A Comparative Study of Effect of Malathion (An Organophosphate) on Electrophoretic Banding Patterns Of Esterases in Gill and Intestiine Tissue of Fresh Water Fish *Labeo Rohita* (Hamilton)

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ABSTRACT

The present study was under taken to assess the toxicological effect of Malathion (an Organophosphate) on electrophoretetic banding patterns of esterase enzymes in gill and intestine tissues of freshwater fish *Labeo rohita* (Hamilton) 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 enzyme 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 and intestine tissue. All the three esterase bands were found in gill and intestine tissues in control. After the fish is exposed to Malathion (an Organophosphate), in gill tissue Esterase-1 shown resistance, Esterase-2 and Esterase-3 were severely affected, where as Esterase-3 was severely affected than Esterase-2 and Esterase-1 exhibited resistance in Intestine tissue.

Keywords: Electrophoresis, Esterase enzymes, *Labeo rohita,* α-naphthyl acetate, PAGE, Malathion.

DOI: 10.53555/ecb/2022.11.8.161

1. INTRODUCTION

Contamination of surface waters has been well documented worldwide and constitutes a major issue at local, regional, national, and global levels (Cerejeira et al., 2003; Spalding et al., 2003). In fish, different insecticides can be absorbed through gills, skin or alimentary ducts (Schlenk, 2005; Banaee et al., 2011. Banaee et al., 2012). Fishes are particularly sensitive to environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (Banaee et al., 2011).So, the effects of insecticides on fishes are of great concern. There are many pathways by which insecticides leave their sites of application and distribute throughout the environment and enter the aquatic ecosystem. The major route of insecticides to aquatic ecosystems is through rainfall, runoff and atmospheric deposition. Another source of water contamination by insecticides is from municipal and industrial discharges. Most insecticides ultimately find their way into rivers, lakes and ponds (Tarahi Tabrizi, 2001; Honarpajouh, 2003; Bagheri, 2007; Vryzas et al., 2009; Werimo et al.,

2009; Arjmandi et al., 2010) and have been found to be highly toxic to non- target organisms that inhabit natural environments close to agricultural fields.

Different concentrations of insecticides are present in water bodies and found to be toxic to aquatic organisms especially fish (Talebi, 1998; Uner et al., 2006; Banaee et al., 2008). Fishes are highly sensitive to the environmental contamination of water. Hence insecticides, serious pollutants may significantly damage certain physiological and biochemical processes when they enter into the organs and tissues of fish (Banaee et al., 2011). It has been found that different kinds of insecticides can cause serious impairment to physiological and health status of fishes (Begum, 2004; Monteiro et al., 2006; Siang et al., 2007; Banaee et al., 2009; Mamidala, 2014). Since fishes are important sources of proteins and lipids for humans and domestic animals, so health of fishes is important for human beings. Biochemical changes induced by pesticide stress lead to disturbances in metabolism, inhibition of enzymes, retardation of growth, reduce fecundity and Biodiversity of the organism (Murty, A.S.1986). Bioaccumulation of pesticides in tissues of marine organisms may have drastic effect on human (Elia et al., 2006). Isozymes are multiple forms of a single enzyme, which often have different isoelectric points and therefore can be separated by electrophoresis. Electrophoretic studies were done extensively on the different tissues of various animals from which it reveals that the enzyme exhibit in multi molecular forms and functions (Markert and Moller, 1959). Organophosphorus pesticides produce toxicity by inhibiting cholinesterase enzymes in aquatic organisms. These enzymes remove the neurotransmitter acetylcholine (ACh) from the synaptic cleft through hydrolysis (Habig, C et al., 1991) Acetylcholinesterase (AChE) is an indicator of the effect of pollutants on aquatic organisms (Das et al., 1998) Monitoring of AChE inhibition has been widely used in marine ecosystems as an indicator of organophosphate pesticide exposure and effects in exposed animals (Edwards, C.A 1991, Grue, C.E., et al., 1991, Zinkl, J.G., et al., 1991).

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 & 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. 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; 1980; Begum et al., 2008).

This current study is focused on Comparative study of Effect of Malathion (an Organophosphate) on Electrophoretic banding patterns of Esterase enzymes in gill and intestine tissues of freshwater fish *Labeo rohita* (Hamilton).

2. MATERIALS AND METHODS

Labeo rohita (Hamilton) 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 enzymes 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).

3. RESULTS AND DISCUSSION

The electrophoretic esterase enzyme banding patterns in various tissues that are stained by 1-naphtylacetate in freshwater fish *Labeo rohita* were studied in control as well as at various time intervals of Malathion expose were studied, and the results are as follows.

3.1 Gill

Gill tissue of *Labeo rohita* exposed 03 esterase enzyme bands in control. Est-1 with Rm value 0.6 ±0.05, Est-2 with Rm value 0.4± 0.05 and Est -3 with Rm value 0.3± 0.05 were highly stained (+++).

When the fish *Labeo rohita* was exposed to Malathion, at 24H gill tissue showed 03 esterase enzyme bands. Est-1 with Rm value 0.6± 0.05 and Est-2 with Rm value 0.4± 0.05 were highly stained (+++), while Est-3 with Rm value 0.3 ±0.05 was faintly stained with α- napthylacetate substrate (+). At 48H of tissue showed 03 esterase bands. Est-2 was stained highly (+++). Est-1 with stained moderately (++). Whereas Est-3 was stained faintly (+). At 72H tissue showed 01 esterase is enzyme band. Est-1 band with faintly stained (+). The other two esterase enzyme bands i.e. Est-2 and Est-3 did not appear (-). At 96H tissue exhibited 01 enzyme band i.e. Est-1 with faintly stained (+). While Est-2 and Est-3 bands disappeared (-) (Figure-1 and Table-1).

3.2 Intestine

Intestine tissue showed 03 esterase enzyme bands in control. Est-1 with Rm value 0.6± 0.05 was highly stained (+++). Est-2 and Est-3 with Rm values 0.4 ±0.05 and 0.3± 0.05 respectively were moderately stained (++). After 24H, intestine tissue showed 02 esterase enzyme bands i.e. Est-1 and Est-2 were moderately stained (++), while Est-3 disappeared (-). At 48H 02 esterase bands appeared. Est-1 with moderately stained (++). Est-2 was faintly stained (+). While Est-3 was not stained (-). At 72H and 96H tissue exhibited only Est-1 with faintly stained (+). The other two Esterase enzyme banding patterns i.e. Est-2 and Est-3 disappeared (-) (Figure-2 and Table-2).

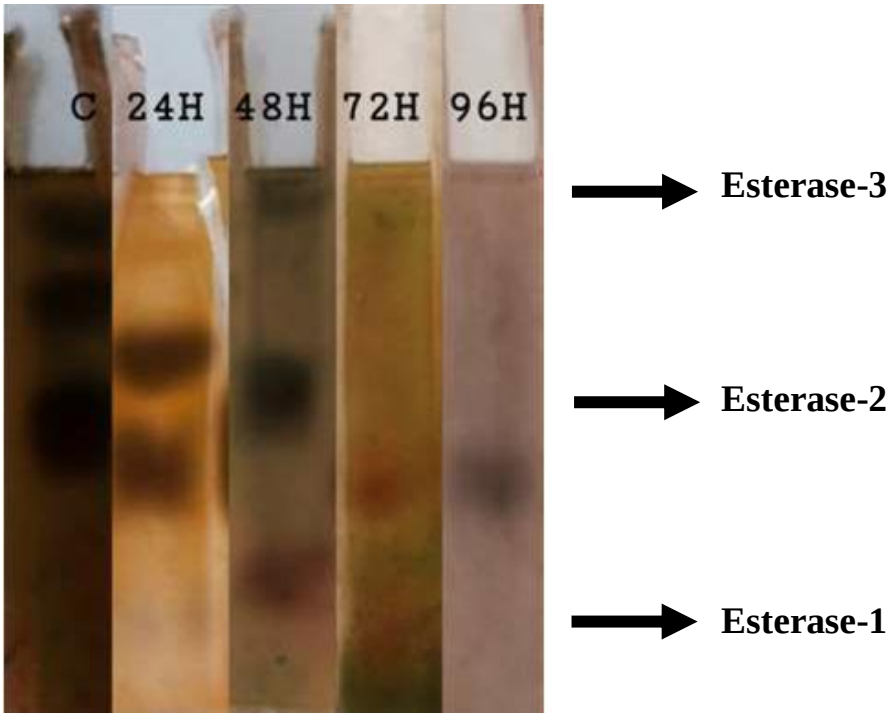

Figure-1: Comparative study of Effect of Malathion (an Organophosphate) in gill tissue of *Labeo rohita* (Hamilton) scored from 1-naphthylacetate as substrate.

Table-1: Electrophoretic banding Patterns showing the intensity of Esterase isozymes in gill tissue of *Labeo rohita* scored from 1-naphthylacetate as substrate. +-faint; ++-moderately stained; +++-deeply stained; Electrophoretic Esterase band disappeared.

DOSE Rm Value/ Esterase	**CONTROL**	**24H**	**48H**	**72H**	**96H**
Esterase-1 Rm 0.6±0.05	+++	+++	++	+	+
Esterase-2 Rm 0.4±0.05	+++	+++	+++	-	-
Esterase-3 Rm 0.3±0.05	+++	+	+	-	-

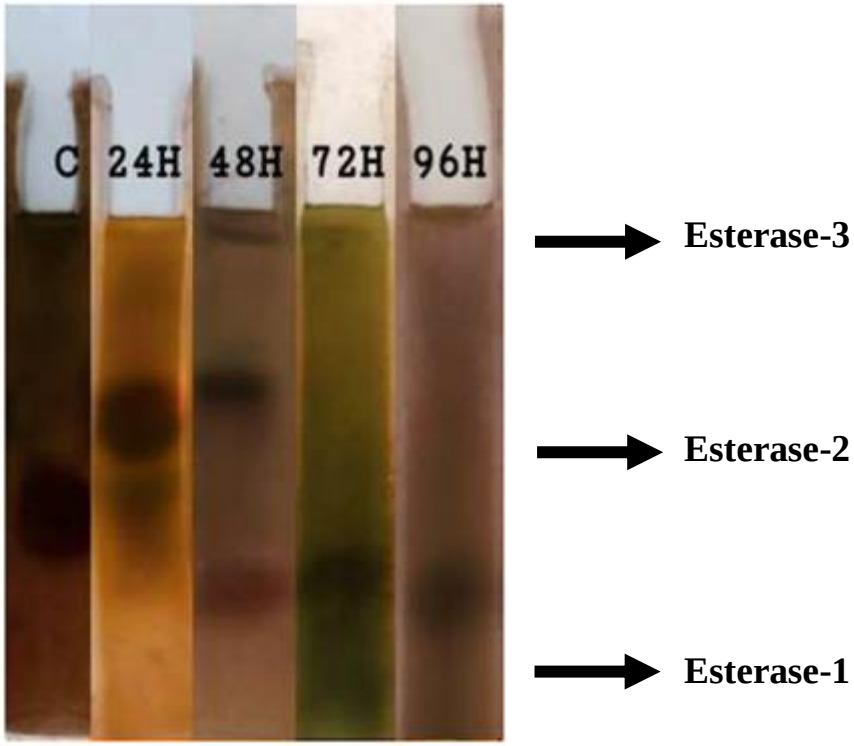

Figure-2: Comparative study of Effect of Malathion (an Organophosphate) in Intestine tissue of *Labeo rohita* (Hamilton) scored from 1-naphthylacetate as substrate.

Table-2: Electrophoretic banding Patterns showing the intensity of Esterase isozymes in Intestine tissue of *Labeo rohita* scored from 1-naphthylacetate as substrate. +-faint; ++-moderately stained; +++-deeply stained; Electrophoretic Esterase band disappeared.

DOSE/Esterase Rm Value	CONTROL	24H	48H	72H	96H
Esterase-1 Rm:0.6±0.05	+++	++	++	+	+
Esterase-2 Rm: 0.4±0.05	++	++	+	-	-
Esterase-3 Rm:0.3±0.05	++	-	-	-	-

The present study i.e. Comparative study of Effect of Malathion (an Organophosphate) on Electrophoretic Banding Patterns of Esterase Enzymes in gill and intestine tissue of fresh water fish *Labeo rohita* (Hamilton). Gill tissue shown three Esterases i.e. Est-1, Est-2 and Est-3 were found in control. Esterase-3 band was faintly stained (+) at 24 hours.Esterase-1 and Esterase-2 bands were darkly stained (+++). Esterase-2 and Esterase-3 bands were disappeared at 72 hours of Malathion

exposure. The Electrophoretic banding pattern of Esterase-1 was unaffected by Malathion (Organophosphate) this band was faintly stained (+) at 96 hours of Malathion Exposure. The results exhibited that Malathion (An Organophosphate) was affected the Electrophoretic banding patterns of Esterase-2 and Esterase-3. Manju Rani *et al.,* 2017 submitted Malathion dimethoate and Chlorpyrifos induce inhibitory effect on AChE activity, while maximum inhibition was induced by Malathion. *Labeo rohita* has been found to be more sensitive to OP contamination. Aetylcholinesterase enzyme is vital for various physiological functions such as orientation towards food, prey location and predator escaping (Miron DS *et al.,* 2005).

Many authors described that AChE activity was decreased as a consequence of expose to Organophosphorous pesticides and Carbamates in birds, mammal and even in humans (Coppage DL *et al.*, 1975). Effect of Triazophos on esterase activity and protein contents of liver, kidney, brain, blood and muscles of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* reported by Ghajala *et al.,* 2016 and the results revealed that sub-lethal concentration of Triazophos inhibited AChE activity, the order of decrease in AChE activity in *Labeo rohita* was recorded as liver > gill > blood > kidney > muscle > brain. In the muscle of *Catla catla* the severe inhibition of AChE was found with Traizophos (Ghajala *et al.,* 2016). An organism develops the resistance against the insecticides by producing high amounts of specific esterases which help in breaking down the insecticide or by binding tightly to it so that the insecticide could not function (Holmes R.S. 1970). Isoenzyme pattern exhibits differences in the various fish populations (Barua S *et al.,* 2004) and also used to develop genetic sexing system (Robinson AS. 1986). Banding pattern of esterases of different tissues has a good potential used in the identification of species. Al-Amin et al. (2005). The results of the present project study coincide with results of Venkateswara Rao et al., 2022, Venkateswara Rao et al., 2023, Bheem Rao et al.,2018; Ch.Shankar et al.,2019.

4. 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. This present study helps in finding a proper solution for aquatic ecosystem pollution that occurs due to pesticides. Research has to continue to find alternatives for OP compounds in agriculture.

Conflict Of Interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Acknowledgements

Authors are thankful to the Head of the Department of Zoology, Kakatiya University, Warangal, Telangana State, for providing laboratory facilities.

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