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HISTOPATHOLOGICAL LESIONS AND OXIDATIVE STRESS IN THE GILL AFTER CHLORPYRIFOS EXPOSURE IN THE FRESHWATER FISH, *PSEUDETROPLUS MACULATUS* (BLOCH, 1795)

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ABSTRACT

The present study aims to evaluate the histopathological lesions and oxidative stress responses of chlorpyrifos in the gill of freshwater teleost fish, *Pseudetroplus maculatus*. Chlorpyrifos was exposed to fish at sublethal concentration (one-tenth of LC_{50}-96 h; 0.661µg/L) for 24, 48, 72 and 96 h maintaining the control group. The weight of gill decreased significantly only after 96 h of chlorpyrifos treatment. The activity of superoxide dismutase increased significantly ($P<0.05$) after 48 h in time-dependent manner, and the catalase activity was significantly ($P<0.05$) increased at 72 and 96 h of chlorpyrifos exposure. The level of hydrogen peroxide increased significantly ($P<0.05$) after 48 h and the lipid peroxidation was found to be increased significantly ($P<0.05$) immediately after 24 h of the toxicant exposure in time-dependent manner. Histopathological lesions in the gill after 96 h of chlorpyrifos exposure include degeneration of gill arches, epithelial uplifting, hyperplasia and aneurysm in primary lamellae, degeneration and necrosis of the secondary lamellae and vacuolization. The present study conclude that acute exposure to chlorpyrifos at 0.661µg/L concentration has the ability to induce oxidative stress as well as damages the gill tissues of fish and those effects are time-dependent.

Keywords: Chlorpyrifos, Histopathology, Gill, Oxidative stress, *Pseudetroplus maculatus*

1.0 INTRODUCTION

Pesticides have been one of the most effective weapons discovered by man to protect agricultural products from pests. Nowadays, it has become the major cause of concern for aquatic environment due to its toxicity, persistency and tendency to accumulate in the organisms (Joseph and Raj, 2010) and it is difficult to remove them from an aquatic ecosystem. The response of aquatic organisms towards the pesticides depends upon the nature of the toxic compounds, exposure period, quality of water and resistance of the species exposed (Fisher, 1991). Fishes are very sensitive to a wide variety of toxicants in their natural environment and various species of fish are forced to uptake and accumulate several toxicants which finally enter into human food chain (Herger *et al*., 1995). Due to the bioaccumulation of toxicants in tissues leads to many physiological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma and Ramamurthi, 1982).

In recent years the use of organophosphate (OP) pesticides are increasing since they are biodegradable therefore persist in the environment only for a short time (Thamizhzhagan *et al*., 2017). Some reports revealed that residues of organophosphates remain essentially 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 and to manage subterranean termites ((Rusyniak and Nanagas, 2004).

The pollution toxicity in aquatic organisms may be associated with increased production of reactive oxygen species (ROS), leading to oxidative stress (Belgekurutas *et al*., 2009). In the aquatic organisms, despite the presence of constitutive or enhanced antioxidant defense systems, increased levels of oxidative damage may occur when exposed to contaminants that stimulate the production of ROS (Livingstone, 2001). Biochemical and physiological indicators such as antioxidant enzymes could be used as biomarkers to identify the possible impact of environmental contaminations on aquatic animals before the health status of ecosystem is assigned (Jiminez, and Stegeman, 1990).

Histopathological investigations have long been recognized as documented biomarkers of stress in fish. It is used as biomonitoring tools or indicators of fitness of an organism in the natural environment and also provides early warning signs of disease (Meyers and Hendricks, 1985). It allows examining specific target organs, including gill, kidney and liver that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al*., 2001).

The teleost fish, *Pseudetroplus maculatus* was selected for the present study due to its wide availability and suitability as model for toxicity testing and also due to sustainability in laboratory conditions. In the light of above information and ideas, the present investigation is aimed to study the effect of sublethal concentration of chlorpyrifos on the antioxidant status of gill of the freshwater teleost fish. In addition, histopathology of gill tissue was performed in order to understand the extent of chlorpyrifos toxicity in tissue damage.

2.0 MATERIALS AND METHODS

2.1 Experimental organism

The Cichlid fish, *Pseudetroplus maculatus* weighing 3.5 ± 0.5g and length 6 ± 0.3cm were collected from local fish farm near Parappanangadi, Malappuram district, Kerala, India. Fishes were acclimatized to the laboratory conditions in well-aerated cement tank of 40 L capacity, which was dechlorinated regularly. Health status of the fishes was continuously monitored throughout the study and no mortality was observed during the experiment. Some preliminary parameters as water temperature (28 ± 2°C), oxygen saturation (70 and 100 %), and pH (6.5 to 7.5) were maintained in all the treatment groups using standardized procedures as described in APHA (1998).

2.2 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.

2.3 Experimental design

After acclimatization, fishes for controls and treatments were grouped in separate tanks retaining ten animals per group. The median lethal concentration (LC_{50}-96 h) of chlorpyrifos in *Pseudetroplus maculatus* was determined in our laboratory by using probit analysis, which is 6.61µg/ L (Raibeemol and Chitra, 2015). Therefore, sublethal concentration (0.661µg/ L; one-tenth) of LC_{50}-96 h was selected and exposed for 24, 48, 72 and 96 h maintaining control group. The experiment was designed as follows.

Group I: Control group maintained for 96 h.

Group II: Chlorpyrifos-treated group maintained for 24, 48, 72 and 96 h.

At the end of every experiment, fishes were caught very gently using a small dip net, one at a time with least disturbance and were decapitated. Gill tissue was dissected, weighed and 1% tissue homogenate was prepared for the biochemical analyses. A 1% (w/ v) homogenate of gill tissue was prepared in ice-cold normal saline with the help of a motor-driven glass Teflon homogenizer on crushed ice for a minute. The homogenate was centrifuged at 800 *g* for 15 min at 4^{o}C to obtain the supernatant, which was then used for the biochemical analyses. Protein was estimated by the method of Lowry *et al*. (1951) with bovine serum albumin as the standard. Activities of superoxide dismutase (Marklund and Marklund, 1974), catalase (Claiborne, 1985), glutathione reductase (Carlberg and Mannervik, 1985), level of hydrogen peroxide generation (Pick and Keisari, 1981), lipid peroxidation (Ohkawa *et al*., 1979) were measured in the supernatant of crude homogenate.

2.4 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.

2.5 Statistical analysis

Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 19.0. Differences were considered to be significant at $p<0.05$ against the control group. Data are presented as mean ± SD for ten animals per group. All biochemical estimations were carried out in duplicate.

3.0 RESULTS

3.1 Biochemical analysis

Changes in the activity of antioxidant enzymes in fish exposed to chlorpyrifos were recorded at the end of experimental period. Chlorpyrifos caused a significant decrease ($P<0.05$) in the weight of the gill after 96h treatment (Figure 1). Activity of superoxide dismutase was significantly increased ($P<0.05$) from 48 h onward in a time-dependent manner (Figure 2) and the activity of catalase was also increased significantly ($P<0.05$) from 72 h onwards (Figures 3) compared to the corresponding control group. Simultaneously, the levels of hydrogen peroxide generation and lipid peroxidation were significantly increased ($P<0.05$) in the treatment groups when compared to the control (Figures 4 and 5).

3.2 Histological analysis

The control group fishes did not display any histological changes in the gill tissue examined. Many histological changes were observed in gill tissue after 96h chlorpyrifos exposure. The major changes are degeneration of gill arches, epithelial lifting, and degeneration of secondary lamellae, hyperplasia and aneurism in primary lamellae and vacoulization of gill arches (Figures A-E).

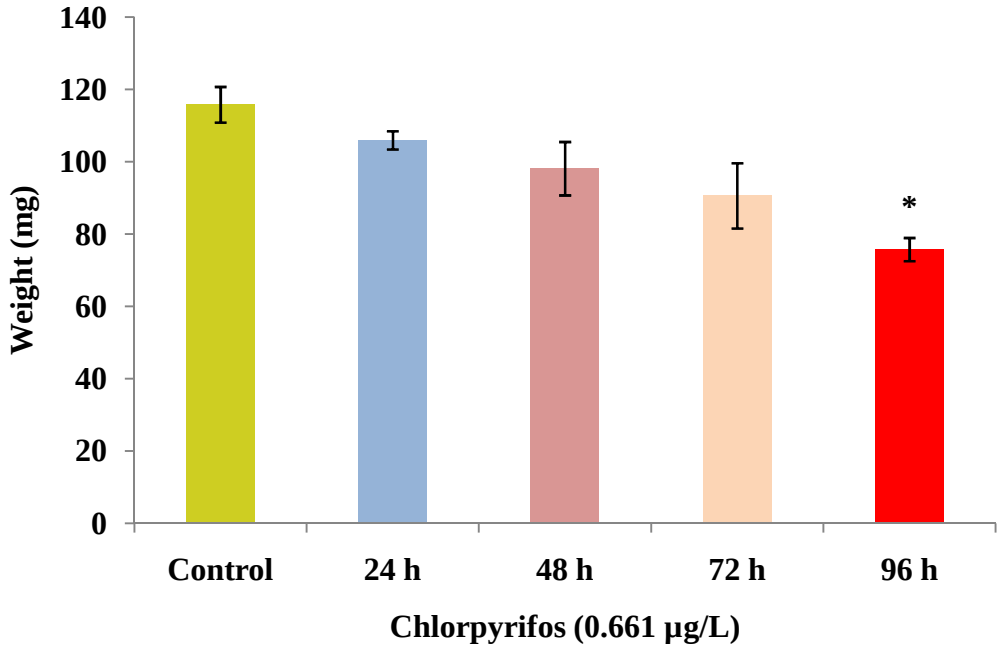

Figure 1: Effect of chlorpyrifos on the weight of gill in the fish, *Pseudetroplus maculatus*

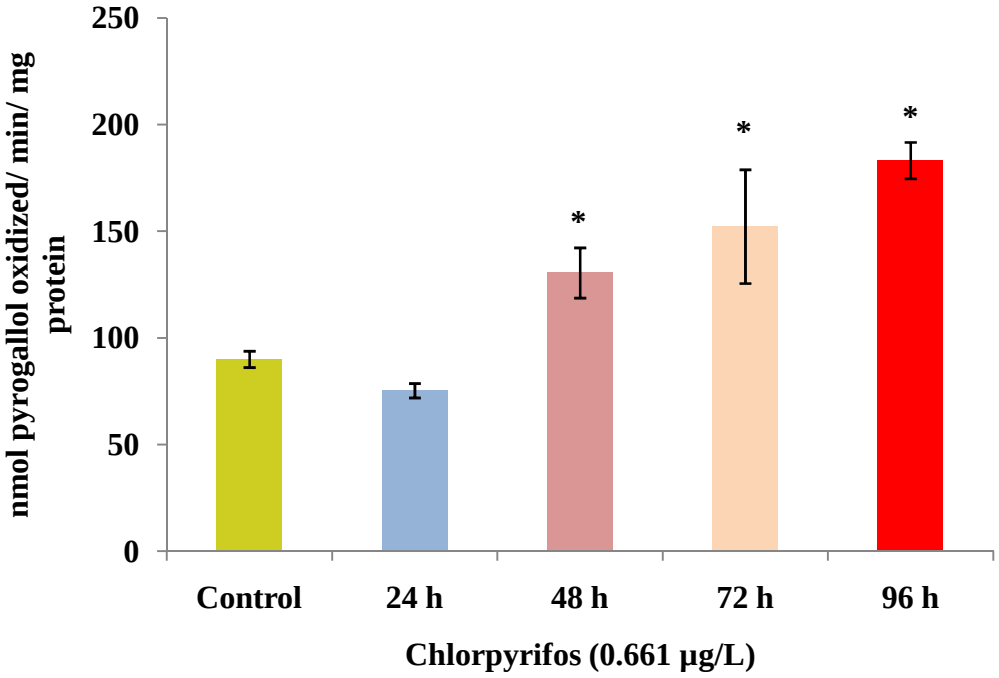

Figure 2: Effect of chlorpyrifos on the activity of superoxide dismutase in the gill of fish, *Pseudetroplus maculatus*

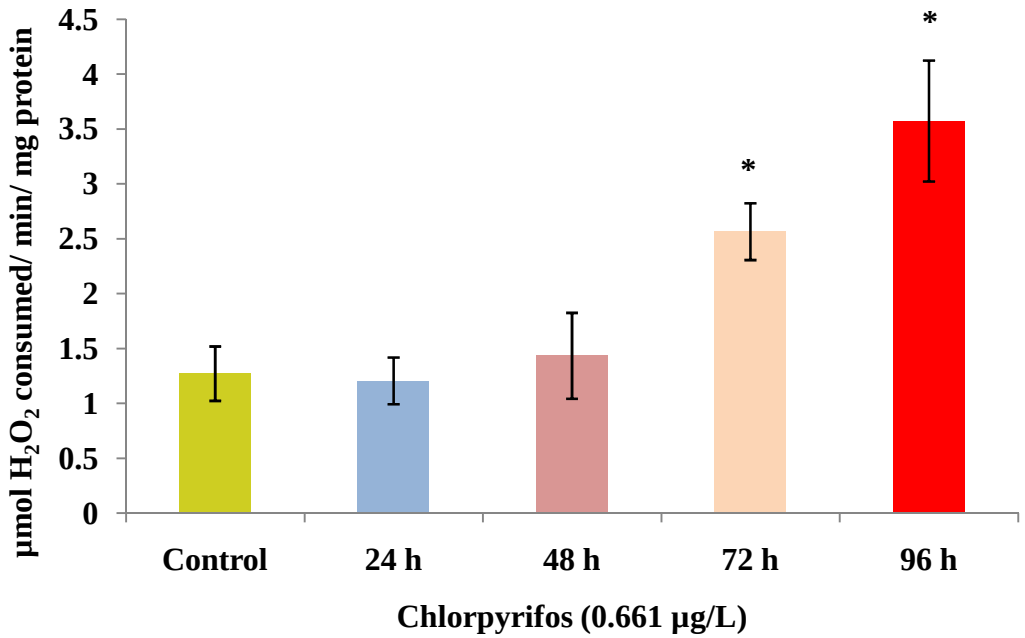

Figure 3: **Effect of chlorpyrifos on the activity of catalase in the gill of fish, *Pseudetroplus maculatus***

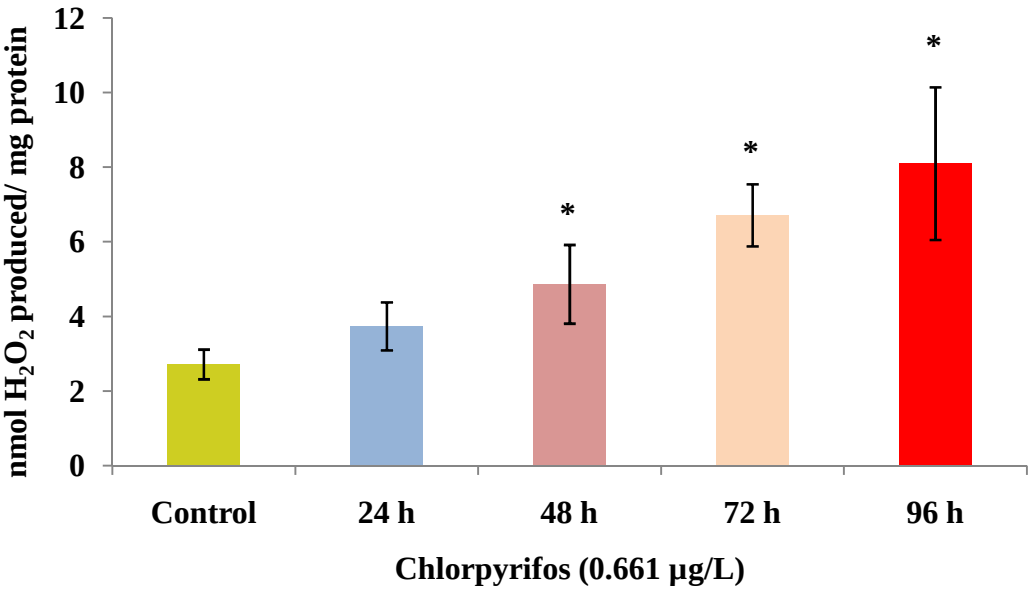

Figure 4: **Effect of chlorpyrifos on the level of hydrogen peroxide in the gill of fish, *Pseudetroplus maculatus***

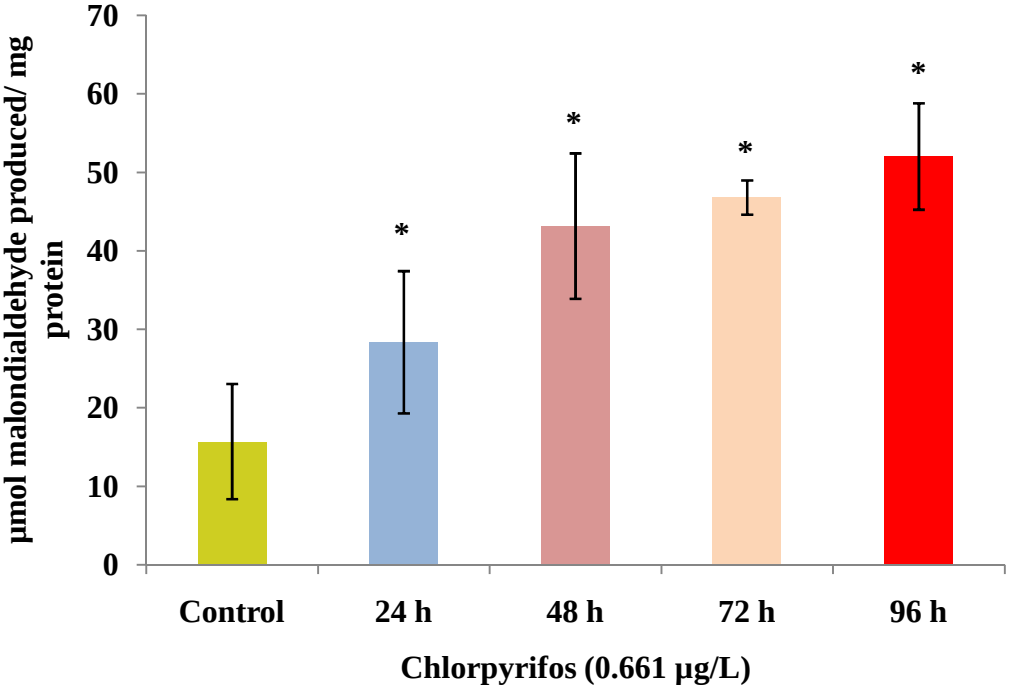

Figure 5: **Effect of chlorpyrifos on the level of lipid peroxidation in the gill of fish, *Pseudetroplus maculatus***

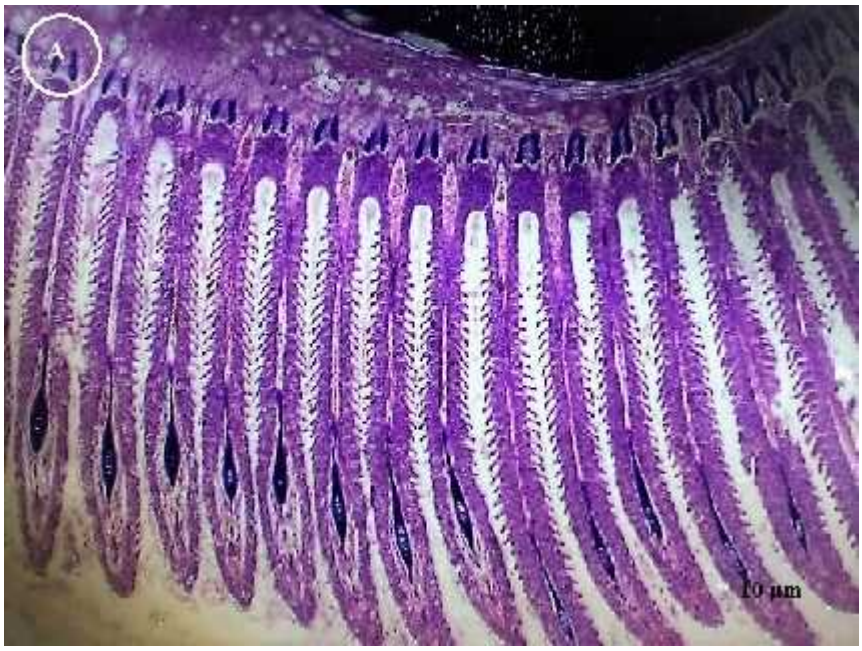

Figure A: **Photomicrograph showing normal architecture of gill tissue in *Pseudetroplus maculatus***

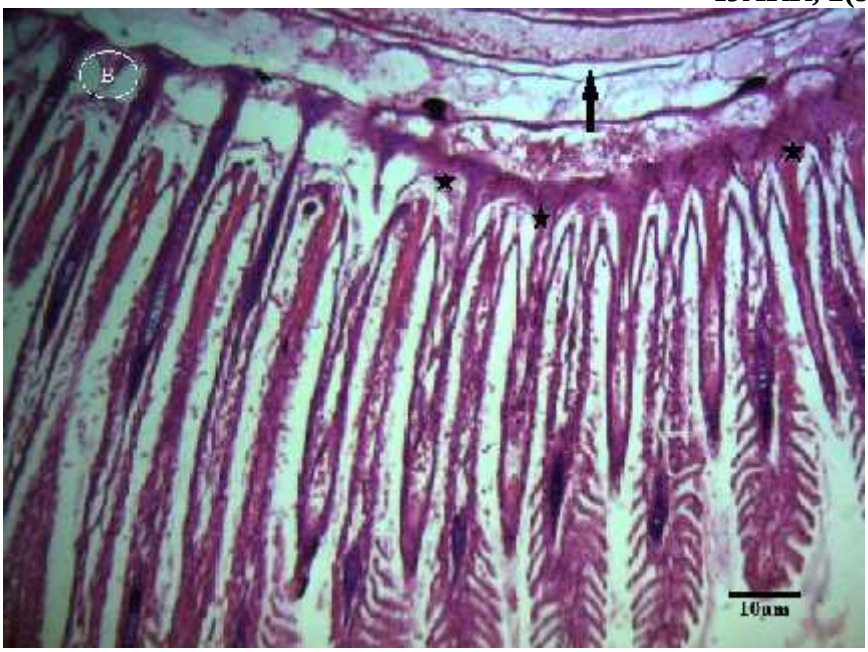

Figure B: **Photomicrograph showing degeneration of gill arches (*) and epithelial lifting (↑) in gill tissue after exposure to chlorpyrifos in *Pseudetroplus maculatus***

Figure C: **Photomicrograph showing degeneration of secondary lamellae (→) in gill tissue after exposure to chlorpyrifos in *Pseudetroplus maculatus***

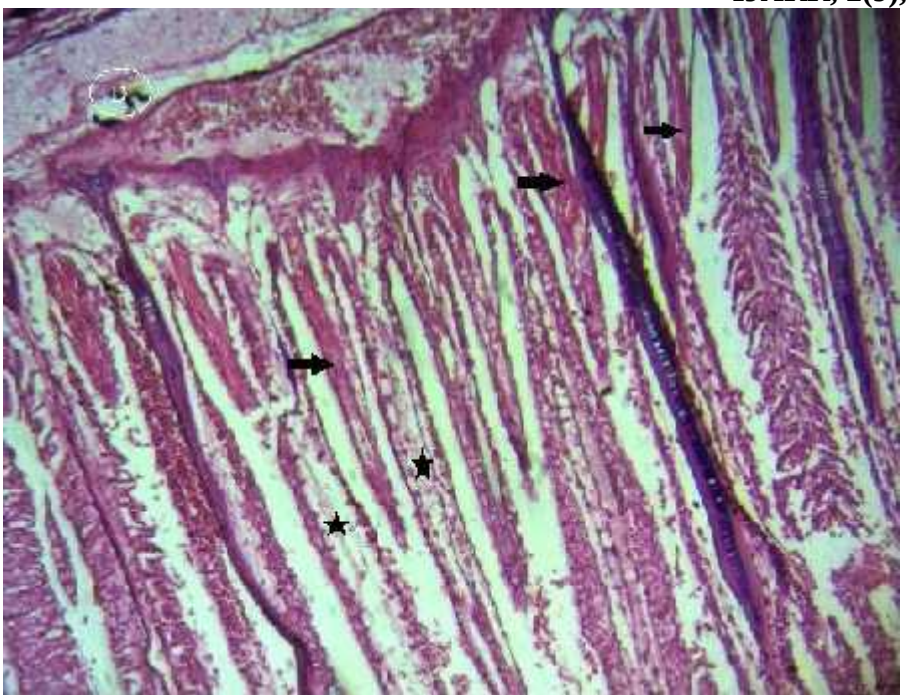

Figure D: Photomicrograph showing Hyperplasia (*) and aneurysm (→) in primary lamellae of gill tissue after exposure to chlorpyrifos in *Pseudetroplus maculatus*

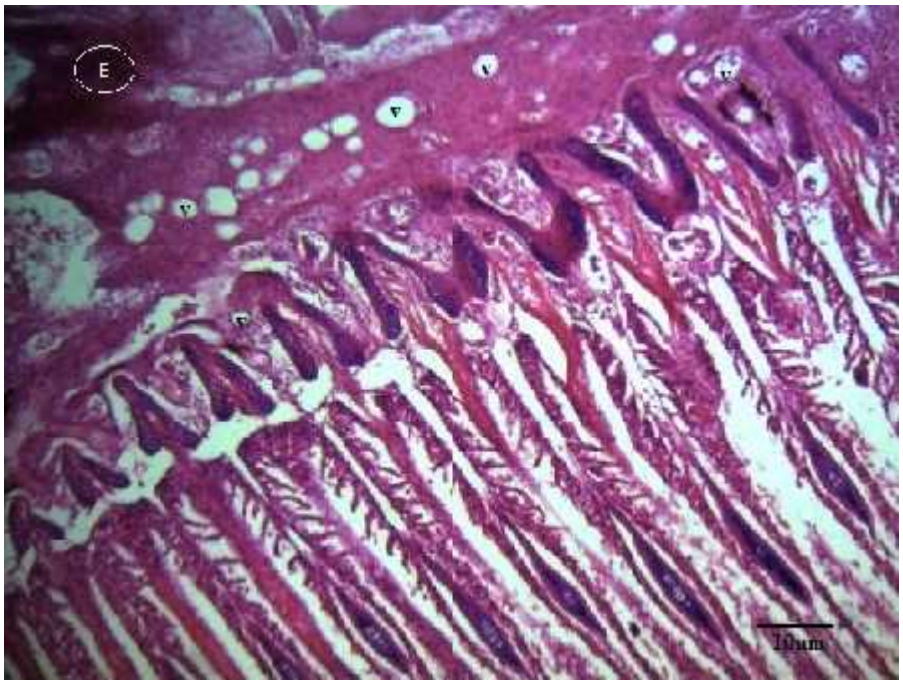

Figure E: Photomicrograph showing vacoulization of gill arches (v) after exposure to chlorpyrifos in *Pseudetroplus maculatus*

4.0 DISCUSSION

Chlorpyrifos is a broad-spectrum organophosphate pesticide widely used in agriculture and domestic purposes (Rao *et al*., 2003). Chlorpyrifos can reach natural waters through air drift or

surface runoff and affect non-target organisms, such as fish and crustacean that are of high economic value to humans (Varo *et al*., 2002). Chlorpyrifos has been shown to induce oxidative stress leading to the generation of oxygen free radicals thereby causing lipid peroxidation and inhibit antioxidative and physiological activities in fish (Kavitha and Rao, 2008). Therefore, in the present study the toxic effects of chlorpyrifos were evaluated by the detection of the activities of antioxidant enzymes and histomorphological changes in the gill tissue of the cichlid fish, *Pseudetroplus maculatus*.

Critical imbalance in the pro-oxidants and antioxidants result in oxidative stress, where excessive generation of reactive oxygen species or depletion of antioxidant enzymes occurs. Antioxidant enzymes are important components in preventing the oxidative stress (Li *et al*., 2006). In this study significant changes in the activities of antioxidant enzymes were observed in gill of fish, *Pseudetroplus maculatus* after chlorpyrifos exposure. Gills are the primary target of waterborne toxicants as it is constantly in contact with the water (Mallatt, 1985). In fish, gill performs multiple vital functions such as osmoregulation, respiration, and excretion.

In the present study, chlorpyrifos exposure showed a significant increase in the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). SOD is a group of metalloenzymes that have an important role in cellular defence against free radical induced damage by catalyzing dismutation of superoxide anion produced in peroxisomes and mitochondria to water and hydrogen peroxide (Kappus, 1985; Zhu *et al*., 2008). Such increase in the activity of SOD may be an adaptive response of the fish against the chlorpyrifos-induced severity of free radical generation (Sulfath *et al.*, 2013).

CAT has been implicated as an essential defensive enzyme against the potential toxicity of superoxide anions that acts specifically on hydrogen peroxide, forming oxygen and water (David *et al*., 2008). The increased activity of CAT after chlorpyrifos exposure could be an attempt of the gill tissue to scavenge the hydrogen peroxide generated as a result of pesticide treatment. Despite of the great effort of antioxidant enzymes to neutralize the harmful effects of free radicals, there was a significant increase in the level of hydrogen peroxide after chlorpyrifos exposure. It clearly indicates the failure of gill tissue to eliminate the hydrogen peroxide generated as a result of chlorpyrifos treatment. The failure of adaptive response of antioxidant enzymes to detoxify chlorpyrifos-induced ROS resulted in the induction of oxidative stress in gill tissue of fish. Similar observations have been found in the gill of the fish, *Pseudetroplus maculatus* exposed to chlordecone (Asifa and Chitra, 2017).

Oxidative damage is related to the formation of ROS, and lipid peroxidation (LPO) is considered as a valuable biomarker of oxidative damage in cellular components (Ferreira *et al*., 2005). Chlorpyifos exposure increased the level of LPO in gill tissues, as evidenced by increase in malondialdehyde production that reveals that toxicant-induced free radicals are not fully scavenged by the antioxidant enzymes. Fish tissues contain large amount of polyunsaturated fatty acids (PUFAs) essential for membrane function. LPO is the process of oxidative degradation of PUFA, and its occurrence has been shown to cause impaired membrane function, structural integrity, and inactivation of several membrane-bound enzymes (Goel *et al*., 2005). Similarly chlordecone exposure also caused increase in the level of lipid peroxidation in gill of fish *Pseudetroplus maculatus* (Asifa and Chitra, 2017).

In the present study, the toxic effects of chlorpyrifos by the alteration in antioxidant enzymes were further confirmed by histopathological analysis. Histology is the most effective and sensitive tool to determine cellular damages in vital organs (Dutta, 1996). The fish exposed to sublethal concentration of chlorpyrifos showed several histological alterations in gill tissue namely, degeneration of gill arches, epithelial lifting, and degeneration of secondary lamellae, hyperplasia, aneurysm in primary lamellae and vacuolization of gill arches. Some of the alterations like epithelial lifting and hyperplasia are examples of defense mechanisms, and these modifications increase the distance between the external environment and the blood and serve as a barrier to the entrance of toxicants (Mallatt, 1985). Epithelial lifting could result in dysfunctional or non-functional gill that result in the adaptive response of gill to prevent the entry of toxicant through the gill surface. The formation of an aneurysm is related to the rupture of the pillar cells due to a bigger flow of blood or even because of the direct effects of contaminants (Martinez *et al*., 2004). The present result is consistent with another study on chlorpyrifos exposure in the fish *Heteropneustes fossilis* (Tiwari *et al*., 2017).

5.0 CONCLUSION

The present observations demonstrate that the administration of sublethal concentration of chlorpyrifos induced oxidative stress and caused histopathological lesions in gill tissue of fish *Pseudetroplus maculatus*.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

None

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