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Scanning Electron Microscopic Studies Of Gills Of *Anabas Testudineus* Exposed To Cypermethrin

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Abstract: This study was to investigate the effects of exposure to a sublethal concentration of 0.5 ppm of Cypermethrin (10% EC) at different durations on the gill morphology of the air-breathing fish *Anabas testudineus*. The experimental fish were divided into control and cypermethrin-exposed groups. All experimental fish species were observed after an interval of 7 and 14 days. Scanning Electron Microscopic (SEM) study revealed that exposure to cypermethrin induced changes in the gill structure. It showed highly active mucous cells, epithelial hyperplasia, and fusion of secondary lamellae.

Index Terms **- Cypermethrin, SEM, *Anabas testudineus*, gills**

I. INTRODUCTION

In recent times the inland fisheries are greatly affected by a wide variety of toxic pollutants and synthetic pyrethroids are one among them. Synthetic pyrethroids are generally used as pesticides because of their high effectiveness and easy biodegradability, low toxicity to birds and mammals and because of their less persistence in the environment. These pesticides are affecting the non- target organisms. Fish, being an essential component of inland fisheries, their productions are easily affected by such pollutants. Cypermethrin (RS)-α-cyano-3-phenoxy benzyl(1RS)-cis, trans-3-(2,2-dichloro vinyl)-2,2-dimethyl cyclopropane-carboxylate) is a synthetic pyrethroid widely used as an agricultural and domestic pesticide. It was first synthesized by Elliot et.al. in 1974. Synthetic pyrethroid pesticides are recently used to control various types of pests to increase agricultural production. The washout from agricultural and domestic environments has resulted in polluting the inland fishery water. Cypermethrin is highly toxic to fish and some aquatic invertebrates (Coats et.al., 1989). Fish are sensitive aquatic organisms and show several responses to changes in their environment. The sensitivity of fish to pyrethroids is mainly due to their relatively slow metabolism inside their body. They are reported to block the sodium channels of nerve filaments. They lengthen the depolarization phase and affect GABA receptors in nerve filaments (Bradbury and Coats, 1989). Fish are the cheapest source of animal proteins but due to aquatic pollutants they are turning unhealthy.

Fish gills are an important part of the fish body. They have direct contact with the aquatic environment and play an important role in gas and ion exchange between the organism and the environment. The structure of fish gills is often used as an indicator of the biological impacts of pollutants as they respond to toxic substances. Pyrethroids show lipophilicity and hence have a high rate of gill absorption even when present at very low concentrations in water.

Scanning Electron Microscope is a useful tool to assess the surface ultrastructure of gills and find if damage occurred in the tissue. It provides a quasi-three-dimensional image of surface ultrastructure and clearly detects any change under stress. Hence this SEM study was carried out to find out the morphological changes in gills of *Anabas testudineus*, if any, on exposure to cypermethrin(10%EC) as early as 7 and 14 days.

The present study is carried out in *Anabas testudineus* as it is an important food fish of India and plays an important role in inland fisheries. Anabas is a common freshwater fish belonging to the family Anabatidnae of the order Perciformes. It is taken as a test fish because it is a food fish and can be easily maintained under laboratory conditions.

II. MATERIALS AND METHODS

2.1 Collection and maintenance of experimental fish

Anabas was collected from nearby ponds by local fishermen and then acclimatized in the laboratory for one week. Healthy fish of comparable body weight and length were selected for this study. The fish were disinfected with 0.1% potassium permanganate solution for 5 minutes to avoid dermal infection. The fish were fed a mixture of artificial pellets and acclimated for 15 days.

Healthy fishes with active movements were only considered for acute, and chronic toxicity tests. Thorough scrubbing and cleaning of each tank were carried out and tap water stored for 12 h was used for complete replenishment in the morning and was used for the bioassay experiment. Each tank is filled with 10 L of water. For 48 hours before testing, the fish were starved, as customary in static tests, to reduce the amount of waste material generated by them.

2.2 Determination of the lethal concentration (LC50)

Bioassay or toxicity tests were carried out for the determination of LC50 values by following the FAO procedure for short-term bioassays (Reish and Oshida, 1987). The duration of the test was 96 hours.

A stock solution of cypermethrin 10% effective concentration (EC) was prepared by diluting 10 ml of insecticide in 100 ml of distilled water, and different concentrations of 1.0, 0.8, 0.6, and 0.5 ppm were used in the experimental waters for the toxicity study of Anabas. The experiment was set in four groups, and healthy fishes (n=10) with an average weight of 10g and an average length of 6cm were maintained in 10 L of experimental water with different concentrations of cypermethrin. Similarly, a control was set up with water devoid of cypermethrin. It was found that the LC50 is 1 ppm.

2.3 Exposure concentration and duration

In natural or experimental conditions, a sublethal concentration of a toxicant is most likely to produce sublethal effects that

alter the morphological, physiological, and histological conditions of the fish, although it may not cause the immediate death

of the individual. For the chronic toxicity studies, the fish were divided into two groups and placed in separate glass aquaria.

Ten fish were used for each group. Group I was maintained in cypermethrin-free water as a control. Group II was exposed to

sublethal concentrations of cypermethrin. A sub-lethal concentration of 0.5 ppm was taken for the experimental study.

Observations were made on the 7^{th} and 14^{th} days of the experiment.

2.4 Processing of gill samples for scanning electron microscopy:

Both control and cypermethrin-exposed fish at the end of the 7^{th} and 14^{th} day of exposure in the chronic test were anesthetized using 2-phenoxyethanol and their gills were collected. Gills were dissected out on the 7^{th} and 14^{th} day, preserved in 10% neutral formalin buffer. They were then dehydrated in a graded series of acetone (30-100%) for 30 minutes each, placed in tetramethylsilane(TMS) solution, kept under refrigeration for 10 minutes, air dried for 5minutes, mounted on stubs with double sided tape and coated with a thin layer of in a sputter cutting unit. Coated specimens were then observed in scanning electron microscope and photgraphed with computer -integrated software.

III. RESULT AND DISCUSSION

Control fish showed gills with normal arrangement of cell components and primary and secondary lamellar organization patterns (Figure 1). In control fish, the four gill arches had a normal structure and they supported many gill filaments or primary lamellae. A row of secondary lamellae was present on the lower and upper sides of each primary lamella. Exposure of fish to a 7-day sublethal concentration of cypermethrin resulted in major ultrastructural changes, including cell hypertrophy and other alterations on the lamellar surface. Scanning electron micrographs showed swelling and fusion of the lamellae, especially at their tips along with mucous deposits on gills. Epithelial lifting and breakdown of the surface epithelium were the major effects of cypermethrin intoxication (Figure 2). Fish treated with a sublethal concentration of cypermethrin for 14 days showed gills with copious amounts of mucous deposited on its surface. Thickening and shortening of the secondary lamellae were accompanied by smothering and obliteration in some places. Swollen and fused tips of lamellae were more pronounced, and there was disruption of surface epithelium in cypermethrin-exposed gill (Figure 3). Oedema and rupture of lamellar epithelium are among the first symptoms that indicate that the fish is suffering from some pathological effects (Thophon S., et al., 2003). The proliferation of mitochondrial-rich cells and stem cells may result in partial or complete fusion of secondary lamellae (Dang Z. et.al.1999). Such adaptive mechanisms of fish comprise defensive responses to reduce the surface area of gills in contact with the toxicant, with the extent of gill damage reflecting the toxic potential and mode of action of the xenobiotic. Parashar and Banerjee., 2002 observed that some gill lesions in *Heteroneustes fossilis* represented direct deleterious effects of the toxicant, while some others were defence responses of the exposed fish.

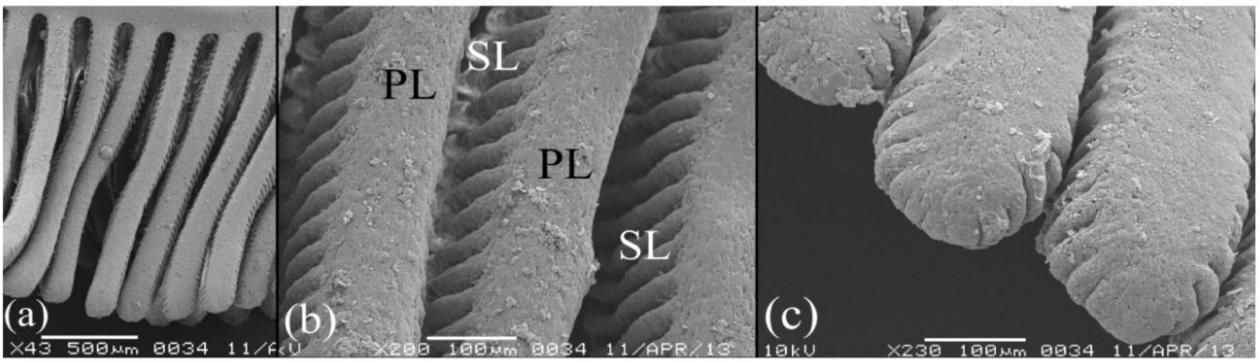

Figure-1 (a- c): Scanning electron micrographs of the gills of control *Anabas testudineus* showing normal gill filament (primary lamellae, PL, and secondary lamellae, SL)

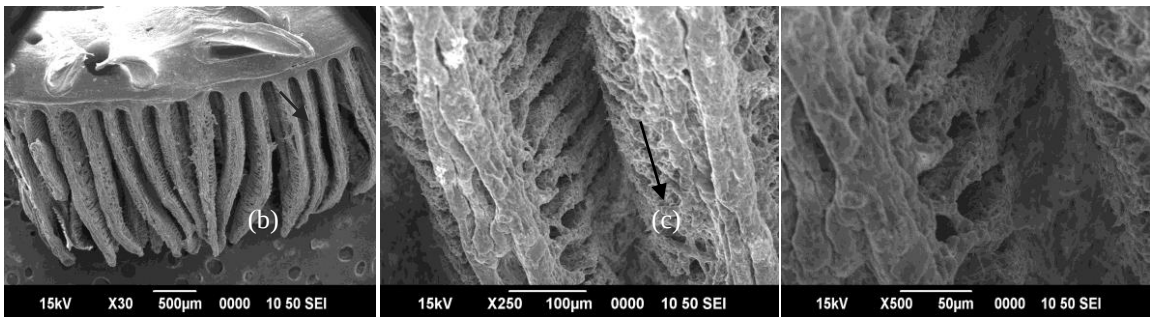

Figure -2(a-c) Scanning Electron Micrograph of the gills of *Anabas testudineus* exposed to sublethal concentration of cypermethrin on the 7th day. Fused tips of the lamellae are prominent. Arrows indicate epithelial lifting and erosion of the gill epithelium.

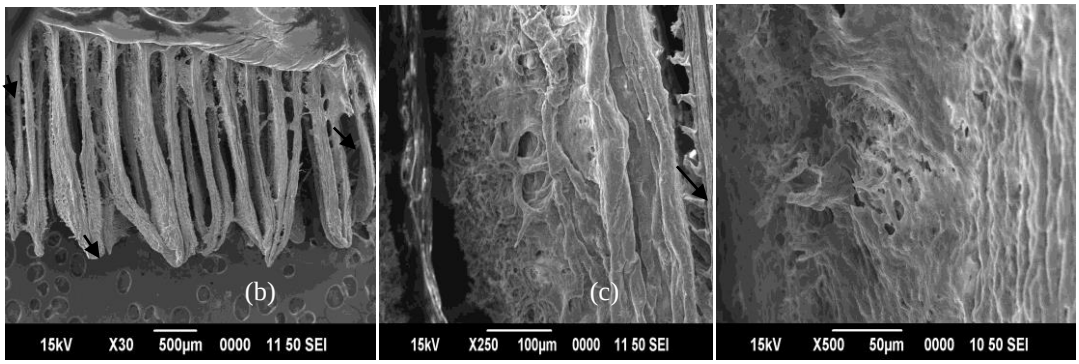

Figure-3(a-c) : Scanning Electron Micrograph of the gills of *Anabas testudineus* exposed to sublethal concentration of cypermethrin on the 14th day. A copious amount of mucus is deposited on the gills; the Second lamellae are swollen and fused, especially at the tip; Epithelial lifting and erosion of gill epithelium are prominent.

The findings of several studies conducted on the gill morphology of freshwater fish indicate that cypermethrin produces lesions. The gill undergoes necrosis and rupture in the respiratory epithelium. It shows hyperplasia, epithelial lifting, mucus secretion, and fusion of secondary lamellae. It is believed to be the defense responses of the fish against stressors (H. M. Dutta, et al., 1997). The structural anomalies of gills are considered as stress responses (D. H. Evans, 1987). Epithelial hypertrophy and hyperplasia, epithelial lifting, edema, fusion of secondary lamellae, necrosis, and desquamation are other symptoms observed. (Velmurugan B et al., 2009). Histological examination of *H. bidorsalis* exposed to cypermethrin showed significant indications, and its effects included gill alteration such as desquamation of the epithelial lining, telangiectasia, hemorrhage, and hyperplasia at secondary lamellae (Olufayo et al., 2012). This study also confirms similar structural changes in Anabas. The present findings were also similar to those when exposed to deltamethrin and permethrin, where swelling of gill filaments and distortion was noted (Devi et.al. 2014). In this study, lamellar hyperplasia, lamellar fusion, and epithelial lifting were observed in gill filaments. It was suggested that when the gill surface when in contact with a toxicant, mucus secretion acted as a protective device, checking further penetration of toxicants . Obliteration of mucus gland openings was because of impairment of protective mucus secretion and a further increase in the vulnerability of the gill epithelium to toxicants. Thus, findings confirm that cypermethrin causes acute toxicity in the fish gill structure.

IV. Acknowledgment

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