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Original Research Article

Toxic Effect of Organophosphorous Pesticide, Phorate on the Biochemical Parameters and Recovery Response of Freshwater Snake Headed Fish, *Channa punctatus*

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ABSTRACT:
Organophosphorous pesticides are mainly used to destroy harmful insects or pests which infect agricultural field, forests and houses. Increasing amount of agricultural, domestic and industrial chemicals into the aquatic environment lead to deleterious effects on the aquatic organisms. The biochemical study is recognized as a valuable tool for evaluating health status, physiological and pathological condition of fish. The present study deals with the level of imbalance evolved by the sub-lethal concentration of phorate on the tissue biomolecule of Channa punctatus. The pesticide, phorate was involved in the metabolic regulation of carbohydrate, protein and lipid in muscles, liver and kidney of Channa punctatus. Although, the mechanism is not clear that how phorate interferes in the metabolic process of these biomolecules. During recovery period, the tissue carbohydrate, protein and lipid content recorded a pattern towards normalcy when compared with 96 h exposed fishes.

Keywords: *Phorate, Organophosphorous pesticides, Channa punctatus, Biomolecules.*

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INTRODUCTION

The major sources of water pollution are industrial effluents, domestic sewage, drainage and pesticides which pollute the river and other water sources (Kalal *et al.,* 2021). These water bodies receive the untreated discharges mainly from textiles, sugar factory, distilleries, paper mills, petrochemicals, tanneries, fertilizers, herbicides and pesticides factories. Pesticides are being used extensively in the control of crop pests, mosquito and vector borne diseases; hence, the production of pesticides has greatly increased in recent years. Pesticides include a wide variety of chemicals with great difference in their mode of action, uptake by the body, metabolism and elimination from the body and toxicity to target and non-target organisms. The synthetic pesticides are of mainly two types Organochlorine and organophosphorus. However, variety of pesticides like organochlorines, organophosphates, carbamates and synthetic pyrethroids are easily available in market and now in use (Shinde, 2021). These pesticides through surface runoff reach into the aquatic

ecosystems and become a global environmental problem. The presence of pesticides in the aquatic system can obviously lead to multi- fold interaction with other forms of pollution because these pesticides enter into the food chain and their subsequent bioaccumulation and biotransformation at different trophic levels have catastrophic effect to the ecosystem (Prakash and Verma, 2014).

Rising temperature of earth surface changes the biological clock of animals especially insects and flowering of plants including weeds (Prakash, 2021; Verma, 2021). Warm atmosphere is suitable for growth and proliferation of both insect pests and weeds, so to control these weeds and pests both developed and developing countries quickly used more pesticides (herbicides and insecticides). With increasing the demand of pesticides, the chemical industries produces more synthetic pesticides and are consequently incessantly release a variety of pesticides into the biosphere.

The indiscriminate use of pesticides causes chemical pollution results potential health hazards to livestock, in particular to fish, frogs, birds and mammals including human beings.The ever- increasing use of pesticides not only brought adverse influence on agro ecosystems but also causes alterations in the physiological processes of many non-target organisms. Pesticides can cause acute and chronic poisoning of fish and may damage their vital organs and causes skeletal deformities.

Now day's farmers are using an assortment of pesticide and insecticide in crop cultivation. After entering the residues of these pesticides into the environment, homeostasis of aquatic ecosystem is disturbed.Thus, aquatic contamination of pesticides causes acute and chronic poisoning of fish and other organisms. India is now the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally. The main use of pesticides in India is for cotton crops (45%), followed by paddy and wheat (Mathur, 1999). In year 2000, the pesticides demand from agriculture sector was gone up to 97,000 tons, and the average pesticide consumption in India is 288 g/ha, which is quite low in comparison to global average of 900 g/ha (Agnihotri, 2000). The increasing dependence on pesticides throughout recent decades has led to pest resistance, disease susceptibility, and loss of biological control and reduced nutrient cycling.

Phorate ($C_7H_{17}O_2PS_3$) is an Organophosphorous pesticide (insecticide), used against insect especially chewing insect, leafhoppers, leaf miners, mites and nematodes. It is a systemic insecticide that acts by inhibiting cholinesterases. Phorate is of moderate persistent in most of the soil and slightly soluble in water. Phorate itself is not persistent in plants but plants metabolize phorate to very potent anticholinesterase agents such as the sulfoxide. Phorate is toxic to fishes and other animals (Singh *et al*., 2010). It acts on the nervous systems of insects by inhibiting the acetylcholinesterase enzyme (Prakash and Verma, 2020a; Prakash, 2020). In India it is consumed about 10763 metric tons during 2005-06 to 2009-10 (Srivastava *et al*., 2016).

The freshwater snake headed fish, *Channa punctatus* is commonly found in small ponds, ditches, lakes and swampy paddy fields. Therefore, they are easily exposed to agricultural runoff. The fish owing to its air breathing habit is more tolerant to adverse environmental conditions than carps.

Several studies have been conducted in assessing the toxicity of organophosphorous pesticides on different fish species (Singh *et al*., 2010; Prakash and Verma, 2014; Verma and Prakash, 2018; Masih, 2021). Perusal of literature reveals paucity of information on acute toxicity of phorate on the biochemical parameters of freshwater edible fishes. Hence the present investigation aimed to study 96 h toxicity of phorate and its effect on the biochemical parameters of freshwater snake headed fish, *Channa punctatus.*

MATERIALS AND METHODS

The healthy *Channa punctatus* 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 $KMnO_4$ for five minutes 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 phorate at 96 hours of exposure were estimated to be 0.30mg/L for air breathing snake headed fish, *Channa punctatus* (Singh *et al*., 2010). Based on 96h LC_{50}, fishes were exposed to sublethal concentrations (0.05mg/L and 0.10mg/L) for the period of 96 hours. A control group was maintained in an identical environment. The fishes were regularly fed with commercial food and the medium was changed daily to remove faeces and food remnants. The fishes were sacrificed from both experimental and control groups after 24, 48, 72 and 96 hours of exposure and dissected out the desired tissues. The muscles, liver and kidney tissues in each group were dissected out and equipped using 10% TCA and homogenate was centrifuged at 3500 rpm for 20 minutes. The supernatant was used for the estimation of the total carbohydrate, protein and lipids. Total carbohydrate was determined by Anthrone method (Roe, 1961), total protein content was determined with the help of Folin Ciocalteau reagent by Lowry's Method (Lowry *et al.*, 1951) and lipid contents was estimated by the Sulpho phosphovaniline reagent method (Barnes and Blackstock**,** 1973). The experimental data were analyzed by student's 't' test to determine the significance of the changes from control.

RESULTS AND DISCUSSION

The total carbohydrate, protein and lipid contents in the different tissues of control and phorate treated fish, *Channa punctatus*, are given in Table 1, 2 and 3, respectively.

Table 1: **Effects of sublethal concentrations of Phorate on Total carbohydrate level (mg/g) in some tissues of Channa *punctatus* at different period of exposure and Recovery Pattern (N=6).**

Tissues	Group	Exposure periods in hours				F. value
		24	48	72	96	
	Control	7.05±0.03	6.45±0.02	6.75±0.13	6.38±0.11	0.048^{NS}
Muscles	0.05mg/l	5.95±0.32	4.78±0.21	4.57±0.23	4.25±0.35	0.128^{NS}
	0.10 mg/l	4.34±0.22	3.79±0.21	3.52±0.27	3.15±0.23	267.38*
	Recovery Pattern	5.25±0.12	5.48±0.11	5.65±0.20	5.75±0.32	0.128*
	Control	17.75±0.05	17.56±0.04	17.14±0.03	17.35±0.06	0.024^{NS}
Liver	0.05mg/l	15.76±0.21	14.43±0.33	15.11±0.27	16.12±0.32	0.124^{NS}
	0.10 mg/l	14,35±0.43	13.54±0.23	13.21±0.31	12.34±0.21	481.36*
	Recovery Pattern	15.06±0.22	15.52±0.24	15.81±0.22	16.32±0.11	0.254*
	Control	1.52±0.23	1.42±0.21	1.38±0,14	1.31±0,27	0.045^{NS}
Kidney	0.05mg/l	1.44±0.24	1.06±0,11	1.05±0.12	0,95±0.23	179.68*
	0.10 mg/l	1.21±0.11	0.93±0.12	0.79±0.13	0.68±0.51	312.32*
	Recovery Pattern	1.30±0.21	1.41±0.21	1.45±0.12	1.50±0.13	211.11*

NS= Non Significant; *=Significant at 5% level of F test ($p<0.05$)

Carbohydrate and protein are the chief nutrients of the animals. They have a variety of functions. The carbohydrate supplies energy in the form of ATP molecules, which are formed during TCA cycle. Even though protein is an important source of energy in fish but stress conditions causes rapid depletion of stored carbohydrate (Verma and Prakash, 2019).

Total carbohydrate in muscles, liver and kidney of pesticide exposed *Channa punctatus* was decreased with increasing the concentration of phorate and duration of exposure period. The results showed hyperglycemia and a significant reduction in carbohydrate content of tissues of phorate exposed fishes. Carbohydrates may be converted into glycogen or shunted in the metabolic pathway to supply the carbon chain for amino acids, or converted into fat, of these various processes formation and breakdown of glycogen appears to occupy a central position (Sathick *et al.,* 2019). The present finding also supported by other scientists who had noticed that carbohydrate content in muscle and liver tissues was declined with increasing concentration and duration of

toxicant exposure in fishes (Verma and Prakash, 2019). The significant decrease of total carbohydrate content in the tissues of phorate exposed fishes was due to utilization of excess energy needed to cope with stress under pesticide exposure. Anoxia or hypoxia increases carbohydrate consumption and thereby induces a sort of respiratory stress on organisms even at a sublethal concentration resulting in additional expenditure of energy. Thus, energy yielding carbohydrate metabolism is, however, disturbed when the fish is exposed to pesticides.

Table 2: **Effects of sublethal concentrations of Phorate on Total protein level (mg/g) in some tissues of Channa *punctatus* at different period of exposure and Recovery Pattern (N=6).**

Tissues	Group	Exposure periods in hours				F. value
		24	48	72	96	
Muscles	Control	24.10±0.05	23.82±0.04	23.52±0.03	23.35±0.02	0.067^{NS}
	0.05mg/l	22.40±0.04	20.05±0.02	19.55±0.03	19.05±0.03	0.134^{NS}
	0.10 mg/l	20.10±0.03	18.35±0,05	16.70±0.06	15.10±0.04	227.36*
	Recovery Pattern	22.60±0.03	22.95±0.02	23.05±0.03	23.25±0.03	157.65*
Liver	Control	21.23±0.03	21.04±0.04	20.53±0.02	19.75±0.01	0.028^{NS}
	0.05mg/l	20.15±0.11	19.25±0.04	18.70±0.12	17.00±0.11	0.131^{NS}
	0.10 mg/l	18.55±0.14	17.50±0.12	16.75±0.08	15.25±0.15	316.32*
	Recovery Pattern	18.70±0.10	19.05±0.03	19.50±0.10	19.70±0.05	212.54*
Kidney	Control	9.75±0.02	9.60±0.04	9.39±0,01	9.25±0.02	0.041^{NS}
	0.05mg/l	8.65±0.06	8.15±0.03	7.50±0.04	7.10±0.05	0.72^{NS}
	0.10 mg/l	7.22±0.11	7.10±0.05	6.80±0.05	6.25±0.04	302.12*
	Recovery Pattern	7.60±0.05	7.85±0.04	8.35±0.02	8.85±0.07	204.12*

NS= Non Significant; *=Significant at 5% level of F test ($p<0.05$)

Proteins are complex substance with high molecular weight and form not only the structural frame work of the cell but also plays a vital role in the biological functions by regulating the metabolic reactions occurring in the body of living organisms. They are useful for the transport and storage. Specific protein transport many small molecules and ions. The primary function of protein is to supply the amino acids needed for the growth, repair and general maintenance of the structural and catalytic mechanism of living beings.

In the present study, the protein content in muscles, liver and kidney of phorate exposed *Channa punctatus* was decreased with increasing its concentration and duration of exposure period. The results indicate that the tissue protein endure proteolysis resulting the production of free amino acids, which are used in TCA cycle for energy production under stresses. In the present study, it was also observed that the rate of proteolysis increased with increase in the concentration of phorate and duration of exposure. It might be due to its conversion into ammoniated residues in order to increase amino acids pool (Prakash and Verma, 2020b) or due to its utilization in the conversion of glucose because during stress conditions fish needs more energy to overcome the stress (Prakash and Verma, 2018). Other workers have also reported decline in protein constituent in different fish tissues exposed to sublethal concentrations of insecticides (Malla Reddy and Bashamohideen, 1988; Singh, 1996). Jagadessan and Mathivanan (1999) reported that the depletion of protein level in the tissues of fish is due to diversification of energy, to meet the impending energy demands during stress condition. In the present study, decrease in protein content may be due to reduced protein synthesis or degradation of protein into free amino acid (proteolysis), which is used for different metabolic activity during stress condition. Since fishes have less amount of carbohydrate so next alternative source of energy is protein to meet increased demand of energy during stress condition.

Table 3: **Effects of sublethal concentrations of Phorate on Total lipid content (mg/g) in some tissues of Channa *punctatus* at different period of exposure and Recovery Pattern (N=6).**

Tissues	Group	Exposure periods in hours				F. value
		24	48	72	96	
	Control	1.85±0.05	1.81±0.04	1.76±0.05	1.74±0.03	0.055^{NS}
Muscles	0.05mg/l	1.70±0.04	1.61±0.06	1.47±0.02	1.40±0.04	0.078^{NS}
	0.10 mg/l	1.52±0.03	1.43±0.05	1.33±0.15	1.29±0.02	215.38*
	Recovery Pattern	1.60±0.05	1.69±0.04	1.72±0.03	1.80±0.05	178.12*
	Control	7.15±0.05	7.10±0.04	7.05±0.03	6.98±0.02	0.037^{NS}
Liver	0.05mg/l	6.10±0.02	5.75±0.11	5.15±0.07	4.85±0.04	0.111^{NS}
	0.10 mg/l	5.29±0.06	5.10±0.06	4.52±0.05	3.92±0.07	341.36*
	Recovery Pattern	5.75±0.03	5.95±0.10	6.25±0.04	6.75±0.03	215.87*
	Control	2.34±0.06	2.28±0.03	2.15±0.04	2.05±0.07	0.042^{NS}
Kidney	0.05mg/l	1.76±0.04	1.63±0.05	1.58±0.03	1.45±0.08	0.63^{NS}
	0.10 mg/l	1.44±0.05	1.37±0.05	1.18±0.04	1.03±0.02	272.0.32*
	Recovery Pattern	1.53±0.03	1.61±0.05	1.74±0.03	1.89±0.04	199.75*

NS= Non Significant; *=Significant at 5% level of F test ($p<0.05$)

Lipid content is an essential organic constituent of the tissues in all the animals, and plays a key role in energy metabolism as they are the best energy producers of the body next to carbohydrates. It acts as reversed depot of energy from where the energy is supplied as and when required (Katti and Sathyanesan, 1983). Besides this, they also play a significant role as messengers in signal transduction pathways and molecular recognition processes (Van Meer *et al*., 2008). Chezhian *et al*. (2010) reported that lipids are vital to embryogenesis, providing two third of energy by oxidation. Hence, any change in lipid metabolism would signal to impairment of these crucial pathways.

In the present study, decline in triglyceride content in muscles, liver and kidney of phorate exposed fishes may be due to inhabitation of lipid synthesis as well as increased utilization of stored lipid as a source of energy to conduct regular metabolic activity during stress condition (Prakash and Verma, 2019). Since lipids form the rich energy reserves whose caloric value was reported to be twice than that of an equivalent weight of carbohydrates or proteins. The concentration of lipid in the normal fish was found to be highest in liver. In the present investigation, the depletion in the triglyceride content in all the tissues of phorate exposed fish may be due to lypolysis of lipid and disturbance in steroid biosynthesis or the mitochondrial injury impaired the function of TCA cycle and the fatty acid oxidation mechanism. Another possible reason to decreased in triglyceride content of phorate exposed fish was that organophosphorus pesticide might interferes with fatty acid oxidation and also inhibits the enzyme acetyl-co-enzyme A synthetase involved in fatty acid oxidation. Some researchers also suggested that the decrease in lipid content in pesticide exposed fishes may be either due to the uptake of lipid by the tissue for utilization at cellular levels or due to increased lipolysis or mitochondrial injury, which affect the fatty acid oxidation mechanism as suggested (Ware, 1980; Rao *et al.,* 1986; Anusha *et al.*, 1996).

When the fishes were transferred to phorate free water, they exhibited a significant increase in biomolecules i.e. carbohydrate, protein and lipid contents of muscles, liver and kidney when compared with 96 h exposed fishes. Thus, the results of the recovery test showed a high regeneration potential of fish and confirmed that the responses of experimental fish, *Channa punctatus* were in all the three tissues reversible when exposure was terminated.

The result of present investigation indicated that organophosphorus pesti cide, phorate was involved in the metabolic regulation of carbohydrate, protein and lipid in muscles, liver and kidney of *Channa*

punctatus. Although, the mechanism is not clear that how phorate interferes in the metabolic process of these biomolecules. The result obtained in this study will serve as an imperative biomarker for monitoring the health status of the fish population; which may be useful indicator of the environment alterations.

CONCLUSION

In view of above results and discussion, present investigation showed the significant changes in total carbohydrate, protein and lipid content of muscles, liver and kidney of fish, which indicates biochemical manifestation due to the toxic action of pesticide. Pesticide induces its effect at cellular or even at molecular level and ultimately causes biochemical alterations. The alteration in biochemical composition of fishes will naturally affect the nutritive value of fish and it will cause a great danger to human beings due to continuous consumption of such contaminated fish.

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