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METHYL PARATHION (50% EC) INDUCED CHANGES IN PROTEIN AND DNA BANDING PATTERNS IN THE FISH *CHANNA PUNCTATUS* (BLOCH)

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Abstract: The acute toxicity tests for 24, 48, 72 and 96 h to the fish *Channa punctatus* were conducted with an organophosphate pesticide methyl parathion 50% EC in both static and continuous flow-through methods. The LC_{50} values obtained for static test were 0.6661, 0.6473, 0.6200 and 0.5949 ppm respectively for static and 0.5736, 0.5592, 0.5364 and 0.521 ppm respectively for continuous flow-through method. The $1/10^{th}$ of static 96 h LC_{50} value was taken as sub-lethal concentration and the fish were exposed to both lethal and sub-lethal concentrations for 8 days and the vital tissue of the fish *viz*., gill, liver, brain, muscle and kidney were isolated and analyzed for study of changes in protein and DNA banding patterns. The Rm values were derived from the electrophoretogram along with the marker. The changes in Rm values indicate that marked variations were taken place in both the protein and DNA bands. The changes in lethal exposure were more prominent compared to sub-lethal exposure. These changes were attributed to the intoxication of the test toxicant methyl parathion. The results obtained were discussed with the available literature.

Key Words: Methyl Parathion, *Channa punctatus*, LC_{50}, Protein, DNA and Electrophoresis

INTRODUCTION

Pesticides are used extensively throughout the world, for protection of crops, public health, for the achievement of increased production of food and fiber in meeting the demand of escalating population as well as control of vector-borne diseases. Indiscriminate use of toxic pesticides coupled with a weak legislative framework is one of the major reasons for high incidence of pesticide poisoning in developing countries (Konradsen *et al.,* 2003 and Remor *et al.,* 2009). These pesticide residues were causing numerous problems with reference to their persistence and metabolism in aquatic bodies associated with the effects on non-target organisms are of great concern. Currently, organophosphate (OP) pesticides are the most commonly used of all the insecticides representing 54% in India. Methyl parathion (*o, o*-dimethyl *o-p*-nitrophenol phosphorothioate, MP) is an important broad spectrum non-systemic organophosphate insecticide (Kidd & James 1991) and used increasingly in agriculture and public health management as an effective replacement of its ethyl analogue, parathion (*o,o*-diethyl *o-p*-nitrophenyl phosphorothioate), which has been banned in many countries because of its higher mammalian toxicity. According to USEPA (2006) the Methyl parathion has been shown to be moderately to highly toxic to freshwater fish and amphibians, causing chronic effects in fish at concentrations less than 80 ppb and affects aquatic invertebrates at less than 0.25 ppb.

MATERIALS AND METHODS

The test toxicant, Methyl parathion (*o, o*-dimethyl *o-p*-nitrophenol phosphorothioate, MP) is an important broad spectrum non-systemic organophosphate insecticide used extensively by the farmers (Kidd & James 1991).

The test fish, *Labeo rohita* (6.5 ± 0.5 cm and 7 ± 0.5 g) brought from a local fish farm were acclimated at 28± 2° C in the laboratory for 10days. Finney's probit analysis (Finney, 1971) was followed to calculate the LC_{50} values. All the precautions laid down by APHA (1998) were followed. The acclimated fish were exposed to sub-lethal concentrations ($1/10^{th}$ of static 96 h LC_{50} value) of commercial grade methyl parathion 50% EC formulations for 8 days. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the analysis of proteins and DNA. The separation of protein and DNA was done based on their molecular weight. The quality and quantity of DNA was analyzed by using 1% agarose gel electrophoresis method (Lammelli, 1957).

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.

$$\text{Relative mobility (Rm)} = \frac{\text{Distance traveled by individual subunit}}{\text{Distance traveled by the marker dye}}$$

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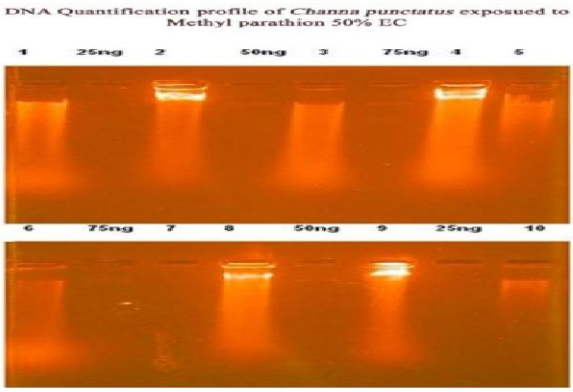
RESULTS AND DISCUSSION

The LC_{50} values of Methyl parathion 50% EC for the fish *Channa punctatus* for 24, 48, 72 and 96 h in static exposure are 0.6661 mg/L, 0.6473 mg/L, 0.6200 mg/L and 0.5949 mg/L and in continuous flow through method are 0.5736 mg/L, 0.5592 mg/L, 0.521mg/L and 0.5364 mg/L respectively. In general, *Channa puntatus* is sensitive towards the test toxicant. These findings are in agreement with Phipps and Holcombe (1985) for channel catfish *Lctalurus punctatus*, Ferrando *et al.,* (1991) for *Angulla angulla,* Holcombe *et al.,* (1982) for *Pimephales promelas*, Phipps and Holcombe (1985) for *Pimephales promelas* and *Carrasius auratus* and Ferguson *et al.,* (1966) for *Gambusia affinis*.

In the electrophoreses analysis of DNA profile, seven base pairs were identified in lambda DNA marker with the Rm values of 0.16, 0.19, 0.22, 0.25, 0.31, 0.33 and 0.48. In the test tissue of gill, brain and liver two base pairs were traced with the Rm values of 0.56, 0.60 in gill, 0.10, 0.15 in brain and 0.55, 0.61 in liver respectively. Whereas in kidney three base pairs with the Rm values of 0.15, 0.55, and 0.60 were identified. No base pair was traced in muscle tissue of control and test organisms. All these bands were traced between 500 and 100 bps. Though the variations in Rm values are minute, these variations have led to depletion in nucleic acid levels of the tissues in exposed organisms.

In protein electrophoresis analysis, several changes were noticed in protein banding patterns of the exposed fish in comparison with those of controls. In protein electrophoretogram, the marker has six protein subunit bands with molecular weights of 116 KD, 66 KD, 45 KD, 35 KD, 25 KD and 18 KD with respective Rm values of 0.12, 0.19, 0.26, 0.39, 0.51 and 0.59. Among the control tissues, brain tissue has highest number of protein subunit bands (15) with different Rm values followed by muscle (13) kidney (11) gill (9) and liver (5) respectively (Fig.1). In control brain fifteen protein subunits were identified whereas in exposed brain only nine protein subunits were identified, out of which only five protein subunit bands matched with the control and the remaining protein Rm values were altered. In control muscle thirteen protein subunit bands were identified whereas, in exposed muscle eleven protein subunit bands were traced, among them eight were matched with the control and remaining protein subunit bands were disappeared. In control kidney eleven protein subunit bands were appeared whereas in exposed kidney five protein subunit bands were identified, out of which, only three bands matched with the control. In control gill nine protein subunits were identified with Rm values of 0.12, 0.13, 0.26, 0.28, 0.30, 0.36, 0.40, 0.43, 0.55 whereas in exposed gill six protein subunits were identified with Rm values of 0.04, 0.08, 0.16, 0.20, 0.26 and 0.29. When the Rm values of control and the exposed gill was compared only one Rm value (0.26) is matching and other Rm values were altered. In control liver five protein subunit bands were identified whereas, in exposed liver seven subunit bands were identified among them only two protein subunit bands were matched with the control.

Figure I: Changes in DNA Profile of the fish *C. punctatus* exposed to methyl parathion 50% EC



1. Control Brain 300 ng
2. Control Gill 500 ng
3. Control Kidney 250 ng
4. Control Muscle 350ng
5. Control Liver 300ng
6. Test Brain 150ng
7. Test Gill 100ng
8. Test Kidney 300ng
9. Test Muscle 250ng
10. Test Liver 300ng

25, 50, 75 ng Lambda DNA

The changes observed in the present study such as appearance or disappearance of base pairs in DNA and protein subunits may be due to the pesticide Methyl parathion intoxication. The inhibition or activation of physiological activities by pesticides is due to the interaction between the animal and the chemical nature of the pesticides. The stress induced biochemical changes are described as secondary responses of the fish. Nucleic acid and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating the different activities of cells. According to Holcombe *et al.,* (1988), the biochemical analysis of DNA, RNA and protein are considered as markers in the toxicity study.

In conclusion, the insecticide used in the present study appears to exhibit different modes of action and seems to be tissue specific. The significant modulation of the insecticide stress suggests that the compound might have direct or indirect interactions with corresponding genes in *C. punctatus*. As these bio-molecules are key regulatory elements, such changes induced by organophosphates might have serious affect on the physiological fitness of aquatic organisms. The alterations of the enzyme activities as observed in different tissues of the test toxicant

treated fish could serve as sensitive biochemical indicators of pesticide methyl parathion pollution in the aquatic environment, which might help in water quality control and management as well as fish productivity with respect to indiscriminate input of organophosphate pesticides from agricultural sites. Nucleic acid and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating the different activities of cells (ref). Their ratios also provide significant information about the way the mechanism by which, these contents regulate the multifaceted activities of cells. In this work, DNA contents were found to be increased in most of the tissues in response to sub-lethal and lethal exposures of methyl parathion. Jyothirmayee *et al.,* (2006) studied the impact of chromium and endosulfan, on the serum protein electrophoretic profile of two important edible fish *Anabas testudineus* and *Clarias batrachus* and reported that these toxicants were transported by serum proteins, and stored in liver, kidney and gill before being excreted. Earlier reports by Natarajan (1981) revealing the enlargement of nuclei in chloride-secreting cells in striatus exposed to Metasystox corroborates the above statement. Ural and Simsek (2006) has demonstrated alterations in the cytoplasmic protein pattern of fish *Clarias batrachus* by performing SDS-polyacrylamide gel electrophoresis of the cytoplasmic protein fractions of the liver and the skeletal muscle exposed to endosulfan and methyl parathion for 1 to 28 days. The drastic increase in the level of total DNA in liver in response to Methyl parathion might be due to increased thymidine uptake in the hepatic DNA, as reported earlier.

Tripathi and Shukla (1990a,1990b) has demonstrated alterations in the cytoplasmic protein pattern of fish *Clarias batrachus* by forming SDS-polyacrylamide gel electrophoresis of the cytoplasmic protein fractions of the liver and the skeletal muscle exposed to endosulfan and methyl parathion for 1 to 28 days. Kumar *et al.,* (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. Munshi (1999) stated that the fish *Heteropneustes fossilis* under exposure to malathion at sub-lethal concentrations for 24,48,72 and 96 h, showed the formation of three low and four high mobility fractions and the disappearance of some protein fraction at different periods of exposure.

Table I: The Rm values of the Electrophoretic DNA profile of the control and exposed tissues (gill, brain, kidney and muscle) of fish *Channa punctatus* exposed to Methyl parathion 50% EC

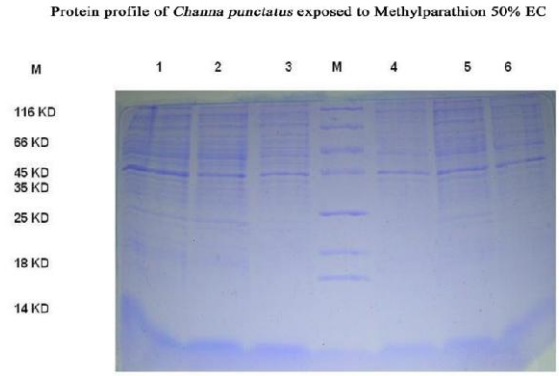
Marker (M)	Lambda DNA (L)	Test Gill (6)	Test Brain (7)	Test Kidney (8)	Test Liver (9)	Test Muscle (10)	Lambda Marker (LM)
0.46	0.05	0.56	0.10	0.15	0.55	-	0.16
0.54	0.14	0.60	0.15	0.55	0.61	-	0.19
0.66	0.18	-	-	0.60	-	-	0.22
-	-	-	-	-	-	-	0.25
-	-	-	-	-	-	-	0.31
-	-	-	-	-	-	-	0.33
-	-	-	-	-	-	-	0.48

Bands in 6,7,8,9 are between 500 bps and 100 bps.

Table II: The Rm values of the Electrophoretic DNA profile of the control and Methyl parathion 50 % EC exposed tissues gill, brain, kidney and muscle of fish *Channa punctatus.*

Control Brain (300ng)	Control Gill (500ng)	Control Kidney (250ng)	Control Liver (300ng)	Control Muscle (350ng)	Test Gill (100ng)	Test Muscle (250ng)	Test Kidney (300ng)	Test Liver (300ng)	Test Brain (150ng)
0.09	0.09	0.11	0.08	0.05	0.10	-	0.07	0.08	0.11
0.55	0.48	0.55	0.45	0.45	0.55	-	0.45	0.48	0.30

Figure II:

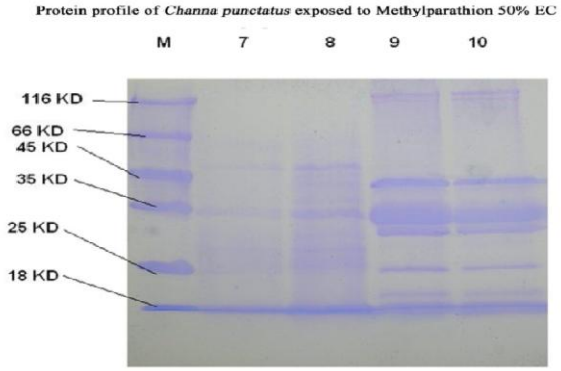


1. Control Gill
2. Control Brain
3. Control Kidney

M. Marker

4. Test Gill
5. Test Brain
6. Test Kidney

Figure III:



M. Marker

7. Control Liver
8. Test Liver
9. Control Muscle
10. Test Muscle

Table III: The Rm values of the Electrophoretic protein fractions of methyl parathion 50% EC in control and exposed Tissues (gill, brain, kidney and muscle) of *Channa punctatus.*

Marker	Con. Gill	Test Gill	Con. Brain	Test Brain	Con. Kidney	Test Kidney	Con. liver	Test Liver	Con. Muscle	Test Muscle
						0.02				
		0.04		0.04						0.04
			0.05							
									0.06	0.06
					0.07				0.07	
		0.08							0.08	
			0.10							
0.12	0.12		0.12	0.12						
	0.13					0.13				
			0.14	0.14						
			0.15			0.15				
		0.16	0.16	0.16	0.16					
			0.17		0.17					
			0.18		0.18					
0.19			0.19	0.19	0.19	0.19				
		0.20	0.20		0.20					
			0.21	0.21	0.21					
			0.22		0.22					
					0.23					
							0.24			
0.26	0.26	0.26		0.26		0.26				
			0.27		0.27	0.27				
	0.28									
		0.29		0.29			0.29			
	0.30				0.30					
			0.32					0.32		
									0.33	0.33
								0.35	0.35	
	0.36									
0.39										
	0.40			0.40				0.40		0.40
			0.41					0.41	0.41	0.41
									0.42	
	0.43								0.43	0.43
							0.44		0.44	0.44
								0.45	0.45	0.45
									0.46	0.46
									0.48	0.48
0.51									0.51	
	0.55						0.55	0.55		
0.59										

In conclusion, the insecticide used in the present study appears to exhibit different modes of action and seem to be tissue specific. The significant modulation insecticide stress suggests that these compounds might have direct or indirect interactions with corresponding genes in *C. punctatus*. As these bimolecular are key regulatory elements, such changes induced by pyrethroids might have serious bearing on the physiological fitness of aquatic organisms. The alterations as observed in different tissues of the insecticide treated fish could serve as sensitive biochemical indicators of pyrethroid pollution in the aquatic environment, which might help in water quality control and management as well as fish productivity with respect to indiscriminate input of pyrethroids from agricultural sites.

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