Effect of Malathion (an Organophosphate) Electrophoretic Banding Patterns of Protein in Gill and Liver Tissues of Fresh Water Fish *Channa Punctatus* (Bloch)

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ABSTRACT

The present study was carried out to determine the effect of Malathion (an organophosphate) on electrophoretic patterns of proteins in the gill and liver tissues of freshwater fish *Channa punctatus* (Bloch). The fish were exposed to 2% sub lethal concentration of pesticide Malathion at different time intervals i.e. 24 h; 48 h; 72 h and 96 h for a period of 10 days. The changes in the tissue proteins of vital organs such as gill and liver were examined on 7.5% of SDS –PAGE. The protein patterns indicated that the gill tissue has higher number of Electrophoretic protein bands compared to liver tissue and control. Gill tissue shown 9 protein bands in control. After exposed to Malathion (Organophosphate) gill tissue exhibited 8 bands at 24h, 6 bands at 48h, 4 bands at 72h and 2 bands at 96h. And the liver tissue showed 8 protein bands, after exposed to Malathion (OP) 8 protein bands at 24h, 5 bands at 48h, 4 bands at 72h and 2 bands at 96h. The results shown that the number of protein bands and their intensity were decreased in gill and liver of test fishes than in control the protein banding patterns were identified by standard marker protein and Rm values were calculated.

Keywords: Electrophoretic Protein patterns, gill and liver tissue, Malathion, SDS –PAGE, Rm value, *Channa punctatus.*

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1. INTRODUCTION

Pesticides have brought tremendous benefits to mankind by increasing food production and controlling the vectors of man and animal diseases. At the same time use of these pollutants has posed potential health hazards to the life of fishes (Malla FA 2009). Pesticides become part of the water column and fish ingest the pesticides, usually through their gills, although sometimes through their scales (Aktar et al., 2009). The pesticides diffuse into their organs and fat tissues and sequestered there cause severe alterations in the tissue biochemistry and histology of fishes (Raj S.J 2015 et al., Muthukumaravel, K. 2013). Fish is highly nutritious, easily digestible and much sought after food. Nutritional value of fish depends on their biochemical composition which is affected by water pollution (Gehan H.Fahmy 2012).Fishes are the major component of aquatic fauna, and chief

source of protein, carbohydrate and fats for humans and domestic animals (P.K.Tripathi *et al*., 2003; Prado, *et al*., 2009).

Fishes are the excellent models for monitoring environmental contamination in aquatic system (G. R. Scott *et al*., 2004; S. C. S. Shinde, 2007). Fishes are very sensitive to a wide variety of toxicants and to the changes in their aquatic environment. Various species of fish show uptake and accumulation of many toxicants such as pesticides (Herger, W; Jung S.J.1995). For this reason, they are known as the bio-indicator species to monitor the water pollution. Organophosphate pesticides are widely used amidst various group of pesticide in intensive agricultural practices to protect the crops from various pest and diseases owing to their high insecticidal property, low mammalian toxicity, low persistence and rapid biodegradability in the ecosystem. Malathion (C10H19O6PS2), one of the earliest organophosphate insecticides is being extensively used as dust, emulsion, and vapour to control wide variety of insect pests under different conditions. Malathion, one of the most extensively studied pesticides, may induce many significant changes in fish i.e., toxicological effects on haematological parameters, physical parameters, biochemical parameters, behavioral changes, neurotoxic, histopathological alterations, respiratory responses, bioaccumulation and chromosomal changes in fishes exposed to the organophosphate pesticide Malathion.

Fish is a good source of protein, lipid and also vitamins particularly Vit-'A' hence becomes a valuable form of food for growing population and can also play an important role on checking malnutrition and undernourishment in developing countries such as India. The fish is rich in essential amino acids and can complement or fulfill the overall protein quality that is required in mixed diet (Lailith Pathak *et al*., 2015; FAO, 2005; Louka, *et al*., 2004; Dempson, *et al*., 2004). Fish being an important component of aquatic ecosystem found to have a high BV and PER (Biological value & protein efficiency ratio (P.K.Tripathi *et al*., 2003; Prado, *et al*., 2009) and its nutritional value depends upon the 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). It was also identified that gill of *C. 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. As gene Controlled proteins form the structural basic source of genetic information at various levels of species organization. Electrophoresis of proteins has been widely applied for direct study of genetic variation in fish population and identification of genetic stocks of Commercially important fishery resources. Tripathi and Shukla, (1990) performed that the SDS-PAGE of the cytoplasm protein fractions of the liver and the skeletal muscle of C*larias batrachus* exposed to Endosulfan and Methyl parathion for 1 to 28 days and observed the appearance of new protein bands at different time intervals after the exposure of the pesticide.

In the present investigation an attempt has been made to study the effects of Malathion (an organophosphate) on electrophoretic banding patterns of proteins in gill and liver tissue of fresh water cat fish *Channa punctatus* through SDS-PAGE.

2. MATERIALS AND METHODS

2.1 Collection of Samples and preparation of OP compound

Adult fishes (weighed about 50-70gms) were collected from local fresh water tanks within the radius of 15km from the laboratory by the netting with the help of local fisher men. They were immediately brought to the laboratory and introduced into a plastic bucket (30X30X60cm) and disinfected with Potassium permanganate and washed thoroughly prior to introduction of fish (to prevent fungal infection). The fishes were acclimatized for about one week in aquaria they were fed with commercial fish food daily until their use. The sub lethal concentrations of the pesticide Methyl parathion (2% E.C) were prepared in 95%

Acetone to yield a concentration of 100mg/ ml which were further diluted with distilled water to get a working solution prepared by the procedure.[APHA] In the present investigation sub lethal concentration of 0.01, 0.04, 0.06 µg of pesticide were taken for 24h,48h,72h and 96 h exposure. A control batch corresponding to each test group was simultaneously experimented to compare the toxicated effect of Methyl parathion in various tissues.

2.2 Preparation of Samples for study

At the end of each exposure period fishes were sacrificed, the tissues such as gill and muscle were dissected out and were used for the further analysis. The tissues were weighed to nearest milligram and were homogenized in 0.01M Tris HCl buffer (pH 7.5) containing 0.9% NaCl. The concentration of tissue homogenates varied from tissue to tissue. The tissues after homogenation were placed in ice jacketed centrifuge tubes. The extracts were centrifuged at 2000rpm for 10min in a clinical centrifuge at room temperature. The supernatant were mixed with equal volume of 20% sucrose solution containing 0.5% bromophenol blue as tracking dye, an aliquot of 0.1ml of this mixture was used for loading the sample on to the gel for electrophoretic separation of protein patterns.

2.3 SDS-PAGE Analysis

Homogenates (10%) of gill and muscle were prepared in Tris-HCl buffer (pH 7.2) and centrifuged at 10,000 rpm for 10min. The pellet was washed with chilled acetone and was dissolved in sample buffer 2ml of 0.5M Tris HCl (pH 6.8), 40% glycerol (1-6ml), 10% SDS (3.2ml), 2-mercaptoethanol (0.8ml), 0.1% (W/V) bromophenol blue (0.4 ml) and heated at 950c for 1min.

2.4 Experimental procedure for preparation of SDS-PAGE

The supernatants were mixed with equal volumes of 20% sucrose containing 0.1% SDS, β-mercaptoethanol and bromophenol blue was used as the tracking dye. An aliquot of 0.1ml (5mg) of the tissue extract was loaded on to the separating gel directly. The electrode buffer 0.025M tris and 0.192M Glycine was used for according to standard procedure [Laemmli, U.K.,1970] whereas 0.074 M Tris, 0.1% SDS adjusted to pH 7.8 with con. HCl. A constant current of 50 volts for the first 15

min followed by 150 volts for the rest of the run was applied to the gel. The current supply was terminated when the tracking dye migrated to a distance of 8.0 cm from the origin.

2.5 Staining Procedure and standardization of protein bands

A solvent containing 0.25% Coomassie brilliant blue in methanol, water & acetic acid (5:5:1) was used for staining the proteins separated on gel by using standard method.[Holmes and Master 1967]. The molecular weight standards used in comparing the variations noticed in the SDS-PAGE, were of Low molecular weight protein standards (15 to 100 KDa) from the SIGMA-Chemical company (USA).

3. RESULTS AND DISCUSSION

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

3.1 Gill

When gill tissue of *Channa punctatus* is exposed to an Organophosphate pesticide, Malathion at different time intervals i.e. 24H, 48H, 72H and 96H the following electrophoretic protein banding patterns were observed on SDS-PAGE stained with Coomassive brilliant blue (Table-1 and figure-1, Table-2 and figure-2).

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.

3.2 Liver

Liver tissue of *Channa punctatus* has shown 08 protein bands in control with Rm values 0.03, 0.06, 0.23, 0.34, 0.64, 0.69, 0.88 and 0.99. At 24H tissue showed 08 protein bands with Rm values 0.03, 0.06, 0.19, 0.23, 0.34, 0.50, 0.69, 0.80. And at 48H 05 protein bands with Rm values 0.03, 0.06, 0.40, 0.69 and 0.99. While at 72H 04 protein bands with Rm values 0.03, 0.14, 0.64 and 0.99 (D65024548), at 96H only 02 protein bands with Rm values 0.64 and 0.99. The results showed in Table.2.A and fig. 2A The protein band with Rm value 0.03 (Zone-A nearer to molecular marker 100-70 KDa) which was observed in control and except at 96H. The protein band with Rm value 0.06 was observed in control and at 24H and at 48H. The same protein bands were absent at 72H and 96H. The protein band with Rm value 0.23 and 0.34 (Zone –B nearer to molecular marker 55-35 KDa) were observed in control and only at 24H, not seen at 48H, 72H and 96H.

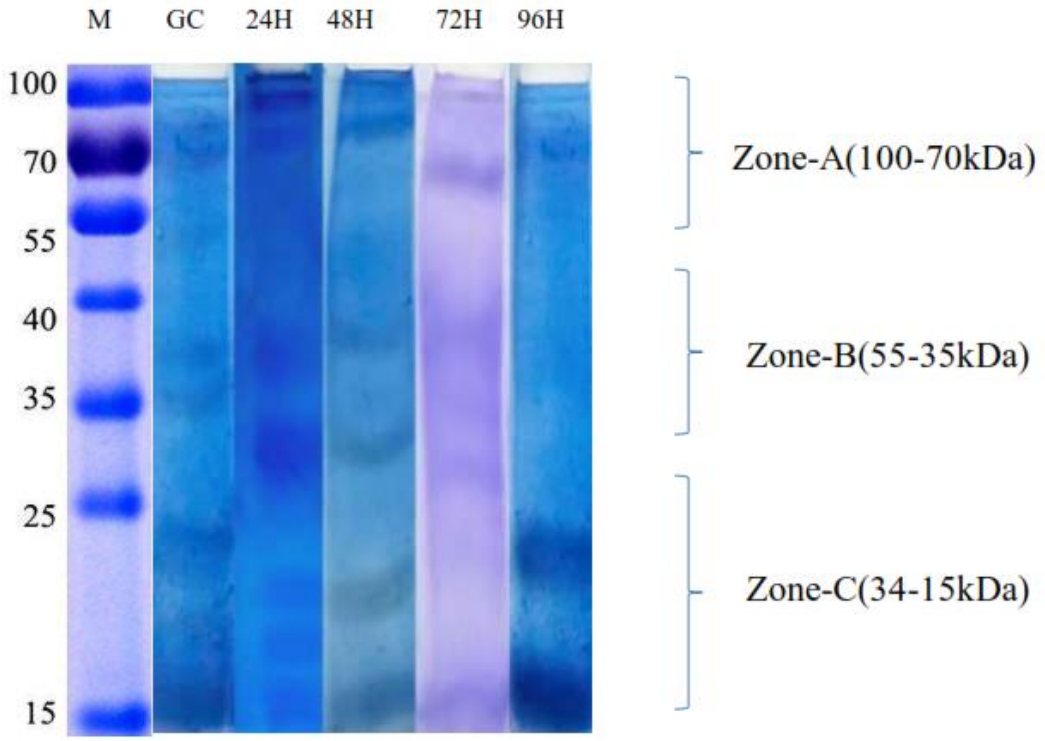

Figure-1. Gill tissue of *Channa punctatus* (Bloch) exposed protein bands in different time intervals after Organophosphate Exposure.

The protein band with Rm value 0.64 (wjpr.net) (Zone –C nearer to molecular marker 34-15) was observed in control and at 72H and96H only. It was affected at 24H and 48H. The protein band with Rm value 0.69 was observed in control, at 24H and 48H only. This reveals that this protein band was influenced at 72H and 96H. The protein band with Rm value 0.88 was identified only in control and not formed at 24H, 48H, 72H and 96H. The protein band with Rm value 0.99 (Zone –C nearer to molecular marker 34-15) was found in control and at 48H, 72H and 96H. Affected this protein band only at 24H. Another protein band with Rm value 0.14 (Zone-A near to molecular marker 100-70)

was appeared only at 72H. This investigation reveals Malathion affected more on Zone-A and Zone-B proteins i.e. high and intermediate molecular weight proteins in liver tissue. Liver tissue of *C. punctatus* expressed Malathion stress inhibiting new electrophoretic protein bands. At 24H 02 bands with Rm value 0.19, 0.80 were exposed. At 48H 01 band with Rm value 0.40 was shown.

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

Table-1: Rm values exhibiting Effect of Malathion (An Organophosphate) On Electrophoretic Banding Patterns of Protein in Gill tissue of *Channa punctatus* (Bloch)

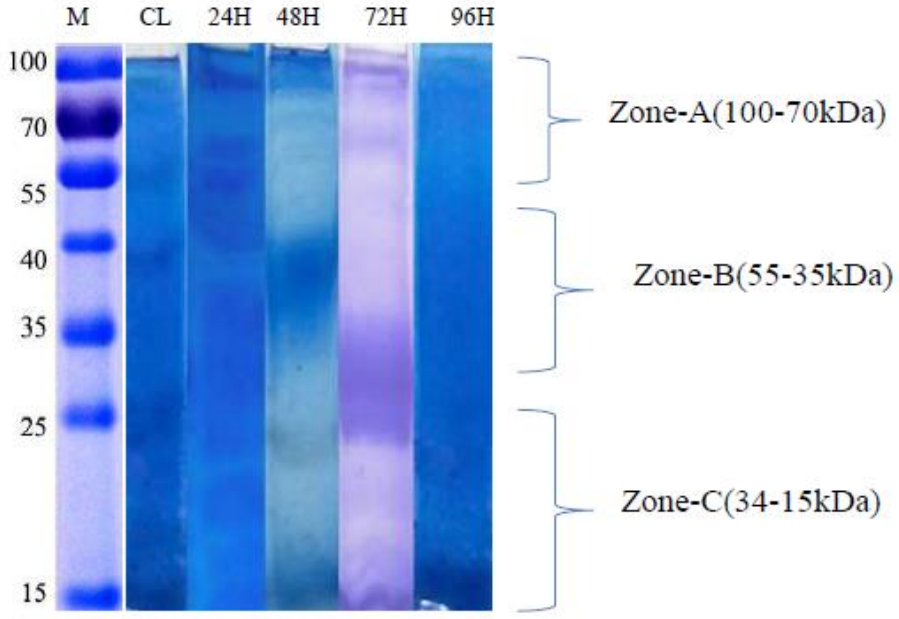

Figure-2. Liver tissue of Channa punctatus exposed protein bands in Different time intervals after Organophosphate exposure

MARKER	CONTROL	24H	48H	72H	96H
0.03	0.03	0.03	0.03	0.03	
	0.06	0.06	0.06		
0.14		0.19		0.14	
0.23	0.23	0.23			
0.34	0.34	0.34			
			0.40		
0.50		0.50			
0.64	0.64			0.64	0.64
	0.69	0.69	0.69		
		0.80			
	0.88				
0.99	0.99		0.99	0.99	0.99

Table-2: Rm values exhibiting Effect of Malathion (An Organophosphate) On Electrophoretic Banding Patterns of Protein in Liver tissue of *Channa punctatus* (Bloch

Protein are the building blocks of the body. They serve as the reserve source of energy. These are the primary effector molecules of all living systems and any adaptive responses to environmental, physiological (or) pathological conditions will be reflected by alterations in protein activity (VJ Florence Borgia *et al.*,2019).Pesticides may inhibit the expression of some genes (or) may activate the others to produce specific mRNAs, which may subsequently be translated into specific proteins called stress induced protein.(Ramadan A 2007., Sandal S. *et al*., 2011., Ksenia Cheshenko *et al*.,2008., Murat S.,et al., 2009). 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. An alteration of protein metabolism was observed in fish exposed to various types of environmental stress like materials and pesticides (Swetha and Gopal 2009; Alexandria *et al*., 2009). The appearance of new protein bandsat different time intervals after exposure of the pesticide demonstrated clearly the alterations in the cytoplasmic protein patterns (Tripathi and Sukla 1990a; 1990b); (Justin Raj *et al.*,2017).

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 (Frigo, D.E.,et al., 2004., Cheshenko, K.,et al., 2008., Senturk, M., et al., 2009).

An alteration of protein metabolism was observed in fish exposed to various types of environmental stresses like metals and pesticides (Agrahari et al., 2009).. Similar trend was reported in the serum proteins of *Channa punctatus* under chronic exposure to organophosphorus and organochlorine insecticides in study by Sahai et al.,(1990) and Ravinder *et al* 1988 in the catfish *Clarias batrachus* exposed to Desis 2.8 E C. In a study by Rita *et al* the fishes (O. *mossambicus)* were exposed to various concentrations of the carbamate pesticide methomyl for different durations revealed a definite pattern of variation in protein fractions.

Studies on *Clarius batrachus* under sub lethal malathion exposure revealed variations in serum proteins, this may be due to the alterations of protein mobility by malathion binding (Mukhopadhyay, P.K. et a., 1980), Kumar and Devi 1992, demonstrated, that malathion, showed profound effect on the protein pattern of *Heteropneustes fossilis* and found new electrophoretic protein bands and some others disappeared after the treatment. Our results coincides with the studies of BheemRao et al., 2018 on effect of Methyl Parathion (OP) on electrophoretic banding patterns of protein in gill and muscle tissue of *Heteropneustes fossilis* (Bloch) and Bheem Rao et al., 2022 on Impact of Methyl Parathion (an Organophosphate) on Electrophoretic protein banding patterns in gill, liver and brain tissues of Fresh water cat fish *Heteropneustes fossilis* (Bloch). And These observations are in agreement with the studies of Dhar and Chatterjee, (1984) in *Channa punctatus* on treatment with pesticides, the electrophoretic patterns of serum proteins has resulted in depletion of several protein fractions and appearance of some new fractions. Many authors reported that the similar observations (Veeraiah *et al*., 2014) were observed the appearance (or) disappearance of some proteins fraction in the tissues of different fishes like *H. fossilis*. Badaway *et al.*, 1998, reported the electrophoretic serum proteinograms of *Clarias gariepinus*. The Fenvalrate induced toxicity on digestive enzyme such as proteinase andamylase of Zebra fish (Justin Raj and Baby Joseph 2014), reported the impact of textile dyes and Acetamiprid toxicity on electrophoretic patterns in liver, brain and gill tissues of fish*Oreochromis mossambicus*, Jyothirmayee *et al*., (2006) studied the alterations in the serum electrophoretic profile of the edible fish *Anabas testudineus* and *Clarias batrachus*. Earlier workers reported that the impact of exposure of different pollutants on the tissues of fishes (Riz kalla *et al*.,2006; Veeraiah *et al*., 2004; Prasad et al., 2014; Arivee *et al*., 2015; V. J. Florence Borgia *et al*., 2019; Yanamala *et al*., 2018; J. Helanchandra *et al*., 2015).

4. 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.

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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