ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 18, Issue, 02, pp.36155-36159, February, 2026
DOI: https://doi.org/10.24941/ijcr.49754.02.2026

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

COMPARATIVE STUDY OF ORGANOPHOSPHATE PESTICIDE (METHYL PARATHION) EFFECTS ON PROTEIN PROFILE IN BRAIN TISSUE OF *HETEROPNEUSTES FOSSILIS* AND *CHANNA PUNCTATUS*

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ARTICLE INFO

Article History:
Received 25th November, 2025
Received in revised form
20th December, 2025
Accepted 18th January, 2026
Published online 27th February, 2026

Keywords:
Protein Bands, Methyl Parathion, SDS-PAGE, Brain Tissue. *Heteropneustes fossilis*, *Channa punctatus.*

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ABSTRACT

The present study focuses on the comparative evaluation of protein profile responses in the brain tissue of *Heteropneustes fossilis* and *Channa punctatus* exposed to the organophosphate pesticide, methyl parathion. Specimens of both species were collected from local ponds located approximately 15 km away from Kakatiya University, Warangal. The protein profiles of brain tissues from control and pesticide-exposed fish were analyzed using the SDS-PAGE technique. In control conditions, the brain tissues of both *H. fossilis* and *C. punctatus* exhibited eight distinct protein bands. However, exposure to methyl parathion induced noticeable alterations in these protein bands, including changes in band intensity and the appearance of new or antagonistic protein bands, which varied between the two fish species.

Citation: Bheem Rao, T. and Anil Kumar, V. 2026. "Comparative study of organophosphate Pesticide (Methyl Parathion) Effects On Protein Profile in Brain Tissue of *Heteropneustes fossilis* and *Channa punctatus*". ***International Journal of Current Research, 18, (02), 36155-36159.***

INTRODUCTION

Pesticide pollution in aquatic ecosystems is a major environmental concern, as fish are particularly vulnerable to these contaminants. According to the World Health Organization (1992), approximately three million cases of pesticide poisoning occur annually, resulting in around 220,000 deaths worldwide. India, being an agricultural country, faces severe pesticide-related water pollution due to the extensive use of various chemical formulations, including organochlorines, organophosphates, and carbamates. These compounds exert diverse toxic effects on fish species (Pandey *et al.*, 1984).The indiscriminate use of pesticides, coupled with inadequate legislative control, has led to numerous cases of severe toxicity, especially in developing countries (Konradsen *et al.*, 2003; Remor *et al*., 2009; Abdel Khalek *et al*., 2017). The harmful impacts of these pollutants are a central focus in the field of ecotoxicology, as they can adversely affect multiple biological systems (Ayadi *et al*., 2015). Moreover, the risk to aquatic organisms is heightened by the biomagnification of synthetic pesticides through the food chain (Murty, 1986; Satyamorthi *et al*., 2017, 2018, 2019; Ravichandran *et al.*, 1918). Many pesticides are known to be 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 developmental abnormalities (Arbuckle and Sever, 1998). Consequently, the widespread use of these toxicants poses significant risks to non-target aquatic organisms such as fish (Anita *et al*., 2016).Proteins are key effector molecules in all living systems, and adaptive responses to environmental, physiological, or pathological stressors are often reflected in changes in protein content or activity (Bradley *et al*., 2002). Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is one of the most widely used techniques in molecular biology, biochemistry, and forensic science. This method separates proteins based on the length of their polypeptide chains, thereby allowing classification according to electrophoretic mobility. SDS-PAGE analysis has proven to be an important biomarker tool in toxicological studies involving fish (Muhammad, 2018).In the present investigation, an attempt has been made to assess the acute toxicity of the organophosphate pesticide methyl parathion and its effect on the electrophoretic protein patterns of brain tissue in two freshwater fish species, Heteropneustes fossilis and Channa punctatus.

MATERIALS AND METHODS

Experimental fish specimens and chemical The freshwater fish *Heteropneustes fossilis* and *Channa punctatu*s 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 Methyl Parathion (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 Methyl Parathion was given to participants in sub lethal doses for 24, 48, 72, and 96 hours. The toxic effects of Methyl Parathion 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 ml 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 mLof 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) wasused 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

RESULTS

Brain Tissue of *Heteropneustes fossilis:* The brain showed 08 protein bands at 24h with R_m values 0.11, 0.23, 0.36, 0.50, 0.63, 0.73, 0.83 and 0.86; whereas it showed 05 protein bands at 48h with R_m value 0.23, 0.31, 0.43, 0.73 and 0.91; while at 72h it showed 04 protein bands with R_m values 0.48, 0.58, 0.63 and 0.83; and at96h it showed single protein band with R_m value 0.58. The results showed in Table 1 and Figure 1

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				

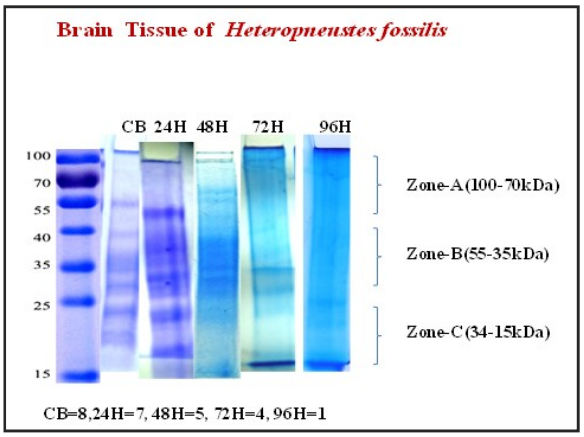

Fig.1 Brain Protein bands in Different time intervals after exposure of Organophosphate

Brain tissue of *H. fossilis* exhibited 10 protein bands in control, whereas when exposed to Methyl parathion at different time intervals such as 24h, 48h, 72h and 96h it showed variations in banding patterns. A protein band with R_m value 0.01 was observed at 72h with decreased in its intensity compared to control. A protein band with R_m value 0.23 and 0.73 were observed at 24h and 48h, which are not found at 72h and 96h, the band exhibited with more intensity at 48h. Two protein bands with R_m value 0.36 and 0.50 were observed at 24h with low intensity compared to control. These bands were completely disappeared at 48h, 72h and 96h. A band with R_m value 0.43 is observed at only 48 h. whereas not found at 24h, 72h and 96h.A protein band with R_m value 0.58 was appeared at 72h and 96h. but intensity was decreased when compared with control. Whereas the band with R_m value 0.58 was completely absent at 24h and 48h. A protein band with R_m value 0.63 was observed at 24h and 72h. Whereas the band with R_m value 0.63 was completely absent at 48h and 96h. The protein band with R_m value 0.73 was observed at 24h and 48h but disappeared at 72h and 96h. A band with R_m value 0.83 was found at 24h and 72h where it was completely disappeared at 48h and 96h. All protein bands were observed in brain with high intensity at 48h when compared with control.

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 Methyl Parathion 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 Methyl Parathion 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.

Table. 2. Rm Values of Brain tissue protein patterns *Channa puntatus*

Control	24H	48H	72H	96H
0.03		0.03		
	0.08			
0.14	0.14		0.14	0.14
0.23	0.23			
		0.34		
0.34				
	0.45			
0.50			0.50	0.50
0.64				
	0.70			
		0.71		
			0.73	
		0.83		
	0.85			
0.99	0.95	0.99	0.99	

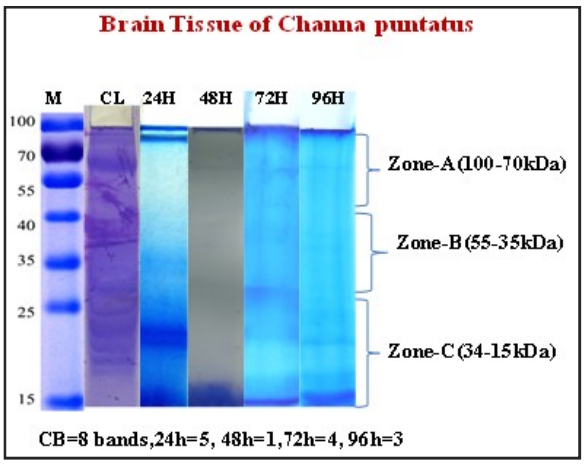

Fig.2 Brain Protein bands in Different time intervals after exposure of Organophosphate

DISCUSSION

Pesticides can influence gene expression by either inhibiting certain genes or activating others, leading to the production of specific mRNAs that are translated into stress-induced proteins (Daniel *et al*., 2004; Ksenia *et al*., 2008; Murat *et al*., 2009). Alterations in protein metabolism have been widely reported in fish exposed to various environmental stressors, including metals and pesticides (Alexssandro *et al*.; Shweta and Gopal, 2009). A consistent decline in the intensity of protein subunits throughout the exposure period indicates the inhibitory effects of endosulfan on kidney and muscle LDH activity.

Sherif *et al*. (2009) observed a reduction in protein band intensity in Diazinontreated Nile tilapia, suggesting that these proteins were significantly affected by pesticide-induced stress. Similar findings have been documented by other researchers. Marinovich *et al*. (1994) reported that Diazinon inhibited protein expression in HL-60 cells after 24 hours of exposure. Jyothirmayee *et al*. (2005) also demonstrated endosulfan-induced alterations in LDH patterns using polyacrylamide gel electrophoresis in freshwater fish Anabas testudineus and Clarias batrachus, results that align closely with the present study. Our findings are further supported by earlier studies confirming the high toxicity of chlorpyrifos and other organophosphates to fish species (Tilak *et al*., 2004; Díaz and Girón, 2014; Okechukwu *et al*., 2013; Reddy *et al*., 2012; Gul, 2005). Comparable observations for other toxicants in different fish species have revealed decreases in protein band intensity and the disappearance of specific protein subunits (El-Sherif *et al*., 2009; Suneetha *et al*., 2010; Bheem Rao *et al*., 2018; Florence Borgia *et al*., 2019). In contrast, some studies reported both the appearance and disappearance of new protein subunits as a response to toxic stress (Firat and Kargin, 2010; Arivu *et al*., 2015; Sobha *et al*., 2017; Venkateswara Rao *et al*., 2023). Collectively, these findings reinforce the results of the present investigation. The observed depletion in total protein content and decreased intensity of protein bands in tissues exposed to malathion indicate protein degradation resulting from pesticide-induced stress. This effect may be attributed to hormonal imbalances, impaired tissue repair mechanisms, or hepatocytic necrosis, all of which can disrupt protein biosynthesis and alter the overall protein profile in fish tissues.

CONCLUSION

The present study concludes that prolonged exposure to methyl parathion poses a continuous health hazard to fish populations. Therefore, regular monitoring of aquatic ecosystems is essential to assess and predict the toxic effects of pesticides on fish and to ensure the protection and sustainability of aquatic life.

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

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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