HISTOPATHOLOGICAL EFFECTS ON *Oreochromis mossambicus* (TILAPIA) EXPOSED TO CHLORPYRIFOS

Aniladevi Kunjamma K.P.*, Babu Philip, Smitha V. Bhanu and Jisha Jose

Department of Marine Biology, Microbiology and Biochemistry, School of Ocean Science and Technology, Cochin University of Science and Technology, Cochin, Kerela (INDIA)

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ABSTRACT

In the present study Tilapia (*Oreochromis mossambicus*) of size 15±3gm, were exposed to sublethal concentration (1/3 of LC_{50} value) of technical grade chlorpyrifos, (LC_{50} is 82ppb=82µg/L) for a period of 21 days, with sampling after 7 days also. The treated fish groups were compared with the control group for the histopathological changes in tissues of gill and liver and marked changes were observed.

Key Words : Histopathological changes, Tilapia, Chlorpyrifos, Gill, Liver

INTRODUCTION

Pesticides are used widely all over the world to control the harmful effects of pests on agriculture productions. The environmental impact of pesticide use has been discussed much due to its widespread use in parallel with the modernization of agricultural operations and indiscriminate permeation of the ecosystem with these pesticides.

It is apparent that human chemical additions have introduced or increased environmental stress for aquatic organisms and fishes, in particular. Pesticides are toxicants capable of affecting all taxonomic groups of biota, including non-target organisms, to varying degrees depending on physiological and ecological factors. Many pesticides are resistant to environmental degradation so that they persist in treated areas and thus their effectiveness is enhanced. This property promotes their long-term effects in natural ecosystems. Dispersal of pollutants in the atmosphere results in treatment of natural terrestrial areas while water run-off transfers pesticide quantities to fresh water areas, and ultimately the oceans and thus their effect comes in aquatic organisms.

The pesticides can be carried by running water towards ground water. The discovery that organochlorine pesticides, such as DDT are highly persistent, bioconcentrate in food chains and can severely affect whole populations or species of wildlife, has led to bans and restrictions in use[1]. Most organophosphorus insecticides are regarded as being non-persistent, but some reports have indicated that esidues of organophosphates remain essentially unaltered for extended periods in organic soils and surrounding drainage systems[2]. The most common termiticidal use of chlorpyrifos involves its application to the

*Author for correspondence

soil surrounding building structures as a barrier against termite invasion. At one time chlorpyrifos was applied as a mosquito larvicide, a use that involved application of formulated product directly to bodies of water[3]. The organic pollutants (xenobiotics) are fat soluble and are therefore readily taken up from the water, sediment and food sources into the tissues of aquatic organisms[4].

There are a number of studies reporting the pathological changes in fishes exposed to different organochlorine, organophosphorus, carbamate and pyrethroid pesticides. The extent of severity of tissue damage of a particular compound as toxicant depends on its toxic potentiality in the tissues of organisms[5]. Lipophilic nature of water-insoluble pesticides enhances its ability to cross the plasma membrane, when the pesticides come in contact with the aquatic organism.

OBJECTIVES

Investigation of aquatic toxicity of the organophosphorus pesticide chlorpyrifos was the objective of the present study. Histopathological changes were observed in the teleost fish *O. mossambicus*, exposed to chlorpyrifos for a period of 7 days followed by 21 days.

MATERIAL AND METHODS

The present study was undertaken using *O. mossambicus* (Tilapia) because of its wide availability and suitability as a model for toxicity testing[6]. The fish of size 15$\pm$3gm were acclimated to the laboratory conditions for 10 days. The test solution of technical grade chlorpyrifos was prepared as 5 gm% stock solution using acetone and diluted further as required. A static acute toxicity bioassay was carried out to determine the LC_{50} value. Fishes without sex determination were exposed to sublethal concentration of technical grade chlorpyrifos of 95% purity, for 21 days. The test solution and the water in which fishes maintained, were renewed daily. Fishes were randomly selected from control groups and treated groups. Sampling was performed after 21days of exposure to the pesticide, with the sampling after 7days also, for histopathological observations.

Gill and liver tissues were collected by sacrificing the fish. The tissues were fixed in 10% formalin for 24 hours. Tissues were dehydrated in ascending grades of alcohol and were cleared in xylene until they became translucent. Tissues were transferred to molten paraffin wax for 1hour to remove xylene completely and impregnated with wax. Then the blocks were cut in a rotary microtome to prepare sections of thickness 4 to 6 microns. The sections were stained with haematoxylin and eosin and mounted in DPX[7].

RESULTS AND DISCUSSION

The structural alterations were observed under light microscope in the sections of gill **(Fig. 1)** and liver **(Fig. 2)** tissues of fish from treated group when compared to those from control group.

Liver, the first organ to face any foreign molecule through portal circulation is subjected to more damage[8]. The parenchymatous hepatic tissue in teleosts, has many important physiological functions and also detoxification of endogenous waste products as well as externally derived toxins, drugs, heavy metals and pesticides[9].

The liver tissues **(Fig. 2)** of pesticide treated fishes showed structural alteration unlike those from control group. Generally, the liver in teleost fish is a compound organ in the form of hepatopancreas. Sinusoids, which are irregularly distributed between the polygonal hepatocytes, were fewer in number and are lined by endothelial cells with very prominent nuclei. Hepatocytes are often swollen with glycogen or neutral fat.

In the present study, in the tilapian liver, treated with sublethal concentration of chlorpyrifos, for a period of 7 days, hepatocytes appeared swollen with granular cytoplasm. Pancreatic acini appeared to loss the normal

Sections showing
Histopathological effects of technical grade chlorpyrifos on
Gill tissue of *Tilapia*

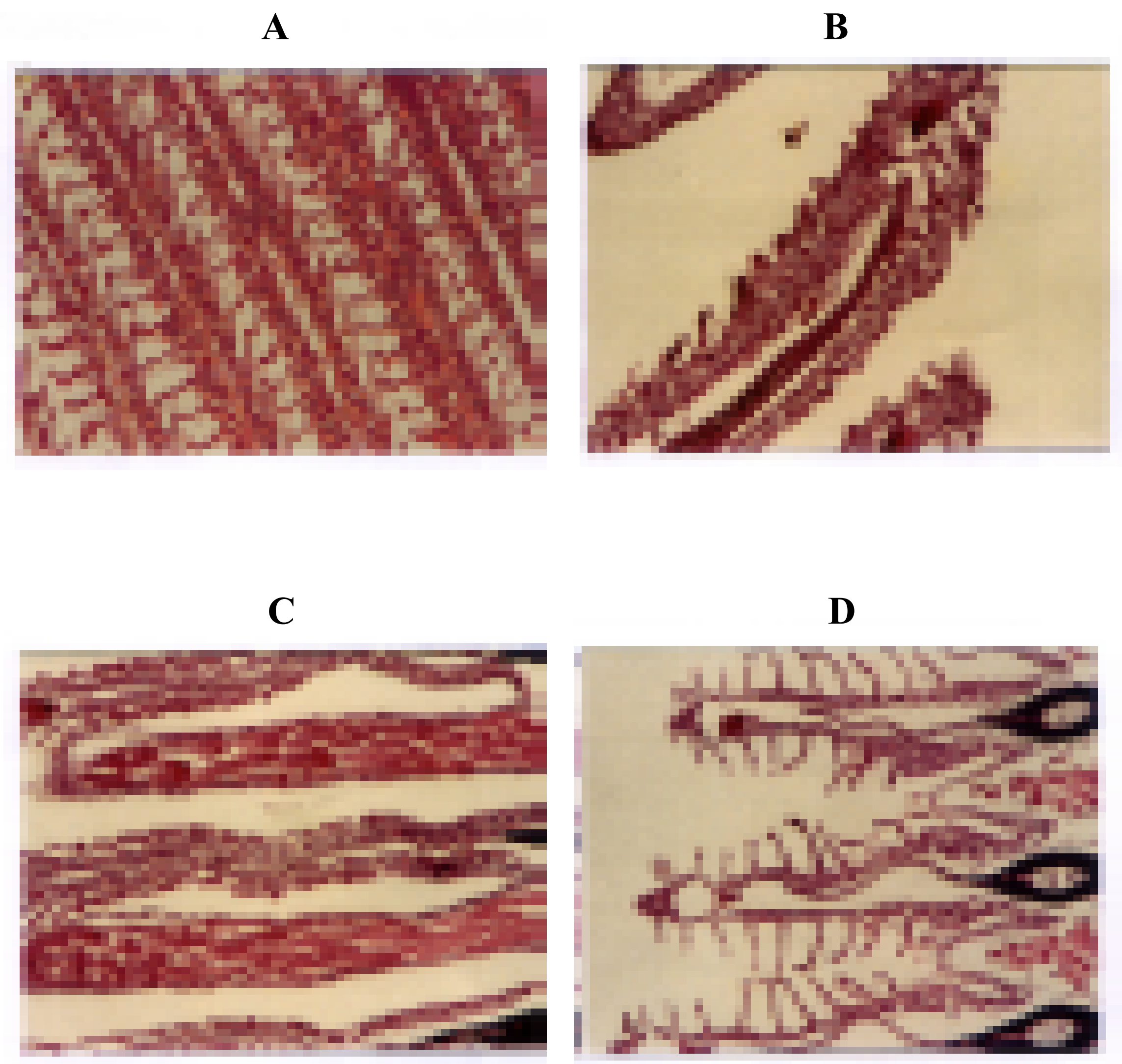

Fig. 1 : A. Control gill **B.** Gill after 7 days of exposure shows clubbing
C and **D.** Gill after 21 days of exposure shows hyperplasia and desquamation

Sections showing
Histopathological effects of technical grade chlorpyrifos on
Liver tissue of *Tilapia*

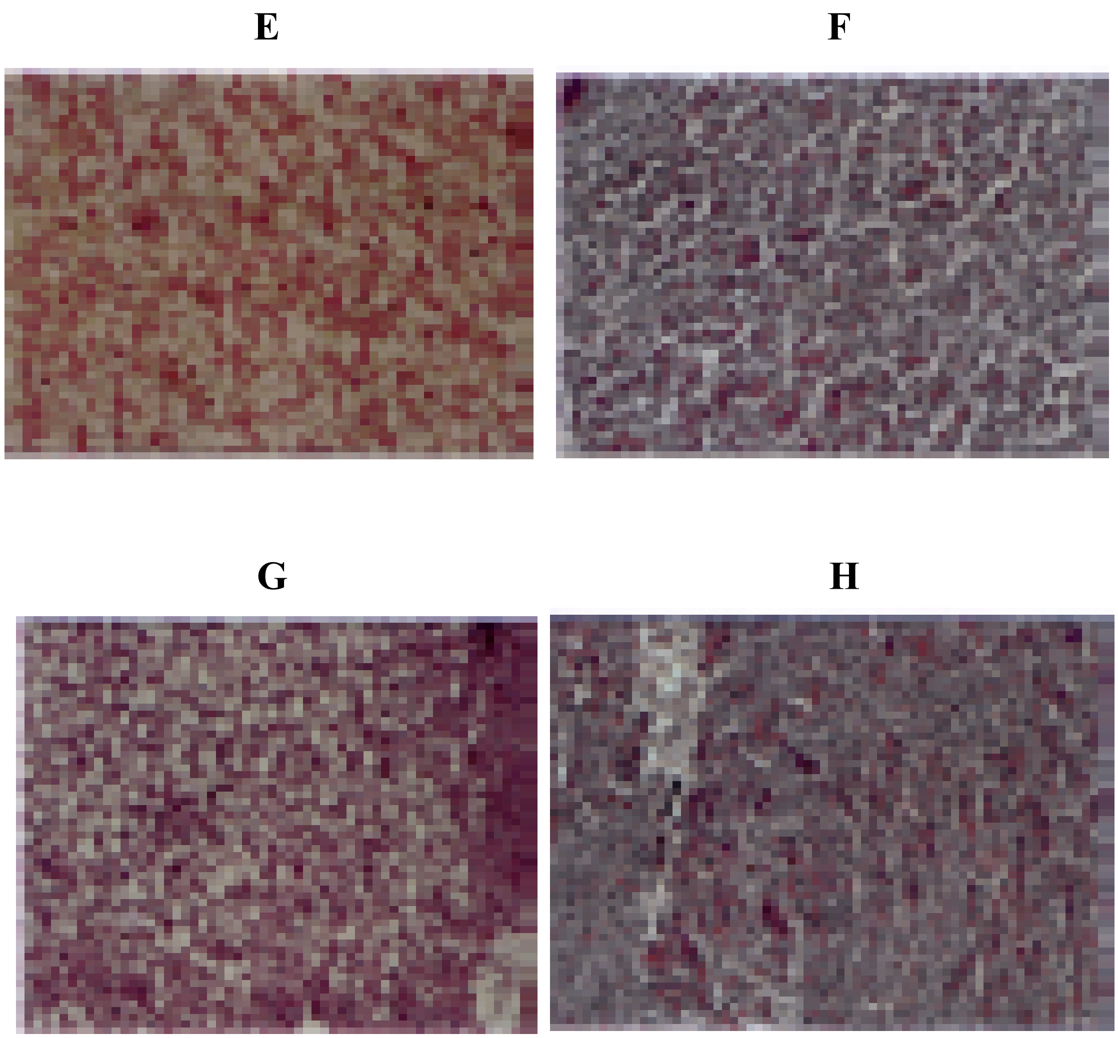

Fig. 2 : E. Control liver **F.** Liver after 7 days of exposure shows necrotic areas
G and **H.** Liver after 21 days of exposure shows vacuolisation (G) and biliary hyperplasia (H)

structure and were found in necrosed state. Cytoplasm of hepatocytes became more basophilic indicating the protein precipitation, which led to necrosis of cell. Detachment of hepatic cells gives the indication of its non-functional condition. The cells became more rounded-off showing acute necrosis and also glycogen depletion.

21 days of long-term exposure to chlorpyrifos, resulted in necrosis of the hepatic tissue. Completely vacuolated areas were observed with fat deposition. Biliary hyperplasia was observed at certain regions of the hepatic tissue. This might be indicating the regenerating hepatic cells to withstand the toxic stress condition.

These observations are in good agreement with reports of Sudha Singh et al.[10] and Tilak et al[11]. A Study by Sudha Singh et al.[10] reported clubbing, vacuolation and also necrosis of pancreatic tissue by the exposure of endosulfan and carbaryl on *Nandus nandus*. Intercellular spaces and spaces around pancreatic mass were also seen. They observed that after a long-term exposure of both endosulfan and carbaryl, the liver tissues were converted into spongy mass. The pancreatic tissues were seen shrunken and scattered due to heavy necrosis of hepatic mass.

Tilak et al.[11] observed the same changes in liver of *Catla catla*. The pathological changes included degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels, necrosis and hepatocyte cell membrane disposition. Hepatic cords appeared in decreased size, nucleus became pyknotic.

Radhaiah and Jayantha Rao[12] reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture in blood vessels and appearance of blood vessels among hepatocytes, pyknotic nuclei in the liver of *Tilapia mossambica* exposed to fenvalerate.

Diffusion of a xenobiotic across the gill epithelium will depend largely on lipid solubility[13]. Generally, the teleost gill arch is a curved osseous structure from which radiate the bony supports of the primary lamellae of which the surface area is increased further by the formation of regular semilunar folds called the secondary lamellae.

On short-term exposure of pesticide, the changes observed in gills were hyperemia, clubbing and edema. After 21 days of pesticide exposure, gills became edematous with prominent clubbing. Separation of primary gill lamellae, and hemorrhage in the blood vessels outside the secondary gill lamellae were observed. Hyperemia of the gill filaments that engorged with blood vessels appeared. Hyperplasia was observed in secondary gill lamellae, which led to fusion of adjacent primary and secondary gill filaments.

The histopathological changes observed in the gills of *O.mossambicus* in the present study are in good agreement with the reports of Rao et al.[14] and Jauch[15].

Rao et al[14] observed the bulging of secondary lamellae at the terminal ends, lesions and erosions at the base of lamellae on 12th day of exposure of *O. mossambicus* to chlorpyrifos. A thick coat of mucus on the gill filaments was persisting on 18th day of exposure. Jauch[15], reported that 96-hour fenthion exposure induced gill lesions, including hyperplasia and desquamation of the epithelium and thrombosis in the secondary gill lamellae.

A study by Gopalakrishnan[16] elucidated the effect of organophosphorus pesticide Dimecron on the gill and liver of the fish *Etroplus*. Dimecron induced branchial congestion in the gill filaments. Edematous fluid lifted the respiratory epithelium in a few secondary lamellae, which were found thickened. The cells between the secondary lamellae were thickened to such an extent that the inter-lamellar spaces occluded, which gave the filament a compact appearance.

CONCLUSION

The present study revealed that the organophosphorus insecticide chlorpyrifos is potent to cause toxic responses, even structural alterations, in aquatic organism like fish. A number of studies have elucidated the aquatic toxicity of different kinds of pesticides. These reports bring discussions on the deteriorating nature and the lethal effects of the pesticides on ecosystem. Pesticides, especially the non-degradable ones, even in minute levels, are causing a stress to aquatic organisms. The toxic responses are reflected by the behavioral, biochemical and pathological changes. But an agricultural effort reducing the use of pesticides and implementing natural remedies for pest-encroachment can become one solution for pesticide pollution.

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