ENDOSULFAN INDUCED CHANGES IN OXYGEN CONSUMPTION AND HAEMATOLOGICAL PARAMETERS IN TILAPIA MOSSAMBICA

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Abstract: Pesticides usage in agricultural fields to control pests is exceptionally toxic to non target organisms and is responsible for loss of biodiversity. Endosulfan is one of the most controversial toxic pesticides on the market today, responsible for many serious pesticide poisoning incidents around the world. The present study is aimed to find out the effects of this potential pesticide on oxygen consumption and on behavioral characters in the blood of a fresh water fish, *Tilapia mossambica*. The toxicity of endosulfan on Tilapia mossambica and the LC_{50} values for 96 hours were determined. The oxygen consumption rate of the fish was studied under sub lethal concentration at12, 24, 48, 72 and 96 hours intervals. It was found that rate of oxygen consumption was declined along with the increase of dose and duration. Fish behavioral data were recorded and it showed abnormalities after exposure to the various endosulfan concentrations. We found few behavioral changes in experimental fish such as increase in spontaneous swimming activity, latency to respond towards mirror, aggressive response towards a perceived object, hypersensitivity, violent and jerky and movements, loss of balance, higher operculum movement, frequent jumping and swimming at the water surface, spiraling, escape attempts from the aquarium were noticed. Decrease in both in respiratory rate and in body weight ($P<0.05$) was observed in all treated groups. This suggests that endosulfan contamination has the potential to affect the physiological and behavior of free-living fishes. Results also indicate that endosulfan is potentially toxic to the fish *Tilapia mossambica* and the stress response of fish are dependent on concentration and duration. Alternatives to reduce the effects pesticides were also given in this paper.

Keywords: Pesticide, Endosulfan, *Tilapia mossambica,* oxygen consumption

Introduction: Pesticide pollution is an area of global concern due to their greater toxicity and persistence in the aquatic environment. Increasing population and urbanization has resulted in rapid erosion of land surface and demand more food which forces higher yield of crop per hectare. To meet up this demand, farmers have started using larger volumes of pesticides which enhanced the faster growth of pesticides consumption [1]. But most of the pesticides or insecticides reach a place other than their target spaces. They can travel by both wind and water for long distances and potentially poisoning not only to the human health but also to other species in the wild. Therefore, the regulation of these chemicals are very important as it causes a great lose to biodiversity [2].

Endosulfan is highly toxic and controversial agrichemical. It is an endocrine disruptor and can potentially accumulate in the tissues. Being highly toxic even EPA (1985) also kept this chemical pesticide in category.1 [3]. Because of these threats to both human health and to the environment, a global ban on the manufacture and use of endosulfan was approved under the Stockholm Convention in April 2011. As a result more than 80 countries including the European Union had already banned it. But unfortunately it is still used extensively in India, China, and few other countries. According to a 2012 report by Research and Markets, India is now the second largest manufacturer of pesticides in Asia after China and ranks sixth globally. The entire global market for pesticides is worth around $44 billion and is projected to grow to $65 billion in the next few years [4].

Fish is a sensitive indicator of the quality of aquatic environment. Fish are responding to various stressors by a series of biochemical and physiological stress reactions comparable to those of higher vertebrates. They are genetically adopted to live in diverse water environments but unexpected change in water quality affects the physiology and metabolic activities of the fish [5]. . Therefore, the present study is aimed to find out the impact of this toxic endosulfan on oxygen consumption and behavioral changes in locally available fresh water fish, Tilapia mossambica.

Materials and Methods: The toxicant endosulfan Thiodan 35 EC® (Endosulfan 35%EC, Bayer Crop Science Ltd., Ankaleshwar, and Gujarat) has been used for the present study. It is a broad spectrum pesticide and acaricide containing the technical Endosulfan 35 % w/w, solvents and emulsifiers' etc. 65% w/w.Tilapia is a genus of cichlid fishes prevalent to freshwater habitats. Living healthy specimens of fishes ranging in weight 10 gm to 15 gm and in length from 6 cm to 10 cm (irrespective of the sex and age) were procured from local fresh water sources and acclimatized for lab conditions for 10 days. During this period, fish were fed every day with commercially available fish food twice a day (oil cake mixed with rice flour). The water in aquarium was changed daily. A stock solution of commercial grade endosulfan was prepared using double distilled water. Successive dilutions of the stock solution were also prepared using earlier aerated and stored tap water. Each fish was weighed before and after the experiment and placed in its respective test chamber. After acclimation for 10 days, healthy fish were selected from stock and transferred to another glass tank. Feeding was stopped one day before the commencement of the experiment. Some fishes were used for the determination of LC_{50} value and it was found to be 0.05 ppm for 96 hrs. Acclimatized fishes were divided into 4 groups and exposed to sub lethal concentration of endosulfan with different sub lethal concentrations. Group I control, Group II, Group III and Group IV were treated with 0.001(1/20) ppm.0.002 ppm (1/40) and 0.0.025 ppm (1/50) respectively during 12,24,48,72 and 96hrs of exposure. The amount of oxygen consumed by the fish was expressed in ml/gm/hr of the tissue. Water was changed for every 24 hrs to maintain the concentration of pesticide. The bioassays were done in the morning and behavioral changes were noted. The mortality and survival was also recorded. Oxygen consumption was estimated in static system through respiratory apparatus developed by Job [6] and the amount of dissolved oxygen (DO) in water was estimated by modified Winkler method [7].The difference in the dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish. Observations of behavioral symptoms such as movement, respiration, swimming, food intake and response to the outer effects were also recorded. The data were expressed as mean ± S.D. Mean value for each group of fish was tested for significance by student's t-test to establish the validity of the findings.

Results and Discussion: The respiratory potential and the oxygen consumption of an organism is the important physiological biomarker to assess the toxic stress as it reflects the energy expenditure during metabolism [8]. The rate of oxygen consumption (ml oxygen consumed per gram wet weight of fish per hour) of control and treated fishes are presented in Table 1. The average rate of oxygen consumption in control fish after 96hours was found to be 0.39.06±0.146 ml/hr/ g tissue). Rates of oxygen consumption after exposure to 0.01, 0.02 and 0.025 ppm of endosulfan after 12, 24, 48, 72 and 96 hours in Tilapia mossambica varied with pesticide concentration and duration. At 24 hours in all experimental groups the rate of oxygen consumption was elevated significantly. This elevation could be explained in terms of acceleration of oxidative metabolism during the initial hours of exposure, as a result of sudden response to the toxic stimulus of pesticide. Respiratory distress is one of the early symptoms of pesticide poisoning [9]. The decline in respiratory rates is possibly by variation of energy metabolism [10]. In the present investigation we found a significant negative dose-response of endosulfan in this fish. With increasing exposure duration, there was a parallel decrease in the oxygen consumption up to 96hours (Table 1). The maximum oxygen consumption was observed at 0.001 ppm whereas the minimum content was recorded at 0.025ppm.There is no significant difference in oxygen consumption between control and experimental fish exposed to 12hours duration. The rate of oxygen consumption was gradually decreased along the increase in dose and exposure time significantly.

The experimental data of the present study reveals that oxygen consumption decreases with the time of exposure to the pesticide and is well supported by Pillai and Diwan [11].The decreased oxygen consumption in endosulfan-exposed fish may be due to the absorbance of a greater amount of pesticide through the gills which are in direct contact with the toxic medium as in the freshwater crab *Trichodactylus borellianus* [12] and in a teleost fish *Macrognathus aculeatum* [13]. The decrease in oxygen consumption at sub lethal concentration of the toxicant appears to be lowering of energy requirements [14]. The study of Shreena et al [15] also reveals that low concentration of pesticide (dimethoate) in Tilapia mossambica can cause stress and create respiratory disturbance. The other ingredients present in the pesticide may also contribute the cumulative effects. The depletion of the oxygen consumption is due to the inadequacy of the respiratory action may be due to rupture in the respiratory epithelium of the gill tissue as in some other species [16], [17].

Table 1. Mean rate of oxygen consumption in *Tilapia mossambica* ((ml/gm. /lit/hr.) at different endosulfan (EC 35) concentrations and at different time periods with standard error of mean values.

Experimental Groups	Fish No.	12 Hrs	24hrs	48hrs	72hrs	96hrs
Control,Group I	10	0.39 ±0.002	0.37±0.001	0.42±0.001	0.44±0.003	0.41±0.01
Group II(0.01ppm)	10	0.48 ±0.001*	0.29 ±0.003*	0.23 ±0.001*	0.19 ±0.001*	0.21 ±0.005*
Group III(0.02ppm)	10	0.46 ±0.002*	0.28 ±0.002 *	0.22 ±0.001*	0.20 ±0.001	0.19 ±0.002
Group IV(0.025ppm)	10	0.48 ±0.001	0.27 ±0.001	0.21 ±0.003**	0.18 ±0.002	0.17 ±0.004***

* Significance at $p<0.05$, ** Significance at $p<0.01$, *** Significance at $p<0.001$

Experimental fishes from different groups showed behavioral abnormalities approximately 1 h. after exposure such as hyper swimming activity, hypersensitivity, erratic and violent movement, loss of balance, hyperactivity, increase operculum movement, regular jumping, swimming at the water surface, spiraling, convulsion, escape attempts from the aquarium, hitting to the walls of the aquarium before finally sinking to the bottom. The exposed fishes exhibit shivers and gradual declining of reflexes leading to imbalance in posture and loss of equilibrium. Finally, they surrender to poison with mouth and operculum wide open, molting of body color changes from silvery white to pale white. At lower concentration, however, the changes in behavior are conspicuous. Results also showed fish loss appetite of food. Abnormalities behavior observed in all treated groups but the severity of signs increased with time and high concentration of pesticide.

Conclusions: The conclusion may be drawn from the above study that endosulfan is highly toxic and can cause the death of the fish by affecting the rate of oxygen consumption. Long term exposure of organisms to pesticides means a continuous health hazard for the population. So, human population is at high risk by consuming these toxicated fishes. This implies that one should take the necessary precaution in the application of pesticides to protect the life of fish and other non target species. It is expected that modern approaches using molecular biology techniques will change toxicological applications that are cheaper and do not require the use of organisms to detect environmental stressors. As pesticides are highly toxic to non-target organisms like fish, it has become vital to formulate rigorous rules against indiscriminate use of this pesticide. It is, therefore, a subject of great public health significance to regularly monitor the pesticide residues in foods and humans.

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