Histopathological and Biochemical studies of hepatopancreas in *Katelysia opima* (Gmelin) exposed to Cypermethrin

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Abstract

The experimental clams, *Katelysia opima* of medium size (4.0 – 4.4 cms) were collected from Bhatye estuarine region, Ratnagiri coast, Maharashtra state and were acclimatized in laboratory condition for 48 hours. Well acclimatized clams were grouped in tens and static bioassay tests were conducted for 96 hours by using Cypermethrin (25% EC). After studying 96 hours (acute) and 30 days (chronic) toxicity of Cypermethrin to *Katelysia opima* in different seasons, hepatopancreas of control, LC_0 and LC_{50} groups from acute exposure and $1/10^{th}$ concentration of LC_{50} groups from chronic exposure was removed from each group and used for histopathological and biochemical analysis. The glycogen content was significantly ($p< 0.001$) increased in LC_0, LC_{50} and chronic groups during all three seasons. The protein content was significantly ($p< 0.001$) decreased in LC_0 whereas increased in LC_{50} group. Lipid content was observed significantly increased during winter in LC_0 group and decrease in LC_{50} group during all the seasons. In chronic group, hepatopancreas had shown low lipid content in summer, The disconnection of digestive and secretary cells with basement membrane, infiltration of amoebocytes in to tubules, accumulation of haemocytes, vacuolization in the cytoplasm of digestive cells and karyolysis or necrosis were the common features of Cypermethrin (acute and chronic) exposure in hepatopancreas. The tissue damage was observed more in LC_{50} and chronic group than control and LC_0 group during monsoon.

KEYWORDS: *Katelysia opima,* Cypermethrin, hepatopancreas *etc*.

Introduction :

Cultural evolution has led to the use of pesticides, for the control of pests and in turn resulted in the pollution of aquatic systems. It is well known that the chemical pollutants present in water may induce severe ecological consequences, generating reorganizations of the biocenosis, changing it and consequently affecting the aquatic ecosystem integrity (Vosyliene and Jankaite, 2006). The pollution of rivers, streams and larger water bodies like lakes with chemical contamination in the form of industrial effluents and pesticides has become one of the most critical environmental problems of the century. The frequent use of pesticides for various industrial, agricultural and domestic purposes is a veritable sources of pesticide introduction into the environment. But sub-lethal pollution, which results in chronic stress conditions also have a negative effect on aquatic life (Adedeji *et al*., 2008).

Cypermethrin is a synthetic pyrethroid insecticide. It acts as a fast acting neurotoxin in insects. Cypermethrin is mainly used to control insect pests of mango and paddy. *Katelysia opima* (Gmelin) are abundant in estuaries along the Ratnagiri coast, Maharashtra and are known to accumulate contaminants. Exposure of organism to pesticide provide a wide variety of effect involving many organs system (Patil and Dhande, 2000), such effects mainly include behavioural, physical and biochemical

processes of organisms, though they may remain unnoticed before significant damage. Therefore, histopathological and biochemical studies have gain importance in ecotoxicological studies. Hence, the present investigation was undertaken to elucidate the Cypermethrin toxicity by observing the histopathological and biochemical changes in hepatopancreas of *K. opima.*

Materials and Methods:

The experimental clams, *Katelysia opima* used for the present study were collected from Bhatye estuarine region, Ratnagiri coast, Maharashtra state. 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 condition 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). The toxicity tests were repeated three times and LC_0 and LC_{50} 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 Finney (1971). All the experiments were carried out on freshly collected clams in three different seasons i.e. summer (March & April, 2010), monsoon (June & July, 2008) and winter (December & January, 2011). Based on the LC_{50} values, $1/10^{th}$ concentration of the LC_{50} of Cypermethrin was selected for sub-lethal toxicity (30 days) studies. All the experiments were done in triplicates. The water parameters like temperature, salinity, pH and dissolved oxygen were measured using standard procedures. After studying 96 hours (acute) and 30 days (chronic) toxicity of Cypermethrin to *Katelysia opima* in different seasons, hepatopancreas of control, LC_0 and LC_{50} groups from acute exposure and chronic exposure were removed from each group and were blotted with filter paper to remove excess moisture. The tissues were then dried in an oven at 60° C, powdered and used for biochemical analysis. The total glycogen content was estimated according to the method proposed by De-Zwaan and Zandee (1972), using glucose as standard. Protein was determined by the method proposed by Lowry *et. al.* (1951) using Bovine Serum Albumin (BSA) as a standard. The method used for determination of lipid was Sulpho-phospho-vanilline method proposed by Barnes and Blackstock (1973). The results are expressed as milligram content per 100mg of dry tissue. Triplicate values of each biochemical constituents were subjected to statistical confirmation using student's' test (Dowdeswell, 1959).

For histopathological studies hepatopancreas of control, LC_0 and LC_{50} groups from acute exposure and chronic exposure were removed and fixed in neutral buffer formalin for 48 hr for proper fixation. Tissues were washed in distilled water, then dehydrated in Ethyl alcohol, cleared in Xylol and embedded in tissue mat (at 58-60°C melting point) and then they were sectioned at 5 to 6 µm thickness on a rotary microtome (Erma, Japan). Theses Sections were stained with Ehrlich's Hematoxylene and alcoholic Eosin stain and mounted in DPX. All the observations for microphotography were done under Trinocular research microscope attached to the camera (Carl Zeiss, model: Axiostar).

Results :

The stained sections of the hepatopancreas of control group did not show major differences in three different seasons. The digestive tubule showed various phases in each season. It consists of ducts and digestive tubules indistinctly connected

and separated by interlobular connective tissue consisting of collagen fibers. Each tubule is bounded by thin muscle fibers which form the basement membrane. Each digestive tubule consists of digestive cells or columnar cells, secretary cells or pyramidal cells. Each of these cells possesses a basal nucleus with prominent nucleoli. The lumen of the tubule increases or decreases on the basis of the amount of food particles accepted for digestion. During this process, fragmentation spherules are budded off from the apex of digestive cells into the lumen. The differences from one season to another or from the one tubule to another were present in the form of fragmentation spherules related with the lumen size. During monsoon, fragmentation spherules are numerous in the lumen as compared to summer and winter. (Plate 1, 2 , 3 : Fig. a)

Acute Exposure:

LC_0 group: During summer and monsoon, tubules showed swelling. The basement membrane of each tubule was ruptured at some places or either got separated from the tubule. The digestive and secretary cells at few places detached from the basement membrane and from each other also. A characteristic feature noticed was the presence of wandering haemocytes in the lumen of gastric diverticula. Tubular damage involved disruption and dislocation of the collagen layer, Loss of identity of tubules and heavy vacuolization of the two types of cells. Similar types of effects were observed during all the three seasons in hepatopancreas but severity was more observed in summer and monsoon than in winter. (Plate 1, 2 , 3 : Fig. b)

LC_{50} group: Hepatopancreas of LC_{50} group showed severe histological changes. Basement membrane and muscular layer got ruptured at places with loss of connective tissue. The collagen layer had disintegrated exposing the muscular layer. There was partial or complete disintegration of digestive and secretary cells. These cells mostly showed karyolysis or necrosis. The secretary cells were shrunken and their nuclei were swollen in appearance. The amoebocytes increased in number in inter lobular spaces and many of them are surrounded by the tubule cells. Haematocytes accumulated more in between the tubules and this was more pronounced in LC_{50} group. Comparing the severity of effects, it was more pronounced during summer and monsoon. (Plate 1, 2 , 3 : Fig. c)

Chronic exposure: The hepatopancreas exposed to sub-lethal concentrations of Cypermethrin for a month had lost basic characteristics giving a highly bulged and enlarged appearance. The collagen layer had disintegrated exposing the muscular layer. The cells were found to be dislodged. In general, there was a reduction of tubules per unit area. Digestive and secretary cells detached from the basement membrane at many places. The number of amoebocytes increased. Vacuolization was more prominent. Darkly stained nuclei of tubule cells indicate karyolysis. Similar effects were present during winter and monsoon. During monsoon, fragmentation spherules were many in the lumen of each tubule. (Plate 1, 2 , 3 : Fig. d)

Biochemical changes:

Glycogen:

Acute Exposure: In the hepatopancreas of control group the glycogen level was found to be 14.666±0.174, 12.11± 0.067 and 15.6± 0.055 mg/100mg dry tissue (Table. I) during summer, monsoon and winter season respectively. In LC_0 and LC_{50} group the glycogen content was 19.23±0.589 and 21.786±0.150, 18.372±0.286 and 17.675±0.072, 16.3±0.039 and 19.095±0.032 mg/100mg dry tissue. As compared to

control group in LC_0 and LC_{50} group, there was significant increase of glycogen *i.e.* 31.11 and 48.5; 51.7 and 45.95 and 4.48 and 22.4% during summer, monsoon and winter season respectively.

Chronic Exposure: In the hepatopancreas of control group the glycogen level was found to be 15.508± 0.129, 11.72 ± 0.064 and 13.825± 0.055 mg/100mg dry tissue (Table. II) during summer, monsoon and winter season respectively. In chronic group the glycogen content was 21.244± 0.102, 18.7 ± 0.068 and 15.345± 0.032 mg/100mg dry tissue during summer, monsoon and winter season respectively. As compared to control group in chronic group, there was significant increase of glycogen *i. e.* 36.99, 59.55 and 10.99 % during summer, monsoon and winter season respectively.

Protein:

Acute Exposure: In the hepatopancreas of control group the protein content was found to be 15.458± 0.303, 13.172± 0.103 and 11.519± 0.076mg/100mg dry tissue (Table. I) during summer, monsoon and winter season respectively. In LC_0 and LC_{50} group the protein content was 14.887± 0.271 and 11.775 ± 0.265, 15.639 ± 0.064 and 14.279 ± 0.089, 12.406 ± 0.063 and 13.776 ± 0.079 mg/100mg dry tissue. As compared to control group in LC_0 and LC_{50} group, there was significant increase of protein *i.e.* 18.72 and 8.4 % during monsoon and 17.7 and 19.59 % during winter. Significant decrease of 3.69 and 23.82 % was observed during summer

Chronic Exposure: In the hepatopancreas of control group the protein level was found to be 14.512± 0.096, 12.166 ± 0.074 and 11.903 ± 0.0024 mg/100mg dry tissue (Table. II) during summer, monsoon and winter season respectively. In chronic group the protein content was 18.413 ± 0.163, 17.199 ± 0.691 and 14.731± 0.0037mg/100mg dry tissue during summer, monsoon and winter season respectively. As compared to control group in chronic group, there was significant increase of protein *i. e.* 26.88, 41.36 and 23.75 % during summer, monsoon and winter season respectively.

Lipid :

Acute Exposure: In the hepatopancreas of control group the lipid content was found to be 8.288 ± 0.168 , 10.456 ± 0.033 and 9.23 ± 0.015mg/100mg dry tissue (Table. I) during summer, monsoon and winter season respectively. In LC_0 and LC_{50} group the lipid content was 10.378 ±0.047 and 7.572± 0.049, 12.884 ± 0.028 and 8.088 ± 0.048, 11.828 ± 0.019 and 8.43± 0.015 mg/100mg dry tissue. As compared to control group in LC_0 group, there was significant increase of lipid *i.e.* 25.31, 23.22 and 28.14 % during summer, monsoon and winter season respectively. In LC_{50} group, significant decrease of 8.63, 22.64 and 8.66 % was observed during summer, monsoon and winter respectively.

Chronic Exposure: In the hepatopancreas of control group the lipid content was found to be 8.303± 0.094, 11.422± 0.0019 and 9.216± 0.0024 mg/100mg dry tissue (Table. II) during summer, monsoon and winter season respectively. In chronic group the lipid content was 6.547 ± 0.132, 8.103 ± 0.0015 and 8.446 ± 0.003 mg/100mg dry tissue during summer, monsoon and winter season respectively. As compared to control group 8.36 .75 % during summer, monsoon and winter season respectively.

Discussions:

Any particular alteration of cell may indicate the presence of disease or the toxic substance. The extent of damage induced by the toxicant to a particular organ can also be judged at a cellular level. Pathological and biochemical disturbances in aquatic organisms like mollusc due to pesticide toxicity are well documented (Waykar and Lomte, 2002; 2004). Histopathological changes are mostly confined to organs directly directly involved in their metabolism and detoxification (Rashatwar and ilyas, 1994).

Hepatopancreas is very important target organ. The above results revealed that the structural changes occurred due to cypermethrin in all the three seasons. The disconnection of digestive and secretary cells with basement membrane, infiltration of amoebocytes in to tubules, accumulation of haemocytes, vacuolization in the cytoplasm of digestive cells and karyolysis or necrosis were the common features of cypermethrin (acute and chronic) exposure. High vacuolation of digestive cell is known to be one of the manifestations of stress response which is indicative of increased lysosomal number. The cells of digestible tubule show atrophy and thinning of epithelium. There is tendency to generalize such changes to stressor effects mainly by genobiotic or prolonged starvation (Pipe *et al*. 1985). Structural assay of cellular damage have shown that, there was enlargement of cells of digestive tubules resulting in bulbous epithelial structure or total atrophy resulting in thinning. The basement membrane of each tubule was ruptured at some places. The presence of completely damaged cells of digestive tubule is an indication of atrophy which would eventually lead to sloughing off of cells. In the present study, the tissue damage was observed in LC_{50} and chronic group than control and LC_0 group. The severe damage was observed in monsoon.

In the present study seasonal changes in glycogen content in different organs of *Katelysia opima* were recorded. It varies according to tissue, season and physiological status of clam. Hepatopancreas showed maximum level during summer whereas minimum level during monsoon. When clams were exposed to LC_0 concentration of acute test, significant increase in glycogen content was observed in hepatopancreas in all seasons. Clams treated with chronic concentration, the glycogen content was seen to increase during summer and monsoon, with a decrease in winter. In general, there was significant increase in glycogen content in hepatopancreas in clams of LC_0 and chronic group. The significant increase in glycogen level indicate that, it accumulates Cypermethrin and combat with Cypermethrin level.

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. In LC_{50} group, hepatopancreas exhibited high protein content in monsoon and winter. In chronic group of clams, significant increase in protein content was observed. This is because according to Umminger (1970), protein is the source of energy during chronic conditions of stress. Number of studies has reported a decline in protein contents of fishes treated with pesticides (Ram and Sathyanasan, 1984; Ganguly *et al*., 1997; Ramani, 2001). 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 pesticidal stress.

In adult bivalves, lipids are mainly stored in gonads. In hepatopancreas, the increase in lipid content was observed in LC_0 whereas LC_{50} group showed decrease in lipid content in all the three seasons. A physiological change that takes place when organisms are exposed to lethal and sub-lethal levels of Cypermethrin stress include rate of feeding as well as respiration and excretion. The net result could be a change in energy available for growth and reproduction. When the clams were subjected to LC_0 concentration, hepatopancreas showed marked increase in lipids during all seasons. The decrease in lipid levels in LC_0 and LC_{50} groups perhaps due to an increase in lipolysis to meet higher energy demand to overcome the toxicant stress. The observed increase in lipids is probably related to the anoxic endogenous oxidation process providing required energy for survival under Cypermethrin stress with simultaneous generation of acetyl Co-A.

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Fig. a Section of Hepatopancreas Control –10 x 10 T: Hepatic Tubule
Fig. b Section of Hepatopancreas LC_0 – 10 x 10 SC: Secretary cells N: Nucleus
Fig. c Section of Hepatopancreas LC_{50} – 10 x 10 B : Basement membrane
Fig. d Section of Hepatopancreas Chronic – 10 x 10 DC: Digestive cells

Plate 1

Fig. a Fig. b Fig. c Fig. d

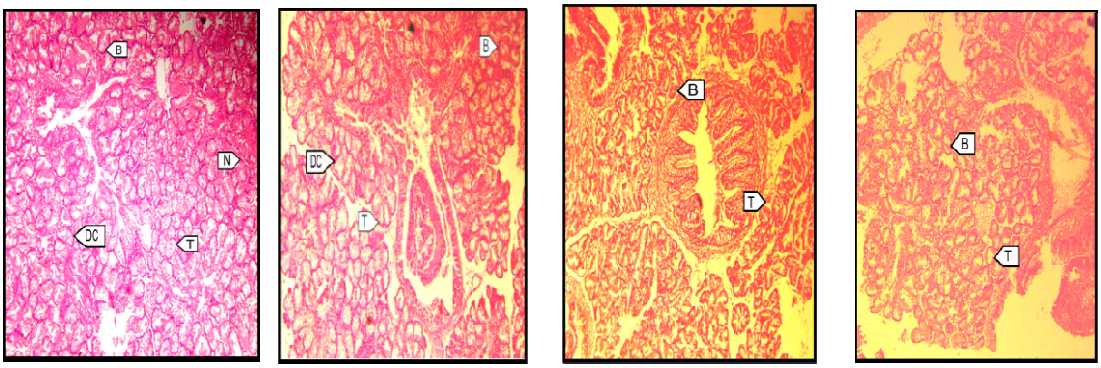

Plate 2

Fig. a Fig. b Fig. c Fig. d

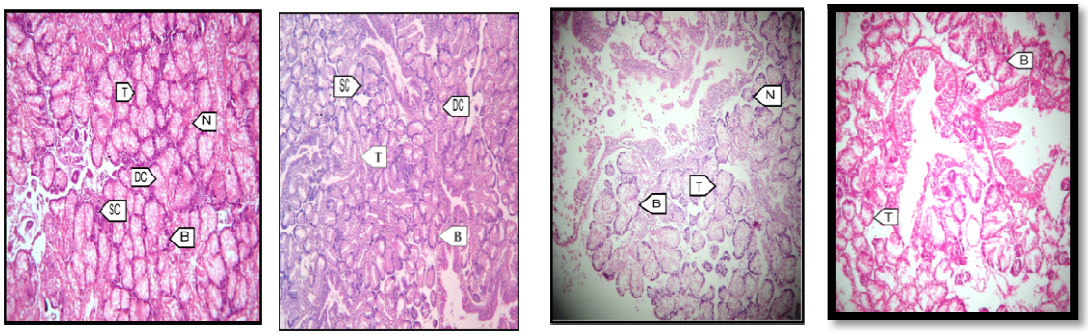

Plate 3

Fig. a Fig. b Fig. c Fig. d

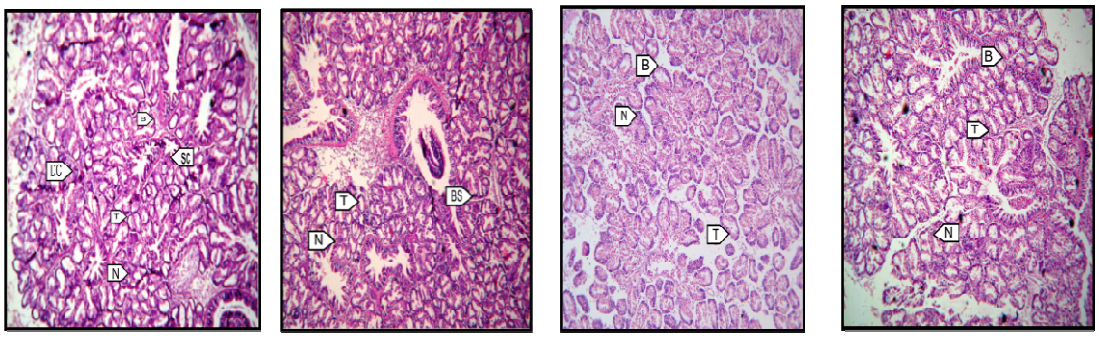

Table No. I
Cypermethrin induced alterations in biochemical constituents of *Katelysia opima* after acute exposure. (Results expressed in mg/100mg dry wt. basis)

	SEASON	CONTROL	LC_0 GROUP	LC_{50} GROUP
Glycogen	SUMMER	14.666 ± 0.174	19.23 ± 0.589 (31.11) ***	21.786 ± 0.150 (48.5) ***
	MONSOON	12.11 ± 0.067	18.372 ± 0.286 (51.7) ***	17.675 ± 0.072 (45.95) ***
	WINTER	15.6 ± 0.055	16.3 ± 0.039 (4.48) ***	19.095 ± 0.032 (22.4) ***
Protein	SUMMER	15.458 ± 0.303	14.887 ± 0.271 (-3.69) ***	11.775 ± 0.265 (-23.82) ***
	MONSOON	13.172 ± 0.103	15.639 ± 0.064 (18.72) ***	14.279 ± 0.089 (8.4) ***
	WINTER	11.519 ± 0.076	12.406 ± 0.063 (17.70) ***	13.776 ± 0.079 (19.59) ***

Lipid	SUMMER	8.288 ± 0.168	10.378 ±0.047 (25.31) ***	7.572 ± 0.049 (-8.63) ***
	MONSOON	10.456 ± 0.033	12.884 ± 0.028 (23.22) ***	8.088 ± 0.048 (-22.64) ***
	WINTER	9.23 ± 0.015	11.828 ± 0.019 (28.14) ***	8.43 ± 0.015 (-8.66) ***

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

Table No. II
Cypermethrin induced alterations in biochemical constituenyts of *Katelysia opima* after chronic exposure. (Results expressed in mg/100mg dry wt. basis)

TISSUE	SEASON	CONTROL	CHRONIC GROUP
Glycogen	SUMMER	15.508 ± 0.129	21.244 ± 0.102 (36.99) ***
	MONSOON	11.72 ± 0.064	18.7 ± 0.068 (59.55) ***
	WINTER	13.825 ± 0.055	15.345 ± 0.032 (10.99) ***
Protein	SUMMER	14.512 ± 0.096	18.413 ± 0.163 (26.88) ***
	MONSOON	12.166 ± 0.074	17.199 ± 0.691 (41.36) ***
	WINTER	11.903 ± 0.0024	14.731 ± 0.0037 (23.75) ***
	SUMMER	8.303 ± 0.094	6.547 ± 0.132 (-21.14) ***

Lipid	MONSOON	11.422 ± 0.0019	8.103 ± 0.0015 (-29.05) ***
	WINTER	9.216 ± 0.0024	8.446 ± 0.003 (-8.36) ***

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