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ORIGINAL ARTICLE

Studies on Protein Profile in Gill of *Channa punctatus* under Stress of Cypermethrin

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ABSTRACT

The population of the world is increasing continuously since time immemorial and utilization of natural resources is also on increase. The rise of human population has crossed all strides in the beginning of 20th century with the result scientists had to employ all tactics to feed the rising population. During this course some of the synthesized chemicals not only helped the mankind but at the same time became reasons for his agony. A good number of chemicals in the form of pesticides residues for quite some time however left many problems which were related to welfare of human beings. The present study reveals detailed protein profile in gill of Channa punctatus under stress of cypermethrin.

Key words: *Protein Profile, Gill, Channa punctatus, cypermethrin*

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INTRODUCTION

To overcome the problem of food requirement biologically safe pyrethroids are in vogue. However, their indiscriminate use is not free of problems. Unfortunately pesticides have diverse effects on the specific target species. Undesirable side effects are wide spread and include injury to non-target organisms, ecosystem imbalance and environmental contamination by persistent pesticides.

One of the most important non-target inhabitants of the ecosystem affected by pollution is fish of both fresh water and marine. High level of pollution often causes high mortality of aquatic organisms particularly large fishes and also produces probable mutations that may not only affect to particular fish species production but also can be one of the causes of extinction of some species. As synthetic pyrethroids are one of the major sources of pollution, it becomes very important to know their effects on genetic material with addressing to fishes. Fish are economically very important and influence the human being in various ways. Their food value is now well known as it provides the much needed protein, vitamin A and D and other elements. Fish food is easily assimilable, richly nutritive and a well balanced diet, so that it has been adopted as one of the main sources of food by the fisheries sector and occupies a very important place in the socio-economic development of the country. It needs a special attention for its development, particularly the pollution control for conservation of aquatic diversity and maintaining sustainable fish production. This important commodity can not only provide the much needed protein food but it can also provide vast rural population with a subsidiary occupation which would also give them an additional income. The fisheries sector is an important source of livelihood for a large section of economically backward population of the country. In coastal states fisheries are an important source of food supply.

Gills are the first to come in contact any with toxicant, which reach in surrounding media. The fish take it directly from the water, particularly by the gills. After that induces a depressive effect on tissue respiration leading death by hypoxia. Gills include a variety of highly vascularized respiratory appendages in fishes, the flow of blood through gills and water outside are in opposite direction. Such mechanism provides quick oxygen uptake and compleate saturation of blood with oxygen. After that blood reaches in muscles and other vital tissues are also important sites to assess extent of toxicity in the organism.
Keeping these points in view the present study highlights the toxic effect of cypermethrin on gill tissue of fresh water fish *Channa punctatus*.

MATERIALS AND METHODS

COLLECTION OF MATERIAL AND TREATMENTS FOR LABORATORY EXPERIMENTS:

The live specimen of *Channa punctatus* commonly known as 'soli' were brought for the present study from ponds in surrounding vicinity of Agra and fish market of Agra. The selection of *Channa punctatus*as experimental fish went in for reason of its easy availability, its hardy nature in terms of survival despite pollutant treatments proposed which might indicate an advantage of long stay of toxic effects in soft tissues. Above all, fish has an economic food value.For experimental purpose fishes almost of the same size and weight so as to refer to similar age group as constant factor were used for noticing effects of treatments by several insecticides. The fishes were washed in 0.1% $KMnO_4$ solution to smear dermal infection if any. Then they were washed with ordinary water and smeared to aquaria filled with water.The latter was already equipped with sand and *Hydrilla* plants, overcrowding was avoided. The fishes were fed with readymade fish food after every 24 hrs. The water was changed to smear the faecal matter and excess food after every 24 hrs. If any mortality occurred the fish was removed immediately to avoid depletion of oxygen. Normally, the fish to be used for experiments were left for fifteen days. So they might acclimatize to the prevailing ecological conditions. For the analysis of insecticide toxicity, insecticide was used in commonly occurring chemical compound cypermethrin25%EC is a synthetic pyrethroid insecticide.

Test compound	:	Cypermethrin25%EC
CAS number	:	52315-07-8
Trade name	:	Super killer
Chemical formula	:	$C_{22}H_{19}Cl_{12}NO_3$
IUPAC number	:	(R,S)-alpha-cyano-3-phenoxylbenzyl I(IRS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate

Cypermethrin 25%EC is a synthetic pyrethroid insecticide used to control various pests.
The diluent water that was used for keeping experimental fishes was subjected to analysis for various physico-chemical characteristics as per procedure given in "APHA (2000) standard methods for the examination of water and waste water". The following data shows the physico-chemical parameters and their average values.

BIOCHEMICAL ANALYSIS:

1. Total Protein:

Total protein was estimated by Biuret method described by Henry *et al*. (1974).

Principle: Proteins react with cupric ions of biuret in an alkaline medium to form a violet blue coloured complex which appears as a result of the reaction between –CO and $–NH_2$ groups of protein cupric ions. The intensity of the coloured complex so developed is proportional to the total protein concentration in the sample.

Reagents:

1. Biuret reagent
2. Protein standard

Calculations:

$$\text{Total protein (mg/dl)} = \frac{\text{O.D. of Test}}{\text{O.D. of standard}} \times 5.7$$

2. Albumin:

Principle: Albumin in serum binds with dyes bromocresol at pH-4.2 to form a green colour complex. The optical density is measured colorimetrically at 600nm (use red filter).

Reagents:

1. Buffered, dye reagent
2. Albumin standard

Procedure:

Three test tubes were marked as test 'T', standard 'S' and blank 'B'.

Test: 4.5ml buffered dye reagent and 0.03ml serum were taken in test tube marked as 'T', mixed well and allowed to stand for one minute at room temperature.

Standard: 4.5 ml buffered dye reagent and 0.03 albumin standard were taken in a test tube marked as 'S' mixed well and allowed to stand for 1 minute at room temperature.

Blank: 4.5ml buffered dye reagent was taken in a test tube marked as 'B' and allowed to stand for 1 minute at room temperature. The optical density (O.D.) of test and standard were measured colorimetrically at 600nm (used red filter) against the blank.

Calculation:

The serum albumin was calculated by the following formula-

$$\text{Serum Albumin (ingm/dl)} = \frac{\text{O.D. of test}}{\text{O.D. of Standard}} \times 4.0$$

3. Globulin:

The globulin was calculated by the following formula from the protein and albumin-

$$\text{Globulin} = \text{Protein} - \text{Albumin}$$

4. A/G ratio:

The A/G ratio was calculated by the following formula-.

$$\text{A/G} = \frac{\text{Concentration of Albumin}}{\text{Concentration of Globulin}}$$

Statistical Calculations: In the present investigation, the formulae were used for different statistical calculations after Fischer and Yates (1950) using statistical software.

RESULTS AND DISCUSSION

Table 1: Total protein (mg/dl) in gill of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

S.No.	Experimental set	No. of fishes	(Mean±S.E.)
1.	Control	5	35.82±0.32
2.	Acute (4 days)	5	25.00±0.12 [a]
3.	Sub-lethal (20 days)	5	22.33±0.40 [b]
4.	Chronic (45 days)	5	15.24±0.20 [d]
5.	Recovery	5	32.10±0.80 [a]

Graph 1: Showing total protein (mg/dl) in gill of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

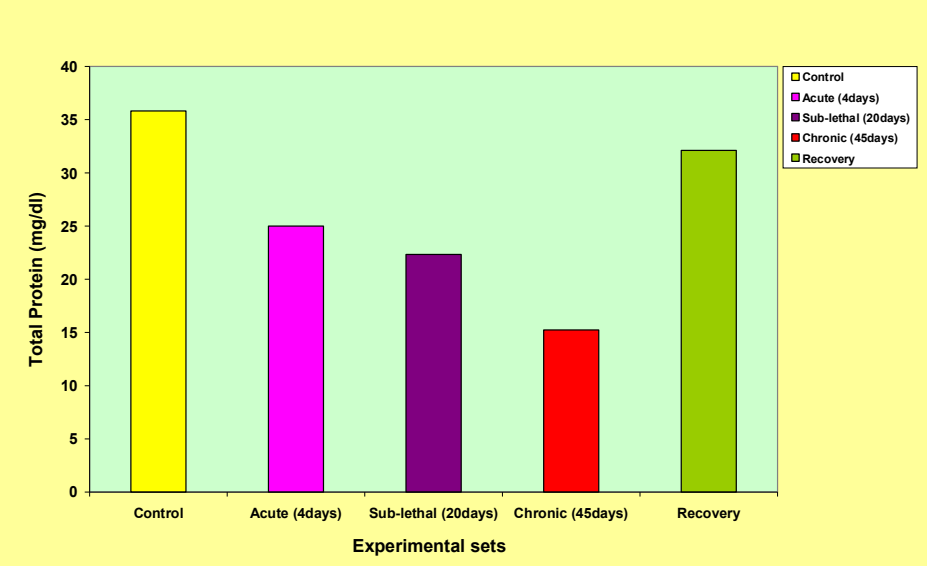


Table 2: Albumin (mg/dl) in gill of *Channa punctatus*after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

S.No.	Experimental set	No. of fishes	(Mean±S.E.)
1.	Control	5	22.85±0.67
2.	Acute (4 days)	5	18.20±0.15 [a]
3.	Sub-lethal (20 days)	5	12.90±0.55 [b]
4.	Chronic (45 days)	5	10.01±0.33 [c]
5.	Recovery	5	23.00±0.66 [a]

Graph 2: ShowingAlbumin (mg/dl) in gill of *Channa punctatus*after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

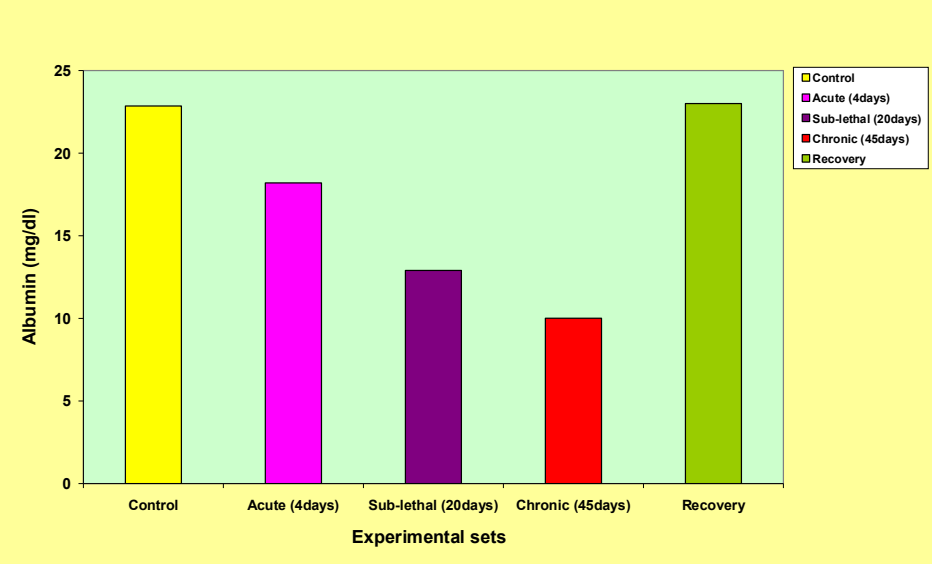


Table 3: Globulin (mg/dl) in gill of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

S.No.	Experimental set	No. of fishes	(Mean±S.E.)
1.	Control	5	12.97±0.04
2.	Acute (4 days)	5	9.43±0.08 [a]
3.	Sub-lethal (20 days)	5	6.80±0.08 [b]
4.	Chronic (45 days)	5	5.23±0.01 [c]
5.	Recovery	5	10.0±0.02 [a]

Graph 3: Showing Globulin (mg/dl) in gill of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

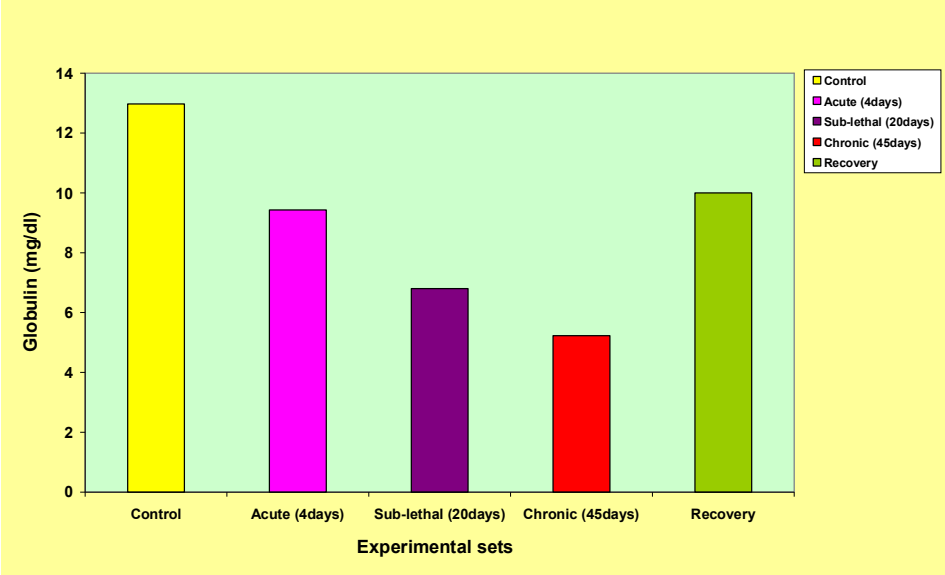


Table 4: Albumin/Globulin ratio in gill of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

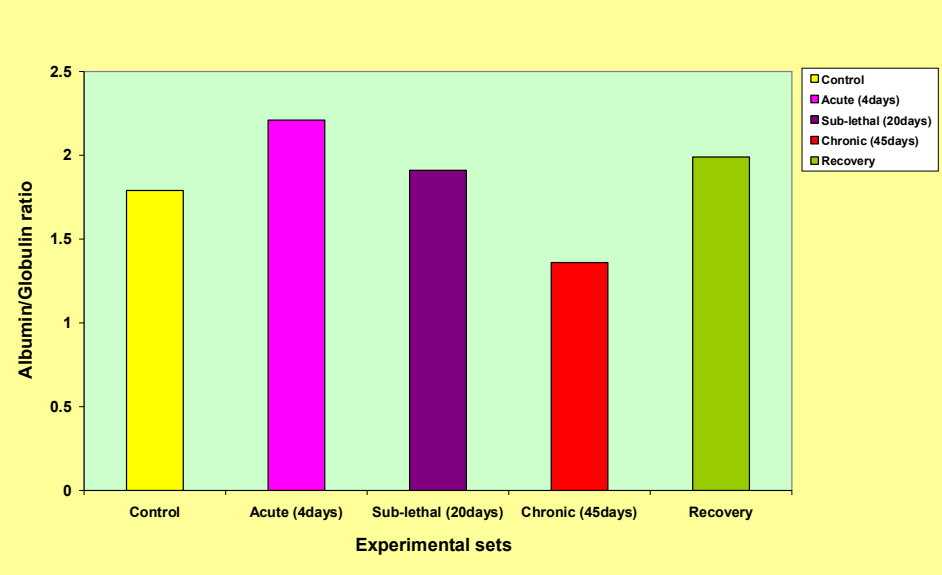
S.No.	Experimental set	No. of fishes	(Mean±S.E.)
1.	Control	5	1.79±0.08
2.	Acute (4 days)	5	2.21±0.06 [a]
3.	Sub-lethal (20 days)	5	1.91±0.10 [a]
4.	Chronic (45 days)	5	1.36±0.12 [b]
5.	Recovery	5	1.99±0.04 [a]

a- Non-significant (P>0.05); b- Significant (P<0.05); c- Highly significant (P<0.01); d- Very highly significant (P<0.001)

Proteins are the important organic substances required in tissue building and repair. Protein content has been decreased with increased duration of exposure, however decrease was more in gill tissue and at recovery it comes to normal level. Proteins are the mainly involved in the architecture of the cell. During chronic period of stress they are also a source of energy (Umminger, 1977). Behavioral response of fish exposed to sublethal concentration of cypermethrin showed that they were under stress condition. During stress condition, fish needed more energy to detoxify the toxicants and to overcome stress. Since fish have a very little amount of carbohydrates, the next alternative source of energy is protein to meet the increased energy demand. The

depletion of protein fraction in muscle and gill tissues may have been due to their degradation and possible utilization degraded products for metabolic purposes.

Graph 4: Showing Albumin/Globulin ratio in gill of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)



In the experimental tissues such as muscle and gill the protein content was decreased. Other workers such as Malla Reddy and Bashamohideen (1995); Singh *et al*., (1996) have also reported decline in protein constituent in different fish tissue exposed to sublethal concentrations of insecticide. Umminger (1970) observed decrease in the protein content in the fish *Fundulusbeteralitus* and stated that the aquatic inhabitants exposed to toxic conditions utilized protein as energy source.Ramalingam and Ramalingam (1982) have reported that the protein level in liver and muscle of *Sarotherodonmossambicus* decreased because of the exposure of the pesticide DDT. Borah and Yadav (1985) studied the effect of the insecticide rogor in the muscle and gill of *Heteropneustes fossilis* and found a decrease in protein content. Malla Reddy and Mohideen (1988) have recorded the reduction of protein content in the branchial tissue of the *Cyprinuscarpio*because of the toxic impact of fenvalerate. Susan, *et al*., (1999) have also reported a significant decrease in protein content under sublethal concentration of pyrethroid fenvalerate in the gill of the fish *Catlacatla*. In the present investigation, reduction in total protein content was noted in the tissues muscle and gill of the test fish *Channa punctatus* exposed to cypermethrin. This was possibly due to the direct effect of the insecticide on protein metabolic demands following exposure to the toxic stress of cypermethrin.

Albumin, globulin and A/G ratio have been decreased with increased duration of exposure, however decrease was more in gill tissue and at recovery it comes to normal level. Similar to the present findings, Ansari and Kumar (1988) reported decline in protein (both albumin and globulin) and nucleoprotein (DNA and RNA) in zebrafish after diazinon treatment. Further, Mauck*et al*. (2005) reported toxicity of natural pyrethrins, S-bioallethrin, dimethrin and d-transallethrinalong with albumin and globulin decline in *Oncorhynchuskisutch* and *Salmogairdner*. Singh and Agarwal (2006) reported changes in albumin, globulin and A/G ratio after treatment of cypermethrin, permethrin and fenvalerate in *Channa striatus*. This decline in albumin, globulin and A/G ratio correlated with decrease in protein content as these are the integral contents of protein itself. The use of biochemical analysis has been advocated to provide an early warning of potentially

damaging changes in stressed fish. Enzymes are such biochemical macromolecules that control metabolic processes of organisms, thus a slight variation in enzyme activities would affect the organism (Roy, 2002). Thus by estimating the enzyme activities in an organism we can easily identify disturbances in its metabolism.

In toxicological studies of acute exposure, changes in concentration and enzyme activities often directly reflect cell damage in specific organ (Casilla, *et al*., 1983). In this study, we monitored the disturbances of metabolism in *Channa punctatus* by exposing concentration of insecticide. A significant decrease in all enzymatic activity was noted in gill and muscle tissue of *Channa punctatus* exposed to higher concentration of insecticides, whereas decrease was not significant at the lower concentration.There is a close relationship between animal's behavior coordination of brain, loss of body equilibrium and nervous function and the metabolic and physical state of an organism (Sambasiva Rao, 1999). The results suggest that different insecticides caused significant alteration in fish metabolism at the tissue level. No such behavioral symptoms and death occurred in control groups, indicating that no factor other than insecticides were for altered fish behavior and mortality.

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