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ASSESSMENT OF TOXICOLOGICAL EFFECTS OF ORGANOPHOSPHATE PESTICIDE (MALATHION) 0N PROTEIN PATTERNS OF MUSCLE AND BRAIN TISSUES OF CHANNA PUNCTATUS (BLOCH) BY USING SDS-PAGE

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Abstract

This study employed SDS-PAGE to analyze the impact of the organophosphate insecticide Malathion on the protein profiles of the freshwater fish Channa punctatus across a variety of tissues, including muscle and brain. The findings revealed that there are 10 protein bands responsible for regulating brain functions and 8 responsible for regulating muscle functions. Malathion, at a sublethal dose of 2% Organophosphate, was applied to Channa punctatus throughout the course of many time periods. The study's results show a decrease in the bands' frequency and intensity over time, as well as the formation of certain new pesticide-opposing protein bands. Pesticide's impact on protein concentration in skeletal muscle and brain tissue, as detected by 7.5% SDS-PAGE. Standard marker proteins were used to determine protein banding patterns, and Rm values were then determined. Electrophoretograms of both skeletal muscle and brain from the present investigation displayed protein band heterogeneity with several variations.

Keywords: Protein Patterns, Intestine, Muscle, Malathion, SDS –PAGE, Rm Value.

1. INTRODUCTION

While pesticides have a crucial role in reducing vector populations and boosting agricultural output, they also have a number of negative consequences for aquatic ecosystems (Malla F.A. et al., 2009).Many pesticides used in agriculture have detrimental effects on fish and other non-target creatures. Pesticides in water can be ingested by fish through their gills or, less frequently, their scales (Aktar et al., 2009). Pesticides often have devastating effects on fish metabolisms, leading to their deaths. Diffusion of the pesticides throughout the body of the fish leads to detrimental changes in tissue biochemistry and histology (Muthukumaravel, K., et al., 2013, Raj, S.J.et al., 2015). Pesticides, both at deadly and more usually at sublethal levels, have been linked to alterations in ion concentrations, organic components, enzyme activity, endocrine activity, and osmoregulation in fish (Ganesan, R.M., et al., 1989).As a result of its potential utility in fishery management and disease research, fish blood has attracted the attention of toxicologists and environmentalists in recent years (Mulcahy, 1975). Due to the proximity of the gills to the water medium, any adverse alteration in the water medium could be

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reflected in the circulatory system of the fish. The findings of such studies may provide clues about the state of fish populations and water quality. Pesticides are chemicals that are sprayed on crops to kill off insects, fungi, and bacteria. Use of pesticides in agricultural settings to control insect populations can have serious consequences for non-target organisms like fish, including a reduction in metabolic function and, in extreme cases, death (Shankar KM et al., 2013). Pesticides can be ingested or absorbed through the skin and gills of fish. Because of their watery habitat, fish are particularly susceptible to the impacts of water pollution. The physiological and biochemical mechanisms that govern a fish's health and vigour are profoundly affected by contaminants such as pesticides (Banaee M et al., 2009). Fish has a high BV and PER (Biological value & protein efficiency ratio; P. K. Tripathi et al., 2003; Prado, et al., 2009) due to its high protein content and low fat content, respectively (Gehan H. Fahmy, 2012). Both G. R. Scott et al. (2004) and S. C. S. Shinde (2007) agree that fish can be used as a model to determine how pervasive water contamination is in a given area. Pesticides after entering the Fish tissue undergo bioaccumulation and consequently fish can be utilised as bioindicator of aquatic ecosystem (Herger, W; Jung S.J.1995). Malathion, an organophosphate chemical, alters fish metabolism in a number of different ways, including physiological, behavioural, haematological, and possibly chromosomal. Proteins serve as the body's structural components. Since enzymes are proteins, cellular metabolism relies heavily on proteins. Enzymes, being proteins, have a special role in cellular metabolism (Deshmukh. D. R. 2017). Because fish protein provides all of the essential amino acids in the proper ratios, it has a high nutritional value and a high biological value. It has been shown that eating fish can make up for a person's inadequate protein intake and even enhance their overall protein quality (FAO, 2005). Since it is so crucial to survival, the human body contains the most protein of any nutrient by far (Sudhakar et al., 2011). In this study, we used SDS-PAGE to examine the effects of the organophosphate insecticide Malathion on the protein banding pattern in the gut and muscle of the freshwater fish Channa punctatus (Bloch).Fish toxicology research often employs the SDS-PAGE technique as a biomarker (Muhammad, OI).

2. MATERIALS AND METHODS

Collection of Samples and Preparation of OP Compound

Adult fish weighing between 50 and 70 grammes were caught using nets by local fishermen from fresh water tanks within 15 kilometres of the lab. In order to prevent fungal infection, they were hastily transferred to the lab and stored in a plastic bucket measuring 30 by 30 by 60 centimetres. The tanks were cleaned and disinfected with potassium permanganate before the fish were added. For about a week, the fish were fed commercial food in aquaria to help them adjust to their new home. The dilution of Malathion (2 E.C.) to 100 mg/ml in 95 acetone produced a sub deadly concentration. After that, distilled water (APHA) was added to further dilute the solution. Insecticide Malathion was given to participants in sub lethal doses for 24, 48, 72, and 96 hours. The toxic effects

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of Malathion on various tissues were compared by employing a control batch for each experimental group.

Preparation of Sample for Study

The fish were killed after each exposure period, and their muscle and brains were removed for study. Materials were weighed to the closest milligramme and then homogenised in a 0.1 M Tris HCl (pH 7.5) buffer containing 0.9% sodium chloride. The concentration of tissue homogenates was found to be quite varied. Tissues were homogenised, and those tubes were placed in cold baths till storage. The samples were spun in a clinical centrifuge for 10 minutes at room temperature, 2000 rpm, to separate the components. Supernatant was concentrated to 0.1 ml, and a 20 mM sucrose solution containing 0.5 mM bromophenol blue was used to distinguish protein patterns on the electrode surface.

SDS-PAGE Analysis

For 10 minutes at 10,000 rpm, gill and muscle tissue in Tris-HCl buffer (pH7.2) was centrifuged to yield homogenates (10). The pellet was heated in 2 mL of sample buffer at 950 degrees Celsius for 1 minute after being washed briefly in cold acetone. The buffer was composed of 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.

Experimental Procedure for Preparation of SDS-PAGE

A 20% sucrose solution containing 0.01% SDS, 1-mercaptoethanol, and bromophenol blue was added to the supernatants for easier monitoring. A tissue extract aliquot (0.1ml, or 5mg) was used to cover the dividing gel. In accordance with accepted practise (Laemmli), a solution of 0.074MTris, 0.1%SDS, pH7.8 with con. Was utilized. Electrode buffers were HCl and a solution of 0.025 M Tris and 0.192 M Glycine, respectively. The gel was exposed to a 50 -volt continuous current for the first 15 minutes and a 150-volt constant current for the remaining duration. As soon as the tracking dye was more than 8.0 cm from the source, power was disconnected.

Staining Procedure and Standardization of Protein Bands

To stain protein gels, scientists commonly use a 5:5:1 mixture of methanol, water, and acetic acid (Holmes and Master) containing 0.25 percent Coomassie brilliant blue solution. "The SIGMA-Chemical company in the United States provided the low molecular weight protein standards (15–100KDa) that were utilized to examine the SDS-PAGE discrepancies.

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3. RESULTS

Muscle

The Rm values of the 10 protein bands found in muscle were (in order) 0.03, 0.14, 0.18, 0.23, 0.34, 0.55, 0.64, 0.75, 0.89, and 0.99 in the control. Muscle exposed to Malathion for 24 hours showed 8 protein bands with relative molecular masses (Rm) of 0.03, 0.14, 0.23, 0.41, 0.50, 0.74, 0.80, and 0.89. Six protein bands with Rm values of 0.03, 0.14, 0.34, 0.58, 0.64, and 0.99 were seen in exposed tissue after 48 hours. Muscle subjected for 96 hours showed three protein bands with Rm values of 0.14, 0.57, and 0.99, while tissue exposed for 72 hours showed four protein bands with Rm values of 0.31, 0.58, 0.88, and 0.99. The Rm value 0.03 Zone-A (100-70 KDa) protein band observed in the control also appeared in 24H and 48H, but was absent in 72H and 96H. At all time points except for 72 hours, we saw the protein band with a Rm value of 0.14, which was found in the control samples (100-70 KDa).Pesticides have a greater effect on the protein band with Rm value 0.18, which was only found in the control group. The protein band which is seen in control with Rm value 0.23 Zone -B (55-35 KDa) was appeared just at 24H and vanished. Rm value 0.34 Zone -B (55-35 KDa) protein band was present in control and at 48H but not at 24H, 72H, 96H.At 24H, 48H, 72H, and 96H, the protein band with a Rm value of 0.55 Zone -B (55-35 KDa) is not present, although being visible in the control. The Rm value 0.64 Zone -C (34-15 KDa) protein band was present in the control and at 48H, but not at 24H, 72H, or 96H.

At all-time points, there was a complete absence of the protein band (Rm = 0.75) seen in the control. The protein band of Rm value 0.89 was seen in control and only at 24H. Especially noticeable at 48H, 72H, and 96H.The Zone -C (34-15 KDa) protein band with a Rm value of 0.99 was seen at all-time points with the exception of 24H. Therefore, high, low, and intermediate molecular weight proteins were found to be vulnerable to Malathion toxicity.

Brain

Control brain samples revealed 8 protein bands with relative molecular masses (Rm) of 0.03, 0.14, 0.23, 0.46, 0.64, 0.75, 0.84, and 0.99. Seven protein bands with Rm values between 0.08 and 0.95 were detected in the tissue after 24 hours. Five protein bands with Rm values between 0.03 and 0.34, 0.71 and 0.83, and 0.99 were detected in 48H tissue. Four protein bands with Rm values of 0.14, 0.50, 0.73, and 0.99 were detected in 72H tissue. There were only two protein bands visible in the 96H tissue, with Rm values of 0.14 and 0.50. Both the control and Malathion-exposed samples showed the presence of a protein band with a Rm value of 0.03 (Zone-A, 100-70 KDa). Rm value 0.14 (Zone-A 100-70KDa) protein band was present in control and all time points except from 48H. Control also had a protein band at 24H with a Rm value of 0.23, corresponding to the Zone-B size range (55-35 KDa). The protein band with Rm value 0.46, 0.64 Zone-B (55-35 KDa), 0.75 and 0.84 were appeared exclusively in control and were not visible at 24H, 48H, 72H and 96H. At 48H and 72H post-translational modification, but not at 24H and

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96H, a protein band with Rm value 0.99 Zone -C (34-15 KDa) was seen. Zone -C proteins, i.e. high molecular weight proteins, were found to be particularly vulnerable to Malathion's toxicity". This tissue revealed previously undetected pesticide-induced protein banding.

4. ROUTES OF PESTICIDES FISH EXPOSURE

Routes of Pesticides Fish Exposure

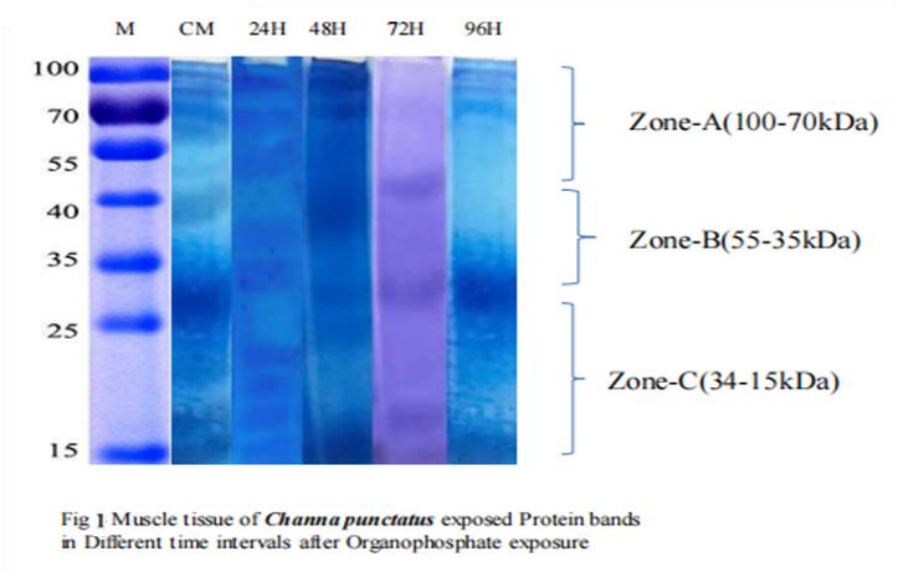

Fig 1 Muscle tissue of ***Channa punctatus*** exposed Protein bands in Different time intervals after Organophosphate exposure

Table 1: Rm values of Muscle Tissue of Channa Punctatus Exposed to Malathion (Organophosphate) at Different Time Intervals

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

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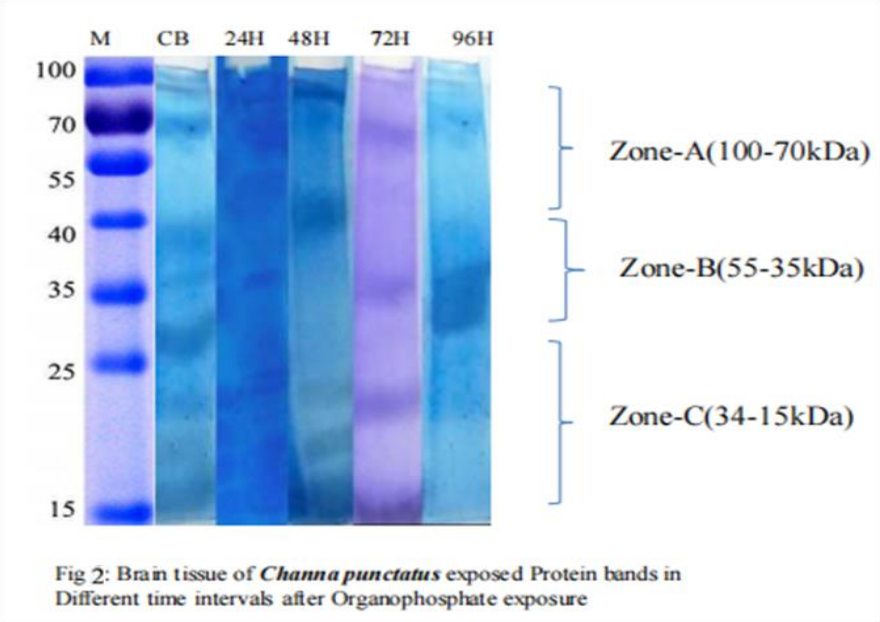

Fig 2: Brain tissue of ***Channa punctatus*** exposed Protein bands in Different time intervals after Organophosphate exposure

Table 2: Rm values of Brain Tissue of Channa Punctatus Exposed to Malathion (Organophosphate)

MARKER	CONTROL	24H	48H	72H	96H
0.03	0.03		0.03		
		0.08			
0.14	0.14	0.14		0.14	0.14
0.23	0.23	0.23			
0.34			0.34		
	0.46	0.45			
0.50				0.50	0.50
0.64	0.64				
	0.75	0.70	0.71	0.73	
	0.84	0.85	0.83		
		0.95			
0.99	0.99		0.99	0.99	

5. DISCUSSION

There were found to be 10 protein bands in skeletal muscle that regulated activity and 8 protein bands in the brain that regulated cognition, according to the current study.Muscle tissue from fish exposed to OP chemical for 24 hours included Malathion, as well as

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several other protein bands with Rm values of 0.41, 0.50, 0.74, and 0.80 that are antagonistic to the pesticide. Six protein bands were detected after 48 hours, and a new band with a Rm value of 0.58 matching to a pesticide was also discovered. Three protein bands and two new bands with Rm values of 0.31 and 0.88 were detected after 72 hours. Only three bands were detectable after 96 hours; the protein band, with a Rm value of 0.57, is unique. Protein bands with a Rm of 0.03 can be seen in the control sample after 24 and 48 hours. A protein band with a Rm value of 0.14 is detectable at all-time points examined (control, 24h, 48h, and 96h). The protein band at Rm = 0.18 is only seen in the control condition. A protein band with a Rm of 0.23 can be seen in both the control and 24-hour samples. A band with a Rm of 0.34 was played for both the control and 48h groups. The control was the only sample to exhibit a protein band with a Rm value in the range of 0.55 and 0.75.The Rm values in the control and 48h conditions were calculated to be 0.64 and 0.99, respectively. The Rm value of the band was 0.89 under the control and 24-hour conditions. These results demonstrate the toxicity of Malathion against proteins containing various levels of Mwt. The Rm value of 0.03 was seen in protein bands in both the control and 48h brain tissue. The 0.23-Rm protein band is visible at the control and 24-hour time periods, but it is obscured at the 48-hour time point. Only the control sample showed protein bands with Rm values of 0.64, 0.75, and 0.84. In the control, 48-hour, and 72-hour conditions, a Rm value of 0.99 band was displayed. At 24 hours, Rm values of 0.08, 0.45, 0.70, 0.85, and 0.95 were revealed; at 48 hours, Rm values of 0.34, 0.71, 0.83, and 0.99 were revealed; at 72 hours, Rm values of 0.50 and 0.73 were revealed; and at 96 hours, Rm value 0.50 was revealed. Stress induced proteins (Frigo, D.E., et al., 2004, Cheshenko, K., et al., 2008, Senturk, M., et al., 2009) are one type of protein that could be produced as a result of the pesticides repressing or stimulating certain genes.Proteins are essential to animal tissues because they store excess chemical potential energy. Adaptive responses are reflected in changes in protein activity or content since proteins are the primary effector molecules in all biological systems. The protein composition of an organism can be altered by pesticides, making protein analysis crucial (Suneetha K et al., 2009). Electroporation of industrial effluent (EIE) was employed to study the sublethal toxic effects on the freshwater fish Cyprinus carpio. It has been shown that following Malathion exposure, some protein fractions in the serum of Heteropneustes fossilis emerge and disappear (Munshi et al., 1999). Badawy et al. (1998) performed an electrophoresis of Clarias gariepinus serum proteins. According to (Bano and Hasan, 1998), declining serum albumin levels are associated with functional repercussions on the liver. Serum proteins of cobalt parahydroxy-benzoate-exposed animals are both overexpressed and inhibited. A capoeta capoeta capoeta (Yilmaz et al., 2008). Toxins like chromium and endosulfan are transported by serum proteins and eventually eliminated from the body via the gill, liver, and kidney (Jyothirmayee et al., 2009). The blood proteins of the edible fish Anabas testudineus and Clarias batrachus were shown to be transporting these toxicants. Several species of Cirrhinus mrigala (Pechiammal K et al., 2006) saw similar results when exposed to various pollutants. The proteolysis of proteins and the likely metabolic utilisation of proteolysis byproducts are both stimulated by protein limitation (Klassan CD, 1991). In the face of impending toxic

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stress, the body must discover alternative fuel sources when protein is in short supply (Jha BS et al., 2002). Protein banding patterns in SDS-PAGE samples of Channa punctatus gill and liver tissues were shown to be affected by malathion (Venkateswara Rao et al.,).Muscle and gill rakers in the Oreochromis niloticus SDS-PAGE following exposure to Quinalphos (Rose Marry Mathew et al., 2022). Exposed Channa punctatus have lower protein levels in their liver, muscle, kidney, stomach, brain, and gills (Sastry et al., 1984).Some protein bands were lost and others were gained in fish tissues exposed to pesticides, according to research by Dhar and Chatterjee (1984). Protein subunit reductions produced by Endosulfan and Fenvalrate were similarly observed in the freshwater fish Labeo rohita, as shown by electrophoretic examinations (Justin Raj et al., 2017; Suneetha K et al., 16) and SDS-PAGE analysis. (Krishnamurthy Yogesh et al., 2022) looked into the effects of Endosulphon's acute toxicity on the protein profiles of Cyprinus carpio using SDS-PAGE. Kareema et al. (2022) reported on the use of SDS-PAGE to examine the acute toxicity of chlorpyriphos and its sub-lethal effects on the protein patterns of Cyprinus carpio (L).Attenuation of tissue protein bands and loss of individual protein subunits are two impacts of toxicants that have been observed across multiple fish species. El-Sherif et al. (2009), Suneetha et al. (2010), Bheem Rao et al. (2018), and Florence Borgia et al. (2019) are only some of the researchers who have looked into this question. Several research (Firat and Kargin, 2010; Arivu et al., 2015; Sobha et al., 2017) have seen the emergence and disappearance of new protein subunits. Consequences Fish (Channa p.) exposed to Oleondrin and Endosulfan had considerably lower amounts of protein in their liver, muscle, gut, gills, and blood (Tiwari et al,). Carp (Cyprinus carpio) have revealed sublethal effects of Endosulfan on hematological and serum biochemical parameters (Jenkins et al.,).The pesticide Chlorantraniliprole (Bantu Nagaraju) was shown to have an effect on the biochemical components (protein on SDS-PAGE) of Channa punctatus (Bloch, 1793).Sublethal effects on metabolic enzymes and protein composition were seen in non-target Danio rerio (K.A. AI.Ghanim) Zebra fish when exposed to synthetic pyrethroid insecticides. Consistent with previous research, our results.

6. CONCLUSION

This study shows that Malathion, an organophosphate, is a persistent threat to fish populations. Keeping an eye on the water supply and planning for the potential effects of pesticides on fish populations is crucial.

7. Conflict of Interest

The authors promise that they have no personal or professional ties that would make them biased in the results of this scientific study.

8. Acknowledgements

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