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ESTIMATION OF PROTEIN PROFILE IN MUSCLE TISSUE OF *CHANNA PUNCTATUS* FOLLOWING TREATMENT OF CYPERMETHRIN

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Abstract

Fish, both freshwater and marine, are one of the most vital non-target members in the environment impacted by pollution. The high mortality rates experienced by aquatic organisms, especially large fishes, as a result of high levels of pollution are only one of the many negative consequences of this type of pollution. The effects of synthetic pyrethroids on fish genetics are of great importance, as these chemicals are a major contributor to environmental degradation. Fish have a significant impact on the human economy and way of life. Their nutritional worth as a source of protein, vitamin A and D, and other nutrients is now widely recognised. Given that fish meal is readily digestible, nutritionally dense, and a nutritionally balanced diet, it has become a staple in the fisheries industry and plays a crucial role in the country's economic and social growth. This investigation provides the first comprehensive protein profile of *Channa punctatus* muscle after cypermethrin administration.
Keywords: Protein Profile, Muscle tissue, *Channa punctatus*, cypermethrin.

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Introduction

The toxicity of pesticides in the different organisms varies with the type of pesticide and the target organism. Some pesticides are extremely toxic to fish at a very low concentration and to aquatic invertebrates at even lower concentration. Some of those kill the organism relatively quicker while others have gradual effects on activity, feeding, general physiology and reproduction. In the former case the effect is quite obvious due to high mortality of organism while in later case it is difficult to detect in the beginning and effects are visible in the long term only. So it is necessary to assess the level of toxicity of a particular pesticide against the target species to make guidelines for safe use of pesticide. The muscles are the main edible parts of fish. Fish muscles provide proteins, fats and vitamins (A and D), a large amount of phosphorous and other elements are also present in it. The white muscles are highly nutritive and edible part of fish and on the other hand most of the pesticides are lipophilic in nature and stored in fish muscles.

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. Many of the country's economically disadvantaged people rely on the fisheries industry for their survival. Fishing is a major industry in many coastal states. It is fashionable these days to use biologically safe pyrethroids to solve the food shortage problem. The trouble arises, though, when they are used without any discernment. Unfortunately, the effects of pesticides might vary depending on the species being treated. Injury to non-target organisms, ecosystem imbalance, and environmental contamination from persistent pesticides are only some of the many unintended consequences.

For this study, we chose the synthetic pyrethroid cypermethrin since it is widely used to combat pests in both homes and farms, despite being extremely harmful to aquatic life. Since pyrethroids have a higher toxicity ratio than other major kinds of insecticides, they are increasingly being used in almost all pest management initiatives. The past ten years have seen significant advancement in the creation of more stable and highly active pyrethroids, suggesting that their widespread use is imminent. When pyrethroids are sprayed on crops, they end up in the soil and eventually make their way into bodies of water. They have a negative impact on fish and other aquatic life in water bodies. Fish that can be eaten by humans and other animals. Thus, they biotransformed onto the next trophic level, where they caused negative effects on life.

Cypermethrin has an especially negative effect on fish. Most fish have an LC_{50} of less than 5 parts per billion, meaning that even minute amounts won't kill more than half of the population. This is due in part to the sensitivity of fish neurological systems to pyrethroids and in part to the fact that fish are not as efficient at breaking down pyrethroids as mammals and birds. It is with these considerations in mind that the present study draws attention to the harmful effect of cypermethrin on the muscular tissue of the fresh water fish *Channa punctatus*.

Materials and Methods

Collection of material and treatments for laboratory experiments

In order to conduct this research, we collected live *Channa punctatus*, also known as soli, from ponds and the Agra fish market. Because of its widespread availability and apparent resilience in the face of suggested pollution treatments, the cynodon dactylifera species of *Channa punctatus* was chosen as the experimental fish. Fish also has nutritional benefits and can be used as an inexpensive source of protein. Fishes of almost same size and weight were employed in the experiments so that a constant factor could be age group. Several pesticides were used, and the results were recorded. Fish with cutaneous infections were rinsed in a 0.1% $KMnO_4$ solution. They were then rinsed with tap water and smeared over aquarium glass. The latter was pre-stocked with sand and Hydrilla plants, so crowding wasn't an issue. Once every 24 hours, the fish were given prepackaged fish meal. Every 24 hours, the water was changed to flush off the waste and unused food. If a fish died, it was taken out of the tank right away to prevent the oxygen level from dropping too low. The fish used in tests were typically kept for fifteen days before being employed. As a means for adapting to local ecological norms. Commonly used insecticide cypermethrin25%EC is a synthetic pyrethroid insecticide, and it was the chemical of choice for this study's toxicity investigation (Finney, 1971).

Test compound: Cypermethrin 25%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

Synthetic pyrethroid pesticide cypermethrin25%EC is effective against a wide range of pests. Following the protocol outlined in "APHA (2000) standard methodology for the testing of water and waste water," the diluent water used to house the experimental fishes was analysed for a number of physicochemical properties. The average values of the physicochemical parameters are displayed below.

Biochemical Analysis

(i) Total Protein

The Biuret technique, as reported by Henry *et al.*, was used to calculate the total protein content (1974).

The -CO and $-NH_2$ groups of protein cupric ions react with biuret in an alkaline media to produce a violet blue coloured complex. The total protein concentration in a sample correlates with the brightness of the resulting coloured complex.

Reagents:

(I) Biuret reagent
(II) Protein standard

Calculations:

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

(ii) Albumin:

Principle: At a pH of 4.2, the albumin in serum will bond with dyes containing bromocresol to generate a green colour complex. Colorimetric analysis at 600 nm is used to determine the optical density (use red filter)

Reagents:

(i) Buffered, dye reagent
(ii) 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 : 4.5ml 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-

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

(iii) Globulin

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

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

(iv) A/G ratio

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

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

Statistical Calculations: In the current inquiry, the equations were employed for various statistical calculations based on Fischer and Yates's (1963) work, and these calculations were performed using statistical software.

Results and Discussion

Table 1: Total protein (mg/dl) in muscle tissue 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	47.55±0.48
2.	Acute (4 days)	5	32.50±0.20 [a]
3.	Sub-lethal (20 days)	5	29.20 ±0.18 [c]
4.	Chronic (45 days)	5	25.64±0.25 [d]
5.	Recovery	5	45.00±1.00 [a]

Table 2: Albumin (mg/dl) in muscle tissue 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	26.00±0.50
2.	Acute (4 days)	5	19.25±0.16 [a]
3.	Sub-lethal (20 days)	5	18.10±0.10 [a]
4.	Chronic (45 days)	5	14.99±0.52 [b]
5.	Recovery	5	26.55±0.66 [a]

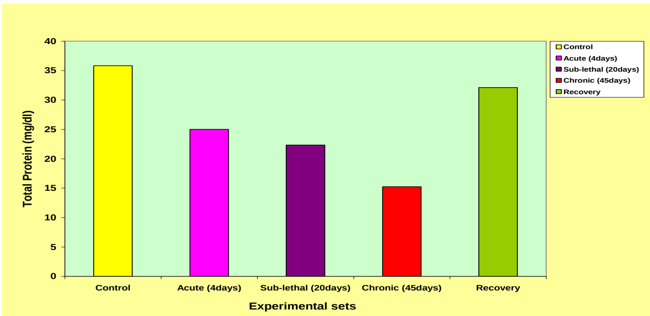
Table 3: Globulin (mg/dl) in muscle tissue of *Channapunctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

S.No.	Experimental set	No. of fishes	(Mean±S.E.)
1.	Control	5	21.55±0.05
2.	Acute (4 days)	5	13.25±0.12 [b]
3.	Sub-lethal (20 days)	5	11.10±0.10 [b]
4.	Chronic (45 days)	5	10.65±0.09 [b]
5.	Recovery	5	18.45±0.01 [a]

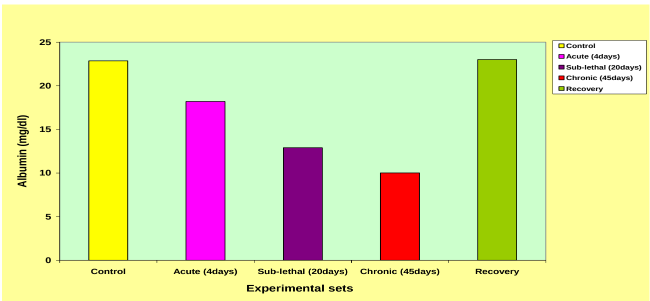
Table 4: Albumin/Globulin ratio in muscle tissue of *Channapunctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)

S.No.	Experimental set	No. of fishes	(Mean±S.E.)
1.	Control	5	1.20±0.01
2.	Acute (4 days)	5	1.45±0.05 [a]
3.	Sub-lethal (20 days)	5	1.63±0.03 [c]
4.	Chronic (45 days)	5	1.40±0.09 [b]
5.	Recovery	5	1.23±0.03 [a]

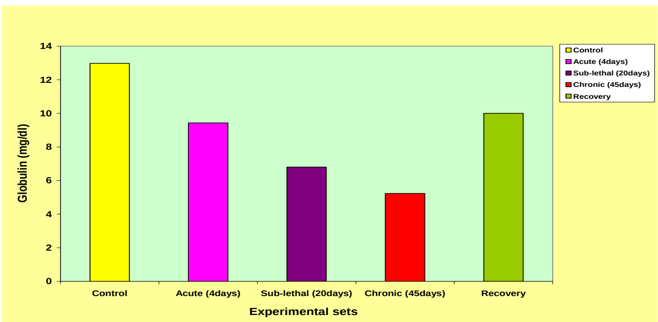
a- Non-significant (P>0.05); b- Significant (P<0.05); c- Highly significant (P<0.01); d- Very highly significant (P<0.001)



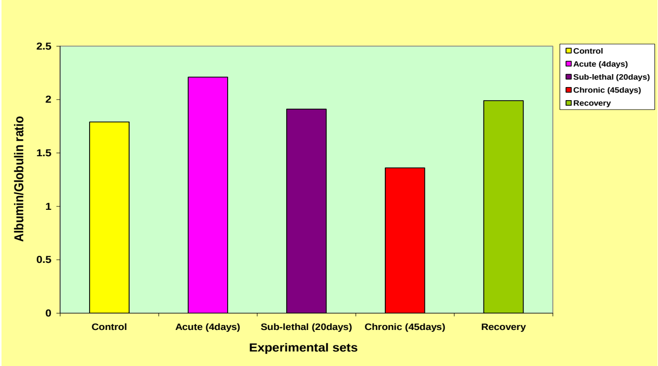
Graph 1: Showing total protein (mg/dl) in gill of *Channa punctatus* after acute (4days), sub-lethal (20days) and chronic (45days) treatment of cypermethrin (25%EC)



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



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



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

In today's advanced technical society, we can expect solutions to every conceivable problem based on the use of various tools, procedures, chemicals, etc., all of which have been developed by humans. Synthetic compounds, more generally known as pesticides, have allowed us to swiftly respond to threats to our crops, stored food products, and human health. As research into the field of pesticides advanced, a plethora of new compounds with targeted features entered the market and quickly became widely used. Companies that cared the least about the consequences to the environment also completed the majority of the follow-up research. In deep water, such as ponds and rivers, these chemicals and other types of pollution will inevitably circulate. We cultivate fish primarily in ponds and rivers. Therefore, maintaining a healthy fish population is a priority for us. Here, we have generated biochemical data and analysed it with stats programmes. This will draw attention to the way that routinely used pesticides contribute to environmental contamination. Pesticides are bad for fish, and people who consume fish that have been contaminated with these chemicals may be putting themselves at risk. Pesticides can have negative consequences on human health, as we might infer from studies of their effects on fish.

Proteins are crucial chemical compounds for cellular function and tissue maintenance and repair. Exposure time correlates negatively with protein concentration, however this decline is most pronounced in gill tissue and recovers to normal levels once the animal has been able to avoid further exposure. Proteins play a pivotal role in shaping the cellular framework. As an added bonus, they provide vitality throughout extended bouts of stress (Umminger, 1977). Fish subjected to sublethal concentrations of cypermethrin exhibited behavioural changes indicative of stress. Fish required a higher metabolic rate to eliminate the toxicants and get through stressful situations. Protein is the second best source of energy after carbohydrates, which fish have very few of, so they can help you out here. Protein breakdown and the subsequent consumption of degradation products for metabolic functions may account for the depletion of protein fraction in skeletal muscle and gill tissues.

There are three main routes through which fish and other aquatic creatures are exposed to pesticides. Pesticides can be ingested in three ways: (1) dermally, through direct absorption through the skin when swimming in pesticide-contaminated waters; (2) respiratoryly, through direct uptake of pesticides through the gills during respiration; and (3) orally, through drinking pesticide-contaminated water or feeding on pesticide-contaminated prey. Secondary poisoning refers to the ingestion of an animal that has already been exposed to a pesticide. For instance, if fish consume a lot of insects that have been poisoned by insecticides and are themselves dying, the fish may perish.

The amount of protein in tissues used in the experiments, including muscle and gill, was reduced. Sublethal pesticide exposure has been linked to a loss of protein content in several fish tissues, as observed by a number of researchers including Malla Reddy and Bashamohideen (1995) and Singh et al. (1996). Protein was used as an energy source by aquatic dwellers subjected to hazardous conditions, as noted by Umminger (1977), who noted a drop in protein content in the fish Fundulus beteralitus.

Muscle and gill tissue from test fish *Channa punctatus* subjected to cypermethrin showed decreased total protein content in the current study. This may have happened because cypermethrin's toxic stress increased the body's desire for proteins. Exposure time decreases albumin, globulin, and the A/G ratio, however the effects are most pronounced in gill tissue and reverse themselves after the fish have recovered. Ansari and Kumar (1988) also observed a decrease in zebrafish protein (both albumin and globulin) and nucleoprotein (DNA and RNA) levels following diazinon therapy, which is consistent with the current results. Natural pyrethrins, S-bioallethrin, dimethrin, and d-transallethrin were shown to be poisonous to Oncorhynchus kisutch and Salmo gairdner, as reported by Mauck et al. (2005), who also noted a decrease in albumin and globulin levels. Channa striatus treated with cypermethrin, permethrin, and fenvalerate resulted in alterations in albumin, globulin, and the A/G ratio, as reported by Singh and Agarwal (2006). As albumin, globulin, and the A/G ratio are all constituent parts of protein, their reduction parallels that of protein content.

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