



# Impact of Monocrotophos on the Gill Ultrastructure of the Freshwater Fish *Oreochromis mossambicus*

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## Abstract

The impact of monocrotophos on the gill ultrastructure of *Oreochromis mossambicus*, fingerlings was investigated on exposure to 10% sublethal concentration of LC<sub>50</sub> value (0.0045 ppm) for a period of 10, 20 and 30 days. Fusion and clumping of secondary gill lamellae were observed in the SEM analysis of the gill of 10 days exposed fish. In the gill tissues of 20 days exposed fish, erosion of epithelial cells, more mucus secretions and acute destruction to denticular structures were observed. Where as in the gill tissues of 30 days treated fish, swelling and fusion of lamellae, severe erosions of epithelial layer and high mucus secretion were noticed, the gill rocker ventricular structures were also totally damaged and were uprooted from their bases.

## Keywords

*Oreochromis mossambicus*,. Monocrotophos, Gill, SEM.

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## 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 effluent of 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.

Gills of fish are critical organs towards their respiratory and osmo-regulatory functions. Respiratory distress is one of the early symptoms of pesticide poisoning (Mc Donald, 1983). According to Skidmore & Tovell, (1972) these toxicants in the gills appear to breakdown the adhesion between branchial epithelial 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 gaseous and ionic exchange between the organism and environment. They also serve as an important way for the uptake of toxic compounds into the organism (Witeska et al, 2006). Thus, the gills are the very first site where pesticide induced lesions may occur which may result in an impairment of gaseous and ionic exchange. Subsequently, pesticides enter the blood and they could affect the blood cells as well as other organs through transportation.

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 & Kudo, 1979) and (Kendall & Dale, 1979) have made extensive studies on the ultra structure of normal gills of *Salmo gairdneri*. Very few workers have observed the morphological changes in the gills following exposure to pollutants. Crespo, (1982) and (Temminck et al., 1983) studied in detail about the morphological changes in gills of *S. canicula* and *S. gairdneri* induced by zinc and chromate. Muthukumaravel et al. (2008) studied the ultra structure of copper sulphate affected gills of *O. mossambicus*. In order to have an overall pathological picture, the study on the extensive gill surface needs special attention. In the present study mode of action of pesticides in surface architecture of the gill of *Oreochromis mossambicus* has been investigated using scanning electron microscopy.

## MATERIALS AND METHODS

The fingerlings of *O. mossambicus* (Weight: 10g; Length 8 cm) were collected from the Sellikuruchi Lake, which is located in Pudukkottai Ullur Village, Pattukkottai Taluk in Thanjavur district, Tamil Nadu. They were acclimatized for 15 days in large cement tanks (Temperature -  $28 \pm 2^\circ\text{C}$ ; total hardness -  $518 \pm 23$  mg/l; DO -  $5.6 \pm 0.2$  mg/l; salinity -  $1.2 \pm 0.13$  ppt

and pH -  $7.8 \pm 0.04$ ) previously washed with 1% potassium permanganate. The water was renewed every 24 h. The  $\text{LC}_{50}$  value of monocrotophos for 96h was determined by using Probit method (Finney, 1971).

For scanning electron microscopic study, *O. mossambicus* was reared in sublethal concentrations (10% of 96 hours  $\text{LC}_{50}$  - 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 sputter coated with gold in a gold coating unit (thickness 100Å) 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 fish *O. mossambicus*, primary gill lamellae appeared normal mucous free and uniform branching of secondary lamellae from primary lamellae were visualized. (Figure 1) SEM showed the presence of denticle of gill rakers (Figure 2).

### Histological alterations of gill in pesticides treated fish under SEM observation

Fusion and clumping of secondary gill lamellae were observed in the 10 days exposed fish (Figure 3). After 20 days of exposure to pesticides, erosion of gill epithelial cells, high amount of mucus secretions and acute destruction of denticular structures were observed (Figure 4). In the 30 days treated fish, the significant changes observed in the gill of *O. mossambicus* were swelling, fusion of lamellae, severe erosions of epithelial layer and abundant mucus secretion, (Figure 5). The gill raker denticular structures were completely damaged and were uprooted from their bases (Figure 6).

## PLATE-1

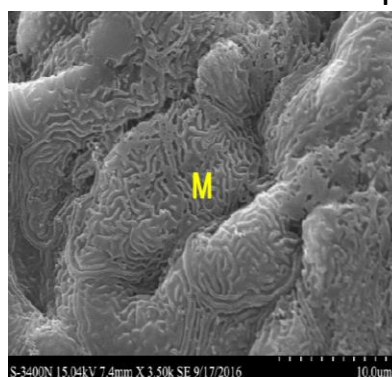


Figure 1

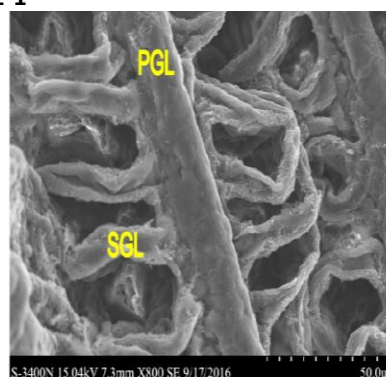


Figure 2

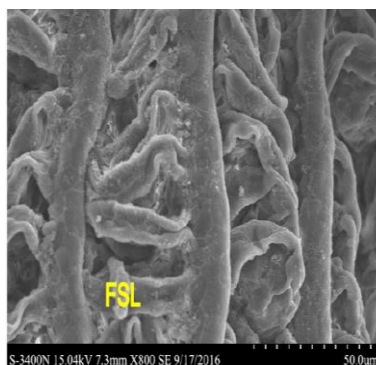


Figure 3

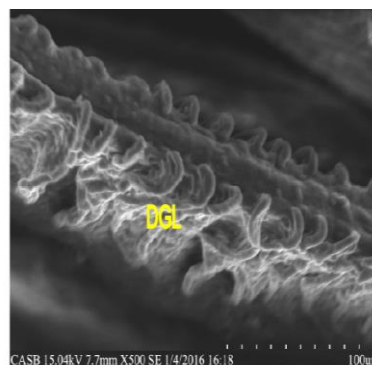


Figure 4

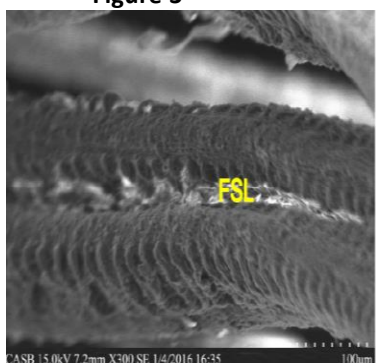


Figure 5

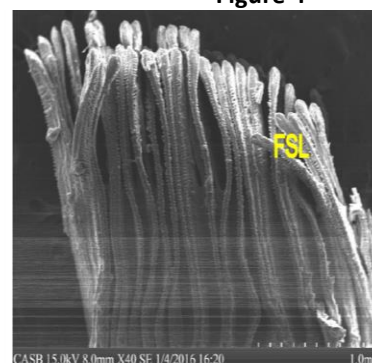


Figure 6

**Figure 1-6. Histological alterations of gill in pesticides treated fish under SEM observation (Figure 1. Scanning electron microscopes of the gill of *O. mossambicus* control. Normal architecture of gill Primary gill lamella (PGL), secondary gill lamellae (SGL). Figure 2. Fusion of secondary lamellae (FSL) gill of 10 days textile effluent treated fish. Figure 3 & 4. Fusion of secondary lamellae (FSL) and Degeneration of secondary lamellae (DSL) gill of 20 days Pesticide treated fish. Figure 5 & 6. Fusion of secondary lamellae (FSL) and Degeneration of secondary lamellae (DSL) gill of 30 days Pesticide treated fish).**

The gills have been carrying out many important functions in the fish, such as respiration, osmoregulation and excretion. Since it remains in close contact with the external environment and being highly sensitive to water quality, it has been considered as the primary target organ of contaminants (Fernandes, 2003). The gills of toxicity exposed fish showed degenerative, necrotic and proliferative changes in gill filaments and secondary lamellae and congestion in blood vessels of gill filaments. These 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 such as proliferation of the epithelial cells, partial fusion of some secondary lamellae and lifting of epithelial cells are the defense mechanisms toward toxicants. Since these alterations are results in the increase of the distance between the external environment and the blood in the gills Thus it serves as a barrier for the entry of contaminants (Fernandes, 2003; Mallatt, 1985). 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 gaseous 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 exposed to different pollutants (Olurin et al., 2006). In this respect, Camargo & 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 lineatilis* caged in Cambe stream, Brazil, polluted by industrial, domestic and agricultural wastes. Triebkorn 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 observed during malathion stress. Secretion of mucus over the gill curtails the diffusion of oxygen (Rudragouda Marigoudar et al., 2009), which may ultimately reduce the oxygen uptake by the animal. Gills would be destroyed due to xenobiotic chemicals (Grinwis et al., 1998). The membrane functions are disturbed by a changed permeability, oxygen uptake rate would even rapidly decrease. On the other hand, the metabolic rate (in relation to respiration) of fish could be increased under chemical stress (Hartl et al., 2001). Kalavathy et al., (2001) reported that the dimethoate is efficiently absorbed across the gill and diffused into the blood stream and becomes toxic to fish.

## CONCLUSION

In the chronically pesticide treated fish gills of *O. mossambicus* swelling, fusion of lamellae, severe erosions of epithelial layer and high mucus secretion were visualized. The gill rocker ventricular structures were completely damaged and were uprooted from their bases.

## ACKNOWLEDGMENT

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