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Quinalphos-induced alteration on the antioxidant defense system in subcellular fraction of gill in fresh water fish, *Oreochromis mossambicus* (Peters, 1852)

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Abstract

Quinalphos (O, O-diethyl-O-quinoxalin-2-yl phosphorothioate), an organophosphorous insecticide at sublethal concentration (0.5 µg/ L) was exposed to freshwater fish, *Oreochromis mossambicus* for 24, 72 and 96 h. Body weight of the animal and the weight of gill remained unchanged throughout the exposure period. Activity of antioxidant enzymes superoxide dismutase, catalase and glutathione reductase decreased significantly ($P<0.05$) in mitochondrial, microsomal and nuclear fractions of gill in concomitant manner at 24, 72 and 96 h after quinalphos exposure. The level of lipid peroxidation increased significantly ($p<0.05$) only at 96 h in mitochondrial fractions and no significant changes were observed at all treatment groups in microsomal fractions. Whereas nuclear fractions showed a significant increase in the level of lipid peroxidation at the end of 24, 72 and 96 h of quinalphos treatment when compared to the corresponding control group. Inhibition in the activity of alkaline phosphatase was highly significant ($P<0.05$) in mitochondrial, microsomal and nuclear fractions and this may be due to the decreased state of inter and intracellular membrane transport and possibly owing to the toxicity of quinalphos. The present study suggests that quinalphos altered the pro-oxidant/ antioxidant balance in subcellular fractions of fish gill and therefore prove acute toxicity of quinalphos at sublethal concentration.

Keywords: Quinalphos, *Oreochromis mossambicus*, gill, oxidative stress, lipid peroxidation, alkaline phosphatase.

Introduction

Quinalphos is an organothiophosphate chemical used chiefly as a pesticide in agriculture. It is a synthetic, non-systemic, broad spectrum insecticide and acaricide, which also act as a cholinesterase inhibitor and are widely used to protect corn, cotton and fruit trees against insects. Owing to the intensive use of such pesticides in agriculture could find a path to the aquatic ecosystem through rainfall run-off and atmospheric deposition. The insecticides cause contamination on water bodies also soundly affects on the growth, survival and reproduction of aquatic animals. Historically it was proved that fish have a significant role in assessing toxic effect of any environmental contaminants in aquatic environments. There are several aquatic toxicity tests that provide both qualitative and quantitative information on adverse effects of environmental contaminants in aquatic organisms. One of the most commonly used standardized toxicity tests in regulatory ecotoxicology is acute toxicity tests lasting for 24 to 96 h to test the effects of chemicals or industrial effluents in the aquatic bodies (OECD, 1992). Fish are considered as a food of excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals. Apart from this, fish are also used as a bio-indicator that gives clues for the fitness of the whole ecosystem. Therefore, if the exposure to quinalphos disrupts the health status of aquatic organisms as fish, then it would certainly reflects the toxic effect of the compound at individual, population or at ecosystem level.

Several researches specify that the effects of environmental contaminants in aquatic organisms may be associated with increased production of reactive oxygen species (ROS), leading to oxidative stress. In the aquatic environment, despite the presence of constitutive or enhanced antioxidant defense systems, increased levels of oxidative damage will occur in organisms exposed to contaminants that stimulate the production of ROS (Livingstone, 2001). One of the pollution-mediated mechanisms of toxicity is the increased production of ROS and consequent oxidative stress that occurs in vital organs as gill, liver, brain or reproductive tissues. The use of biochemical measurements as the evaluation of antioxidant enzyme activities has the advantage to understand the pro-oxidant and antioxidant balance in the cells or tissues. In the present study quinalphos, an environmental contaminant was exposed to freshwater fish, *Oreochromis mossambicus* at sublethal concentration for 24, 72 and 96 h and the alteration in the antioxidant defense system was studied in subcellular fractions of gill tissue. Hence the study contributes the basic information on the ecological risk assessment of quinalphos and its toxic effect in subcellular fractions of fish gill antioxidant defense system.

Materials and Methods

Standardization procedures

Fresh water fish, *Oreochromis mossambicus* weighing 3.5 ± 0.5 g and length 4.5 ± 1 cm were collected from a fish farm, Kaloos Aquarium, Kottakal, Malappuram District, Kerala. Prior to the experiments fishes were acclimatized to the laboratory conditions in dechlorinated and well-aerated cement tanks of 40 L capacity for four weeks. The physico-chemical features of the tap water were estimated as per APHA (1998) maintaining water temperature ranged from 28 ± 2°C, oxygen saturation of water between 70 and 100 % and pH maintained at 6.5 to 7.0. The LC_{50} values of quinalphos for 96 h were determined by probit analysis (Finney, 1971), which was 5 µg/ L (Surya et al., 2015). One-tenth of quinalphos concentration (0.5 µg/ L) was chosen as sublethal concentration and it was used in the present study.

Grouping of animal

Quinalphos - *O*, *O*-Diethyl *O*-2-quinoxalinyl phosphorothioate of 97% purity obtained from SISCO Research Laboratories Ltd., Mumbai, India was used in the experiment. Animals were grouped into four maintaining ten fishes per group and are treated as follows:

Group I: Control group (without toxicant)
Group II: Quinalphos at 0.5 µg/ L for 24 h
Group III: Quinalphos at 0.5 µg/ L for 72 h
Group IV: Quinalphos at 0.5 µg/ L for 96 h

Preparation of subcellular fractions

Fish was caught very gently using a small dip net, one at a time with least disturbance. At the end of each exposure period, fishes were decapitated and gill tissue was dissected. Different sub-cellular fractions were obtained by the differential centrifugation method as described by Palade and Siekevitz (1956). A 1% (w/v) homogenate of gill tissue was prepared in ice-cold 0.25 M sucrose solution with the help of a motor-driven glass Teflon homogenizer on crushed ice for a minute. The homogenate was centrifuged at 1000g for 10 minutes at 4°C to obtain the nuclear pellet. Mitochondrial pellet was obtained by centrifuging the post-nuclear supernatant at 10,000g for 10 minutes at 4°C. As microsomal membranes can sediment prematurely during traditional pre-clearances (6,000 – 10,000g), it is evidently not necessary to use ultracentrifugation (100,000g) to collect microsomes. In fact, it is possible to sediment quantitatively all major microsomal-type membranes at 21,000g in a normal bench-top microcentrifuge. The obtained nuclear, mitochondrial and microsomal fractions were used for the following biochemical estimations.

Biochemical parameters

Protein was estimated by the method of Lowry et al (1951) with bovine serum albumin as the standard. Activity of antioxidant enzymes as superoxide dismutase (Marklund and Marklund, 1974), catalase (Claiborne, 1985), glutathione reductase (Carlberg and Mannervik, 1985) was estimated. Level of lipid peroxidation (Ohkawa et al., 1979) and the activity of alkaline phosphtase (Bessey et al., 1946) were also evaluated.

Statistical analyses

All biochemical estimations were carried out in duplicate and are presented as mean ± SD for ten animals per group where the differences were set significant at $p<0.05$ against the control group which were denoted with asterisk (*) symbol in the figures. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 19.0.

Results and Discussion

The pollution of aquatic ecosystem with chemical contaminants has become one of the most critical environmental issues of recent years. An increasing variety of industrial, agricultural and other environmental contaminants that are entering into the aquatic ecosystems are being taken up into the tissues of invertebrates as well as vertebrates. Aquatic environment is subjected to temporal and spatial variations in quality due to the indiscriminate dumping and release of wastes containing several environmental contaminants, including quinalphos into the freshwater might lead to environmental disturbance that could be considered as a potential source of stress to the biotic community.

Fishes constitute good test-organisms and bio-indicator to monitor the water quality, especially small freshwater fishes, since they can be maintained in laboratory and can be easily exposed to toxic substances in a similar way of the other higher vertebrates, and in this way, could be used to assess the presence of substances that have the potential to cause toxic effects in humans. *Oreochromis mossambicus* is a very important fresh water fish species in south India. Fish gills are the main target of several aquatic pollutants, being its epithelium, an excellent model to examine the effects of dissolved substances in the tissues; besides this, gills are the most seriously affected organs due to their constant contact with the water (Mishra et al., 1985). In the present study the acute toxic effect of quinalphos in antioxidant enzymes at subcellular fractions of gill tissue was evaluated.

Exposure to quinalphos at the sub lethal concentration (0.5 µg/ L) for 24, 72 and 96 h did not altered the body weight and the weight of gill when compared to corresponding control group (Figs. 1 & 2) thereby proves that quinalphos did not cause systemic toxicity to the animal.

Figure1. Effect of quinalphos on the body weights of freshwater fish, *Oreochromis mossambicus*

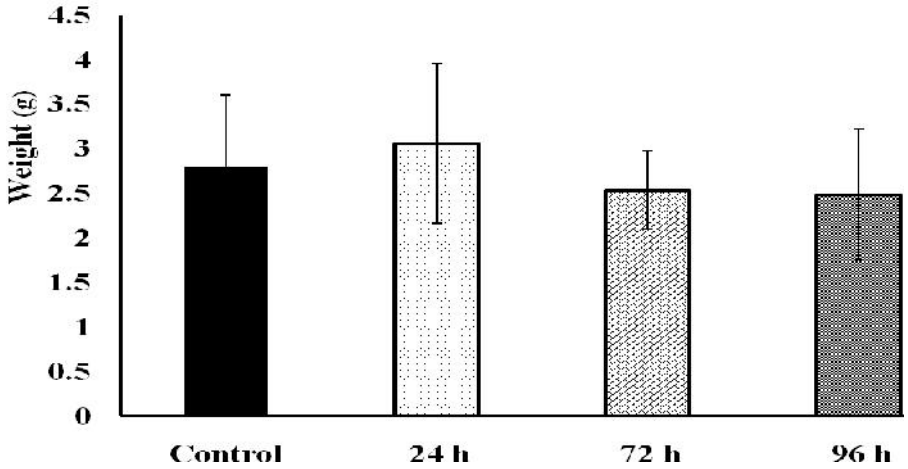
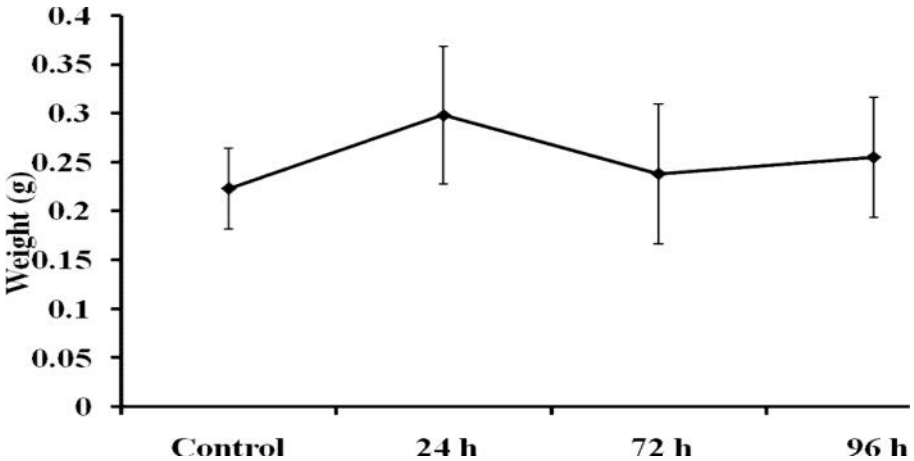


Figure 2 Effect of quinalphos on the weight of gill in the freshwater fish, *Oreochromis mossambicus*



Gills participate in many important functions as it is the site of gas exchange, ionic regulation, acid-base balance, and nitrogenous waste excretion by fishes. All these processes are controlled either by passive or active transport of various solutes across the epithelium. As gills remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are thus considered as the primary target of the environmental pollutants (Poleksic and Mitrovic-Tutundzic, 1994).

In the present study quinalphos exposure significantly ($p<0.05$) decreased the activities of superoxide dismutase, catalase and glutathione reductase in a time-dependent manner in the subcellular fractions as mitochondrial, nuclear and microsomal fractions of gills of fishes as compared with the corresponding group of control animals (Figs. 3-5).

Figure 3 Effect of quinalphos on the activity of superoxide dismutase in subcellular fractions of gill in the freshwater fish, *Oreochromis mossambicus*

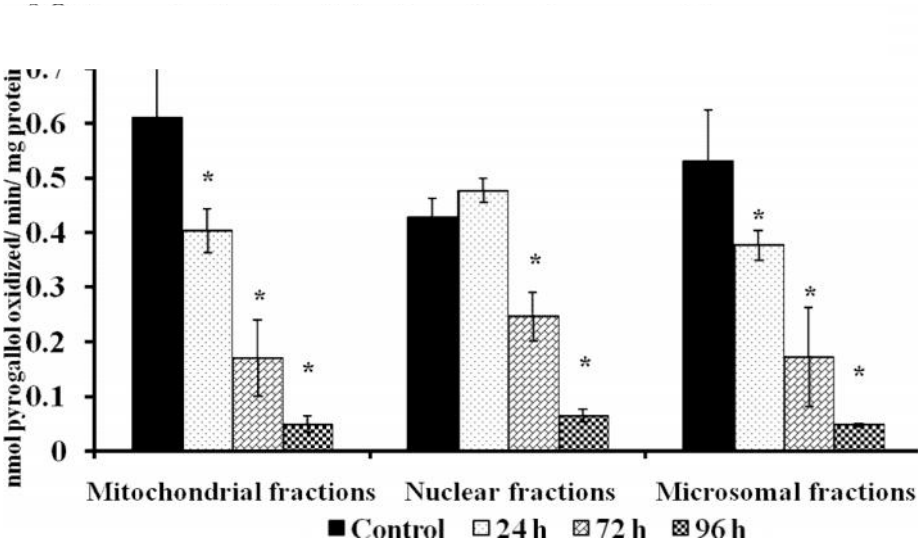


Figure 4 Effect of quinalphos on the activity of catalase in the subcellular fractions of gill in the freshwater fish, *Oreochromis mossambicus*

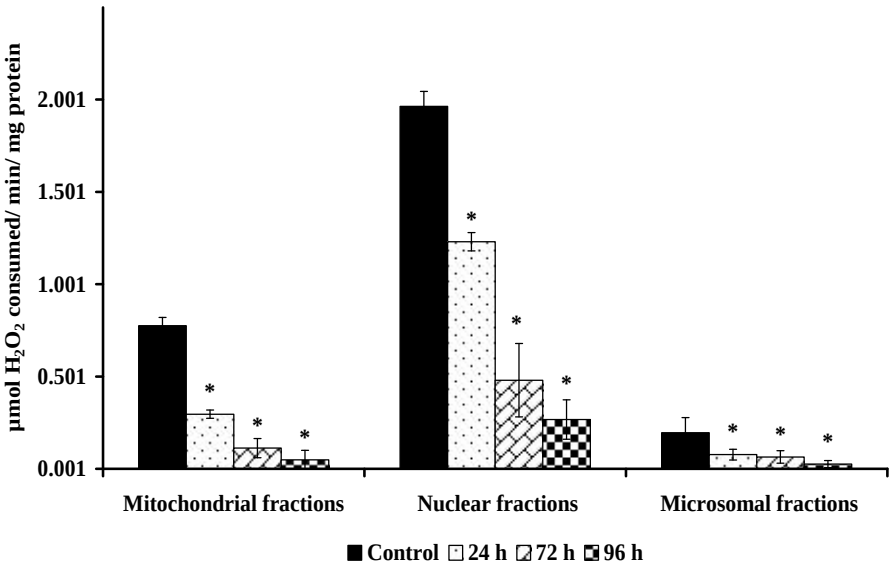
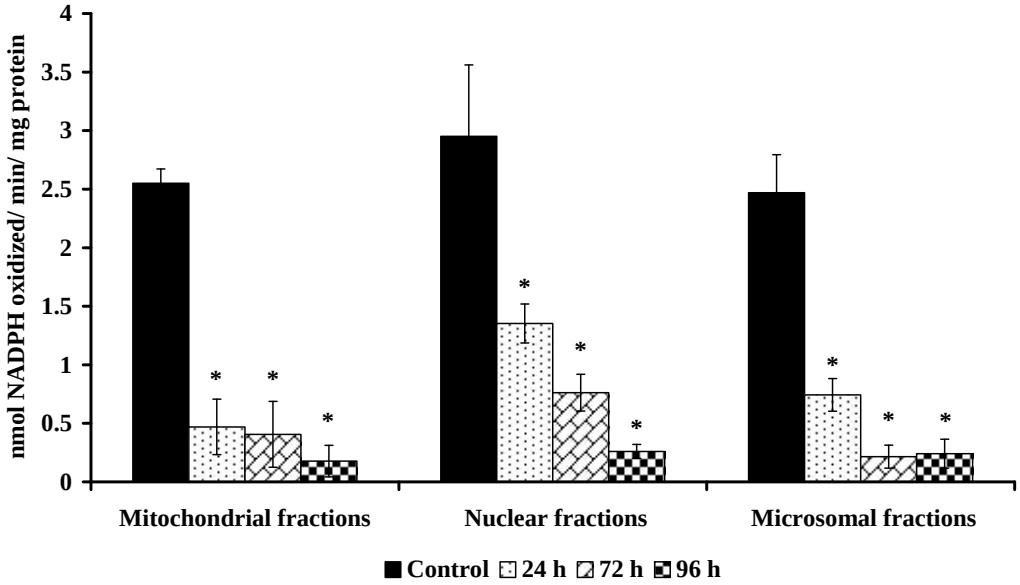


Figure 5 Effect of quinalphos on the activity of glutathione reductase in the subcellular fractions of gill in the freshwater fish, *Oreochromis mossambicus*



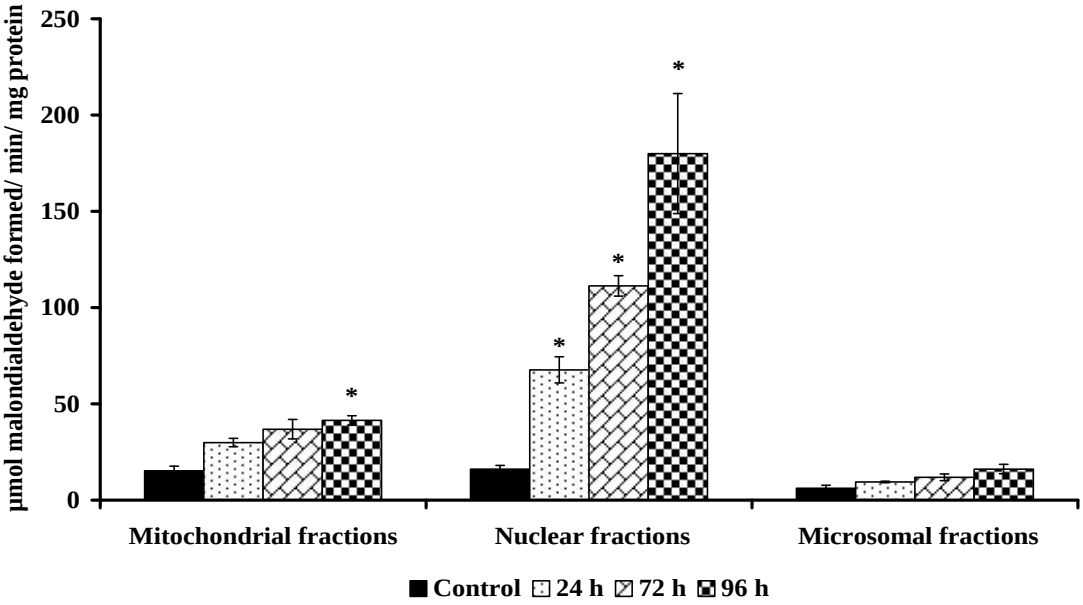
Aerobic organisms, which derive their energy from the reduction of oxygen, are susceptible to the damaging actions of the small amounts of superoxide anion ($\bullet O_2$), hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2) that inevitably formed during the metabolism of oxygen, especially in the reduction of oxygen by the electron transfer system of mitochondria. These three species, together with unstable intermediates in the peroxidation of lipids, are referred to as Reactive Oxygen Species (ROS). Damage from ROS as a result of an imbalance between radical-generating and radical-scavenging systems results in a condition called oxidative stress (Sikka, 2001).

Free radicals/ ROS generated in tissues and sub-cellular compartments are effectively scavenged by the antioxidant defense system, which constitutes antioxidant enzymes such as, superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase. Superoxide dismutase (SOD) catalysis the dismutation of superoxide to hydrogen peroxide (H_2O_2) and oxygen (O_2). The conversion of H_2O_2 to $2H_2O$ is by the enzyme glutathione peroxidase and the conversion of H_2O_2 to O_2 and H_2O is by the enzyme catalase. Since the reaction catalyzed by glutathione peroxidase requires glutathione (GSH) as substrate and depends in part on the ratio of glutathione disulfide (GSSG): GSH, the concentrations of these reactants and their ratio, which is a reflection of the redox state of the cell, are important to ROS detoxification. Glutathione peroxidase/ reductase directly act as antioxidant enzymes to inhibit lipid peroxidation (Sikka, 2001).

In the present study a decrease in the activity of superoxide dismutase has been shown to increase the level of superoxide anion, which is known to inactivate catalase activity (Kono and Fridovich, 1982). Similarly, catalase or glutathione peroxidase has been shown to eliminate hydrogen peroxide from the cell leading to the inactivation of superoxide dismutase and generation of lipid peroxides (Bray et al., 1974).

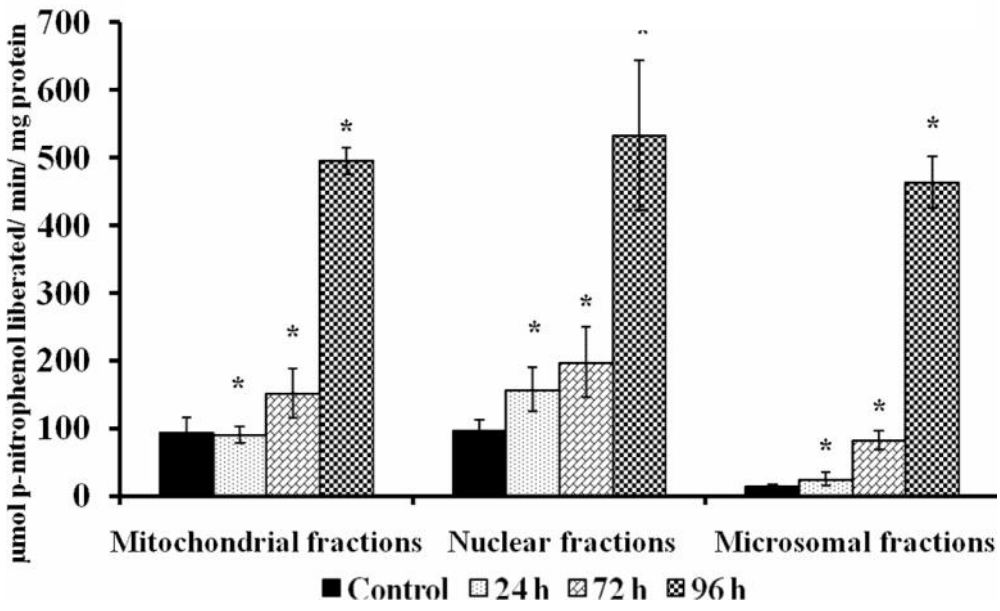
In the present study quinalphos increased ($p<0.05$) the level of lipid peroxidation in all subcellular fractions of gills in the test animal when compared with the corresponding control group (Fig. 6). Increased lipid peroxidation may indicate an increased oxygen free radical generation (Thiele et al., 1995). Accumulation of hydrogen peroxide at low concentration is not reactive, but at a higher concentration promotes the formation of highly toxic hydroxyl radicals in the presence of Fe^{2+}, which attack almost all biomolecules including membrane lipids causing formation of lipid peroxides (Halliwell and Gutteridge, 1985).

Figure 6 Effect of quinalphos on the level of lipid peroxidation in subcellular fractions of gill in the freshwater fish, *Oreochromis mossambicus*



There was a significant ($p<0.05$) increase in the activity of alkaline phosphatase in the mitochondrial, nuclear and microsomal fractions at the end of 24, 72 and 96 h of quinalphos-treated fishes as compared with the control group (Fig. 7).

Figure 7 Effect of quinalphos on the activity of alkaline phosphatase in subcellular fractions of gills in the freshwater fish, *Oreochromis mossambicus*



Alkaline phosphatase is a protein stress marker enzyme that catalyzes the hydrolysis of phosphorus compounds and the transfer of phosphoryl groups to an acceptor molecule. The rate of catalytic activity of the enzyme is inversely proportional to the concentration of the inorganic phosphate in the ambient environment (Dyhrman and Palenik, 1999). Inhibition or induction of stress marker enzyme could be considered as an important marker to indicate the state of fish health and their physiological conditions. Alkaline phosphatase is a brush border hydrolytic lysosomal enzyme, which splits various phosphorous

esters at an alkaline pH and is released by the lysosomes for the hydrolysis of foreign material. It is well known that phosphatases are involved in carbohydrate metabolism, growth and differentiation, protein synthesis, synthesis of certain enzymes, secretory activity, and transport to phosphorylated intermediates across the cell membranes (Vijayavel et al., 2006). In the present study the fish exposed to sub lethal concentration of quinalphos increased the activity of alkaline phosphatase in mitochondrial, nuclear and microsomal fractions of gill tissue in time-dependent manner. This increase in the enzyme activity could be possibly to overcome the depletion of energy caused due to pollutant stress in gills. Thus the result showed that the fish is unable to bear the stress caused due to quinalphos at organ level during sub lethal intoxication.

Conclusion

To abridge, the acute exposure of quianlphos at sub lethal concentration altered antioxidant defense system at sub cellular level in gill of freshwater fish *Oreochromis mossambicus* as revealed by the decreased activity of antioxidant enzymes and increased level of lipid peroxidation. Therefore, the adverse toxic effect of quinalphos is mediated through reactive oxygen species generation in gill tissue as it is the prime target organ of toxicity exposure.

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