

RESEARCH ARTICLE

Histopathological changes induced by Quinalphos and Cypermethrin in gills of the Catfish, *Heteropneustes fossilis* (Bloch)

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ABSTRACT

Pesticide pollution in aquatic environment is toxic which brings histopathological changes in the tissues and organs of fish. Extensive and indiscriminate use of pesticides, insecticides, fertilizers, accidental spillage or discharge of untreated effluents into natural waterways have harmful effects on the fish population. Gills are the main respiratory organs of fish. Pesticides present in the water cause structural changes in the gills. In the present study, the gills of *Heteropneustes fossilis* showed various changes in the structure on exposure to sublethal concentrations 1.267 mg/l and 0.056 mg/l of Quinalphos and Cypermethrin insecticides for 4, 15, and 30 days. Damage of epithelium of gill lamellae, presence of cell debris in interlamellar space, separated epithelial layer from pillar (pillar) cells, presence of blood cells due to damaged blood vessels in between epithelial cells, vacuolation and hypertrophy of epithelial cells and pillar cells of the respiratory lamellae and disorganized pillar cells and swelling in mucous glands were observed.

Key words: Histopathology, gill, *Heteropneustes fossilis*, Quinalphos, Cypermethrin

INTRODUCTION

Aquatic pollution has been increasing at alarming rate due to the indiscriminate use of various types of pollutants affecting non-target organisms like fishes, invertebrates and human beings. Agricultural pesticides and insecticides are further making the problem of pollution more serious both from the points of view of public health as well as aquaculture (Jhingran, 1991). Various pollutants, such as heavy metals, pesticides, industrial and sewage effluents are accumulated in the water bodies like reservoirs and lakes. After all, the aquatic environment is the ultimate sink for pollutants that rendering in long term adverse effect on fishes. Agrochemicals including pesticides have an important role in ensuring food supply and better health for a growing world population. Though the agrochemicals are necessary in modern agriculture and their application contributed much to the

success of agricultural production, their vital role in upsetting the ecological balance cannot be ignored. Indiscriminate use of pesticides, careless handling, accidental spillage or discharge of untreated effluents into natural waterways have harmful effects on the fish population (Dutta, 1995). The overall effect of pollutants resulted in reduced adult survival and lowered population abundance.

The problems are aggravated due to the capacity of toxic substance to get biomagnified in fish tissues, which otherwise exist in water in extremely low concentrations. The nutritive and medicinal value of fish has been recognized from times immemorial. Therefore the fresh water catfish, *Heteropneustes fossilis* has been included in the present work. The present study deals with the histopathological effects induced by a organophosphorous insecticide Quinalphos and a synthetic pyrethroid Cypermethrin on gills of the catfish, *Heteropneustes fossilis*.

MATERIAL AND METHOD

After collection of fish, *Heteropneustes fossilis* were dipped in 0.1% potassium permanganate solution to avoid dermal infections. They were acclimatized for 10 days to laboratory conditions in glass aquaria filled with chlorine free water.

For determination of 96 hr LC₅₀ values of quinalphos and cypermethrin, toxicity tests were performed as per standard methods by APHA-AWWA-WPCF (1976). The fishes with total length ranging from 18.8 to 20.2 cms and weight ranging from 40 to 60 gms were chosen for experiments. Further, the fishes were exposed separately to sublethal concentrations 1.267 mg/l and 0.056 mg/l (1/5th of 96 hr LC₅₀) of respective insecticides for 30 days. The fishes from both control and exposed groups were sacrificed at the interval of 4 days, 15 days and 30 days of exposure.

The methodology on Animal Tissue Techniques given by Humason (1979) was adopted for studying the histopathological changes in the gill tissues of the fish, *Heteropneustes fossilis* exposed to Quinalphos and Cypermethrin.

For histological studies, the test fishes from both control and exposed groups were taken after 96 hours

(i.e., 4 days), 15 days and 30 days of exposure to the toxicants. After exposure each fish was removed from the test container and sacrificed. Gills were dissected out from each fish and fixed for 24 hours in aqueous Bouin's fixative. Thereafter, the tissues were thoroughly washed in running tap water for 24 hours to remove yellow colour of picric acid. Then the tissues were decalcified before dehydration by treating them with 2% nitric acid in 70% alcohol for 72 hours.

Then they were dehydrated in different grades of alcohol, cleared in xylene and embedded in paraffin wax (melting point 58°C – 60°C). Paraffin blocks were prepared and cut at 5-6 micron thickness. Tissue sections were stained with Ehrlich's Haematoxylin and counterstained with Eosin. The stained sections of all tissues were observed under the microscope and the photomicrographs of the sections were prepared by using Micro Imaging Computerised system (LABO made DIGI-3 model) attached with C.C.D. camera.

RESULTS AND DISCUSSION:

Histology of gills of control fish:

Gills are the main respiratory organs of fish, *Heteropneustes fossilis*. Each gill arch supported by bone, bears two hemibranches (which forms a complete gill or holobranch) in the form of two rows of leaf-like folds, called gill filaments or primary gill lamellae. The filaments of the two rows are free at their distal ends but they are joined proximally by an inter branchial septum. Each gill filament (leaf-like fold) of is supported by a gill ray which is partly bony and partly cartilaginous. Gill rays are connected with the gill arch and provide passage for the efferent branchial vessels. The gill filament is covered by a multilayered epithelium which consists of mucus secreting cells and chloride cells. The mucous glands are oval or pear-shaped and secrete thin film of mucus to protect the respiratory epithelium from dehydration. Chloride cells are generally found in the interlamellar epithelium of the filament and perform important function in osmoregulation.

A primary gill lamella is borned on the gill ray and the secondary gill lamellae form a series of transverse plates on the sides of the primary. These lamellae of a gill filament are the main sites of gaseous exchange (i.e., intake of O₂ and release of CO₂).

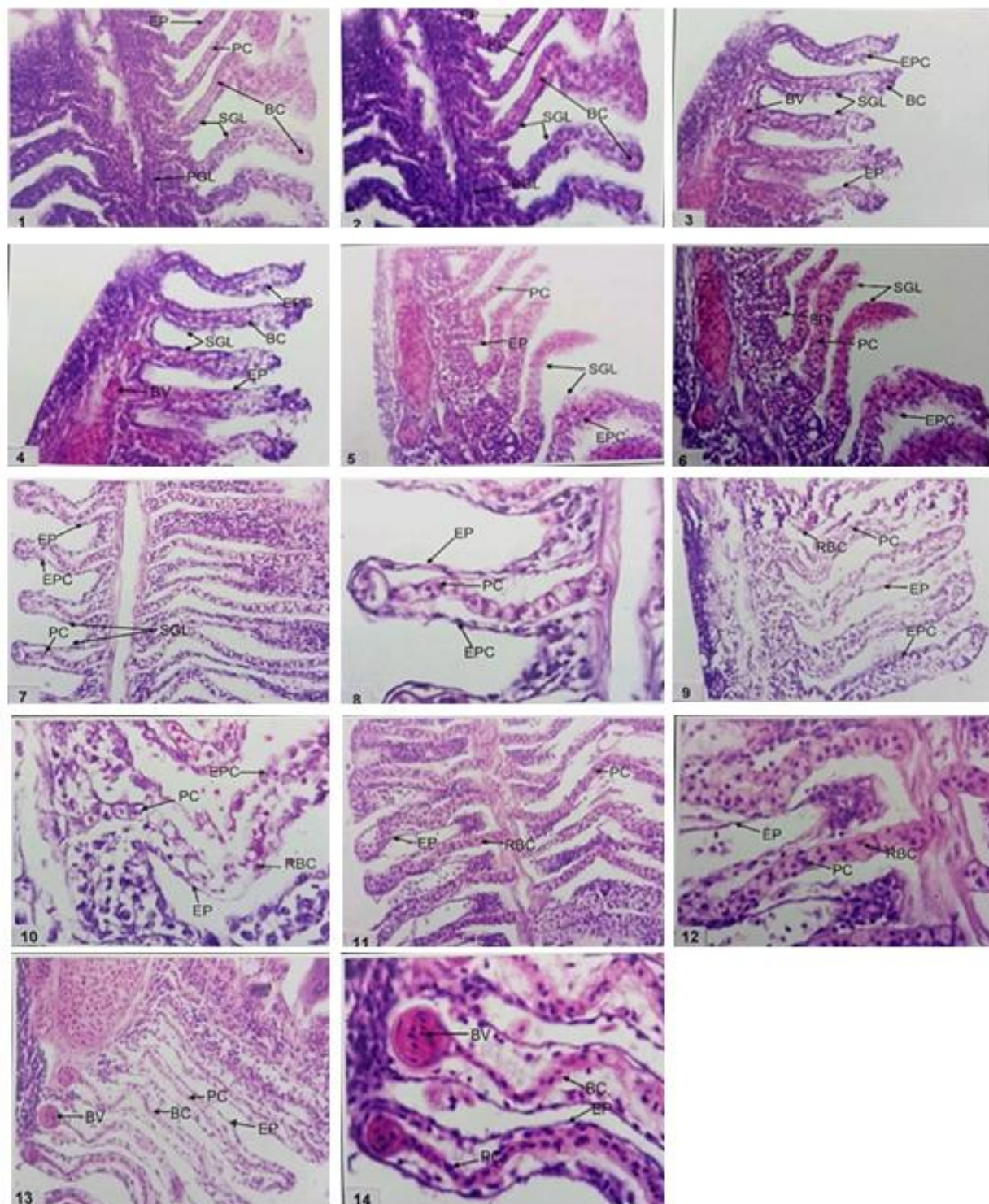


Fig. 1 & 2: T. S. of gill of control fish, *H. fossilis*. (Haematoxylin Eosin 400x & 1000x respectively).

Fig. 3 & 4: T. S. of gill of *H. fossilis* exposed to 1.267 mg/L of Quinalphos for 4 days (HE 400x & 1000x).

Fig. 5 & 6: T. S. of gill of *H. fossilis* exposed to 1.267 mg/L of Quinalphos for 15 days (HE 400x & 1000x).

Fig. 7 & 8: T. S. of gill of *H. fossilis* exposed to 1.267 mg/L of Quinalphos for 30 days (HE 400x & 1000x).

Fig. 9 & 10: T. S. of gill of *H. fossilis* exposed to 0.056 mg/L of Cypermethrin for 4 days (96 hours) (HE 400x & 1000x).

Fig. 11 & 12: Section of the gill of *Heteropneustes fossilis* exposed to 0.056 mg/L of Cypermethrin for 15 days (HE 400x & 1000x).

Fig. 13 & 14: Section of the gill of *Heteropneustes fossilis* exposed to 0.056 mg/L of Cypermethrin for 30 days (HE 400x & 1000x).

Abbreviations: EP- Epithelium, EPC- Epithelial cells, PC- Pilaster (Pillar) cells, RBC- Red blood cells, PGL- Primary gill lamellae, SGL- Secondary gill lamellae, BC- Blood capillary, BV- Blood vessel.

The free edges of the secondary lamellae are extremely thin, covered with epithelium and contain a vast network of blood capillaries supported by pillar cells (pillar cells). The pillar cells serve as 'pillars' to keep the two epithelial layers of the lamella separate and prevent them from collapsing. They also form a contractile apparatus to regulate the blood flow in the lamella (Fig. 1 & 2).

Histopathological changes in gills of fish exposed to Quinalphos:

In the 4 days Quinalphos treated gill, epithelial cells are broken at many places. Epithelium gets separated from basement membrane and vacuolation is seen in the cells. Blood vessels are damaged and at some places due to which blood cells are seen to come out. Hypertrophy (i.e., increase in size) of epithelial cells is observed (Fig. 3 & 4).

In the 15 days Quinalphos treated gill, epithelial cells are damaged due to which they are seen in debris. Blood cells are also intermingled. At certain places only rows of pillar cells are observed. Epithelial layer is damaged and separated from pillar cells (Fig. 5 & 6).

In the 30 days Quinalphos treated gill, epithelial cells are separated from the pillar cells. Epithelial cell debris is observed due to damage to the cells. The level of damage is more due to prolonged exposure. Vacuolation in pillar cells is observed. Pillar cells are damaged at certain places (Fig. 7 & 8).

Histopathological changes in gills of fish exposed to Cypermethrin:

On 4 days exposure to Cypermethrin, the gill shows destruction of epithelium to greater extent. Blood vessels are damaged, hence blood cells are seen in between the epithelial cells. Hypertrophy of pillar cells is seen. At some places, the epithelium gets separated off completely from the supporting cells, i.e. from pillar cells. Epithelial cells show vacuolation and hypertrophy (Fig. 9 & 10).

On 15 days exposure to Cypermethrin, epithelial cells of gill lamellae are destroyed to greater extent. The supporting cells (pillar cells) are also damaged. Disintegration of pillar cells is observed, only blood cells are there in the lamellae (Fig. 11 & 12).

On 30 days exposure to Cypermethrin, epithelium of lamellae is damaged more and more. Epithelial lining

is separated from pillar cells. Pillar cells are disorganized. Blood capillaries are also damaged (Fig. 13 & 14).

The pesticide pollution in aquatic environment is toxic which brings histopathological changes in the tissues and organs of fish. Histopathology deals with the study of histological structures of the ailing tissues. It appears to be a very sensitive parameter in terms of toxic levels and is decisive in determining cellular changes that may occur in the target organs. Histopathological study is useful in assessing the toxic effects on fish and may help to identify the actual cause of death and its causative agents. Gills are the vital organs of the fish responsible for respiration, accessory excretion and regulation of water-salt balance.

In the present investigation, the gills of *Heteropneustes fossilis* exhibited various changes in the structure on exposure to Quinalphos and Cypermethrin. Destruction of epithelium of gill lamellae was seen and also the cell debris was observed in the interlamellar space. Epithelial layer was separated from pillar (pillar) cells. Blood vessels were damaged due to which blood cells were noticed in between epithelial cells. Epithelial cells and pillar cells of the respiratory lamellae showed vacuolation and hypertrophy. Pillar cells were disorganized and mucous glands showed swelling. The pesticides present in the water cause structural changes in the gills as reported by many workers. Similar changes have been reported in the gills of the pesticide exposed fishes by Ratnakar and Awasthy (1979), Gabhane (1984), Singh and Sahai (1984), Plaha (1988), Gupta (1989), Dutta (1995), Lonkar (1996), Jain (2000), Susan and Tilak (2003), Tilak *et al.* (2005) and Cengiz (2006). It was noted that the alterations in the gill architecture were more pronounced in the Cypermethrin exposed fish than those in mixture and Quinalphos.

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