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Effect of Malathion (An Organophospahte) on Electrophoretic Banding Patterns of Esterase Isozymes in Gill, Liver, Brain Tissue of Fresh Water Fish *Channa Punctatus* (Bloch)

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ABSTRACT:
This investigation was conducted to compare the electrophoretetic banding patterns of esterase isozyme in the gill, liver, and brain tissues of the freshwater fish Channa punctatus (Bloch) at 24 hours, 48 hours, 72 hours, and 96 hours after exposure to Malathion (an Organophosphate) to those at the control group. Quantitative analysis of the esterase isozymes was performed using 7.5 native polyacrylamide gel electrophoresis (PAGE) stained with -naphthyl acetate as substrate. The relative mobilities of three esterase Isozyme bands in gill, liver, and brain tissue were determined to be 0.60.05, 0.40.05, and 0.30.05, respectively; these bands were designated Est-1, Est-2, and Est-3, respectively. Control samples from the gill, liver, and brain all had all three esterase bands. Both Est-2 and Est-3 Esterase Isozyme bands in gill and liver tissue were eliminated when fish were subjected to Malathion (an Organophosphate) for 72 and 96 hours, respectively. Malathion induced greater damage in Est-1 and Est-3 brain tissue than in Est-2.

Keywords: Electrophoretic banding patterns, Esterase Isozymes, *Channa punctatus,* α-naphthyl acetate, PAGE, Malathion (an Organophosphate), different time intervals.

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INTRODUCTION

Channa punctatus is a native species of fish that can be farmed and consumed for its high nutritional value. Protein and other essential nutrients can be found in fish. The high protein content and low fat content of fish meat have a beneficial effect on cholesterol levels. When compared to other types of meat, fish is quite inexpensive. Fish meal is a good source of vitamins A and D, among others. There's a lot of vitamin D in the cod liver oil. Indicators of exposure to and toxicity from one or more pollutants are known as biomarkers (Peakall, D., 1992). A common method for gauging the effects of agrochemicals like organophosphate (OP) and carbamate insecticides on neural tissue is the measurement of acetylcholinesterase (Ache, EC 3.1.1.7) inhibition (Sultatos, 2006). Multiple isoforms of the same esterase enzyme exist, and

they can be distinguished by electrophoresis based on their isoelectric points. Studies using electrophoresis on tissues from many organisms demonstrate the wide variety of molecular shapes and functions exhibited by enzymes (Markert C.L et al. Among the several lipid hydrolyzing enzymes, esterases have been shown to have an important role in genetics and toxicology (Callaghan et al., 1994). Esterases are used to determine the relative genetic distance across populations since the esterase Isozyme banding pattern has a genetic foundation (Turner B.J. 1973). Since Esterases can be used to assess the hazardous potential of pesticide residues like Malathion (OP), they are highly relevant as biomarkers (P. Swapna et al., 2017). Bioaccumulation of pesticides is harmful to aquatic ecosystems and human health (Jenssen B.M., 2003). Pesticides were found to have a negative impact on the marine edible fish *Lepturacanthus savala's* survival behaviour, eating habit, growth, and mortality (Nagma Tamkeen et al., 2020). Alcohol carboxylic acid esters are formed and broken down by enzymes known as esterases. Esterases have been organised by Aldridge (1953), Holmes and Masters (1967; 1968), and others. Electrophoretic banding patterns of Esterase Isozymes in gill, liver, and brain tissue of the freshwater fish *Channa punctatus* (Bloch) were analysed to determine the impact of Malathion (OP) on these organs.

MATERIALS AND METHODS

Fish of the genus *Channa punctatus* weighing between 50 and 70 grammes were caught in a river close to Kakatiya University. Fish were transported to the lab with meticulous attention to oxygenation and cleanliness. By feeding them natural planktons, the fish were able to adjust to the laboratory environment. Fish were exposed to sub-lethal levels of Malathion (OP) after a week. The gill, the liver, and the brain were the tissues chosen for this investigation. Homogenization in 0.01N Tris. HCl buffer (Ph =7.5) containing 0.9% NaCl followed tissue collection, blotting to remove blood clots and other adhering tissues, and weighing to the closest milligramme. The homogenates were spun at room temperature for 10 minutes at 2000 rpm in a clinical centrifuge. The tracking dye, 0.05% bromophenol blue in a 20% sucrose solution, was added to the supernatant. To load the sample onto the separating gel for the separation of esterase patterns, 0.1 ml of this mixture was utilised. Several authors (Holmes RS, Masters CJ. 1967, Reddy. M.T. and Lakshmipathi, V. 1988) have confirmed this. Thin-layer (1.5 mm), 7.5% polyacrylamide gels were used to separate esterase isozymes. The gel was made using the Clark-1959 method. The gel was made using Clark-1964's method. The electrode buffer consisted of Tris (0.05M) and glycine (0.38M) solution (PH=8.3). And then we loaded gel with the tissue samples. For the first 15 minutes of the electrophoresis, a steady current of 50 volts was given. After that, the voltage was increased to 150 volts for the duration of the run. When the migration of the tracking dye reached 5cm from the origin, the electrophoretic run was stopped. Gels were stained in a manner similar to that used to visual is esterase in other studies (Raju and Venkaiah, 2013; Bheem Rao et al., 2018; Shankar et al., 2019; Venkateswara Rao and Venkaiah, 2022). Esterase activity was detected by using - naphthyl acetate as a stain (M.T. Reddy and V. Lakshmipathi, 1988).

RESULTS

Table 1 shows the results obtained from the current study, which examined the effect of Malathion (an organophosphate) on the electrophoretic banding patterns of Esterase Isozymes in the gill, liver, and brain of *Channa punctatus* (Bloch) at 24, 48, 72, and 96 hours. Esterase activity on 7.5% native polyacrylamide gel is scored using - naphthylacetate as the substrate. Deeply stained (DS), moderately deeply stained (MDS), and faintly stained (FS) esterase activity was obtained and arbitrarily assessed in various *Channa punctatus* (Bloch) tissues based on visual evaluation of staining intensity.

Gill

In normal gill tissue, three isoenzyme bands of esterase were present. The 0.60.05 Rm value of Est-1 bands, the 0.40.05 Rm value of Est-2 bands, and the 0.30.05 Rm value of Est-3 bands. There

were three strongly stained esterase bands (+++).

At 24 hours post-exposure to Malathion, considerable staining (++) was observed in three esterase isoenzyme bands in gill tissue; Est-1 and Est-2. Est-3, on the other hand, only had a faint stain (+). There were three bands of Esterase isoenzymes in gill tissue after 48 hours. There was a very light staining (+) on Est-1, Est-2, and Est-3. After 72 hours of Malathion exposure, just one faintly stained (+) band of Est-1 was visible in gill tissue, while both Est -2 and Est -3 were completely absent (-). The banding patterns of Est-1, Est-2, and Est-3 vanished entirely after being exposed to Malathion for 96 hours (Table 1. and Figure 1).

Liver

Esterase isoenzyme 03 was detected at normal levels in liver tissue. Highly stained (+++) were Est-1 with a Rm value of 0.6 0.05, Est-2 with a Rm value of 0.4 0.05, and Est-3 with a Rm value of 0.3 0.05.

Liver tissue exposed to Malathion for 24 hours exhibited bands of the esterase isoenzyme 03 at the 24-hour mark. "The staining on Est-1 was strong (+++), while that on Est-2 and Est-3 was modest (++). Tissue from a 48H time point exhibited bands of the esterase isoenzyme at 03. The staining intensity of Est-1 was ++, while that of Est-2 and Est-3 was +. Liver tissue exposed to pesticides for 72 hours showed a single band of esterase isoenzymes. There was a slight smear on the Est-1 band (+). Moreover, the Est-2 and Est-3 bands vanished (-). Liver tissue was negative for esterase isoenzyme bands after 96 hours of Malathion exposure. Table II and Figure II show that all traces of Est-1, Est-2, and Est-3 were eliminated.

Brain

There were three bands of an esterase isoenzyme in normal brain tissue. The Rm values for Est-1 were 0.6 0.05, Est-2 were 0.4 0.05, and Est-3 were 0.3 0.05, all of which are very strongly stained (+++).

At 24 hours, Malathion-exposed fish tissues showed three esterase isoenzyme bands: Est-2 was strongly (+++) stained, while Est-1 was just slightly stained (++). A very light (+) stain was found on Est-3. 48 hours later, tissue contained two enzyme bands. Both Est-1 and Est-2 have a substantial amount of staining (+++). Although Est-3 had disappeared. Two esterase bands were seen at 72H. Est-2 was moderately discoloured (++). There was a slight smear on the Est-1 band (+). However, the Est-3 band vanished. Tissue 01 esterase band expression was detected after 96 hours of Malathion exposure. Table III and Figure III show that Est-2 was strongly stained (++), but Est-1 and Est-3 were undetectable.

Figure 1: Esterase Isozyme band intensity of gill tissue of *Channa punctatus* after exposed to Malathion (an Organophosphate)
C: Control
24H: Banding pattern of Esterase Isozymes at 24 hours of Malathion exposure.
48H: Banding pattern of Esterase Isozymes at 48 hours of Malathion exposure
72H: Banding pattern of Esterase Isozymes at 72 hours of Malathion exposure
96H: Banding pattern of Esterase Isozymes at 96 hours of Malathion Exposure

Table 1: Effect of Malathion on gill tissue of *Channa punctatus*

Est; Rm/ dose	CONTROL	24H	48H	72H	96H
Est-1 0.6±0.05	+++	++	+	+	-
Est-2 0.4±0.05	+++	++	+	-	-
Est-3 0.3±0.05	+++	+	+	-	-

+++: Deeply stained
++: Medium deeply stained
+: Faintly stained
-: Not stained (No esterase Isozyme band is found)

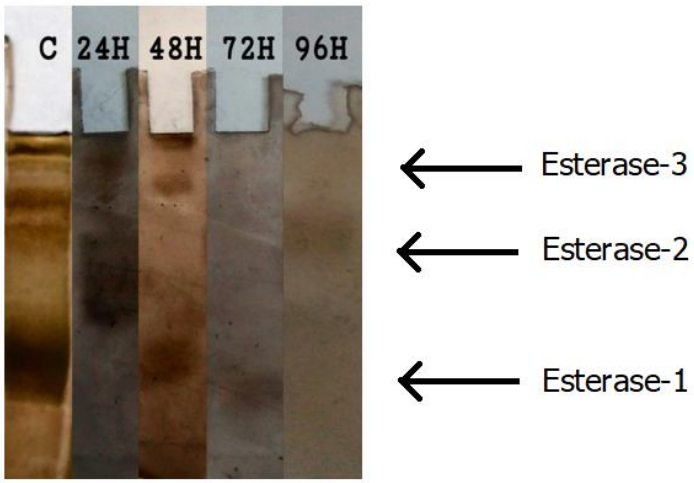

Figure 2: Esterase Isozyme band intensity of liver tissue of *Channa punctatus* after exposed to Malathion (an Organophosphate)

Table 2: Effect of Malathion on liver tissue of *Channa punctatus*

Est; Rm value/ dose	Control	24H	48H	72H	96H
Est-1 0.6±0.05	+++	+++	++	+	-
Est-2 0.4±0.05	+++	++	+	-	-
Est-3 0.3±0.05	+++	++	+	-	-

+++: Deeply stained
++: Medium deeply stained
+: Faintly stained
-: Not stained (No esterase Isozyme band is found)

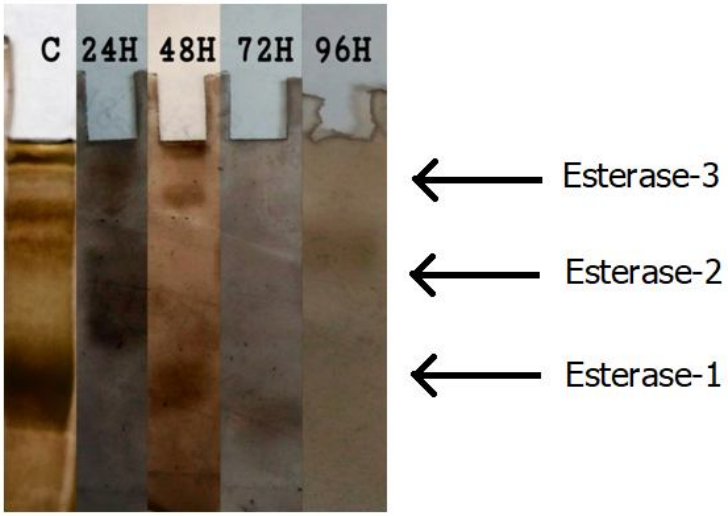

Figure 3: Esterase Isozyme band intensity of brain tissue of *Channa punctatus* after exposed to Malathion (an Organophosphate)

Table 3: Effect of Malathion on brain tissue of *Channa punctatus*

Est; Rm Value/ Dose	Control	24H	48H	72H	96H
Est-1 0.6±0.05	+++	++	-	-	-
Est-2 0.4±0.05	+++	+++	++	++	+
Est-3 0.3±0.05	+++	+++	++	+	-

+++: Deeply stained
++: Medium deeply stained
+: Faintly stained
-: Not stained (No esterase Isozyme band is found)

DISCUSSION

The present study examined the effects of malathion on the expression of particular esterase isozymes in the gill, liver, and brain of the freshwater fish *Channa punctatus* using electrophoretic banding analysis. Fish subjected to Malathion for 24 hours, 48 hours, 72 hours, or 96 hours showed a significant reduction in the number of isoenzyme bands and their intensity. In normal conditions, three Esterases were found in gill tissue. Esterase -3 band began degrading immediately after Malathion exposure, at around 24 hours. Although the Esterase-1 Isozyme band did not disappear until 96H, the Esterase-2 and Esterase-3 bands disappeared at 72H. All three Esterase Isozymes were found in normal amounts in liver tissue. The Esterase-2 and Esterase-3 Isozyme bands disappeared after 72 hours of exposure to Malathion, but the Esterase-1 band disappeared after 96 hours. All the Esterases were under control in brain tissue. Esterase-1 Isozyme band disappeared immediately after Malathion

exposure, and Esterase-3 Isozyme band disappeared 72 hours later. After 96 hours of Malathion exposure, only one weakly stained Isozyme band was seen, however the Esterase-2 Isozyme band remained unaffected.

RabeyaAkter et al.'s (2020) research on the pesticide envoy 50 SC and its toxic effects on the gill, liver, and kidney of stinging catfish (*Heteropneustes fossilis*) found that it caused dramatic changes in the tissue structures of these organs. At greater concentrations of this organophosphate pesticide, a decrease in red blood cell (RBC) count and AChE activity in the brain revealed the reasons for abrupt behaviour, increased oxygen use, and fish mortality. The inhibition of acetylcholinesterase (AChE) activity is a more reliable biomarker of exposure to and effects of organophosphate and carbamate pesticides in fish than any other pollutant tested. Activity of acetylcholine esterase in freshwater fish as affected by a combined insecticide Pesticide dose and duration both had an effect on Danio rerio (Zebra fish) by inhibiting their Acetylcholine activity (Rajini A. et al., 2015). Inhibition of too many acetylcholinesterase molecules causes the rapid onset of Chlorpyrifos toxicity's acute symptoms (Christensen K et al., 2009). Both invertebrates and vertebrates need cholinesterases (ChEs), namely acetyl cholinesterase-AChE - (EC3.1.1.7), to control the amount of acetylcholine released during nerve impulses. Potential biomarkers for monitoring Organophosphorous pesticides in aquatic environments can be derived from changes in enzymatic parameters (B.D. Abhijith et al., 2016). A accumulation of acetylcholine, caused by inhibiting this enzyme, produces continual and excessive excitation of nerve/muscle fibres, leading to tetany, paralysis, and death. Impairment of numerous cellular activities in aquatic creatures has been linked to an increase in the formation of reactive oxygen species (ROS) due to exposure to pesticides (Uner et al., 2006; Monteiro et al., 2009; Modesto et al., 2010; De Menezes et al., 2011). Antioxidant systems can prevent or mitigate the damage caused by free radicals (Lushchak, 2011; Singh et al., 2011). According to research by Tripathi and Shasmal (2011), pesticides have a direct effect on metabolic enzymes by reducing their enzymatic activity during exposure to organophosphorous pesticides. In addition to interfering with the immune system's normal function, OP pesticides have been shown to elicit immunotoxic effects via anti cholinergic and non-cholinergic pathways (Galloway et al., 2003). The esterase enzymes in fish were found to be impacted by parathion in a study conducted by (Rajaiah and Venkaiah, 2007). In addition to being utilised to create a genetic sexing system (Robinson AS. 1986), changes in isoenzyme patterns have been observed across different fish populations (Barua S et al., 2004). Malathion inhibits acetyl cholinesterase competitively in four tissues of T. mosambica (Kabeer and Ramanarao, 1980). Tovar-Juarez E et al., 2016 evaluated inhibition of brain AChE activity by organophosphate and carbamate in the fish species *Profundulus punctatus* and Poecilliabutleri from the coaton water shed of S. Mexico". Malathion has been shown to be toxic to Channa punctatus (Bloch, 1973) and has been linked to variations in tissue esterase (Hadaiar Rahman, 2009). Liver and kidney AchE esterase activity was shown to be decreasing (Shahid Mahboob KA et al., 2014). Consistent with our findings, Shankar et al. (2019) found that Cholphyrifos affected Esterase Isozymes in electrophoretic banding patterns in muscle and brain tissue of fresh water fish *Heteropneustes fossilis*. It has been documented that Esterase Isozyme displays a distinct electrophoretic banding pattern across a variety of *Puntius sophore* (Cyprinidae: Cypriniformes) tissues. Wayne S. Leibel (1988) published the results of an evaluation of Esterase activity in Surgeon fish tissues. *Heteropneustes fossilis* larvae and adults were studied to compare tissue-specific esterase isozyme banding pattern (Rowshan Ara Begum, 2011).

CONCLUSION

The results of the present study, titled “Effect of Malathion, an Organophosphate, on Electrophoretic banding Patterns of Esterase Isozymes in Gill, Liver, and Brain Tissue of Fresh Water Fish *Channa punctatus*,” show that the banding pattern of Esterase Isozymes differs depending on the tissue being analysed. Different authors' research on esterase isozymes confirms that the banding patterns of esterases in diverse fish tissues vary widely. Because of

this, the Esterase Isozymes can be employed as molecular, genetic markers and for species identification.

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

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