Effect of Cypermethrin Toxicity in the Gills of the Fish *Oreochromis mossambicus*

Thayappan Karthigayani¹, Maghil Denis¹, Annadurai Rexlin Andrew Remy¹* and Narayanasamy Shettu²

¹Department of Zoology, University of Madras, Chennai – 600 025, Tamil Nadu, India.
²Department of Zoology, Pachaiyappa's College, Chennai – 600 030, Tamil Nadu, India.

*Corresponding Author e-mail: remyandrew@gmail.com
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Abstract

Cypermethrin a potent insecticidal pyrethroid and a major pollutant present in agricultural and domestic wash out water that enter aquatic food chain and have toxic effect on aquatic organisms. Fish are considered as a tool to assess the environmental quality by studying the effect of pollutants on its physiological function and most importantly its survival. In the present study the fish tilapia *Oreochromis mossambicus* was taken as an experimental animal to study the toxic effect of cypermethrin at different concentrations and time of exposure up to 192 hours. The lethal concentration LC₅₀ was calculated by arithmetic logic method and found to be 0.04 ppm at 96 hours. The histological alterations on the gills were observed and recorded at different time intervals up to 192 hours. The gills showed significant alterations in structure and histology at the sublethal concentrations and thereby reduction in the oxygen diffusion capacity leading to distress in breathing in the fish. The findings were significant as the toxic effect of cypermethrin was found to increase with time of exposure suggesting its deleterious effect on aquatic organisms not only directly but also by bio accumulations.

Keywords: *Oreochromis mossambicus*, Cypermethrin, LC₅₀, Toxicity

INTRODUCTION

The hazardous effect of pollutants has its impact on all living organism. The water pollutants include industrial, agricultural and urban discharges that have deleterious effect on aquatic organisms especially when these contaminants are slight decomposers and exhibit high potential for accumulation with synergistic effect (Bernet et al., 1999) Fish are often used as indicators of such biological impacts of pollutants as they respond to low concentrations of toxic substances (Ayas et al., 2007) Alteration in the histology of the tissue such as gill or intestine that are directly related to the contaminants serve as important bio monitoring tools or bio markers to assess the toxicity (Thophon et al., 2003)

However, also in short term effects, fish being an essential component of the inland fisheries are particularly sensitive to a wide variety of pesticide chemicals and their productions are easily affected by such toxic pollutants (Tilak et al., 2005). Of late, synthetic pyrethroid insecticides are extensively used in place of organochlorine, organophosphorous insecticides and carbamates to control various types of pests to
increase agricultural production (Malla Reddy and Bashamoideen, 1989). These chemicals are potentially toxic to fish and other aquatic organisms (Malla Reddy and Bashamoideen, 1989), as the lipophilicity of pyrethroids have a high rate of gill absorption and in turn have damaging effect on the gills (Doharty et al., 1987; polat et al., 2002).

Cypermethrin is a potent pyrethroids insecticide and first synthesized by Elliott et al., (1974), is a highly active synthetic insecticide and used against a wide range of pests in agriculture, public health and animal husbandry. Considerable amount of literature is available on histopathological changes induced by organophosphorous, organochlorine and organocarbamate pesticides in fishes. Thus the present study was an attempt to be made to find the effect of synthetic pyrethroids, cypermethrin on the survival rate and histopathological alterations of gills in fish tilapia, Oreochromis mossambicus.

MATERIALS AND METHODS

Tilapia fish
The Mozambique tilapia, O.mossambicus as is an African tilapia cichlid fish, used as food and thereby introduced in aquaculture for commercial production and may be (erroneously) called "Java tilapia" in trade was chosen for the present study on toxicity of cypermethrin.

Collection and maintenance of tilapia
About 300 fishes with the average body length and the body weight of the fishes varying between 3 to 5 cm and 400 to 800mg respectively, was obtained from an aquarium in Chennai, India and maintained in the laboratory in aerated aquarium tanks with ambient temperature of 26 ± 2°C. The fish were fed with a mixture of artificial pellets and acclimated for a period of 15days.

Chemical
Cypermethrin is a synthetic pyrethroid molecule used to control pests especially the moth pests of cotton, fruits and vegetable crops. Technically Cypermethrin is a mixture of eight different forms of isomers. Cypermethrin is highly photo stable and at temperatures below 220°C. Used as a pesticide as a neurotoxin of insects, in agriculture and for domestic purposes as ant chalk against ant and cockroach.

Analytical grade chemical was purchased from Sigma Chemicals Co. (USA)

Molecular Structure
Molecular structure of Cypermethrin is given below (Dr. Raymond J. Heitzman Compton, Newbury Berkshire, United Kingdom)

(RS)-alpha-cyano-3- phenoxybenzyl(1RS)-cis-,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (IUPAC chemical name);

Molecular formula: C22H19Cl2NO3
Molecular weight: 416.3 g/mol
Solubility -0.009mg/l at 20°C (WHO 1989)

Structure of cypermethrin

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

Stock solution of Cypermethrin 10% Effective Concentration (EC) was prepared by diluting 1ml insecticide in 100ml of distilled water, and was diluted to different concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.08 ppm, which were used as experimental waters for toxicity study of tilapia. The experiment was set in triplicate and healthy fishes (n=10) with an average weight of 450mg and an average length of 3.5 cm were maintained in 10 litre of experimental water having different concentrations of Cypermethrin.
Similarly a control was set up with water devoid of Cypermethrin. Feeding was stopped one day prior to the experiment and also during the experimental period, as recommended by Ward and Parrish (1982) and Reish and Oshida (1987). The LC$_{50}$ values were calculated as average from the three replicates for each experimental concentration of water by arithmetic graph method (Reish and Oshida, 1987).

**Histological Techniques**

The method for histological study followed was that (McManus and Mowry, 1964). The gills of the control and the experimental fishes were dissected and fixed in Bouin’s fluid for 24 hrs. The fixed gills were dehydrated in graded ethanol series, cleaned in xylene and embedded in paraffin wax (congealing point 58-60°C). The longitudinal and transverse sections each of 6µm thickness were stained in haemotoxylin and eosin (HandE). The sections were deparaffinised in xylene each and rehydrated using a graded ethanol series and the dehydrated sections were blotted once again and cleared in two changes of xylene with the first change of ten minutes duration and second change of fifteen minutes duration and further blotted and mounted in DPX (Diestrene Plasticizer Xylene) to be examined under light microscope.

**Data analysis**

The data obtained were statistically analyzed by statistical package SPSS (version 16). The data were subjected to one way ANOVA and Duncan’s multiple range tests to determine the significance of difference at 5 % Probability level.

**RESULTS**

**Median Lethal Concentration (LC$_{50}$ -96 hours)**

For the tilapia fish, *O. mossambicus*, at 96 hours LC$_{50}$ value for cypermethrin was found at 0.04 ppm for Replicate I, II and III and the ppm for Replicate III. The arithmetic graphic for LC$_{50}$ value (96 hrs) for cypermethrin in *O. mossambicus* was plotted for three replicates (Figures 1) mean values obtained for all the replicates was 0.040 ppm.
Effect of cypermethrin on breathing activity of the fish

The entire body responses of the fish *O. mossambicus* such as surfacing, darting movements, and the gill opercular activity was observed for the different concentration of cypermethrin at different time intervals (24, 48, 72 and 96hrs). When the fish as were exposed to 0.01 ppm, 0.02 ppm and 0.03 ppm of cypermethrin at sublethal concentrations no change in behavioural response was observed. However in higher concentrations above lethal concentration 0.04, 0.05, 0.06, 0.07, 0.08 the opercular movements changed and frequent surfacing, for gulping and erratic movements were observed. The fishes often came to the surface and positioned themselves in an erect posture with the abundant discharge of mucous at the gill surface.

Effect of Cypermethrin on the histology of gills

Histology of gills in control fish

Like any other teleost fish, the *O. mossambicus* have seven pairs of gill arches. In the front, four pairs, with slender gill filaments form the two lines facing towards the back and these two lines are joined at the base forming the gill septum. Numerous secondary semi circular gill lamellae line both sides of gill filaments. The primary gill lamella consists of centrally placed rod like supporting axis with blood vessels on either side. The secondary gill lamellae are highly vascularised and covered with a thin layer of epithelial cells (Figure 4A).

Fish exposed to Cypermethrin

The observation of experimental fish exposed to 0.008 ppm of Cypermethrin at 24 hrs duration showed that the gill structure, such as primary gill lamellae (PGL), secondary gill lamellae (SGL) and epithelial cells (EC) in the gill lamellae remain unaffected with blood cells having prominent nucleus without any change (Figure 4B). However after 48 hrs, secondary gill lamellae became club shaped and damaged (Figure 4C). The sublethal exposure of cypermethrin at 72 hrs duration resulted in necrosis of the primary gill lamellae and severe necrosis in the secondary gill lamellae were observed at 96hrs (Figure 4D). The gill epithelium, curled, bulged with the fusion of secondary gill lamellae (Figure 4E and 4F) and at 192 hrs thickening and shortening of secondary gill lamellae was observed, the epithelial cells bulged and necrosis was visible (Figures 4G, 4H and 4I).

DISCUSSION

Cypermethrin, are used as agricultural and domestic pesticide that continuously pollute the inland fishery water as wash out from agricultural and domestic water by its toxic effect on aquatic organisms. The present study was undertaken to study the toxic effect of cypermethrin on the survival rate of the fish *tilapia, O. mossambicus* that was assessed by the LC50 value calculated as 0.04ppm at 96hrs exposure. The result implicated, that the fish was unable to withstand the exposure of cypermethrin with time and thereby the toxicity of the insecticide was possible on long exposure. It has already been reported that pesticide, xenobiotic and other chemicals are accumulated over a period of time in natural waters which may ultimately result in toxicity risk to aquatic organisms (Madherb *et al.*, 2002). The toxicity of cypermethrin though appears at concentration of 0.04ppm, takes long time for accumulation and the pesticide is stable in water that does not degenerate in aquatic bodies but only on soil surface. In aerobic conditions the soil half-life of cypermethrin is 4 days to 8 weeks. This clearly brings out the fact that the toxicity in fish under natural conditions may appear after the accumulation of the pesticide occurs over long period of time as the concentration of pesticide in wash out water are less than 0.001ppm (Tilak *et al.*, 2005).

The histological studies on the gills of the fish support the toxic nature of cypermethrin. At sub-lethal levels of cypermethrin concentration less than 0.004 in experimental water the fish survive without any sign of asphyxiation or breathing difficulty and opercular movement were normal. However above sublethal concentration the oxygen demand of the fish which normally occurs through gills seems to be affected and the fish gulped for air by darting to the surface. The histological observation of the primary and secondary gill lamellae including the gill membranes showed a slight change which possibly affects the oxygen diffusion capacity of the gills. Hence the supply of the
Figure 4A: Longitudinal section showing the normal organization of the gill structure of control fish, *Oreochromis mossambicus* Primary Gill Lamellae (PGL), secondary Gill Lamella (SGL) and Central Axis (CA). Bouin, 6 µm, Haematoxylin Eosin (HE). X110.

Figure 4B: Longitudinal section showing the organization of the gill structure of control fish *Oreochromis mossambicus* with PGL, SGL, with Epithelial Cells Bouin, 6 µm, HE. X110.

Figure 4C: Longitudinal section of the gill of the *Oreochromis mossambicus* exposed to 24 hrs sublethal exposure of cypermethrin at 0.008 ppm concentration showing less affected and less damages in the gill structure. Epithelial cells (EPC). Bouin, 6 µm, HE. X110.

Figure 4D: Longitudinal section of the gill of the fish *Oreochromis mossambicus* exposed to 48 hrs sublethal exposure of cypermethrin at 0.008 ppm concentration showing club shaped gill lamellae (CSGL) and damages in the secondary gill lamellae (DSGL) Bouin, 6 µm HE. X 950.

Figure 4E: Longitudinal section of the gill of the fish *Oreochromis mossambicus* exposed to 72 hrs sublethal exposure of cypermethrin at 0.008 ppm concentration showing necrosis in the primary gill lamellae (NPGL) and severe necrosis in the secondary gill lamellae (SNGL). Bouin, 6 µm, HE. X 950

Figure 4F: Longitudinal section of the gill of the fish *Oreochromis mossambicus* exposed to 96 hrs sublethal exposure of cypermethrin at 0.008 ppm concentration showing lifting up of epithelium, curling, bulging and of secondary gill lamellae (indicated in arrows). Bouin, 6 µm, HE. X 950.
The present study was an attempt to find the toxicity of cypermethrin on tilapia and results conclusively showed that the cypermethrin is the pesticide which may not have toxic effect up to sub lethal concentrations and bio accumulation of the pesticide in the tissues of the aquatic organisms or any organism of the aquatic food chain may have deleterious effect on the aquatic biodiversity.

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