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INVESTIGATION OF PROTEIN PATTERNS BY USING SDS-PAGE IN INTESTINE TISSUE EXPOSED TO MALATHION (OP) OF TWO FRESH WATER FISHES *CHANNA PUNCTATUS* AND *LABEO ROHITA*

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Abstract

Pesticides find a perennial application in agriculture, combating pest and enhancing productivity. But on the rear end, these pesticides find avenues to enter water bodies thereby affecting aquatic flora and fauna. These pesticides cause significant variation in protein particularly in fish. Present study focused on the effect of Malathion (LC50) induced protein profiling and alteration in the intestine tissue of freshwater fishes *Channa punctatus* and *Labeo rohita* at different time periods i.e. 24H, 48H, 72H and 96H. And results shown that 7 protein bands in *Channa punctatus* and 9 protein bands in *Labeo rohita* controls the functions of intestine tissue of both the fishes, and also observed that prominent decrease in protein in the test period. SDS-PAGE analysis revealed decreased in intensity and the formation/deletion of bands of the treated tissues with Malathion when compared to control was due to pesticidal stress. More research should be performed to conclude the lethal and sub-lethal impact of the particular pesticide used on the survival and performance of non-target invertebrate and vertebrate animals including fish health.

Keywords: *Channa punctatus, Labeo rohita* Pesticide, Malathion, Protein Profile, SDS-PAGE, Intestine Tissue

1. INTRODUCTION

The increasing use of pesticides in agriculture including commercial and household production of vegetables for the control of pests causes chemical pollution of aquatic environment. The chemical pollution causes potential health hazards to live stock, especially to fish, frogs, birds and mammals (K.Sunitha et al., 2010). Pesticides are frequently applied to agricultural commodities to enhance quality and quantity of food. The unrestricted, heavy use of synthetic chemical pesticides results in deleterious effects, odour of water, taste, lethal effect on various non-target organisms in aquatic environment and direct or indirect effect to users (Kalavathy et al.,2001,Satyamoorthi et al.,2019, Kumaresan et al.,2019). Fish and various wildlife populations have been seriously affected by environmental pollutants. The frequent use of industrial chemicals and pesticides affect water bodies and soil. Also, pesticide affect mammals, fishes and birds have been reported previously by various research groups. The utilization of these pesticides has increased yield of crop and significantly reduced post-harvest losses (Ravichandran et al., 2017, Arasu et al., 2016, Arasu et al., 2917a, Arasu et al., 2017b, Ravichandran et al., 2016. Moreover, the continuous use of these pesticides causes adverse effect on human health. More than 1400 pesticides are currently in use worldwide, as

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herbicides, insecticides, infanticide and fungicides (Turinek et al., 2009). Among the synthetic pesticides, organophosphate ranked fist than other pesticide consumption (AI-Sharbati et al., 1998). Most of the used agrochemicals are not easily degraded but remain in the aquatic environment for considerable period of time, adversely affecting fishes and other aquatic fauna (Ramaswamy et al., 2007).The physiological and biochemical alterations observed in an animal under any physiological stress can be correlated with the structural and functional changes of cellular proteins. Proteins occupy a unique position in the metabolism of cell because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways (Lehninger, 2008; Harper, 2006).Current agricultural practice compelled the utilization of pesticides in a large scale with the hope of high crop yield, resulting in pushiness of these organics in the food chain causing detrimental effects on biomarkers of aquatic fauna (Yu Zhang et al., 2012)One of the most widely used methods in many scientific fields, including molecular biology, biochemistry, forensic sciences, etc., is sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), which can separate proteins on a gel based on the length of their polypeptide chains. As a result, SDS-PAGE is a frequently used technique in many disciplines to categories proteins according to their electrophoretic mobility. SDS-PAGE analysis is a crucial indicator for toxicological investigations on fish, according to Muhammad (2018). Protein electrophoresis has been successfully employed in numerous studies to identify intra-and inter-specific variation among species.

2. MATERIALS AND METHODS

Preparation of SDS -polyacrylamide gel and Preparation of Samples

The samples were then subjected to SDS-PAGE under 6% stocking gel and 10 % separating gel (Laemmli, 1970).Electrophoretic study of protein patterns in intestine tissues of *Channa punctatus* and *Labeo rohita* was carried out in the above mentioned tissues.

Preparation and Casting of Gel.

The gel plates were assembled according to the manufacture's instruction and the volume of the gel mould, was determined. In a conical flask, the acryl amide mixture for 10% resolving gel was prepared (10ml acryl amide + 7.5ml resolving gel buffer, pH 8.8 + 12.3 ml distilled water + 150 µl ammonium per sulphate -freshly prepared (10% stock and 50 µl TEMED (N,N,N,N' Tetra Methylene diaminine).The components were mixed and without delay the acryl amide mixture was poured into the glass mould till the lower mark. A layer of distilled water or iso propyl alcohol was overplayed to facilitate proper polymerization. After polymerization (30 minutes) the layer was removed and the gel top was washed with distilled water. Stocking Gel6% stocking gel was then prepared using 2ml acryl amide (30 % stock) + 3ml stocking gel buffer (pH 6.8) + 4.9 ml distilled water + 75µl APS (10% stock) + 25 µl TEMED. The components were mixed properly and poured over the resolving gel, the comb was immediately inserted and the gel was allowed to polymerize.

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Loading of Sample

Intestine tissue samples of *Channa punctatus* and *Labeo rohita* were prepared using required volume of sample (100 µg protein / lane) + equal volume of sample buffer (7.25 ml distilled water + 1.25 ml stocking gel buffers + 1ml Glycerol + 0.5ml ß mercaptoethanol + 150 mg SDS and a pinch of Bromophenol blue).The samples were heated in boiling water bath for 2 minutes. Denatured the protein and kept on ice to retain the denatured stage. The comb was then removed from the mould and the wells were washed with distilled water. The gel was mounted on electrophoretic apparatus. Electrophoretic buffer (Tris 3gm, Glycine 14.4gm and SDS 1gm in 1000ml distilled water, pH 8.3) was added to the top and bottom reservoir of the electrophoretic apparatus. The samples were loaded along with marker proteins into the lanes/ wells).Electrophoresis The apparatus was attached to power supply unit, 8v/cm for gel (70v) and 15v/cm for resolving gel (150-200 v) was applied. The electrical contact between the two buffer tanks was through the slab gel, care was taken to avoid air bubbles, while adding electrode buffer in the tanks as the air bubbles, inhibit electrophoretic mobility. The gel was run until the Bromophenol blue dye reached the bottom of the resolving gel. The power supply was turned off and the gel was removed from the sandwiched plates from the apparatus and placed on a paper towel. The plates were removed using a spatula and the orientation was marked. The temperature for electrophoresis was kept, constant in an air conditioned room at 25oC Staining and Destaining. This Gel was immersed in 5 volume of staining solution (200 mg Coomassie Brilliant Blue R + 250 + 50 MeOH+ 7ml Acetic acid solution + 30 ml MeOH + 63 ml distilled water) every half an hour. This was followed by two to three washes. The gel was then stored in 7% Acetic acid solution. When visualized under the illuminated, the protein zones were visible as dark blue bands after 24 to 48h of destaining. The results were recorded by observing the relative electrophoretic mobility of protein zones for each sample and the run was repeated for the samples that did not show clear cut zones. The protein profiles of the gel obtained out of the prepared cell-lysates are manually observed and compared with the various protein bands in the standard, control and the treated. Subsequently treated with staining and de-staining solution and identified the additional bands with varied KDa protein.

3. RESULTS

Intestine tissue of ***Channa punctatus*** had shown 07 protein bands in control with Rm value 0.06, 0.16, 0.40, 0.64, 0.80, 0.90 and 0.99. At 24H this tissue showed 07 protein bands with Rm value 0.06, 0.34, 0.50, 0.60, 0.73, 0.83 and 0.95. At 48H it showed 04 protein bands with Rm value 0.03, 0.23, 0.64, and 0.99. At 72H tissue shown 03 protein bands with Rm value 0.34, 0.88 and 0.99 and at 96H tissue exposed 03 protein bands with Rm value 0.71, 0.85 and 0.91.The protein band with Rm value 0.06 was seen in control and only at 24H and affected this protein band at48H, 72H and 96H. The protein band with Rm value 0.16 and 0.40 were seen in control, but not appeared at any time interval of 24H, 48H, 72H and 96H. The protein band with Rm value 0.64 was exhibited in control and only at 48H. While protein bands with 0.99, 0.64, 0.33 and 0.23 appeared only at 48H. The protein band with Rm value 0.99 was exhibited in control and at 48H, 72H of Malathion exposure. It reveals that Malathion toxic stress was high on Zone –A and Zone-B proteins i.e. low molecular weight and intermediate

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molecular weight proteins. It was also observed that intestine exhibited Malathion toxic stress opposing new protein bands at 24H, 04 bands with Rm value 0.60, 0.73, 0.83, 0.95. At 48H 02 bands with Rm value 0.03, 0.23. At 72H 01 band with Rm value 0.88, at 96H 03 bands with Rm value 0.71, 0.85, 0.91.

And the Intestine tissue of ***Labeo rohita*** had shown 09 protein bands with Rm value 0.07, 0.14, 0.35, 0.45, 0.59, 0.71, 0.78, 0.85, 0.99. At 24H tissue had shown 07 protein bands with Rm value 0.01, 0.23, 0.34, 0.64, 0.82, 0.89, 0.99. Among these protein bands, the bands with Rm value 0.01, 0.23, 0.34, 0.64, 0.82, 0.89 were newly expressed which were not shown in control. At 48H tissue exhibited 05 protein bands with Rm value 0.14, 0.38, 0.71, 0.85, 0.95. While the Rm value 0.38, 0.85, 0.95 a new protein bands were appeared. At 72H tissue shown 04 protein bands with Rm value 0.10, 0.34, 0.64, 0.99. While protein bands with Rm value 0.10 was a new protein band. At 96H tissue exposed 02 protein bands with Rm value 0.64, 0.99. The protein band with Rm value 0.03 (slow moving Zone –A with known MWt: 100-55 KDa) was not identified in control and in dose exposure. The protein band with Rm value 0.14 (Zone-A with MWt: 100-55 KDa)) was appeared in control and 48H and absent in 24H, 72H and 96H. The protein band with Rm value 0.23 (intermediate moving Zone –B with Molecular weight: 55-35 KDa) was showed at 24H and absent in 48H, 72H, 96H. One protein band with Rm value 0.34 (Zone –B, MWt: 55-35 KDa) was shown at 24H, 72H and not appeared at 48H, 96H. A protein band with Rm value 0.50 (Zone-B: MWt: 55-35 KDa) was totally vanished when exposed to Malathion. The protein band with Rm value 0.64 (Zone-C; MWt: 35-15 KDa) was not appeared in 48H. Another protein band with Rm value 0.99 (Zone-C: MWt: 35-15) was identified in control, and except 48H. Hence Malathion shown high toxicity upon Zone-A and Zone – B proteins.

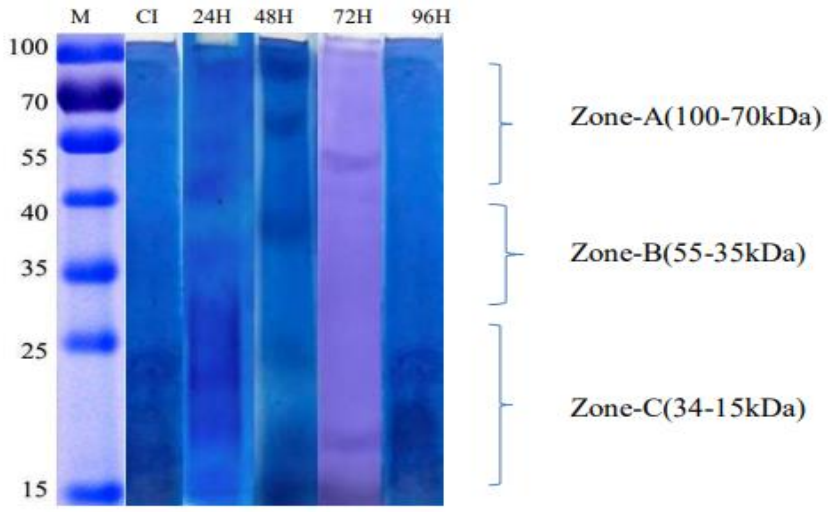

Fig 3:Intestine tissue of ***Channa punctatus*** exposed Protein bands in Different time intervals after Organophosphate exposure

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Table 1: Intestine tissue of *Channa punctatus* exposed protein bands molecular weight in kDa at different time intervals of Malathion expose

MARKER	CONTROL	24H	48H	72H	96H
0.03			0.03		
	0.06	0.06			
0.14					
	0.16				
0.23			0.23		
0.34		0.34		0.34	
	0.40		0.41		
0.50		0.50			
		0.60			
0.64	0.64		0.64		
		0.73			0.71
	0.80	0.83			0.85
	0.90	0.95		0.88	0.91
0.99	0.99		0.99	0.99	

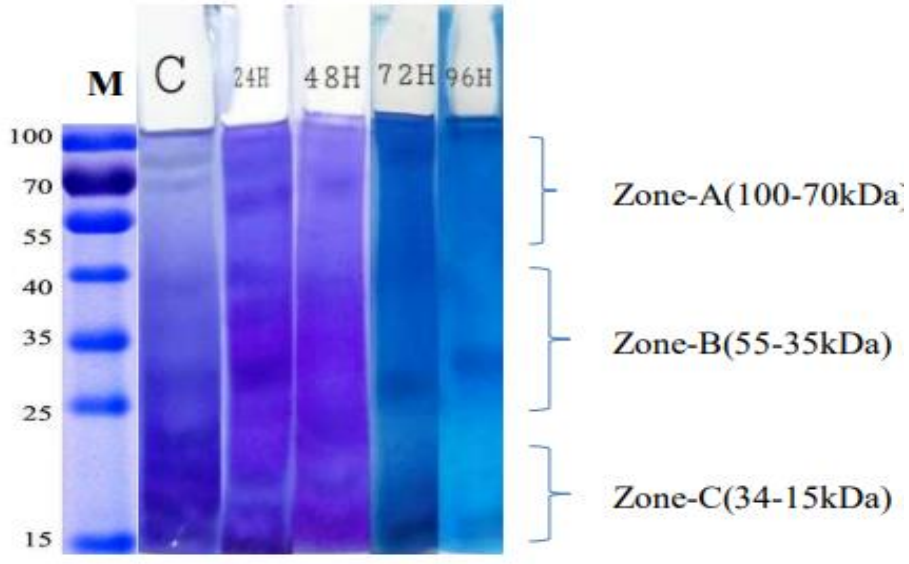

Fig 3: Intestine tissue of ***Labeo rohita*** exposed Protein bands in Different time intervals after Organophosphate exposure

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Table 2: Intestine tissue of *Labeo rohita* exposed protein bands Molecular weight in kDa at different time intervals of Malathion expose

MARKER	CONTROL	24H	48H	72H	96H
0.03		0.01			
	0.07			0.10	
0.14	0.14		0.14		
0.23		0.23			
0.34	0.35	0.34		0.34	
			0.38		
	0.45				
0.50					
	0.59				
0.64		0.64		0.64	0.64
	0.71		0.71		
	0.78	0.82	0.85		
	0.85	0.89	0.95		
0.99	0.99	0.99		0.99	0.99

4. DISCUSSION

Muhammad et al., 2018, used SDS-PAGE analysis as an important biomarker for various toxicological studies in fishes. Protein is an indispensable constituent required in tissue building and is important source of energy during chronic conditions of stress (Remia et al., 2008). Bakthavalsalam (1980) and Ramani (2001), reported decline in protein content of different fish organs exposed to pesticides. The inhibition in synthesis of proteins might be due to tissue necrosis which leads to loss of intracellular enzymes or other proteins (Jyothirmayee et al., 2005).Inhibition/activation of genes by the pesticides also might result in synthesis of stress induced proteins (Suneetha et al., 2010).Our results coincides with J.Helan et al., (2015).Jyothirmayee, S.,(2006) ,Tripathi and Shukla (1990a, 1990b).It is well documented that pesticides alter the total protein content in different tissues of fish (Ahmad, 2012).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; Muratet 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).

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Our result is in consistent with Martinez, Raynard, Bernard, and Chapman (2011), who worked on *Clarius batracus,* Karuppasamy, 2000 who researched on Phenyl mercuric acetate (heavy metal) induced low protein level in muscles and liver of *Channa punctatus* and Cypermethrin exposure resulted in significant decrease in protein in endangered cyprinid fish Tor putitora (Ullah et al., 2014) and *Colisa fasciatus* (Singh & Singh, 2010). A pesticidal mixture used against *Clarias batrachus* induced changes in protein content (Jha & Verma, 2002). Bibi et al. (2014) proposed decreased protein contents in *Cyprinus carpio* due to karate. In another study, monocrotophos declined lipid, protein and carbohydrate content in *Labeo rohita* (Muthukumaravel, Sivakumar, Kumarasamy, & Govindarajan, 2013).Present investigation clearly shows that a high energy demand is the reason behind enhanced breakdown of proteins in blood thereby reducing the serum proteins' content. It is also found that a high proteolytic activity or increased production of protease enzyme or low protein genesis could cause decrement in protein content in tissues of fish under stress. David, Mushigeri, Sivakumar, & Philip (2004) also demonstrated the similar remarks in *Cyprinus carpio* and Oreochromis mossambicus exposed to Cypermethrin and λ-cyhalothrin respectively. The present research results are in consonance with Venkateswara Rao et al., 2023, Venkateswara Rao et al., 2023, Venkateswara Rao et al., 2023, Venkateswara Rao et al., 2023.

5. CONCLUSION

The present study reports that the variability of patterns of protein describes the electro morphs of an individual. It can be conclude that each tissue has specific protein banding pattern which may be used for the development of genetic molecular marker for proper identification of fish species.

6. Acknowledgements

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7. Conflict of interest

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

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