may induce long term stress effects in young fish affecting the populations of *O. mossambicus.*

Acknowledgement

Thanks are due to Ms. S. K. J. Liyanage for technical assistance.

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