Section A -Research paper

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Electrophoretic Esterase Enzyme Banding Pattern Alterations in Intestine, Muscle Tissue of Fresh Water Fish *Channa Punctatus* (Bloch) Exposed to Malathion (An Organophospahte)

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ABSTRACT

The present study was under taken to assess the Electrophoretic Esterase Enzyme banding pattern alterations in intestine and muscle tissue of fresh water fish Channa punctatus (Bloch) exposed to Malathion(an Organophosphate) at different time intervals i.e. 24H, 48H, 72H and 96H 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 Isozyme bands were detected and named as Est-1; Est-2 and Est-3 with different relative mobilities such as 0.6± 0.05; 0.4± 0.05; 0.3± 0.05 in gill, liver and brain tissue. All the three esterase bands were found in intestine and muscle tissues tissues in control. After the fish is exposed to Malathion (an Organophosphate),

Key words: Electrophoretic banding pattern alterations, Esterase Isozymes, *Channa punctatus*, α-naphthyl acetate, PAGE, Malathion (an Organophosphate), different time intervals

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INTRODUCTION

In spite of its high nutrient value and also less cholesterol *Channa punctatus* is most preferred edible indigenous fish in India. The fish meal contain vitamin A and D and omega 3 fatty acids. Fish is the best bio Indicator of water pollution. Among Organophosphate pesticides Malathion is the most used worldwide. Esterases are the lipid hydrolyzing enzymes which has lot significance in genetics and toxicology (Callaghan, 1994). Studies of R.M Shahjahan, 2008 reveals that the Electrophoretic banding patterns of Esterase Isozymes are different from species to species and also tissue to tissue, hence Esterases could be used to identify the different species. The Electrophoretic banding pattern of Esterases is gene linked hence these are can be used to estimate the genetic distance of various species (Turner B.J.1973).Esterases do not require cofactors and this property makes them attractive biocatalysts (Godinho L.F, 2011). Adapting the strategies of substrate specificity and sensitivity to various inhibitors, the Esterases were classified into four categories (Holmes and Masters, 1967, Holmes et al., 1968) viz, Carboxylesterases (E.C.3.1.1.1=aliesterases), Arylesterases (E.C.3.1.1.2=Arom esterasses), Acetylesterases (E.C.3.1.1.6=C esterases) and Cholinesterases (including Acetylcholenesterases E.C. 3.1.1.7 and pseudocholinesterases E.C.3.1.1.8). Aldridge (1953) classified Esterases into Esterase A and Esterase B by using organophosphate inhibitors. Hart.N.H&Cook,M 1976; Verma A.K. and Frankel J.S., 1980; Horitos and Salmastakis 1982; Lakshmipathi.V

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&Reddy.T.M 1989, 1990 identified the deviations in the sensitivity of fish esterases according to Holmes and Masters classification and these scientists classified the esterases into four types i.e. ER-esterases that are resistant to all inhibitors, Ese- Esterases that are inhibited by Eserine only, Esdp- Esterases that are inhibited by OP and p CMB both, CHsp esterases(Cholinesterase like enzymes) are inhibited by all three inhibitors. Isozyme analysis has been used to estimate the genetic variation between different species of fish (Barua et al., 2004). Many researchers have studied the effect of pesticide on acid phosphatase activity in fish (Joshi et al., 1981; Jaroli et al., 2005; Sreenivasan et al., 2011)

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 (Debnath, 1978; Sahib et al., 1980; Begum et al., 2008).

The current research work investigated the Electrophoretic Esterase enzyme banding pattern alterations in intestine and muscle tissue of fresh water fish *Channa punctatus* (Bloch) when exposed to Malathion (OP).

MATERIAL & METHODS

Channa punctatus fishes weighing about 50-70 gm were collected from river nearby Kakatiya University campus, Warangal, Telangana State, India. The fish were brought to laboratory by taking the stringent care about aeration and hygiene. The fish were acclimatized to lab conditions by feeding with natural planktons. After a week the fishes were exposed to sub lethal concentration of Malathion (OP). Three tissues were selected for the study i.e. intestine and muscle. After collecting the tissues blotted to free from blood clots and other adherent tissues and weighed to the nearest milligram and were homogenized in 0.01N Tris.HCL buffer(Ph =7.5) containing 0.9% of Nacl. The homogenates were centrifuged at 2000 rpm for 10 min on a clinical centrifuge at room temperature. The supernatant were mixed with equal volumes of 20% sucrose solution containing 0.05% bromophenol blue as the tracking dye. An aliquot of 0.1 ml of this mixture was used for loading the sample on to the separating gel for separation of esterase patterns. (Holmes RS, Masters CJ.1967, Reddy. M.T. and Lakshmipathi, V. 1988.). Esterase Isozymes were separated on thin layer 1.5mm polyacrylamide gels (7.5%). The gel mixture was prepared according to Clark-1964. Tris (0.05M), glycine (0.38M) buffer (PH=8.3) was used as the electrode buffer. And the tissue samples were loaded on gel. A constant current of 50 volts for the first 15 min followed by 150 volts for the rest of the run was supplied during the electrophoresis. The electrophoretic run was terminated when the tracking dye migrated to the distance of 5cm from the origin. Esterases were visualized on the gels by adapting the staining procedures of (Raju and Venkaiah 2013; Bheem Rao et al., 2018; Shankar et al., 2019; Venkateswara Rao and Venkaiah 2022, Venkateswara Rao and Venkaiah 2023). They were stained for esterase activity with α- naphthyl acetate as substrate. (Reddy. M.T. and Lakshmipathi, V. 1988). . The relative mobility (Rm) activity of zone was determined according to Klebe (1975)

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RESULTS

Intestine

Intestine tissue showed 03 esterase isoenzyme bands in control. Est-1 with Rm value 0.6± 0.05 and Est-2 with Rm value 0.4± 0.05 were deeply stained (+++). While Est-3 with Rm value 0.3± 0.05 was moderately stained (++).After Malathion exposure, at 24H tissue showed 03 esterase bands. Est-1 and Est-2 and Est-3 were stained moderately (++). At 48H of Malathion expose tissue showed 03 esterase enzyme bands. Est-1 and Est-3 were faintly stained (+), while Est-2 was stained moderately (++) . At 72H tissue showed 02 esterase enzyme bands. Est-1 and Est-2 were stained faintly (+), and Est-3 was disappeared. At 96H tissue showed only one esterase enzyme band. Est-2 with stained faintly (+). The other two esterase isoenzyme bands i.e. Est-1 and Est-3 were vanished (Table I and Figure. I).

Muscle

Muscle tissue showed 03 esterase isozyme bands in control. Est-1 band with Rm value 0.6±0.05 was highly stained (+++), while Est-2 with Rm value 0.4± 0.05 and Est-3 with Rm value 0.3± 0.05 were moderately stained (++).

At 24H of Malathion exposure, the muscle tissue showed 03 esterase enzyme bands. Est-1 band was highly stained (+++). Whereas Est-2 and Est-3 enzyme band was moderately stained (++) and the intensity also reduced. At 48H tissue exhibited 02 enzyme bands. Est-1 and Est-3 were moderately stained (++) and Est-2 was vanished (-). At 72H tissue showed 02 enzyme bands. Est-1 and Est-3 were faintly stained (+). But Est-2 band was disappeared (-). At 96H tissue shown 01 enzyme band i.e. Est-3 which was faintly stained. The other two esterases i.e. Est-1 and Est-2 were vanished (Table II and Figure.II).

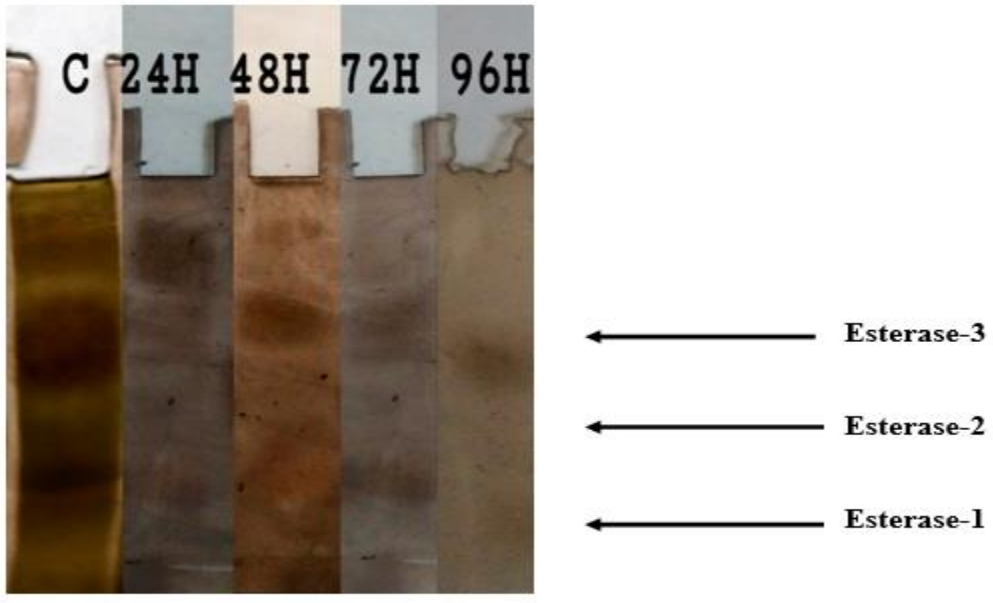

Fig.1: Electrophoretic Esterase enzyme Banding Pattern Alterations in intestine tissue of fresh water fish *Channa punctatus* (bloch) exposed to malathion(an Organophosphate)

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Esterase/Dose	Control	24 H	48H	72H	96H
Esterase-1 Rm 0.6±0.05	+++	++	+	+	-
Esterase-2 Rm 0.4±0.05	+++	++	++	+	+
Esterase-3 Rm 0.03±0.05	++	++	+	-	-

Table 1: Electrophoretic Esterase enzyme Banding Pattern Alterations in intestine tissue of fresh water fish *Channa punctatus*(bloch) exposed to malathion(an Organophosphate)

+++: Deeply stained
++: Medium deeply stained
+: Faintly stained
-: No staining, Esterase enzyme was absent

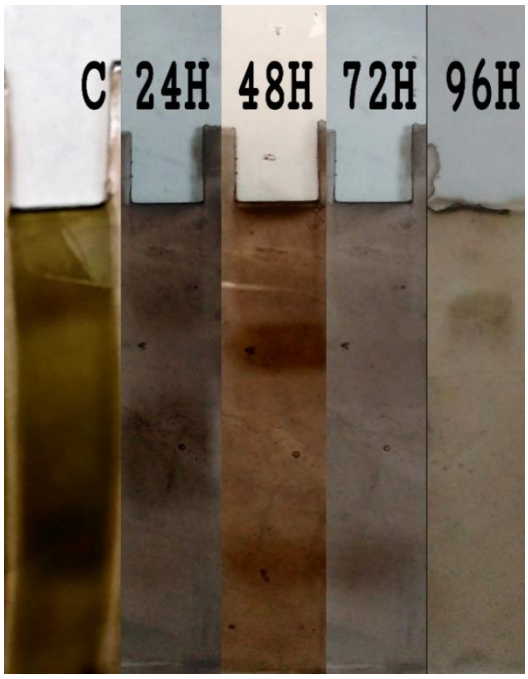

Figure-II: Electrophoretic Esterase enzyme Banding Pattern Alterations in Muscle tissue of fresh water fish *Channa punctatus*(bloch) exposed to malathion(an Organophosphate)

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Esterase/Dose	Control	24 H	48H	72H	96H
Esterase-1 Rm 0.6±0.05	+++	+++	++	+	-
Esterase-2 Rm 0.4±0.05	++	++	-	-	-
Esterase-3 Rm 0.03±0.05	++	++	++	+	+

Table-II: Electrophoretic Esterase enzyme Banding Pattern Alterations in Muscle tissue of fresh water fish *Channa punctatus*(bloch) exposed to malathion(an Organophosphate

+++: Deeply stained
++: Medium deeply stained
+: Faintly stained
-: No staining, Esterase enzyme was absent

DISCUSSION

The alterations of Electrophoretic Esterase Isozyme banding pattern in intestine and muscle tissue in Fresh water fish Channa punctatus (Bloch) exposed to Malathion (An Organophosphate) was investigated in the present research. Effect of Malathion on Esterase –I is more pronounced in intestine tissue, the intensity of the band and disappearance of the band started at 48hours of Organophosphate exposure. Whereas the disappearance of Esterase-1 started at 72 hours of exposure. The effect of Malathion on Esterase-2 was more in muscle tissue than intestine tissue. The intensity of the enzyme band and disappearance of the Esterase band started from 48 hours, whereas the Esterase bands were unaffected even at 96hours of exposure, still single Esterase band was seen at 96 hours. Effect of Malathion on Esterase-3 was seen more in intestine tissue than muscle tissue, the Esterase bands were vanished at 72 hours, whereas single Esterase-3 was seen even at 96 hours. Correlation between esterase activity and cypermethrin was observed in different tissues of selected species. Sahib et al. (1980) investigated the impact of Malathion on acetylcholine esterase in the tissues of the fish Tilapia mossambica. Eight esterase bands were observed in different tissues of Oreochromis. aureus (Hongtudo et al. 1993), six esterase bands in the brain of Channel cat fish Ictalurus punctatus (Knowles et al. 1968), seven esterase bands in blunt snout lorean fish (Sifa et al. 1993), five esterase bands in Nile tilapia (Shahjahan et al. 2008) and Pangasius hypophthalmus (Begum et al. 2008). Esterase Zymograms of four Surgeon fish species were investigated (Wayne S.Leibel, 1987). Banding pattern of esterases of different tissues has a good potential used in the identification of species. Al-Amin et al. (2005) reported that isozyme banding pattern of the intestine could be used for identification of two species of Pangasius (P. sutchi and P. pangasius). Between the two species the intestine of P. sutchi and P. pangasius possesses 4 and 6 bands, respectively. Furthermore, two species of Anabas (A. testudineus and A. oligolepis) was

identified using esterase bands of liver, kidney, skeletal muscle, heart and egg (Ramaseshaiah & Dutt, 1984). The findings in the present study may be extended to use as genetic marker in various fields of physiology, taxonomy and toxicology in Nile tilapia (O. niloticus).Different forms of esterases found in different tissues of Punctius sophore was analyzed (Hawajahan et al., 2016; Ghazala 2016) 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. Three Esterase Isozyme bands were identified (Venkateswara Rao et al., 2022), Effect of Malathion on Electrophoretic Esterase isozyme in fresh water fish Channa punctaus is studied (Venakteaswara Rao et al., 2023).Effect of Chloropyrifos was reported(Shankar et al.,2019). Three Electrophoretic esterase Isozyme bands were investigated (Venakteraswara Rao et al., 2023). Effect of Malathion on Electrophoretic Esterase Isozymes was studied (venkateswara Rao et al., 2023). Effect of Malathion on Electrophoretic banding patterns of estaerase was studied (Venakteswara Rao et al., 2023 Bulletin of Pure and Applies Biology., Final proof received).

CONCLUSION

The present study reports that Expression of tissue specific esterase isozymes showed differential banding pattern that could be used in toxicological study and also could be used for the development of molecular markers for the identification of different species of fish.

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CONFLICT OF INTEREST

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

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