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Original Article

Sub Lethal Effect Of Phorate On The Acetylcholinesterase Production In The Indian Major Carp Rohu *Labeo Rohita* (Linn.)

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ABSTRACT

An experiment was undertaken to study the effect of sub lethal effect of phorate on the acetylcholinesterase production in the Indian Major Carp rohu *Labeo rohita* (Linn.). The fishes were exposed to sub-lethal concentration of phorate at a dose of 18.846 µg/l. On 10, 20 and 30 days of exposure of sub-lethal test concentration of phorate, the acetylcholinesterase level in the liver, muscle, gill and brain of the fishes were estimated. Compared with control fish, the liver acetylcholinesterase content decreased to 37.21%, 52.41% and 66.51% respectively on 10, 20 and 30 days of exposure of phorate in the fishes. Compared with control fish, the AChE content decreased to 50.34%, 62.64% and 72.71% respectively on 10, 20 and 30 days of exposure of phorate in the fishes on comparison with control. Compared with control fish, the AChE content in gill decreased to 69.07%, 74.79% and 77.58% days respectively on 10, 20 and 30 of exposure of phorate in the fishes. Compared with control fish, the AChE in content in brain decreased to 54.44%, 62.85% and 67.41% respectively on 10, 20 and 30 days of exposure of phorate in the fishes. There was significant decrease in the AChE in all the tissues studied after exposure to sublethal concentration of phorate.

Keywords:
Labeo rohita, Phorate, Sub-lethal concentration, Acetylcholinesterase.

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Introduction

Agricultural use of pesticides is a subset of the larger spectrum of industrial chemicals used in modern society. Pesticides are increasingly becoming the predominant environmental contaminants due to their extensive use particularly in the developing countries (Parvez and Raisuddin, 2005). While agricultural use of chemicals is restricted to a limited number of compounds, agriculture is one of the few activities where chemicals are intentionally released into the environment because they kill living things. The ecological effects of pesticides (and other organic contaminants) are varied and are often inter-related (Ongley, 1996). There is overwhelming evidence that agricultural use of pesticides has a major impact on water quality and leads to serious environmental consequences (Ongley, 1996) by disruption of predator-prey relationships, threatening the long-term survival of major ecosystems, loss of biodiversity and also, pesticides can have significant human health consequences (Parvez and Raisuddin, 2006). The acute poisoning by pesticides through run-off water from agricultural fields is a serious water pollution problem resulting in the incidence of poisoning of fish and other forms of aquatic life and their long-term effects in the environment (Jyothi and Narayan, 1999). Effects at the organism or ecological level are usually considered to be an early warning indicator of potential human health impacts.

Phorate is one of the organophosphate pesticides which play an important role in the global agriculture scenario. Phorate is a broad spectrum, non-bioaccumulative organophosphorus insecticide and acaricide, an indirect inhibitor of cholinesterase with good contact, stomach and fumigant action against target organisms. Phorate is used to control mites, aphids, greenbugs, thrips, leafhoppers, sorghum shootfly, leafminers, corn rootworms, psyllids, cutworms, Hessian fly, foliar nematodes, wireworms, flea beetles, whiteflies, pine tip moth and others. It is extremely toxic to mammals, fishes and other non-target organisms.

Rohu *Labeo rohita* (Linn.), an important species of Indian major carps, is cultured in Indian subcontinent intensively. Pollution research on *L. rohita* is very scanty and research on this area will be more relevant to India. The objective of the present study was to investigate the effect of sub lethal effect of phorate on the acetylcholinesterase production in the Indian Major Carp rohu *L. rohita.*

Materials and Metods

Sub Lethal Exposure

The commercial formulation of phorate (thimet; 10%, w/w, granules) was obtained from Cyanamid India Ltd (Bombay, India) and it was used for the present study. Sub-lethal test concentration (18.846 µg/l) used for this study was based on 96 h LC50 values of phorate for *L. rohita* as arrived in an earlier experiment by the author. A group six fish of 8.2±0.98 cm were exposed to phorate at a dose of 18.846 µg/l in a glass tank of hundred litre capacity. Three such tanks were maintained. The water was refreshed every day to compensate for the pesticide lost in the exposure medium. Renewal assay for static test was applied (APHA, AWWA, WPCF, 1981). Control and experimental fish were fed daily with a commercial fish food approximately 3% of their body weight. No mortality was observed during the experiments.

Acetylcholinesterase estimation

Before the start of sub lethal exposure to the fishes, six acclimatized fishes were randomly selected and sacrificed for biochemical studies. On 10, 20 and 30 days of exposure of sub-lethal test concentration of phorate, one fish from each tank was removed and decapitated. The fishes were sacrificed by severing the spinal cord behind the head and the liver, muscle, gill and brain tissues were dissected out immediately on an ice-cold plate, washed in physiological saline solution (0.59% NaCl), and stored at - 76◦C until analysis.

Tissues were homogenized for 1.5 min in cold 0.25MpH 7.4 sucrose buffer (1:5, w/v) using a glass–teflon homogenizer and then centrifuged at 9500×g for 30 min at Sorvall RC2B centrifuge. All processes were carried out at 4◦C. Supernatants were used to determine AChE activity. AChE activity was determined by the method of Ellman *et al.*, (1961). Enzymatic activity was spectrophotometrically determined by measuring the increase in absorbance of the sample at 412 nm in the presence of 2.55 mL, 0.1MpH 8.0 phosphate buffer, 100 µL, 0.015Macetylthiocholine iodide as substrate, 50µL, 8.52mM ethopropazine as butyrlcholinesterase inhibitor, 100 µL, 0.01M 5,5µ- dithiobis-2-dinitrobenzoic acid (DTNB) and 200 µL tissue homogenate at 30◦C for 5 min. The enzymatic reaction rate was quantified against a blank without substrate for each activity measurement. Rate of activity expressed was µmol/mg protein.

Statistical analysis

The statistical analysis of data was done to ascertain the significance of test results using One Way ANOVA and the results are tabulated.

Results

The effect of exposure to a sub lethal concentration of phorate (1.846µg/l) on acetylcholinesterase level in a gram of wet tissues of liver, muscle, gill and brain of the fish *L. rohita* was studied in 10, 20 and 30 days of exposure and the results are shown in the table 1 and 2 and figure 1.

Table 1. Effect of sub lethal concentration of phorate on the biochemical changes in different tissues of *Labeo rohita*

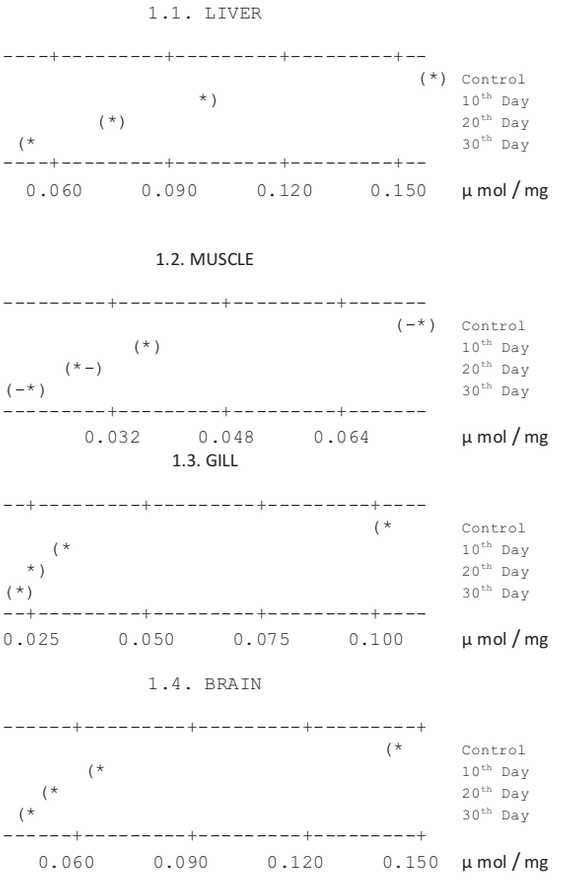
AChE in µmol / mg protein								
Days	Liver	%COC	Muscle	%COC	Gill	%COC	Brain	%COC
Control	0.15900±0.00316		0.07450±0.00267		0.10183±0.00147		0.14267±0.00258	
10th Day	0.09983±0.00349	-37.21	0.03700±0.00290	-50.34	0.03150±0.00308	-69.07	0.06500±0.00228	-54.44
20th Day	0.07567±0.00216	-52.41	0.02783±0.00117	-62.64	0.02567±0.00216	-74.79	0.05300±0.00179	-62.85
30th Day	0.05317±0.00117	-66.56	0.02033±0.00197	-72.71	0.02283±0.00147	-77.58	0.04650±0.00105	-67.41

%COC – Percent Change Over Control

Table 2: One way ANOVA showing the differences in the level of acetylcholinesterase in different tissues of *Labeo rohita* among the control and treatments

Variable	Source	df	SS	MS	F	P
Liver AChE	Treatment	3	0.0373708	0.0124569	1766.94	<0.001
	Error	20	0.0001410	0.0000071		
	Total	23	0.0375118			
Muscle AChE	Treatment	3	0.0104042	0.0034681	669.08	<0.001
	Error	20	0.0001037	0.0000052		
	Total	23	0.0105078			
Gill AChE	Treatment	3	0.0256595	0.0085532	1849.33	<0.001
	Error	20	0.0000925	0.0000046		
	Total	23	0.0257520			
Brain AChE	Treatment	3	0.0357731	0.0119244	2950.36	<0.001
	Error	20	0.0000808	0.0000040		
	Total	23	0.0358540			

Fig.1. One way ANOVA showing individual 95% Confidence Limit for mean Acetylcholinesterase (µ mol AChE per mg protein) based on Pooled Standard Deviation



Due to the exposure of sub lethal concentration of phorate, invariably all the tissues studied showed decrease in the acetylcholinesterase (AChE) content. The AChE content in liver of control fish was 0.15900±0.00316 µmol/mg protein. Compared with control fish, the liver acetylcholinesterase content decreased to 37.21%, 52.41% and 66.51% respectively on 10, 20 and 30 days of exposure of phorate in the fishes. The table 2 shows that the difference in the liver AChE level among the control and treatments was highly significant (F_3,20 = 1766.94; P<0.001).

The AChE content in muscle of control fish was 0.07450±0.00267 µmol/mg protein. Compared with control fish, the AChE content decreased to 50.34%, 62.64% and 72.71% respectively on 10, 20 and 30 days of exposure of phorate in the fishes. The table 2 shows that the difference in the muscle AChE level among the control and treatments was highly significant (F_3,20 = 669.08; P<0.001).

The AChE content in gill of control fish was 0.10183±0.00147 µmol/mg protein. Compared with control fish, the AChE content in gill decreased to 69.07%, 74.79% and 77.58% days respectively on 10, 20 and 30 of exposure of phorate in the fishes. The table 2 shows that the difference in the gill AChE level among the control and treatments was highly significant (F_3,20 = 1849.33; P<0.001).

The AChE content in brain of control fish was 0.14267±0.00258 µmol/mg protein. Compared with control fish, the AChE in content in brain decreased to 54.44%, 62.85% and 67.41% respectively on 10, 20 and 30 days of exposure of phorate in the fishes. The table 2 shows that the difference in the brain AChE level among the control and treatments was highly significant (F_3,20 = 2950.36; P<0.001).

Discussion

Biochemical changes are very sensitive to sub lethal concentrations of many stress agents. Their main disadvantage is that often they are specific to special responses. It is therefore possible not to observe any change in the experiment if the appropriate biochemical system is not chosen. For this reason, it is referable to choose general parameters (glucose, glycogen etc.) to determine a stress situation in the organisms under study (Giesy *et al.*, 1981; Patel and Eapen, 1989).

AChE activity measurement in fish has been used for monitoring the neurotoxicity of organophosphate pesticides. Organophosphate pesticides cause dose and time-dependent AChE inhibition and the rate of inhibition differs depending on species and age (Ansari et al., 1987; Keizer *et al.*, 1995; Szegletes *et al.*, 1995; Pan and Dutta, 1998). OP insecticides are known to inhibit acetylcholinesterase, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate (Soreq and Zakut, 1993). The inhibitory effects of OP insecticides are dependent on their binding capacity to the enzyme active site and by their rate of phosphorylation in relation to behavior and age (Dutta *et al.*, 1995). The toxic effect of some organophosphate pesticides (e.g., paraoxon, sarin) is not limited to inhibition of cholinesterase: following the cholinergic crisis changes in non-cholinergic neurotoxic parameters, such as specific damage to cell membranes, are observed (Ongley, 1996).

The fishes tested with phorate in this study revealed significant inhibition of AChE activity in the liver, muscle, gill and brain tissues of *L. rohita*. In agreement with our reults, Dembele *et al.* (2000) indicated that one-half LC_{50} of diazinon in C. carpio showed 49% inhibition compared to the AChE activity of the control group brain tissue. It has been generally accepted that a 20% or greater inhibition in AChE activity indicates exposure to organophosphate insecticides. Although 50% or greater depression is indicative of a life-threatening situation, available investigations show that fish are capable of tolerating over 90% AChE inhibition (Day and Scott, 1990).

Balint *et al.* (1995) reported 90–92% inhibition following methidathion exposure; and Pan and Dutta (1998) indicated 91.4% depression after diazinon treatment. Rath and Misra (1981) reported that the degree of AChE inhibition in liver of Tilapia mossambica in relation to the interacting effects of aging and sublethal concentrations of dichlorvos showed a positive correlation with insecticide concentration and the time of exposure. Ansari and Kumar (1984) obtained similar results after malathion exposure.

In the present study there was a sharp decrease of protein level in all the tissues of the experimental animals as a result of phorate toxicity (Table 1). In the present study the protein decreased in all the tissues of *L. rohita* on exposure of sub lethal concentration of phorate

(Table-2). Among the organs in gill a maximum of 77.58% of decrease in carbohydrate was noted on the 30th day when compared to the control.

Similarly, AChE inhibition in brain was observed earlier when fish were exposed to other organophosphate insecticides like chlorpyrifos and profenofos (Kumar and Chapman, 2001; Venkateswara Rao *et al.*, 2003a,b). It is apparent from the earlier results that the fish can survive even after 70% inhibition of true cholinesterases in brain AChE when exposed to parathion (Fulton and Key, 2001). The inhibition of AChE enzyme leads to the accumulation of ACh at synaptic junctions and is regarded as a marker for assessing the organophosphate pesticides.

Inhibition of AChE was accompanied by an increase in acetylcholine levels (Brzezinski and Ludwicki, 1973). This condition can lead to increase of catecholamines which can affect the activity of enzymes involved in glycogenolysis and glycogen synthesis. Continuous stress may affect the synthesis site of AChE or decrease the levels of excess AChE. Mortality of fish may result due to inhibition of other enzymes, especially those taking part in carbohydrate and protein metabolisms. The inhibitory effect on AChE activity indicates that insecticides might interfere in vital processes like energy metabolism of nerve cells (Ansari and Kumar, 1984). OPs not only inhibit AChE but also affect other metabolic activities in the animals and the combined effect leads to death (Ansari *et al.*, 1987). The inhibition of AChE activity in fish can be dangerous since it will affect feeding capability, swimming activity, identification, avoidance of predators and spatial orientation of the species (Balint *et al.*, 1995; Pan and Dutta, 1998) as observed in this study throughout the experiments. Symptoms exhibited by L. rohita after exposure to phorate, such as erratic swimming and convulsions, have also been observed in other fish species exposed to other organophosphate pesticides (Hai *et al.*, 1997; Sancho *et al.*, 2000).

The present study reveals that the organophosphate pesticide, phorate, is found to influence the metabolism of *L. rohita* and causing significant reduction in the acetylcholinesterase levels in various tissues. *L. rohita* being a commercially important fish, increased phorate levels in its living environment will drastically affect the growth and other physiological activities of the fish thereby decreasing its population and yield.

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