The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10532344

ORGANOPHOSPHATE PESTICIDE (MALATHION) EFFECTS ON PROTEIN PROFILE OF BRAIN TISSUE OF CHANNA PUNCTATUS AND LABEO ROHITA

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Abstract

Present study is carried out on the evaluation of the comparative study of protein profile response in liver tissue of *Channa punctatus* and *Labeo rohita* is exposed to pesticide, Malathion. Specimens were collected from local ponds 15km distance away from Kakatiya University, Warangal. . Pesticide tested was Malathion (OP). Protein profiles of the brain tissue of controlled and exposed fish were analyzed by SDS-PAGE technique. The functions of Brain tissue of Channa punctatus and Labeo rohita are regulated by 8 protein bands. However after the pesticide, Malathion expose the alterations in the protein bands, their intensity and the formation of new protein antagonistic bands were differ in both the Fish

Keypoints: Protein Profile, Malathion, SDS-PAGE, Brain Tissue.

1. INTRODUCTION

Pesticide pollution of the aquatic biota is a serious issue, and fish are more at risk of these pollutants. Every year, roughly 3 million incidences of pesticide poisoning occur, resulting in 220,000 global deaths (WHO 1992). India is an agriculture country. Pesticides are the major reason for water pollution. Farmers use different types of chemicals, which include organochlorines, organophosphates, and carbamates. The different types of chemicals shown different effects on fish (Pandey et al., 1984). Indiscriminate use of the pesticides and poor legislative framework lead to the severe toxic incidences is observed in developing countries (Konradsen *et al.,* 2003 and Remor *et al.,* 2009). (Abdel khalek et al., 2017). The effects of these pollutants are of interest to the field of Ecotoxicology as they can cause damage to various biological systems (Ayadi et al., 2015). The pesticide hazard to aquatic organism is further increased by biomagnification of the synthetic pesticides from water by aquatic organisms (Murty, 1986, Satyamorthi et al., 2018, 2019, Ravichandran et al., 1918, Satyamootyhi et al, 2017).Many of these substances are carcinogenic (Garaj-vrhovac and Zeljezic 2000; Kumar et al. 2009; Nwani et al. 2010), and have been associated with cancer development (Leiss and Savitz 1995), or may induce developmental abnormalities (Arbuckel and Server 1998). However, due to widespread use of pesticides, it may cause adverse effects on the non-target organism, fish (Anita et al., 2016).Protein sub-units were separated on SDS PAGE and the molecular weight of the individual protein sub-units were determined by their relative mobility, which is calculated by using the following formula. Proteins are the principal effector molecules in all living systems, and any adaptive responses to environmental, physiological,

The Seybold Report
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or pathological factors will be reflected in changes in protein activity or content (Bradley et al. 2002).Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), One of the most popular techniques in many scientific fields, such as molecular biology, biochemistry, forensic sciences, etc. can separate proteins on a gel, Depending on the length of their polypeptide chains. Thus, SDS-PAGE, an effective technique is widely employed in various disciplines to classify proteins based on electrophoretic mobility. According to Muhammad (2018), SDS-PAGE analysis is an important biomarker for toxicological studies in fish

The current study has been undertaken to examine the acute toxicity of Malathion (OP) and its impact on electrophoretic protein patterns within the tissues of liver of fresh water fishes *Channa punctatu*s and *Labeo rohita*

2. MATERIALS AND METHODS

Experimental fish specimens and chemical

The freshwater fish Channa punctaus and Labeo rohita are edible and commercially valuable. Live fish of size 7-9 cm and weight 50-70g weight were caught from the local ponds nearby Kakatiya University, Warangal, Telangana State, India and were washed with 0.05 percent potassium permanganate (KMnO4) for 2 minutes to prevent the skin infections. The fishes were acclimatized in the laboratory conditions for two weaks. During the acclimatization period, fish were supplemented with commercial fish pellets and rice bran twice a day. To reduce the ammonia content in the water, feces and other waste constituents were drained off daily. 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 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 homogenized 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 homogenized, 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

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10532344

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.

3. RESULTS

Brain Tissue of *Channa Punctatus*

The brain of *Channa punctatus* had shown 08 protein bands in control with Rm values 0.03, 0.14, 0.23, 0.46, 0, 64, 0.75, 0.84, 0.99. At 24H, tissue had shown 07 protein bands with Rm values 0.08, 0.14, 0.23, 0.45, 0.70, 0.85 and 0.95. At 48H, tissue has shown 05 protein bands with Rm values 0.03, 0.34, 0.71, 0.83 and 0.99. At 72H, tissue has shown 04 protein bands with Rm values 0.14, 0.50, 0.73 and 0.99. And at 96H, tissue showed only 02 protein bands with Rm values 0.14 and 0.50. The protein band with Rm value 0.03 (Zone-A, 100-70 KDa) appeared in control and 48H of Malathion exposure. The protein band with Rm value 0.14 (Zone-A 100-70KDa) found in control and at all the time intervals, except at 48H. The protein band with Rm value 0.23 Zone-B (55-35 KDa) appeared in control also seen at 24H. The protein band with Rm value 0.46, 0.64 Zone-B (55-35 KDa), 0.75 and 0.84 were appeared only in control and were not seen at 24H, 48H, 72H and 96H. The protein band with Rm value 0.99 Zone –C (34-15 KDa) was appeared in control and at 48H, 72H but not in 24H and 96H. It shows Malathion toxicity was severe upon Zone –C proteins i.e. high molecular weight proteins. This tissue exposed new protein bands, which were pesticide, affected. 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.

Brain Tissue of Labeo Rohita

The brain tissue of Labeo rohita had shown 08 protein bands in control with Rm values 0.03, 0.14, 0.23, 0.46, 0, 64, 0.75, 0.84, 0.99. At 24H, tissue had shown 07 protein bands with Rm values 0.08, 0.14, 0.23, 0.45, 0.70, 0.85 and 0.95. At 48H, tissue has shown 05 protein bands with Rm values 0.03, 0.34, 0.71, 0.83 and 0.99. At 72H, tissue has shown 04 protein bands

ISSN 1533-9211

DOI: 10.5281/zenodo.10532344

with Rm values 0.14, 0.50, 0.73 and 0.99. And at 96H tissue showed only 02 protein bands with Rm values 0.14 and 0.50. The protein band with Rm value 0.03 (Zone-A, 100-70 KDa) appeared in control and 48H of Malathion exposure. The protein band with Rm value 0.14(Zone-A 100-70KDa) found in control and at all the time intervals, except at 48H. The protein band with Rm value 0.23 Zone-B (55-35 KDa) appeared in control also seen at 24H. The protein band with Rm value 0.46, 0.64 Zone-B (55-35 KDa), 0.75 and 0.84 were appeared only in control and were not seen at 24H, 48H, 72H and 96H. The protein band with Rm value 0.99 Zone –C (34-15 KDa) was appeared in control and at 48H, 72H but not in 24H and 96H. It shows Malathion toxicity was severe upon Zone –C proteins i.e. high molecular weight proteins. This tissue exposed new protein bands, which were pesticide, affected. 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.

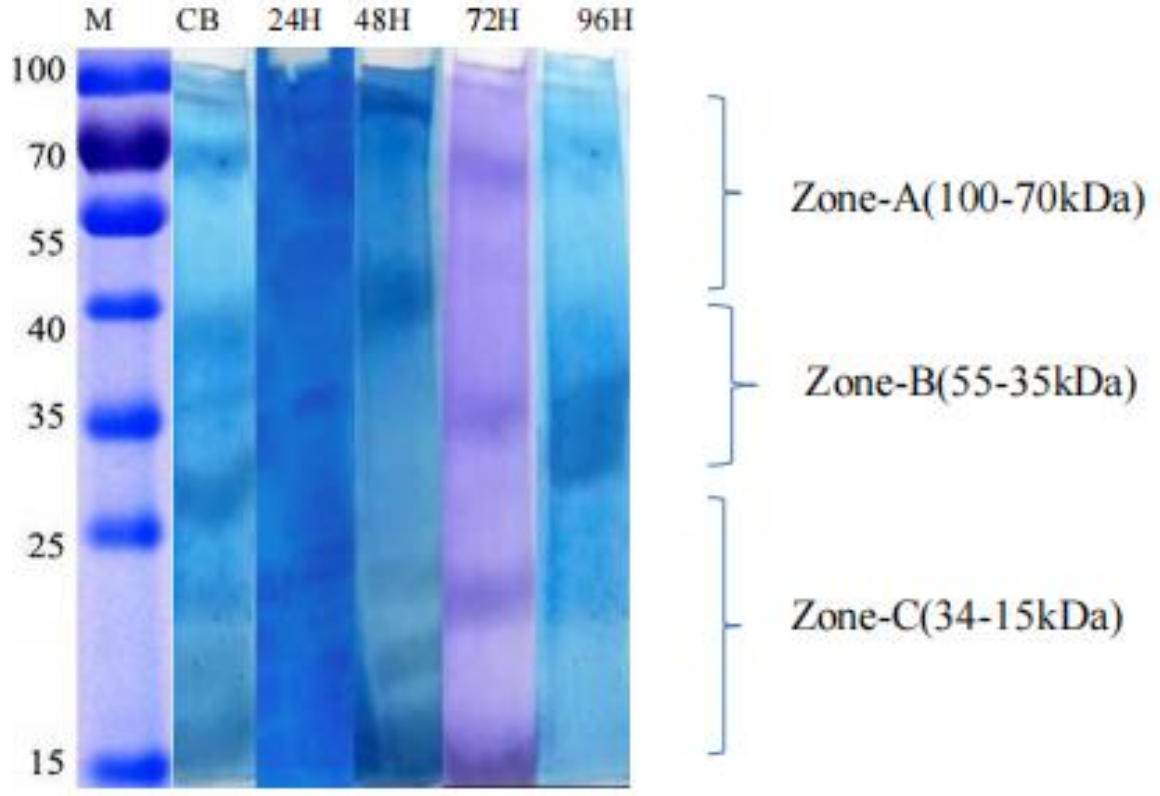

Fig 5: Brain tissue of ***Channa punctatus*** exposed Protein bands in Different time intervals after Organophosphate exposure

Figure 1: Protein Profile in Brain Tissue of *Channa Punctatus* Exposed to Malathion (OP) at Different Time Intervals

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10532344

Table 1: Rm Values of Protein Profile in Brain Tissue of Channa Punctatus Exposed to Malathion (OP) at Different Time Intervals

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	

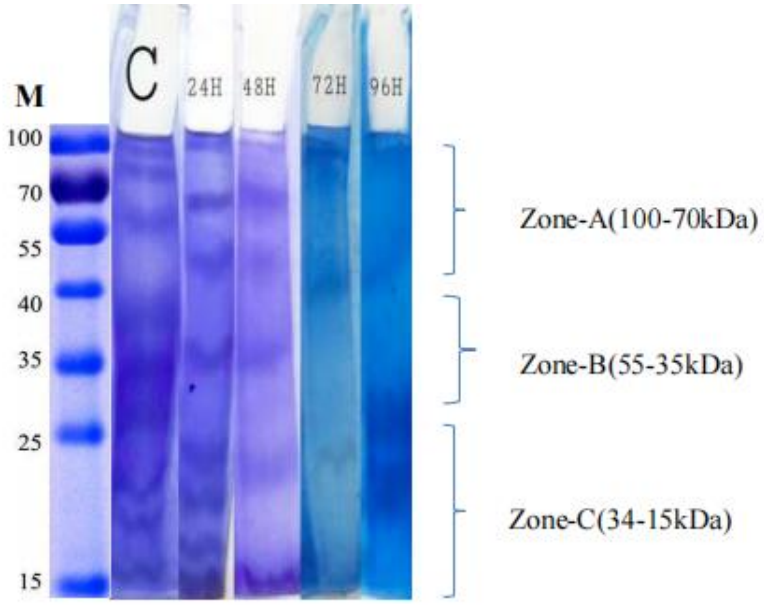

Fig 5: Brain tissue of ***Labeo rohita*** exposed Protein bands in Different time intervals after Organophosphate exposure

Figure 2: Protein Profile in Brain Tissue of Labeo Rohita Exposed to Malathion (OP) at Different Time Intervals

The Seybold Report
ISSN 1533-9211
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Table 2: Rm Values of Protein Profile in Brain Tissue of Labeo Rohita Exposed to Malathion (OP) at Different Time Intervals

MARKER	CONTROL	24H	48H	72H	96H
0.03	0.03	0.03		0.03	0.03
	0.09				
0.14	0.14	0.14	0.14		
0.23	0.23				
		0.29	0.30		
0.34				0.34	
	0.40				
0.50	0.50	0.50	0.50		
0.64	0.64				0.64
	0.76		0.75	0.71	
		0.80			0.80
	0.84	0.90			
0.99	0.99	0.99	0.99	0.99	

4. DISCUSSION

The pesticides may inhibit the expression of some genes (or) activate the others to produce specific mRNAs, which may subsequently be translated into specific proteins called stress induced proteins (Daniel et al., 2004; Ksenia et al., 2008; Murat et al., 2009). An alteration of protein metabolism was observed in fish exposed to various types of environmental stresses like metals and pesticides (Alexssandro et al.; Shweta and Gopal,2009).The protein subunits showed a steady decreasing trend in intensity of all the fractions throughout the exposure period demonstrating an inhibitory effect of endosulfan on kidney and muscle LDH. Sherif et al. (2009) observed slight reduction or decrease in intensity of proteins in Diazinon treated fish Nile Tilapia, which indicates that these proteins were highly affected by the stress caused by the pesticides. A number of authors have reported similar observations. Marinovich et al. (1994) found that Diazinon could induce inhibition of proteins in HL 60 cells at 24 hr exposure. Jyothirmayee et al. (2005) had done polyacrylamide gel electrophoresis for endosulfan induced changes in LDH pattern in freshwater fish Anabas testundineus and Clarias batrachus and the results are coinciding with our current research. Our results are also in good consonance with the previous reports validating the high toxicity of chlorpyrifos to various fish species. (Tilak et al.,2004; Díaz and Girón, 2014; Okechukwu et al.,2013 ;Reddy et al.,2012; Gul, 2005).Similar observations for other toxicants on different fishes, including a decrease in the

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.10532344

intensity of protein banding pattern in the tissues and the fading/disappearance of some protein subunits. (El-Sherif et al., 2009; Suneetha et al., 2010; Bheem Rao et .al., 2018; Florence Borgia et al., 2019). Some observations show both the appearance 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, Venkateswara Rao et al., 2023. All these reviews uphold our current examination, depletion in total protein and decreased expression of protein patterns in tissues exposed to Malathion implies a degradation of proteins due to the toxic stress of pesticides, and also it could be due to hormonal imbalance, impaired tissue repair which affects the protein levels in tissues, or maybe hepatocytic necrosis of cells which subsequently dysfunction the protein biosynthesis.

5. CONCLUSION

Thus, Present study has concluded that the long-term exposure of Methyl parathion becomes a continuous health hazard for the fish population. Therefore, it is required to monitor the aquatic system and predict the toxic effect of pesticides on fish.

6. Conflict of Interest

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

7. Acknowledgments

The authors are thankful to the Head Dept. of Zoology, Kakatiya University for providing the laboratory facilities to carry out this work.

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