RESPIRATORY AND BEHAVIOURAL DYSFUNCTION AS AN INDICATOR OF MALATHION TOXICITY IN TILAPIA

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Abstract: Malathion is widely used organophosphate insecticide and also most important source of environmental pollution in the developing countries including India. In the present investigation we aimed to study the toxic effects of Malathion on behavioral and respiratory dysfunction in Tilapia mossambica. The toxicity tests were conducted by static renewal bioassay method on the juveniles of fish Tilapia mossambica (Peters). The LC50 value of Malathion for Tilapia was found out to be 3.6mg/L (for 24.7gm bodyweight). One third (120 µg/1) one tenth (36 µg/1) and one fifth (7.1µg /1) and of the LC50 values were selected for sub lethal studies. Behavioral patterns and oxygen consumption were observed in all (1/3rd and 1/5th) sub lethal concentrations during 12,24,48,72 and 96 hours exposure. Fish behaved irregular, erratic, and dartic movements, by followed hyper excitability, loss of balance, finally settles to the bottom of the test chamber. Increase in the oxygen consumption was observed in low sub lethal concentrations for less duration (12 hr and 24hr exposure in $1/5^{th}$ and $1/10^{th}$ exposure). However a sharp decrease in oxygen consumption was observed in 1/3rd and 1/5th sub lethal concentrations in all fishes of all durations. Fish under sub lethal concentration were found to be under stress and duration of the exposure is found to be an important factor for inducing the toxic effects. Hence, respiratory dysfunction and swimming behavior can serve as marker index of Malathion toxicity.

Key words: Behavior, Malathion, oxygen consumption, Tilapia mossambica

Introduction: In an agricultural country like India, insecticides represent the major components of aquatic chemicals as pollutants. Environmental contaminants such as metals, pesticides, and other chemicals cause severe risks to many aquatic organisms. For that reason, an immense deal of previous research has described physiological mechanisms of toxicity in animals exposed to pollutants. On the contrary, effects of pollutants on fish behavior are not regularly studied. Since behavior links physiological function with ecological processes, behavioral markers of toxicity appear ideal for evaluating the effects of aquatic pollutants on fish populations. Toxicant exposure often completely reduces the performance of behaviors that are necessary for fitness and survival in natural ecosystems, frequently after exposures of lesser magnitude than those causing significant mortality. Unfortunately, the behavioral toxicity of many contaminants is still unknown, necessitate their future study.

Physiological effects of toxicants in the literature include disruption of sensory, hormonal, neurological, and metabolic systems, which are likely to have profound implications for many fish behaviors. On the other hand, little toxicological research has required to integrate the behavioral effects of toxicants with physiological processes. The most commonly observed links with behavioral disruption include cholinesterase (ChE) inhibition, altered brain neurotransmitter levels, sensory deprivation, and impaired gonadal or thyroid hormone levels[1].

Oxygen uptake is widely used in physiology as a biological indicator that integrates the overall metabolic activity of an animal in response to specific environmental factors [2] because it reflects energy expenditure and, ultimately, the food requirements. The changes in the oxygen consumption of fish as an index of toxicity to various pesticides have been studied by several investigators. [3], [4], [5], [6], [7].

Malathion is an organophosphorus insecticide widely used in agriculture as well as houses for the control of insect vectors. However, it is a major source of environmental pollution in the developing countries. Once Malathion is introduced into the environment it may cause serious threats to aquatic organisms and known to cause severe metabolic disturbances in non target species including fish [8]. Therefore, the present study is aimed to determine the acute toxicity of Malathion, with reference to its effects on swimming behavior and dysfunction

in the oxygen consumption to the freshwater fish *Tilapia mossambica (Peters).*

Materials and Methods:

Sample Collection and Maintenance: Healthy and active *Tilapia mossambica (Peters)* juveniles of same size, length and weight approximately (22gm-25gm) (irrespective of age and sex) were procured from the local fishermen at Jhabua, Madhya Pradesh, India. Fish were brought to the laboratory in large aerated containers and were acclimatized for 20 days in 100 L glass aquaria containing dechlorinated tap water. They were fed with commercial dry feed pellets (Nova, Aquatic P. Feed). Physico-chemical characteristics of water were analyzed following the methods mentioned in APHA [9]. Water was changed every day and a 12-12 h photoperiod was maintained during both acclimatization and test periods. The fish were fed on a regular basis with commercial fish food pellets during acclimatization and test periods, but feeding was stopped two days prior to exposure to the pesticide.

Acute toxicity test: Malathion was procured from the local market (50% EC. Coramandal Fertilzers, Vellore, India). A stock solution was prepared in acetone and mixed in water to obtain required dilutions. Required quantity of Malathion was drawn directly from this stock solution using micropipette. Required dilutions of the acetone formulation were made with tap water. The LC50 value for 96 hours of Malathion was determined by procedure of Finney [10].

A group of 10 healthy fishes in each group were exposed to different concentrations of Malathion to calculate the medium lethal concentration LC_{50} value using probit analysis method. It was found to be 3.609 mg/L. The fish, in bathes of 10, were exposed to varying concentrations of Malathion with 20 liters of water using three replicates for each concentration. One third (120 µg l-1) one tenth (36 µg l-1) and one fifth (7.1µg l-1) and of the LC50 values were selected for sub lethal studies. Behavioral patterns and oxygen consumption were observed in all experimental and control groups. During the experimental period the control and Malathion exposed fish were kept under constant observation to study swimming behavior and whole animal oxygen consumption.

Whole body animal oxygen consumption: The whole body animal oxygen consumption was measured for all sub lethal concentrations along with the control by following the methods Welsh and Smith [11] and modified by Saroja [12]. Difference in the dissolved oxygen content of the water before and after experiment yielded the amount of oxygen consumed by the fish during the period of experimentation. The oxygen consumed by normal and treated fish was determined. After experimentation, the fishes were individually weighed and their unit metabolism was calculated and expressed as milliliters of oxygen consumed per gram wet weight of fish per hour. The data were analysed by T test for the study of significance.

Results and Discussion:

Acute toxicity study of Malathion : The findings of research related to acute toxicity study of Malathion in *Tilapia mossambica* (Peters) in terms of treatment (concentration of malathion) and cumulative response (mortality) at 12, 24, 48, 72 and 96 h and corresponding LC50 values with 95% confidence limits are presented in Table 1.

Table.1.Determination of Lethal Concentration $(LC)_{50}$ of Endosulfan for Tilapia mossambica. (Normal laboratory conditions)

Sr. No.	No. of Animal	Malathion con.ug/L	Mortality				% Mortality
			24h	48h	72h	96h	
1	10	Control	–	–	–	–	–
2	10	2.0	–	–	–	2	20
3	10	3.0	–	–	–	3	30
4	10	4.0	–	–	1	5	50
5	10	5.0	–	1	1	5	50
6	10	6.0	–	1	2	6	60
7	10	7.0	1	2	2	6	60

8	10	8.0	1	1	2	7	70
9	10	9.0	1	1	3	9	90
10	10	10.0	1	2	3	9	90

Newhart (2006) reported the LC50 assessments of Malathion for different species of fish and found that it ranges from 0.06 to 7620 µg/L. The difference in the lethal potential of Malathion can also be related to the variations in exposure and tolerance related to its accumulation, biotransformation and excretion. Differences in metabolic pathways among species may also result in varied patterns of bio-transformation, leading to more or less toxic metabolites [13]. The degree of toxic effects of pesticides also depends on length and weight, corporal surface/body weight ratio and breathing rate [14]. Oh et al [15] also reported that factors like inhibition of acetylcholine esterase, detoxication and absorption ability of liver and kidney of an organism are responsible for toxicity of the pesticide.

The LC50 values corresponding to 12, 24, 48, 72 and 96 h of exposure of Malathion were 7.681, 5.929, 4.767, 4.066, and 3.609 mg/L, respectively. In the present study among the range for 24, 48, 72 and 96 h LC50, there was significant difference between 24 and 96 h LC50 values. The Malathion used in this study was 99% pure with 'a' and 'b' isomer proportion of 70:30, respectively. Low value of 96th hour LC50 (median lethal concentration) of 3.609 mg/L indicates that Malathion is highly toxic to fish. The 96 h LC50 value of Malathion found in the present study was comparatively higher than the many values reported earlier, which may perhaps be due to the toughness of the test species, size of animal, and rearing system. Hii et al [16] also found significant differences between 24 and 96 h LC50 value as they renewed 56% of the test solution each day. In nature, fish were able to tolerate a short-term exposure to four times the concentration of pesticide that kills all the fishes in 24 h [17].

Behaviour of the Control and Exposed Fish: Behavior provides an exclusive outlook linking the physiology and ecology of an organism and its environment [18]. Behavior is both a sequence of physiological actions, operating through the central and peripheral nervous systems and the collective expression of genetic, biochemical and physiologic processes essential to life. Behavior allows an organism to adjust to external and internal stimuli in order to best meet the challenge of surviving in a changing environment. Conversely, behavior is also the result of adaptations to environmental variables. The escaping response by fishes is one form of phenotypic adaptation allowing fishes to survive in altered environment [19]. Every change in the behavior and physiology of fish indicates the worsening of water quality, as fish serve as the biological markers of water quality. In the present investigation, the control fish exhibited normal and natural behavior i.e. they were active with their well synchronized movements. They were even aware by the smallest disturbance, but in the fish treated with malathion, exhibited irregular, erratic and darting swimming movements and loss of equilibrium which may due to inhibition of an enzyme acetyl choline esterase (AChE) activity leading to accumulation of acetylcholine in the end bulbs of neuron at synapses ending up with hyper stimulation [20]. They gradually became sluggish, hyper excited, restless and secreted excess mucus all over the body. Mucus secretion in fish forms a barrier between body and contaminated environment thus perhaps weakens the contact of toxicant so as to minimize its irritating effect, or to eliminate it through epidermal mucus. Similar observations were made by Rao et al [21] and Parma de Croux et al [22]. It is also noticed that opercular movements of gill elevated at first in all exposure periods but decreased further progressively in all experimental groups. The increased gill opercular movements observed at first may perhaps balance the increased physiological activities under stressful conditions [23]. Gulping of air at surface by the exposed fish possibly a protective behavior which helps to keep the animal away from contact of toxic medium. One more reason might be more demand of higher oxygen level during the exposure period [24]. Finally, fish sunk to the bottom of the aquarium with the lowest opercular movements and died with their mouth opened. However in sub lethal exposure,

fish body became bending towards abdomen position compared to control fish and was found under stress, but that was not fatal. Bending of fish indicate less quantity of food consumed by the fish due to pesticide stress [25]. The incidence of morphological aberrations such as peeling of scales, yellowing, injury of skin, cracks and necrosis of fins, eye deformities, scoliosis (caudal bleeding), injured skull, lower lip expansion and plentiful amount of mucus secretion all over the body were shown in all treated groups.

Respiratory dysfunction: As fish live in the water, any changes in the water quality may be reflected in the breathing activity and respiratory gas exchanges [26]. The oxygen consumption is regularly used as a biomarker of pollution associated stress in biological early warning systems. However, respiratory responses were found to be less sensitive when compared to biochemical biomarkers, but also might be successfully used in bioassay of toxicity testing. A change in respiration rate is one of the common physiological responses to toxicants and is easily detectable through changes in oxygen consumption rate, which is frequently used to evaluate the changes in metabolism under environmental deterioration. It is clearly evident from the above studies (Table.2) that Malathion affected oxygen consumption of *Tilapia mossambica* under sub lethal concentrations. Fish exposed to various sub lethal concentration shown increased oxygen consumption on first day but started decreasing along with dose and duration of exposure. In one tenth and one third sub lethal concentration exposure, rate of oxygen consumption increased in first few hours but the end of the first day onwards it shown decreasing trend with duration. Even it was found that the rate of oxygen consumption was slowly decreased in control group which may be due to the starved conditions and the reduced metabolic rates of the starved fish [27]. Initially the fish were in more stress during first hour and later they shown signs of recovery. From beginning to end of the experimental period the fish showed painstaking respiratory distress with rapid opercular movements leading to the higher amount of toxicant uptake. It is well known that pesticides can cause respiratory distress and even failure by affecting respiratory centers of the brain (or) tissue involved in breathing[28],[29]. Fish exposed to sub lethal concentration showed increased oxygen consumption in Group II but decreased in other treated groups along with increased concentration of pesticide. The fluctuated response in respiration may be endorsed to respiratory distress as a result of the demolition of oxidative metabolism as in *Tilapia mossambica* [30] due to cypermethrin toxicity.

Gills are the most significant respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply. Whichever damage to these central organs causes a series of negative events, which eventually lead to respiratory distress [31]. Elevated secretion of mucus layer over the gill lamellae has been observed during experimental period. Secretion of mucus over the gill limits the diffusion of oxygen [30], which may finally decrease the oxygen uptake by the fish. The pollutants present in the water may cause structural anomalies of gill [32] or the membrane functions are disturbed by an altered permeability [33] may hamper the oxygen uptake rate. In contrast, the metabolic rate (in relation to respiration) of fish could be increased under toxic stress. Kalavathy *et al.* [25] reported that the dimethoate is competently absorbed across the gill and diffuse into the blood stream resulting toxic to fish. The analysis of data from the present investigation evidenced that Malathion is highly toxic and had intense impact on both behavior and oxygen consumption in *Tilapia mossambica* in all sub lethal concentrations. Consequently it has led to the distorted fish respiratory physiology. Differences in the oxygen consumption rate in experimental fish treated with Malathion are perhaps due to damaged oxidative metabolism and pesticide induced respiratory stress. Hence, dysfunction of behavior and respiration can serve as an index of pesticide toxicity. The outcome of our research suggests that the altered rates of respiration of Tilapia may also serve as a quick biological monitor to evaluate the impacts of Malathion on other biotic communities in the aquatic sources.

Table 2. Oxygen consumption (ml of oxygen consumed/gm/hr wet wt. of fish/) of the fish, *O.mossambica* following exposure to different sub lethal concentrations of Malathion.

S.no	Hours of exposure	Control	Sub-Lethal concentrations				
			1/5	1/10	1/15	1/20	1/30
A	12	1.236+/- 0.3677	1.176+/- 0.1537	1.176+/- 0.1537	1.176+/- 0.1537	1.176+/- 0.1537	1.176+/- 0.1537
B	24	1.132+/- 0.1281	1.098+/-0.8010	1.098+/-0.8010	1.098+/-0.8010	1.098+/-0.8010	1.098+/-0.8010
C	48	0.914+/-0.1718	0.912+/- 0.0858	0.912+/- 0.0858	0.912+/- 0.0858	0.912+/- 0.0858	0.912+/- 0.0858
D	72	0.856+/-0.1137	0.850+/- 0.0607	0.850+/- 0.0607	0.850+/- 0.0607	0.850+/- 0.0607	0.850+/- 0.0607
E	96	0.758+/-01146	0.758+/- 0.0828	0.758+/- 0.0828	0.758+/- 0.0828	0.758+/- 0.0828	0.758+/- 0.0828

Conclusion: The analysis of data from the present investigation evidenced that Malathion is toxic and had profound impact on behavior and respiration in *Tilapia mossambica* in different sub lethal concentrations. The changes in the oxygen consumption in Malathion treated fish is probably due to impaired oxidative metabolism and Malathion induced respiratory stress. Hence, respiratory dysfunction and swimming behavior can serve as index of Malathion toxicity.

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