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Effect of Methyl Parathion (an Organophosphate) on Electrophoretic Patterns of Esterases in Gill and Muscle Tissues of Freshwater Cat fish. *H. fossils* (bloch)

Bheem Rao T[1], Venkanna Lunavath[2]

[1,2] *Department of Zoology, Kakatiya Government College, Hanamkonda, Telangana State, India*
E-mail: thatipartibheemrao@gmail.com

ABSTRACT

The present study was under taken to assess the toxicological effect of Methyl Parathion (an Organophosphate) on esterase isozyme banding patterns in gill and muscle 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 0.60; 0.40; 0.30 in gill tissue and 0.60; 0.40; 0.30 in Muscle. All the three esterase bands were found in gill and muscle tissues. Among the three esterases Est-1 at 24hrs and Est-2 at 48 hrs and 72hrs in gill tissue, Est-2 at 24hrs in muscle tissue 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, Methyl Parathion

INTRODUCTION

Fish is the main source of animal protein in the diet of the people of India, because more than 80% of animal protein in our diet comes from fish alone (Ruby *et al*., 1978). *Heteropneustes fossilis* (Stinging catfish) is one of the most popular indigenous catfish having considerable potential for aquaculture and commercial importance in India and Bangladesh. 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.

Fresh water ecology is polluted due to continuous use of agrochemicals especially pesticides. 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].

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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.*

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 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.

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 AND DISCUSSION

The different tissues of *H. fossilis* after exposure of Methyl parathion at different time intervals exhibited following results.

Gill:

The gill showed two esterase isozymes at 24h with R_m value 0.60 and 0.40; while at 48h it showed two esterase isozymes with R_m value 0.60 and 0.40; and at 72h it showed one esterase isozymes with R_m value 0.60 and it showed two esterase isozymes at 96h with R_m value 0.60 and 0.40.

Table.1. Electrophoretic banding patterns showing the variation of intensity of Esterase isozymes in Gill tissue of *H.fossilis*

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

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

The gill exhibited three esterase bands in control i.e. Est-1(0.6), Est-2(0.4) and Est-3(0.3). Out of three bands Est-1 was deeply stained, Est-2 was moderately stained and Est-3 was faintly stained. Est-1 was gradually decreased and Est-2 and Est-3 were disappeared at 24h, 48h, 72h and 96h.

Muscle:

The muscle showed only one esterase isozyme at 24h with R_m value 0.40, whereas at 48,72 and 96hr it showed a single esterase isozyme with R_m value 0.40.

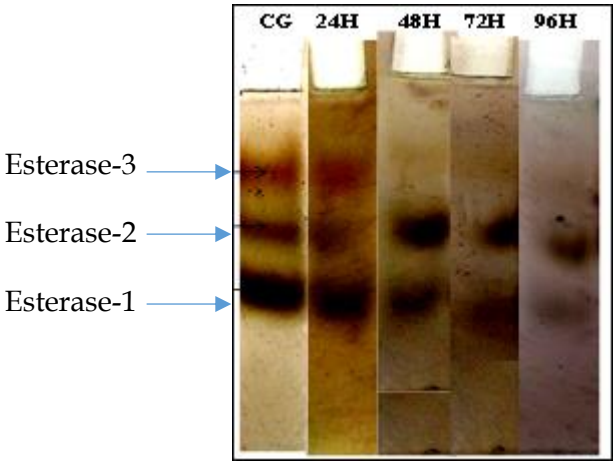

Figure-1. Electrophoretic patterns of Esterase showing band intensity of Gill after exposure of Methyl parathion

Three esterase bands were found in muscle with R_m values of Est-1(0.6), Est-2(0.4) and Est-3(0.3). where Est-1 was moderately stained Est-2 and Est-3 were faintly stained. These were gradually decreased in Est-2 at 24h, 48h, 72h, 96h. Est-1 and est-3 was completely disappeared at 24h, 48h, 72h and 96h.

Table.1. Electrophoretic banding patterns showing the variation of intensity of Esterase isozymes in Muscle tissue of *H.fossilis*

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

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

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 and stomach, mentioned two organs have a relation with food digestion. Liver esterases could be associated with digestion and metabolism of different esters (Swapna & Ravinder Reddy, 2015). Distribution of different kinds of esterases indicates that CE esterases were present in all the channiformes fishes. But CE esterase were not noticed in perciformes fishes in brain tissue. ArE

esterases were found in all the perciformes fishes but ArE esterases were not noticed in channiformes fishes indicated that channiformes order is distinct from perciformes order fishes. (Rajaiah *et al.,* 2010). Slowest bands with higher moleculer weight were expressed than the others and spreading area of a particular band was not fixed in all the fishes (Mohammed Abdur Rashid & Reza hahjahan,2012). Expression of tissue specific esterase isozymes showed differential banding pattern that could be used in toxicological study (Sabekum Nahar & Md. Abdur Rashid, 2013).

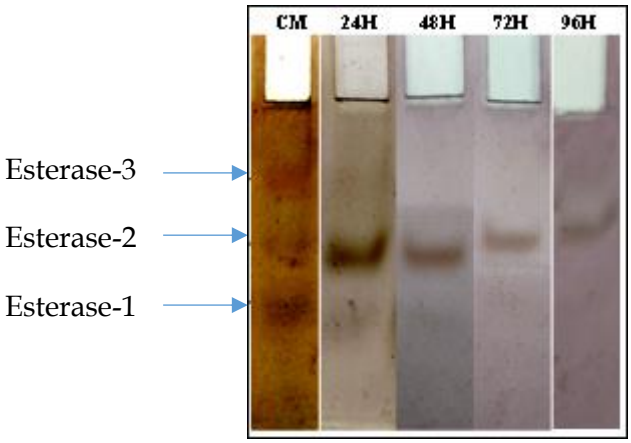

Figure-2. Electrophoretic patterns of Esterase showing band intensity of Muscle after exposure of Organophosphate

Electrophoretic banding pattern of esterase isozyme in different tissues of *Puntius sophore* (Cyprinidae:cypriniformes) (Hawajahan *et al*., 2016). Comparative study of esterases found invariuos tissues of snail *Lamelliderns corrianus* (Fresh water mussel) indicate that each esterases had its own characteristic patterns of esterases (Swapna *et al*., 2014). Variation was observed in the expression of esterase isozymes in the developmental stages of mosquito fish *Gambusia afficinis* (Md.Abdur Rashid 2012). Characterization of esterases was made on the basis of its responses towards certain inhibitors. Among the liver esterase fractions of *C. punctatus* (bloch) (Arunachalam *et al.,* 1985). Liver, heart and muscle tissues are better applied in studying the isoenzymatic profiles for fish physiology and systematics. (Mohammad Salem *et al*., 2012).

Expression of tissue specific esterase isozymes showed differential banding pattern that could be used in rapid inexpensive identification of studied species (Shams Noor *et al*., 2013). It is possible that the enzymes may play a critical role in processing and potentiating toxins secreted by venomous glands (Venkaiah & Lakshmipathi, 2006). Whether esterases present in the secretions have a role in de-esterifying these esterin venomous glands (Venkaiah & Lakshmipathi, 1998; Vijayagiri *et al*, 2012).

The comparative study of various classes of esterases contributing to tissue enzyme activity indicates that ArE esterases are the major contributing to tissue enzyme activity in the tissue of *Arion hortensis* (Swapna *et al*., 2017). The esterase activity was most aboundant in liver and stomach, mentioned two organs have a relation with food digestion (Swapna & Ravinder Reddy, 2016). All the samples treated with both α-napthyl acetate and β-napthyl acetate as substrate but absent in some slot when stained separately with both α-napthyl acetate and β-napthyl acetate, so the enzymes activity depends on the substrate in Mosquito fish, *Gambusia affinis* (Md.Monjurul Ahasan *et al*., 2011). Genetic variation and structure of different populations of the studied species before undertaking any stock improvement and conservation program (Mohammad Arif Hossain *et al*., 2013). Banding pattern of esterases of different tissues has a good potential used in the identification of species (Reza Md.Shajahan *et al.,* 2008). ChEs in blood in different fish species showed reduced AChE and BuChE(activites after exposure to triazophos (Ghazala *et al*., 2016). Esterases are used for development and application of molecular markers as for example species identification, selection and toxicological status (Md.Abdur Rashid *et al.,* 2013).After exposure to an organophosphate on *Cyprinus carpio* the increased levels of transaminase enzyme due to subsequent liver damage (Sanjay Singh *et al*., 2016). The inhibition patterns suggests that the esterase enzymes are sensitive to organophosphate compounds, Paraoxon (Raju & Venkaiah, 2013).

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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Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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