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Effect of sublethal concentration of methyl parathion on the behavioural activities of the climbing perch (*Anabas tesudineus* : Bloch)

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ABSTRACT

Pesticides are among most hazardous chemicals causing risks to environment, fisheries or people exposed to them. Due to the biodegradability and short residence time in the environment, organo-phosphate pesticides are increasingly used in recent years. The present study analysed the alterations in various behavioural patterns of the climbing perch in a novel environment like open field as a result of exposure to sublethal concentration of pesticide methyl parathion. We also tested whether there is any equivalent change in the total protein content of telencephalon. Our results suggest that the exposure to pesticides influences the behavioural activities of the organism and makes marked changes in normal functioning of brain centers involved in the control of the exploratory behaviour of the fish. The knowledge of sublethal effects of xenobiotic compounds is very important to delineate the fish health status and provide a future understanding of ecological impacts.

Key words: Methyl parathion, climbing perch, telencephalon, xenobiotics.

INTRODUCTION

The lack of information about the risks of poisoning causes the improper and excessive use of these chemicals in different combinations. Methyl parathion, an organophosphate insecticide, widely used to avoid agricultural losses due to fruit flies, bugs, and other insect attacks. These chemicals along with other industrial effluents may reach other ecological compartments such as lakes and rivers through rains and wind, affecting many other organisms away from the first target [1, 12]. Only 1% of these chemicals and heavy metals reach at the specific target [2]. In addition, it is frequently used in aquaculture to prevent fingerlings loss due to predation by odonata nymph and chironomidae. The injuries of insecticides to aquatic environment are fatal and fish are able to bio accumulation due to direct exposure to chemicals or ingestion of

contaminated prey and food [9]. The disproportionate use of synthetic chemical has shown to be causing significant loss to aquatic ecosystem affecting the protein and lipid metabolism of fishes [10, 11].

According to Aguiar [2], methylparathion inhibits acetylcholine esterase activity in fish, resulting neuronal system blockage. Methyl parathion seems to be not accumulated or persisted on environment as such, since plants and animals are able to metabolize it quickly [5]. It is transformed into a molecule very similar to acetylcholine, directly responsible for animal poisoning. This molecule binds to acetylcholine esterase which in turn leads to continuous excessive stimulation of nerve muscle fibres [7].

The behaviour (activities) of an organism represents the final integrated results of diversity of biochemical and physiological processes. Recent development in ecotoxicology has been shown that observations of behaviour can constitute a sensitive approach in sublethal toxicity monitoring. The effects of lethal and sub lethal concentrations of soap and detergent effluents on the behavioral responses of the laboratory populations of *Clarias gariepinus* fingerlings and adults have determined by Adewoye [1]. The present study analyses the alterations in various exploratory behavioural patterns (such as locomotion, resting, speed, air gulping, jumping) in a novel environment like open field as a result of the exposure to sublethal concentration of pesticide methyl parathion. We also tested whether there is any equivalent change in total protein content of the telencephalon since it is considered to be a key factor of locomotion and spatial cognition.

MATERIALS AND METHODS

2.1 Stocking of test fish:

One hundred and fifty medium sized *Anabas tesudineus* (standard length 10 $\pm$ 2.3 cm and weight 20 $\pm$ 3.2 kg) were collected from a few culture ponds at Irinjalakuda, Thrissur. They were brought to the laboratory and stocked pond water in two large tanks of 500-l capacity for two weeks for acclimation. During this period they were fed twice daily with artificial pellet food (Higashi, Aqua Feed, Japan). After the period of acclimatization sixty fish were taken from the stocking tank and housed in groups of ten in six separate identical glass aquaria having capacity of 20-l.

2.2 Pesticide:

Pesticide Methyl parathion under the trade name Methyl parathion Metacid-50 (Methyl parathion ai 50% w/w, Emulsifier 10% w/w, Solvent balance w/w) was used for the present study. The sublethal concentration selected for the present study was 2.7 mg/l (33.33% of LC50).

2.3 Open field:

The activities of the fish were evaluated by measuring the behaviour displayed in an arena called ‘open field’ [6]. For the present study a rectangular open field was used which designed using white rectangular tubs (40cm x 20 cm) of 15-l capacity. The bottom of the tub was marked into 15 equal cells (8cm x6.5cm) containing 3 rows and 5 columns.

2.4 Behavioral analysis:
Behavioural patterns of ten pesticide treated fish and ten untreated fish were observed individually for 10 minutes and the data were collected on the third, fifth and seventh days. The test fish was gently introduced into the open field apparatus and after five minutes of acclimation period the behavioral patterns - locomotion, resting, speed, air gulping, jumping - were recorded, sitting behind a black screen with slits. Speed of exploration was determined by counting the number of cells crossed by the test fish during one minute (The average value of speed was taken from the values observed in first, fourth and eighth minute after the commencement of the observations).

2.5 Determination of total protein content in forebrain:
After the behavioral studies the fishes were sacrificed using 2-phenoxy ethanol and their forebrain was dissected out. The weight forebrain was determined and the amount of protein present in brain was estimated according to the method adopted by Lowery *et al*., 1951 (Lowery *et al*., 1951).

2.6 Statistics :
The data were analyzed using the non-parametric test – The Kruskal – Wallis or H test. For small samples, H is approximately distributed as Chi- Square with k-1 degrees of freedom.

RESULTS AND DISCUSSION

The present study shows that methyl parathion has severe effects on the locomotory movements of *Anabas testudineus*. A marked increase in exploratory activity that was positively correlated with duration of exposure was observed in pesticide treated fish where as the control fish showed an almost constant rate of movement inside the 'open field' (Fig:1). The speed of exploratory activity of the fish exposed to methyl parathion decrease with increase in the duration of exposure to the pesticide (Fig. 2). The air gulping behaviour of the test fish also showed a marked reduction as the days of exposure increased (Fig: 3). Both the control fish and the fish exposed to methyl parathion exhibited escape tendency on the third, fifth and the seventh day. However, the escape attempts of the fish exposed to methyl parathion by jumping out of the experimental of arena were found to be higher with the duration of exposure to the pesticide as compared to the behaviour of the control fish (Fig. 4). The level of protein content in the fore brain of *Anabas testudineus* exposed to the pesticide methyl parathion for seven days were examined. The fore brain protein index was determined by dividing the weight of the protein content of the forebrain with that of the total weight of the forebrain. The experimental fish showed a significant decrease in protein content from that of control fish (Fig:5).

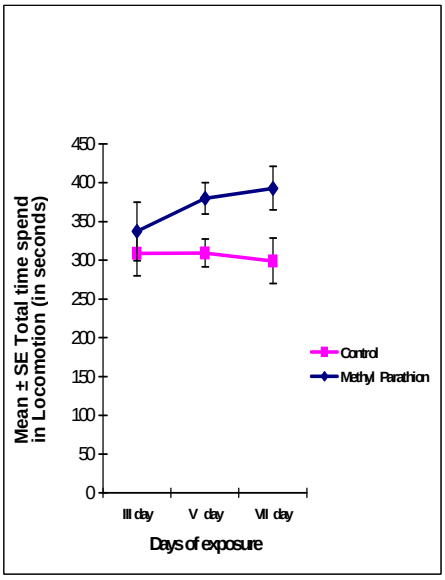

Fig:1 Effect of sublethal concentration of methyl parathion exploratory movements of *Anabas testudineus*

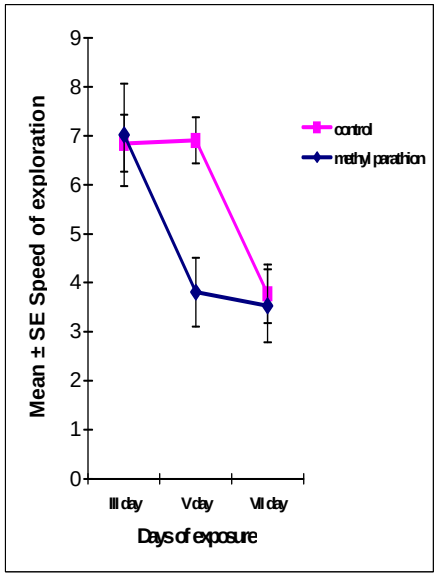

Fig: 2 Effect of sub lethal concentration methyl parathion on the on the speed of exploration *Anabas testudineus*

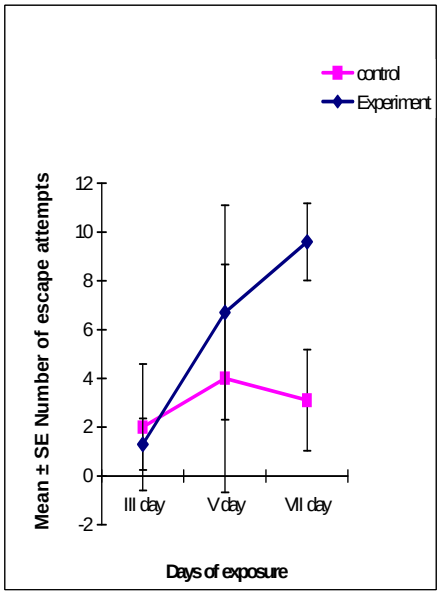

Fig: 3 Effect of sub lethal concentration of methyl-parathion on the air-gulping behaviour of *Anabas testudineus*

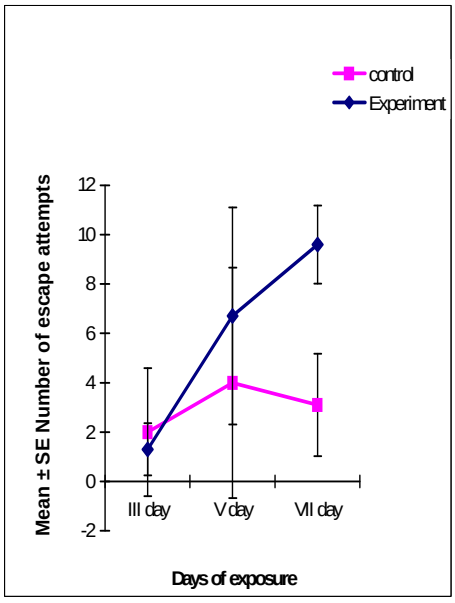

Fig: 4 Effect of sub lethal concentration of methyl-parathion on the number of Escape attempts of *Anabas testudineus*

Table 1: Kruskal-wallis (x^2): Probability values

Locomotion	Control	0.694116	N.S P>0.05
	Exp.	23.90182	P<=0.001
Speed of locomotion	Control	2.234341	N.S P<0.05
	Exp.	21.01974	P<=0.001
Escape behaviour	Control	0.195367	N.S P>0.05
	Exp.	12.06854	P<0.01
Air gulping	Control	1.607394	N.S P>0.05
	Exp.	9.119095	P<0.05
Protein index	Control	2.381011	N.S P>0.05
	Exp.	24.85431	P<=0.001

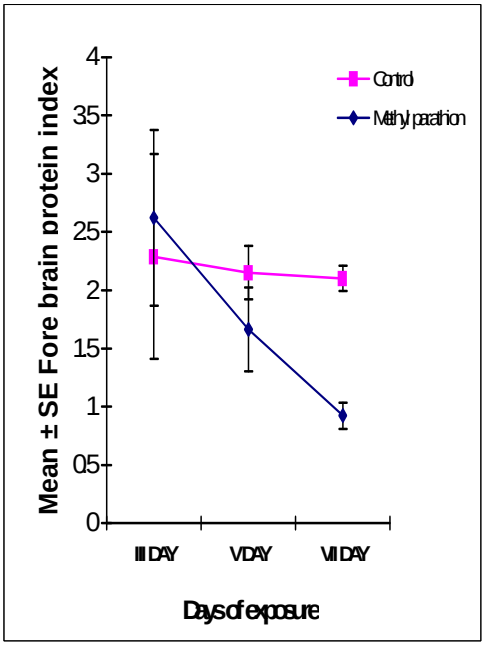

Fig:: 5 Effect of sub lethal concentration of methyl Parathionon the fore-brain protein content of Anabas testudineus

The component wise analysis of the behavioural patterns shows that the pesticide methyl parathion has definite influence on the exploratory behaviour of the fish. The increase in exploration of the test fish may be an outcome of the influence of the xenobiotic on the brain centers that control the exploratory behaviour or may be due to the increased motivation for escaping from the noxious medium [13]. The results obtained from the analysis of the impact of pesticide on the escape behaviour (reflected in the attempt to jump out of the open field) of the *Anabas testudineus* also support the above hypothesis.

The stress produced by the pesticide on the physiology of fish is also reflected in the frequency of air gulping. This behaviour is said to positively correlate with oxygen in the body of the air gulping fishes [14]. In addition, presence of any noxious chemicals can alter the rhythm of air gulping.

In mammals hippocampus, is intimately involved with the processing of information about the environment. Since there is no hippocampus in fishes, Vergas [15] suggested that the lateral pallium of telencephalon is one of the major centers that control exploratory activity of fishes. The present study indicates that the total protein content of the fore brain of the *Anabas testudineus* reduced considerably as a result of the exposure to methyl parathion during the initial five days. Eventhough the pesticide treated fish recovered and the behavioral patterns made to that of the control fish, the reduced protein content of the telencephalon remained as such on seventh day. It is possible that in addition to hippocampus some other centers of the brain like reticular arousal system (RAS) [4] may also have involved in the control of exploratory behaviour in fishes.

This type of alterations is detrimental to the fish as the change in the exploratory behaviour can influence the latent learning. Even though pesticide pollution may not directly kill aquatic

organisms like fishes, the sublethal concentration of pesticide in water can adversely affect several vital behavioural patterns of the fish reducing the overall fitness of the organism.

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