Effect of Methyl Parathion (An Organophosphate) on Electrophoretic patterns of esterases in Liver, Intestine and Brain tissues of fresh water Cat Fish. *H. fossilis* (Bloch)

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Abstract: The present investigation was aimed at determining the effect of Methyl Parathion (an Organophosphate) on esterase isozyme banding patterns in different tissues such as Liver, Intestine and Brain of fresh water 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 0.60; 0.40; 0.30 in liver tissue and named as Est-1; Est-2, Est-3 with different relative mobilities such as 0.60; 0.40; 0.30and 0.15 in intestine tissue and 0.60; 0.40; 0.30 in brain tissue and four different esterase bands were detected All the three esterase bands were found in liver and brain tissues. Among the three esterases Est-1 at 24hrs and Est-2 at 72 hrs and 96hrs in liver tissue, Est-2 at 24hrs, 72hrs and 96hrs Est-4 at 72hrs in intestine are found to be more abundant with highest intensity. The intensity of Est-2 Est-3 was faintly stained in brain tissue, Est-3 in liver tissue Est-4 in intestine tissues were faintly stained. The results revealed that, there are various altrations due to methyl parathion exposure in esterases patterns of the tissues such as brain, intestine, liver of H. fossilis which showed homology in protein bands with minor variations.

Keywords: Esterase, isozymes, PAGE, *H.fossilis*, Methyl Parathion.

INTRODUCTION

The fish H.fossilis is commonly known as Stinging Catfish (for poisonous pectoral spine), locally called Shing or Shingi (Rahman, AKA.) Ingilayee, mapujella and marpu (A.P), shing, (Bangladesh). Singee and sheene (Assam), singhi (West Bengal) Kamacha singhi, Bitchu, Tailia, and singee(U.P), Lahoord and Nulli (punja), singee and singhi (Osrissa), Thaylee and Thaimeen (T.N). It is a very wide range (Pakistan, India, Sri Lanka, Nepal, Bangladesh, Myanmar, Thailand and Laos) and has been introduced elsewhere. Whilst it is heavily utilized for food and for medicine in many parts of its range, and it may be threatened by over exploitation and habitat loss and degradation (especially from pollution and dams), it is considered least concern at present. Related synonym is Saccobranchus microcephalus (Gunther, 1864). The Greek word Sacco means a sack, a bag and branchus means respiratory organ, gill pertaining to additional respiratory sack along with gill. It is commonly known as Stinging Catfish (for poisonous pectoral spine), as suggested by its common name - stinging catfish, Heteropneustes fossilis can deliver a Painful sting via the spines on its pectoral fins. In the above scenario we investigate the Effect of Methyl Parathion (An Organophosphate) on tissue specific esterase patterns in Indian cat fish Heteropneustes fossilis (Bloch).

The stinging cat fish H. fossilis (Bloch) is locally called as Ingilayee or Marpujella. It is an important air sac cat fish indigenous to many Asian countries. It inhabits in fresh water and able to tolerate brackish water too. It is very popular not only for its good taste but also highly nutritional and medicinal point of view. H. fossilis is found mainly in ponds, ditches, swamps, and marshes, but sometimes occurs in muddy rivers. It is omnivorous. It is in great demand due to its medicinal value (Froese et al., 2011). The stinging catfish is able to deliver a painful sting to humans. Poison from a gland on its pectoral fin spine has been

known to be extremely painful. It is also farmed and found in the aquarium trade (Froese et al., 2011). Fish reproduction is a periodic phenomenon and is controlled by environmental (exogenous) as well as internal (endogenous) regulatory mechanism. It acts of breeding occur under optimal environmental conditions that are favorable to the survival of the young ones. Environmental stimuli are detected by sensory organs, relayed to brain, that triggers endogenous mechanism into action.

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 organophosphate methyl parathion at different time intervals to calculate the medium lethal concentration less value using probit analysis method

Toxicological Studies:

The toxicity tests were conducted in accordance with standard method. An organophosphate methyl parathion 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 Methyl Parathion 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 Liver Intestine 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.

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. 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.

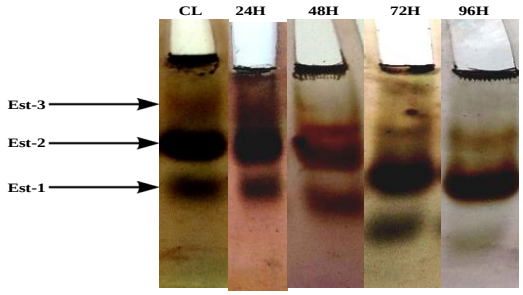

Fig.2. Esterase band intensity of Liver after exposure of organophosphate

Table.I. Electrophoretic banding patterns showing the variation of intensity of Esterase isozymes in Liver tissue of *H.fossilis*

Est (Rmvalues) / Dose	Est-1 (0.6)	Est-2 (0.4)	Est-3 (0.3)
Control	++	+++	+++
24H	+	+++	++
48H	+	+++	++
72H	+	+++	++
96H	+	+++	++

+ = Faint; ++ = Moderate ; +++ = Deeply stained

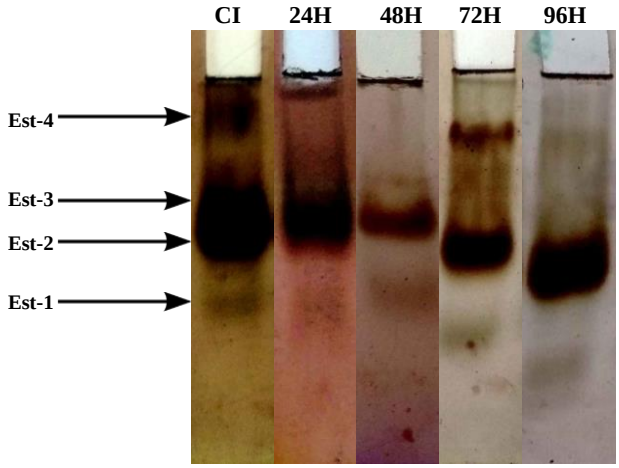

Fig.3. Esterase band intensity of Intestin after exposure of organophosphate

Tab.II. Electrophoretic banding patterns showing the variation of intensity of Esterase isozymes in Intestine tissue of *H. fossilis.*

Est(Rmvalues) / Dose	Est-1 (0.6)	Est-2 (0.4)	Est-3 (0.3)	Est-4 (0.15)
Control	++	+++	+++	++
24H	+	+++	+++	-
48H	+	+++	-	-
72H	+	+++	-	++
96H	+	+++	-	-

+ = Faint; ++ = Moderate ; +++ = Deeply stained

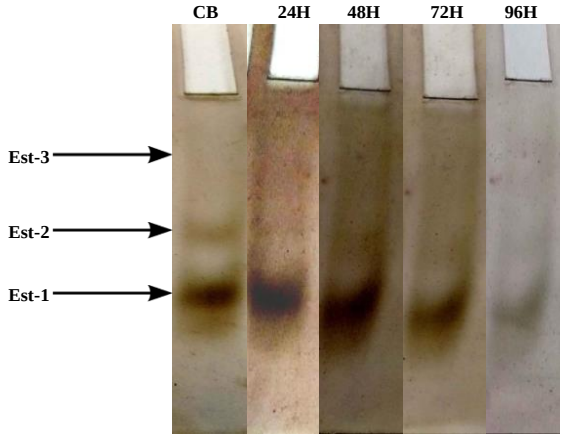

Fig.5. Esterase band intensity of Brain after exposure of Methyl Parathion

Table.III. Electrophoretic banding patterns showing the variation of intensity of Esterase isozymes in Brain tissue of *H.fossilis*.

Est(Rmvalues) / Dose	Est-1 (0.6)	Est-2 (0.4)	Est-3 (0.3)
Control	+++	++	+
24H	+++	+	-
48H	++	-	-
72H	++	-	-
96H	+	-	-

+ = Faint; ++ = Moderate; +++ = Deeply stained

RESULTS

Liver

The liver showed three esterase isozymes at 24h with R_m value 0.60, 0.40 and 0.30; whereas it showed three esterase isozymes at 48h with R_m value 0.60, 0.40 and 0.30; while at 72h it showed three esterase isozymes with R_m value 0.60, 0.40 and 0.30; and at 96h it showed two esterase isozymes with R_m value 0.60, 0.40 and 0.30.

The liver exhibited three esterase bands in control i.e. Est-1(0.6), Est-2(0.43) and Est-3 (0.3). Est-1 was moderately stained, Est-2 was deeply stained and Est-3 is faintly stained. Est-1 and Est-3 was gradually decreased and Est-2 was not affected in 24h, 48h, 72h and 96h.

Intestine

The intestine showed three esterase isozymes at 24h with R_m value 0.60, 0.40 and 0.30; while at 48h it showed two esterase isozymes with R_m value 0.60 and 0.40; whereas at72h it showed three esterase isozymes with Rm value 0.60, 0.40 and 0.30; and at 96h it showed two esterase isozymes with R_m value 0.60 and 0.40.

The intestine exhibited four esterase bands at control viz., intestine, Est-1(0.6), Est-2(0.4), Est-3(0.3), ESt-4(0.15). Out of four bands Est-1 was faintly stained, Est-2 and Est-3 were deeply stained and Est-4 was moderately stained. Est-1 was gradually decreased and Est-2 was not affected but Est-3 was disappeared at 48, 72 96hr and present in 24h. Est-4 was present in 72h but disappeared at 24h, 48, 96h.

Brain

The brain showed two esterase isozymes at 24h with R_m value 0.60 and 0.40, whereas at 48, 72, and 96hr it showed single esterase isozyme with R_m value 0.60 .

The brain exhibited three esterase bands with R_m values Est-1(0.6), Est-2 (0.4) and Est-3(0.3). In this Est-1 was deeply stained, Est-2 moderately stained and Est-3 faintly stained. Est-1 band was slightly increased in 24h and gradually decreased in 48, 72 and 96hr. whereas Est-2 and Est-3 were completely disappeared.

DISCUSSION

In the present study among three esterases Est-2 is found in liver and intestine to be more abundant with deeply stained (+++). And Est-1 was deeply stained (+++) in brain. The intensity of Est-2 were deeply stained (+++) in liver and intestine and moderately in brain. The Est-3 was deeply stained (+++) in liver tissue and moderately stained (++) in liver and intestine. The liver tissue showed in all the three esterases zone i.e (Est-1; Est-2; and Est-3) were deeply (+++) stained. In Est-2 &3 esterases zone of liver, intestine were deeply (++) stained. Est -1 esterase zone was moderately (++) stained. Est -3 and Est-4 were moderately (++) stained in intestine and brain.

Esterases 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. Esterases (EST, 3.1.1.1) are obiquitous in living organisms. Several esterases have been isolated from various tissues of microbes, plants and animals and investigated for their biochemical properties.

The present study reports that the variability of patterns of esterase isozymes describes electro morphs of an individual, representating expression of tissue specific esterase isozymes, which showed differential banding patterns that could be used in toxicological study. It can be concluded that the tissue wise variation in the banding patterns of esterase may be used for the development of genetic molecular markers. Thus, Present study has concluded that the long term exposure of Methyl parathion becomes a continuous health hazard for the fish population. Therefore it is required to monitor the aquatic system and predict the toxic effect of pesticides on fish. After exposure of Methyl parathion we observed that esterase activity in different tissues of *H. fossilis* was gradually decreased with increasing the time intervals. Similar results were observed by mores *et al*., 2000.The esterase activity was most abundant in liver to compare with other tissues such as intestine and brain.

From the above Table I, II and III 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. The binding patterns of esterases in different tissues have good potentiality for species identification. AChE esterase activity was observed to be reducing in liver and kidney (Shaid Nahboob KA.,Ghazala Ghazala 2016).The tissue and species specific distribution of esterase were earlier reported from two catfishes and toad (Shahijahan R M., Karim A., Begum RA., Alam MS., Begum a 2008). AChE Esterase activity revealed that subleathal concentration of Methyl Parathion inhibited esterase activity, the order of decrease AChE esterase activity in *H.fossilis* was recorded as Liver > Intestine > Brain. An organism develops the resistance against the insecticide could not function (Holmes RS 1970). Isozyme patterns exhibits differences in the various fish populations (Barua S et al.,2004) and also used to develop genetic sexing system (Robinson AS,1986). The results of present study is coincide with results of Venkateswara Rao et al.,2022, Venkateswara Rao et al.,2023, Shankar et al 2019).

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. The long term exposure of methyl parathion becomes a continuous health hazards for the fish population. Therefore it is required to monitor the aquatic system and predict the toxic effect of pesticides on fish.

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