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Protein Profile Changes In Gill Tissue Of Two Fresh Water Fishes Channa Punctatus And Labeo Rohita Exposed To Malathion (Organophosphate) In Sds-Page A Comparative Study

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Abstract:
The impact of the Organophosphate insecticide Malathion on the protein profiles of the freshwater fishes *Channa punctatus* and *Labeo rohita* in gill tissues was studied in SDS-PAGE. The findings revealed that there are 10 protein bands regulating the gill functions in *Channa punctatus* and 9 protein bands are responsible for regulating gill functions in *Labeo rohita.* Malathion, at a sub lethal dose of 2% Organophosphate, was applied to *Channa punctatus* and *Labeo rohita* in the test period i.e. 24h, 48h, 72h,96h. It is discovered that the protein bands intensity and the number of the bands were decreased gradually in the test time and some new protein bands were also detected. Pesticide's impact on protein concentration in gill tissue of both *Channa punctatus* and *Labeo rohita* are detected by 7.5% SDS-PAGE. Standard marker proteins were used to determine protein banding patterns, and Rm values were then determined. Electrophoretogram of gill tissue of *channa punctatus* and *Labeo rohita* from the present investigation displayed protein band heterogeneity with considerable variations.

Keywords: Protein patterns, gill tissue, Malathion, SDS –PAGE, Rm value, *Channa punctatus, Labeo rohita.*

1. Introduction

Fish is the most important organism in the aquatic medium, fish meat possess high biological value (BV) and high protein efficiency ratio (PER) (P.K.Tripathi *et al*., 2003; Prado, *et al*., (2009). The nutritional value of fish is determined by its biochemical composition (Gehan H.Fahmy 2012). Fish can be used as an excellent model for monitoring environmental contamination affected by water pollution (G. R. Scott *et al*., 2004; S. C. S. Shinde, (2007).Malathion is an OP insecticide extensively applying in agriculture and houses for the control of pest or vectors. 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).The current study has been undertaken to examine the acute toxicity of Malathion (OP) and its impact on electrophoretic protein patterns within the gill tissues of both Channa punctatus and Labeo rohita. Proteins are the principal effector molecules in all living systems, and any adaptive responses to environmental, physiological, 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.

2.Materials and Methods

2.1 Collection of Samples and Preparation of OP Compound:

Fifty to seventy-gramme adult fishwere gatheredfrom freshwater tanks within a 15-kilometreradius of the lab with the help oflocalfishermen using nets. They were quicklytransported to the lab

and placed in a plasticbucket that measured 30 x 30 x 60 centimetresto prevent fungal infection. Before adding fish, they were properly cleaned and disinfected withpotassium permanganate. The fish were givencommercial meals daily in aquaria forapproximately a week to get used to theenvironment. When Malathion (2 E.C.) wasdiluted to 100 mg/ml in 95 Acetone, it had a sublethal effect. Following this, the solution wasfurther diluted with distilled water (APHA). Sub lethaldoses of the insecticide Malathion wereadministered to individuals for 24, 48, 72, and 96hours in the present study. Malathion's harmfulimpact on different tissues was compared usinga control batch corresponding to each test group.

2.2 Preparation of Samples for Study

The fish were killed after each exposure period,and their brains and muscles were harvested for analysis. After being weighed to the nearest milligram, materials were homogenized through a 0.01M Tris HCl buffer (pH 7.5) containing 0.9NaCl. Tissue homogenate concentration was found to be highly variable. The tissues were homogenized and then kept in centrifuge tubeswith cold baths. "To separate the components,the samples were spun for ten minutes at room temperature at 2000 rpm in a clinical centrifuge. From a volume of 0.1 ml of the supernatant, protein patterns were separated on the electrodesurface using a 20 mM sucrose solutioncontaining 0.5 mM bromophenol blue as atracking dye.

2.3 SDS-PAGE Analysis

Homogenates (10) were obtained from centrifuging gill and muscle tissue in Tris-HClbuffer (pH 7.2) at 10,000 rpm for 10 minutes. Following a quick wash in cold acetone, thepellet was heated in 2 mL of sample buffer for 1 minute at 950 degrees Celsius. The bufferconsisted of 0.5 mL of Tris HCl (pH 6.8), 40% glycerol (1-6 mL), 10% sodium dodecyl (3.2 mL),2% mercaptothion (0.8 mL), and 0.1 mL of w/v bromophenol blue (0.4 mL).

2.4 Experimental Procedure for Preparation of SDS-PAGE

To facilitate tracking, the supernatants were combined with a 20% sucrose solution containing0.1% SDS, -mercaptothions, and bromophenol blue. The dividing gel was covered with analiquot of tissue extract (0.1 ml, or 5 mg). A 0.074M Tris, 0.1% SDS, pH 7.8 with con. solution was used, as per standard procedure(Laemmli) . HCl was used as an electrode buffer, while a solution of 0.025 M Tris and 0.192 M Glycine was used instead. For the first 15 minutes, the gel was subjected to a 50-volt continuous current, and for the remaining time, it was subjected to a 150-volt constant current. The supply was cut off as soon as the tracking dye moved 8.0 cm away from the source.

2.5 Staining Procedure and standardization of protein bands

Protein gels are typically stained with a 0.25 percent Coomassie brilliant blue solution in a
5:5:1 mixture of methanol, water, and acetic acid(Holmes, Master). Low molecular weight protein standards, ranging from 15 to 100 KDa, were purchased from the SIGMA-Chemical firm in the United States and used to analyze the SDS-PAGE variances.

3.Results

The Electrophoretic protein banding pattern in gill tissue of *Channa punctatus and Labeo rohita was* studied, and the results are given below.

The gill of *Channa punctatus*had shown 09 electrophoretic protein bands in control with Rm values 0.03, 0.14, 0.23, 0.42, 0.50, 0.70, 0.75, 0.82 and 0.99. After exposure to Malathion at 24H, it showed 08 protein bands with Rm values 0.03, 0.06, 0.42, 0.55, 0.60, 0.79, 0.85 and 0.99. At 48 H tissue showed 06 protein bands with Rm values 0.03, 0.10, 0.34, 0.58, 0.80 and 0.99. At 96H showed only 02 bands with Rm values 0.70 and 0.99 were present. It was also observed that the protein band near to Zone –A between 100-70 KDa, which coincides with Rm values 0.03 appeared in control, 24H, 48H and this band disappeared in 72H and 96H, a band of Rm value 0.14 was appeared in control and 72H,

while this band is vanished in 24H, 48H and 96H. The protein band in Zone –B between 55-35 KDa with Rm value 0.23, 0.34 and 0.50 were present only in control, a band of Rm value 0.34 appeared only at control & 48H, and these were disappeared when exposed to OP. A protein band with Rm value 0.50 was disappeared in control 24H, 48H, 72H and 96H it was absent. The protein band in Zone –c between 34-15 KDa which coincides with Rm value 0.64, 0.99 were not appeared in control and at different time intervals. Rm value 0.99 was present in control, and different time intervals except 72H of pesticide expose. It shows that toxic effect of Malathion was high on Zone –A and Zone B proteins i.e. high and intermediate molecular weight proteins in gill tissue.It was also identified that gill of *Channa punctatus* shown pesticide opposing new protein bands: At 24H it shown 06 new bands with Rm value 0.06, 0.55, 0.60, 0.79, 0.85, 0.99. At 48H this tissue exhibited 03 new protein bands with Rm value 0.10, 0.58, 0.80. At 72H and 96H these new protein bands were not appeared.

Gill tissue of *Labeo rohita*shown 09 protein bands in control with Rm values 0.14, 0.23, 0.29, 0.50, 0.64, 0.72, 0.81, 0.89, and 0.99. When gill of was exposed at 24H it shown 07 protein bands with Rm values 0.14, 0.23, 0.34, 0.43, 0.75, 0.84, 0.99. It was also observed that some other new protein bands were appeared which were not exhibited in control viz, 0.34, 0.43, 0.75, 0.84**.** At 48H tissue showed 06 protein bands with Rm value 0.03, 0.15, 0.50, 0.64, 0.85, 0.99. It was noticed that 03 new protein bands exhibited with Rm value 0.03, 0.15, 0.85. At 72H tissue showed 04 protein bands with Rm value 0.14, 0.76, 0.85, 0.90. Among these, the protein bands with Rm value 0.76, 0.85, 0.90 were identified as new protein bands. At 96H gill shown 02protein bands with Rm value 0.80, 0.99. While the protein band with Rm value 0.80 was a new protein band The protein band with Rm value 0.03 (near slow moving Zone-A; molecular weight: 100-70 KDa) was expressed only at 48H of Malathion exposure. The protein band with Rm value

0.14 (Zone-A; M.wt:100-70 KDa) was not appeared at 48H. Another protein band with Rm value 0.34 (Zone-B: M.wt:55-35 KDa) was expressed at 24H. The protein band with Rm value 0.50 (Zone-B: M.wt:55-35 KDa) was exhibited in control, at 48H. The protein band with Rm value 0.64 (fast moving Zone-C: M.wt:35-15 KDa) was expressed in control, at 48H.Another protein band with Rm value 0.99 (Zone-C : M.wt:35-15 KDa) was not expressed in 72H. It shows toxic effect of Malathion was more pronounced on Zone-B i.e. intermediate proteins.

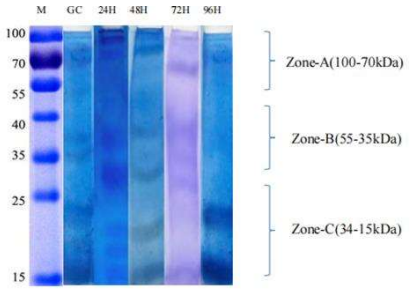

Fig.1 Gill tissue of ***Channa punctatus*** expressed Protein bands in Different time intervals after Malathion (Organophosphate)exposure

MARKER	CONTROL	24H	48H	72H	96H
0.03	0.03	0.03	0.03		
		0.06			
0.14	0.14		0.10	0.14	
0.23	0.23				
0.34			0.34		
	0.42	0.43			
0.50	0.5				
		0.55	0.58		
		0.60		0.60	
0.64					
	0.70				0.70
	0.75	0.79			
	0.82	0.85	0.80		
0.99	0.99	0.99	0.99	0.96	0.99

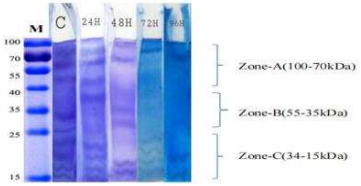

Fig.1: Gill tissue of ***Labeo rohita*** exposed Protein bands in Different time intervals after Organophosphate exposure

MARKER	CONTROL	24H	48H	72H	96H
0.03			0.03		
0.14	0.14	0.14	0.15	0.14	0.14
0.23	0.23	0.23			
	0.29				
0.34		0.34			
		0.43			
0.50	0.50		0.50		
0.64	0.64		0.64		
	0.72	0.75		0.76	
	0.81			0.85	0.80
	0.89	0.84	0.85	0.90	
0.99	0.99	0.99	0.99		0.99

4. Discussion

The gill tissue of *Channa punctatus* exhibited 09 protein bands in control. After the Organophosphate, Malathion exposure at 24h 08protein bands, 48h 06 protein bands, 72h 03 protein bands and at 96h 02 protein bands were exposed. At 24h 06 protein bands, 48h 03 protein bands, 72h 01 protein band and at 96h 01 protein bands were new which are pesticide antagonistic that indicates the gill tissue of *Channa punctaus* able to resist the pesticide toxicity up to only 48h of exposure, at 72h and 96h the resistance against the pesticide toxicity have reduced. Whereas the gill tissue of *Labeo rohita* shown 09 protein bands in control. At 24h 04 protein bands, 48h 02 protein bands, 72h 03 protein bands, 96h 01 protein band are new protein opposing bands, this indicates gill tissue exhibited the resistance to Malathion up to 72h of exposure. The comparative study of protein profile in gill tissue of two fresh water fishes

Channa punctatus and *Labeo rohita* indicated that *Labeo rohita* exhibited more protein bands than *Channa punctatus.* But the pesticide opposing new protein profile is more in *Channa punctatus.*Pollutant management in the aquatic ecosystem can be done using Bioassays. The purpose of using Bioassays is to monitor the levels of toxicity effects in the targeted biotope and to identify the low concentrations of toxicants that cause adverse effects (Kelso et al., 1990).These studies are critical in raising awareness about the potentially negative impacts of pesticides on the environment (Adedeji et al., 2008).Data obtained from Acute toxicity provides water quality guidelines for regulatory purposes (Sundaram et al.,1994) the present study reveals that Malathion (OP) is toxic to fish.Our results are in good consonance with the previous reports validating the high toxicity of pesticides to various fish species.(Tilak et al.,2004; Díaz and Girón, 2014; Okechukwu et al.,2013 ;Reddy et al.,2012; Gul, 2005.,Slaninova (2009)). The gill is the respiratory site, influenced by any change in environmental water quality (Lyndon and Houlihan1998).Similar observations for other toxicants on different fishes, including a decrease in the 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).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. Venkateswara Rao et al., 2023., Venkateswara Rao et al., 2023 studied the effect of Malathion on protein banding

pattern in various tissue of *Channa punctatus* and *Labeo rohita* and the results are coinciding with the current results.

Conclusion

The current study found that administering sublethal concentrations of Malathion to fish is harmful and causes changes in their protein patterns. The considerable alteration of protein subunits shows that Malathion may interact with peptide sequences in both *Channa punctatus* and *Labeo rohita*directly or indirectly thereby altering the structural and functional confirmations of cellular proteins. Changes in these characteristics may provide an early warning signal for determining pesticide toxicity and its impact on aquatic species. It would be very advantageous in assessing the associated environmental risk of these pesticides and thus establish subsequent management strategies for safeguarding aquatic organisms and their associated fauna.

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