Studii şi Cercetări	Mai 2016	Biologie	25/2	77-83	Universitatea"Vasile Alecsandri" din Bacău

LETHAL AND SUBLETHAL EFFECTS OF PROFENOFOS AND CARBOSULFAN ON PROTEIN PATTERN OF INDIAN MAJOR CARP, *LABEO ROHITA* (HAMILTON)

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Key words: *SDS-PAGE, liver, proteins, intensity, patterns*

INTRODUCTION

Environmental pollution has globally emerged as one of the most serious threats to the environment. A series of environmental pollutants such as pesticides, heavy metals, sewage and industrial effluents etc in both freshwater and marine water [1]. Pesticides are frequently used against a pests, in the field to increase the crop production, these are highly toxic to fish and other species in the environment, these are ubiquitous in the environment and had significant economic and public health impact [2], they are substances released into the environment in large amounts with the potential to cause adverse effects on human and animals. Indiscriminate usage of pesticides in agriculture results in leaching of these harmful chemicals into the adjoining water, thereby affecting aquatic organisms [3].

Fish are the important aquatic organisms and are vulnerable to such environmental stress. However, assessing the potential toxic effects and quantifying the risks associated with the exposure to chemical mixtures in ecosystems still remains a major challenge for environmental scientists, risk assessors and regulators [4, 5]. Pesticide residues reach waterbodies and represent a risk for the non-target organisms and finally their way into the food chain threatening the ecological balance [6]. They absorb rapidly via different routes and accumulate in liver, kidney and fat[11]. The pesticides have been recognized as one of the serious pollutants of the aquatic ecosystems with deleterious effects on the living resources. Although the effects of pesticides on fish are extensively studied and has also been reviewed [7, 8]. Analysis of biochemical parameters could help to indentify target organs of toxicity as well as general health status of animals and there is a need for information from the physiological angle [9]. Many pesticides have been reported to produce a number of biochemical changes in fish both at lethal and sublethal levels. Changes in ion concentrations, organic constituents, enzyme activity and endocrinal activity as chemo regulators in fish have been attributed to pesticides [10]. It may also provide an early warning signal in stressed organism. The source of these parameters is the indicators responding to environmental effects and can also serve as markers for the xenobiotic exposure [11].

Hence, an attempt has been made to know the presence of organophosphorus and carbosulfan pesticides induced protein patterns in fish. The freshwater fish, *Labeo rohita* were exposed to sublethal concentrations of profenofos and carbosulfan pesticides for 15 days and changes in the patterns of vital organs such as brain, liver, gill, Kidney and muscle were studied under SDS-PAGE. The protein subunits were identified by running marker proteins parallel and Rm (relative mobility) values were calculated accordingly.

MATERIALS AND METHODS

Freshwater fish*, Labeo rohita* of size 6±7 cm and 6.5±7.5 g weight were brought from a local fish farm Nandivelugu, Guntur district of Andhra Pradesh, India and acclimated at 28 ± 2^0C in the laboratory for 15 days. During the acclimation period fish were fed twice a day with commercial fish pellets and rice bran. At the same time water was renewed with freshwater every two days. The same water supply was used during acclimation period and subsequent lethal and sublethal toxicity tests. Such acclimated fish were exposed to sublethal and lethal concentrations ($1/10^{th}$ 96 hr LC_{50} i.e.10 µg l^{-1}; 0.12 mg l^{-1} and 96 hr LC_{50} i.e. 100 µg l^{-1}; 1.2 mg l^{-1}) of profenofos and carbosulfan for 15 days. The water used in the experiments and water had following physic-chemical characteristics[12].

Protein electrophoresis

A change in protein fractions was done using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to Laemmli [13] method.

Sample preparation

1% homogenates of brain, muscle, liver, kidney and gill were prepared in 10% TCA and centrifuged at 8000 rpm for 10 min in cooling centrifuge. The pellet

was washed twice with ice cold acetone, again centrifuged at 8000 rpm for 10 min.

The following results were in (mg L^{-1})

Variable	Datum
Turbidity	8 silica units
Electrical conductivity at 28°C	816 Micro ohms/cm
pH at 28°C	8.1
i) Phenolpthalene	Nil
ii) Methyl orange as $CaCO_3$	472
Total Hardness	320
Calcium Hardness	80
Magnesium Hardness	40
Nitrite nitrogen (as N)	Nil
Sulphate (as SO_4)	Trace
Chloride (as Cl)	40
Fluoride (as F)	1.8
Iron as (Fe)	Nil
Dissolved oxygen	8-10 ppm
Temperature	28 ± 2°C

The pellet was dissolved in sample buffer (0.5M Tris-HCL, pH 6.8-2ml, 40% glycerol-1.6 ml) and boiled in water bath at 95°C for 10min.

Preparation of Gel Slab

The glass plate's sandwich was assembled by using two clean glass plates and 1mm Teflon gel solution 12.5%(1.5M Tris-HCL, pH 8.8-2 ml, 30% Acrymalide-3.2ml,10% SDS-0.5 ml double distilled water-1.8 ml, TEMED-0.015 ml, Ammonium persulphate-0.5ml) was prepared and poured in between the clamped glass plates. To avoid entrapment of any air bubbles, the gel solution was overlaid with distilled water. The plates were left undisturbed for 30 min for polymerization of the gel. After gel polymerization overlaid water was removed and rinsed with stacking gel buffer, Now the 5% stacking gel solution (0.5M Tris HCL, pH 6.8-2 ml, 30% acrylamide -0.8 ml,10% SDS-0.5ml, double distilled water 1.2 ml,TEMED-0.015 ml,1.5% APS 0.5 ml) was prepared and poured over the polymerized resolving gel, comb was inserted carefully. The gel slab was left undisturbed for 15min, after polymerization comb was loaded into the wells and gel was run at 60V.

Staining method

The Coomassie Brilliant Blue Staining (CBBS) was based on the method of[14]. Incubate the gel in staining solution of 40% methanol, 10% acetic acid 0.025% and Coomassie Brilliant Blue R-250, which has been filtered through Whatman #1 paper. Incubate the gel for 6 hr to overnight in the staining solution with shaking on a rotary shaker. Destaining solution is the same as staining solution, but not containing the Coomassie R-250 dye powder. Add destaining solution to the gel and incubate for 30-60 min. The gel was washed several times in double distilled water and the electrophoretogram gel was preserved in water.

Determination of molecular weight of the protein subunits separated on SDS-PAGE

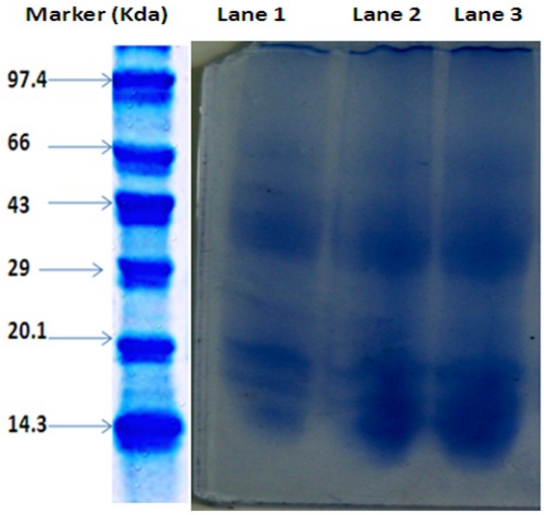
To determine the molecular weight of the individual subunits of the protein, the relative mobility of the individual subunits was calculated by using the following formula

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

A standard curve is prepared by plotting migration distances ('X'-axis) of known protein standards against their molecular weights ('Y'-axis) on semi log graph paper. From the migration distance of an unknown protein, the molecular weight of the protein is being calculated from the standard curve.

RESULTS AND DISCUSSION

The relative mobility's of the protein fractions in different tissues of the freshwater fish *Labeo rohita* exposed to profenofos and carbosulfan in sublethal concentrations for 15 days are given in the Table 1-5and Figure 1-5. The electrophoretogram (Fig.1) represents the decrease in the intensity of gill protein subunits compared to control.



Lane 1: Gill control, Lane 2: Profenofos exposed, Lane 3: Carbosulfan exposed

Fig. 1. Changes in protein subunits in gill tissues of fish L. rohita exposed to profenofos and carbosulfan

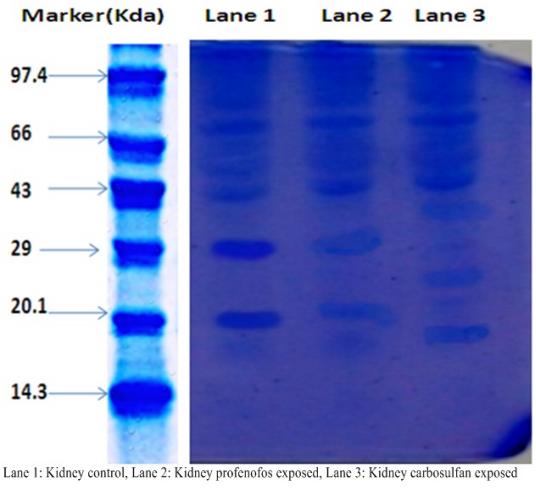
Under the profenofos exposure tissue samples, gill protein subunits showed more decreased intensity in banding pattern compared to the carbosulfan exposed tissue sample. The Rm value of protein subunit 0.52 nearer to 66 daltons (Kda) was absent in carbosulfan and Rm value of protein subunit 0.79 in between molecular weight of 29 daltons and 20.1 daltons was absent in both profenofos and carbosulfan treated fish tissue 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 exposed samples, the profenofos treated brain protein subunits showed more decreased intensity in banding pattern compared to carbosulfan treated tissue sample.
The Rm value of 0.44, 0.48, 0.52 and 0.63 protein subunits in between the molecular weight 66 daltons and 29 daltons were completely disappeared in the profenofos treated samples.

Table 1. Relative mobility values for gill control, profenofos exposed gill and carbosulfan exposed gill

Marker	Lane1 Control	Lane 2 Profenofos exposed gill	Lane 3 Carbosulfan exposed gill
-	0.13	0.13	0.13
-	0.15	0.15	0.15
0.26	-	-	-
-	0.25	-	0.25
-	0.31	0.31	0.31
0.44	-	-	-
-	0.52	0.52	-
0.66	-	-	-
-	0.47	-	0.63
0.73	-	-	-
-	0.68	-	0.71
0.86	-	0.71	0.82
-	0.72	0.82	-
0.94	0.79	-	-
-	0.80	0.90	0.98
-	-	0.98	-
-	0.88	0.88	-
-	-	0.92	0.92

The electrophoretogram (Fig.3) represents liver protein subunits of carbosulfan exposed sample and profenofos exposed samples, decrease in the intensity of liver protein subunits compared to control.

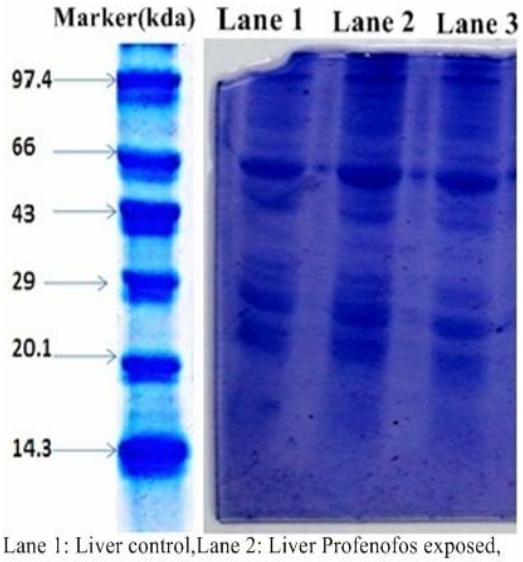


Lane 1: Kidney control, Lane 2: Kidney profenofos exposed, Lane 3: Kidney carbosulfan exposed

Fig. 2. Changes in protein subunits in kidney tissues of fish L. rohita exposed to profenofos and carbosulfan

Table 2 Relative mobility values for kidney control, profenofos exposed kidney and carbosulfan exposed kidney

Marker	Lane-1 Control	Lane -2 Profenofos exposed kidney	Lane- 3 Carbosulfan exposed kidney
-	0.13	0.13	0.13
-	0.29	0.29	-
0.26	-	-	-
-	-	0.32	0.31
-	-	0.41	0.44
0.66	0.45	-	0.53
-	0.50	-	-
0.73	-	-	-
-	0.64	0.68	0.53
-	-	-	-
-	0.93	-	0.79
0.94	-	0.86	-
-	-	-	-
	1.03	-	0.96
	-	0.98	-



Lane 1: Liver control,Lane 2: Liver Profenofos exposed, Lane 3: Liver Carbosulfan exposed

Fig. 3. Changes in protein subunits in liver tissues of fish L. rohita exposed to profenofos and carbosulfan.

In the pesticide treated tissue samples, the profenofos treated liver protein subunits showed more decreased intensity in banding patterns compared to carbosulfan treated tissues samples.

The Rm (relative mobility) value of protein subunit 0.49 molecular weight nearer to 43 daltons was absent in carbosulfan treated tissue samples. Whereas Rm values of protein subunits of 0.16, 0.38, 0.42 and 0.69 were absent in both profenofos and carbosulfan treated samples.

The electrophoretogram (Fig.4) represents the decrease in the intensity of muscle protein subunits compared to control. In the both pesticides treated tissue samples, profenofos treated muscle protein

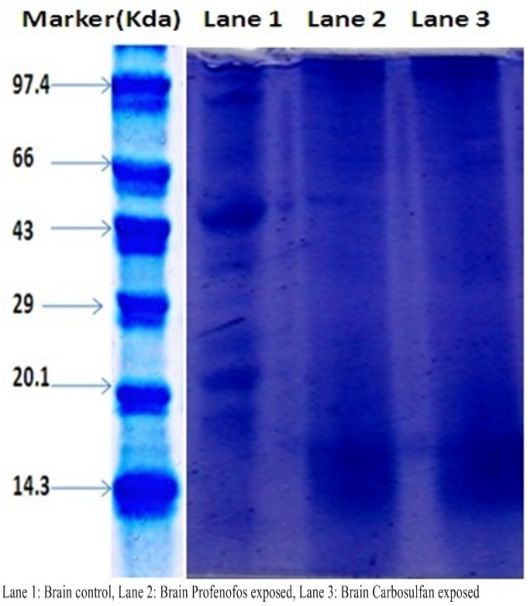
subunits showed slight decreased intensity in banding pattern compared to carbosulfan pesticide treated sample.

The Rm(relative mobility) values of protein subunits 0.35, 0.43, 0.49 and 0.86 subunits in between molecular weight 97 daltons to 66 daltons were absent in both profenofos and carbosulfan treated samples.

The electrophoretogram (Fig.5) represents the decrease in the intensity of kidney protein subunits compared to control. In the profenofos treated kidney protein subunits showed slight decreased intensity in banding pattern compared to carbosulfan pesticide treated sample.

Table.3 Relative mobility values for liver control, profenofos exposed liver and carbosulfan exposed liver

Marker	Lane -1 Control	Lane -2 Profenofos exposed liver	Lane -3 Carbosulfan exposed liver
-	0.12	0.12	0.12
-	-	-	-
0.26	-	-	0.18
-	0.35	0.31	0.34
-	0.43	0.43	-
-	0.38	-	-
0.45	-	-	-
	-	0.51	0.51
-	0.66	0.66	0.66
0.66	-	-	-
-	-	-	-
-	0.69	-	-
-	0.72	0.72	0.72
-	0.76	0.76	0.76

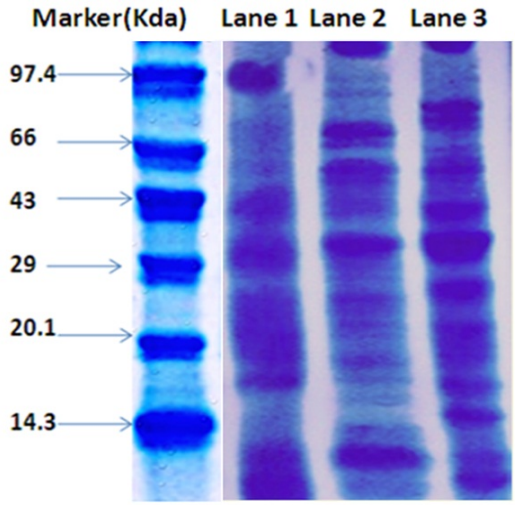


Lane 1: Brain control, Lane 2: Brain Profenofos exposed, Lane 3: Brain Carbosulfan exposed

Fig. 4. Changes in protein subunits in brain tissues of fish L. rohita exposed to profenofos and carbosulfan

Table 4. Relative mobility values for brain control, profenofos exposed brain and carbosulfan exposed brain

Marker	Lane-1 Control	Lane -2 Profenofos exposed brain	Lane -3 Carbosulfan exposed brain
-	0.9	0.9	0.9
-	0.14	0.14	0.14
0.26	-	-	-
-	0.29	0.29	0.29
0.44	0.36	0.36	-
0.66	-	0.43	.43
-	-	-	-
0.73	0.44	-	-
-	0.48	-	0.48
-	0.52	-	0.52
-	0.63	0.63	0.63
0.86	-	0.46	-
-	-	-	-
-	0.73	0.73	0.73
-	0.79	0.79	0.79



Lane 1: Muscle control, Lane 2: Muscle Profenofos exposed, Lane 3: Muscle Carbosulfan exposed

Fig. 5. Changes in protein subunits in muscle tissues of fish L. rohita exposed to profenofos and carbosulfan

The Rm values of protein subunits 0.29, 0.45,0.50 and 0.64 subunits in between molecular weight 97 daltons to 66 daltons, The Rm(relative mobility) values 0.29 were absent in carbosulfan treated samples,0.45,0.50 Rm values were absent both in profenofos and carbosulfan treated samples.

In the present study SDS-PAGE was performed for the tissues of liver, brain, muscle, gill and kidney of *L. rohita* exposed to profenofos and carbosulfan. When compared to control the protein subunits of pesticides exposed tissues showed decrease in intensity and some protein subunits were disappeared. Inhibition of proteins may be due to

tissue necrosis which leads to losses of intracellular enzymes or other proteins.

Table 5. Relative mobility values for muscle control, profenofos exposed muscle and carbosulfan exposed muscle

Marker	Lane-1 Control	Lane- 2 Profenofos exposed muscle	Lane- 3 Carbosulfan exposed muscle
-	-	0.07	0.07
-	0.13	0.13	0.13
0.26	-	-	-
-	0.35	-	-
0.44	0.41	-	-
-	0.43	-	-
-	0.49	0.49	0.49
-	-	-	-
0.66	0.66	0.66	0.66
-	0.69	0.69	0.69
-	0.78	0.78	0.78
	-	-	-
0.86	-	-	-
	0.81	-	-
-	0.95	0.95	0.95

The proteins showed more decrease in intensity or significant fading in profenofos exposed tissue samples than carbosulfan. The variations in protein subunits band patterns due to change in the turnover (synthesis or degradation) of various proteins.

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 [15, 16]. An alteration of protein metabolism was observed in fish exposed to different types of environmental stresses [23]. Cytoplasmic proteins of the liver and the skeletal muscle of *Clarius batrachus* exposed to endosulfan and methyl parathion for 1 to 28 days. The appearances of new proteins after exposure to pesticide demonstrated clearly alterations in the cytoplasm proteins [17, 18.19] separate the sarcoplasmic proteins by SDS-PAGE, the proteins showed that the intensity of protein bands changed due to increased pressure level and holding time. High pressure processing causes denaturation, aggregation, fragmentation of sacrocplasmic proteins in sea bass (*Dicentrachus labrax*) fillets.

[20] reported that increased concentration of pesticide chloropyrifos 0.025 ml l^{-1} to 0.01ml l^{-1} to the gold fish *Carassius auratus (var*.,) *auratus* showed marked changes in protein banding pattern of liver tissue. Decreased protein bands in different tissues of fish *Labeo rohita* exposed to endosulfan and fenvalerate [21].

Investigated[22] the plasma protein bands in Nile tilapia by SDS-PAGE, the number of protein bands decreased in fish exposed to different concentrations (0.20 ppm, 0.002 ppm, 0.004 ppm, 0.008 ppm and 0.02 ppm) of herbicide butataf for 30 days. The number of plasma protein bands decreased due to increased with the increasing toxicity of butataf (12-9) bands, when compared to control (13bands). [24] Observed that slight reduction or decrease in intensity of proteins in diazinon treated fish Nile Tilapia, which indicates that these Nile tilapia, which indicates that these proteins were highly affected by the stress compared by the pesticides. Similar observations were made by [25, 26, 27].Therefore some bands disappeared due to chronic toxicity of both pesticides profenofos and carbosulfan. These results were agreed with [27] copper exposed to fish, *Oreochromis niloticus,* disappearance of some protein fractions and electrophoretic mobilities in different tissues.

CONCLUSIONS

The results of the comparative study of profenofos and carbosulfan clearly indicate the toxic nature of both pesticides on protein fractions in different tissues of *Labeo rohita*. It also reveals that the profenofos may be more effective than carbosulfan to non target organism such as fish.

ABSTRACT

In the present study SDS-PAGE was performed for the tissues of liver, brain, muscle, gill and kidney of *L. rohita* exposed to profenofos and carbosulfan. When compared to control the protein subunits of pesticides exposed tissues showed decrease in intensity and some protein subunits were disappeared. Inhibition of proteins may be due to tissue necrosis which leads to losses of intracellular enzymes or other proteins. The proteins showed more decrease in intensity or significant fading in profenofos exposed tissue samples than carbosulfan. The variations in protein subunits band patterns due to change in the turnover (synthesis or degradation) of various proteins.

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