Impact of Methyl Parathion on Proteins and Esterases patterns in Brain tissue of fresh water Cat fish *Heteropneustes fossilis* (Bloach) through polyacralamide Gel Electrophorus.

ABSTRACT:

The current study sought to determine the effect of Methyl Parathion (an Organophosphate) on proteins and esterase isozyme banding patterns in the brain tissue of freshwater catfish Heteropneustes fossilis (Bloch) at various time intervals (24, 48, 72, and 96 hours) and compare it to the control. The protein banding patterns were examined using a standard maker, and Rm values were calculated and obtained from the study. The findings demonstrated that methyl parathion exposure caused diverse changes in protein patterns in tissues such as the liver, muscle, gut, and brain of Heteropneustes fossilis, resulting in similarity in protein bands with small variances. All three esterase bands were present in brain tissue. Among the three esterases discovered in liver tissue, Est-1 at 24 hours, Est-2 at 72 hours, and Est-4 at 72 hours were determined to be the most prevalent and intense. Est-2 and Est-3 were weakly stained in brain tissue; Est-3 in liver tissue; and Est-4 in gut tissues. The findings demonstrated that methyl parathion exposure causes diverse alterations in esterase patterns of tissues, such as H. fossilis brain tissue, which showed similarity in protein bands with small variations.

Keywords: Proteins, Esterase, isozymes, PAGE, *Heteropneustes fossilis*, Methyl Parathion.

INTRODUCTION

Pesticide pollution poses a significant threat to the aquatic biota, particularly fish. Pesticide poisoning kills 220,000 people worldwide each year, with around 3 million cases reported (WHO 1992). India is an agricultural country. Pesticides are the main cause of water pollution. Farmers utilise a variety of chemicals, including organochlorines, organophosphates, and carbamates. Pandey et al. (1984) found that different compounds have distinct impacts on fish. In poorer nations, indiscriminate pesticide usage and inadequate legislation have resulted in significant toxicities (Konradsen et al., 2003; Remor et al., 2009; Abdel Khalek et al., 2017). Ecotoxicology studies the impact of contaminants on different biological systems (Ayadi et al., 2015). Aquatic species can biomagnify synthetic pesticides from water, increasing their hazard (Murty, 1986; Satyamorthi et al., 2018, 2019; Ravichandran et al., 1918; Satyamootyhi et al., 2017). Several of these compounds are carcinogenic (Garaj-vrhovac and Zeljezic 2000; Kumar et al., 2009; Nwani et al., 2010), linked to cancer development (Leiss and Savitz, 1995), and may

cause developmental defects (Arbuckel and Server, 1998; Konradsen et al., 2003; Remor et al., 2009; Abdel Khalek et al., 2017). However, because pesticides are widely used, they may have negative impacts on nontarget organisms, such as fish. (Annita et al., 2016).

Protein subunits were separated using SDS PAGE, and their relative mobility was used to compute their molecular weight using the formula below. 1998). (Konradsen et al., 2003; Remor et al., 2009). (Abdel Khalek et al, 2017). Proteins have a crucial role in adaptive responses to environmental, physiological, and pathological variables (Bradley et al. 2002). SDS-PAGE, a widely used technique in domains including molecular biology, biochemistry, and forensics, separates proteins based on their polypeptide chain lengths. SDS-PAGE is a popular technique used in many fields to classify proteins based on their electrophoretic mobility. Muhammad (2018) suggests that SDS-PAGE analysis is a valuable indicator for toxicological investigations in fish. The current study was conducted to assess the acute toxicity of Methyl parathion (OP) and its impact on electrophoretic protein patterns within the tissues of the brain of freshwater fishes. Heteropneustes fossilis.

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The fish H.fossilis is generally known as Stinging Catfish (for its toxic 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 (Orissa), Thaylee and Thaimeen (Tamil Nadu). It is widely distributed (Pakistan, India, Sri Lanka, Nepal, Bangladesh, Myanmar, Thailand, and Laos) and has spread elsewhere. While it is highly used for food and medicine in many parts of its range, and it may be threatened by overexploitation as well as habitat loss and degradation (particularly from pollution and dams), it is now regarded as of least concern. A related synonym is Saccobranchus microcephalus (Gunther, 1864). Heteropneustes fossilis. The Greek term Sacco means sack, a bag, and branchus denotes respiratory organ, with gill referring to an additional respiratory sack. It is commonly known as Stinging Catfish (for the toxic pectoral spine). As the name implies, Heteropneustes fossilis can deliver a painful sting via the spines on its pectoral fins. In the scenario above, we look at the effect of Methyl Parathion (an organophosphate) on tissue-specific esterase patterns in the Indian catfish Heteropneustes fossilis (Bloch).

The stinging catfish H. fossilis (Bloch) is known locally as Ingilayee or Marpujella. It is an important air sac catfish native to many Asian countries. It lives in freshwater but can also survive in brackish water. It is extremely popular not only for its delicious taste, but also for its excellent nutritional and therapeutic value. H. fossilis is primarily found in ponds, ditches, swamps, and marshes, but it sometimes appears in muddy rivers and it is omnivorous.

It is in high demand due to its therapeutic properties (Froese et al., 2011). The stinging catfish may administer a painful sting to humans. Poison from a gland on its pectoral fin spine has been reported to cause significant agony. It is also grown and sold in aquariums (Froese et al., 2011). Fish reproduction is a periodic process that is governed by both environmental (exogenous) and internal (endogenous) regulatory mechanisms. Breeding occurs under optimal environmental conditions that promote the survival of the younger ones. Environmental stimuli are registered by sensory organs and communicated to the brain, triggering an endogenous process into action.

MATERIALS AND METHODS

1. Proteins:

Experimental fish specimens and chemical The freshwater fish Heteropneustes fossilis and Channa punctatus are both edible and economically useful. Live fish weighing 50-70 g and measuring 7-9 cm were gathered from local ponds near Kakatiya University in Warangal, Telangana State, India, and washed with 0.05 percent potassium permanganate (KMnO4) for 2 minutes to prevent skin diseases. The fish were acclimatized in laboratory settings for two weeks. During the acclimatization period, the fish were fed commercial fish pellets and rice bran twice a day. To lower the ammonia concentration of the water, faeces and other waste elements were emptied daily. The diluting of Methyl Parathion (2 E.C.) to 100 mg/ml in 95 acetone resulted in a sublethal concentration. The solution was then further diluted by adding distilled water (APHA). Participants were given sublethal dosages of the insecticide Methyl Parathion over 24, 48, 72, and 96 hours. The harmful effects of Methyl Parathion on different tissues were compared by using a control batch in each experimental group.

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Preparation of Sample for Study

After each exposure period, the fish were killed and their muscles and brains were collected for research. Materials were weighed to the nearest milligramme and homogenised in a 0.1 M Tris HCl buffer (pH 7.5) with 0.9% sodium chloride. The concentration of tissue homogenates was observed to vary significantly. Tissues were homogenised in tubes and stored in cold baths. To separate components, the samples were spun in a clinical centrifuge for 10 minutes at room temperature and 2000 rpm. The supernatant was concentrated to 0.1 ml, and a 20 ml sucrose solution with 0.5 mM bromophenol blue was used to identify protein patterns on the electrode surface.

SDS-PAGE Analysis

To get homogenates, gill and muscle tissue were centrifuged in Tris-HCl buffer (pH7.2) for 10 minutes at 10,000 rpm. After a quick wash in cold acetone, the pellet was heated in 2 mL of sample buffer at 950 degrees Celsius for one minute. The buffer contained 0.5 mL of Tris HCl (pH 6.8), 1-6 mL of 40% glycerol, 3.2 mL of 10% sodium dodecyl, 0.8 mL of 2% mercaptoethanol, and 0.4 mL of 0.15% w/v bromophenol blue.

Comment [h3]: Align

Experimental Procedure for Preparation of SDS-PAGE

To facilitate monitoring, the supernatants were treated with a 20% sucrose solution containing 0.01% SDS, 1-mercaptoethanol, and bromophenol blue. To cover the dividing gel, a tissue extract aliquot of 0.1ml (5mg) was utilised. To follow standard practice (Laemmli), a solution of 0.074MTris, 0.1%SDS, pH 7.8 with con. was used. Electrode buffers were HCl, 0.025 M Tris, and 0.192 M Glycine. The gel was exposed to a 50-volt continuous current for the first 15 minutes, followed by a 150-volt constant current for the rest of the time. When the tracking dye was more than 8.0 cm from the source, power was disconnected.

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Staining Procedure and Standardization of Protein Bands

To stain protein gels, scientists typically employ a 5:5:1 mixture of methanol, water, and acetic acid (Holmes and Master), with 0.25 percent Coomassie brilliant blue solution. The SIGMA-Chemical company in the US produced the low molecular weight protein standards (15-100 KDa) used to analyse SDS-PAGE differences.

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2. Esterases

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The freshwater catfish H. fossilis were gathered from local freshwater tanks within a 15-kilometer radius of the laboratory using netting with the assistance of a local fisherman. Fish with an average length of 15 ± 1cm and weight of 50 ± 5gm were brought to the laboratory and transferred into plastic buckets (30X30X60cm) and disinfected with potassium permanganate and rinsed thoroughly before to introduction (to prevent fungal infection). The fish were acclimatised for 10 to 15 days before the experiment. They were frequently fed commercial fish food, and the medium (tap water) was changed on a daily basis to remove faeces and food leftovers. The healthy fishes were divided into five batches of six each and were treated to varied concentrations of organophosphate methyl parathion at different time intervals to compute the medium lethal concentration less value using the probit analysis method.

Preparation of samples for study:

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At the end of each exposure period, fish were sacrificed, and tissues such as the liver and brain were dissected and blotted to remove blood clots and other adherent tissues before being weighed to the nearest milligramme and homogenised 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 and 0.01% bromophenol blue as a tracking dye. An aliquot of 0.1ml of each solution was placed directly onto the separating gel.

Electrophoretic study and staining of gels:

Esterase patterns were isolated using 7.5 percent polyacrylamide gels with a thickness of 1.5 mm and an area of 8X8 cm. The gel mixture was produced as follows. Gelling was allowed for 45 minutes. After loading (10-20 µl) on the gel, the samples were filled with electrode buffer comprising Tris (0.05M) and glycine (0.38M). The pH was adjusted to 8.3 with 1N Hcl, and the gel plates were attached to the electrophoretic tank. Power was given at 50 volts for the first 15 minutes, then at a constant 150 volts for the remainder of the run during electrophoresis. The electrophoretic run ended when the tracking dye travelled 8.0 cm from the origin. Esterases were visualised on the gels using the staining method.

RESULTS

1.Proteins

Brain

At 24 hours, the brain showed 08 protein bands with Rm values 0.11, 0.23, 0.36, 0.50, 0.63, 0.73, 0.83, and 0.86; at 48 hours, it showed 05 protein bands with Rm values 0.23, 0.31, 0.43, 0.73, and 0.91; at 72 hours, it showed 04 protein bands with Rm values 0.48, 0.58, 0.63, and 0.83; and at 96 hours, it showed a single protein band with Rm value 0.58. The results are shown in Table 1 and Figure 1.

H. fossilis brain tissue had 10 protein bands in the control group, but when exposed to Methyl parathion at different time intervals such as 24h, 48h, 72h, and 96h, the banding patterns varied. At 72 hours, a protein band with Rm value 0.01 was seen, however its intensity had decreased compared to the control. A protein band with Rm values of 0.23 and 0.73 was identified at 24 and 48 hours, but not at 72 and 96 hours; the band was more intense at 48 hours. At 24 hours,

two protein bands with Rm values of 0.36 and 0.50 were seen with lower intensity than the control. These bands vanished completely at 48, 72, and 96 hours. A band with a Rm value of 0.43 is noticed at 48 hours but not at 24 hours, 72 hours, or 96 hours.A protein band with a Rm value of 0.58 developed at 72 and 96 hours, however its intensity was reduced when compared to the control. Whereas the band with Rm value 0.58 was completely absent at 24 and 48 hours. At 24 and 72 hours, a protein band with a Rm value of 0.63 was seen. The band with a Rm value of 0.63 was completely absent at 48 and 96 hours. The protein band with Rm value 0.73 was visible at 24 and 48 hours, but faded at 72 and 96 hours. A band with a Rm value of 0.83 was discovered at 24h and 72h, and it totally faded by 48h and 96h. When compared to the control, all protein bands were seen in the brain with high intensity after 48 hours.

Table. 1. Rm Values of Brain tissue protein patterns *H. fossilis*

Control	24H	48H	72H	96H
0.01			0.01	
0.05				
	0.11			
0.15				
0.23	0.23	0.23		
		0.31		
0.36	0.36			
		0.43		
			0.48	
0.50	0.50			
0.53				
0.58			0.58	0.58
0.63	0.63		0.63	
	0.73	0.73		
	0.83		0.83	
	0.86			
		0.91		
1.0				

Brain Tissue of ***Heteropneustes fossilis***

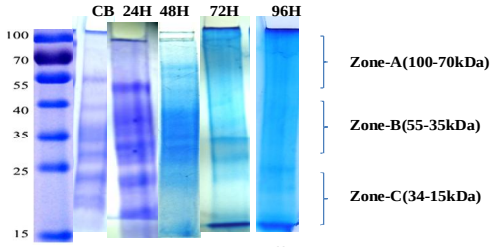

Fig.1 Brain Protein bands in Different time intervals after exposure of Organophosphate

CB=8,24H=7, 48H=5, 72H=4, 96H=1

2.Esterases

Brain

At 24 hours, the brain revealed two esterase isozymes with Rm values of 0.60 and 0.40, but at 48, 72, and 96 hours, it showed a single esterase isozyme with Rm value 0.60.

The brain contained three esterase bands, with Rm values of Est-1 (0.6), Est-2 (0.4), and Est-3 (0.3). Est-1 was heavily stained, Est-2 moderately stained, and Est-3 faintly stained. Est-1 band grew somewhat in 24 hours and steadily declined in 48, 72, and 96 hours, whereas Est-2 and Est-3 disappeared altogether. (Table II, Figure II)

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

Est (Rmvalues) / Dose	Est-1 (0.6)	Est-2 (0.4)	Est-3 (0.3)
Control	+++	++	+
24H	+++	+	-
48H	++	-	-
72H	++	-	-

96H	+	-	-

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

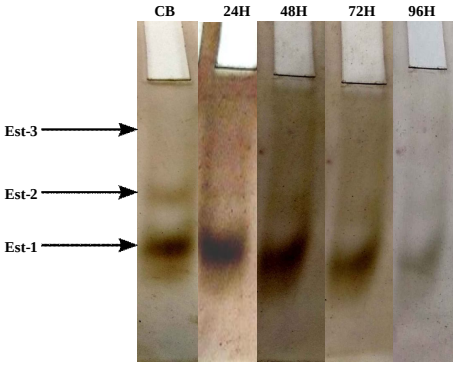

Fig.II.. Esterase band intensity of Brain after exposure of Methyl Parathion

DISCUSSION

In the current investigation, Est-2 was shown to be more plentiful and strongly stained in the liver and gut than the other two esterases (+++). And Est-1 was heavily stained (+++) in the brain. Est-2 was highly stained (+++) in the liver and colon, but only marginally in the brain. Est-1 esterase zone was strongly stained (++). Est-3 and Est-4 were moderately (++) stained in the brain. Pesticides may block (or increase) the expression of some genes, resulting in the production of specific mRNAs that can then be translated into specific proteins known as stress induced proteins (Daniel et al., 2004; Ksenia et al., 2008; Murat et al., 2009.

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Protein metabolism was altered in fish subjected to a variety of environmental challenges, including metals and pesticides (Alexssandro et al.; Shweta and Gopal, 2009).Throughout the exposure period, the protein subunits showed a consistent decrease in intensity across all fractions, indicating that endosulfan inhibited kidney and muscle LDH. Sherif et al. (2009) detected a small drop or decrease in protein intensity in Diazinon-treated Nile Tilapia, indicating that pesticide stress had a significant impact on these proteins. Several authors have reported

similar observations. Marinovich et al., (1994) discovered that Diazinon could inhibit proteins in HL 60 cells after 24 hours of treatment.

Jyothirmayee et al., (2005) conducted polyacrylamide gel electrophoresis on endosulfan-induced changes in LDH patterns in freshwater fish Anabas testundineus and Clarias batrachus, which aligns with our current research. Our findings are consistent with earlier studies indicating chlorpyrifos' significant toxicity to numerous fish species. (Tilak et al., 2004; Díaz & Girón, 2014; Okechukwu et al., 2013; Reddy et al., 2012; Gul, 2005). Other toxicants on different fishes produced similar results, including a decrease in the strength of the rotein banding pattern in tissues and the fading/disappearance of several protein subunits. El-Sherif et al. (2009); Suneetha et al. (2010); Bheem Rao et al., (2018); and Florence Borgia et al. (2019). Some observations suggest the emergence and disappearance of new protein subunits (Firat and Kargin, 2010; Arivu et al., 2015; Sobha et al., 2017); Venkateswara Rao et al., 2023; Venkateswara Rao et al., 2023.

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All of these reviews support our current findings, which show that depletion in total protein and decreased expression of protein patterns in tissues exposed to Malathion indicates protein degradation due to pesticide toxic stress, as well as hormonal imbalance, impaired tissue repair, which affects protein levels in tissues, or possibly hepatocytic necrosis of cells, which then disrupts protein biosynthesis.

Esterases are a class of hydrolytic enzymes that exist in various forms and have broad substrate specificity. Esterases are a varied category of enzymes that catalyse the hydrolysis of organic esters. Esterases (ESTs, 3.1.1.1) are ubiquitous in living organisms. Several esterases have been isolated from different tissues of microorganisms, plants, and animals and studied for their biochemical features.

The current work shows that the variety of esterase isozyme patterns characterises an individual's electro morphic, representing expression of tissue-specific esterase isozymes with differential banding patterns that could be exploited in toxicological studies. It can be concluded that tissue-specific variation in esterase banding patterns could be exploited to build genetic molecular markers. Thus, the current study revealed that long-term exposure to Methyl parathion creates a continual health risk for the fish population.

As a result, it is necessary to monitor the aquatic system and predict pesticides' hazardous effects on fish. After exposure to Methyl parathion, we discovered that esterase activity in several tissues of H. fossilis steadily decreased with increasing time intervals. Mores et al. (2000) obtained similar results.When compared to other tissues such as the intestine and brain, the liver had the highest esterase activity level.

Tables I, II, and III show that the intensity of esterase bands varies by tissue and species, even inside the same individual's body. The binding patterns of esterases in different tissues have a high potential for species identification. AChE esterase activity was found to be decreasing in the liver and kidney (Shaid Nahboob KA, Ghazala Ghazala 2016).Esterase's tissue and species-

specific distribution has previously been documented from two catfishes and a toad. AChE Esterase activity demonstrated that a sublethal quantity of Methyl Parathion suppressed esterase activity in H.fossilis, with the order of reduction being Liver > Intestine > Brain. An organism that develops resistance to a pesticide may be unable to operate.Isozyme patterns vary among fish populations (Barua et al., 2004) and have also been utilised to build genetic sexing systems (Robinson, 1986). The findings of this study are consistent with those of Venkateswara Rao et al., (2022), Venkateswara Rao et al., (2023), and Shankar et al., (2019).

CONCLUSION:

The current work reveals that changes in protein bands found in several tissues of insecticide-treated fish may serve as a sensitive biochemical biomarker of OP pollution in the aquatic environment and may aid in water control and management. Thus, the current study indicated that long-term exposure to methyl parathion creates a persistent health risk for the fish population. As a result, it is necessary to monitor the aquatic system and predict pesticides' hazardous effects on fish. Individual electromorphs are described by the variety of their esterase isozyme patterns. It can be concluded that each tissue has a distinct esterase banding pattern that can be exploited to develop genetic molecular markers for accurate fish species identification. Long-term exposure to methyl parathion creates ongoing health risks for the fish population. As a result, it is necessary to monitor the aquatic system and predict pesticides' hazardous effects on fish.

Comment [h10]: As above

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Comment [h11]: Rearrange