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Changes in protein subunits induced by endosulfan and fenvalerate in fresh water fish *Labeo rohita* through SDS-PAGE

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Abstract: *Fresh water fish Labeo rohita was exposed to two pesticides i.e., endosulfan an organchlorine and fenvalerate a synthetic pyrethroid. The 1/10th of 24 hr LC_{50} of endosulfan (0.0687 μ g l^{-1}) and fenvalerate (0.0 474 μ g l^{-1}) were selected as sub lethal concentrations. The fish were exposed to sublethal concentrations for one week and the changes in the tissue proteins of vital organs such as brain, liver, gill and muscle were studied under SDS-PAGE. The protein subunits were identified by running marker proteins parallel and R_m values were calculated accordingly. The changes in the protein banding pattern are more pronounced in the fenvalerate exposure than endosulfan.*

Key words: *SDS-PAGE, Electrophoretogram, R_m values*
PDF of full length paper is available online

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.

Endosulfan is an organocholroine insecticide widely used in agriculture to control pests on fruits, vegetables, tea and non food crops such as tobacco and cotton. In India, it has been identified as one of the main pesticides found in the waters of major rivers in a study conducted by central pollution control board of India (CPCB, 2000). In India alone, the agricultural consumption of endosulfan was estimated to be 5,200 metric tons in 1994-1995 (Shafiq-ur-Rehman, 2006). Although endosulfan increases crop yields by reducing agricultural damage, it may also be toxic to nontarget organisms like fish by altering the physiology, metabolism, behaviour and fecundity of fish, ultimately affecting the survival of the population (Tripathi and Verma, 2004; Altinok and Capkin, 2007).

Fenvalerate is one of the pyrethroid insecticide and most widely used in agricultural crops such as cotton, paddy, jowar, maize, soyabean, tomato, lady's finger, cauliflower, tobacco and tea. But the use of this insecticide also tend to affect the biology of non-target species along with pests (Veeraiah and Durga Prasad, 1998; Anita Susan *et al.,* 1999; Tilak *et al.,* 2003; Tripathi and Verma, 2004; Sakr *et al.,* 2005; Babuvelmurugan 2007; Ramaneswari and Rao, 2008; Majumdar and Gupta, 2009).

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Understanding of the protein components of cell becomes necessary in the light of the radical changes that take place in protein profiles during pesticide intoxication. Both the protein degradation and synthesis are sensitive over a wide range of conditions and show changes to a variety of physical and chemical modulators. 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).

In the present investigation an attempt has been made to study endosulfan (35% EC) and fenvalerate (20% EC) induced changs in protein submits the fresh water fish *Labeo rohita*.

Materials and Methods

The freshwater fish *Labeo rohita* (Hamilton) is an edible and commercially valuable fish. Live fish of size 6-7 ±1cm and 6-8 g weight were brought from a local fish farm and acclimatized at 28 ± 2°C in the laboratory for one week. The stock solutions for endosulfan 35% emulsifiable concentrate (EC) and fenvalerate 20% emulsifiable concentrate (EC) were prepared in 95% acetone to yield a concentration of 100 mg 100 ml^{-1} which were further diluted with distilled water to get a working solution. The water used for acclimatization and conducting experiments was clear unchlorinated ground water.

In the present investigation, sub lethal concentration 0.06876 and 0.04749 µg l^{-1} of 24 hr LC_{50} value of two pesticides were taken for exposure for one week, simultaneously another batch of fish in fresh water served as the control.

SDS- PAGE analysis: 10% homogenates of brain, muscle, liver and gill were prepared in 10mM Tris-HCl buffer (pH-7.2) and centrifuged at 10,000 rpm for 15 minutes. To the supernatant ice cold 10% trichloroacetic acid was added and centrifuged. The pellet was washed with chilled acetone and it was dissolved in sample buffer (0.5M Tris-HCl pH-6.8-2 ml, 40% glycerol-1.6ml, 10% SDS-3.2ml, 2-mercaptoethanol-0.8ml, 0.1%(w/v) bromophenol blue-0.4 ml) and heated at 95°C for 2 minutes.

The SDS-PAGE was performed to analyze protein profile in brain, muscle, liver and gill of control and pesiticide exposed tissues by using standard method (Laemmli UK *et al.,* 1970). The concentration of acrylamide was 12% and sample extract was loaded in each lane of the gel. The electrophoresis was carried at

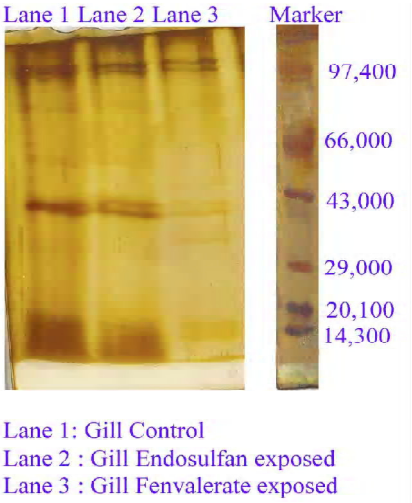

Fig. 1: Changes in protein subunits in gill tissues of *Labeo rohita,* exposed to endosulfan and fenvalerate

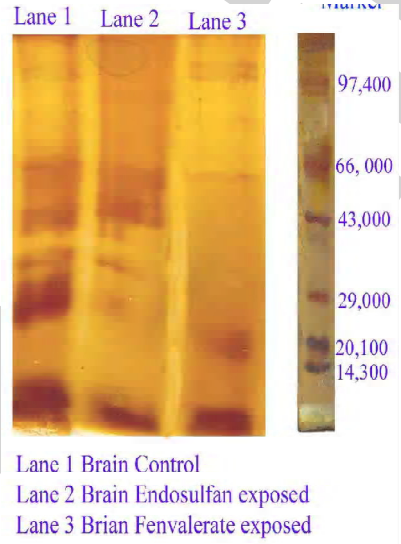

Fig. 2: Changes in protein subunits for brain control, endosulfan exposed gill and fenvalerate exposed gill tissues of *Labeo rohita*

Table - 1: R_m values for gill control, endosulfan exposed gill and fenvalerate exposed gill

Marker	Lane-1 Control	Lane-2 Endosulfan exposed gill	Lane-3 Fenvalerate exposed gill
—	0.11	0.11	0.11
—	0.12	0.12	0.12
0.13	—	—	—
—	0.22	0.22	0.22
0.33	-	-	—
—	0.36	0.36	—
—	0.42	0.42	0.42
0.45	—	—	—
—	0.46	0.46	0.46
—	0.48	0.48	0.48
—	0.59	0.59	0.59
0.66	—	—	—
0.70	—	—	—
—	0.71	—	—
—	0.81	0.81	0.81
0.82	—	—	—
-	0.86	0.86	0.86

Table - 2: R_m values for brain control, endosulfan exposed brain and fenvalerate exposed brain

Marker	Lane-1 Control	Lane-2 Endosulfan exposed brain	Lane-3 Fenvalerate exposed brain
—	0.08	0.08	0.08
—	0.10	0.10	0.10
0.13	—	—	—-
—	0.20	0.20	0.20
—	0.32	0.32	0.32
0.33	—	—	—
—	0.39	0.39	—
—	0.43	0.43	—
0.45	—	—	—
—	0.55	0.55	—
—	0.62	0.62	—
0.66	—	—	—
—	0.68	0.68	0.68
0.76	—	—	—
0.82	—	—	—
—	0.87	0.87	0.87

80V for 3 hrs by watching the movement of the tracking dye and the gel was analyzed by silver staining (Sammons *etal.,* 1981). The relative mobility of the individual subunit was calculated by using the following formula.

$$\text{Relative mobility } R_m \text{ value} = \frac{\text{Distance travelled by individual subunit}}{\text{Distance travelled by the marker dye}}$$

Results and Discussion

The electrophoretogram (Fig. 1) represents the decrease in the intensity of gill protein subunits compared to control. In the pesticide exposure tissue samples, the fenvalerate exposed gill protein subunits showed more decreased intensity in banding pattern compared to the endosulfan exposed tissue sample. The R_m value

Fig. 3: Changes in protein subunits for liver control, endosulfan exposed liver and fenvalerate exposed liver tissues of *Labeo rohita*

Table - 3: R_m values for liver control, endosulfan exposed liver and fenvalerate exposed liver

Marker	Lane-1 Control	Lane-2 Endosulfan exposed liver	Lane-3 Fenvalerate exposed liver
—	0.11	0.11	0.11
0.13	—	—	—
—	0.16	—	—
—	0.25	0.25	0.25
—	0.30	—	—
0.33	—	—	—
—	0.35	—	—
—	0.38	0.38	0.38
—	0.42	0.42	0.42
0.45	—	—	—
—	0.47	0.47	0.47
—	0.54	—	—
—	0.60	0.60	0.60
—	0.73	0.73	0.73

of protein subunit 0.36 nearer to molecular weight 66,000 Daltons was absent in fenvalerate and R_m value of protein subunit 0.71 in between molecular weights of 29,000 and 20,100 daltons was absent in both endosulfan and fenvalerate exposed samples when compared to control.

The electrophoretogram (Fig. 2) represents the decrease in the intensity of brain protein subunits compared to control. In the pesticide exposure tissue samples, the fenvalerate exposed brain protein subunits showed more decreased intensity in banding pattern compared to the endosulfan exposed tissue sample. The R_m values of 0.39,0.43,0.55 and 0.62 protein subunits in between molecular weight 66,000 and 29,000 daltons were completely disappeared in the fenvalerate exposed tissue samples.

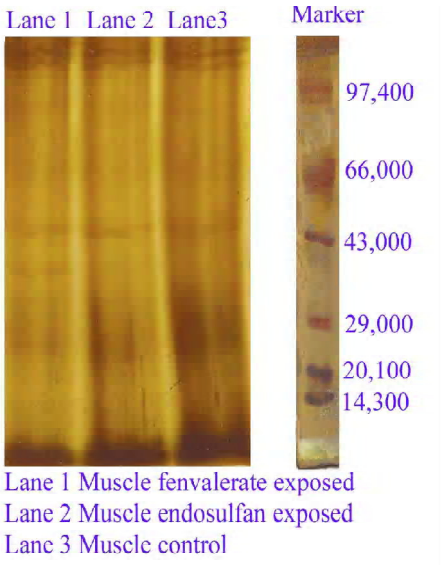

Fig. 4: Changes in protein subunits for muscle control, endosulfan exposed muscle and fenvalerate exposed liver tissues of *Labeo rohita*

Table - 4: R_m values for muscle control, endosulfan exposed muscle and fenvalerate exposed muscle

Marker	Lane-1 Control	Lane-2 Endosulfan exposed muscle	Lane-3 Fenvalerate exposed muscle
—	0.04	0.04	0.04
—	0.06	0.06	0.06
0.13	—-	—-	—-
—	0.15	—-	—-
—	0.24	—-	—-
—	0.32	—-	—-
0.33	—-	—-	—-
0.45	0.45	0.45	0.45
—	0.55	0.55	0.55
—	0.61	0.61	0.61
0.66	0.66	0.66	0.66
—	0.73	0.73	0.73
0.76	—	—	—
0.82	—	—	—
—	0.96	0.96	0.96

The electrophoretogram (Fig. 3) represents the liver protein subunits of endosulfan exposed sample and fenvalerate exposed samples showed decrease in the intensity of liver protein subunits compared to control. In the pesticide exposure tissue samples, the fenvalerate exposed liver protein subunits showed more decreased intensity in banding pattern compared to the endosulfan exposed tissue sample. The R_m value of protein subunit 0.47, molecular weight nearer to 43,000 daltons was absent in fenvalerate exposed sample.Where as R_m values of protein subunits of 0.16, 0.30, 0.35 and 0.54 were absent in both endosulfan and fenvalerate exposed tissue samples.

The electrophoretogram (Fig. 4) represents the decrease in the intensity of muscle protein subunits compared to control. In the pesticide exposure tissue samples, the fenvalerate exposed muscle protein subunits showed slight decreased intensity in banding pattern compared to the endosulfan exposed tissue sample. The R_m values of protein subunits 0.15, 0.24, 0.32 subunits in between molecular weight, 97,000 to 66,000 daltons were absent in both fenvalerate and endosulfan exposed samples.

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; Murat *et 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).

Tripathi and Shukla (1990a, 1990b) performed SDS-PAGE of the cytoplasmic proteins of the liver and the skeletal muscle of C*larias batrachus* exposed to endosulfan and methyl parathion for 1 to 28 days. The appearance of new proteins after exposure of the pesticide, demonstrated clearly alterations in the cytoplasm proteins.

In the present study SDS polyacrylamide gel electrophoresis was performed for the tissues of liver, brain, gill and muscle of *Labeo rohita* exposed to endosulfan and fenvalerate. When compared to control the protein subunits of pesticide exposed tissues showed decrease in intensity and some protein sub units were disappeared. The proteins showed more decrease in intensity (or) significant fading in fenvalerate exposed tissue samples than endosulfan. This indicates that fenvalerate may be more toxic than endosulfan. The variations in protein subunit band patterns may be due to change in the turn over (Synthesis /degradation) of various proteins.

A number of authors have reported similar observations. Marinovich *et al.* (1994) found that Diazinon could induce inhibition of proteins in HL 60 cells at 24 hr exposure. The inhibition of proteins may be due to tissue necrosis which leads to losses of intracellular enzymes or other proteins. Jyothirmayee *et al.* (2005) had done polyacrylamide gel electrophoresis for endosulfan induced changes in LDH pattern in freshwater fish *Anabas testundineus* and C*larias batrachus.* The protein subunits showed a steady decreasing trend in intensity of all the fractions throughout the exposure period demonstrating an inhibitory effect of endosulfan on kidney and muscle LDH. Sherif *et al.* (2009) observed slight reduction or decrease in intensity of proteins in Diazinon treated fish *Nile Tilapia*, which indicates that these proteins were highly affected by the stress caused by the pesticides. The study has suggested that the changes in the protein banding are more pronounced in fenvalerate than endosulfan.

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