Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 10	No. 1	pp. 63-66	2011

Original Research Paper

Cypermethrin Induced Histological Alterations in Estuarine Clam, *Meretrix meretrix* (Linn.)

Madhuri Prabhupatkar, A. S. Kulkarni, M. V. Tendulkar, S. M. Nikam and A. S. Injal
Department of Zoology, Gogate-Jogalekar College of Arts, Science and Commerce, Ratnagiri-415 612, Maharashtra, India

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Key Words:
Cypermethrin
Estuarine clam
Meretrix meretrix
Histological alterations
LC_{50}

ABSTRACT

Cypermethrin is a synthetic pyrethroid class of insecticide. Toxic effects of cypermethrin were explored by selecting *Meretrix meretrix* as an animal model. After acute (96 hours) exposure to Cypermethrin, tissues of *Meretrix meretrix* like gill, mantle, foot, hepatopancreas, male gonad and female gonad, were investigated for histological alterations. Pesticide stress showed histological changes, deterioration of tissue and damage at cellular and subcellular levels. There was a remarkable damage in all tissues. Gills showed damage in epithelial cells and connective tissue in LC_{50} group. Mantle of clams from LC_0 group showed considerable damage to the dorsal as well as ventral epithelium, connective tissues and blood spaces. Hepatopancreas of clams from LC_{50} group showed swelling of tubules. Male gonad showed severe damage; follicle wall was ruptured; sperms were clumped in a mass. Female gonad showed deterioration of ooplasmic material. LC_{50} group showed more damage than LC_0 group. Foot of LC_{50} group showed significant damage i.e., folding of marginal epidermis, shrinkage of epidermis, loss of connective tissues took lace as compared to control group of the clam. Theses histological changes were more prominent in gill and hepatopancreas as compared to other tissues.

INTRODUCTION

Cypermethrin is one of the recent synthetic pyrethroids being used to control many pests including moth pests of cotton, fruit and vegetable crops. It is also used for crack, crevice and spot treatment to control the insect pests in stores, warehouses, industrial buildings, houses, apartments, buildings, green houses, laboratories, and on ships, buses, trucks and aircrafts. Cypermethrin is available in emulsifiable concentrate and wettable powder formulations.

In Ratnagiri, cypermethrin is mainly used to control mango hoppers, mango mealy bugs, jassids and other insect pests of mango. It is also used to control various insect pests of paddy like brown plant hopper (BPH), green leaf hopper (GLH), white backed plant hopper (WBPH) etc. Because of its excessive use, cypermethrin is getting concentrated in aquatic bodies like rivers and estuaries and might get biomagnified in food chains.

Clams are abundant in major estuaries and creeks of Ratnagiri coast and are known to accumulate contaminants without getting killed easily and have relatively long life span. Due to these facts, clams can act as ideal bioindicator of estuarine pollution. They can also be collected easily during the low tide.

Histology has been successfully used as a diagnostic tool in medical and veterinary sciences ever since mid 19th century. Any particular alteration of cell may indicate the presence of disease or the toxic substance. The histology provides very important and useful data, concerning changes in cellular or subcellular structure of an organ much earlier than external notification. The extent of damage induced by the toxicant to a particular organ can also be judged at a cellular level. These studies are useful to investigate the extent of pollution and nature of lethality of pollutants. Kumbhar (2001) observed cellular alterations in target organs of estuarine clams, *M. meretrix, K. opima* and *P. laterisulca* under cadmium stress. Akarte & Muley (1987) noted significant cellular alterations in soft tissues of *K. opima*. Scanty references are available regarding induced histopathological changes in estuarine clams. Therefore, *Meretrix meretrix* (Linn.) was used as an experimental model for this study.

MATERIALS AND METHODS

The experimental clams (*Meretrix meretrix*) were collected from Bhatye estuarine region of Ratnagiri coast. Clams of medium size (4.0-4.8 cm) were selected and brought to the laboratory and stocked in the plastic containers containing filtered, aerated estuarine water for 48hr. Well-acclimatized clams to the laboratory condition were grouped in ten and kept into plastic containers containing five-litre filtered estuarine water. Static bioassay tests were conducted for 96 hr using cypermethrin (25 EC). For every experiment, a control group of clams was also run simultaneously. The toxicity tests were repeated for three times and LC_0 and LC_{50} values were determined.

Pooled tissues of live clams from control, LC_0, LC_{50} and chronic group were fixed in neutral buffer formalin for 48 hr for proper fixation. Tissues were washed in distilled water and then dehydrated in ethyl alcohol, cleared in xylol and embedded in tissue mat. Embedded tissues were sectioned at 5-6 µm thickness on rotary microtome. Sections were stained with Harris hematoxylene and alcoholic eosin and mounted in DPX. All the observations for microphotography were made under the Leica Galen lll microscope.

RESULTS

The present study provided understanding of basic cellular architecture and alternations after acute (96 hr) exposure to cypermethrin. The tissues like gill, mantle, foot, hepatopancreas, male gonad and female gonad were investigated under light microscope.

Gills

Control group: The stained section of gill of clams from control group showed clear structure of normal gill. A cross section of gills reveals that each gill lamella is a flattened structure clothed by a cuboidal epithelium containing isolated gland cell of oval shape with clear cellular layer of connective tissue and blood lacunae. The lamella always bears few frontal cells at its extreme edge which carry rather short cilia. In *Meretrix meretrix*, frontal cilia are long and distinct (Fig. 1).

LC_0 group: Gills of LC_0 group showed pathological blacking in fresh condition. Epithelial cells and connective tissue was damaged, widening of inter-filamental junction was more prominent (Fig. 2).

LC_{50} group: Gills showed pathological blacking in fresh condition. Epithelial cell connective tissue, cuboidal epithelium and blood lacunae are completely damaged (Fig. 3).

Mantle

Control group: The mantle in clam is characterized by the presence of both an inner and outer epithelium. The epidermis of dorsal surface of mantle is continuing with shell. Subepidermal gland is absent and the interior consists primarily of connective tissue, blood lacunae and nerves. Dorsal mantle epithelium is thin when compared to ventral epithelium. The single layered epithelium is composed of columnar cells interspersed with mucocytes and gland cells. The mucocytes of epithelia are flask shaped with homologous cytoplasm. The mantle edge is an important area of growth and secretion. It is provided with clusters of gland cells that open into the mantle edge throughout its circumference (Fig. 4).

LC_0 group: Dorsal epithelium as well as ventral epithelium showed considerable damage. Subepidermis gland as well as connective tissue is considerably damaged. Mucocytes are detached from outer epithelium (Fig. 5).

LC_{50} group: Exposure of clams to cypermethrin had produced pronounced microscopic cellular alterations in mantle. Mantle showed severe damage to dorsal as well as ventral epithelial cell, connective tissue and blood spaces. Connective tissue is detached from dorsal epithelium and showed severe damage (Fig. 6).

Foot

The foot is glandular structure with marginal epidermis. The sole is covered with microscopic cilia which consist of uniform ciliated cells. The marginal granular cleft is shallow laterally, often quite deep centrally. It is lined by columnar epidermis through which open masses of subepidermal polygonal gland cells were distinct (Fig. 7).

LC_0 group: Clams of LC_0 group showed considerable damage in foot. Folding of marginal epidermis and shrinkage of epidermal cell took place. These cells were ruptured at places. Pedal gland cells were more swollen and also ruptured (Fig. 8).

LC_{50} group: As compared to LC_0 group, clams showed considerable damage to foot. Folding of marginal epidermis, shrinkage of epidermis, loss of connective tissue took place (Fig. 9).

Hepatopancreas

Control group: The stained structure of hepatopancreas of control animals consists of ducts and digestive tubules indistinctly connected separated by interlobular connective tissue consisting of collagenous fibres. Each tubule is bounded by thin muscle fibres, which forms the basement membrane. Each of these cells posses a basal nucleus with prominent nucleolus. The cells in digestive tubules are responsible for absorption and intracellular digestion of food material. Large numbers of amoebocytes are found in the interlobular connective tissue. The lumen of the tubule increases or decreases on the basis of amount of food particles accepted for digestion. During this process fragmentation spherules areas are budded off from the apex of the digestive cells into the lumen (Fig. 10).

LC_0 group: Hepatopancreas of clams from LC_0 group showed swallowing of tubules. The basement membrane of each tubule was ruptured at the same place or either got separated from tubule. The digestive and secretary cell at few places detached from basement membrane and from each other as well (Fig. 11).

LC_{50} group: Hepatopancreas of LC_{50} group showed severe histological changes. Basement membrane, muscular layer

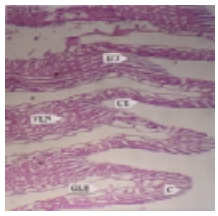
Fig. 1

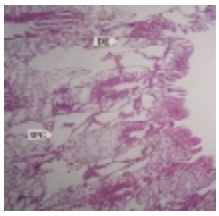
Fig. 2

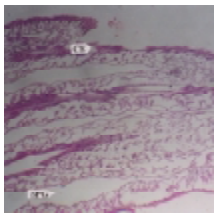
Fig. 3

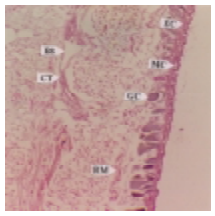
Fig. 4

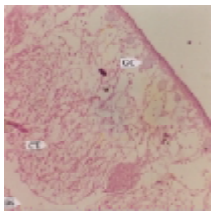
Fig.5

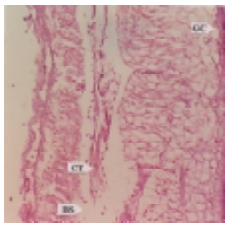
Fig. 6

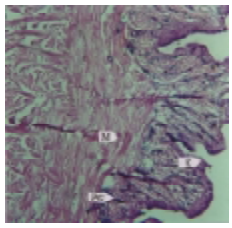
Fig. 7

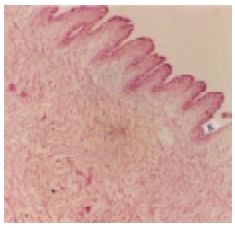
Fig. 8

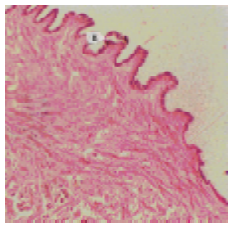
Fig. 9

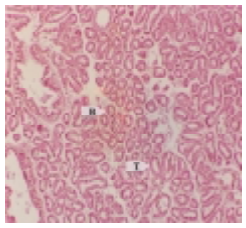
Fig.10

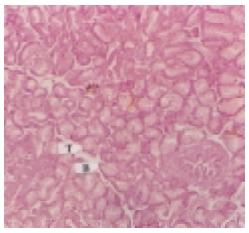
Fig. 11

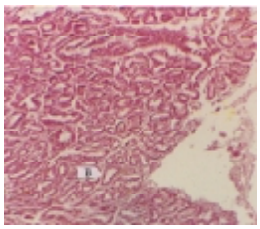
Fig. 12

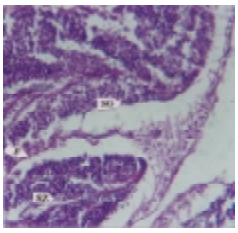
Fig. 13

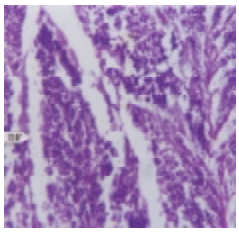
Fig. 14

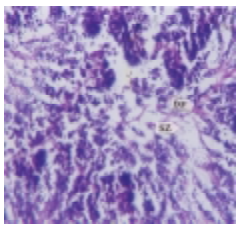
Fig. 15

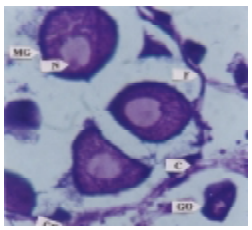
Fig. 16

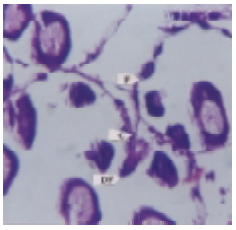
Fig. 17

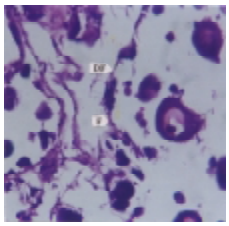
Fig. 18

Figs. 1-18: Histological alterations in estuarine clam, *Meretrix meretrix* in control and after exposure to cypermethrin.

got ruptured with places with lot of tissue there was partial or completely under disintegration of digestive and secretary cells (Fig. 12).

Male Gonad

Control group: Male gonad of *Meretrix meretrix* showed the development of spermatogonia, spermatocytes and spermatid arranged in array. All these components were distinctly stained. Intrafollicular connective tissue is intact and distinct. Lipid globules and nutritive cells are present but not clearly visible (Fig. 13).

LC_0 group: Clams showed considerable damage to the male gonad. Follicle wall ruptured at many places and follicular content much scattered. The spermatid lost continuity and was detached (Fig. 14).

LC_{50} group: In LC_{50} group follicle wall ruptured at many places and follicular content much scattered. Sperms are clumped in a mass. The spermatid lost their integrity in development and showed dissolution (Fig. 15).

Female Gonad

Control group: Female gonad of *Meretrix meretrix* showed developmental condition. The female follicles showed follicle wall with prominent germ cells and vitellogenic oocytes of different sizes. Many free mature eggs processed dense cytoplasm and distinct nucleus located in centre having prominent nucleus. The staining of these different parts in female follicles was distinct. There are few relict gametes in the female follicles containing very few mature eggs with distinct nucleus and nucleolus. Very few vitellogenic oocytes

and germ cells appeared along the follicle wall (Fig. 16).

LC_0 group: LC_0 group of clams showed considerable damage to the female gonad. The follicle wall ruptured at places with shrinkage of germ cell along the wall. Deterioration of ooplasmic material, nucleus and nucleoli was observed in mature eggs. The germ cells and vitellogenic oocytes lost their shape and detached from follicle wall (Fig. 17).

LC_{50} group: As compared to LC_0 group, these clams showed considerable damage to female follicles. The female follicle wall was distorted but mostly showed continuity. The follicle wall was shrunkened. The oogonia showed prominent nuclei but the cytoplasm was opaque. These are likely to undergo degeneration (Fig. 18).

DISCUSSION

Pesticide stress showed histological changes, deterioration of tissue and damage at cellular and subcellular levels. Pesticide stress alters the normal structure of the tissue. There was remarkable damage in all the tissues.

Gills showed damage in epithelial cells and connective tissue in LC_{50} group. LC_{50} group showed more damage than LC_0 group. Epithelial cells, connective tissue and cuboidal epithelial cells were completely damaged, and erosion was prominent at the distal end of gill filament. This damage of epithelial lining may be attributed to the cypermethrin toxicity. Lethal concentration of cypermethrin has devastating effect on gill, which result in death of the clams. Dalela et al. (1979) observed similar type of histological alterations in the gills of *Channa gachua* after acute and subacute exposure to Endosulfan and Rogor.

After acute exposure mantle of clams from LC_0 group showed considerable damage to the dorsal as well as ventral epithelium. It also showed damage to subepidermal gland and connective tissue. Because of intimacy of organ, mantle showed severe damage to dorsal as well as ventral epithelial cells, connective tissue and blood spaces.

In LC_{50} group, foot showed significant damage, folding of marginal epidermis; shrinkage of epidermis, loss of connective tissue took place. This may be due to higher concentration of cypermethrin and rapid penetration of cypermethrin into the foot tissue. Muley (1985) reported loss of cilia, folding of marginal epithelium and damage to pedal gland cells and connective tissue in foot of *Viviparous bengalensis* after acute exposure to LC_0 and LC_{50} concentration of follithion, lebacid, mercuric chloride and mercuric sulfate.

Hepatopancreas of clams from LC_{50} group showed swelling of tubules. The basement membrane of each tubule was ruptured at some places. Kumbhar (2001) observed similar type of histological alterations in the hepatopancreas of *Meretrix meretrix* after acute and subacute exposure to cadmium.

Cypermethrin caused severe damage to male gonad. In sublethal and lethal concentration, follicle wall was ruptured at many places. Sperms were clumped in a mass. This was due to rapid penetration of cypermethrin deep into gonadal tissue.

Female gonad of control group showed developmental condition. Many free mature eggs possessed dense cytoplasm and distinct nucleus and nucleolus. After acute exposure of cypermethrin, sublethal group showed considerable damage. The follicle wall ruptured at places with shrinkage of germ cells along the wall. Female gonad showed deterioration of ooplasmic material. Nucleus and nucleoli were observed in mature egg. LC_{50} group showed more damage than LC_0 group. In lethal concentration of cypermethrin, the follicle wall was shrunken, the oogonia showed prominent nuclei but the cytoplasm was opaque. Muley (1985) observed similar changes in both LC_0 and LC_{50} groups of *Viviparous bengalensis* after acute exposure to follithion and lebaycid.

CONCLUSION

The cypermethrin at various concentrations showed histological alterations and damage to the organs at cellular level in the clam *Meretrix meretrix*. Severe damage at cellular level was observed in various tissues like gill, mantle, foot, hepatopancreas, male gonad and female gonad after acute exposure to cypermethrin. As compared to control group of clams, these histological changes were more prominent in gills and hepatopancreas after acute exposure than other tissues.

REFERENCES

Akarte, S.R., Muley, D.V. and Mane, U.H. 1987. Effect of cythion-malathion 50EC (W/W) on the estuarine clams, *Katelysia opima* (Gmelin). Biology of Bathetic Marine Organisms: Techniques and Methods as Applied to the Indian Ocean. Thompson, M. F., Sarojini, R., Nagabhushanam, R. (Eds.) 1986, No. 12, pp. 501-510.

Dalela, R.C., Bhatnagar, M.C., Tyagi, A.K. and Verma, S.R. 1979. Histological damage of gill in *Channa gachua* after acute and subacute exposure to endosulfan and rogor. Microskopie (Wien), 35: 301-307.

Kumbhar, S.N. 2001. Cadmium induced toxicity to estuarine clams from Ratnagiri coast of Maharashtra. Ph.D. Thesis, Shivaji University, Kolhapur.

Muley, D.V. 1985. Effect of pollutants on freshwater molluscs from Godaveri river at Paithan. Ph.D.Thesis, Dr. B.A. Marathawada University, Aurangabad, Maharashtra, India.