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EFFECT OF CHLORPYRIFOS ON ESTERASE ISOZYME BANDING PATTERNS IN MUSCLE AND BRAIN OF FRESH WATER CAT FISH *HETEROPNUESTES FOSSILIS*

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ABSTRACT: The present study was under taken to assess the toxicological effect of Chlorpyrifos (an Organophosphate) on esterase isozyme banding patterns in muscle and brain tissues of freshwater cat fish *Heteropneustes fossilis* (Bloch) at different time intervals i.e. 24,48,72 and 96hrs and was compared with control. The esterase isozymes were quantitatively analyzed by using 7.5% native polyacrylamide gel electrophoresis (PAGE) stained with α-naphthyl acetate as substrate. Three different esterase bands were detected and named as Est-1; Est-2 and Est-3 with different relative mobilities such as 0.35; 0.43; 0.30 in muscle tissue and 0.60; 0.40; 0.30 in brain. All the three esterase bands were found in muscle and brain tissues. Among the three esterases Est-1 in brain tissue at 24hrs and Est-2 in muscle at 24 hrs is found to be more abundant with highest intensity. The intensity of Est-3 was faintly stained in both the tissues.

KEYWORDS: Esterase, isozymes, PAGE, *H. fossilis*, Chlorpyrifos.

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1.INTRODUCTION

Fresh water ecology is polluted due to continuous use of agrochemicals especially pesticides. The pesticides like Organophosphates, Organochlorides and carbamates are regularly used in agricultural pest management for food production through their excessive and indiscriminate use in public health operations [1]. They ultimately find their way into aquatic habitats such as rivers, lakes and ponds. The environmental quality is determined by assessing the toxicity of different chemicals in agriculture which cause serious hazards to fish and other aquatic organisms [2], [3]. Pesticide toxicity to fish has been investigated in several studies [4] hence toxic studies are required for the

physiology and metabolic activities of fish. Among the aqua fauna fish form an important group due to their nutritive value. For centuries pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vector [5]. Among these pesticides organophosphate compounds (OP's) are commonly used as insecticides [6]. Chlorpyrifos (0,0-diethyl-0-3,5,6-trichlor 2-pyridyl phosphorothiate) is a broad spectrum organophosphate insecticide. Once Chlorpyrifos introduced in to the environment that may be highly toxic for aquatic animals [7]. Enzymes play an important role in metabolism of living organisms. They are exceeding efficient and very specific reaction catalyzed and substrate utilized [1]. Esterases (Est-3.1.1.2) are a group of hydrolytic enzymes occurring in multiple forms with broad substrate specificity. Esterases comprise a diverse group of enzymes catalyzing the hydrolysis of organic esters [8]. Esterase enzymes are multiple forms of a single enzyme which have different iso-electric points and therefore can be separated through electrophoresis. Electrophoretic studies were done extensively on various tissues of different animals from which it reveals that the enzyme exists in multiple molecular forms and functions [9]. Many researchers have studied the effect of pesticide on acid phosphatase activity in fish [10],[11],[12]. Esterases are also used as bio-indicators to measure the toxic potency of pesticide residue usually applied in agriculture. The residual effect of pesticide in aquaculture specifically in fish which in-turn cause death of fish [13],[14],[15]. Electrophoretic banding patterns of esterases were identified in different tissues of fishes like *Punctius* and *H. fossilis*[16],[17]. However very little information is available on the alterations in enzyme activities due to Chlorpyrifos in the *H. fossilis.* In the present investigation an attempt has been made to study the toxicological effect of Chlorpyrifos on esterase isozyme banding patterns in muscle and brain of fresh water cat fish *H. fossilis.*

2. MATERIALS AND METHODS

The fresh water cat fish *H. fossilis* were collected from local fresh water tanks within the radius of 15km from the laboratory by netting with the help of local fisher man. The fishes having an average length of 15 ± 1cm and weighed about 50±5gm were brought to the laboratory and transferred in to a plastic buckets(30X30X60cm) and disinfected with potassium permanganate and washed thoroughly prior to introduction of fish (to prevent fungal infection). The fishes were acclimatized for about 10 to 15 days prior to experimentation. They were regularly feed with commercial fish food and the medium (tap water) was changed daily to remove feaces and food remnants. The healthy fishes were grouped into five batches containing six each and were exposed to different concentrations of insecticide Chlorpyrifos at different time intervals to calculate the medium lethal concentration less value using probit analysis method [18].

Toxicological Studies

The toxicity tests were conducted in accordance with standard method [19]. The pesticide Chlorpyrifos was dissolved in acetone to yield a concentration of 100mg/ml which were further

diluted with distilled water to required concentrations. The fishes (five batches) were exposed to sub lethal concentrations (0.5ppm to 1ppm) of Chlorpyrifos for 24, 48, 72 and 96 hrs respectively, and recorded the mortality rate of fishes. Another group of fish was maintained as control without pesticide.

Preparation of samples for study

At the end of each exposure period fishes were sacrificed, the tissues such as muscle and brain were dissected out and was blotted to free from blood clots and other adherent tissues and weighed to nearest milligram and were homogenized in 10% 0.01M Tris-HCl buffer (pH 7.4) containing 0.9% NaCl. The homogenates were centrifuged and the supernatants were diluted 1:1 with 20% sucrose containing 0.01% bromophenol blue as tracking dye. An aliquote of 0.1ml of these solution was loaded directly on to the separating gel [20],[21].

Electrophoretic study and staining of gels

Esterase patterns were separated on thin layer (1.5mm thickness, 8X8 cm) polyacrylamide gels (7.5%). The gel mixture was prepared according [28]. Gelling was allowed for 45minutes. After (10-20 µl) loading on the gel, the samples were overload with electrode buffer containing Tris (0.05M), glycine (0.38M), pH was 8.3 adjust with 1N Hcl and gel plates were connected to the electrophoretic tank. Power supplied 50 volts for the first 15minutes followed constant 150 volts for the rest of the run during electrophoresis. The electrophoretic run was terminated when the tracking dye migrated to the distance of 8.0 cm from the origin. Esterases were visualized on the gels by adopting the staining procedure [20],[21].

3. RESULTS AND DISCUSSION

The results obtained on the toxicological effect of Chlorpyrifos on esterase banding patterns at different time intervals i.e. 24, 48, 72 and 96hrs exposure in muscle and brain of fresh water cat fish *H. fossilis* and the details about relative mobility of individual esterase isozyme banding patterns are presented in tab.1 and 2; fig.1 and 2 respectively. α- naphthyl acetate was used as substrate to score the intensity of esterase bands on 7.5% poly acryl amide gels. The variation in the intensity of the banding pattern of esterase isozyme in *H. fossilis* were observed. When only α-naphthyl acetate was hydrolyzed, the bands in the gels turned black and named as α-esterases. The results showed differences at different time intervals of cat fish *H. fossilis.*

Muscle

Muscle exhibited 3 esterase isozyme bands i.e.Est-1, Est-2 and Est-3 on the zymogram with R_m values 0.65,0.43 and 0.30 respectively. Est-1 (+++) was deeply stained and Est-2, Est-3 (++) were moderately stained in control tissue. In muscle tissue Est-2 was deeply stained (+++) at 24hrs and 48hrs at different time intervals compared to 72 and 96hrs. Est-3 was moderately stained (++) at 24hrs and faintly stained at 48hrs and 72hrs. Est-1 was completely disappeared at 24hrs, 72and 96hrs, but it was faintly stained at 48hrs. Est-1, Est-2 and Est-3 were completely disappeared at 96

hrs.

Brain

Brain exhibited 3 esterase bands i.e. Est-1, Est-2, Est-3 respectively (tab.2 and fig.2.).On the zymogram with R_m values 0.60, 0.40, 0.30.Among the three esterase bands Est-1was faintly stained (+) and Est-2 was deeply stained (+++) and Est-3 was moderately stained (++) in control tissue. Est-1 was deeply stained (+++) at 24 and 48 hrs and moderately stained (++) at 72hrs and completely disappeared (-) at 96hrs. Est-2 was moderately stained (++) at 24 and 48 hrs and completely absent (-) at 72 and 96hrs. Est-3 was moderately stained (++) at 24 and 48hrs, faintly stained at 72hrs and completely disappeared at 96hrs. Est-1, Est-2 and Est-3 were completely disappeared at 96 hrs.

From fig.1 and 2 highest relative mobility value in brain tissue was 0.60 (Est-1) close to the anode (+), slowest relative mobility was 0.30 (Est-3) on the cathode (-). In muscle tissue the highest relative mobility value was 0.65 (Est-1) on the anode (+), slowest relative mobility was 0.30 (Est-3) on the cathode (-).

Table 1: Effect of Chlorpyrifos on esterase banding patterns in Muscle tissue of *H. fossils*

	Control	24H	48H	72H	96H
Est-1 (0.65)	+++	-	+	±	-
Est-2 (0.43)	++	+++	+++	±	-
Est-3 (0.30)	++	++	+	+	-

Table 2: Effect of Chlorpyrifos on esterase banding patterns in Brain tissue of *H. fossils*

	Control	24H	48H	72H	96H
Est-1 (0.60)	+	+++	+++	+	-
Est-2 (0.40)	+++	++	++	-	-
Est-3 (0.30)	++	++	++	+	-

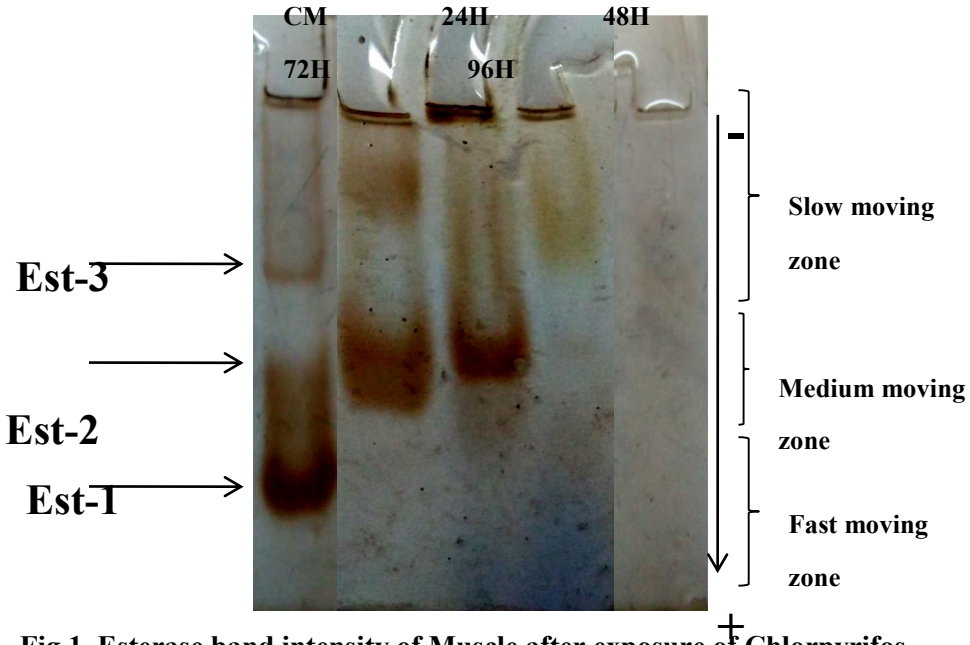

Fig.1. Esterase band intensity of Muscle after exposure of Chlorpyrifos

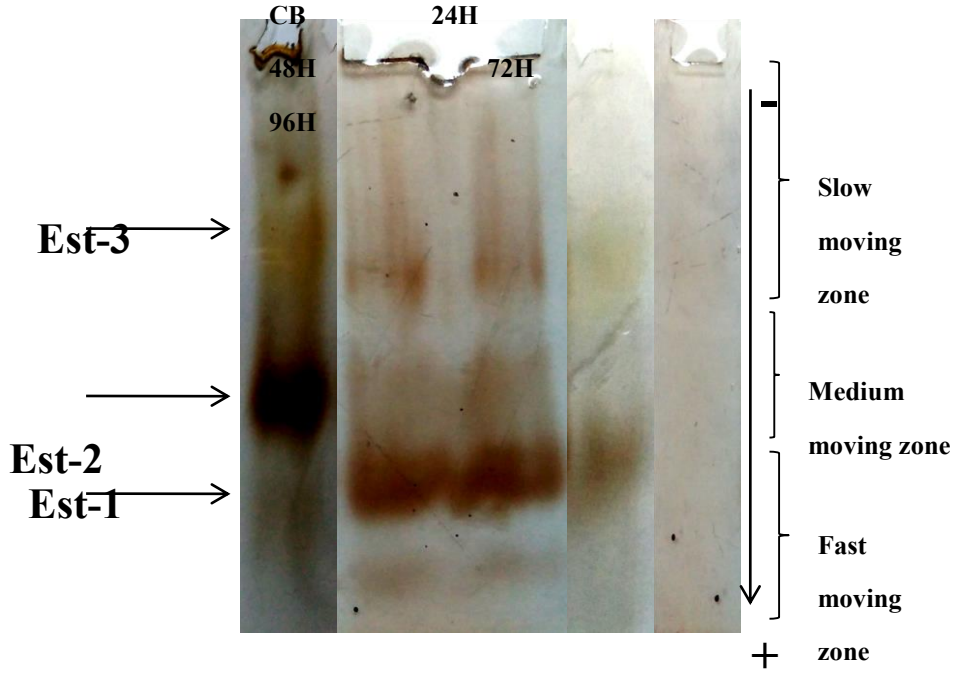

Fig.2. Esterase band intensity of Brain after exposure of Chlorpyrifos

DISCUSSION

From the table.1and 2; fig.1 and 2, it was observed that the intensity of esterase bands was differing from tissue to tissue and species to species even in the different region of the body of the same individual. It is found that the Chlorpyrifos disturb the chemical components of the fish which leads to cell damages and finally death of fishes. The banding pattern of esterases in different tissues have good potentiality for species identification. AchE esterase activity was observed to be reducing in liver and kidney [22]. The tissue and species specific distribution of esterases were earlier reported

from two catfishes and toad [23],[24]. Tissue esterase patterns of muscle and brain of Channiforms and Perchiforms were reported [25]. Different forms of esterases found in different tissues of *Punctius sophore* was analyzed [26],[27] and reported that the effect of Triazophous on esterase activity and protein contents of liver, kidney, brain, blood and muscle of *Catla catla*, *Labeo rohita*, *Cirrihinus mrigala.*

4. CONCLUSION

The present study reports that the variability of patterns of esterase isozyme describes electromorphs of an individual. It can be conclude that each tissue has specific esterase banding pattern which may be used for the development of genetic molecular makers for proper identification of fish species.

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CONFLICT OF INTEREST

No interest of conflict.

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