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COMPARATIVE STUDY OF ORGANOPHOSPHATE PESTICIDE (METHYL PARATHION) EFFECTS ON ESTERASE ISOZYME IN GILL TISSUE OF *HETEROPNEUSTES FOSSILIS* AND *CHANNA PUNCTATUS*

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ABSTRACT

The present study was under taken to assess the toxicological effect of Methyl Parathion (an Organophosphate) on esterase isozyme banding patterns in gill tissue of freshwater cat fish *Heteropneustes fossilis* (Bloch) and *Channa puntatus* 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 gill tissue of *Heteropneustes fossilis* and 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 gill tissue of *Channa puntatus* . Est-1 is deeply stained, Est-2 is moderately stained, Est-3 is faintly stained and in both fishes. Two fishes exhibits Decreasing of Esterase bands intensity is observed.

KEYWORDS: Esterase, isozymes, PAGE, *H. fossilis*, *Channa puntatus* Methyl Parathion, Gill Tissue.

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.

RESULTS

Gill tissue of *Heteropneustes fossilis*

The gill showed two esterase isozymes at 24h with Rm value 0.60 and 0.40; while at 48h it showed two esterase isozymes with Rm value 0.60 and 0.40; and at 72h it showed one esterase isozymes with Rm value 0.60 and it showed two esterase isozymes at 96h with Rm value 0.60 and 0.40. Est-1 showed more intensity compared with others.Est-1 showed deeply stained bands Est-2 Showed moderate bind intensity and Est-3 showed faintly stained bands.

Table 1: Esterase Band intensity in Gill tissue of *Heteropneustes fossilis* after exposure to Organophosphate Methyl Parathion at different time intervals.

EST/Rm Value	EST-1(0.6)	EST-2(0.4)	EST-3(0.3)
Control (Gill)	+++	++	+
24H	++	++	+
48H	+	++	-
72H	+	+	-
96H	+	+	-

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

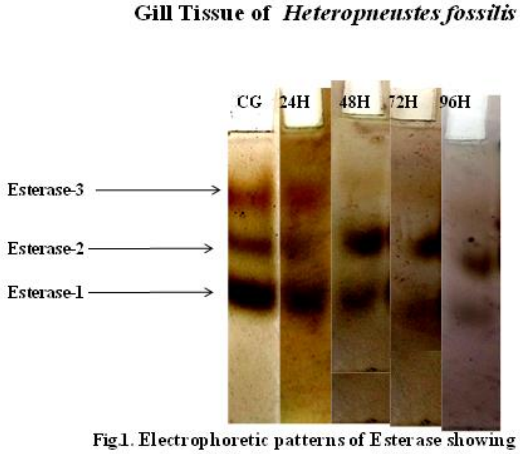

Fig.1. Electrophoretic patterns of Esterase showing band intensity of Gill after exposure of Methyl Parathion

Gill tissue of *Channa puntatus*

The gill showed three esterase isozymes Controll but, at 24h gill showed two esterase isozymes with Rm value 0.60 and 0.40; while at 48h it showed two esterase isozymes with Rm value 0.60 and 0.40; and at 72h it showed two esterase isozymes with Rm value 0.60, and 0.40 and it showed one esterase isozyme at 96h with Rm value 0.60. Est-2 showed more intensity compared with others. Est-2 showed deeply stained bands Est-1 Showed moderate bind intensity and Est-3 showed faintly stained bands.

Table 2: Esterase Band intensity in Gill tissue of *Channa puntatus* after exposure to Organophosphate Methyl Parathion at different time intervals.

EST/Rm Value	EST-1(0.6)	EST-2(0.4)	EST-3(0.3)
Control (Gill)	++	++	+
24H	+	+++	-
48H	+	++	-
72H	+	+	-
96H	+	-	-

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

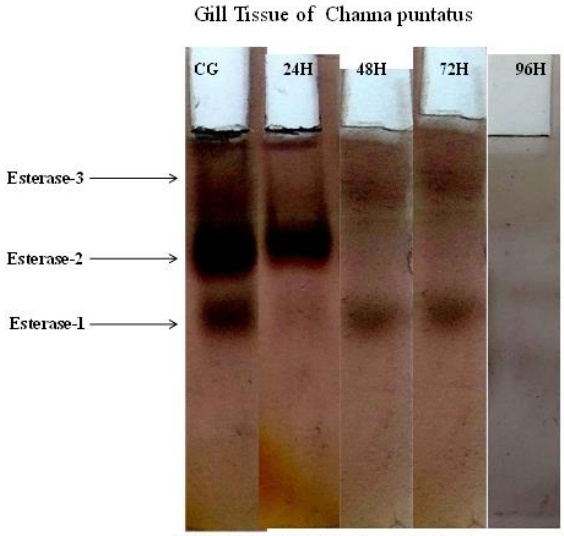

Fig.2. Electrophoretic patterns of esterases after exposure of Methyl Parathion

DISCUSSION

In the present study among three esterases Est-2 is found in and liver to be more abundant with deeply stained (+++). And Est-1 was moderatly stained (++) in all tissues The intensity of Est-2 were deeply stained (+++) in kidney, heart and liver and. The Est-3 was deepaly stained (+++) in liver tissue and moderately stained (++) in kidney,heart. 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, kidney were deeply (++) stained. Est -1 and Est-3 esterase zone was moderately (++) stained.

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 and II 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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