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Full Length Research Paper

Malathion toxicity in Nile tilapia, *Oreochromis niloticus* - A haemotological and biochemical study

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Deliberate or accidental contamination of ponds by widely utilized organophophorous (OP) insecticides, such as malathion is a potential problem for aquaculture in tropical countries. The aim of the study was to investigate the toxic effects of malathion (0, 0.5, 1.0, 1.50 and 2 mg L^{-1}) toxicity in the Nile tilapia *(Oreochromis niloticus;* 15.0 cm length and 50.0 g weight) by correlation of acute toxicity (LC_{50}) studies with biochemical and hematological parameters. Tilapia were very sensitive to malathion (96 h LC_{50} 2ppm). The LC_{50} for malathion (48 and 96 h) was 2.0 and 1.5 ppm, respectively. The fish shows quick response to malathion. In comparison with controls, sublethal levels of these pesticides led to a significant decrease (*P*<0.05) in final body weight. The erythrocyte count, haematocrit value and haemoglobin content of Nile tilapia were Glycogen, and protein in fish muscle gradually decreased with increased pesticide concentrations. On the other hand, total production, net returns and profitability of reared fish decreased with increase in concentrations of pesticides.

Key words: Tilapia, malathion, toxicity, mortality, haemotological, biochemical.

INTRODUCTION

Pollution of the aquatic environment is a serious and growing problem (Sasaki et al. 1997). Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment led to various deleterious effects on the aquatic organisms (McGlashan and Hughies, 2001). Aquatic organisms, such as fish, accumulate pollutants directly from contaminated water and indirectly via the food chain (Sasaki et al., 1997). Heavy metals toxicity has been extensively studied in fish (Chan et al., 1999; Heing and Tate, 1997). Application of chemical fertilizers containing trace of heavy metals causes contamination of fish with these metals (Chaisemartin, 1983). The effects of either organophosphorous or chlorinated pesticides have been extensively studied and confirmed in fish (Rao, 2006a; Rao, 2006b; Pandcy et al., 2006; Capkin et al., 2006).

Pesticides may enter water bodies as a result of spray drift and leaching from the soil in concentrations, which may exert adverse effects on fish populations. In tropical environments, insecticides are also added to aquaculture ponds to control mosquitoes and other pests (Kumar et al., 1993). In the assessment of the hazards of pesticides to fish, studies have been largely restricted to the direct effects of individual compounds; however, under field conditions, the metabolism and toxicity of pesticides could be modulated by simultaneous exposure to combinations of pesticides with other pollutants.

Malathion, a slightly toxic compound in EPA toxicity class III, is a General Use Pesticide (GUP) and is one of the earliest organophosphate insecticides developed (introduced in 1950). Most of the organophosphorus pesticides are characterized by their direct inhibitory action or are rapidly converted to inhibitors of acetylcholinesterase. It is a widely used organophosphorus insecticide because of its relatively low toxicity to mammals and high selectivity for insects compared with other organophosphorus insecticides. There are many earlier findings that clearly warned of the genotoxic potential of technical-grade malathion (Flessel et al., 1993) in a wide range of organisms including fish species (Kushwaha et al., 2000; Barat et al., 1998). The large-scale use of technical-grade malathion in various eradication programs has raised concern over its potential to cause genetic damage (Thompson et al.,

1989) and there is need for further investigation to determine its ‘‘safe level’’.

The effects of malathion is also of growing concern and have been extensively studied in various fish species, including its accumulation, behavior effects, morphology effects, reproductive effects, and biochemical effects. Malathion may occur in natural water sources through run-off from agricultural fields or directly through careless application, which may exert adverse effect on untargeted organisms, such as fish and other aquatic animals, since fish are often at the top of the aquatic food chain and capable to concentrate the pesticides (Tsuda et al., 1997; Sapozhnikova et al., 2004). The bioconcentration factor (BCF, the ratio of the chemical concentrations in the organism and in water during steady state or equilibrium) in killifish (*Oryzias latipes*) was found to be 11 (Tsuda et al., 1997), and a high frequency of malathion have been found in fish samples, such as mackerel and sardine with some samples at concentrations higher than 1.13 mg/L (Abou-Arab et al., 1996). In laboratory studies, malathion has been observed to cause different types of deformities in early life stages of fish, including deformed notochord, yolk sac edema, bent body (Lien et al., 1997). Fish exposed to malathion showed abnormal behavior and dose- and time-dependent increase in mortality were also observed (Pandey et al., 2005). In the protogynous *Monopterus albus*, acute or chronic exposure of females to malathion reduced *in vitro* production of sex steroids (both testosterone and estradiol) and aromatase activity, and affected the number of animals with intersex gonads (Singh, 1993). Malathion was also documented to affect the natural and acquired immunity of Japanese medaka, including a dose-dependent reduction of the production of antibodies to sheep red blood cells and a mild decrease in the superoxide production by kidney phagocytes (Beaman et al., 1999).

The contamination of aquatic ecosystems by heavy metals and pesticides has gained increasing attention in recent decades. The acute and chronic toxicity of pesticides to fish has been widely summarized (Eaton, 1970; Johnson, 1965; Jacob et al., 1982). Chronic exposure and accumulation of these chemicals by aquatic biota can result in tissue burdens that produce adverse effects not only in the exposed organisms, but also in organisms including human beings (IARC, 1990, 1993). The present study was conducted to determine the acute toxicity of organophosphorus pesticide malathion to the freshwater fish, *Oreochromis niloticus*. This species was selected for bioassays because it can easily be raised under laboratory conditions.

MATERIALS AND METHODS

Experimental diets

Healthy specimens of *O. niloticus* were obtained from local fish hatchery and their initial morphometric characteristics were recorded. *O. niloticus* was selected because of its ability to withstand stress and its high commercial value in the Kingdom. The fingerlings were stocked in 50-L glass aquaria containing deep tube-well water stored in an overhead tank. The fish was acclimatized to this condition for 1 week before using in any trial during which the time they were provided with artificial feed (35 % crude protein) was obtained locally. The fish of both sexes were stocked without discrimination. The fish was inspected for disease conditions and general fitness. Water was changed daily. Altogether 18 aquaria were arranged according to randomized block design with three replicates. Each aquarium was stocked with ten fishes. Five different treatment groups with three replicates were used. The fish fed three times daily. Feeding was ended while aeration continued during the 96 h test periods.

Malathion toxicity studies

Six sets of ten fishes each were subjected to serial dilutions of the stock solution of malathion (from 0.5 - 2 mg/l) in triplicates. Two sets of control (each consisting 10 fishes), which contains no toxicants were set up. The test was performed by following semistic (renewal) bioassay method in which the exposure medium was exchanged after every 24 h to maintain toxicant strength and level of dissolved oxygen, as well as minimizing the ammonia excretion levels during this experiment. Initially, the fish was observed at 1 h intervals for the first 6 h after which they were observed at 3 h intervals. All toxicity studies were conducted using technical grade malathion, which was dissolved in acetone (250 mg /ml) and stored in dark bottles at 0–5°C. This stock was appropriatel y diluted and mixed with aquarium water just before use in the experiments. Acute lethal bioassays of malathion toxicity to tilapia fingerlings were carried out using static tests in accordance with the standard procedure outlined by the United Nations Food and Agriculture Organisation (Reish and Oshida, 1986). Size-graded groups of 15 fish were exposed under continuous aeration to water containing 0, 0.5, 1, 1.5, and 2 mg L^{-1} malathion or an equal concentration of the vehicle, acetone. PBO-exposed fish were continuously exposed to 2 mg L^{-1} PBO in addition to malathion. Fish were not fed during the experiment. For LC_{50} calculation, mortality of fish exposed to each concentration was recorded every 12 h for 96 h, dead fish being removed every 3 - 8 h.

Hematological and biochemical analyses

Blood samples were collected from both the control and experimental fishes that survived the 96 h toxicant exposure period. The blood samples were taken by puncturing posterior caudal vein using Ethylene-diaminetetraacetate (EDTA) as anticoagulant (Schmitt et al., 1999). Blood, 2.0 ml, was decanted in heparinzed bottles for determination of blood parameters. The microhaematocrit method of Snieszko (1960) was used to determine the hematocrit (PCV). Hemoglobin (Hb) concentration was measured with Hb test kit using the cyanmethemoglobin method (Larsen and Snieszko, 1961). Red blood cell (RBC) and white blood cell (WBC) counts were counted under light microscope with an improved Neubauer haemocytometer (Mgbenka et al., 2003; Shah and Altindag, 2004, 2005). The derived hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae as described by Jain (1986):

MCV will be calculated in femtoliters = PCV/RBC × 10; MCH was calculated in picograms = Hb/RBC × 10; and MCHC = (Hb in 100 mg blood / Hct) × 100.

Fish from the experimental and control groups were vivisected without

Table 1. Physicochemical characteristics of water samples used in the experiment.

Parameter	Unit	Minimum	Maximum
Temperature	℃	31	36
pH	-	7.6	7.8
Dissolved oxygen	mg O_2 L^{-1}	6.8	8.9
Chemical oxygen demand	mg O_2 L^{-1}	75	128
Biochemical oxygen demand	mg O_2 L^{-1}	5.5	9.6
Total dissolved solids	g L^{-1}	41.4	44.7
Chloride	g L^{-1}	12.8	16.1
Salinity	g L^{-1}	28.5	30.4
Total hardness	mg L^{-1} as $CaCO_3$	6220	6562
Total alkalinity	mg L^{-1}	158	180
Ammonia nitrogen	mgN L^{-1}	0.16	0.77
Nitrite nitrogen	mgN L^{-1}	0.10	0.14
Nitrate nitrogen	mgN L^{-1}	0.11	0.16
Total pesticides	mg L^{-1}	0.54	0.88

anesthesia after an interval of 24, 48, 72 and 96 h (eight fish in each interval for each concentration). The heart was carefully removed; standard procedures were used for the estimation of glycogen (Hassid and Abraham, 1957), protein (Lowry et al., 1951) and cholesterol (Zlatkis et al., 1953). The results were expressed as mg/g wet weight of the tissue.

Statistical analysis

Treatment effects were compared with the least significant difference method using MstatC software of Michigan State University, MI, USA. Significance difference has been presented as probability (P) values. Treatments were compared (LSD) to determine significant variation (P > 0.05) among the dietary levels (Gomez and Gomez, 1984). Duncan's multiple range (DMR) test was employed for comparing the mean mortality values after estimating the residual variance by repeated measures ANOVA.

RESULTS AND DISCUSSION

The physiochemical characteristics of test water are listed in Table 1. Temperature ranged from 35 to 36℃ during experimentation. The pH of the water ranged from 7.6 to 7.8, which was slightly higher than neutral. Dissolved oxygen ranged from 6.8 to 7.9 mg/L. The body weight of malathion exposed fish showed a slight but progressive decrease in the time course when compared with normal fish (Table 1), suggestive of the loss of some body constituents. Since the loss of weight was probably associated with the susceptibility to pesticides (Gish and Chure, 1970), the prolonged exposure of fish to the same concentration of malathion may prove to be fatal. Studies with other organophosphorus compound like methyl parathion on the fish *Tilapia mossambica* (Siva Prasada, 1980) and malathion and lindane on the same species (Basha, 1980) showed a decrease in the body weight. Since symptoms of pesticide toxicity normally involve respiratory distress (Ferguson and Goodyear, 1967), the decreased oxygen consumption of the malathion exposed fish is probably due to the absorbance of more pesticide through the gills.

The LC_{50} values were determined using different concentrations of pesticide for the present mortality 10 fishes (Table 2) for different time of exposure. The LC_{50} of malathion for 48 and 96 h was found to be 2.0 and 1.5 mg L^{-1} (Figure 1). These values were relatively low when compared with those obtained for 48 h. The literature of Pickering et al. for Fathhead fish (25 ppm) and gold fish (0.79 ppm) shows that the value of Killifish (1.8 ppm) studied by Tsuda et al. (1997) was lower. Vittozi and De Angelis (1991) summarized the 96 h LC_{50} values of malathion 0.091 to 22.09 ppm for different species. The difference in toxicity to the different aforementioned species might be due to differences in absorption pesticide, their accumulation, biotransformation and excretion. Differences in metabolic pathways among species may result in different patterns of biotransformation leading to more or less toxic metabolites (Johnson and Taledo, 1993). The magnitude of toxic effects of pesticides also depends on length and weight, corporal surface to body weight ration and breathing rate (Singh and Narain, 1982; Murthy, 1986 and Alkahem et al., 1998).

Hematological parameters

The variations of the mean values for the red blood cell parameters after exposure are shown in Table 3, which demonstrated a significant ($P < 0.05$) decrease in RBC count, Hb and Ht for the groups in the group exposed to malathion. Compared to the control specimens, fish after an acute exposure to diazinon had lower erythrocyte count ($p < 0.01$), haemoglobin content ($p < 0.01$) and lower haematocrit value ($p < 0.01$). Values recorded for MCV, MCH and MCHC were comparable in both groups

Table 2. Mortality of *Oreochromis niloticus* at different concentration of malathion for 24, 48, 72 and 96 h exposure period.

Exposure of 24 h malathion						Exposure of 48 h malathion				
S/N	Conc. of malathion conc. in ppm	Log conc.	Fish exposed	Fish dead	Mortality (%)	Conc. of malathion conc. in ppm	Log conc.	Fish exposed	Fish dead	Mortality (%)
1	0.5	-	10	0	0	0.5	0.0383	10	1	10
2	1.0	0.0381	10	1	10	1.0	0.0399	10	2	20
3	1.5	0.0496	10	2	20	1.5	0.0489	10	3	30
4	2.0	0.0584	10	3	30	2.0	0.0602	10	5	50

Exposure of 72 h malathion						Exposure of 96 h malathion				
S/N	Conc. of malathion conc. in ppm	Log conc.	Fish exposed	Fish dead	Mortality (%)	Conc. of malathion conc. in ppm	Log conc.	Fish exposed	Fish dead	Mortality (%)
1	0.5	1.0031	10	2	20	0.5	0.6084	10	4	40
2	1.0	1.0782	10	3	30	1.0	0.7881	10	6	60
3	1.5	1.1472	10	5	50	1.5	0.9056	10	8	80
4	2.0	1.2082	10	8	80	2.0	1.00	10	10	100

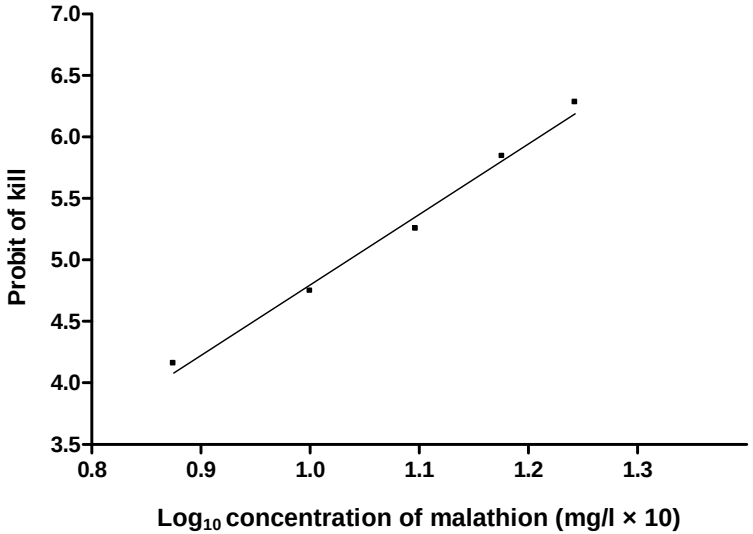

Figure 1. Graph showing the relationship between probit of kill and $\log_{10}$ concentrations of malathion to find the Lc_{50}.

Table 3. Blood parameters values of *Oreochromis niloticus,* for various concentrations of malathion.

Blood parameter	0.5 mg / L	1.0 mg / L	1.5 mg / L	2.0 mg / L	Control
Haematocrit (% V)	39.2 ± 1.8[a]	26.7± 1.1[a]	23.5 ± 1.6[b]	21.7 ± 2.5[b]	40.2 ± 2.7
WBC (× 10^3 mm^3)	2.5 ± 0.3[a]	1.9 ± 0.5[a]	1.5 ± 0.4[c]	1.1 ± 0.3[c]	2.9± 0.9
RBC (× 10^{-7}µL)	15.9 ± 2.2[a]	13.1± 1.6[a]	10.7 ± 2.5[b]	8.1 ± 1.4[c]	16.1 ± 2.5
Hb (× 10^2 g /dL)	79.5 ± 4.3[a]	72.1± 4.8[a]	61.2 ± 5.4[b]	48.7 ± 3.3[c]	85.5 ± 4.2
MCV (× 10^{-7}Fl)	2.4 ± 0.4[a]	1.9 ± 0.3[a]	1.5 ± 0.2[b]	0.8 ± 0.3[c]	2.2 ± 0.5
MCH (× 10^{-7}pg)	6.2 ± 1.3[a]	5.6 ± 0.9[b]	4.4 ± 1.5[c]	3.6 ± 0.8[d]	5.7± 1.8
MCH (pg)	411.6 ± 7.2[a]	388.5± 8.7[a]	325.4 ± 7.6[b]	272.6 ± 8.23[c]	282.6 ± 11.2

Values are expressed as the mean ± S.E. Means in the same horizontal column followed by different superscript are significantly different (≤= 0.05) according to Duncan's new multiple range test.

under study. Haematological parameters of fish are highly variable between and within species and seasons (Luskova, 1997), with the values of individual indicators differ in relation to temperature, season, sex, food, and type of culture (Sopi*ƒ*ska, 1985, Thomas et al., 1999). Blood parameters may also show within-population differences (Allen 1993; Thomas et al., 1999), which explain wide variations within the control during the experiment. Values of the haematological parameters recorded in the control were close to those typical of the healthy carp (Singh et al., 2010).

The hematological report shows that the mean PCV, WBC, HCT and Hb of O*. niloticus* in the control were recorded as 39.2%, 3.7 × 10^7 mm^3 , 24.7 g/dl and 85.5 g/dl, respectively (Table 3). In the present study, a gradual decrease in these parameters was observed in the experimental fish as the concentration increases with span of time of exposure to Malathion in water under the various treatments. The decrease was very significant ($P < 0.05$) at higher concentrations of Malathion (1.5 and 2.0 mg/L). Similar findings were reported in *C. garipenus* by Ololade and Oginni (2010). The reduction in WBC count of the treatment groups may be due to the release of epinephrine during stress, which is capable of causing the contraction of spleen and a decrease of leucocytes count, which can result in the weakening of the immune system (Svoboda, 2001; Witeska, 2003). Thus, the significant reduction in these parameters might be an indication of severe anemia caused by exposure of the experimental fish to Malathion in the water. Erythrocyte swelling is related to intracellular osmotic disorders and stress. Erythrocyte haemolysis is associated with blood serum acidification and intracellular alkalisation (Nikinmaa and Huestis, 1984). Maheswaran et al. (2008) observed an increase in hematocrit levels in different fish species after zinc treatments. They attributed such an increase in hematocrit values to increase in the size of the erythrocytes as being demonstrated for chromium and zinc treated rainbow trout. Our findings were substantiated by Maheswaran et al. (2008). They further discussed that decrease or increase in certain blood parameters can be associated with the nature of species and the toxicants in different studies. Annune et al. (1994a) reported a significant increase in RBC count of *C. gariepinus* when subjected to Zn treatment. They attributed the red blood cell elevation to blood cell reserve combined with cell shrinkage as a result of osmotic alterations of blood by the action of the metal. In another study, a non-significant decrease in red cells for *O. niloticus* was observed (Annune et al., 1994b; Singh et al. 2010). Our results are supported by previous research work that various heavy metals such Malathion and toxins enter the aquatic system and exerts a specific toxic effect on fish blood and tissues (Mousa and Khattab 2003). The decreased number of white blood cells (leucopoenia) may be as a result of bioconcentration of the tested metal in the kidney and liver. Other authors have associated the cause to hindering of granulopoiesis or lymphpoiesis, induced by primary or secondary changes in haematopoietic organs (Tomaszewski, 1997; Al-Akel et al., 2010). In the present study, for the values obtained for the hematological indices, no significant change was recorded in the mean corpuscular volume (MCV) and mean corpuscular hemoglobin content (MCHC) among all the treatment groups. It has been observed that there was significant change in the mean corpuscular hemoglobin (MCH) especially at higher concentrations (that is, 1.5 and 2.0 mg/L). However, slight fluctuations were recorded in the MCV and MCHC when compared with the control. Ololade and Oginni (2010) reported that cell released from the spleen, which is an erythropoietic organ would have the lower MCV values when compared with the control. A similar observation was made for *Cyprinus carpio* after cadmium exposure (Al-Akel et al., 2010; Singh et al., 2010). The significant change ($P < 0.05$) in the MCH of the experimental fish when compared with the control may be due to the reduction in cellular blood iron. These results were upheld by the findings of Hodson et al. (1978).

The changes in glycogen and protein levels in heart muscles of fish after the treatment with malathion are presented in Table 4. While analyzing the changes in the glycogen, protein and cholesterol, it obvious that they fluctuated during different intervals of treatment.

Table 4. Concentration of glycogen and protein in liver and muscle of *Oreochromis niloticus* exposed to malathion.

Concentration of malathion (mg/L)	Glycogen concentration (mg/l)		Protein(g / L)	
	Muscle	Liver	Muscle	Liver
Control	7.76±1.1[a]	5.95± 0.98[a]	12.44± 1.88[a]	14.22± 2.41[a]
0.5	6.54± 0.86[b]	5.84± 0.87[a]	11.84± 2.12[a]	13.61± 2.18[a]
1.0	6.05 ±0.92[b]	5.12±0.74 [b]	11.05± 1.86[b]	12.21± 2.44[b]
1.50	5.65±0.77[c]	4.56± 0.81[c]	10.66± 1.55[c]	11.42± 1.85[c]
2.0	5.33±0.94[d]	4.22±0.55[d]	9.88± 1.68[d]	10.23± 1.98[d]

Values are expressed as the mean ± S.E. Means in the same horizontal column followed by different superscript are significantly different (≤= 0.05) according to Duncan's new multiple range test.

Conclusion

Chemical determination of any persistent toxicant concentration in water, as well in sediment may not provide information on the severity of contamination, especially in the case of sublethal levels. Biological monitoring using a series of assays having different endpoints in a ''key species'' could allow a sensitive approach to predict the potential risk of persistent contaminants like pesticides, which is helpful in formulating the ''safe levels'' of such bioaccumulative chemicals having genotoxic potential. Acute toxicity studies are the very first step in determining the water quality requirements of fish. These studies obviously reveal the toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. Therefore, studies demonstrating the sensitivity of genotoxic effects of pesticides in aquatic organisms, particularly in fish are needed. Thus, it can be concluded from the present study that fish are highly sensitive to malathion and their mortality rate is dose dependent.

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