IMPACT OF MALATHION ON THE BIOCHEMICAL COMPOSITION OF THE FRESHWATER FISH, OREOCHROMIS MOSSAMBICUS

K. C. CHITRA & SUNNIYA ABDU
Department of Zoology, University of Calicut, Thenhipalam, Kerala, India

ABSTRACT

The aim of the present study was to evaluate the effect of malathion on the antioxidant status in the liver of adult freshwater fish, Oreochromis mossambicus. Malathion at sublethal concentration (0.125 mg/ L) was used as the test dose and the treatment was given for 24, 48 and 96 h to ten animals in each group maintaining a control group. Malathion generates reactive oxygen species in the liver of the fishes which is evident by the time-dependent reduction in the antioxidant enzymes and concomitant increase in the lipid peroxidation. However, there were no significant changes in the antioxidant parameters in brain of malathion-treated fishes. Thus the present study reveals that the antioxidant enzyme potential is very poor in liver when compared with that of brain. On the other hand, the present study also showed a significant decrease in the marker enzyme, alkaline phosphatase in the liver after 96 h of malathion treatment. Alkaline phosphatase, a hydrolyase enzyme are involved in the mediation of membrane transport and transphosphorylation.

A decreased alkaline phosphatase activity in liver of malathion-treated animal indicate the decreased state of inter and intracellular membrane transport and possibly this could be due to the toxicity of malathion. In the malathion-treated fishes, the hepatocytes have lost their normal architecture and large number of these cells appeared with pyknotic nuclei. The intrahepatic blood vessels were dilated and congested with blood, and inflammatory leucocytic infiltrations were also observed. Numerous hepatocytes showed marked cytoplasmic vacuolization. Thus malathion induces toxic stress to the exposed fishes, which is obvious by the histopathological changes in the liver. Thus the present study reveals that malathion induced oxidative stress in liver of Oreochromis by inducing reactive oxygen species generation.

KEYWORDS: Malathion, Liver, Antioxidant Enzymes, ROS, Alkaline Phosphatase, Oreochromis

INTRODUCTION

Pesticides are widely used to kill a very narrow range of undesirable organisms but also they are capable of harming non-target organisms inhabiting treated ecosystems. Pesticides reach and remain in aquatic ecosystems through run-off and trophic transfers. On reaching aquatic ecosystems, such chemicals can cause serious harm to man dependent on water bodies. Human population growth and industrial development have been the major causes of coastal contamination around the world during recent years (Murty, 1986). The ecological effects of pollutants in aquatic ecosystems and their bioavailability and toxicity are closely related to species distribution, both in the solid and the liquid phase of the aquatic ecosystem. Pollutants are transferred to the plankton, aquatic plants, mollusks and fish.

Fishes being one of the most ancient forms of aquatic life as a food item have been reported to have a nutritional advantage of being able to provide high proportions of their dry weight as proteins of relatively good quality due to the presence of essential amino acids and also being easily digestible unlike those of other livestock. Pollution of aquatic environment from industrial, domestic and agricultural waste has exposed these important aquatic organisms to contaminants which not only endanger their lives but also eventually enter the food chain leading to serious public health
hazards. Fishes feed extensively on different varieties of food and so it is important to control the concentration of likely pollutants as pesticides in the aquatic habitat, so as to reduce their minimal ingestion by fishes thereby rendering them unfit for human consumption (Agnihotri and Chattopadhyay, 1992).

In health, agriculture and government, the word “organophosphate” refers to a group of insecticides or nerve agents acting on the enzyme acetyl cholinesterase (AChE); Organophosphorus pesticides irreversibly inactivate this enzyme which is essential to nerve function in insects, humans and many other animals. Since acetylcholinesterase is the enzyme that degrades acetylcholine following stimulation of a nerve, its inhibition allows acetylcholine to accumulate and result in initial excessive stimulation followed by depression.

Organophosphorus pesticides degrade rapidly by hydrolysis on exposure to sunlight, air and soil photocatalysis proved to be an effective and inexpensive tool for the removal of organic and inorganic pollutants from water. However, small amounts of pesticides can be detected in food and drinking water. Metabolism is one of the most important factors that govern bioconcentration, bioaccumulation and detoxification of pesticides. Organophosphates undergo chemical changes in the environment as well as in the human body.

Malathion is a pesticide that is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as fruit fly eradication. Malathion is rapidly and effectively absorbed by practically all routes including the gastrointestinal tract, skin, mucous membranes, and lungs. Malathion binds to the enzyme acetylcholinesterase (AChE) at nerve endings and under normal circumstances, AChE binds to the neurotransmitter acetylcholine (ACh) at the nerve junction, effectively ending the stimulation of the next neuron. When AChE is bound by malathion’s metabolite malaoxon, ACh accumulates at the nerve junction and results in overstimulation of the nervous system (Tomlin, 2006). Bioactivation of malathion is necessary for it to exert its toxic effect.

Bioactivation is primarily mediated by cytochrome P450 enzymes in the liver, which create the active metabolite malaoxon through oxidative sulfuration (Savage et al., 1988). If they are released in environment in adequate before degradation, they kill non-target and other aquatic species, some toxic substances bio accumulate in tissues of fish and other species thus posing health risks to human beings on the consumption of the exposed fish. The nutritive value of fish also gets reduced and fish population gradually diminishes because of the pesticides.

Most studies on the effects of malathion are confined to various toxicological effects on different animals. There is vast amount of scientific information available on the toxicity effect of malathion on Oreochromis in India, but limited information is available on the effect of this pesticide on the antioxidant system of Oreochromis mossambicus. Based on the above views the present work have been undertaken to study the toxic effects of insecticide malathion on the antioxidant status of the fresh water fish, Oreochromis mossambicus.

MATERIAL AND METHODS
Collection and Maintenance of Animal

Fresh water fish, Oreochromis mossambicus weighing 10 ± 2 g and length 9 ± 1 cm were collected from a fish farm, KKF Nursery, Manjeri, Vaniyambalam. Fishes were acclimatized to the laboratory conditions for four weeks with constant supply of water and good lighting system. They were maintained in well-aerated tubs (40 L capacity), which was dechlorinated and sustained with fresh water flow and waste water discharge. During the period of acclimatization and experiment, fish were fed everyday with standard fish pellets. Bath was changed every 24 h, which was dechlorinated, respectively.
Impact of Malathion on the Biochemical Composition of the Freshwater Fish, *Oreochromis mossambicus*

**Preliminary Tests**

The physico-chemical features of the tap water were estimated as per APHA (1998). Water temperature in the test ranged from 28 ± 2°C during the experiment, oxygen saturation of water ranged between 70 and 100 %, pH is 6.5 to 7.5 which were monitored using a standardized procedures. The LC$_{50}$ values in the respective time intervals were determined by probit analysis, with a confident limit of 5 % level (Finney, 1971). Preliminary tests were conducted to provide guidance on range of concentration of pesticide to use in the bioassay. The specimens were not fed a day prior to and during toxicity tests to reduce faecal and excess food contaminating the test solution. Five specimens were placed in each tub of replicates so that ten fishes were maintained in each test and aerated using tubed motorized pumps. Monofilament netting was used to cover the tanks to prevent the specimens from jumping out of test solutions. The behaviour of specimens was observed and death was also recorded throughout the study.

**Evaluation of Median Lethal Concentration (LC$_{50}$)**

The concentration of the pollutant at which 50 percentage of the test animals dies during a specific period or the concentration lethal to one half of the test population is referred to as median lethal concentration (LC$_{50}$) or median tolerance limit. For determining LC$_{50}$ concentration separate circular plastic tubs of 40 L of water capacity were taken and different concentrations of malathion were added. Then, 10 fish were introduced into each tub. A control tub with 40 L of water and 10 fish were also maintained (no toxicant). The lethal concentration for 50 % killing (LC$_{50}$) values was computed on the basis of probit analysis (Finney, 1971) for 96 h, which was 0.5 mg/ L. One-fourth of the dosage (0.125 mg/ L) malathion was chosen in the present study.

**Chemicals**

Technical grade organophosphate insecticide, malathion (diethyl (dimethoxy thiophosphorylthio) succinate, 50% EC) was obtained from Jayakrishna Pesticides, Salem, Tamil Nadu. Malondialdehyde, NADPH and glutathione oxidized were obtained from SISCO Research Laboratories, Mumbai, India. Thiobarbituric acid and pyrogallol were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and obtained from local commercial sources.

**Treatments**

There were four groups, three tanks with toxicant doses and a control tank. Single dose with three durations were used in present study. Ten fish specimens were used for every test and also in control. The first group of fish was maintained in pesticide free water and used as control and the second group was treated with malathion at 0.125 mg/ L for 24 h; the third group was treated with malathion at 0.125 mg/ L for 48 h and the fourth group was also treated with malathion at 0.125 mg/ L for 96 h. Biochemical estimation of liver and brain was performed at the end of 24, 48 and 96 hours of malathion treatment, at the same time the control group was also maintained.

**Killing of Animals**

The fish was caught very gently using a small dip net, one at a time with least disturbance. At the end of each exposure time, fishes were decapitated. Liver and brain were dissected and stored at 4ºC until the analyses were performed.

**Tissue Processing and Biochemical Analysis**

A 1% (w/ v) homogenate of liver and brain 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 8000 g for 15 min at 4ºC to obtain the supernatant, which was then used for the biochemical analyses.
Protein was estimated by the method of Lowry et al. (1951) with BSA as the standard. Activity of superoxide dismutase was estimated by the method of Marklund and Marklund, 1974. The activity of catalase was measured by the method of Claiborne, 1985. Glutathione reductase was assayed by the method of Carlberg and Mannervik (1985). Glutathione peroxidase was assayed by the method of Mohandas et al. (1984). Levels of hydrogen peroxide generation were assayed by the method of Pick and Keisari (1981), levels of lipid peroxidation were measured by the method of Ohkawa et al., 1979. Alkaline phosphatase was measured as described by Bessey et al., 1946.

**Histology of Tissues**

Liver tissue was collected by sacrificing the fish. The tissue was fixed in 10% formalin for 24 hours. Tissue was dehydrated in ascending grades of alcohol and was cleared in xylene until they became translucent. Tissue was transferred to molten paraffin wax for 1 hour to remove xylene completely and then 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. The structural alterations were observed under light microscope in the sections of liver of fish and was compared with those of control tissues. Photomicrographs were taken using Cannon shot camera fitted to the Carl Zeiss Axioscope 2 Plus Trinocular Research Microscope.

**Statistical Analyses**

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan’s Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at p<0.05 against control group. Data are presented as mean ± SD for ten animals per group. All biochemical estimations were carried out in duplicate.

**RESULTS**

Administration of malathion at the sublethal dose of 0.125 mg/ L showed a significant decrease in the body weight after 48 and 96 h of exposure (Figure 1). Administration of malathion significantly (p<0.05) decreased the activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase with concomitant increase in the levels of hydrogen peroxide and lipid peroxidation in the liver of fishes after 96 h of exposure. However, the activities of antioxidant enzymes and the levels of hydrogen peroxide and lipid peroxidation remained unchanged after 24 and 48 h in the liver as compared with the control groups (Figures 2 - 7). Exposure to malathion for 24, 48 and 96 h did not alter the activities of antioxidant enzymes and the levels of hydrogen peroxide and lipid peroxidation in the brain of the test animals when compared with the corresponding control groups (Figures 2 - 7). There was a significant (p<0.05) decrease in the activity of alkaline phosphatase in the liver of malathion-treated fishes after 96 h of exposure as compared with the control groups (Figure 8).

Malathion at sub lethal dose showed a disruption in the cellular integrity of hepatocytes which is revealed by the histological changes that was observed after 96 h. In the malathion-treated fishes, the hepatocytes have lost their normal architecture and large number of these cells appeared with pyknotic nuclei and some cells undergone necrosis. The intrahepatic blood vessels were dilated and congested with blood, and inflammatory leucocytic infiltrations were also observed. Numerous hepatocytes showed marked cytoplasmic vacuolization with detachment of cells and fat deposition (Figure 9a-d).

**DISCUSSIONS**

Earlier studies of malathion have demonstrated the toxic effects of the pesticide on aquatic organisms, such as
Impact of Malathion on the Biochemical Composition of the Freshwater Fish, *Oreochromis mossambicus*

Fish. In the present study the development of oxidative stress conditions, in different fish tissue following malathion exposure was investigated as a possible mechanism for pesticide toxicity for the selected dosage. Aquatic organisms have been recognized for their sensibility to oxidative pollutants, such as malathion. Previously, the different concentrations of malathion found in several fish organs and tissues suggested an organ-specific bioaccumulation of this pesticide (Sapazhonikova et al., 2004).

Taking into account its organ specific toxicity and the general lack of knowledge on the antioxidant defence system, the aim of the study was to investigate the consequences of malathion exposure in the liver and brain on these parameters. Furthermore, the content of malondialdehyde (MDA), a lipid peroxidation product resulting from ROS formation, was also examined. In addition, alkaline phosphatase, a biomarker enzyme of liver was also investigated.

The body weights of fishes were monitored throughout the experiments in order to evaluate the effect of toxic compounds on the general health status of the animals. Malathion decreased the body weight of the test species after 48 and 96 h of administration and this may be due to several internal and external environmental factors such as rejection of feed, treatment induced anorexia or systemic toxicity.

Liver is the first organ to face any foreign molecule through portal circulation is easily subjected to more damage. The parenchymatous hepatic tissue has many important physiological functions and helps in detoxification of endogenous waste products as well as extremely derived toxins, drugs, heavy metals and pesticides (Roberts, 2001).

The liver tissues of malathion-treated fishes showed structural alteration unlike those from control group. Generally, the liver of fish is a compound organ in the form of hepatopancreas. In the present study, sinusoids, which are irregularly distributed between the polygonal hepatocytes, were fewer in number and are lined by endothelial cells with very prominent nuclei.

Detachment of cells and some necrotic cells gives the indication of its non-functional condition. The cells became more rounded-off showing acute necrosis. Completely vacuolated areas were also 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.

On the other hand, the present study also showed a significant decrease in the marker enzyme, alkaline phosphatase in the liver after 96 h of malathion treatment. Alkaline phosphates serve as diagnostic tool to assess the toxicity stress of chemicals in the living organisms (Harper, 1991). Alkaline phosphatase is a hydrolytic lysosomal enzyme and is released by the lysosomes for the hydrolysis of foreign material. Subsequently the enzyme activity may begin to drop either as a result of having partly or fully encountered the toxin or as a result of cell damage.

Alkaline phosphatase is involved in the mediation of membrane transport and transphosphorylation. A decreased alkaline phosphatase activity in liver of malathion-treated fish at 96 h of exposure indicate the decreased state of inter and intracellular membrane transport and possibly this could be due to the toxicity of malathion.

Administration of malathion at the dose of 0.125 mg/ L for 96 h significantly (p<0.05) decreased the activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase, whereas the levels of hydrogen peroxide and lipid peroxidation increased significantly in the liver of fishes when compared to the corresponding group of control fishes. Similar observations were observed in the freshwater fish, *Labeo rohita* when exposed to malathion chronically for 15 days duration (Patil and David, 2013). However, the activity of antioxidant enzymes remained unchanged after 24 and 48 h of malathion exposure in the liver of the animals as well as in brain of the fishes after 96 h.
Oxidation is a reaction which can produce free radicals. An antioxidant is a molecule that is capable of inhibiting oxidation by removing these free radical intermediates, and inhibits other oxidation reactions. Pesticides may induce oxidative stress leading to generation of free radicals and alteration in antioxidant or oxygen free radical scavenging enzyme system (Banerjee et al., 1999). The alteration of the cytochrome P450 system has been shown to increase oxygen free radical production in the liver (Traverso et al., 1999). The ability of cytochrome P450 system to induce the generation of ROS in hepatic and other tissues also has been reported (Bondy and Naderi, 1994). Cytochrome mono-oxygenase 3A4 (CYP3A4) gene product in human liver has been shown to involve in the metabolism of most drugs, a variety of endogenous steroids, and environmental procarcinogens.

Thus in the present study a reduction in the activity of antioxidant enzymes and an increase in hydrogen peroxide and lipid peroxidation levels reflects enhanced oxidative damage to the cell membranes (Akhgari et al., 2003). Lipid peroxidation is a degenerative process, which affects the polyunsaturated fatty acids of membrane phospholipids. The general mechanism of this process involves the formation of toxic aldehydes, which react with protein and non-protein substances resulting in widespread changes in cellular membranes. It also suggest that fish exposed to malathion generates covalent adducts between proteins and the carbonyl groups of the malondialdehyde.

It is well known that antioxidants are essential for maintaining the redox status of fish cells and tissues (Cowey and Cho, 1993). If the antioxidants are insufficient, oxidative stress may occur, leading to an altered physiological condition of the animal, and ultimately to death if essential tissues are affected (George et al., 2000). The difference in the activities of enzymes in different tissues is due to difference in the free radical production.

CONCLUSIONS

The results of this study suggest that malathion exposure for 96 h induces oxidative stress in the liver and not in the brain of Oreochromis. Taking into account that the enzymatic activities of antioxidant enzymes differed widely among the organs of this freshwater fish, it was concluded that the response of antioxidant scavenging enzymes to malathion was organ specific. Therefore, it is suggested that the activities of these antioxidant enzymes function as indicators of environmental exposure of freshwater fish to malathion.

REFERENCES

APPENDICES

Figure 1: Effect of Malathion on the Body Weight of *Oreochromis mossambicus*

Figure 2: Effect of Malathion on the Activity of Superoxide Dismutase in Liver and Brain of *Oreochromis mossambicus*

Figure 3: Effect of Malathion on the Activity of Catalase in Liver and Brain of *Oreochromis mossambicus*
Impact of Malathion on the Biochemical Composition of the Freshwater Fish, *Oreochromis mossambicus*

Figure 4: Effect of Malathion on the Activity of Glutathione Reductase in Liver and Brain of *Oreochromis mossambicus*

Figure 5: Effect of Malathion on the Activity of Glutathione Peroxidase in Liver and Brain of *Oreochromis mossambicus*

Figure 6: Effect of Malathion on the Level of Hydrogen Peroxide Generation in Liver and Brain of *Oreochromis mossambicus*
Figure 7: Effect of Malathion on the Level of Lipid Peroxidation in Liver and Brain of Oreochromis mossambicus

Figure 8: Effect of Malathion on the Activity of Alkaline Phosphatase in Liver of Oreochromis mossambicus

Figure 9a: Histology of Liver of the Control Fish (X 400 Magnification)
Figure 9b: Histology of Liver of the Malathion-Treated Fish after 24 hours of Exposure (X 400 Magnification); N – Necrosis

Figure 9c: Histology of Liver of the Malathion-Treated Fish after 48 hours of Exposure (X 400 Magnification); V – Vacuolated Area

Figure 9d: Histology of Liver of the Malathion-Treated Fish after 96 hours of Exposure (X 400 Magnification); F – Fat Deposition; D – Detachment of the Cells

Figures 9a-d: Histopathalogy on the Liver of Fish, Oreochromis mossambicus at 24, 48 and 96 hrs of Treatment and Control Tissues