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Toxic effect of malathion on acetylcholinesterase activity of liver, brain and gills of freshwater catfish *Heteropneustes fossilis*

Sudhish Chandra
P.G. Department of Zoology, B.S.N.V. College, Lucknow

Abstract

The toxic effects of malathion were evident in the inhibition of acetylcholinesterase activity of liver, brain and gills of freshwater catfish *Heteropneustes fossilis*. Maximum inhibition of 77.12% and 72.83% were recorded in brain and gills respectively after 72 hour of exposure to 4.80 mg/l pesticide. However, in liver highest inhibition of 67.81% in enzyme level was noticed at 6.50 mg/l pesticide concentration after 24 hours of exposure, beyond which fish could not survive. Pesticide repressed the enzyme activity so intensely that it showed no sign of return to normalcy. The fish also elicited dissociated behaviour with increasing concentrations of pesticide toxicity.

Keywords:- *AchE, Malathion, Toxicity, Concentration, Inhibition*

Introduction

Fish inhabiting a diverse environment are sensitive to toxic environment, being exposed via extensive delicate respiratory surface. Wide variety of pesticides and due to their persistance and nonbiodegradable nature, they got accumulated in different tissues of the fish and affect their growth and survival (Kaur and Toor, 1977; Kulshrestha and Arora, 1985). Acetylcholinesterase is the enzyme responsible for terminating the action of acetylcholine at cholinergic synopsis (Ronald *et al.,* 1999). It acts as a key transducer at cell membrane level, an alteration in the level of its functional activity may result in microdeformation and configurational change. Acetylcholinesterase is the target enzyme for organophosphate pesticides which is inhibited by these pesticides and its inhibition leads to blockage of neurotransmission (O'Brien, 1976) leading to physiological alterations in non targeted organisms of the aquatic system.

Present observation deals with the effect of organophosphate pesticide malathion on the Acetylcholinesterase (AchE) activities of liver, brain and gill tissues of freshwater stinging catfish *Heteropneustes fossilis,* highly popular and expensive table fish, due to its higher nutritional quality and medicinal value.

Materials and Method

Freshwater stinging catfish *Heteropneustes fossilis* (length 20.5 – 24.0 cm, weight 160 – 200 grams) were collected from local resources, transported to laboratory and treated with $KMnO_4$ (2 mg/l). Apparently healthy looking fishes were acclimatized for seven days in glass aquaria under standard laboratory conditions and fed them with small pieces of goat liver, but starved for a period of 24 hours prior to the experiment. Static bioassay tests were followed as given by Doudoroff *et al.,* 1951; APHA, 1992. The pesticide was commercially formulated product (Cynamid India Ltd.), extensively used in India. Pesticide concentrations were selected on the basis of 80 – 100% survival of fishes for that period. Control fish in

experiment were also maintained in identical conditions.
The controled and exposed fishes were taken out from each preparation after every 24 hours interval and washed with distilled water. Liver, brain and gill tissues were taken out and 2.5% homogenate was prepared in 0.7% saline solution, at nearly freezing temperature. Biochemical analysis was performed following the method of Hestrin (1949), using Bausch and Lomb spectronic - 20 spectrophotometer at 540 µm against blank. The physico-chemical properties of the tap water used in the experiment were as follow: temperature (22±1.4 ^{0}C), pH (7.4±0.02), Dissolved oxygen (6.6±0.8 mg/l), alkalinity (106±10.0 mg/l) and hardness (118±12.4 mg/l).

Results

Acetylcholinesterase activity was significantly inhibited by malathion toxicity in *H. fossilis*. The results obtained on enzymal activity of liver, brain and gill tissue of fishes, exposed to different concentrations of

Table-1: Effect of Malathion toxicity on Acetylcholinesterase levels of *H. fossilis*

Pesticide conc. (mg/l)	**Acetylcholinesterase (µ moles Ach hydrolysed/ 100 mg f. wt./ hr.) Mean ± Standard Deviation**			
	Time of exposure in hours			
	24	**48**	**72**	**96**
LIVER – Control – 69.60±8.15				
3.55	28.80±7.05	32.60±6.40	38.40±3.82	41.36±5.05
4.80	36.00±7.75	29.60±2.82	23.20±3.72	
5.80	30.00±3.72	26.40±5.05		
6.20	22.40±5.40			
BRAIN – Control – 75.20±9.21				
3.55	31.20±6.56	32.80±6.10	29.60±5.05	30.00±6.32
4.80	36.80±7.83	33.40±4.25	17.20±4.07	
5.80	44.00±8.84	15.20±5.05		
6.20	18.80±6.09			
GILLS – Control – 64.80±6.64				
3.55	24.40±5.04	27.20±2.70	32.80±1.91	37.60±3.20
4.80	43.20±7.21	32.80±3.72	17.60±4.36	
5.80	30.60±3.82	25.60±4.10		
6.20	28.00±4.60			

No. of observations – 8 in each experiment

pesticide have been summarized in Table-1. At 3.55 mg/l pesticide concentration AchE levels were inhibited 58.62%, 53.16%, 44.82% and 39.08% in liver, 58.51%, 56.38%, 60.63% and 60.10% in brain and 62.34%, 58.02%, 49.38% and 41.97% in gills, after exposure for 24, 48, 72 and 96 hours respectively, from control levels. The maximal inhibition was found in liver after 24 hours, in brain after 72 hours and in gills after 24 hours. At 4.80 mg/l concentration the enzyme level continuously fell by 48.27%, 57.47% and 66.66% in liver, 51.06%, 55.58% and 77.12% in brain and 33.33%, 49.38% and 72.83% in gills after exposure to 24, 48,

72 hours respectively, versus control levels. At 5.80 mg/l concentration AchE activity decreased significantly by 56.89% and 62.06% in liver, 41.48% and 79.78% in brain and 52.77% and 60.49% in gills after exposure for 24 and 48 hours respectively, as compared to control. At the highest concentration of 6.50 mg/l, all the fishes died after 24 hours of exposure, when decline of 67.81% in liver, 75.00% in brain and 56.79% in gills had occurred from initial control levels.

Pesticide repressed the enzymal activity so intensely that it showed no sign of return to normalcy and finally proved fatal to fish after 96 hours, even at the lowest concentration of pesticide used. Exposure at higher concentration of pesticide, fish appeared restless, hyperactive showing erratic movements, rapid opercular movements, gulping air at the surface, followed by loss of balance, became calm and finally died.

Discussion

Esterases and transaminases are the target enzyme involving every tissue. Interaction of these enzymes with toxicant is a complex phenomenon involving interplay between several metabolic pathways. Repressed enzyme levels caused by toxicant and other associated compounds have been attributed to decreased neurosecretory and hepatosecretory activities (Praveen *et al.*, 2004). The toxicant on entering into the fish body affect their metabolism, leading to physiological, pathological and biochemical disorders (Bais and Arasta, 1995; Arasta *et al.*, 1999; Karuppasamy, 2000; David *et al.*, 2003). Organophosphate pesticide are known to metabolise into their corresponding oxygen analogue which are potent inhibitors of acetylcholinesterase in invertebrates and vertebrates (Natarajan, 1984). Varied Lc_{50} have been reported with different organophosphate pesticides in different fishes under varying situations (Kumar *et al.*, 1995; Thannipon *et al.*, 1995; Rawat and Bhargava, 1997; Nath *et al*., 2000; Jeyarthi Shanti and Jebanesan, 2001; Sharma *et al*., 2001 and Singh, 2003) noticed marked decline in acetylcholinesterase activity. Present investigation clearly indicated significant inhibition in AchE enzyme levels in liver, brain and gill tissues of *H. fossilis*, due to the toxicity of malathion, even at lowest concentration. Sudheer Kumar *et al*., (2006) reported maximal inhibition in AchE in brain followed by muscle, gill and liver in *Tilapia mossambicus*, exposed to chlorophyriphos. Progressive decrease in AchE activity in Nile *Tilapia* by Thannipon *et al.*, (1995) exposed to monocrotophos. Fish *H. fossilis* exposed to different concentrations of malathion for different periods revealed comparatively higher abatement in brain showing a maximal inhibition of 77.12%, followed by gills (72.83%) and liver (67.81%). Gills and body surface being the primary sites of absorption (Murphy, 1971; Kumar *et al*., 2000) were the first to be affected by toxicant. In order to get rid off the toxic environment fish exhibited, increased opercular movements copious mucus secretion followed by restlessness, hyperactivity and erratic movements showing fish in acute stress. Intensity in such evident behavioral changes are dose dependent (Matsmura, 1980) and their median survival time seems to be directly related to body size, weight and age. Profuse secretion of mucus by gills with oedema and fusion of hyperplastic secondary gill lamellae were observed by Mishra *et al*., (2005). It appears that fish *H. fossilis* exposed to lowest concentration of pesticide did not reach up to the exhaustion, rather they were able to accommodate and acclimatize with the developed stress.

Biochemical changes due to the utilization of organic resources of fish owing to the shift in respiratory metabolism in toxic environment, possibly yielded excess energy to compensate the stress. A shift in cellular respiratory metabolism towards anaerobiosis as a prelude towards adaptability to cope with the enhanced energy demand (Obula Reddy and Neerja, 2001). Chandra (1988) opined that malathion may

lead to inhibition of cholinesterase at neuro effector sites in adrenal medulla, leading to hypersecretion of adrenalin which resulted in hypermetabolic conditions to meet stress condition. Tandon and Dubey (1983) reported increased aldolase activity, an important gluconeogenic enzyme to withstand the stress condition caused by pesticide malathion and dimicron in *Clarias batrachus*. Marked inhibition in AchE in different tissues of fish following exposure to higher concentration of sumithion has been reported by Koundiya and Ramamurthy (1978), Dubey (1980), Natrajan (1984), Thannipon *et al*. (1995) and Sudheer Kumar *et al*. (2006). It has been observed that the cause of death in organophosphate pesticide poisoning is always asphyxiation. The pesticide penetrates brain which results in the failure of respiratory centre of brain after that of respiratory organs (O'Brien, 1976). In these observations AchE levels were prominently hindered in brain and gills at lower but maximal inhibition occurred in liver at highest malathion concentration. Since the fishes have ability to detoxify xenobiotics in their liver like mammals, probably that is why liver might have the last target of toxicant in accumulating more acetylcholine. The dissociated behaviour of fish *H. fossilis* may be related due to the accumulation of acetylcholine which resulted in neuromuscular paralysis of the bronchial pump and oxygen diffusion across the gill and lead to excitatory effects like tremors and convulsions.

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