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IDENTIFICATION AND SPECIFICATION OF STRESS PROTEINS IN THE LIVER TISSUE OF *Carassius auratus* EXPOSED TO CHLORPYRIFOS

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ABSTRACT

In recent times the enormous usage of pesticides has led to a huge deterioration of soil and consequently the aquatic ecosystem and its inhabitants. Fishes are commonly the target animals due to pesticide contamination in water bodies at large. It is being understood that the fishes create a stress response towards the entry of toxic chemicals by means of synthesizing a group of novel proteins called stress proteins which play a major role in the stress- resistance. In the present study, the ornamental fish *Carassius auratus* (*var.*,) *auratus* was exposed to various concentrations of the organophosphorous pesticide, chlorpyrifos in various exposure periods and the nature of stress proteins synthesized were investigated.

INTRODUCTION

The menace of environmental pollution is widespread nowadays and is disrupting the various ecosystems and their inhabiting living organisms in many ways. The abundant usage of pesticides to protect the crops from its voracious pests and in increase the crop yield on another side enters the aquatic ecosystem agricultural runoff and seepage and ultimately affects the aquatic organisms, particularly the fishes at large. Organophophorous pesticides are most extensively used chemicals in modern agriculture which lead to elevated risks of environmental degradation and the aquatic fauna in particular. The pesticidal stress in fishes shows severe disorders including behavioral changes and it is being understood that fishes synthesize a type of specialized proteins in the cells to counteract the toxic effects of these involved chemicals called shock proteins or stress proteins (Iwama *et al*., 2004; Multhoff, 2007; Keller *et al*., 2008; Osman *et al*., 2010). In general, these proteins help to protect the cells during the stress condition and later quicken recovery (Mao *et al*., 2005; Heinz *et al*., 2012). Extensive literature is available on the impact of various extrinsic factors on the production of stress proteins in different animal groups. But scanty information is available on stress protein production towards pesticide stress. Keeping in view of the above facts, the present investigation was initiated to study the impact of an organophosphorous pesticide chlorpyrifos on the production of stress proteins in the ornamental fish, *Carassius auratus.*

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MATERIALS AND METHODS

Experimental fish

The gold fish *Carassius auratus (var.,) auratus* is a freshwater fish in the family Cyprinidae of order Cypriniformes. It was one of the earliest fish to be domesticated, and is one of the most commonly kept aquarium fish.

Experimental pesticide

Chlorpyrifos (IUPAC name: *O, O-diethyl O-3,5,6-trichloropyrin-2yl phosphorothioate*) is an organophosphate insecticide. Chronic exposure of chlorpyrifos to animals leads to neurological effects, developmental disorder, and autoimmune disorders.

Collection and Maintenance

The fishes were collected from an aquarium at Chidambaram Town. From the collection, fishes of 25 to 30 g were chosen and reared separately in plastic troughs to get them acclimatized in the laboratory condition. The oxygen content

in the water was maintained throughout the experiment by an aerator, so that oxygen was not acting as a limiting factor. The fishes were fed with pelletized meal containing groundnut oil cake, Bengal gram powder and rice bran. The fishes were acclimatized to laboratory conditions such as feeding and temperature for a period of 15 days prior to the initiation of the experiment.

Determination of Lethal Concentration (LC_{50})

In the present study, an attempt was made to study the formation of stress protein when the animals were exposed to chlorpyrifos. Primarily the LC50 of chlorpyrifos was fixed. The percentage of mortality for a period of 96hrs was observed and the data is incorporated in the

Table 1. Percentage mortality of *Carassius auratus* exposed to various concentrations of chlorpyrifos at different exposure periods

Conc. In (ml)	Mortality percentage (Hours)					Remarks
	12	24	48	72	96	
0.01	0	0	0	0	0	Non- lethal dose
0.05	0	0	0	0	0	Non- lethal dose
0.1	0	0	0	0	0	Non- lethal dose
0.2	0	25	0	0	50	LC_{50}/96hrs
0.25	0	0	50	0	75	
0.5	25	25	50	100	0	
1.0	100	0	50	0	0	

Table given under. 50% mortality for a period of 96 hrs was observed in the concentration of 0.2 ml/l. Therefore the LC_{50} of chlorpyrifos for this particular species was fixed as 0.2 ml/l (Finney, 1964).

Experimental Design

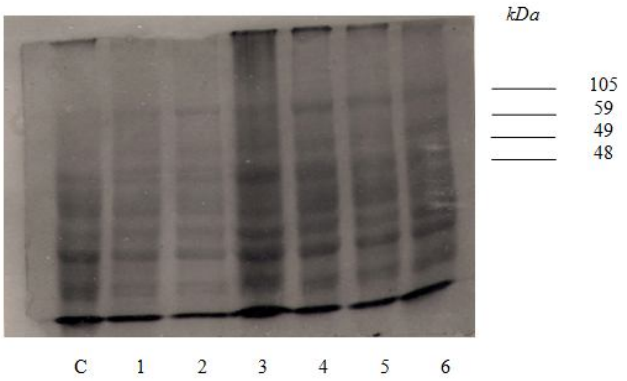
To study the sublethal doses of chlorpyrifos on the changing protein profile in freshwater fish *Carassius auratus*, fishes weighing about 25 to 30g were grouped into 3, representing 3 different pesticide concentrations such as 0 ml/l (control), 0.025 ml/l and 0.1 ml/l as the 1/8 and 1/2 of the lethal concentration 0.2 ml/l. For the study of change in the tissue profile, fishes were exposed to acute shock towards the pesticide, chlorpyrifos for a period of 15 mins, 60 mins and 180 mins. After the stipulated time of pesticide exposure, fishes were sacrificed and the liver tissue was collected for the analysis of stress proteins using gel electrophoresis technique.

Electrophoresis technique

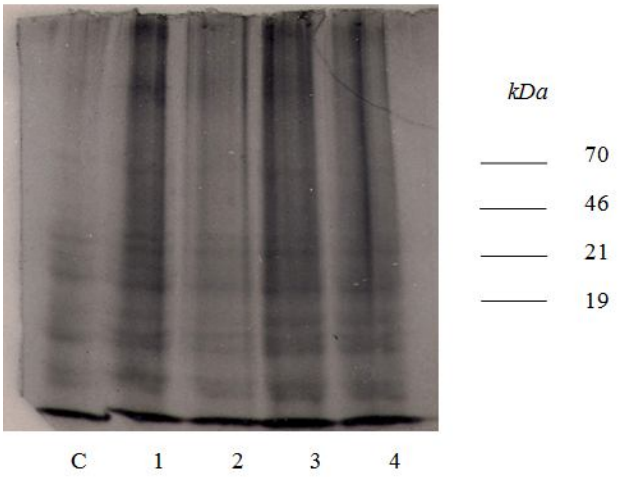
SDS polyacrylamide gel electrophoresis was carried out to study the protein profile in the liver tissue. PAGE has been extensively employed with denaturing agents like SDS and mercaptoethanol. SDS and mercaptoethanol break up a majority of macromolecular complex proteins into their rod-like linear polypeptides. These molecules were subjected to electrophoretic migration according to their molecular size and molecular weight.

RESULT

The liver tissue samples of chlorpyrifos treated *Carassius auratus* exhibited different protein profiles on electrophoretic analyses. The protein profile of pesticide exposed liver tissues showed entirely varied response (Plate 1). Liver tissue exposed to acute shock to the pesticide (0.025 ml/l) expressed new proteins with the molecular weight of 105 kDa, 59 kDa and 48 kDa when compared with the untreated ones. Among these protein fractions, 59 kDa and 48 kDa were found to be entirely new proteins whereas 105 kDa was found increasing in the pesticide exposed fishes over that of the control group. Based on the duration of exposure the synthesis of the two protein fractions, 59 kDa and 48 kDa increased in direct proportion. The quantity of proteins expressed lower with lesser period of exposure (15 mins) and increased remarkable with the higher period of exposure. Raised dosages of chlorpyrifos from 0.025 ml/l to 0.01 ml/l to the fish showed marked changes in the protein profile of liver tissue (Plate 1 and 2).



Changes in the protein profile of the liver of ***Carassius auratus*** exposed to chlorpyrifos (0.025 ml/L) for a period of 15 and 60 mins.



Changes in the protein profile of the liver of ***Carassius auratus*** exposed to chlorpyrifos (0.1 ml/L) for a period of 60 and 180 mins.

DISCUSSION

The results signified the synthesis of new proteins and the intensification of still other polypeptides during pesticide stress and these new proteins are termed as stress proteins (Table 2). This specific cellular stress response involves shutting off the general transcription and activating the transcription of selected set of genes coding for the stress proteins. These stress proteins are involved in protecting animals from damage as a result of exposure to a wide variety

Table 2. Proteins expressed in the liver tissue of *Carassius auratus* exposed to chlorpyrifos

Tissue tested	Protein MW (kDa)	Concentration of chlorpyrifos in ml/l (0.025)			Concentration of chlorpyrifos in ml/l (0.1)		
		Duration of exposure (mins)			Duration of exposure (mins)		
		0	15	60	0	60	180
Liver	105	√	√√	√√	–	–	–
	70	–	–	–	–	√	√
	59	–	√	√	–	–	–
	48	–	√	√	–	–	–
	45	–	–	–	√	√√	√√
	21	–	–	–	√	√√	√√
	19	–	–	–	–	√	√

– = Absence of polypeptides; √ = Presence of polypeptides;
√√ = Increased synthesis of polypeptides

of environmental stressors. The observed results show that stress protein synthesis is highly tissue specific. According to Brenda (1994) indicate that tissue specificity is probably a result of the differences in gene expression among specialized cell types and the extent of tissue damage. The protein fractions in the liver tissue are unique in appearance in the pesticide induced fish. Similar reports were made with salinity stress in *Cyprinus carpio* (Ranjini, 2001) and *Mystus vittatus* (Sujatha, 1997). The appearance of low molecular weight proteins in the liver intensified with the increasing time of exposure to chlorpyrifos. Similar observations were made by Theodarakis *et al*. (1992); Dyer *et al*. (1993); Sanders *et al*. (1995); Keller *et al*. (2008) and Eder *et al*. (2009). This regulation of induction stress proteins may be understood by the study of stress response to environmental stressors. It also signifies the major role played by stress proteins in the cells undergoing stress due to the pesticides. Though several stress proteins are expressed during pesticide stress, it is of immense need to characterize the functions of each of these newly synthesized proteins which may serve as a continuation of the present investigation and specific stress proteins may be engaged as biomarkers for detecting complete environmental pollution (Wang *et al*., 2007).

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