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Regulation of AChE System of Freshwater Fish, *Cyprinus carpio*, under Fenvalerate Toxicity

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The indiscriminate and excessive use of synthetic pyrethroid insecticides to control various types of pests to increase food supply are known to cause deleterious effects on non-target organisms like fishes (Casida et al. 1983). Acetylcholinesterase (AChE) is an enzyme that modulates the amount of neurotransmitter substance acetylcholine (ACh) at neuron junctions (O' Brien 1967) and inhibition of AChE activity was regarded as a significant parameter in assessing complex toxicogenic effects of various toxicants including pyrethroid insecticides (Bandyopadhyay 1982; Bradbury et al. 1987). The literature pertaining to the toxic effects of pyrethroids on AChE system is very scanty. Therefore, an attempt has been made to study the impact of fenvalerate on AChE system in the selected tissues such as gill, brain, liver and muscle of freshwater fish, Cyprinus carpio.

MATERIALS AND METHODS

The collection and maintenance of the fish, C. carpio was described earlier (Malla Reddy and Bashamohideen 1988). The technical grade (90%) was obtained from Gujarat insecticides limited, India and the stock solution was prepared in acetone. The LC_{50} value of fenvalerate to C. carpio (48 hrs) was found to be 0.030 ppm (Malla Reddy 1988). 0.01 ppm was selected as sublethal concentration and fish were exposed to 6, 12, 24 and 48 hrs. Control fish were maintained under identical conditions without pesticide in the medium. After each exposure period, the tissues were isolated and chilled in ice box and used for estimation of acetylcholine (ACh) and acetylcholinesterase (AChE). 5% homogenate of gill, liver, muscle and 1% homogenate of brain were prepared in 0.25 M sucrose solution.

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Homogenates were centrifuged at 2500 g for 10 mins at 4°C to remove cell debris. The clear cell free extract was used for enzyme assay. ACh content was estimated by the Hestrin method modified by Augustinson (1957). AChE was determined by the method of Metcalf (1957). The recation mixture contains 1 ml of 0.1M phosphate buffer (pH 7.2) and 0.5 ml of enzyme. The mixture was incubated at 37°C for 30 mins and the reaction was stopped by the addition of 2 ml of alkaline hydroxalamine hydrochloride and 1 ml of hydrochloric acid. AChE activity was expressed as µ moles of ACh hydrolysed/mg protein/hr. Protein in the homogenate was estimated by the method of Lowry et al (1951). Some behavioural changes were observed in the fenvalerate exposed fish after 48 hrs. The mean values of control and fenvalerate exposed fish of ACh and AChE were subjected to statistical treatment using analysis of variance (Pillai and Sinha 1968). If the difference between any two values exceed critical difference (CD), then the values are considered as significant. The CD values are given in the tables 1 and 2.

RESULTS AND DISCUSSION

The AChE activity levels decreased whereas ACh content increased in all the tissues of fish, C. carpio under fenvalerate exposure (Tables 1 & 2).

The tissue specific AChE activity recorded maximum inhibition at 48 hrs of exposure. The lyotrophic inhibition of AChE and elevation of ACh content pattern is as follows: brain > muscle > gill > liver. Some behavioural changes were also observed in fish exposed to fenvalerate. The colour of gill changed from red to pale red and the body became dark in colour, reduction in opercular movement and irregular and erratic swimming movements were observed. The animal lost its geotaxic movements and hence showed lateral line swimming. Similar behavioural changes were also observed in freshwater fish, Tilapia mossambica treated with fenvalerate (Radhaiah and Jayantha Rao 1988). Darkening of the body during exposure to fenvalerate may be due to the decrease in blood supply to the body surface. In support of this, certain blood parameters like red blood corpuscular count, haemoglobin concentration and packed cell volume decreased in fish, C. carpio exposed to fenvalerate (Malla Reddy and Bashamohideen 1989). The general ill health and the overall decline in physical ability may be due to the disorders in the central nervous system.

Acetylcholinesterase is an enzyme that modulates the amount of neurotransmitter substance at neuron junctions (O'Brien 1967) and it is also concerned with

Table 1. Acetylcholinesterase (AChE) levels in the tissues of control and fenvalerate exposed fish, C. carpio.

Tissues	Control	Sublethal exposure periods (hrs)				
		6	12	24	48	CD
Gill	4.41 ±0.28	4.09 ±0.12 (7.26)	3.76 ±0.06 (14.74)	3.22 ±0.07 (26.98)	2.59 ±0.06 (41.27)	0.17
Brain	8.89 ±0.17	7.29 ±0.07 (17.99)	6.54 ±0.12 (26.43)	5.00 ±0.19 (43.76)	3.24 ±0.06 (63.55)	0.16
Liver	2.98 ±0.13	2.71 ±0.05 (9.06)	2.49 ±0.08 (16.44)	2.26 ±0.03 (24.16)	1.98 ±0.04 (33.56)	0.09
Muscle	5.26 ±0.18	4.76 ±0.04 (9.50)	4.31 ±0.08 (18.06)	3.78 ±0.05 (28.14)	3.08 ±0.09 (41.44)	0.13

Each value is mean ± SD of six individual estimations; Values in the parenthesis indicate per cent decrease over control; Values are significant at 5% level.

the ionic content (Vander Kloot 1956). The inhibition of AChE and elevation of ACh content may be due to the decreased ionic composition in the tissues of C. carpio under fenvalerate stress (Malla Reddy 1988). The greater decrease in AChE with a concomitant increase in ACh content in the brain tissue is an implication of greater inhibition in the integratory activity of the central nervous system and ACh accumulated in brain and other tissues may cause uncontrolled hormonal release and the toll of an animal may be possible by the degeneration of many biochemical and physiological functions (Corbett 1974). Bandyopadhyay (1982) observed significant inhibition of AChE in the brain tissue of rat by permethrin under in vivo and in vitro conditions. Yellamma and Ravikumar Reddy (1987) reported the inhibitory activity of AChE in ventral nerve cord of Periplaneta americana under lethal and sublethal concentrations of fenvalerate.

Thus it is inferred that exposure to fenvalerate causes inhibition of AChE activity and the accumulation of ACh at synaptic junctions in C. carpio. This may lead to

Table 2. Acetylcholine (ACh) content in the tissues of control and fenvalerate exposed fish, C. carpio.

Tissues	Control	Sublethal exposure periods (hrs)				
		6	12	24	48	CD
Gill	25.43 ±3.68	30.49 ±6.21 (19.89)	33.26 ±3.09 (26.86)	40.57 ±3.31 (59.53)	46.07 ±2.36 (81.86)	4.70
Brain	54.88 ±1.00	65.41 ±6.11 (19.19)	81.50 ±6.45 (48.51)	97.94 ±7.67 (78.46)	104.04 +10.21 (89.58)	8.29
Liver	15.73 ±1.98	18.02 ±1.46 (14.56)	22.03 ±2.45 (40.05)	23.55 ±2.43 (49.71)	28.03 ±1.39 (78.19)	2.37
Muscle	28.12 ±1.30	34.41 ±2.75 (22.37)	36.62 ±2.75 (30.23)	45.18 ±3.55 (60.67)	51.65 ±2.84 (83.68)	3.41

Each value is mean ± SD of six individual estimations; Values in the parenthesis indicate per cent increase over control; Values are significant at 5% level.

behavioural changes and create a wide spread disturbance in the normal physiology ultimately causing the death of the organisms. The physiological and ecological significance of fenvalerate induced disturbances in AChE of freshwater fish may be used as valuable indices for determining the environmental pollution by fenvalerate.

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