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Research Article

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Histopathological alteration in gill of the freshwater fish *Pseudetroplus maculatus* (Bloch, 1795) under chlorpyrifos toxicity

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Abstract

Sublethal toxicity effects of organophosphorous pesticide, chlorpyrifos (O, O-Diethyl-O-3,5,6-trichloro-2-pyridy phosphorothioate) was investigated in the freshwater teleost fish, *Pseudetroplus maculatus*. Fishes were exposed to one-tenth of LC_{50} concentration, ie., 0.661 µg/ L of chlorpyrifos for 24, 48, 72 and 96 h maintaining ten fishes in each group, along with the control group. At the end of every treatment period, animal was killed and the gill tissue was collected for the histopathological analysis. Control tissue showed normal gill architecture with compact lamellar gill epithelium, gill arches, primary and secondary lamellae. Gill of chlorpyrifos exposed groups were compared with that of control tissue and it was observed that no notable changes on the histology of gill at 24 h. Some structural alterations such as curling of secondary lamellae, fusion of adjacent secondary lamellae and hyperplasia of primary lamella were observed after 48 h of pesticide exposure. Interestingly, the complete destruction of secondary lamellae with vacoulization of primary lamellae was observed at the end of 72 h exposure. However, after 96 h of treatment period necrosis of the secondary lamellae and aneurism were noticed. Thus the pathological effect of chlorpyrifos was well documented from the present results and the intensity of gill tissue damage was observed in time-dependent manner. The present study illustrates that even at sublethal concentration of chlorpyrifos exposure at short-time duration could cause severe pathological lesions in fish and is of great threat to aquatic ecosystem.

Keywords: *Pseudetroplus maculatus*, Pesticide, Chlorpyrifos, Histopathology, Gill, Acute toxicity

Introduction

All living organisms attain ability to adapt themselves to change in the environment such as temperature, humidity, oxygen supply or toxicant exposure. There are wide variety of toxicants present in the environment in the form of metals, nanoparticles, pesticides, insecticides etc. Such toxicants may reach waterbodies such as freshwaters, rivers, lakes or streams in variety of ways. Thus the pesticides on reaching the aquatic ecosystem greatly influences the non-target organisms, especially fish (Herger *et al.*, 1995). Highly effective pesticides could bring multiple changes in the organism by altering the rate of growth and survival, nutritional value, behavioural pattern etc. As the major part of world's food is dependent on fish source, it is essential to protect the health status of fish population.

Organophosphates are more frequently used pesticide among the different classes because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment (Freedman, 1995). Some reports revealed that residues of organophosphates remain unaltered for extended periods in organic soils and surrounding

drainage systems **(**Harris and Miles, 1975). Chlorpyrifos (O, O-diethyl-O-3, 5,6-trichlor-2-pyridyl phosphorothioate; CPF) is a broad spectrum organophosphate insecticide widely used to control foliar insects in agricultural crops (Rusyniak and Nanagas, 2004) and subterranean termites (Venkateswara Rao *et al*., 2005). It is the second highest selling organophosphate insecticide and is more toxic to fish than anyother organochlorine compounds (Tilak *et al*., 2001). From the surface water chlorpyrifos has been shown to be absorbed through the gill, skin and digestive system of fish and get distributed in various tissues through the blood where it accumulates particularly in fatty tissues due to its lipophilic property (Shkoukani *et al*., 2013). Due to the accumulation of pesticides in different tissues results in many physiological, biochemical and pathological changes in fishes, which produces many complex and damaging effects in different organs, tissues or cells (Nagarathnamma and Ramamurthi, 1982).

Histopathological investigations have long been recognized as a biomarkers of toxicity stress in fish. Histopathological changes are often the result of the integration of a large number of interactive physiological processes, therefore, it helps to identify target organs of toxicity and mechanism of action (Ramalingam *et al*., 2000). The present study was thus focused to investigate the histopathological alteration in gill of the freshwater fish, *Pseudetroplus maculatus* after exposure to chlorpyrifos at sublethal concentration.

Materials and Methods

Test organism

Pseudetroplus maculatus, freshwater cichlid fish weighing 3.5 ± 0.5 g and length 6 ± 0.3 cm collected from local fish farm near Parappanangadi, Malappuram district, Kerala, India, were adjusted to the laboratory conditions for 15 days before experiment. Fish were fed with standard fish pellets during and at the time of experiment, and are maintained in large cement tank containing dechlorinated water and well aeration. The physiochemical features of the tap water were analysed by maintaining water temperature at 28 ± 2°C, dissolved oxygen at 8.5 and pH at 7.6 according to the method as described in APHA (1998).

Chemical

Chlorpyrifos (O, O-diethyl O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate) of technical grade (97%) was obtained commercially from Hikal Chemical Industries, Gujarat, India

Experimental design

Sublethal concentration of chlorpyifos i.e., 0.661µg/L (one-tenth of LC_{50}-96 h) was chosen for the present study and the animal was exposed to toxicant for 24, 48, 72 and 96 h along with the control group. Ten fishes were maintained in each group and after the end of each duration, both control and treated fishes were sacrificed for histopathological study**.**

Histopathological analysis

After the end of every treatment, fishes from both control and treated groups were sacrificed and gill was removed. Tissue was then fixed in buffered formalin, dehydrated in ascending alcohol series and cleared in xylene. Tissue was embedded in molten paraffin wax and sections of 5-6 µm thickness were made with a rotary microtome. Preparations were stained with eosin- hematoxylin and mounted in DPx and the stained sections were observed under trinocular research microscope and photographed.

Results

In the present study a variety of histopathological changes were observed in the gill of *Pseudetroplus maculatus*. The severity and frequency of organ lesions were more pronounced according to the the duration of the treatment. Control gill tissue showed normal architecture with compact lamellar gill epithelium, gill arches, primary and secondary lamellae (Figure A). Gill of chlorpyrifos exposed groups were compared with that of control tissue and it was observed that no notable changes on the histology of gill at 24 h (Figure B). Some structural alterations such as curling of secondary lamellae, fusion of adjacent secondary lamellae and hyperplasia of primary lamella were observed after 48 h of pesticide exposure (Figure C). Interestingly, the complete destruction of secondary lamellae with vacoulization of primary lamellae was observed at the end of 72 h exposure (Figure D). However, after 96 h of treatment period necrosis of the secondary lamellae and aneurism were noticed (Figure E).

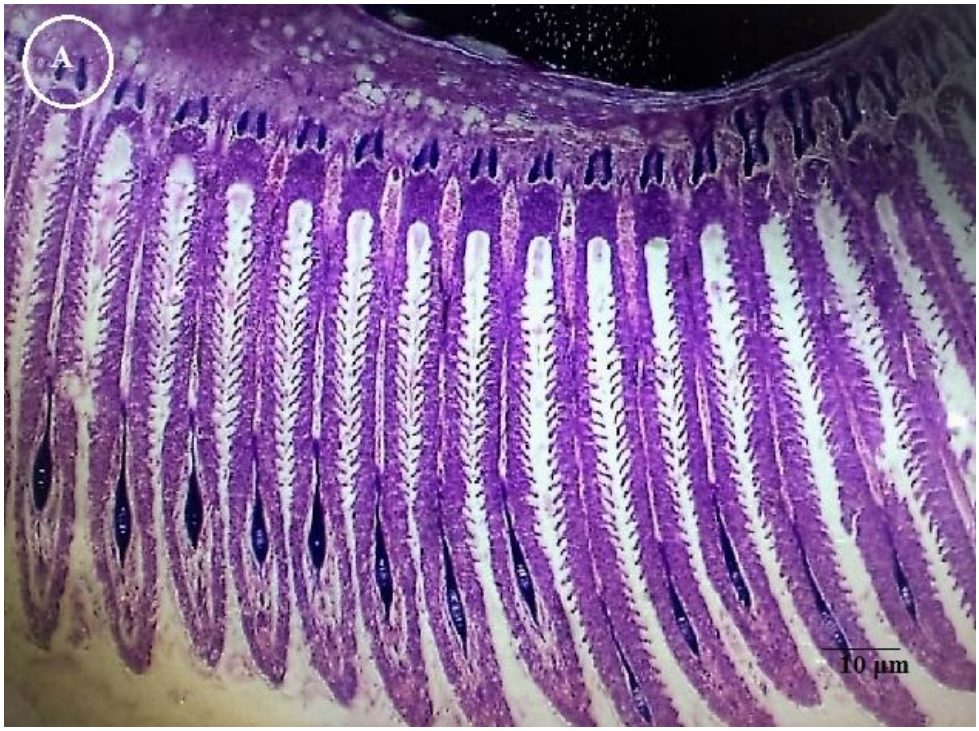

Figure A. Photomicrograph showing normal architecture of gill with compact lamellar gill epithelium, gill arches, primary and secondary lamellae

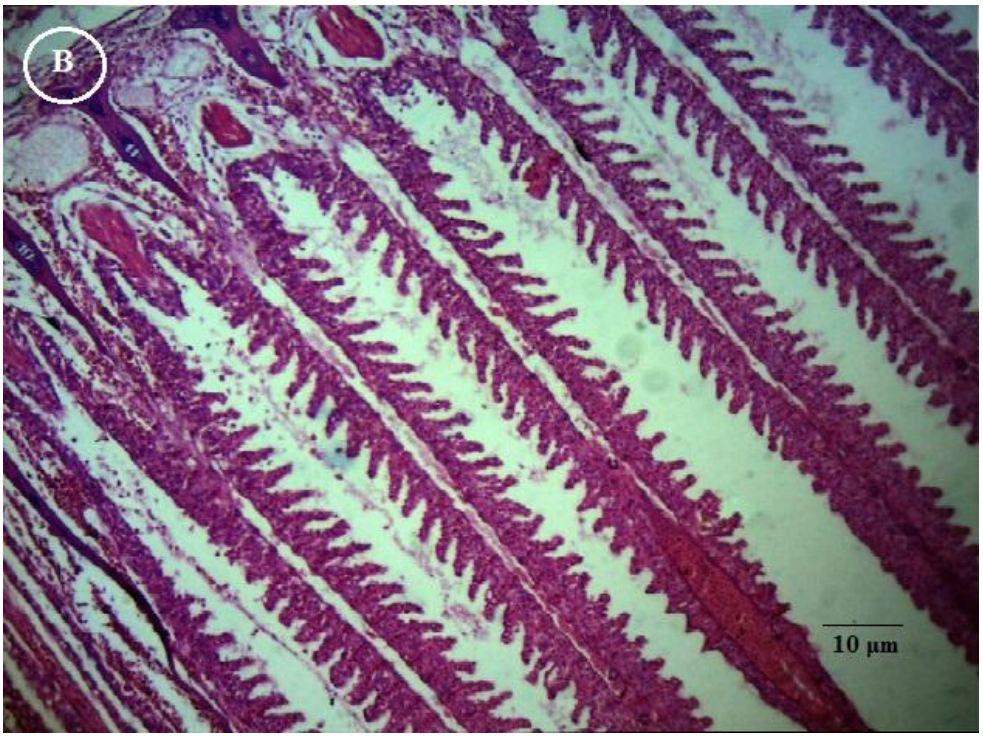

Figure B. Photomicrograph showing no notable changes on the histology of gill at 24 h of chlorpyrifos exposure

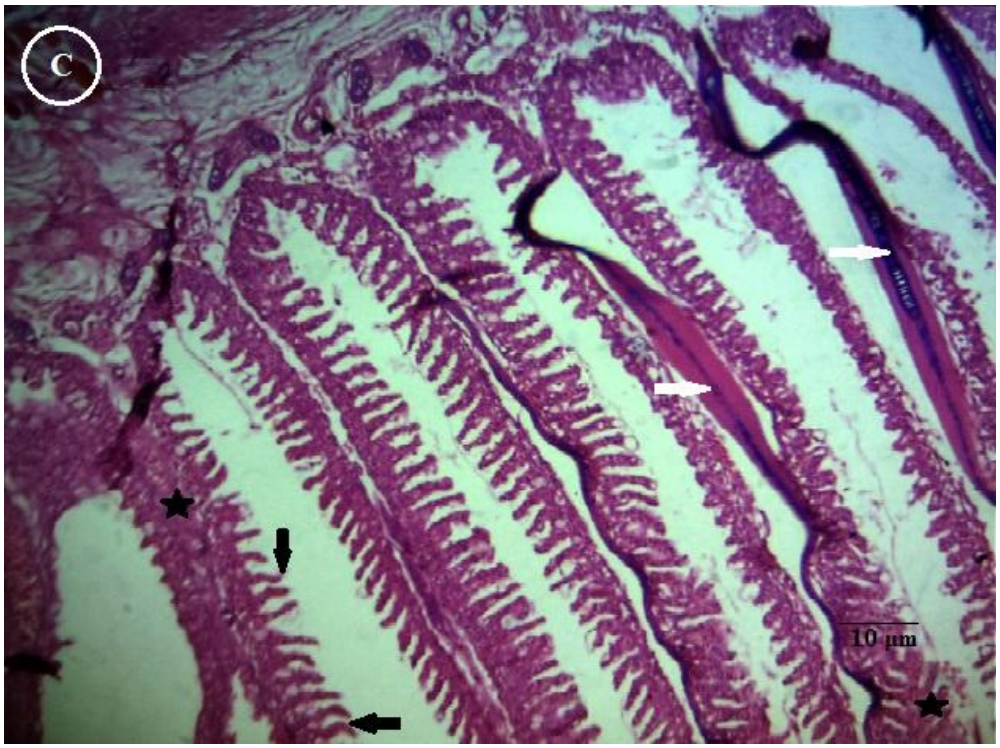

Figure C. Photomicrograph showing curling of secondary lamellae (black arrows), fusion of adjacent secondary lamellae (asterisks) and hyperplasia of primary lamella (white arrows) after 48 h of chlorpyrifos exposure

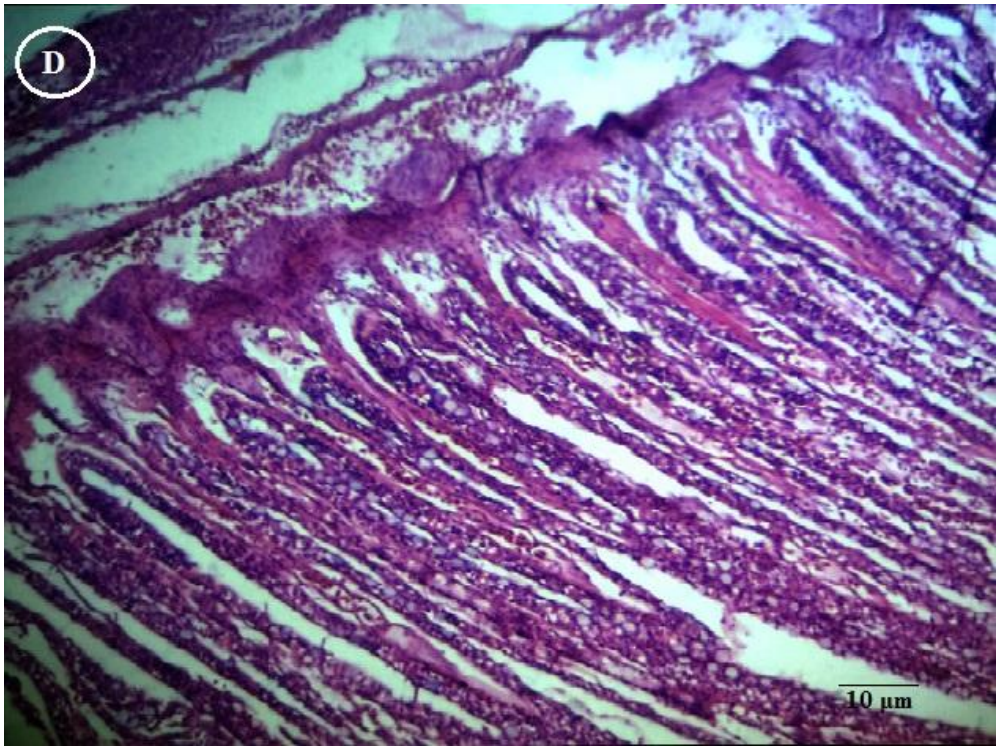

Figure D. Photomicrograph showing complete destruction of secondary lamellae with vacoulization of primary lamellae after 72 h of chlorpyrifos exposure

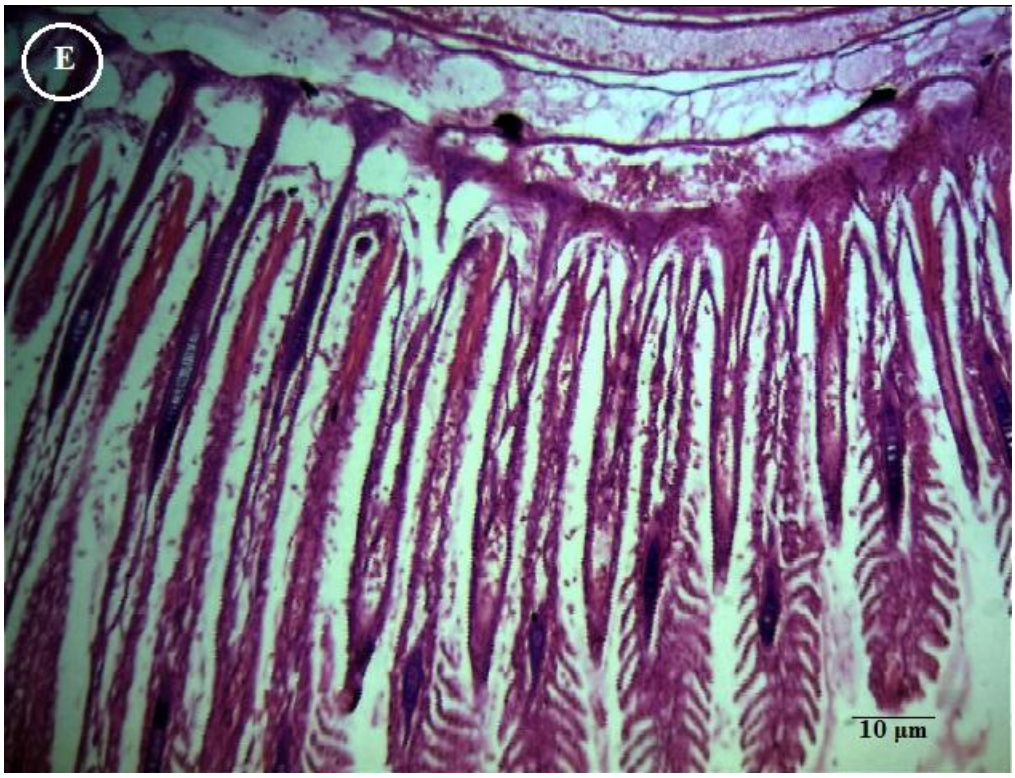

Figure E. Photomicrograph showing necrosis of the secondary lamellae and aneurism after 96 h of chlorpyrifos exposure

Discussion

Chlorpyrifos is considered as highly toxic pesticide to both marine and freshwater organisms. Several literatures suggested the toxicity effect of chlorpyrifos with specific behavioural defects, morphological deformities, neurotoxic effects by altering acetyl cholinesterase activity, induction of oxidative stress, as endocrine disruptor, also genotoxic and mutagenic effects in various aquatic organisms. The present study was undertaken to evaluate chlorpyrifos-induced histomorphological alterations in gill of *Pseudetroplus maculatus*. Histological analysis is the most effective and sensitive tool to determine cellular change that may occur in vital organs (Dutta, 1996). It proves a good indicator to study chemical toxicity and can correlate to the health status of animals in the exposed aquatic ecosystem. Fish when exposed to sublethal concentration of various pesticides or other chemical contaminants in the environment may result in various histological alterations in tissues (Altinok and Capkin, 2007). It is well known that gills are the primary target of waterborne toxicants as it is constantly in contact with the water (Mallatt, 1985). Some of the alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, partial fusion of some secondary lamellae are examples of defense mechanisms, and these results increase the distance between the external environment and the blood and serve as a barrier to the entrance of toxicants (Mallatt, 1985).

The major deformalities found in the present study were curling of secondary lamellae, fusion of adjacent secondary lamellae and hyperplasia of lamellar epithelium, necrosis and aneurism. The present study was in agreement with similar observations reported under chlorpyrifos and di (2-ethylhexyl) phthalate (DEHP) toxicity for 96 h in the freshwater fish, *Oreochromis mossambicus* treated with (Kunjamma *et al.*, 2008., Revathy and chitra, 2015). Gill morphologythus proved as a useful indicator in environmental monitoring. The present observation clearly illustrates that chlorpyrifos after acute exposure at sublethal concentration can result in structural changes in gill tissues of test organism. Besides, it can be assumed that chlorpyrifos entered into the body of fish through skin, gill or by direct engulping of water can also cause deterioration of vital organs. Therefore, reducing the use of harmful pesticides is the only way to safeguard from the aquatic pollution.

Conclusion

Chlorpyrifos is the second widely used pesticide in the world, so the extensive use can affect aquatic life and its ecosystem. It may also lead to harmful consequences to humans on consumption of those toxicant exposed fish.

Acknowledgments

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