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RESEARCH ARTICLE

IMPACT OF MONOCROTOPHOS ON THE GILL ULTRASTRUCTURE OF THE FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS*

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ABSTRACT

The freshwater fish *Oreochromis mossambicus* was exposed to 10% sublethal concentrations of (LC_{50} for 96h ppm - 0.0028 ppm) monocrotophos for a period of 10, 20 and 30 days to study the effect of monocrotophos on the ultrastructure of gill. The scanning electron microscopic studies revealved many morphological changes in the gill of *Oreochromis mossambicus* such as fusion of secondary gill lamellae, degeneration of secondary lamellae and necrosis due to chronic exposure of pesticide monocrotophos.

INTRODUCTION

Pesticides are one of the most potentially harmful chemicals liberated into the environment in an unplanned manner. Pesticides drained to the aquatic environment are primarily of agricultural origin and which may also stem from effluent from manufacturing plants. Since there is great concern about toxic hazards in the aquatic ecosystem due to pesticides, either from surface run-off from paddy fields or through direct application into ponds for control of parasites, it is necessary to study the cellular changes in the fish tissue associated with this toxicity. In fish, gills are critical organs for their respiratory and osmo-regulatory functions. Respiratory distress is one of the early symptoms of pesticide poisoning (Mc Donald, 1983). According to Skidmore and Tovell (1972), in the gills these toxicants appear to breakdown the adhesion between epithelial branchial cells and the underlying pillar cells; this is accompanied by a collapse of the structural integrity of the secondary lamellae and subsequent failure of the respiratory functioning of the gills.

Fish gills comprise a large part of fish body that contacts the external environment and they play an important role in the gas and ion exchange between the organism and environment. They are also an important way of uptake of toxic compounds into the organism (Witeska *et al*., 2006).

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Thus, the gills are the very first site where pesticides induced lesions may occur which may result in an impaired gas and ion exchange. Subsequently, pesticides enter the blood in which they may affect the blood cells. Current interest in the field of pesticide detoxification lies on observations under scanning electron microscope, since such observations would lead to a better understanding of the morphological changes, induced in the gills at ultra structural levels, as well as the functions of various cells in the gills. Kimura and Kudo (1977) and Kendall and Dale (1979) have made extensive studies on ultra structure of the normal gills of *Salmo gairdneri*. Very few workers have observed the morphological changes in the gills following exposure to pollutants. Crespo (1982) and Temmink *et al.* (1983) studied in detail the morphological changes in gills of *Salmo canicula* and *S. gairdneri* induced by zinc and chromate. Muthukumaravel *et al.* (2008) studied ultra structure of gills of *O. mossambicus* affected by copper sulphate. In order to have an overall pathological picture, the study of the extensive gill surface needs special attention. In the present study mode of action of pesticide, monocrotophos in surface architecture of the gill of *Oreochromis mossambicus* has been investigated using scanning electron microscopy.

MATERIALS AND METHODS

The fish, *Oreochromis mossambicus* (Weight :10g ; Length 8 cm) were collected from the Udhayamarthandapuram Lake (N10° 26' 49.4" - E 79° 33' 12.8") is a bird sanctuary and it is located in Tiruthuraipoondi Taluk in Tiruvarur district, Tamil Nadu. They were acclimatized for 15 days in large

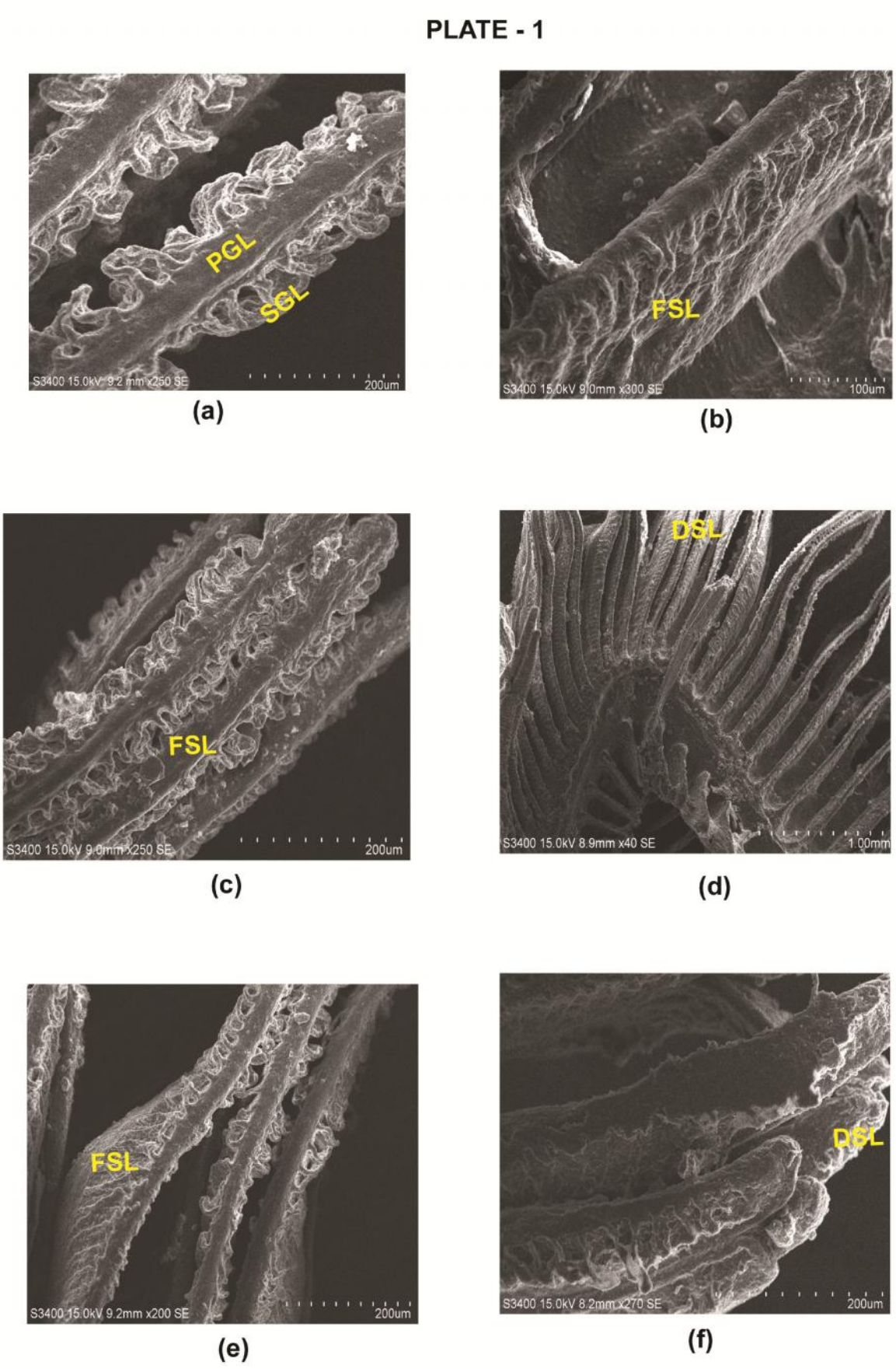

Plate 1. (a) Scanning electron microscopic structure of the gill of *Oreochromis mossambicus* control. Normal architecture of gill: Primary gill lamella (PGL), secondary gill lamellae (SGL). (b) Fusion of secondary lamellae (FSL) gill of 10 days monocrotophos treated fish. (c & d) Fusion of secondary lamellae (FSL) and degeneration of secondary lamellae (DSL) gill of 20 days monocrotophos treated fish (e & f) Fusion of secondary lamellae (FSL) and degeneration of secondary lamellae (DSL) gill of 30 days monocrotophos treated fish.

cement tanks (Temperature – 28 ± 2°C**;** total hardness – 518 ± 23 mg/l; DO - 5.6 ± 0.2 mg/l; salinity - 1.2 ± 0.13 ppt and pH - 7.8 ± 0.04) previously washed with 1% potassium permanganate. The water as renewed every 24 h. The LC_{50} of monocrotophos for 96h was found out by using Probit method (Finney,1971). For Scanning electron microscopic study, *Oreochromis mossambicus* were reared in 10% sublethal concentration (10% of 96 hours LC_{50} - 0.0045 ppm.) for a period of 10, 20 and 30 days. The gill arches were dissected out, washed repeatedly in 0.2M phosphate buffer and then fixed in 3% gluteraldehyde. The dehydration was done in acetone grades and was followed by critical point drying. Ultimately dried gills were mounted on the stub and were sputter coated with gold in a gold coating unit (thickness 100A^o) and were examined and photographed using JEOL JSM 6360 scanning electron microscope (SEM) Japan.

RESULTS AND DISCUSSION

Scanning electron microscopic (SEM) study of control gills

In the control *Oreochromis mossambicus*, primary gill lamellae appeared normal and mucous free and uniform branching of secondary lamellae from primary lamellae were visible (Plate 1. a).

Ultrastructural alterations of gill in monocrotophos treated fish under SEM observation

The damages, fusion and clumping of secondary gill lamellae were observed after 10 days of exposure (Plate 1. b). On exposure to pesticides for 20 days, erosion of epithelial cells, more mucus secretions and destruction to secondary lamellar structures were observed (Plate 1. c & d). In fish treated up to 30 days, the changes observed in the gill of *Oreochromis mossambicus* were swelling, fusion of lamellae, severe erosions of epithelial layer (Plate 1. e & f).

The gills, which participate in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to quality of the water are considered the primary target of contaminants (Fernandes and Mazon, 2003). The gills of fish showed degenerative, necrotic and proliferative changes in gill filaments and secondary lamellae and congestion in blood vessels of gill filaments. The pathological changes may be a reaction to toxicant intake or an adaptive response to prevent the entry of the pollutants through the gill surface. The observed alterations like proliferation of the epithelial cells, partial fusion of some secondary lamellae and epithelial lifting are defense mechanisms, since in general, these results in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Mallat, 1985 and Fernandes and Mazon, 2003). The cellular damage observed in the gills in terms of epithelial proliferation, separation of the epithelial layer from supportive tissues and necrosis can adversely affect the gas exchange and ionic regulation (Dutta *et al*., 1993). The observed edematous changes in gill filaments and secondary lamellae probably due to increased capillary permeability (Olurin *et al*., 2006). The present results are in agreement with those observed in other fish species of different pollutants (Olurin *et al*., 2006). In this respect, Camargo and Martinez (2007) observed hyperplasia of the epithelial cells, fusion of secondary lamellae, lifting of the lamellar epithelium and blood congestion in the gills of *Prochilodus lineatlls* caged in Cambe stream, Brazil, polluted by industrial, domestic and agricultural wastes. Also, Triebskorn *et al*. (2008) noticed epithelial lifting, proliferation of epithelial cells of primary and secondary lamellae, hyperplasia of mucous cells and necrosis of epithelial cells in the gills of fishes from river Mures, Western Romania, polluted by heavy metals, faecal coliforms and *streptococci* bacteria.

Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs cause a chain of destructive events, which ultimately lead to respiratory distress. Pronounced secretion of mucus layer over the gill lamellae has been during malathion stress. Secretion of mucus over the gill curtails the diffusion of oxygen (David *et al.*, 2002), which may ultimately reduce the oxygen uptake by the animal. If gills would be destroyed due to xenobiotic chemicals (Grinwis *et al.,* 1998) or the membrane functions are disturbed by a changed permeability (Hart *et al.*, 2001), oxygen uptake rate would even rapidly decreased. Kalavathy *et al.* (2001) reported that the dimethoate is efficiently absorbed across the gill and diffused into the blood stream resulting toxic to fish.

Conclusion

In the present study, it can be stated that pesticide monocrotophos exposure during sublethal treatment produces severe toxic effects on the respiratory organ of the freshwater fish *Oreochromis mossambicus*. The findings of the present study indicate that ultrastructural changes observer serve as "biomarkers" for assessing pesticide toxicity in aquatic environment.

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