



Effect of Organophosphate insecticide (Rogor) on Protein content of *Channa striatus* from Sukhana River, Aurangabad (M.S).

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Abstract

The pesticidal effects on biochemical parameters of fresh water fishes are well illustrated from the recent research in the field of toxicology. Among the major biochemical components proteins are of prime importance as they are determine nutritive value of fresh water fishes.

Activity of a few biomarkers have been investigated on fresh water fish *Channa striatus* exposed to three sub – lethal concentrations of rogor (1/5, 1/10 and 1/15 of 96 hrs LC₅₀ value). The alteration in protein contents of muscle, liver and kidney were investigated. The protein levels were found to be depleted in all the tissues after exposure to rogor over the control. Therefore the detailed result and observations are summarized in the present investigations.

Keywords: Organophosphate insecticide, Sukhana river, rogor, *Channa striatus*, proteins.

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Introduction

Freshwater represents only a small portion of the total global resources with the rest held in ground water and soil stores, atmospheres, lakes and marshes. Seasonal rainfall often occurs as high intensity storm at short duration and results in runoff that washes slit, organic and inorganic materials, which has been accumulated in catchments, into the water bodies (Chourpagar 2011).

Now days, the application of pesticides become an essential part in agricultural practices. The benefits due to use of pesticides are numerous, but at the same time they cause considerable harm to the ecosystem. Pesticides contaminate through agricultural runoff the streams, lakes and ponds during rainy season and adversely affect the non target aquatic flora and fauna. The pesticide derivatives are known to alter the physico-chemical properties of the water; these in turn interact with various physiological activities of organisms. Biochemical constituents such as glycogen, protein and lipid are considered as sensitive indicators of metabolic activities (Patil *et al.*, 2008, Chourpagar 2011).

Biochemical and physiological alterations being intimately associated with overall physiological disturbances, their study has been found wide usage in chemical diagnosis. Biochemical profiles have also been gaining progressive importance in toxicological research. Some of the biochemical changes have been already acquired the status of reliable and sensitive indicators of general or specific physiological disturbances under environmental stress (Mahalingam *et al.*, 2009; Hongwei *et al.*, 2010). Study of biochemical anomalies now occupies an established position in the universally accepted research framework concerning toxicology.

The effects of pesticides on the physiological and biochemical parameters have been known from the recent research. The biochemical characteristics are particularly the quantitative and qualitative occurrence of the major biochemical components viz. proteins, glycogen and lipid, etc. of fish are of prime importance as they determine nutritive value of fish. Occurrence of polluted water bodies has exposed biota and particularly fishes an unlimited extent of danger. Pesticides affect fish to variable degree depending on the concentration of pollutants in water and thereby make fish unsuitable as food or a constant hazard is posed knowingly or unknowingly if such fish are consumed by human population. Therefore, it becomes necessary to evaluate nature and extent of alterations in biochemical components of fish,

so that safety measures or ways to overcome the altered conditions in fish can be suggested.

Alterations in biochemical values in fish give an indication and help to understand the mode of action of pollutants. Despite the fact that like other living organisms, fish has its own detoxification mechanism to encounter the toxic effects, however, the toxic substances once enter the body certainly damage and weaken the mechanism concerned. The damage may be at cellular or molecular level but ultimately it will lead to physiological, pathological and biochemical disorders (Gaikwad, 2003).

It has been reported that, protein, carbohydrate and lipid contents decreased significantly in muscles, liver and intestine of *Cyprinus carpio* exposed to sublethal concentrations of textile mill effluent (Rajan, 1990).

Materials and Methods

For biochemical study, the live specimens of *Channa striatus* were collected from Sukhan River near Nipani, 25 km away from Aurangabad (M.S.) and brought to the laboratory. The fishes were maintained in glass aquaria and acclimatized for four weeks. After acclimatization healthy fishes, showing normal activities were selected for biochemical estimations.

The fishes were maintained in sufficiently large aquaria so to prevent overcrowding, the acclimatized fishes were given artificial air by aerator. All the necessary precautions were taken to keep the aquaria clean and away from any mechanical disturbances. Glass of size (3* 1* 1* feet) were used as test container. Artificial aeration during the toxicity tests was avoided.

The fishes, *Channa striatus* were selected for the tests. The fishes, *Channa striatus* ranged from 14 to 16 cm in length and 30 to 32 gm in weight. The fishes were washed with 0.1% KMnO₄ solution to remove dermal infections.

The test fishes, *Channa striatus* were exposed to three sublethal concentrations of rogor for 30 days of 1/5, 1/10 and 1/15 ppm. Simultaneously, a control aquarium was also maintained. On 30th day's exposure, fishes from each experimental group were sacrificed, liver, kidney and muscle were dried in oven at 550C to 600C till constant weight was obtained and blended into dry powder. These powders were used for the estimation of various biochemical components such as protein, lipid and glycogen. The methods adopted for estimations were as follows.

Quantitative analysis of biochemical constituents:

Estimation of protein:

For the estimation of protein in the tissue of freshwater fishes *Channa striatus* the Biuret method of (Lowry's et al., 1951) was adopted.

Principle:

The first step involves formation of a copper-protein complex in alkaline solution. This complex then reduces a phosphomolybdic-phosphotungstate reagent to yield an intense blue colour. Folin's reagent is stable only at acidic pH; however the reduction indicated above occurs only at pH 10. Therefore, when folin's reagent is added to alkaline copper protein solution, mixing must occur immediately so that the reduction can occur before the phosphomolybdic-phosphotungstate (Folin's) reagent breaks down.

Reagents:

Lowry's 'A' Solution:

Dissolve 2 gm of Na_2CO_3 in 100 ml of 0.1N NaOH.

Lowry's 'B' Solution:

B1: 1% $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ in 50 ml distilled water.

B2: 2% Sodium citrate solution.

Lowry's 'C' Solution:

1ml of B1+ 2ml of B₂ diluted to 100 ml with Lowry's 'A' Solution.

Folin's Phenol Reagent:

This reagent is diluted by distilled water in proportion to 1:1.

10% TCA (Trichloro Acetic Acid):

Weight 10 gm of TCA and dissolved in little distilled water and make up to 100ml.

Procedure:

100 mg of tissue was homogenized in 5 ml of cold distilled water. 5 ml of 30% TCA was immediately added to precipitate the protein. Precipitate was collected after centrifugation at 3000 rpm for 15 minutes. The supernatant was discarded. The pellet was repeatedly washed with distilled water to remove the traces of TCA. Precipitated protein was dissolved in 0.1 NaOH and estimated by the method of (Lowry et al., 1951) using folin phenol reagent. The protein was expressed in terms of mg/100 mg dry weight of the tissues.

Results

In the present study, changes in the biochemical constituents in body tissues of test fishes, *Channa striatus* were exposed to rogor for long term (30 days) exposure at different sublethal concentrations have been recorded for protein. Rogor induced changes in biochemical constituents which have been represented in the form of percentage in alterations of biochemical constituents. The data were supported by various statistical analyses, such as variance, standard deviation and standard errors of the mean were calculated. Analysis of Variance (ANOVA) test was used to find out significance. The level of significance have been used in the present study ($P < 0.1$, $P < 0.05$, $P < 0.01$, $P < 0.001$) according to (Mungikar, 2003).

Channa striatus*:*Protein:**

Protein recorded in control group of fishes, *Channa striatus* were 25.2163 (± 0.5094) mg in muscle, 18.4689 (± 0.4213) mg in liver and 15.9723 (± 0.2024) mg in kidney. The fishes, *Channa striatus* exposed to three sublethal concentrations of rogor for long term (30 days) exposure, showed that there were significant decrease in level of protein content in muscle, liver, kidney and at 1/5 (3ppm), 1/10(1.50 ppm) and 1/15(0.99ppm) rogor exposure.

In muscle recorded values were (18.1315 ± 0.4213) 28.0960 %, (20.5605 ± 0.4213) 18.4631 % and (22.6522 ± 0.4048) 10.1680 % as compared with their control values.

In liver recorded values were (11.8564 ± 0.2337) 35.8032 %, (14.2854 ± 0.5094) 22.6510 % and (16.9169 ± 0.3092) 8.4027 % as compared with control values.

In kidney recorded values were (13.4083 ± 0.5094) 16.0528 %, (13.9481 ± 0.2024) 12.6732 % and (14.5553 ± 0.4048) 8.8713 % as compared with their control values. These variations recorded in (Table No. 1 and Fig.1).

Table No 1: Effect of rogor on protein content in different tissues of fresh water fish, *Channa striatus* after exposure to sublethal concentrations for 30 days.

Sr. no.	Tissue	Control	3ppm (1/5)	% changes	1.50ppm (1/10)	% changes	0.99ppm (1/15)	% changes
1	Muscle	25.2163 ± 0.5094	18.1315** ± 0.421375	28.0960	20.5605* ± 0.42137	18.4631	22.6522S ± 0.40484	10.1680
2	Liver	18.4689 ± 0.42137	11.8564** ± 0.2337	35.80322	14.2854* ± 0.509418	22.6510	16.9169 NS ± 0.3092	8.4027
3	Kidney	15.9723 ± 0.20242	13.40833** ± 0.509418	16.0528	13.94812* ± 0.202422	12.6732	14.5553 S ± 0.40484	8.8713

1. The values are expressed in mg/100 mg dry weight (mean $\pm$ S.D.) 2) $\pm$ indicates S.D. 3) * P < 0.05, *P<0.01, ***P<0.001, NS= Not significant.

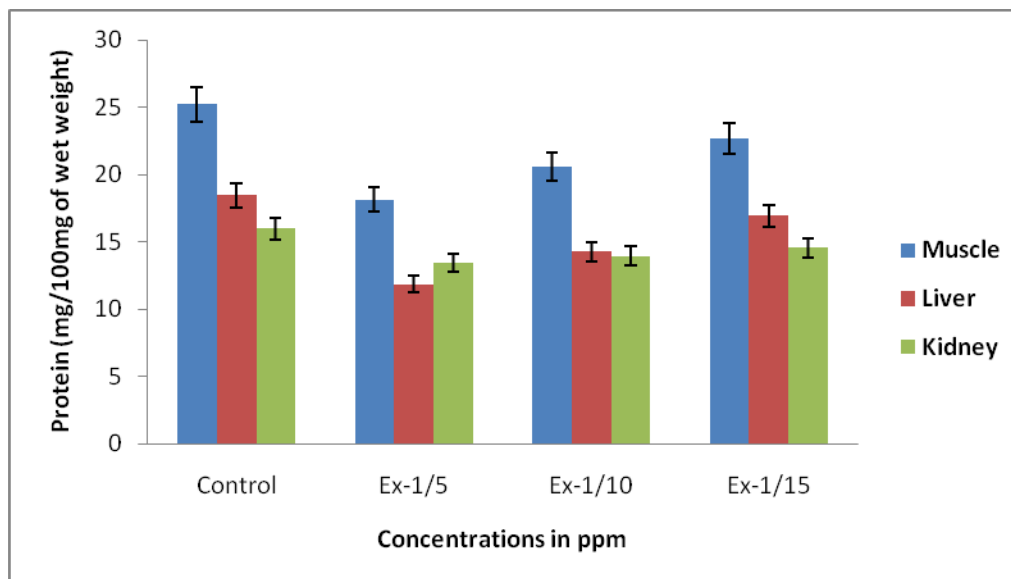
Analysis of variance (ANOVA) Protein for Table No. 1

S.E Treatment	1.130028	-----
C.D Treatment	P=0.05	2.765179
	P=0.01	4.189014

S.E = Standard Error

C.D = Critical Difference

Fig No.1: Protein content in freshwater fish, *Channa striatus* after exposure to sublethal concentration for 30 days.



Discussion

It is well known fact that fishes have been the major source of protein to the low income group people due to its low price and availability (Dinakaran *et al.*, 2009). The nutritional quality of the fish protein is favourable over that of muscle flesh of mutton, chicken, duck etc.

The present study was aimed to understand alterations in protein contents in muscle, liver and kidney of the freshwater fishes *Channa striatus* under rogor pesticide stress.

Protein:

Proteins are important organic substances required in tissue building and repair. Under extreme stress conditions, protein supplies energy in metabolic pathways and biochemical reactions (Winer, 1971). Proteins are mainly involved in the architecture of the cell. During chronic period of stress they serve as a source of energy (Tripathi *et al.*, 2003). Protein can be involved in the compensatory mechanisms of stressed organisms (Sharma and Gupta, 2009).

Protein recorded in control group of fishes, *Channa striatus* are 25.2163% mg in muscle, 18.4689 mg in liver and 15.9723 mg in kidney. The fishes, *Channa striatus* exposed to three sublethal concentrations of rogor for long term (30 days) exposure, showed that there are significant decrease in level of protein content in muscle, liver and kidney at 1/5 (3ppm), 1/10(1.50 ppm) and 1/15(0.99ppm) rogor exposure. In muscle values recorded are 28.0960 %, 18.4631 % and 10.1680 % as compared with control values. In liver values recorded are 35.8032 %, 22.6510 % and 8.4027 % as compared with control values. In kidney values recorded are 16.0528 %, 12.6732 % and 8.8713 % as compared with control values. These variations are recorded (Table No. 1) and graphically represented (fig.1).

Changes in the protein content in muscle, liver and kidney of *Channa striatus* exposed to rogor insecticide for 30 days exposure showed (Table no 1) and graphically represented (fig. no.1). The protein content decreased highly significant at 1/5, $P < 0.01$ in liver (35.8032) followed by muscle (28.0960) and kidney (16.0528).

There is significant decrease at 1/10, $P < 0.05$ in liver (22.6510), muscle (18.4631) and kidney (12.6732). While non significant decrease in protein was observed at 1/10 in muscle, liver and kidney.

Though muscle is rich in protein, it forms mechanical tissue intended for mobility and it does not participate in metabolism. Muscle rich in proteins, forms mechanical tissue intended for mobility and do not participate in metabolism. Liver being the centre for various metabolisms is also rich in proteins. In all the tissues of the exposed fishes, the total protein content is found to be reduced.

The decrease in the protein content is observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or due to directing the free aminoacids for the synthesis of proteins, or for the maintenance of osmo and ionic regulation (Schmidt Nielson, 1975). It could also due to the production of heat shock proteins or destructive free radicals or could be a part of pesticide induced apoptosis.

During stress, an organism needs sufficient energy which can be supplied from reserve material i.e. glycogen, protein, cholesterol and lipid etc. If the stress is mild, then only stored glycogen is as source of energy but when stress is strong then energy stored in the form of lipid, protein cholesterol may be used. The toxicant also affects the metabolic or physiological activities in the animals particularly those organs in which phosphorylation, oxidation, and hydrolization processes are carried out. The liver is the main site for all the activities and also for detoxification of toxic materials. Metabolic products are broken down in liver cells due to which the liver cells may get damaged more severely than any other cells. This causes changes in biochemical composition. These changes are studied by earlier workers.

Activities of a few biomarkers have been studied on freshwater fish *Channa punctatus* treated with

monocrotophos for acute exposure to 18.56 ppm at 96 hr and subacute exposure viz. 0.46 ppm, 0.96 ppm and 1.86 ppm for 30 days. Biomarkers such as total protein, lipid peroxidation and acetylcholinesterase have been measured in different tissues of fish viz. gills, liver, brain and muscles. The protein levels are found to be depleted in all the tissues after pesticide exposure to lethal and sublethal concentration over the control, where as the lipid levels showed an increase under the stress of pesticide monocrotophos. The increased lipid level may be due to inhibition of lipase activity and other biomarkers of lipid metabolism. A significant inhibition of brain acetylcholinesterase (AChE) indicating its effects on nervous system have also been observed. These parameters can be used as biomarkers to predict the early toxicity of monocrotophos added to aquatic ecosystem Agrahari et al., (2006).

The decrease of protein content in gill, liver, muscle and brain of *Labio rohita*, *Mystus vittatus* and *Channa punctatus* exposed to monocrotophos observed by (Rao and Ramaneshwari, 2000).

Decline in the protein level in liver, muscle, gill and brain of carbamide exposed *Labio rohita* (Rajyashree, 1996). Cythion exposures have been reported to reduce the protein level in brain and liver of *Channa punctatus* (Ram and Sathyanesan, 1985). Decrease in total protein level in liver and muscle of *Channa punctatus* exposed to monocrotophos for 15, 30 and 60 days studied by (Sastry and Dasgupta, 1991). Monocrotophos reduced the protein content of fish brain, *Tilapia mossambica* (Joshi and Desai, 1983; Richardson, 1981). A significant decrease is reported in the protein content in almost all tissues in *Channa punctatus* when exposed to sublethal and lethal concentration of fenvalerate (Tilak et al., 2003).

Depletion of protein may be due to the excretion of proteins by kidney due to kidney failure or impaired protein synthesis as a result of liver disordered. (Dhapate et al., 2006) exposed fish, *Nemacheilus botia* to sub lethal concentrations of endosulfan and shows alteration in the protein content of muscle and kidney. The protein content is found to be decreased between 7 and 15 days.

Conclusion

In conclusion, it can be concluded that rogor poisoning may lead alterations in protein *Channa striatus*. Fishes with low protein values (due to rogor) are not fit for human consumption. This implies that one should take the necessary precaution in the application of insecticides/pesticides for protection of fishes life and other aquatic fauna.

In the present study protein content in different tissues showed decreased values in treated fishes *Channa striatus*. Decrease in protein content may be attributed to the impairment to protein synthesis or increase in the rate of its degradation to amino acid. The fall in protein level during rogor exposure may be due to increased catabolism, (Begum and Vijayaraghavan, 1995) and decreased anabolism of protein, (Khare and Singh, 2002). The reducing trend of protein content may be attributed to metabolic utilization of ketoacids to gluconeogenesis pathway for the synthesis of glucose or for the maintenance of osmotic and ionic regulations (Schmidt 1975). The present study shows that the rogor is toxic to the freshwater fishes *Channa striatus*, it has been noticed during the course of study, *Channa striatus* found more sensitive to the rogor.

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