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Toxic effects of an organophosphorus pesticide, malathion (50% EC) on the freshwater fish, *Oreochromis mossambicus* (Peters, 1852)

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Abstract
Malathion is a wide spectrum aliphatic organophosphorus pesticide commonly used for agricultural purposes. In the present study, an attempt was made to study the acute toxic effects of malathion in the adult freshwater fish, *Oreochromis mossambicus*. The median lethal concentration or LC_{50}-96 h was determined by exposing the fish to seven different concentrations of malathion i.e., 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 µg/ L concentrations for 96 h along with the control group, without toxicant. The weight of the animals was recorded before and after exposing to the pesticide, which showed no significant changes after the treatment period. Behavioural modifications of the fish such as frequent surfacing and air engulping, irregular erratic swimming, aggressiveness, rapid opercular lifting along with slight haemorrhage and mucous deposition throughout the body were noticed. The study proved that an increase in the malathion concentration for 96 h caused increase in the rate of mortality in which 50% mortality occurred at 0.5 µg/ L concentration, which was taken as the median lethal concentration or LC_{50}-96 h. The data obtained was subjected to Probit analysis at 95% confidence limit also proved 0.5 µg/ L concentration as 96 h-LC_{50} value of malathion in the fish, *O. mossambicus*.

Keywords: Malathion, acute toxicity, mortality, behaviour, *Oreochromis mossambicus*

1. Introduction

Aquatic ecosystems, both freshwater and marine, are continuously exposed to different types of toxic chemicals and wastes. Some pollutants are directly discharged into the water bodies, which forms the point source pollution whereas some are leached from many miscellaneous sources such as rainwater runoff, untreated sewage effluents, agricultural products etc referred as non-point source pollution [1]. The use of pesticides to control terrestrial pests in agriculture and forestry are known to contaminate water bodies thereby affect flora and fauna of the aquatic ecosystems. Pesticides either directly enter into the aquatic organisms like fish due to the lipophilic properties or enter into zooplanktons which finally get accumulated in the tissues of animals through the food chain. Thus fish are more susceptible to the environmental changes yet they have the ability to adapt themselves when exposed to the low concentration of pollutants [2]. However, prolonged exposure to low concentration or exposure at high concentrations of toxicants for short period may prove to be fatal.

Malathion is a broad-spectrum, non-systemic general-purpose insecticide used to control sucking and chewing insects on fruits and vegetables. It is also used to control flies and mosquitoes, and sometimes directly used on fish to eliminate parasites [3]. Malathion is also used as an ingredient in shampoo products to eradicate head lice. Thus the occurrence of malathion was wide including soil, sediments, and also detected in ground and surface water [4] with half-life ranging from 2 to 18 d [5]. Toxic effects of any pollutants can be evaluated by employing several parameters including biochemical, physiological, endocrine and reproductive effects on the test species. Meanwhile quantitative parameters such as percentage of mortality against the test concentrations are used as a sensitive measure to determine acute toxicity of the chemicals. Several studies have reported the median lethal concentration of malathion in different fish species with LC_{50} value ranging from 0.592 ppb in *Oreochromis mossambicus* [6], 101 ppb in Brown trout [7], 0.25 ppm in *Clarias batrachus* [8], 8.22 ppm in *Clarias gariepinus* [9], 5 µg/ L in *Labeo rohita* [10] and 16 µg/ L in *Ophiocephalus punctatus* [11] when exposed for 96 h.

All laboratory bioassays used malathion as the single test substance exposed to single test species at specific period of time, particularly for 96 h. But the available data revealed that the acute toxicity or median lethal concentration of malathion varies among the fish species. Thus an attempt was made to determine the lethal toxicity of malathion in the adult freshwater fish, *Oreochromis mossambicus* using standard procedure prescribed under OECD guidelines to assess change in 96 h-LC_{50} value, along with behavioural modifications during the treatment period.

2. Materials and Methods

2.1 Preliminary tests

The physicochemical features of the tap water were estimated and maintained in a range such as water temperature at 26 ± 2 °C, oxygen saturation between 70 and 100% and pH from 7.4 to 7.6 according to the standard protocol as prescribed in the APHA guidelines [12].

2.2 Test animal

Adult freshwater fish, *Oreochromis mossambicus*, weighing 14 ± 2 g and length 9 ± 1 cm were collected from CP fish farm, Kottackal, Malappuram District, Kerala, India. Fishes were brought to the laboratory without stress and acclimatized for two weeks prior to the experiment in dechlorinated, aerated, static water stored in a glass tank of 40 L capacity maintaining photoperiod of 12 h light: 12 h dark conditions throughout the study. Fish were fed with standard fish pellets twice a day during the acclimatization period where the food remains and faeces were removed on daily basis to avoid stress to the animal.

2.3 Malathion treatment

Malathion 50% EC (O, O-dimethyl, S-(1, 2- dicarboethoxy ethyl) phodophorodithioate; 50% emulsifiable concentrate; E.C.), was obtained from Hikal Chemical Industries, Gujarat, India. The experiment was conducted in replicates using ten fish specimens exposed to each concentration in a static condition, without food and aeration for 96 h.

2.4 Acute toxicity test

After acclimatization, fish were exposed to seven different concentrations of malathion such as 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 µg/ L for 96 h along with control group, without the toxicant. The above test concentrations were chosen after repeated range-finding experiments. The required test concentration was drawn directly using a micropipette without any diluents and added to each experimental tank. Fish were introduced into each tank with different malathion concentration for 96 h, and behavioural changes were observed for 1 h after the immediate introduction of fish, and for 30 min after every 24 h. Mortality of fish was observed at 24 h interval for 96 h, in which fish was considered as dead when there was no opercular movement and unresponsive condition on probing with the glass rod. The dead specimen was immediately removed so as to prevent contamination. Median lethal concentration (LC_{50}-96 h) that kills 50% of test animal and its 95% confidence interval was predicted based on the different concentrations of malathion, and the corresponding observed mortalities to logarithmic scale and probit. The concentration for 50% mortality was directly read and its subsequent mathematical regression equation was calculated by plotting mortality in Y-axis and concentrations in X-axis using standard regression formula [13].

2.5 Statistical analyses

The statistical package SPSS 17.0 was used to test probit analysis using log10 concentration transformation, and the graph of mortality against concentration of test chemical was plotted using MS-Excel.

3. Results and Discussion

The present study was performed strictly following the Organization of Economic Co-operation and Development (OECD) guidelines of fish acute toxicity testing [14]. Accordingly, the freshwater fish, *Oreochromis mossambicus* was exposed to different concentrations of malathion in a static condition for a period of 96 h. The mortality of fish was recorded on daily basis and it was found that the rate of mortality increases with increase in concentration of the toxicant as shown in Table 1.

Table 1: Effect of malathion on the mortality rate for 96 h in the fish, *Oreochromis mossambicus* (n=10 in replicates)

Malathion concentration (µg/ L)	Mortality		Hour of mortality
	Number of animals	Percentage	
0.00	0	0	96 h
0.25	2.0	20	96 h
0.50	5.0	50	96 h
0.75	6.0	60	96 h
1.00	7.0	70	96 h
1.25	10.0	100	96 h
1.50	10.0	100	72 h
1.75	10.0	100	24 h

Acute toxicity testing is an important tool for assessing the effect and fate of toxicant in aquatic ecosystems as well as it indicates the quality of water [15]. Physico-chemical parameters play an important role in assessing the toxic effects of pollutants, thus the present study maintained some important physico-chemical features of water such as temperature, salinity, pH, oxygen saturation and other chemical analysis to ensure the quality of water for the maintenance of fish in stress-free environment.

Fish exposed to 0.25 µg/ L concentration showed 20% mortality whereas 0.50, 0.75, 1.00 and 1.25 µg/ L concentrations showed 50%, 60%, 70% and 100% mortality after 96 h. Increase in the concentration of malathion for 1.50 and 1.75 µg/ L concentrations showed 100% mortality after 72 and 24 h, respectively (Table 1; Figure 1).

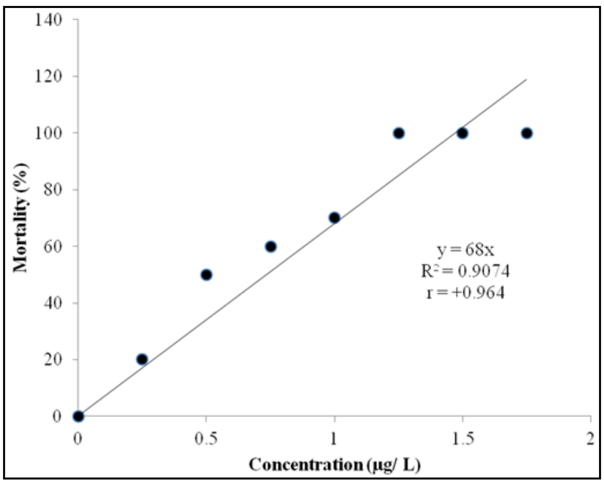

Fig 1: Median lethal concentration or LC_{50}-96 h of malathion in the freshwater fish, *Oreochromis mossambicus*

The data obtained was then used for calculating the slope of dose-response curve at 95% confidence limit, which showed the regression equation as Y = 68X with r^2 value 0.9074 having a high degree of positive correlation (r = +0.964). A graph was plotted against the different concentration of malathion and percentage of mortality of animal, where the median lethal concentration of malathion calculated using Finney's Probit analysis was 0.51 µg/ L concentration (Table 2).

Table 2: Probit analysis of 95% confidence limits for effective concentrations of malathion in *Oreochromis mossambicus*

Probability	95% Confidence Limits for Concentration		
	Estimate	Lower Bound	Upper Bound
.010	.112	.029	.200
.020	.134	.039	.228
.030	.150	.047	.247
.040	.164	.054	.263
.050	.175	.061	.277
.060	.186	.067	.289
.070	.196	.073	.300
.080	.205	.079	.311
.090	.213	.085	.321
.100	.222	.091	.330
.150	.260	.119	.372
.200	.295	.147	.410
.250	.329	.175	.447
.300	.363	.206	.483
.350	.397	.238	.521
.400	.433	.272	.561
.450	.470	.309	.604
.500	.510	.350	.652
.550	.554	.394	.706
.600	.601	.442	.770
.650	.655	.494	.848
.700	.717	.552	.946
.750	.791	.617	1.074
.800	.882	.691	1.249
.850	1.001	.780	1.506
.900	1.173	.898	1.929
.910	1.219	.928	2.050
.920	1.271	.961	2.192
.930	1.331	.998	2.361
.940	1.401	1.040	2.567
.950	1.486	1.090	2.825
.960	1.592	1.150	3.164
.970	1.732	1.228	3.641
.980	1.938	1.339	4.394
.990	2.314	1.529	5.921

The weight of the animal recorded before and after the malathion exposure showed no significant changes, and was found similar to the fish from the control group (data not shown). The disparity observed in the median lethal concentration of malathion in the freshwater fish, *Oreochromis mossambicus* on comparison with similar study [6] could be due to the change in physicochemical parameters of tap water or the test conditions maintained in the laboratory.

Fish behaviour is considered as a link between the physiological functions and ecological factors including several endogenous or exogenous chemical stressors. Aquatic organisms exposed to environmental contaminants are suspected to cause behavioural modifications, and are used as a sensitive biomarker to understand the health status and viability of fish in the natural population [16]. The present study noticed several changes in the behaviour of fish when exposed to malathion at different concentrations. Immediately after the introduction of fish to the toxicant showed irregular erratic swimming when compared to the fish from the control group. Swimming is one of the most common and relevant measure used to address the response of fish to the toxicant, which ultimately reflects the fitness of the affected fish population [17]. Similar observations were documented on exposure of fullerene C_{60} nanomaterial to the freshwater fish, *Anabas testudineus* [18].

In the present study, fish exposed to malathion also showed frequent surfacing and air engulping in order to escape from the exposed toxicant, which resulted in aggressive behaviour by hitting on the walls of the experimental tanks. It was followed by the increase in excessive mucous secretion that was formed as a layer throughout the body of animal in order to minimize the irritating effect of the toxicant. Mucous secretion also functions to inhibit the diffusion of oxygen during gaseous exchange, and this could be the reason for rapid opercular lifting in the malathion-exposed groups. In another study, fish exposed to bisphenol A also demonstrated severe mucous secretion in the cichlid fish, *Etroplus maculatus* [19]. In the high concentrations of malathion-exposed groups such as 1.25, 1.50 and 1.75 µg/ L concentrations there was a slight haemorrhage in the fins and eyes of fish along with the bulging of eyes (Figure 2), and this could be probably due to the disruption in the physiological adaptation of the fish against the polluted environment.

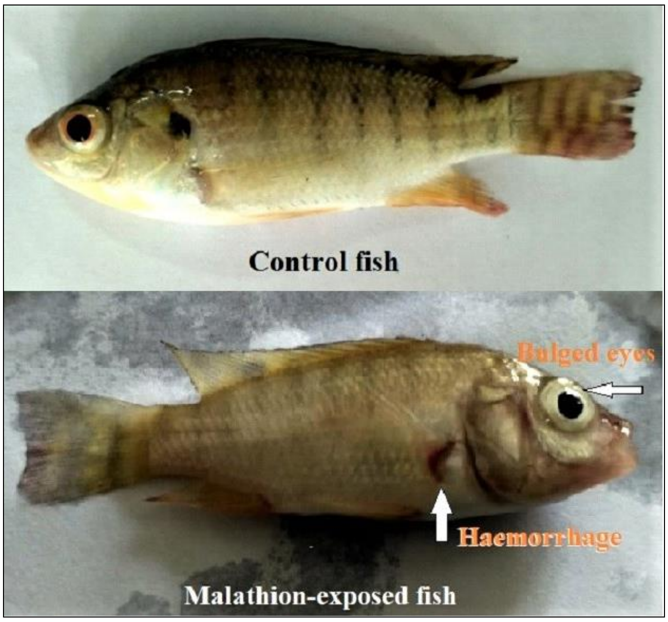

Fig 2: Behavioural changes in malathion-exposed fish, *Oreochromis mossambicus*

Finally, the exposed fishes showed loss of equilibrium and settled at the bottom of tank without movement showing respiratory distress followed by the death of the fish. Similar behavioural response has been shown by the freshwater fish, *Etroplus maculatus* exposed to another organophosphorus pesticide chlorpyrifos thereby proved neurotoxic effects of the pesticide [20]. The mortality of fish was further confirmed by touching the caudal peduncle using glass rod that showed no visible movement including opercular lifting, and the dead fish were immediately removed from experimental tanks to prevent stress and contamination.

4. Conclusion
The present study demonstrated that the acute exposure of malathion caused 50% fish mortality at 0.5 µg/ L concentration, which was mathematically proved by probit analysis. Thus median lethal concentration or 96 h-LC_{50} value for malathion in the freshwater fish, *Oreochromis mossambicus* was 0.5 µg/ L concentration.

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