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Effect of Organophosphorus Pesticides on Biomolecules of Fresh Water Fish, Heteropneustes fossilis (Bloch)

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Abstract

The harmful effect of any pollutant which entered into water bodies can be assessed by investigating the health of aquatic fauna. The present investigation was aimed to study the effect of sublethal concentration of chlorpyrifos on glycogen, protein and lipids and nucleic acids contents in different tissues of freshwater cat fish, *Heteropneustes fossilis* after 24, 48, 72 and 96 hours exposure. The result showed the declined level of glycogen, protein, lipids and nucleic acid contents in the tissues of experimental group of fishes as compared to control. The overall results revealed that sublethal concentration of chlorpyrifos effects on the nucleic acids contents in vital tissues of *Heteropneustes fossilis* leading to decrease in the content of biomolecules.

Keywords: Chlorpyrifos; *Heteropneustes fossilis*; Biomolecules; Organophosphrous pesticides.

Introduction

The assessment of environmental status has become an important issue in striving for a sustainable society and use of natural resources. The pollution of water is the greatest and most problematic source due to industrialization, civilization and green revolution. Pesticides are the biological toxicants, which are being used by the man to kill the pests for increasing the yield of many crops and insect vectors to control the spread of disease (Prakash and Verma, 2014). But over the years, there is a growing concern about indiscriminate use of pesticides in agriculture. The widespread use of pesticides in agriculture not only brought adverse influence on agro-ecosystems but also caused alteration in physiological processes of non-target organisms (Verma and Prakash, 2018). These pesticides through surface runoff reach into the water bodies like ponds and rivers which alter the physicochemical properties of water and are toxic to aquatic organisms and cause deleterious effect or even death to the aquatic animals (Prakash, 2020).

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The enormous use of pesticide becomes the predominant environmental contaminants. Once these pesticides are released in the environment they are metabolized in a short time whereas other persists over longer period and can accumulate in the soil and water and badly influence the biodiversity, which is the 'foundation of human life' on earth and for human's survival and sustainable development (Ashok and Sadguru, 2020). From soil and water, these ultimately reach the tissue of the living organisms including human and causes deleterious effects on their health. The pesticides are known to cause behavioural changes in fish,

impairments in incubation and post-embryonic development of fishes affecting chances of their survival (Anna et al. 2001). Recently this problem of aquatic pollution has a great attention for the researchers (Dwivedi, 2020) for the evaluation of hazardous effects of these toxic pesticides on the physiology of living organisms.

Organophosphorus is some of the most used pesticides in the world. These are used in agriculture, home, gardens and veterinary practices. These are highly toxic to fish and non-target aquatic organisms and are powerful nerve noxious, since they inhibit AChE activity (Klaverkamp and Hobden, 1980).

Several studies have been conducted in assessing the toxicity of organophosphorus insecticides on different fish species (Palanikumar et al., 2014; Prakash and Verma, 2014; Verma and Prakash, 2018; Kadam and Patil, 2016; Kaur and Mishra, 2019 and Prakash, 2020). A perusal of literature reveals the paucity of information on acute toxicity of Chlorpyrifos on freshwater fishes such as *Heteropneustes* ***fossilis*** which are nutritional and popular fish in the study area. In the present study, an attempt was made to examine the toxic effects of chlorpyrifos on the serum biomolecules of freshwater cat fish, *H. fossilis*.

Biochemicals are the most assessable body contents for checking the toxicity of any chemicals. Any alteration in biochemical parameters can result in serious outcomes in the form of various diseases in both the animal and its consumers (Prakash and Verma, 2020a). Chlorpyrifos is one of the widely used organophosphorus pesticides throughout the world. However, limited efforts have been made to study its toxicity in different tissues of fish. Therefore the present study aims to determine the acute toxicity effect of Chlorpyrifos, an organophosphate pesticide on freshwater cat fish, *Heteropneustes fossilis*.

Materials and Methods

The healthy *Heteropneustes fossilis* ranging from 8.5-9.5 cm in length and 9.0-10.0 g in weight were collected from local fish ponds and washed with 1% solution of KMnO4 for five minute and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. Fishes were acclimated to laboratory conditions for 10 days at room temperature. The LC_{50} values of Chlorpyrifos at 96 hours of exposure were estimated to be 23.10 ppm for air breathing cat fish, *H. fossilis* (14.01±1.03cm in size). At 95% confidence level, the lower and upper limits were 17.46 ppm and 28.74 ppm for *H. fossilis* (Zahan et al, 2019). Based on 96 LC_{50}, fishes were exposed to sublethal concentration (10 ppm) for the period of 24, 48, 72 and 96 hours. A control group was maintained in an identical environment. The fish were regularly fed with commercial food and the medium was changed daily in order to provide fresh oxygenated water and to maintain the concentration of chlorpyrifos.

The fishes were sacrificed immediately at the end of 24, 48, 72 and 96 hours of exposure from both experimental and control groups and subjected to analysis for changes in content of biomolecules. The muscles, liver and kidney were homogenized in 0.25 M sucrose solution and centrifuged at 1000x g for 10 minutes. The supernatants were filtered and the filtrates were used for analysis of nucleic acids. The amount of glycogen, protein, lipids and nucleic acids content was estimated by method given by Caroll (1956), Lowry (1951), Barnes and Blackstock (1973) and Ceriotti (1955), respectively. The data in this paper have been presented with mean ± mean standard error and the statistical significance of difference between control and experimental group was calculated by student's t- test.

Results and Discussion

After 96 hours of exposure of *Heteropneustes fossilis* to sublethal concentrations of chlorpyrifos, the glycogen, protein, lipid and nucleic acids contents in muscles, liver and kidney were significantly declined in chlorpyrifos exposed fishes (Table 1).

In the present study, the significant decline in glycogen content in muscles, liver and kidney of chlorpyrifos exposed fishes suggests enhancing conservation of glycogen to glucose to meet an increased energy requirement under stress conditions. During stress condition, fish needs more energy to detoxify the toxicant and to overcome stress. During stress, elevated circulating catecholamines increase the glycogenolysis (Verma and Prakash, 2019). Shah and Dubale (1985) and Tripathi et. al. (2003) reported that glycogen content was decreased in liver and kidney of fresh water teleost *Channa punctatus* due to toxic effect of organophosphorus pesticides. The increased glycogenolysis indicated a general disturbance in carbohydrate metabolism, which might have an adverse effect on the life of exposed animals.

Protein plays a vital role in the biological functions and serves as building blocks for cellular mass. In the present study, the significant decrease in protein content in muscles, liver and kidney in

Table 1: Effect of Chlorpyrifos on the biomolecules of freshwater cat fish, *Heteropneustes fossilis.* (Each value is of mean of five readings ± Standard Deviation)

Tissue	Control	Experimental Duration (In hours)			
		24	48	72	96
		Glycogen (mg/gm wet wt of tissue)			
Muscles	0.85±0.12	0.81±0.09	0.75±0.11	0.67±11*	0.58±0.13**
Liver	20.12±0.14	19.67±0.12	18.64±0.13	17.62±0.14*	16.54±0.08*
Kidney	7.24±0.12	7.07±0.13	6.58±0.14	5.67±0.13*	4.52±0.11**
		Protein (mg/gm wet wt of tissue)			
Muscles	175.12±1.12	170.14±1.11	164.25±0.98	159.10±0.87*	148.11±1.24**
Liver	90.78±1.01	84.15±1.12	78.14±1.14*	68.16±0.87*	58.47±1.11**
Kidney	57.14±0.82	55.11±0.54	50.13±0.68*	45.11±0.45*	40.12±0.47**
		Lipid (mg/gm wet wt of tissue)			
Muscles	8.12±0.31	7.84±0.21	7.17±0.24	6.41±0.47*	5.87±0.29**
Liver	71.12±1.21	67.14±1.22	63.11±1.19*	57.17±1.18*	49.14±1.12**
Kidney	14.18±0.41	13.10±0.39	12.14±0.28*	11.12±0.34*	10.01±0.31**
		RNA content (mg/gm wet wt of tissue)			
Muscles	2.15±0.08	2.07±0.06	1.97±0.07	1.78±0.05*	1.69±0.08**
Liver	3.25±0.13	3.01±0.11	2.71±0.14	2.11±0.09*	1.92±0.07**
Kidney	1.71±0.04	1.67±0.10	1.58±0.12*	1.47±0.16*	1.32±0.12**
		DNA content (mg/gm wet wt of tissue)			
Muscles	0.87±0.11	0.71±0.07	0.62±0.12*	0.54±0.11**	0.48±0.12**
Liver	1.94±0.15	1.72±0.14	1.54±0.14*	1.41±0.13**	1.30±0.14**
Kidney	0.74±0.12	0.62±0.11	0.53±0.09*	0.41±0.08**	0.38±0.14**

*Significant at P< 0.05 ; ** significant at P< 0.01.

chlorpyrifos exposed fishes may be inhibition of protein synthesis or due to excessive proteolysis in these tissues during stress condition (Prakash and Verma, 2020b). Shah and Dubale (1985) reported that protein content was decreased in liver and kidney of fresh water teleost *Channa punctatus* due to toxic effect of malathion. Singh and Khare (1999) reported that protein content in the liver was decreased in pesticide exposed fish, *Channa punctatus*. As the liver has multiple metabolic functions, such damage can have serious effects on the metabolism (Srivastava and Prakash, 2019). Protein was depleted in the tissues of pesticides exposed fishes because during stress conditions fish needs more energy to overcome the stress (Prakash and Verma, 2018). Neff (1985) reported that when an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted.

Lipids also contribute to energy production as they are having high caloric values and play a vital role during the biochemical adaptations of animals to stress conditions. In the present study the significant decrease in lipid content in muscles, liver and kidney of chlorpyrifos exposed fishes may be due to inhibition of lipid synthesis as well as increased utilization of stored lipid as source energy to conduct regular metabolic activity during stress condition (Prakash and Verma, 2019). Another possible reason for decline in lipid content of pesticides exposed fish was that organophosphorus pesticides may be interfered with fatty acid oxidation and also inhibits the enzyme acetyl-co-enzyme A synthetase involved in fatty acid oxidation.

Nucleic acid content is considered as an index of capacity of an organism for protein synthesis and proteins are regulator of all biological activities. All the enzymes activities are controlled by the process of transcription. When transcription process is curtailed, absence of RNA and protein synthesis occurs. As a result, metabolism is impaired. Hence

any variation in DNA content reflects on protein synthesis and there by protein level in body of an animal. Chlorpyrifos appears as a potential inhibitor of DNA synthesis, which might result in reduction of RNA level. In the present study decrease in the nucleic acid (Both RNA & DNA) content was observed in all tissues of *Heteropneustes fossilis* exposed to chlorpyrifos. Similar reports were observed by Duraj and Selvarajan (1992), Borah and Yadav (1995), Gautam et. al., (2002), Tripathi and Verma (2004), Tilak et. al., (2005) and Thirumurugan (2011). They concluded the decrement in DNA and inhibition of transcription in RNA enzymes was the possible reason for the depletion of RNA and protein contents. Hence, under toxic effect of chlorpyrifos, both nucleic acid and protein content were depleted in these tissues. These findings support the result of present study. The overall results reveals that sublethal concentration of chlorpyrifos affects on the nucleic acids contents in vital tissues of *Heteropneustes fossilis* leading to decrease in glycogen, protein and lipid synthesis and cellular degradation (Kaur and Mishra, 2019).

Conclusion

It can be concluded that because of electrophilic nature, the organophosphorus pesticides may attack many enzymes responsible for the normal metabolic pathway. Thus, it is possible that the enzyme necessary for the DNA synthesis might have been inhibited by chlorpyrifos and ultimately the disruption of DNA synthesis affected RNA and enzyme synthesis which are responsible for the metabolism of biomolecules.

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