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Oxygen consumption and behaviour surveillance in the freshwater fish *Rasbora daniconius* exposed to dimethoate

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Abstract
Dimethoate, is highly contaminating aquatic ecosystems as a toxic pollutant, it was used for acute toxicity test on freshwater fish *Rasbora daniconius.* In the present investigation were the toxicity tests were conducted by static renewal bioassay method on the test fish. The LC_{50} value of dimethoate to *Rasbora daniconius* was found out to be 9.136 ppm of the LC_{50} value was selected for sublethal studies. Behavioural patterns and oxygen consumption were observed in sublethal and lethal concentration of 0 hours to 96 hours. Diemthoate is highly toxic to the animal tested. The treated fish also shows fading of their body colour, like erratic movement, jerky movement, fast swimming surfacing activity, followed hyper excitability, loss of balance, finally settles to the bottom of the test chamber. In the present investigation the oxygen consumption of fish clearly shows that the rate of consumption increases in the control group in throughout the study period at different exposure. In the lethal and sublethal concentration the rates of oxygen consumption is increases in the 48 and 72 hours and suddenly falls down in 72 hours and slightly decrease in 96 hours. Fish under sublethal concentration were found to be under stress but not fatal.

Keywords: *Rasbora daniconius*, Dimethoate, oxygen consumption, Behaviour.

1. Introduction
Pesticides are indicated to cause respiratory distress or even failure by affecting respiratory centers of the brain or the tissue involved in breathing. The effect of toxicants on the respiration of fishes and invertebrates has received aspread attention and were reviewed by researchers [1]. observed that one of the earliest symptoms of acute pesticide poisoning is respiratory distress. This serves as a tool in evaluating the susceptibility or resistance potentiality of the animal.
The behavioural changes taking place in the fish to the toxicant is a result of an inbuilt mechanism to overcome the physiological stress. The hyper excitability of the fish invariably in different concentrations of pesticides may be due to the hindrance in the nervous system [2]. Aquatic organisms are particularly sensitive to environmental contamination and pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes [3]. When fishes were exposed to different concentrations of pesticides they showed different behavioral changes which extends from mild to chronic [4, 5] reported that the respiration is a vital phenomenon of the life and the rate of oxygen consumption in turn controls the metabolic activities and changes in respiratory rates have been used as the indicator of the stress in pollutant exposed organisms. The oxygen consumption was gradually decreasing with increasing exposure periods as observed by [6, 5] in *Oreochromis mossambicus* exposed to sublethal concentrations of Quinolphenols. The rate of oxygen consumption was gradually decreases in all the exposure periods. Toxicants in the environment mainly enter into fish by means of their respiratory system [7].
From an environmental perspective, endosulfan, a persistent organic pollutant, has high aquatic toxicity and may be toxic to fishes even at recommended levels [8]. Several studies have indicated that pesticide pollution may occur at higher concentrations in small, shallow ponds [9]. Respiratory distress is one of the early symptoms of pesticide poisoning [10]. In accordance with the fluctuations in the physico-chemical characteristics of the ambient waters, the air breathing fishes are equipped with dual mode gas exchange machinery, employing

respiration using highly vascularised air breathing organs and brachial integument exchange of gases with water. These days' pesticides are used indiscriminately, which affect aquatic environment including fishes. One of the early symptoms of acute pesticide poisoning is the alteration or failure of respiratory metabolism [1]. Changes in oxygen uptake of fishes in response to pesticide exposure are varying in different fishes exposed to a variety of pesticides [11]. The effect of pesticides on oxygen consumption has been extensively studied in a number of water breathing fishes [12], [13, 14]. However, these investigators estimated only the changes in aquatic respiration even though the air breathing fishes like, *Mystus vittatus* [15] and *Channa punctatus* [16] were used in their investigations.

Aquatic animals have to pass large quantities of water over their respiratory surface and are subjected to relatively greater risk of exposure to the toxic substances [17]. Oxygen consumption forms important physiological parameters to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general [18].

Pesticides are known to modify the behavior of animals when exposed to toxic levels. The effects have been found to be multifarious and known to differ at different concentrations; viz. pesticide intoxication may induce ineffective feeding of fish [19]. The behavioral changes may be caused by the changes in the nervous system caused directly or through metabolic or physiological activities. The present study was aimed to determine the acute toxicity of dimethoate, with reference to its effects on behaviour and dysfunction in the oxygen consumption to the freshwater fish, *Rasbora daniconius*.

2. Material and Methods

The test fishes were collected from Manjara River, Latur district is in the Marathwada region in Maharashtra in India, located between 17°52' North to 18°50' North and 76°18' East to 79°12' East in the Deccan plateau. It has an average elevation of 631 meters (2070 ft) above mean sea level. The collected fishes immediately brought to laboratory without any injury. For disinfected the collected fishes are placed in 0.1% solution of potassium permagnate ($KMnO_4$) for 2 minutes so as to avoid any dermal infection. The fishes were then washed with freshwater and then acclimatized for two weeks in glass aquaria.

Healthy fishes sorted in two groups for behavioural studies, each groups of 10 fish per aquarium. Group 'A' served as control and was kept in dechlorinated tap water. Group 'B' was exposed to lethal concentration of dimethoate at 96 hours LC_{50} value 9.136 ppm. Visual observations are made after every 24 hours upto 96 hours. Behavioural studies were made by following the activities of fish, *Rasbora daniconius*, like responds of pollutant, body movement, and jerky movement, opercular movement, swimming movement, dashing against the wall of aquarium, secretion of mucous, equilibrium, colour change, galping and surfacing activity.

For oxygen consumption, fishes were regularly fed and stopped one day before the experiments. Healthy fishes divided in three batches batch A, B, and Batch C. Each batch 10 fishes were taken for the study. Batch A was considered as control, batch B was exposed to the lethal concentration of dimethoate at 96 hours Lc_{50} value 9.136 ppm and Batch C was exposed to sublethal concentration of dimethoate 1/10th of 96 hours Lc_{50} value 0.9136 ppm. The test fishes *Rasbora daniconius*, were exposed lethal and sublethal concentrations of dimethoate for duration of 96 hours and rate of oxygen consumption was determined after 24, 48, 72 and 96 hours of exposure. The oxygen consumption of control and experimental sets were measured by standard Winkler's method suggested by [20]. Each experiment repeated five times and their standard deviation were calculated. The average rate of oxygen consumption at different concentrations of toxicants was determined and values expressed oxygen consumed by the fish in ml/lit./hr/gm body weight.

3. Results

1. Control Group 'A'

In the present investigation in control Group 'A' contains ten fishes. In this group no mortality occurs throughout experimental period. A number of changes were observed in the behavior of Rasbora *daniconius.* Fishes were behaving normally, normal opercular movement, and normal skin colour. They were usually very active and showed fast well coordinated movements. They were quite alert even at the slightest possible disturbance or external stimulus like touching them with a glass rod.

2. Experimental Group 'B'

In the present investigation in experimental group'B'contains 10 fishes were exposed to dimethoate at lethal concentration i.e. LC50 of 96 hours following changes are observed. In this group fishes were behaving abnormally as compared to the fishes of control group. These were increased opercular movement, abnormal swimming movement, and sluggish, lethargic, loss of orientation and tendency of muscular tetany. The treated fish also shows fading of their body colour, like erratic movement, jerky movement, fast swimming surfacing activity, etc. In this group fast swimming and surfacing associated with gulping activities were observed. At 24 hours exposure the fishes initially increase in gulping activity and followed by decrease. Hyper excitation followed by dashing of the fishes to the wall of aquarium was another common observation. Fishes exhibited loss of equilibrium and despigmentation at the end of experiment.

In the present investigation the effect of sub lethal and lethal concentration of dimethoate on the rate of oxygen consumption at 0.0 hours is 0.4571 $\pm$ 0.016, 0.5142 $\pm$ 0.022 and 0.4142 $\pm$ 0.039 ml of oxygen /lit/hr/gm body weight of fish at control, sub lethal concentration and lethal concentration respectively. The consumption rate of fish showed decreases in lethal concentration.

The rate of oxygen consumption in control fish showed at 24 hours exposure period is 0.6142 $\pm$ 0.0180.671 $\pm$ 0.038_and 0.6857 $\pm$ 0.018 ml of oxygen /lit/hr/gm body weight of fish at control, sub lethal and lethal concentration respectively. The rate of oxygen consumption in 48, hours 0.6428 $\pm$ 0.027, 0.7428$\pm$ 0.039 and 0.8285 $\pm$ 0.038 ml of oxygen /lit/hr/gm body weight of fish at control, sub lethal concentration and lethal concentration respectively. The rate of oxygen consumption of test fish at 72 hours 0.6714 $\pm$ 0.022, 0.4857 $\pm$ 0.034 and 0.3857$\pm$ 0.031 ml of oxygen /lit/hr/gm body weight of fish at control, sub lethal concentration and lethal concentration respectively. The rate of oxygen consumption at 96 hours exposure period 0.7142 $\pm$ 0.022, 0.3428 $\pm$ 0.369 and 0.2428 $\pm$ 0.023 ml of oxygen /lit/hr/gm body weight of fish at control, sub lethal concentration and lethal concentration respectively oxygen consumption by a test fish.

The rate of oxygen consumption changes in control, sublethal and lethal concentration at 0.0 hours is + 0.057 and - 0.042, at 24 hours + 0.057 and + 0.071, at 48 hours + 0.1 and + 0.185, at 72 hours - 0.185 and - 0.285, 96 hours - 0.371 and - 0.471respectively. The effect of sublethal and lethal concentration of dimethoate on oxygen consumption depicted in table No.1 and graphically represented in fig. No.1.

Table 1: Effect of sublethal and lethal concentration of dimethoate on rate of oxygen consumption of *Rasbora daniconius.*

Exposure period	Control	Sublethal concentration	Change	Lethal concentration	Change
0.0 Hours	0.4571 ± 0.016	0.5142 ± 0.022	+ 0.057	0.4142 ± 0.039	- 0.042
24 Hr.	0.6142 ± 0.018	0.6714 ± 0.038	+ 0.057	0.6857 ± 0.018	+ 0.071
48 Hr.	0.6428 ± 0.027	0.7428 ± 0.039	+ 0.1	0.8285 ± 0.038	+ 0.185
72 Hr.	0.6714 ± 0.022	0.4857 ± 0.034	- 0.185	0.3857 ± 0.031	- 0.285
96 Hr.	0.7142 ± 0.022	0.3428 ± 0.369	- 0.371	0.2428 ± 0.023	- 0.471

Values are exressed in ml/liter/hr/gm of body weight of fish.
Each value is the mean of five observations (±S.D.)
(+) or (-) indicates percent variation over control.

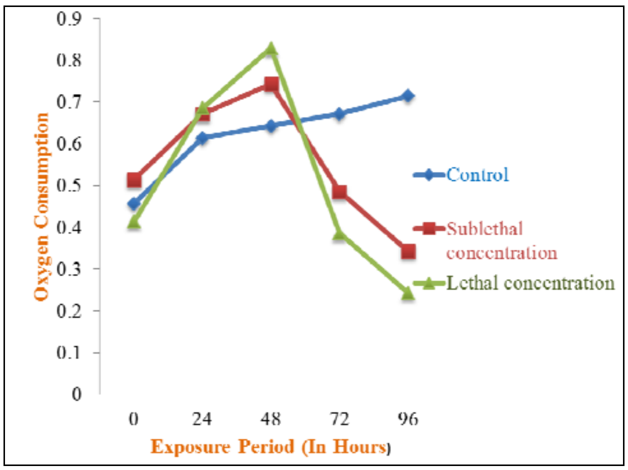

Fig 1: Effect of sublethal and lethal concentration of dimethoate on rate of oxygen consumption of *Rasbora daniconius.*

4. Discussion

Behavioral responses meet the criteria as rapid tool for bioassay testing and could be easily standardized using dimethoate as reference toxicant. The development of behavioral methods in fish as important tools in aquatic toxicology has now reached the stage where they have been standardized. Behavioral responses represent an integrated response of fish species to toxicant stress [21]. Changes in spontaneous locomotor activity and respiratory responses are sensitive behavioral indicators of sublethal exposure in fish [22]. A guide covering some general information on methods for qualitative and quantitative assessment of the behavioral responses of fish (locomotory activity, feeding, and social responses) during standard laboratory toxicity tests to measure the sublethal effects of exposure to chemical substances [23] and a guide covering information on methods to measure and interpret ventilatory behavioral responses of freshwater fish to pollutants are available. Behavioral end points have been slow to be integrated in aquatic toxicology because, until recently, there was a poor understanding of how alterations in behavior may be related to ecologically-relevant issues such as predation avoidance, prey capture, growth, stress resistance, reproduction and longevity [21, 24] working on acute toxicity and behavioural responses of common carp *Cyprinus carpio* (Linn) to an organophosphate (Dimethoate) and reported that the fishes appear excited, the swimming becomes erratic and the schooling is disprupted, surfacing frequency and gulping of surface water with occasional coughing is increased. In the present investigation similar results were observed [25]. studied on effect of dimethoate on mortality and biochemical changes of freshwater fish *Labeo rohita* (Hamilton) and reported that test organism showed normal behavior in control group but jerky movements, hyper secretion of mucus, opening mouth for fasting, losing scales in experimental group. They stated that behavioral characteristics are obviously sensitive indicators of toxicant effects. Similar behavioral observations were made in the present investigation.

Studied toxicity of ammonia, nitrate and nitrite on heamatological change of carps and reported that the demand of oxygen uptake due to stress and the results show for non-carrying capacity of blood to transport oxygen [26]. According to [27], the gill movement increased at the initial phase and gradually decreased towards the lethal phase. It is presumed that the toxicant directly or indirectly affects the respiration of fish. In the present study the dimeothate is directly affected on the oxygen consumption and the behaviour of the fish. The rate of oxygen consumed by the affected fish was very low. The decrease in whole animal oxygen consumption might be due to the damage in the structural integrity of the cells of respiratory organs [28]. reported that the symptoms of pesticide toxicity normally involve respiratory distress, the decreased oxygen consumption of the cypermethrin exposed fish is probably due to the absorbance of more pesticide through the gills [29]. have stated that cypermethrin induces respiratory and behavioral responses of the freshwater teleosts, *Labeo rohita* (Hamilton) observed that the fish exposed to a lethal concentration depicted increased oxygen consumption. [30] have observed respiratory responses and behavioural anomalies of the carp *Cyprinus carpio* under quinalphos intoxication in sublethal doses and observed that the variation in respiration rate is an indicator of stress and is frequently used to evaluate the changes in metabolism under environmental deterioration. The dimethoate induced a remarkable alteration in the rate of oxygen consumption of *Rasbora daniconius* at different exposure. In the present investigation the oxygen consumption of fish clearly shows that the rate of consumption gradually increases in the control group in throughout the study period at different exposure. In the lethal concentration the rates of oxygen consumption is increases in the 48 and 72 hours and suddenly falls down in 72 hours and slightly decrease in 96 hours. In sub lethal concentration similar trend were observed in the experimental period. The changes in oxygen consumption of fish may be due to the time of exposure and concentration of pesticide and inhibition for metabolic activity, disturb in the gill at cellular level.

5. Conclusion

In the present investigation, the rate of oxygen consumption of *Rasbora daniconius* exposed to lethal concentration of dimethoate showed considerable alterations. The rate of oxygen consumption is found to be increased initially upto 48 hours then decreased up to end of the experiment when fish exposed lethal concentration of dimethoate. Oxygen consumption clearly shows that the rate of respiration decreased as concentration of toxicant and time of exposure period increased. It may be due to toxic stress and due to disturbance of the gills. The behavioural study helps to analyse normal morphological behaviours of the fish. In the present investigation increased opercular movement, abnormal swimming movement, and sluggish, lethargic, loss of orientation, fading of their body colour, like erratic movement, jerky movement, fast swimming surfacing activity, dashing of the fishes to the wall of aquarium and loss of equilibriumin fishes were recorded. In the present investigation the above observations were made after exposure of dimethoate at 96 hours of LC_{50}, it might be due to the acculumation of toxicant in different tissues, probably caused by brain damage and reduction in conduction of nerves impulses and potential of nervous system. In the control group fishes remain active and healthy.

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