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Quinalphos induced Histoarchitectural Alterations in Fresh Water Fish, *Oreochromis mossambicus* (Peters)

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Abstract: ***Acute toxicity of commercial grade organophosphate insecticide, Quinalphos (25% EC) on gill, liver and muscle of fresh water fish, Oreochromis mossambicus was studied. The estimated LC_{50} value of quinalphos with 95% confidence levels was found 0.56µl/L, at 96 hr. $1/10^{th}$ and $1/25^{th}$ of 96 hr LC_{50} values were taken as sub lethal concentrations. After 5 days of exposure period fishes were sacrificed and gill, liver and muscle were dissected out and histopathological changes were studied. Several histopathological alterations were observed in all the tissues which were dose dependent as the cellular changes were more expressed with increase in concentration. Histopathological studies can be used as suitable biomarker in the evaluation of the health of the organism exposed to pesticides.***

Keywords: Quinalphos, LC_{50}, Histopathology and *Oreochromis mossambicus*

1. Introduction

Pesticides are a diverse group of widely varying chemicals ranging from simple to complex substances used to control pests causing economic injury. The use of pesticides was increased epidemically with the development of industries and agricultural growth. Increased use of pesticides in the field affects the aquatic environment to a great extent. In aquatic environment, aquatic organisms especially fishes, takes these dissolved pesticides and it gets accumulated in their tissues. Approximately less than 0.1% of the pesticides, applied in the fields, reach the target organisms while the remaining 99.9%enters into the environment and it pollutes the environment [1].

Quinalphos is an organophosphorus pesticide extensively used in agriculture for pest eradication. It is a synthetic, non-systemic, broad spectrum insecticide and acaricide, extensively used because of its low bioaccumulation and high rate of biodegradation [2]. Quinalphos is commonly used in the crops including wheat, rice, coffee, sugar cane, peanuts, fiber crops, vines, grapes, and cotton for controlling caterpillars, aphids, mealy bugs, mites, bollworms, leaf hoppers. It is a hard insecticide which has become a matter of concern because of its potential hazardous effect.

Fishes are very sensitive to different chemicals or pesticides and their tissues are prone to pathological effects [3]. The accumulation of pesticides in the tissues of organism may cause chronic illness and severe damages to the fish population. Histopathology is shown to be a suitable technique in the evaluation of the health of the organisms exposed to pesticides and it can be used as a bio monitoring tool for the toxicology studies. One of the major advantages of histopathological study is that it helps to examine specific target organs [4], and in the present paper histopathological studies were carried out to see the changes in the gill, liver, and muscle of *Oreochromis mossambicus* exposed to quinalphos.

2. Materials and Methods

Test Organism

The cichlid fish, *Oreochromis mossambicus* were collected from local fish farm Kottakal, Malappuram District, Kerala. The fishes were acclimatized in the laboratory condition for 3 to 4 weeks in dechlorinated tap water in well aerated glass tank. The physico-chemical features of the tap water were estimated by following the APHA [5]. Healthy fishes weighing 3.2±0.4g and 4.6±0.5 cm length was selected for the study.

Experimental design: An acute toxicity test was conducted by static renewal bioassay method to determine toxicity of quinalphos on fish. The fish were exposed with different concentration of quinalphos for 96hrs. The mortality of the fishes was noted in every 24hrs. After the 96hrs of exposure period the mortality data was subjected to Probit analysis. [6]

The fishes were grouped in to three for the purpose of experiment. Group 1 serve as a control (without pesticide), Group 2 and 3 serves as test (with pesticide). Two sublethal concentrations were selected for the present experiment ($1/10^{th}$ and $1/25^{th}$ of 96hrs LC50) and the pesticide exposed for 5days.

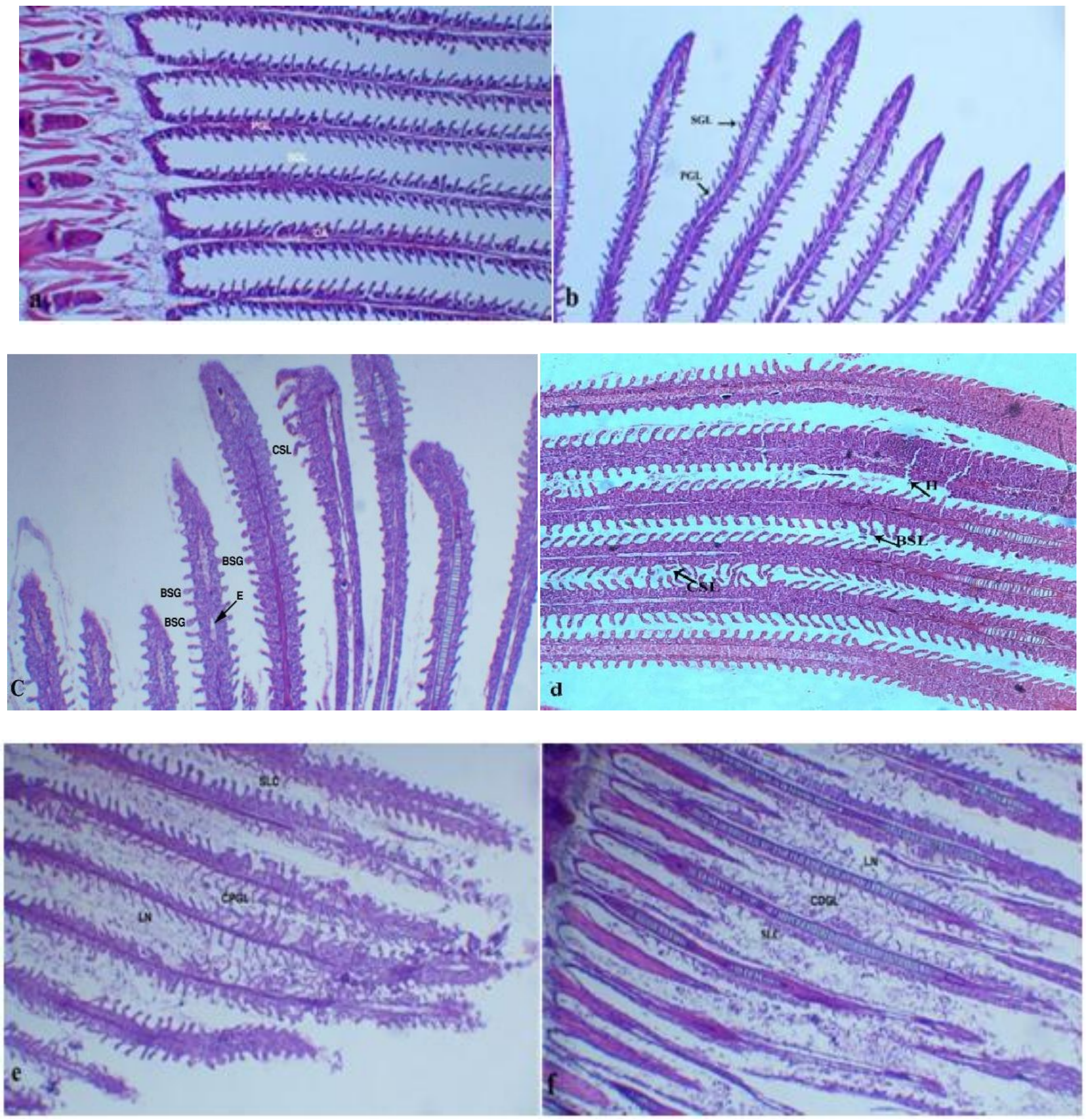
After the stipulated period of exposure fishes were sacrificed and tissues like gill, liver and muscle were dissected out. Tissues were immediately fixed in Bouin's fluid. After the period of fixation, tissues were passed through different grades of alcohol. They were cleared using xylene and embedded in paraffin wax. Sections were cut at 6µ thicknesses, stained with hematoxylin-eosin. Slides were mounted and photographs were taken using a computer aided microscope (Lawrence and mayo N-800M).

3. Results

The results of histological studies of gill, liver and muscle tissues of *Oreochromis mossambicus* of control and those exposed to sub lethal concentration of quinalphos are given in figure 1, 2 and 3. The structural alterations were observed under light microscope in the sections of tissues and compared with the control group. The tissues of fishes from quinalphos-treated groups appeared to have structural differences from those of control group fishes.

Gill

The gill tissue of control was shown in Figure 1a & 1b. Generally, the gills of *O. mossambicus* comprised two sets of four holobranchs, forming the sides of the pharynx. Each holo branch consisted of two hemibranchs projecting from the posterior edge of the branchial arch or gill arch in such a way that the free edges diverged and touched those of the adjacent holobranchs. Close examination of the hemibranchs of a fish gill shows that they consists row oflong thin filaments, the primary lamellae (PGL), which project from the arch like the teeth of a comb. The surface area of each primary lamellae is increased further by the formation of regular semi lunar folds across its dorsal and ventral surface-secondary lamellae. The dorsal and ventral rows of secondary lamellae (SGL) on each primary lamellae are staggered so that they complement the spaces in the rows of lamellae of adjacent filaments.



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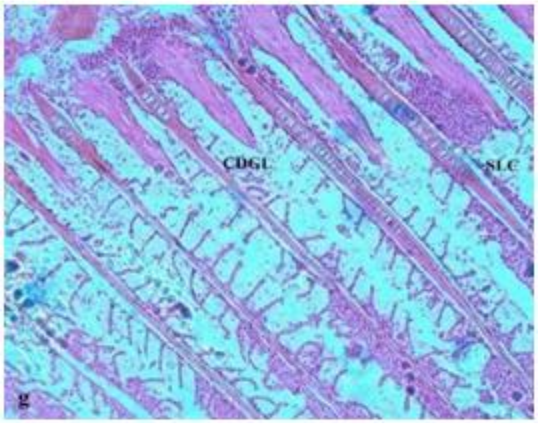

Figure 1: Histopathological observations of gill exposed to quinalphos on *Oreochromis mossambicus* (H & E stained). (a and b - Control (10X), c and d - Fish exposed to 1/25 th of LC50 value for 5 days (10X), e, f and g - Fish exposed to 1/10 th of LC50 value for 5 days (10X and 20X). PGL- Primary gill lamellae, SGL- Secondary gill lamellae, CA- Central axis, CSL- Curved secondary lamellae, BSG- Ballooning of secondary lamellae, E- Edema, SLC- Sloughed off cells, LN- Lamellar necrosis, FSL-Fused secondary lamellae, CDGL- Completely degenerated gill lamellae

In the present study, the gills of *O. mossambicus* in control group showed a normal structure and gill of the treated group showed several pathological changes throughout the experiment.

The changes observed in the gill of *O. mossambicus* exposed to lower concentration (1/25 th of LC50) were shown in figure 1c and 1d. Histological changes such as curved secondary lamellae (CSL), Ballooning of Secondary Lamellae (BSL), Edema (E) and Hemorrhage (H).

In the higher concentration (1/10th of LC50) treated groups primary and secondary gill lamellae lost their basic structure. Changes include edema, Lamellar Necrosis (LN), Sloughed off Cells (SLC), epithelial lifting, fused secondary lamellae (FSL) and Completely Degenerated Gill Lamellae (CDGL). It was shown in figure 1e, 1f and 1g.

Liver

The control fish liver showed normal histoarchitecture with continuous mass of polygonal shaped hepatocytes with granular cytoplasm and centrally placed definite nucleus. Hepatocytes (H) were large in size arranged in well-organized hepatic cords and separated by narrow blood sinusoids (Figure 2a).

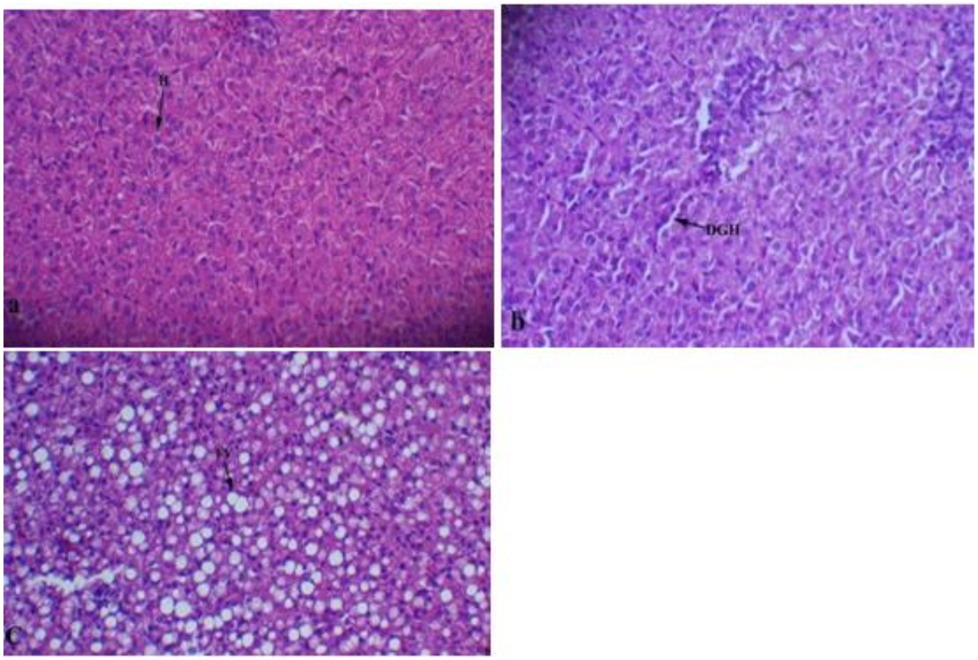

Figure 2: Histopathological observations of liver exposed to quinalphos on*Oreochromis mossambicus* (H & E stained). (a-control, b- Fish exposed to 1/25 th of LC50 value of quinalphos for 5 days (10X), c-Fish exposed to 1/10 th of LC50 value of quinalphos for 5 days (10X), H- Hepatocytes, DGH- degeneration of hepatic tissue, FV- Formation of vacuole

Liver tissue exposed to lower concentration (1/25 th of LC50) showed histopathological alterations compared to the control. Mild necrosis and degeneration of hepatic tissue (DGH) were observed and shown in figure 2b.In the liver tissue exposed to higher concentration(1/10th of LC50) of pesticide cloudy swelling of hepatocytes, vacuolar degeneration (FV) and necrosis were seen (figure 2c).

Muscle

The skeletal muscles of fish are very active helping in their navigation through water. Histological study of muscle tissue of the control fish showed muscle bundle, muscle fiber and nuclei.

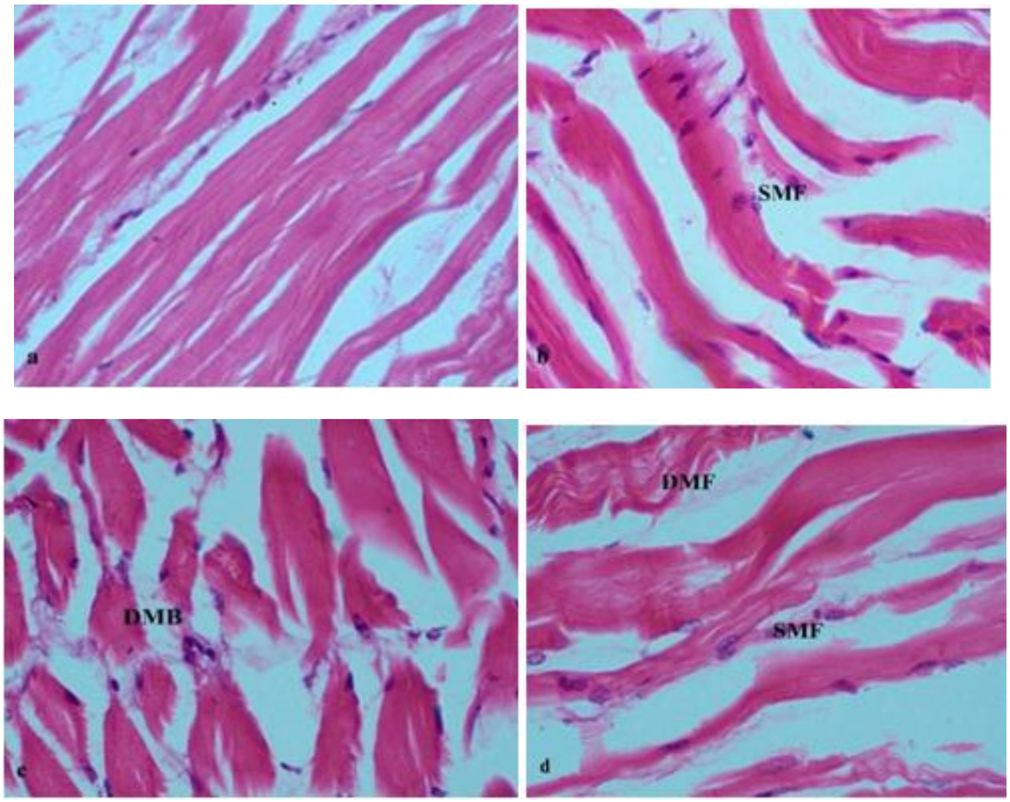

Figure 7: Muscle of control fish, *Oreochromis mossambicus* (H & E stained, 20x)

DMB- Degeneration in the muscle bundle, DMF- Degeneration in the Muscle Filament, AMB- Atrophy of Muscle Bundle, SMF – Splitting of muscle filament

Degeneration in muscle filament (DMF) was spotted in themuscle tissue of *Oreochromis mossambicus* treated with pesticide. In lower concentration treated tissue showed Degeneration in the Muscle Filament (DMF), Atrophy of Muscle Bundle (AMB), and Dystrophic Changes (DC)and it was shown in the figure 10 and 11.

The fish treated with higher concentration showed formation of vacuoles (V), Elongated Muscle Filament (EMF), severe Hemorrhage and degeneration in the muscle bundle (DMB), Elongated muscle bundles (EMF), Hemorrhage (H) also observed (Figure 12 and 13).

4. Discussion

Tissue histology is considered as an indicator of exposure to pollutants and represents a useful tool to assess the degree of pollution, particularly the sub lethal and chronic effects [7]. It can be used as a useful tool to visualize the stress induced structural alterations in the cells and tissues. Histopathological techniques are rapid, sensitive, reliable and comparatively less expensive tool for the assessment of toxicity by particular pollutant.

Gills are the most delicate structure of fish. It is an organ having many functions such as gaseous exchange, acid base balance, and transport of Na^+, Ca^+, Cl^- and nitrogenous secretion. Gills are primary route of entry for any pollutants or toxicants in to the fish's body and it is the first organ that comes in direct contact with the insecticide or any pollutant. Binding of hydrophobic organophosphate with various lipid and protein groups of gill epithelial cells might be the reason for alterations in the structure of gill filaments. Loss of structural integrity of the gills may easily lead to drop in the concentration of blood electrolytes, such as sodium, chloride and calcium [8]. Therefore, histopathological studies in the fish gill exposed to environmental pollutants has been used as an important biomarker [9].

Histological observation of the gill tissue after 5 days of exposure of quinalphos showed a concentration dependent increase in the damages of the tissue was observed. Tissue exposed to a sub lethal concentration of 1/25th of the LC_{50} value of pesticide showed many structural changes such as ballooning of secondary lamellae and curving of secondary lamellae. These two changes are predominant in lower concentration treated group and this might be due to inflammation brought about by pesticide toxicity [10].

Tissue exposed to 1/10th of the LC50 value of the pesticide exhibited severe degenerations. Primary and secondary lamellae lost their actual structure. Hyperplasia of epithelial lining of secondary lamellae was observed which lead to the fusion of secondary lamellae. Many of the secondary lamellae showed necrotic changes and lost their original structure.

Similar histological changes have been observed in the gills of fish exposed to Diazinon [9]. Butchiram *et al* [11] observed necrosis, vacuolar degeneration, lamellar fusion and atrophy of primary and secondary gill lamellae in *Channa punctatus* exposed to Alachlor. Reddy *et al* [3] studied the histopathological effects of pesticide confider on *Labeo rohita*. Sublethal exposure of confider induced damages in the primary and secondary gill lamellae, including shortened and clubbing of the ends of the secondary gill by contaminated sediments exposure. Histo- structures of gill of *Cirrhinas mrigala* were analyzed and comparison between the degree of damage of the alterations in fish organs was performed after exposure to metal contaminated sediments for 7, 14 and 28 days under semi-static water renewal bioassays. Histopathological changes include, hemorrhage, edema, epitheliocystis, degeneration of primary and secondary lamellae, disintegration of secondary gill lamellae, fusion of primary and secondary gill lamellae and necrosis. Bhuvaneshwari *et al* [12] studied the histopathological alterations in gill of Zebra fish due to environmentally relevant concentrations of organochlorine pesticides and observed hyperplasia andnecrosis. Degeneration of gill lamellae was observed by Singh *et al* [13] (2017) on *Channa punctatus*, treated with triazophos. Histopathological study of gill tissues of catfish exposed to chlorpyrifos showed swollen gill lamellae with mild congestion. Degenerative changes were also observed in lower concentration treated fishes. The lamellar fusion was observed in the present study to reduce the surface area of gill available for gaseous exchange [14] Liver plays an important role in detoxification and it is the site of biotransformation for the majority of toxicants [7] [13]. Hepatic damage is well demonstrated histologically as the normal histoarchitecture of the hepatocytes observed in liver of control fish is disrupted on quinalphos exposure. Changes in the liver tissue were concentration and exposure time dependent. In higher concentration treated group most of the hepatocytes get degenerated as indicated by heavy vacuolization.Butchiram*et al* [11] observed the similar changes in the liver of *Channa punctatus*. The changes include degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles and ruptured blood vessels, necrosis and disappearance of hepatocyte cell membrane disposition. Hepatic cords are found to be deceased in size and nucleus became pyknotic. Ram and Singh [15], (1988) observed the changes in the liver of *Channa punctatus*, changes include cytoplasmolysis, nuclear pyknosisand necrosis leading to complete exhaustion and disintegration of hepatocytes.Kadam and Patil [16], studied the histopathological alterations in the snake head fish, *Channa gachua* exposed to dichlorvos. The observations include vacuolation in the cytoplasm, degeneration in the hepatic sinusoids, ruptured blood vessels, necrosis and hepatocytes atrophy. The present study confirms that the liver is the major site of detoxification. The finding of the present study of muscle histology is in consonance with finding of Kaur*et al* [17]. Many pathological alterations were observed in *Clariasbatrachus*exposed to heavy metals which include degeneration of muscle fiber, severe intra muscular edema, necrosis, separation of muscle bundle and degeneration of muscle bundles. Similar results were observed by Dhevakrishnan and Zaman [18], on fresh water fish *Labeo rohita*.

In the present study it has been found that the sub lethal dose of quinalphos induced alterations in the structure of gill, liver and muscle tissues of the fish *Oreochromis mossambicus.* Histological alterations in the organs of experimental fish are increased with increasein dose of the pesticide. The increased severity of the lesion of the tissues was high in 1/10 th of LC50 value treated fish as compared to 1/25th LC50 value treated group.

5. Conclusion

Organophosphorus insecticide quinalphos induced histological alterations in the tissues like gill, liver and muscle of freshwater fish, *Oreochromis mossambicus* was studied and reported. The histopathological changes in the gill, liver and muscle tissues are dose and time dependent alterations. The findings of the present study indicate that sublethal concentrations of quinalphose was toxic to fish population and it will lead to the damage of internal organs of fish which may ultimately lead to the reduction in the population of fish species. The present study also shows that the histological studies serve as biomarkers for assessing pesticide toxicity in aquatic environments.

6. Acknowledgement

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