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Evaluation of Acute Toxicity of Malathion and Its Sub- Lethal Effects on Protein Patterns in Muscle and Brain Tissue of *Channa punctatus* And *Labeo rohita*

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ABSTRACT:
An organophosphorous insecticide, Malathion has extensive agricultural application. There is a serious danger to aquatic life since this pesticide has leaked into the water system and is polluting it. The protein patterns in the brain and muscles of two freshwater fish species, *Labeo rohita* and *Channa punctatus*, were studied in order to determine the acute toxicity from Malathion as well as the sub-lethal consequences of the chemical. The sub-lethal concentration (LC50) is obtained using probit analysis. The protein profiles of fish exposed to sub-lethal levels of Malathion were measured 24, 48, 72, and 96 hours later. Using SDS-PAGE, researchers looked at how proteins in important organs including the brain and muscles have changed. Because Malathion is poisonous, the electrophoretogram shows that the intensity of the protein bands in the muscles and brain is lower than in the control group. Muscle tissue of *Channa punctatus* exhibited 10 protein bands and brain tissue had shown 08 protein bands in control. Whereas the Muscle tissue of *Labeo rohita* shown 10 protein bands and Brain tissue exhibited 10 protein bands in control. According to the current research, the anabolic processes are negatively impacted when Malathion binds to proteins and the machinery that synthesizes them, which is likely to cause the loss of protein bands. Assessing pesticide-induced stress in fish relies heavily on biochemical investigations, such as electrophoretic protein quantification. It is possible that the significant drop in protein fractions would aid in the early diagnosis of pesticide contamination.

Keywords:
Malathion acute toxicity, muscle, brain, SDS –PAGE, *Channa punctatus*, *Labeo rohita,* LC50.

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INTRODUCTION

A variety of agro-chemicals, both those released into rivers on purpose and those used in legally sanctioned farming methods, may have a devastating effect on fish populations (Ayoola, S. O. et al., 2008). Pesticides are being used on a massive scale in agriculture in the hopes of achieving high crop yields, but these organic pollutants are making their way up the food chain and are having a negative impact on aquatic biomarkers (Laurie Boithias et al., 2011). Accumulated pesticides in tissues trigger a cascade of physiological and biochemical reactions that impact the actions of many enzymes and metabolites, ultimately disrupting the metabolic process as a whole (Jackson GA et al., 1968, Mukesh K N et al., 2003, Scott WN, 1967). Because of soil erosion and runoff from agricultural land, heavy rainfall events temporarily but significantly increase the amount of pollutants that enter freshwater surface streams (Yu Zhang, 2010). Insecticides are used all throughout the world to protect crops against pests that destroy plants (Duanping Xu, 2004). Proteins, which are the primary enzymes in a cell, are among the most adaptable macromolecules found in living things. They govern metabolism by selectively accelerating chemical processes, making them one of the most ubiquitous macromolecules. They transport oxygen molecules, serve as a mechanical supply and defense mechanism against invaders, mediate communication between cells, and transmit electrical impulses (Berg JM et al., 2005).In addition to their remarkable functional diversity and involvement in several critical physiological processes, proteins are a diverse group with a broad range of roles (Albert L, et al., 2013). A determination of the physiological levels of animals may be made by analyzing the value of the protein content (Kapilamanoj, et al., 1999, Ramadan AA, 2007). The rate of protein synthesis and breakdown, also known as catabolism, is a dynamic equilibrium that determines the concentration of proteins in tissues (Schimke RT, 1974). Catabolism is the process by which proteins are broken down. In general, the cycle of proteins that occurs in an animal is the same (Grainde B, 1981). It is because fish proteins include high amounts of amino acids such as lysine, arginine, histidine, leucine, isoleucine, valine, threonine, methionine, phenylalanine, and tryptophan that fish flesh has such a high biological value (Srivastava CBL, 1999). The study of protein shapes and activities is known as proteomics, and it is often used to understand proteins (WP Blackstock, 1999, NL Anderson, 1998). Fish and other aquatic creatures are particularly vulnerable to environmental contaminants including metals, herbicides, and other organic materials. They are generally bioindicators of environmental pollution and may be used to assess the quality of the aquatic environment (Leena Grace Beslin). In recent decades, industrial and agricultural wastewater from water drains has become a serious global environmental concern (Popoola, O et al., 2014). SDS-PAGE protein separation is used to assess the distribution of proteins across fractions and determine the relative quantity of proteins in a sample (Popoola, O et al., 2020) Proteins are separated into different groups according to their size by the use of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). An initial step involves unfolding the sample's proteins by denaturation and a reduction in heat. Following this step, the sample is coated with SDS detergent in order to make it negatively charged. (Krian A et al., 2020).

MATERIALS AND METHODS

Collection of Samples and preparation of OP compound

With the cooperation of local fishermen, adult fish weighing between 50 and 70 grams were taken from freshwater tanks located within a 15-kilometer radius of the laboratory. The fish were retrieved using netting techniques. To avoid fungal infections, they were quickly transported to the lab and placed in a plastic bucket (30X30X60 cm). Before the fish were added, the bucket was cleaned with potassium permanganate and rinsed extensively. Before being used, the fish were kept in aquariums for around one week to help them adjust. Every day, they were given commercial fish food.

According to the process, a working solution was generated by diluting the sub lethal concentrations of the insecticide Malathion (2Ï E.C.) with distilled water after being synthesized in 95Ï Acetone to a concentration of 100mg/ml (APHA, 2005). For exposure durations of 24, 48, 72, and 96 hours, sub lethal concentrations of 0.01, 0.04, and 0.06 µg of pesticide were used in this study. In order to investigate the potentially detrimental effects of methyl parathion on a variety of tissues, testing was performed concurrently on a control batch that was identical to each of the test groups.

Preparation of Samples for study

Fish were terminated following each exposure period, and their tissues, encompassing their musculature and gills, were meticulously extracted for subsequent scientific investigations. Following their milligram-level weighing, the tissues were pulverized in a 0.01M Tris HCl (pH 7.5) solution supplemented with 0.9 · NaCl. Variable concentrations of tissue homogenates were detected. "The tissues were deposited in ice-jacketed centrifuge containers following homogenization. At ambient temperature, the specimens were centrifuged for ten minutes at 2,000 revolutions per minute in a clinical centrifuge. To prepare the sample for electrophoretic separation of protein patterns, 0.1 ml of the supernatant was combined with an equivalent amount of 20i sucrose solution that included 0.5i bromophenol blue as a tracking dye.

SDS-PAGE Analysis

Following preparation in Tris-HCl buffer (pH 7.2), muscle and gill homogenates (10η) were subjected to a 10-minute centrifugation at 10,000 rpm. To dissolve the particle, it was initially rinsed with cool acetone. Following that, the sample was subjected to cooking at 950 degrees Celsius for one minute in a buffer containing 0.4 ml of 0.1v (W/V) bromophenol blue, 0.8 ml of 2-mercaptoethanol, and 0.4 ml of 0.5M Tris HCl (pH 6.8) in a sample volumetric ratio (1-6 ml each).

Experimental procedure for preparation of SDS-PAGE

The tracking dye, βmercaptoethanol, was used, and the supernatants were combined in equal parts with 20% sucrose containing 0.1% SDS. The tissue extract was immediately placed onto the separating gel in a 0.1 ml (5 mg) aliquot. The electrode buffer, which consisted of 0.025 M Tris and 0.192 M Glycine, was used in accordance with the normal technique. Additionally, 0.074 M Tris and 0.1% SDS were adjusted to a pH of 7.8 using concentrated hydrochloric acid (Laemmli/1970). The gel was subjected to a steady current of 50 volts for the first 15 minutes of the experiment, and then it was subjected to a current of 150 volts for the remaining length of the experiment. At a distance of eight centimeters from the point of origin, the tracking dye migrated, which resulted in the current supply being cut off.

Staining Procedure and standardization of protein bands

Proteins that had been separated on gel using the normal methodology were stained with a solution that included 0.25% Coomassie brilliant blue in a mixture of acetic acid, methanol, and water in the proportions of 5:5:1 (Holmes and Master 1967). For the purpose of comparing the changes seen in the SDS-PAGE, low molecular weight protein standards ranging from 15 to 100 KDa were obtained from the SIGMA-Chemical firm in the United States.

RESULTS

This study aims to assess the acute toxicity of Malathion as well as its sub lethal effects on protein patterns in the brain and muscle tissues of two freshwater fish species*: Labeo rohita* and *Channa punctatus.* And these are the outcomes that were attained.

Muscle tissue of *Channa punctatus*

Rm values of 0.03–0.99, 0.18–0.23, 0.34–0.55, 0.64–0.64, 0.75–0.89, and 0.99 were among the ten protein bands seen in the control muscle. A total of eight protein bands were seen in the muscle following a twenty-four hour exposure to Malathion. These bands had Rm values of 0.03, 0.14, 0.23, 0.41, 0.50, 0.74, 0.80, and 0.89. In the tissue, sixteen protein bands with Rm values of 0.03, 0.14, 0.34, 0.58, 0.64, and 0.99 were seen after the treatment had been administered for a period of 48 hours. In the tissue, there were four protein bands seen after 72 hours with Rm

values of 0.31, 0.58, 0.88, and 0.99; in the muscle, there were three protein bands observed after 96 hours (D65024548). The protein band which was exposed in control with Rm value 0.03 Zone-A (100-70 KDa) was also appeared in 24H, 48H and not seen at 72H, 96H. It was also observed that the protein band that was displayed in the control with an Rm value of 0.14 Zone-A (100-70 KDa) appeared at all-time intervals with the exception of 72H (wjpr.net). The presence of the protein band with an Rm value of 0.18 was only seen in the control group, which is an indication that the pesticide had a far more significant impact. The protein band, which had an Rm value of 0.23 Zone -B, did not vanish from the control until twenty-four hours had passed. The protein band measured between 55 and 35 KDa. The protein band with an Rm value of 0.34 Zone -B (55–35 KDa) was visible in the control and at 48 hours, but it was not detectable at 24 hours, 72 hours, or 96 hours. The Rm value was 0.34. At 24H, 48H, 72H, and 96H, the protein band with a Rm value of 0.55 Zone -B (55-35 KDa) is shown to be present, but it is not present at any of the other high temperatures. The control and 48-hour samples had a protein band with an Rm value of 0.64 Zone -C (34-15 KDa) at 48 and 24 hours, but not at 72 or 96 hours. The protein band that was discernible in the control (Rm = 0.75), vanished completely at every time point. In the control group, the protein band with an Rm value of 0.89 became apparent only after a span of 24 hours. The influence is higher during hours 48, 72, and 96. With the exception of 24 hours, all time intervals showed the presence of the protein band in Zone -C (34-15 KDa) with an Rm value of 0.99. So, it was determined that Malathion toxicity impacted proteins with high, low, and intermediate molecular weights, or Zones A, B, and C, respectively. This tissue also exhibited toxic stress inducing proteins at various time intervals, at 24H Rm value 0.41, 0.74, 0.80, at 48H Rm value 0.58, at 72H Rm value 0.31, 0.88, at 96H Rm value 0.57 protein bands were appeared new.

Brain tissue of *Channa punctatus*

Eighteen protein bands with Rm values of 0.03, 0.14, 0.23, 0.46, 0.64, 0.75, 0.84, and 0.99 were seen in the brain's control region. There were seven protein bands visible in the tissue at 24 hours, with Rm values of0.08, 0.14, 0.23, 0.45, 0.70, 0.85, and 0.95. There are five protein bands seen in the tissue at 48 hours, with Rm values of0.03, 0.34, 0.71, 0.83, and 0.99. There were four protein bands seen in the tissue at 72 hours, with Rm values of0.14, 0.50, 0.73, and 0.99. After 96 hours, the tissue exhibited only two protein bands, D65024548, with Rm values of 0.14 and 0.50. The control and 48-hour Malathion exposure groups both showed a protein band (Zone-A, 100-70 KDa) with an Rm value of 0.03. A protein band with an Rm value of 0.14 (Zone-A 100-70 KDa) was present in the control and all time periods except for 48 hours. The identical Zone-B (55–35 KDa) protein band with an Rm value of 0.23 was still visible to the control group 24 hours later. The control group exhibited the protein bands with Rm values of 0.46, 0.64, 0.75, and 0.84 for Zone-B (55-35 KDa), respectively; these bands disappeared after 24 hours, 48 hours, 72 hours, and 96 hours. The protein band with a Rm value of 0.99 Zone -C, or 34 to 15 kDa, was present in the control, 48, and 72-hour samples but not in the 24 or 96-hour samples. This proves that Zone-C proteins, also known as high molecular weight proteins, were very sensitive to Malathion. Novel pesticide-affected protein bands were revealed in this tissue. At 24H bands with Rm value 0.08, 0.45, 0.70, 0.85, and 0.95: At 48H 0.71, 0.83: at 72H 0.73 a new protein band were appeared.

Muscle tissue of *Labeo rohita*

Displayed muscle There are ten protein bands; their Rm values are as follows:0.03,0.07,0.19,0.34,0.40,0.59,0.64,0.86, and 0.99. The tissue exhibited the presence of eighteen protein bands after a 24-hour exposure period, each with an associated Rm value of 0.64, 0.89, 0.14, 0.23, 0.34, 0.50, 0.59, 0.64, and 0.99. In contrast to the control, the protein bands exhibiting Rm values of 0.14, 0.23, and 0.89 were conspicuous. The tissue exhibited sixteen protein bands with Rm values of 0.03, 045, 0.64, 0.70, 0.75, and 0.99 after a period of 48 hours. Protein bands exhibiting Rm values of 0.45, 0.70, and 0.75 were notably absent from the control group. Twenty-two hours later, five protein bands with Rm values of 0.02, 0.10, 0.35, 0.64, and 0.99 were discernible. Rm values of 0.02, 0.10, and 0.35 were observed for unique protein bands that were absent from the control. Three bands corresponding to proteins were visible at

96H, with Rm values of0.34, 0.80, and 0.99; however, a fourth band, D65024548, had a Rm value of 0.80 and was not present in the control. At 48 hours after exposure, the control group detected a protein band with a Rm value of 0.03 (close to Zone -A with MWt: 100-70 KDa), however at 24 hours, 72 hours, and 96 hours, the band was not there. Only after 24 hours did the protein band with a Rm value of 0.14 (Zone-A, with a known MWt of 100-70 KDa) become visible. Only after 48 hours did the Rm value of 0.23 protein band (close to Zone -B with MWt: 55-35 KDa) become visible. The control, 24H, and 96H samples showed a protein band with an Rm value of 0.34 (Zone-B with MWt: 55-35 KDa), however the 48H and 72H samples did not. There was an additional molecular marker protein band detected in the control, 24H alone, with an Rm value of 0.50 (Zone-B with MWt: 55-35). Except for 96H, all of the controls showed the same protein band, which had an Rm value of 0.64 and a Zone-C MWt of 35–15 KDa. A protein band with a known molecular weight of 35–15 KDa and an Rm value of 0.99 (Zone –C) was also present in the control group at every time point. It was observed Malathion toxic effect was high upon Zone-A i.e. low molecular weight proteins in muscle tissue

Brain tissue of Labeo rohita

Ten different protein bands with Rm values ranging from 0.03 to 0.99 were seen in the brain. The bands were linked to different peptides and enzymes. After twenty-four hours, seven protein bands were observed, each with the following Rm values: 0.03, 0.14, 0.29, 0.50, 0.80, 0.90, and 0.99. The control group did not exhibit the bands characterized by Rm values of 0.29, 0.80, and 0.90. At 48 hours, tissue analysis identified five distinct protein bands with respective Rm values of 0.14, 0.30, 0.50, 0.75, and 0.99. Nevertheless, the bands denoted by Rm values of 0.30 and 0.75 were novel and absent from the control. Four protein bands were seen in the tissue after 72 hours, with Rm values of 0.03, 0.34, 0.71, and 0.99. The bands that showed Rm values of 0.34 and 0.71 were unique and did not have controlling characteristics among these bands. The tissue contained two protein bands with Rm values of 0.64 and 0.80 after 96 hours. Both the control and 48-hour samples exhibited Zone-A (100-70 KDa) protein band characteristics, as determined by the Rm value of 0.03. At 24 and 48 hours, Zone-A, a protein with a Rm value of 0.14 and a molecular weight range of 100–70 kDa, was observable in the control sample, but not at 72 and 96 hours. The protein band with an Rm value of 0.23 (Zone B, 55–35 KDa) was distinctest in the control group (wjpr.net). Zone-B protein band (MWt: 55-35 KDa, Rm value0.34) was only visible at 72 hours (wjpr.net). A different protein band, Zone-B with an MWt of 55–35 KDa and an Rm value of 0.50, was seen in the control and 24–48 hour samples (wjpr.net). In the control samples as well as the samples that were taken 96 hours later, a protein band with a Rm value of 0.64 was seen (Zone-C: MWt:35-15 KDa). All time periods, with the exception of 96 hours, revealed the existence of a protein band (Zone-C, molecular weight 35–15 KDa) with an Rm value of 0.99. It is established that Malathion affected Zone-B proteins more than other substances. The results are shown in Figure 1, Table 1, and Figure 2 and Table 2.

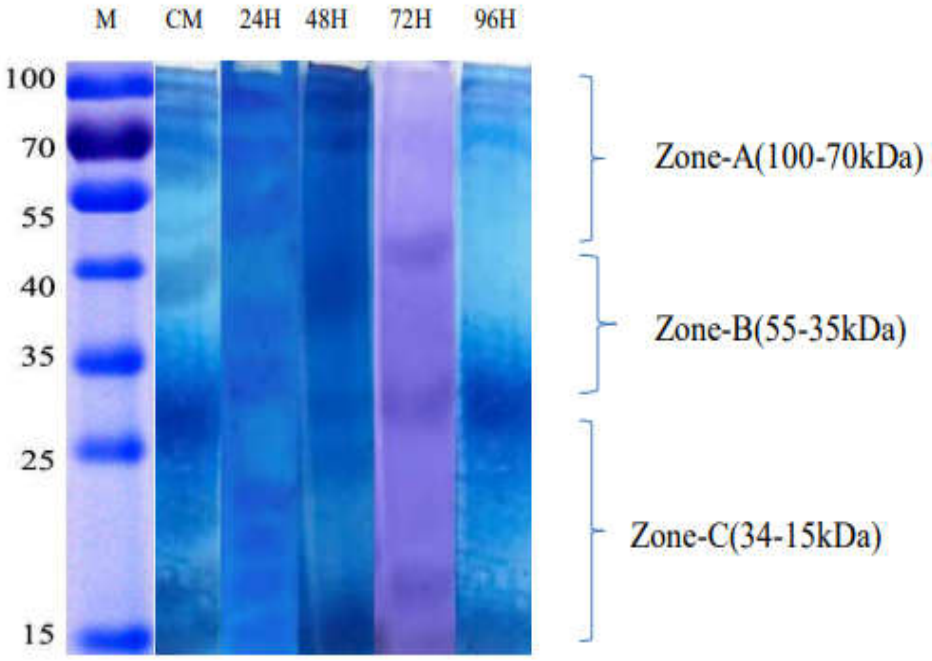

Figure 1: Protien banding pattern of Muscle tissue of Channa punctatus exposed to Malathion at different time intervals

Table 1: Rm values of Protein in muscle tissue of *Channa punctatus* exposed to Malathion

Marker	Control	24H	48H	72H	96H
0.03	0.03	0.03	0.03		
0.14	0.14	0.14	0.14		0.14
	0.18				
0.23	0.23	0.23			
				0.31	
0.34	0.34		0.34		
		0.41			
0.50		0.50			
	0.55				
			0.58	0.58	0.57
0.64	0.64		0.64		
	0.75	0.74			
		0.80			
	0.89	0.89		0.88	
0.99	0.99		0.99	0.99	0.99

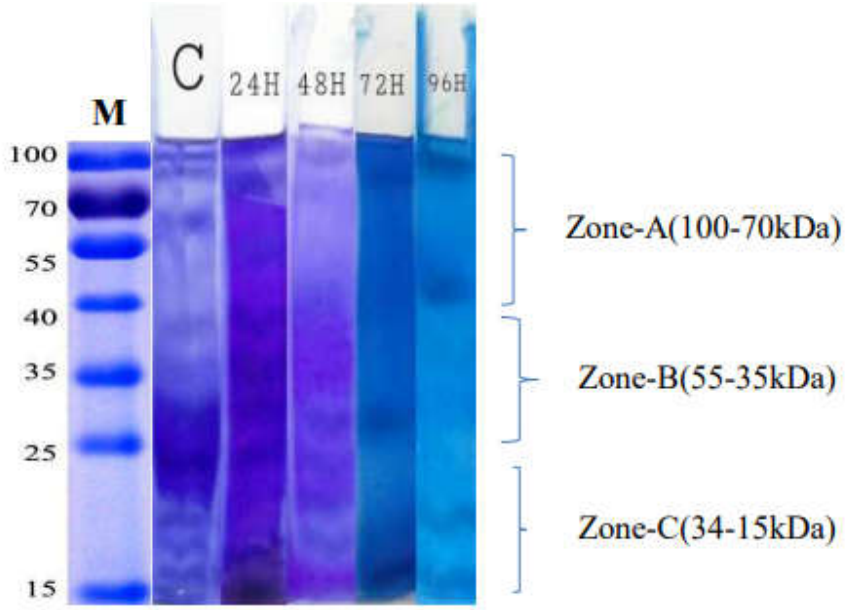

Figure 2: Protien banding pattern of Muscle tissue of Labeo rohita exposed to Malathion at different time intervals

Table 2: Rm values of Protein in muscle tissue of *Labeo rohita* exposed to Malathion

Marker	Control	24H	48H	72H	96H
0.03	0.03		0.03	0.02	
	0.07			0.10	
0.14		0.14			
	0.19				
0.23		0.23			
0.34	0.34	0.34		0.35	0.34
	0.40				
			0.45		
0.50	0.50	0.50			
	0.59	0.59			
0.64	0.64	0.64	0.64		
			0.70		
	0.86	0.89	0.75		0.80
0.99	0.99	0.99	0.99	0.99	0.99

DISCUSSION

There has been substantial use of biomarkers to establish a link between organism exposure to contaminants, tissue contamination inside the body, and the onset of harmful consequences (C. Thanomsit, 2020). Since they may identify impacts on target biota before population, community, or ecosystem level effects are seen, contaminants are viewed as "early warning systems." The numerous stress impacts of these pollutants on auatic creatures and ecosystems are now difficult to measure. Therefore, biomarkers may be an important piece of

evidence in the fight against pollution's negative effects on ecosystem structure and function and in understanding the links between stress and its consequences on coastal resources (Kroon, 2007). The development of new proteins following pesticide exposure clearly confirmed modifications in the cytoplasmic proteins. SDS-PAGE was used to separate sarcoplasmic proteins, and the proteins showed that the strength of protein bands changed in response to an increase in the pressure level and the length of time that they were held (Sherif, 2009, Swetha Agrahari, 2009, Sipra Mohapatra, 2012).

There was a decrease in the amount of protein bands in fish that were subjected to various doses of the herbicide butataf over a period of thirty days, according to a review (Suneetha et al., 2010) that utilized SDS-PAGE to analyze the plasma protein bands in Nile tilapia. Compared to the control group, which had thirteen bands in their plasma protein profile, the number of bands reduced as the toxicity of butataf rose (12–9). (Tripathi.G, 1990) in It was noted that there was a modest drop in protein intensity in Nile tilapia fish treated with diazinon. This suggests that the proteins in these fish were significantly impacted by stress rather than the pesticides. Previous research has reached similar conclusions (Tripathi. G.1990, Vaidehi 2013, Yakeen T.A, 2011). As a result, the persistent toxicity of the herbicides profenofos and carbosulfan caused the disappearance of several bands. According to the results of (Yakeen, 2011). Copper exposure to the fish species *Oreochromis niloticus*, certain protein fractions vanished and the electrophoretic mobilities differed across tissues. Quinalphos exposed *Channa punctatus* had lower protein concentration in the liver, muscles, kidneys, intestines, brain, and gills, according to Sastry and Siddiqui", (1984) Pesticide treatment reduced the number of protein fractions, eliminated some of them entirely, and sometimes introduced new fractions, according to Dhar and Chatterjee, (1984) Electrophoretic protein bands vanished following treatment with malathion, as shown by Kumar and Devi (1992), demonstrating that the compound had a significant impact on the protein pattern of *Heterneustes fossillis*. Electrophoretic patterns in freshwater fish liver, brain, and gill tissues as a result of acetamiprid poisoning Electrophoretic investigations on *Oreochromis massambicus* have shown comparable results (Justin Raj and Baby Joseph,2017).Suneetha K et al ., (2010) found that the freshwater fish *Labeo rohita* had less protein subunits when Endosulphon and Fenvalrate were used. This was determined by using SDS-PAGE. Protein synthesis increases in response to toxicant stress, as shown by Sharma.B (1999) and Saravanan et al. (2011). Malathion poisoning has similar effects on certain fish species, altering their blood biochemical makeup. (R. Kumar et al., 2009, Y. El-Nahhal et al., 2018, S.M. Yonar, 2014). The electrophoretogram of our present research study reveals that Malathion exhibited its toxic effects on both the fishes. Majority o., f the protein bands that were appeared in control are disappeared and some new Malathion toxicity opposing new protein bands were seen during the research. Our results are in consonance with (Venkateswara Rao Mandalapu, et al., 2023, Venkateswara Rao et al., 2023, Venkateswara Rao et al., 2023, Venakateswara Rao et al., 2023, Praveen Statute, 2017).

CONCLUSION

According to the current research, an individual's electromorphs are described by the variety of protein patterns. It follows that different types of fish have distinct protein banding patterns in their tissues, which might lead to the creation of genetic molecular markers for accurate species identification.

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Conflict of Interest
The authors of this article affirm that their financial relationships do not interfere with their ability to provide an unbiased assessment of this research.

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