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TOXIC IMPACTS OF THIODAN 35 EC® ON PROTEIN, GLYCOGEN AND OXYGEN CONSUMPTION IN *TILAPIA MOSSAMBICA*

Rita Ganava[1*], Reena Ganava[2], R. R. Kanhere[3] and Hemant Pandit[4]

[1, 2,] Govt. PG. College, Jhabua.
[3]PSC Exam Controller.
[4]Medicaps College, Indore.

***Author for Correspondence: Dr. Rita Ganava**
Govt. PG. College, Jhabua.

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ABSTRACT
Endosulfan is one of the most disputed toxic pesticides and is responsible for many severe pesticide poisoning incidents around the world. The present study is aimed to find out the effects of this pesticide on whole body oxygen consumption and protein and glycogen content of the liver a fresh water fish, *Tilapia mossambica*. The toxicity tests were conducted by static renewal bioassay method on the juveniles of fish Tilapia mossambica (Peters). The probit analysis showed that the LC50 (lethal concentration) value of endosulfan (Thiodan 35 EC®) for 24, 48, 72 and 96 h were 0.9, 0.5, 0.1 and 0.05ppm for 96 hours. The teleost fish Tilapia mossambicus of 15±1g was exposed to three different sub lethal concentrations of Thiodan 35 EC® for 24,48,72,96 hours. Hepatic metabolic parameters like protein and glycogen were also determined. Results clearly shown that rate of oxygen consumption were declined along with the increase of dose and duration. Decrease in both in respiratory rate and in body weight ($P<0.05$) was also observed in all treated groups. One third (30 µg/L) one tenth (5 µg/L) and one fifth (10 µg /L) of the LC50 values were selected for sub lethal studies. An initial increase in the oxygen consumption was observed in low sub lethal concentrations ($1/5^{th}$ and $1/10^{th}$ exposure) but thereafter a sharp decrease in oxygen consumption was observed in all sub lethal concentrations. Results also clearly revealed that protein, glucose and glycogen content of liver were significantly reduced in all experimental groups treated with sub lethal concentrations of endosulfan. The reduction in glycogen content of liver in all the experimental fishes might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia was observed which has also been reflected in reduction in oxygen consumption. 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 hepatic metabolic markers like glucose and glycogen can be used as an index of Parathion toxicity.

KEYWORDS: Pesticide, Endosulfan, glycogen, *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 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. Many parts of the world have fallen prey to this pesticide that has affected a lot of humans, animals and the environment. The Supreme Court of India had passed an interim order on May 13, 2011, as a major number of victims were reported to be affected in Kasargode (Kerala) and banned the production, distribution and use of endosulfan.

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. They are adopted to live in diverse water surroundings but any unexpected change in water quality affects their physiology and metabolic activities.[5] Liver is the first organ where pesticide is metabolized and detoxified.[6] Therefore, the present study is aimed to find out the impact of this toxic endosulfan on oxygen consumption and metabolic parameters like protein and glycogen of liver in locally available fresh water fish, Tilapia mossambica.

MATERIALS AND METHODS

Chemicals

The pesticide endosulfan Thiodan 35 EC® (Endosulfan 35% EC, Bayer Crop Science Ltd., 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.

Fish sampling and acclimatization

Tilapia is a genus of cichlid fishes and common to freshwater habitats. Living healthy specimens of fishes ranging in weight 15±1g and in length from 8 cm to 10 cm of either sex or 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.

Preparation of stock solution

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.

Calculation of LC50 value

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 three sub lethal concentration of endosulfan with different sub lethal concentrations. One third (30 µg/L) one tenth (5 µg/L) and one fifth (10 µg /L) of the LC50 values were selected for sub lethal studies Group I control, Group II, Group III and Group IV were treated with 1/3,1/10 and 1/5 respectively during 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[7] and the amount of dissolved oxygen (DO) in water was estimated by modified Winkler method.[8] 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.

Total protein estimation

The total Protein content of was estimated according to modified standard method of Lowery et al.[9] An amount of 5% homogenate of liver was isolated and precipitated with 5% trichloro acetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. The precipitate was dissolved in 1 ml of 1 N NaOH solution and 0.2 ml of extract taken into test tube and mixed with 5 ml of alkaline copper solution was added. To this 0.5% ml of 50% folin phenol reagent was added. In the time following 30 minutes, the optical density was measured at 540 nm against a blank. The standard graph was plotted by using Lowry method with bovine serum albumin. The values were expressed as mg/g wet weight of the tissue.

Glycogen estimation

The glycogen was estimated by the standard method of Kemp et al.[10] A 2% homogenate of liver tissue was prepared in 80% methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of trichloro acetic acid (TCA), boiled for 15 minutes at 1000C and then cooled in running water. The solution was made up to 5 ml with TCA to compensate the evaporation and then centrifuged. From this, 2 ml of supernatant was taken into the test tube and 6 ml of concentrated H2SO4 was added and the mixture was boiled for 10 minutes. The mixture was cooled and the optical density was measured at 520 nm. The standard graph was plotted with D-glucose by using the above mentioned method. The glucose was converted to glycogen by the multiplication factor of 0.98[11] and is expressed as mg of glycogen/g wet weight of the tissue.

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

Table1. 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 Thiodan 35 EC®.

S.No	Hours of exposure	Control	Sub-Lethal concentrations		
			1/3	1/5	1/10
A	24	1.132+/- 0.1281	1.198+/-0.110	1.298+/-0.110	0.98+/-0.010
B	48	1.914+/-0.1718	0.902+/-0.0558	0.812+/-0.018	0.812+/-0.058
C	72	1.056+/-0.1137	0.850+/-0.0407	0.780+/-0.0407	0.761+/-0.007
D	96	1.158+/-01146	0.751+/-0.072	0.718+/-0.0328	0.708+/-0.028

Table 2: Effect of sub lethal concentration of Thiodan 35 EC® on total proteins and total glycogen content in liver in *Tilapia mossambicus.*

Hours of exposure	Control(X ± SD)		Treated with Sub-Lethal concentrations(X ± SD)					
			1/3		1/5		1/10	
	Protein(mg/g)	Glycogen (mg/g)	Protein (mg/g)	Glycogen (mg/g)	Protein (mg/g)	Glycogen (mg/g)	Protein (mg/g)	Glycogen (mg/g)
24	151.11	39.18	131.11	38.18	130.2	41.18	141.11	48.08
48	149.57	40.21	130.17	33.14	129.11	31.10	126.14	27.11
72	159.2	40.31	119.12	28.11	115.01	24.2	114.12	20.11
96	148.44	41.21	118.14	22.03	98.14	18.41	89.53	17.81

Results are mean (X ± SD) of 5 observations indicates the standard deviation values and are significant at $P < 0.05$.

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.[12] 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 1/3, 1/5/ and 1/10 endosulfan after 24, 48, 72 and 96 hours in *Tilapia mossambica* varied with 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.[13] The decline in respiratory rates is possibly by variation of energy metabolism.[14] In the present investigation we found a significant negative dose-response of Thiodan 35 EC® in this fish. With increasing exposure and 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 1/3 group.

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.[15] The decreased oxygen consumption in Thiodan 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*[16] and in a teleost fish *Macrognathus aculeatum*.[17] The decrease in oxygen consumption at sub lethal concentration of the toxicant appears to be lowering of energy requirements.[18] The study of Shreena et al[19] also reveals that low concentration of pesticide (dimethoate) in *Tilapia mossambica* can cause stress and create respiratory disturbance. 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.[20, 21]

Experimental fishes from different groups showed behavioral abnormalities approximately 1 h. after exposure such as hyper swimming activity, loss appetite, erratic and darting swimming movement and loss of equilibrium. This abnormal behaviour may due to inhibition of an enzyme acetylcholine esterase (AChE) activity leading to accumulation of acetylcholine in the end bulbs of neuron at synapses ending up with hyper stimulation.[28] Abnormalities behavior observed in all treated groups but the severity of signs increased with time and high concentration of pesticide.

Biochemical analysis of total protein and glycogen

The results are summarized in Table 2**.** The change in the biochemical parameters (increase or decrease) is dependent on the health status and metabolism of the individual. Most of the toxicants including pesticides act as metabolic depressor and influence the metabolism of biologically active molecules such as proteins, glycogen, and carbohydrates and lipids.[22] In the present study total protein content significantly and gradually decreased in

experimental fish gradually along with increase in the pesticide concentration. The depletion in protein content in liver might be due to the damaged or low protein synthesis under the toxic stress condition as reported the earlier workers.[23,24,25] Besides, special catabolic reactions like proteolysis, formation of lipoproteins to repair the cell and to fulfill energy requirement in cells are also responsible for reduction of protein content.[26,27]

Glycogen is a polysaccharide and major reserve food and first source of energy for many animals. It plays a significant role in the glucose cycle that can be quickly transferred to meet an unexpected need for glucose.[29] In the present experiment, results clearly indicated that the glycogen content of liver was decreased with increased Thiodan concentration. The reduction of liver glycogen content in all the experimental fishes might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia which has also been reflected in oxygen consumption. Similar reports were observed in common carp *Cyprinus carpio* exposed to sub lethal concentrations of endosulfon showing a decrease in levels of plasma glucose and slight variation in the serum protein.[30] The depletion in glycogen content may also due to active glycogenolysis and glycolytic pathway to provide excess energy in stress condition.[31] Similar findings were also found in the glycogen content of the liver of *Colisa fasciatus* and *Sarotherodon mossambicus* exposure to various toxicants including Thiodan and Arsenate.[32, 33, 34, 35, 36] In the present investigation the reduction in liver glycogen might be due to the hypoxic condition created by pesticides during the period of experimentation.

CONCLUSION

The conclusion may be drawn from the above study that endosulfan is highly toxic and can cause the death of the fish by affecting protein and glycogen depletion and the rate of oxygen consumption. We also found that the sub lethal exposure of the Thiodan proved to be highly toxic to *Oreochromis mossambicus* which affected the rate of oxygen consumption, depletion in total proteins and glycogen levels in liver. The reduction of liver glycogen content in all the experimental fishes might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia which has also been reflected in oxygen consumption. The depletion in protein content in liver might be due to the damaged or low protein synthesis under the toxic stress condition or due to special catabolic reactions like proteolysis, formation of lipoproteins to repair the cell and to fulfill energy requirement in cells.

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