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RESEARCH ARTICLE

# EFFECT OF CYPERMETHRIN ON BRAIN ACETYLCHOLINESTERASE ACTIVITY IN THE INDIAN MAJOR CARP LABEO ROHITA

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**ABSTRACT**

The toxic effect of cypermethrin on the acetylcholinesterase activity of *Labeo rohita* was studied. The fish were exposed for 24- 48, 72 and 96 hours in 10 and 20% sub lethal concentrations of 96 h $LC_{50}$ of cypermethrin (0.057 ppm). Exposure of fishes to 10% and 20% sub lethal concentrations of cypermethrin caused severe changes in the acetyl cholinesterase activity. Highest activity was observed in the fish exposed to 20% sub lethal concentration during 24 hour. Highest inhibitory effect was noticed in the fish exposed to 20% sub lethal concentration of cypermethrin.



## INTRODUCTION

A great number of insecticides have been used to enhance the agriculture yield. The run-offs from insecticide applied fields are notorious to interfere with the non target and nutria-significant animals and also disrupting the growth and physiology of aquatic animals. In fishes, nervous system shows deep control on their functional activity. Pesticides are generally neurotoxins and thereby paralyzing the insects thus results in their death (Casida *et al.*, 1983; Clark and Matsumura, 1987). Recently emerged synthetic pyrethroids have been utilized extensively in agriculture and horticulture to curtail insect pests . Pyrethroid compounds in insects provoke consecutive impulses which impede the nerve physiology and ultimately results in the death. (Vijverberg *et al.*, 1982). The synthetic pyrethroids have distinct action depending on the presence or absence of Alfa-cyano group. Cypermethrin causes toxicity similar to that of DDT, while other pyrethroids show toxicity by inhibiting both ATPase and acetylcholinesterase (AChE) systems. AChE is an enzyme that regulates neurotransmitter acetylcholine (Ach) at neuron junctions.

The inhibitory effects of insecticides on Acetyl choline esterase activity of fish had been reported by various authors, metacid-50 and carbaryl in *Channa punctatus*(Gosh and Bhattacharya,1992) Elsan in *C.punctatus* (Rao *et. al.*, 1987) cypermethrin in *Channa striatus* (Ahamed *et.al.*, 2015). The present investigation is aimed at estimating impact of cypermethrin on the acetyl cholinesterase activity in the Indian major carp *Labeo rohita.*

## MATERIALS AND METHODS

On exposure to the 10% and 20% sub lethal concentrations of $LC_{50}$ (0.537 ppm) of the pesticide cypermethrin the fish brain was subjected to Acetylcholinesterase activity after 10, 20 and 30 days. Acetylcholinesterase activity was determined by following the method of Ellman *et.al.*.1961.

### Principle

Acetylcholinesterase hydrolyses acetylcholine iodide into thiocholine and acetate. Thiocholine reacts with Dithiodiitrobenzoic acid to form thionitrobenzoic acid which is an yellow coloured compound which is measured at 412 nm and is propotional to the amount of acetylcholinesterase.

1% homogenate of the brain tissue was separately prepared in 0.25 M ice cold sucrose solution. The AChE activity in the extracts was estimated by the method suggested by Ellman *et al.*. (1961). The reaction mixtures contained 1 ml of 0.1 M phosphate buffer (pH 7.2) and 0.5 ml of the tissue homogenate. After 30 min of incubation at 37°C, the reaction was stopped

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by the addition of 2 ml of alkaline hydroxylamine hydrochloride and 1 ml of HCl (1:1 HCl:H2O). The contents were thoroughly mixed and centrifuged at 100 rpm for 15 min. To the supernatant, 0.5 ml solution of 10% ferric chloride solution was added to deepen the colour. Colour of the solution was measured against the blank at 540 nm in a spectrophotometer. The blank contained 1 ml of the buffer solution. Enzyme activity was expressed as µmoles of ACh hydrolysed/ml of protein/h.

## RESULTS AND DISCUSSION

Exposure of *Labeo rohita* to 10% and 20% sub lethal concentration of cypermethrin caused severe changes in the acetyl cholinesterase activity. Highest activity was observed in the fish exposed to 20% sub lethal concentration during 24 h(Table-1). Highest inhibitory effect was noticed in the fish exposed to 20% sublethal concentration of cypermethrin.

Pesticides are widely used in agriculture and there is a need for tool to monitor the impact of these pesticides on fish population. There is a growing concern worldwide over the indiscriminate use of such chemicals, resulting in environmental pollution and toxicity risk to non-targeted organisms (Coppage and Bradiah,1976 and Khader khan,1996). Organophosphate and carbamate insecticides are known to disrupt transmission in the central and peripheral cholinergic nervous systems in vertebrates by inhibiting acetyl cholinesterase activity(Sahib and Rao,1980). These pesticides are produced and used in large amounts and they enter the environment and greater quantities than chlorinated hydrocarbon insecticides (Coppage and Mathews,1974). The possible hazard of AChE inhibiting pesticides in the aquatic environment should not be ignored, since these pesticides act as a nerve poison and neuromuscular junction of the respiratory apparatus (Coppage and Braidach,1976).

Aquatic organisms exhibit a broad range of inhibitory response to organophosphate and carbamate pesticides depending on the compound, exposure time, water conditions and species (Coppage and Mathews,1974). Measuring AChE is probably the best general indicatory of serious organophosphate and carbamate pesticide pollution(Cook *et al.*,1976). Determination of brain AChE activity is widely used to diagnose organophosphate and carbamate poisoning (Hart,1993). In the present study, the Indian major carp *Labeo rohita* was exposed to 10% and 20% sublethal concentrations of the pesticide cypermethrin. Acetyl cholinesterase activity was done in the brain of control and pesticide exposed fish to assess the neuro toxicity.

In the present study, variations in Acetyl cholineesterase enzyme activity was significant on exposure to cypermethrin. A conspicuous raise in acetyl cholineesterase enzyme activity was recorded during 24 h of exposure. Then there is a slash down in the activity was evident. Activation of enzyme during early period and inhibition during later period depend on the concentration of the pesticide. These findings are in harmony with that of earlier report by various authors (Jindal and Kaur, 2009; Rao *et al.*, 2005 and Joseph and Raj,2011).

Similarly Sathyadevan *et al*. (1983) reported an initial elevation in AChE activity of the brain of *C. carpio*exposed to an organophosphorus dimethoate. Kufesak *et al*. (1994) reported an initial elevation in AChE activity in carp on exposure to pesticides. In the major carp *Catla catla* on exposure to deltamethrin, Nissar Ahmed (1994) reported an initial elevation and further inhibition of AchE activity. Subhan (2000) reported an initial elevation and further inhibition of AChE activity in *C. catla* exposed to synthetic pyrethroid fenvalerate. Gupta (1994) ascribed that the inhibition of AchE consequently leads to excessive Ach accumulation at the synapses and neuromuscular junctions, resulting in overstimulation of Ach receptors.

In the present study, the control fish were active with their well coordinated movements, but the pesticide exposed fishes exhibited irregular spiral swimming movements. Venkateswara Rao *et al*.,(2005) observe inhibition of AChE enzyme activity in the brain of Gambusia affinis on exposure to chlorpyrifos. Mushigeri and David, 2005 & Patil and David, 2008 observed darting movements and loss of equilibrium are due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyper stimulation.

In the present study, variations in Acetyl cholineesterase enzyme activity was significant on exposure to cypermethrin. A conspicuous raise in acetyl cholinesterase enzyme activity was recorded during 24 h of exposure. Then there is a slash down in activity was evident. Activation of enzyme during early period and inhibition during later period depend on the concentration of the pesticide. These findings are in harmony with that of earlier report by various authors (Jindal and Kaur, 2009; Kumar and Champman, 2001; Rao *et al.*, 2003 & Joseph and Raj,2011).

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**Table 1** Brain acetylcholinesterase activity of *Labeo rohita* exposed to the sublethal concentrations of Cypermethrin. (Enzyme activity µmoles/min/mg protein).

| S.No | Period of exposure | Control | 10% sub lethal | | 20% sub lethal | |
|---|---|---|---|---|---|---|
| | | | Acetyl cholinesterase | % of Activity of Ach E | Acetyl cholinesterase | % of Activity of Ach E |
| 1 | 24 Hours | 196 | 163±0.14 | 33% | 138±0.37 | 58% |
| 2 | 48 Hours | 189 | 198±0.42 | -09% | 212±0.27 | -23% |
| 3 | 72 Hours | 192 | 218±0.22 | -26% | 243±0.53 | -51% |
| 4 | 96 Hours | 190 | 228±0.34 | -38% | 252±0.36 | -62% |

In the major carp *Catla catla* on exposure to deltamethrin, Nissar Ahmed (1994) reported an initial elevation and further inhibition of AchE activity. Subhan (2000) reported an initial elevation and further inhibition of AChE activity in *C. catla* exposed to synthetic pyrethroid fenvalerate. Gupta (1994) ascribed that the inhibition of AchE consequently leads to excessive Ach accumulation at the synapses and neuromuscular junctions, resulting in overstimulation of Ach receptors.

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