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Effect of phosalone on haematological indices in the tilapia, *Oreochromis mossambicus*

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Abstract: The aim of this study was to assess the effect of phosalone on the tilapia, *Oreochromis mossambicus*. The effect was assessed on the basis of the results of acute toxicity tests and comparison of results of haematological examination of a control and an experimental group exposed to phosalone. The 96 h LC_{50} is the basic value in the acute toxicity test and it was 0.26 mg/L for phosalone against the test fish.

Haematological profile of the healthy fish was carried out with 2 sublethal concentrations, viz 0.0325 mg/L (1/8) and 0.065 mg/L (1/4) of phosalone. The experiment was performed semi-statically with a group of 10 fish in 10 experimental aquaria, 2 control aquaria, and 4 aquaria each with different concentrations.

The results showed that phosalone had some effect on the haematological parameters of tilapia. It was determined that the total RBCs, WBCs, haemoglobin content, and haematocrit value significantly decreased ($P < 0.001$).

The percentages of erythrocyte sedimentation rate, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration showed an increasing trend with respect to the increase in exposure period in both sublethal concentrations. ANOVA showed that the pesticide concentrations had more influence than the duration of exposure in all cases ($P < 0.001$).

Key words: Haematology, pesticide, phosalone, tilapia, toxicity

Introduction

In India, the use of pesticides in agriculture has significantly increased during the past 3 decades. The agriculture run-off in the aquatic environment leads to massive killing of fish and hence warrants close attention. To avoid such damage it is imperative to test the toxicity of the pesticides before they are applied to the agricultural field on a large scale. Phosalone, a member of the organophosphate family of pesticides, is used as an insecticide to protect many fruits, vegetables, nuts, and field crops against a wide range of hemipterian and hymenopterian insects. However, phosalone is known to have harmful effects on aquatic organisms as well as on the environment. There is vast amount of scientific information available on different pesticides' toxicity on different fishes in India (1-3) but limited information is available on the effect of these pesticides on the haemopoietic system of fishes.

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Since haematological parameters are important for reflecting the healthy state of a fish, they have been widely used as indicators of disease or stress due to pollutants. Several studies have been carried out on the impacts of pesticides on the haematology of freshwater teleosts by various authors (4-8). However, there is a paucity of information about the effects of phosalone on the haematology of *Oreochromis mossambicus*.

As phosalone is extensively used during farming operations and *O. mossambicus* is an important animal in freshwater fishery in Kanyakumari district, India, the objective of the present study was to determine the toxicity of phosalone and its effects on haematogical parameters of *O. mossambicus* in order to enrich the present knowledge on pesticide toxicity and to show the toxic effects of the pesticide.

Materials and methods

A group of healthy *O. mossambicus* (15 ± 3 g) was stocked in a large cement tank containing chlorine-free well water for 1 month at normal temperature. The water was changed on alternate days and the fish were fed ad libitum on a formulated fish diet prepared from ground nut oil cake and rice bran in the laboratory. Ten well-acclimatised fish were transferred from the stock to each experimental tank containing 10 L of water exposed to different concentrations of phosalone for 96 h under natural light with constant aeration. Temperature, pH, and dissolved oxygen of the water were maintained at 27 ± 2° C, 7.1 ± 0.5, and 3.9 ± 0.02 mg/L, respectively. The fish were fasted for 24 h prior to the experiment. Twelve various concentrations and a control were used in the basic test. The LC_{50} values in the respective time intervals were calculated using software by transforming mortalities (percentage values) into a probit scale (9).

From the 96 h LC_{50} values 2 sublethal concentrations, viz 0.079 mg/L (1/4) and 0.037 mg/L (1/8), were chosen to expose the fish to for haematological studies. A control group was also maintained. The experiment was performed semi-statically with a group of 10 fish in 10 experimental aquaria, 2 control aquaria, and 4 aquaria each with 2 different concentrations. Fresh test media were provided on alternate days and the fish were fed daily with formulated food. The experimental regime was maintained in the laboratory for 45 days. Haematological examinations of the healthy fish were carried out at 10, 35, and 45 days of exposure.

Blood was sampled by the following method. The fish were caught very gently using a small dip net, one at a time with least disturbance. Each fish was held and wrapped with a clean, dry towel and the posterior half of its body was blotted with a clean coarse filter paper. Then the caudal peduncle of the fish was severed with a single stroke from a heavy, sharp knife. After discarding the first drop of blood, the freely oozing blood was collected in a small watch glass containing a sufficient quantity of anticoagulant heparin solution (1:10 with physiological saline). The blood was thoroughly mixed with the anticoagulant using a thin, blunt glass rod, during the process of collection itself. Collection of blood was stopped as soon as the blood oozing from the cut caudal peduncle began to show the signs of clotting. Samples for analysis were prepared immediately after collecting blood.

Haematological parameters such as total RBC and WBC counts, haemoglobin content (Hb), haematocrit value (Ht), erythrocyte sedimentation rate (ESR), mean corpuscular haemoglobin (MCH), and MCH concentration (MCHC) were estimated. The results were processed statistically using analysis of variance (ANOVA) and are presented as means ± SD.

Results

Acute toxicity

The LC_{50} values of phosalone for the tilapia *Oreochromis mossambicus* in 12 h time intervals are given in the Figure. The data clearly showed the relationship between the concentration of the pesticide and the percentage mortality. Fish exposed to higher concentrations underwent rapid death. The 96 h LC_{50} is the basic value in the acute toxicity test and it was 0.26 mg/L for phosalone against the test fish.

Haematological profile

Results of the erythrocyte profile of the control and experimental tilapia fish studied are given in the Table. The results showed a decrease in total RBCs

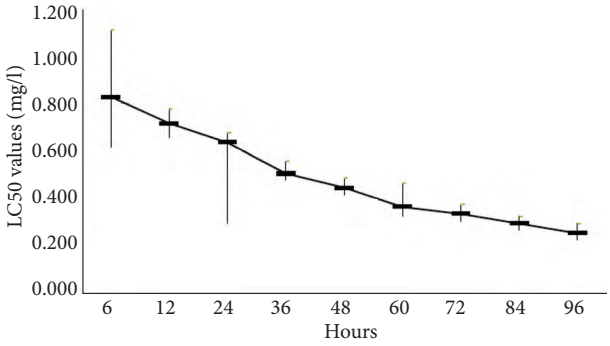

Figure. Acute toxicity test of phosalone in tilapia *Oreochromis mossambicus* (range means 95% confidential interval).

with respect to increases in exposure periods and concentrations. The percentage of total RBCs declined suddenly on day 10 of exposure and showed substantial reductions during further exposure times. The total WBCs showed delayed decreases at both concentrations with a minimum reduction of 2.2%. The maximum percentage of reduction (48.24%) was observed in the haematocrit value at a concentration of 0.065 mg/L after 45 days. Hb percentage also decreased in both sublethal concentrations. Compared to the control fish, those after the acute exposure to phosalone had significantly lower erythrocyte count, Hb, and haematocrit values ($P < 0.001$). The percentages of ESR, MCH, and MCHC showed an increasing trend with respect to increases in exposure period in both sublethal concentrations. The ESR values showed no change at 0.0325 mg/L on day 10 of exposure and increased abruptly to 21.43% after 30 days but rapidly declined to 5.5% on day 45 of exposure, whereas at a concentration of 0.065 mg/L it increased to 30.77% on day 10 of exposure. MCH and MCHC showed an elevated trend with respect to increased exposure periods in both sublethal concentrations. ANOVA showed that the pesticide concentrations have more influence than the duration of exposure in all cases ($P < 0.001$).

Discussion

In the present study, the LC_{50} test of phosalone on tilapia, *O. mossambicus*, showed dose-dependant mortality. The value observed is in agreement with those reported by other authors who have determined the toxicity of phosalone for various fish (10). The increase in exposure duration was associated with a decrease in LC_{50}. This result is corroborated by the results reported by Venugopal et al. (11) and Tilak et al. (12). The static bioassay experiments were conducted under laboratory conditions using malathion on *Clarias batrachus* and the 96 h LC_{50} value was 0.040 ppm (13). From the present study, it is quite evident that phosalone is an important toxic xenobiotic in the aquatic environment.

In the present investigation decreases in the various parameters of blood were observed due to the treatment of *O. mossambicus* to different sublethal doses of phosalone for longer durations. The total numbers of RBCs and WBCs, the haemoglobin content, and haematocrit value registered significant decreases ($P < 0.001$). Such a decline in blood cells and haemoglobin content indicates the haemotoxicity of phosalone to fish.

Phosalone is known to bring about necrotic changes in the liver of fish (14) as evidenced from the present pathological studies. Lower haemoglobin level might decrease the ability of the fish to enhance its activity in order to meet occasional demands like seeking food and escape. The decreases in total erythrocyte count and haemoglobin concentration are often accompanied by decreases in haematocrit. This demonstrates the physiological dysfunction of the haemopoietic system. The increases in MCH and MCHC of the blood conform to the erythrocyte count and their production in the disorder. The increases in MCH and MCHC indicate that the reduction in RBC count may be due to the destruction of red blood cells and reduction in Hb content in each cell (15). The ESR was found to increase in *O. mossambicus* as the concentration of the pesticide and their exposure duration were increased.

The present study has produced a better perception of the possible disruptions to the aquatic biota exposed to low levels of phosalone over a long period in the laboratory (chronic bio-assay) in such a way to reflect what might happen in field conditions. Therefore, the use of the pesticides in farm lands should be controlled to prevent possible contamination by leaching into the aquatic environment. In this way aquatic organisms could be protected from these kinds of toxic chemicals.

Table. Changes in the haematological parameters of *Oreochromis mossambicus* exposed to 2 sublethal concentrations (1/4 and 1/8 of LC_{50}) of phosalone for 45 days. Each value is the mean ± SD of 4 individual observations. The values in the parentheses are percentage changes over the control.

Blood parameters	10 days			35 days			45 days		
	Control	0.035 mg/L	0.065 mg/L	Control	0.035 mg/L	0.065 mg/L	Control	0.035 mg/L	0.065 mg/L
RBC (10^6/mm^3)	1.7 ± 0.17	1.3 ± 0.13 (-23.53)	1.1 ± 0.13 (-35.29)	1.8 ± 0.16	1.2 ± 0.1 (-33.33)	1.0 ± 0.17 (-44.44)	1.7 ± 0.17	1.3 ± 0.12 (-31.58)	1.1 ± 0.1 (-42.11)
Hb (g/L)	81 ±1.2	75 ± 8.3 (-7.41)	71 ±6.4 (-12.35)	85 ± 7.6	73 ± 6.6 (-14.12)	65 ± 5.5 (-23.53)	85 ± 7.6	70 ± 7.7 (-17.65)	65 ± 5.2 (-23.53)
HAEMATOCRIT (%)	30 ± 0.3	26 ± 0.26 (-13.33)	22 ± 1.98 (-26.67)	27 ± 2.4	22 ± 0.22 (-18.52)	18 ± 1.8 (-33.33)	29 ± 2.6	19 ± 2.28 (-34.48)	15 ± 1.65 (-48.28)
ESR (mm/H)	13 ± 1.3	13 ± 1.17 (0.00)	17 ± 1.87 (30.77)	14 ± 1.24	17± 1.7 (21.43)	18 ± 1.8 (28.57)	18 ± 1.62	19 ± 1.7 (5.56)	21 ± 2.1 (16.67)
MCH (g/L)	418 ± 46	509 ± 41 (21.77)	561 ± 44 (34.21)	390 ± 35.9	513 ± 56 (31.54)	578 ± 52 (48.21)	431.2 ± 38	516.1 ± 46.4 (19.69)	591 ± 70 (37.06)
MCHC (g/L)	29.2 ± 3.2	32 ± 3.2 (9.059)	34.7 ± 3.8 (18.84)	33 ± 2.9	37 ±4.4 (12.12)	42 ± 4.6 (27.27)	35 ± 3.1	42.1 ± 4.6 (20.29)	50 ± 6.0 (42.86)
WBC (10^4/mm^3)	4.2 ± 0.37	4.1 ± 0.45 (-2.38)	3.8 ± 0.38 (-9.52)	4.4 ± 0.39	3.7 ± 0.3 (-15.91)	3.4 ± 0.27 (-22.73)	4.4 ± 0.39	3.6 ± 0.32 (-18.18)	3.0 ± 0.27 (-31.82)

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