

Acute Toxicity of Synthetic Pyrethroid Cypermethrin on Protein Content in Estuarine Clam, *Marcia Opima* (Gmelin, 1791)

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Abstract

Cypermethrin is a synthetic pyrethoid mainly used against mango insect pests. Clams are good indicators of pesticide pollution and are known to be tolerant to pesticide accumulation. Acute toxicity experiment of Cypermethrin is carried out on estuarine clam, *Marcia opima*. The clam exhibited significant increase in protein content of gill in LC₀ and LC₅₀ group of clams while foot, male gonad and female gonad exhibited considerable decrease in protein content. Whereas, in LC₀, mantle showed high protein content. In LC₅₀ group, hepatopancreas exhibited high protein content.

Keywords: Cypermethrin; *Marcia opima*; Protein

Introduction

In recent years, human intervention has brought major changes in the aquatic ecosystem. Among all these changes one of the one of such important intervention is that of pesticides. Among the pesticides, pyrethroids are commonly used due to their high effectiveness, low toxicity to birds and mammals, and easy biodegradability [1]. Indiscriminate use of different pesticides in agriculture to prevent crop damage from pests has increased over the years, especially in the developing countries [2]. These pesticides, even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and alter the physico-chemical properties of water [3]. Cypermethrin is a highly potent and broad spectrum synthetic pyrethroid which is used extensively for pest control. Although it is not persistent in the environment, the excess use of this pesticide may result in its entry into natural waters through agricultural run-off and ultimately cause damage to non-target organisms such as fish [4].

Ratnagiri district is famous for mango and paddy production and Cypermethrin is widely used to control the pest of these crops. As mango is the main cash crop and big asset for foreign currency, Cypermethrin is heavily used against mango insect pests. Pesticides are generally used from September to April. A maximum portion of pesticides gets concentrated in the soil nearby. Heavy rain freshets bring Cypermethrin impregnated soil into Bhatye estuary, Ratnagiri (Maharashtra). This estuary acts as a cradle bed for estuarine flora and fauna, including economically important fauna such as fish, oysters and clams. Among important organisms inhabiting estuarine zones, bivalves are sessile and filter-feeder species, able to accumulate contaminants in their tissues. Clams are abundant along the coast of Bhatye estuary and are important because they are commonly used as food. Survey of the literature has revealed that clams are good indicators of pesticide pollution. Clams are known to be tolerant (may be sensitive) to pesticide accumulation (may be presence) and have a relatively long life span. In spite of the above facts, miniscule attention has been paid by researchers with regard to the impact of pesticide pollution on estuarine clams. Considering the nutritive value of clams, wide use of Cypermethrin in the agriculture fields of Ratnagiri, its bioaccumulation and biomagnifications, the estuarine clam *Maria opima* was selected to study the impact of sub-lethal concentrations of Cypermethrin.

The acute toxicity studies are useful in determining a sensitive

species that can be an indicator species for a particular type of pollution and a tool for the logical assessment of acute toxicity amongst various biological systems. Pollution of water bodies by pesticides causes disorders in the metabolic activities and physiological functioning, thereby alters the biochemical constituents in aquatic organisms. Changes in macromolecules like glycogen, protein and lipid are considered to be sensitive indicators of pesticides stress [5].

In estuarine clams, protein is one of the important sources of energy for carrying out various activities, but due to contaminants stress such prime source of energy is affected severely, retarding various processes in the clam body. Therefore, the present work is undertaken to assess the effect of acute toxicity of synthetic pyrethroid Cypermethrin on protein content in estuarine clam, *Marcia opima*.

Materials and Methods

The Bhatye estuary is located in between 17°20' N and 73°20' W, in the Ratnagiri district in the Konkan region and this is the major estuary on the west coast of Maharashtra state. The experimental clams, *Marcia opima* used for the present study were collected from Bhatye estuarine region, Ratnagiri coast, Maharashtra state during January 2011. The clams of medium size (4.0-4.4 cms) were selected, brought to the laboratory and stocked in the plastic containers containing filtered, aerated estuarine water, for 48 hours. Clams well acclimatized to the laboratory conditions were grouped in tens and kept in plastic containers containing 5 liters filtered estuarine water. Static bioassay tests were conducted for 96 hours by using Cypermethrin (25% EC). For every experiment, a control group of clam was also run simultaneously. The volume of the container was maintained at 5 L for each. Observations were made at 12, 24, 36, 48, 60, 72, 84 and 96 hours after introduction into different pesticide concentrations.

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The LC₅₀ value for each time period was estimated by a regression analysis determined for the log of concentrations and percentage survival of the clam. The percentage mortality in various concentrations at particular period were converted into probit values and plotted against the log of concentrations [6]. The toxicity tests were repeated three times and LC₀ and LC₅₀ values were determined. The regression equation between the log of concentration (X) and probit mortality (Y) were determined statistically for acute toxicity using the formula $Y = \alpha + \beta \log(x)$ and 95 % fiducial limits were established according to the procedure outlined by Finney [7].

After the 96-h study period, various tissues (i.e. gills, mantle, hepatopancreas, foot and male and female gonad) of control clam, LC₀ and LC₅₀ groups from acute exposure were removed from each group and were blotted with filter paper to remove excess moisture. The homogenized sample tissues were then dried in an oven at 60 °C until a constant weight was obtained. Tissues were powdered and oven dried tissue powder was used for biochemical analysis. 100 mg of dry powder of each tissue was taken for biochemical analysis. Protein was determined by the method proposed by Lowry et al. [8], using Bovine Serum Albumin (BSA) as a standard. The results are expressed as milligram content per 100mg of dry tissue. Triplicate values were subjected to statistical confirmation using students' test.

Results and Discussions

During experimental period, clams showed no mortality for concentrations of cypermethrin ranging up to 1.86 ppm. The calculated LC₅₀ value was 2.75 ppm and the observed LC₅₀ value was 2.79 ppm. The regression equation established was $Y = 0.6242 + 9.9484x$, the 95% fiducial limit was 1.7632-3.7368 ppm for this season (Table 1).

Changes in protein content of *Marcia opima* after cypermethrin exposure

In Table 2, the control group showed protein content of 11.519 ± 0.076, 19.406 ± 0.063, 21.507 ± 0.093, 21.766 ± 0.052, 30.486 ± 0.069 and 34.065 ± 0.052 mg/100 mg dry tissue, in hepatopancreas, gill, mantle, female gonad, male gonad and foot, respectively. In LC₀ (1.86ppm) group, protein content was presented in ascending order of 12.406 ± 0.063, 20.066 ± 0.052, 20.426 ± 0.063, 22.406 ± 0.063, 29.499 ±

0.074 and 30.644 ± 0.008 mg/100mg dry tissue in hepatopancreas < gill, female gonad < mantle < male gonad < foot, respectively. As compared to control group, LC₀ group showed 3.40, 4.18 and 7.70% significant increase in protein content in gill, mantle and hepatopancreas, respectively and 3.23, 6.15 and 10.04% (p<0.001) significant decrease in protein content in male gonad, female gonad, and foot, respectively. LC₅₀ (2.79ppm) group showed protein content in ascending order of 13.776 ± 0.079, 18.166 ± 0.052, 19.159 ± 0.064, 27.426 ± 0.079, 28.399 ± 0.052 and 28.466 ± 0.105 mg/100mg dry tissue in hepatopancreas < female gonad < mantle < foot < gill < male gonad, respectively. As compared to control group, LC₅₀ group exhibited significant increase of 19.59 and 46.34 % (p<0.001) in hepatopancreas and gill, respectively while 6.62, 10.91, 16.53 and 19.48 % (p<0.001) decrease in male gonad, mantle, female gonad and foot, respectively.

Protein plays vital role in spawning and other metabolic activities. It is the main organic nutrient used to build up different body tissues. In the present study, it was observed that protein content changes according to season, physiological status of the clam and artificial environmental stress. Hepatopancreas, male and female gonad showed low protein content during the experimental period. It may be due to spawning period. Gill is an exceptional case in which, the protein content was high. Nagbhushnam and Talikhedkar [9] found that in *D. cuneatus*, protein content remain relatively high throughout the year except a decline during the breeding period and at the time when the clams were in fully matured condition. Durve and Bal [10], while studying biochemical composition of *C. gryphoides* observed high protein values throughout the year. Further, they stated that period of low value coincided with the spawning season. Protein content in the mantle, hepatopancreas and adductor muscles of bivalve *L. marginalis* was decreased due to mercury toxicity [11]. Also, Mule and Lomate [12] observed decrease in protein concentration in mantle, foot and whole body due to mercury toxicity in gastropod, *Thiara tuberculata*. In gastropods, protein content was decreased due to the herbicide toxicity [13]. During this type of stress condition the protein synthesis and interconversion of amino acids, glucose and fatty acids to liberate energy get affected [14].

In the present study, it was observed that, increase or decrease in protein content depends upon spawning season, environmental stress, concentration of toxicants and particular tissue. The clam exhibited significant increase in protein content of gill in LC₀ and LC₅₀ group of clams while foot, male gonad and female gonad exhibited considerable decrease in protein content. Whereas, in LC₀, mantle showed high protein content. In LC₅₀ group, hepatopancreas exhibited high protein content. This is because protein is the source of energy during chronic conditions of stress, Number of studies had reported a decline in different organs of fishes treated with pesticides [15-17].

A reduction in protein content after the exposure of Cypermethrin may be due to reduced protein synthesis. The reduced protein content may also suggest increased proteolysis and it is also possible that utilization of degraded products for metabolic processes might have increased the pesticide stress. The protein level decreased in all organs except gill in LC₀ and LC₅₀ group. Decrease in protein level may be due to increased proteolytic activity or might be due to anaerobic conditions produced by Cypermethrin. Decrease in fish protein content was due to pesticide stress or utilization of proteins in glycconeogenesis for energy production [13].

In the present study, there was slight increase in protein content of gill in LC₀ and LC₅₀ group. Muley et al.(1996) [18], reported increased level of protein content in gill, muscles and kidney of *Tilapia mosambica*

Y = α + βx	95% fiducial limit (ppm)	Calculated LC ₅₀ (ppm)	Observed LC ₅₀ (ppm)
0.6242 + 9.9484x	1.7632 - 3.7368	2.75	2.79

Table 1: Regression equations, 95% fiducial limits with LC₅₀ values for *Marcia opima* exposed to Cypermethrin.

Tissue	Control	LC ₀ Group	LC ₅₀ Group
Mantle	21.507 ± 0.093	22.406 ± 0.063 (4.18)***	19.159 ± 0.064 (-10.91)***
Gill	19.406 ± 0.063	20.066 ± 0.052 (3.40)***	28.399 ± 0.052 (46.34)***
Foot	34.065 ± 0.052	30.644 ± 0.008 (-10.04)***	27.426 ± 0.079 (-19.49)***
Hepatopancreas	11.519 ± 0.076	12.406 ± 0.063(-17.70)***	13.776 ± 0.079 (19.59)***
Male Gonad	30.486 ± 0.069	29.499 ± 0.074 (-3.23)***	28.466 ± 0.105 (-6.62)***
Female Gonad	21.766 ± 0.052	20.426 ± 0.063(-6.15)***	18.166 ± 0.052 (-16.53)***

Values in parenthesis are percent change. ± = S.D. of five animal. * = p < 0.05, ** = p < 0.01, *** = p < 0.001

Table 2: Cypermethrin induced alterations in the total protein content of *Marcia opima* after acute exposure. (Results expressed in mg/100mg dry wt. basis).

exposed to 0.016 ppb of endosulfan for 168 hours. Such increase in protein level may be assumed to be due to the heavy metal induced new proteins which may have some harmful effects on animal system. An increase in protein content of various tissues which could reflect stimulated protein synthesis of detoxification enzymes. Toxic level of Cypermethrin in the present study showed an alteration in tissue protein suggesting disturbance in physiological activity.

Conclusion

In the present study, increase in protein content may be due to reproductive status or induction of new proteins by pesticides or it might be due to stimulated protein synthesis. Decrease in protein content may be due to reproductive status, unfavorable water parameters or pesticide stress or utilization of proteins in gluconeogenesis for energy production.

Author's contributions

- 1) Madhura Mukadam carried out the toxicological studies and drafted the manuscript.
- 2) Arvind Kulkarni helped to draft, read and approved the manuscript.

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