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Research Article

RESPIRATORY RESPONSE OF *CIRRHINUS MRIGALA* (HAMILTON,1822) FINGERLINGS EXPOSED TO SUB LETHAL CONCENTRATION OF FENVALERATE

[1]Baskaran A, [2]Stephenraj D, [3]Balachandar M, [1]Narayanan M and [1]*Azhagu Raj R

[*1]Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai-627 002, Tamil Nadu, India
[2]Department of Zoology, St. John's College, Palayamkottai,627002,Tamil Nadu, India
[3]Department of Zoology, Loyola College (Autonomous), Chennai- 600 034, Tamil Nadu, India

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ABSTRACT

Pesticides are toxic to aquatic fauna, which form significant components of the food chain. In this study to estimate, the lethal concentration LC_{50} of fenvalerate and sub-lethal concentration of Fenvalerate on the respiratory metabolism of *Cirrhinus mrigala* fingerlings was recorded in laboratory condition. The median lethal concentration of fenvalerate was estimated, by exposing different concentration such as 0.01, 0.05, 0.1, 0.15 and 0.2 ppm of fenvalerate. The profit analysis, it was estimated that the LC_{50} value for 96 hrs for the pesticide fenvalerate was calculated as 0.025 ppm. The rate of oxygen consumption increased from 0.44 mg/g/hrs to 0.67 mg/g/hrs. When the partial pressure increase from 80 to 100 (mm/hg) in the exposed to 0.00083 ppm. The rate of oxygen consumption shoots us in 0.00083 and 0.00125 ppm concentration of fenvalarate, when compared with control. In the present investigation, the decline of oxygen consumption was observed in the subsequence exposure of Fenvalerate.

Keywords: Respirometer, Indian Major Carp, Bioassay, Pesticides and Toxicity.

INTRODUCTION

Pesticide is the most important factors in improving agriculture production. They are used to destroy any organism that isconsidered, as pest. They are persistent and non-biodegradable with their residues lasting in our environment. They kill not only the pest but also the non-target organism like fish and other aquatic fauna and enter into food chains. Among pesticide chlorinated compounds are most prevalent toxicant in the aquatic environment. Fenvalerate is one of the potent pyrethroid insecticides. It acts both as a contact and stomach poison of the pests on crops of cotton, vegetable and fruits. It is extremely toxic to certain aquatic and marine group of animal including fish (Khan 1983). It has undergone rapid development as an important agriculture pesticide and several workers have extensively studied its toxicological properties (Lee *et al.*1985).

A large number of pesticides are mixed regularly in water bodies where fishes encounter with them and develop various metabolic abnormalities. Pesticides accumulate in fishes and in turn hamper human health via ecological and biological magnification (Moore and Waring, 2001; Fulton and Key, 2001). Pesticide represents a diverse group of poisonous chemical, which are primarily used to control insect pest species. They are used environmentally in agriculture for forest protection and in public health program. Acute toxicity of deltamethrin and fenvalerate has already been recorded by group in the fishes (Tandon *et al*., 2005). The present laboratory investigation was aimed to study the medium lethal concentration as respiratory metabolism of freshwater fish, *Cirrhinus mrigala* (Hamilton) response to sub-lethal concentration of fenvalerate.

*Corresponding Author: Dr. R. Azhagu Raj, Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai-627 002, Tamil Nadu, India, Email: drazhaguraj@gmail.com.

MATERIALS AND METHODS

Fish collection and acclimatization

Freshwater fish of *Cirrhinus mrigala* (Hamilton) Cyprinidae and order: Cypriniformes were collected from Fish Hatchery, Kallidaikurichi in Tirunelveli district. The fish were brought in plastic buckets without any mechanical injury and kept in aquaria for a week to get acclimated to the laboratory condition. Water in the tanks was changed every day to ensure sufficient oxygen supply to fish and they were fed with formulated feed rice bran and oil cake.

Experimental design

The experiments were carried out, in five plastic troughs of ten-liter (10L) capacity. The five plastic troughs were grouped in to twelve and each group consists of five troughs. The weight of the fishes was measured, by the help of a physical balance and was kept same for all the five troughs for an experiment, which was around 4.5 gm for an individual fish**.** The percent mortality was noted for LC_{50} value for Fenvalerate (20% EC) was determined and a low sub-lethal concentration corresponding to one tenth of LC_{50} value was taken for experiments. Twelve fishes were kept in each test concentration using plastic troughs of ten-liter capacity. The fishes were exposed to sub-lethal concentration (0.01, 0.015, 0.02 ppm) for fifth day. Control was kept simultaneously.

LC_{50} is a concentration in which 50% of the experimental animal survives. Estimation of LC_{50} by interpolation involves plotting of data in a graph with concentration on X-axis, while percentage survival on Y-axis. A straight line was drawn at two successive concentrations that were lethal to more and less than half of the total number of test animal exposed to toxicant. The concentration at which this line crosses the 50% survival line in the LC_{50} value (Litchfield & Wilcoxon, 1949). After completion of experimental period, the fishes were subjected to sub-lethal concentration (0.1) of the pesticide for determining the rate of oxygen consumption. The oxygen consumption was determined using the Winkler's method (Welsh & Smith 1961).The Winkler test was used to determine the level of dissolved oxygen in water sample.

Routine metabolism

Respirometer used in the present study. The respirometers were of closed type model. They were wide mouth borosil flasks of 575mL capacity closed with one holed rubber stopper fixed with a thistle funnel. The thistle funnel went atleast 2/3 of the flask which server as the inlet. The outlet was a slide glass tube fixed close to the bottom of the flask to which a rubber tube was attached and was kept closed with a pinchcock. The fishes were taken in conical flask and were tightly closed after filling them with tap water. After the introduction of the respective density of fishes into the respirometers, the sampling of water was done with an interval of 30 minutes. At every time of experimentation, required number of healthy experimental animals were led to the respirometer with much care and little stress to them. The respirometers were closed airtight. At the time of sampling the pinch cock was released and a few drops of water was deliberately allowed to flow down as it might be the stagnant water in the rubber tube and the samples were collected in the micro Winkler's bottle.

To compensate water loss from the respirometer, while sampling, a little quantity of water poured into it through the thistle funnel. Before sampling waters from the respirometers. Sampling was done every 30 minutes and continued up to three hours (3hrs) or till the death of the experimental animal inside the respirometer. The experimental sample was analyzed for the dissolved oxygen content using unmodified Winkler's method (Reish *et al.* (1978). In order to minimize the quantity of sampling, Water to analyses the dissolved oxygen content Micro-Winkler's bottle (8.5, 8.7, 8.9, 9 mL) were used. In principle, the concentration of oxygen was measured at the inflow and at the out flow of the animal chamber, by unmodified Winkler's method (Reish *et al.*, 1978).

The concentration of the oxygen is measured by using the formula:

$$PO_2\ (mgo_2/l) = \frac{N \times ml\ of\ Titrant \times 8 \times 1000}{Vs\ \frac{VR-V}{V_R}}$$

Where,

N = Normality of the titrant,

Vs = Volume of the sample used for titration,

V_R = Volume of the Reagent bottle after placing the stopper.

V = Volume of Reagents added.

Respirating animals reduce the inflow oxygen content of the chamber water until a steady state is reached.

$$PO_2 = \frac{X \times 160}{7.77}$$

Where, $X = \frac{O_2\ in\ control\ sample + O_2\ in\ experimental\ sample}{2}$

RESULTS AND DISCUSSION

In the present investigation experiment were conducted, to study to estimate the median lethal concentration of fenvalerate and three sub-lethal concentration of fenvalerate on the respiratory metabolism of *Cirrhinus mrigala* fingerlings. The median lethal concentration of fenvalerate was estimated, by exposing different concentration such as 0.01, 0.05, 0.1, 0.15 and 0.2 ppm of fenvalerate. Data collected from the mortality percentage were subjected to log profit analysis and LC_{50} for 12, 24, 48, 72, and 96 hrs. LC_{50} for 96hrs (Table 1) gives overall log dose profit regression analysis for 12, 24, 48, 72 and 96hrs for the experimental fish *Cirrhinus mrigala*. From the profit analysis, it was estimated that the LC_{50} value for 96 hrs for the pesticide fenvalerate was calculated as 0.025 ppm (Table 2). Pesticide is most potent and prevalent

toxicant in the aquatic environment. The search of alternatives to bio accumulating pesticide phyrithoid came to risky. It is also found to be toxic to sudden aquatic and marine groups of animals including fish (Khan 1983). It has undergone rapid development, as on important agriculture pesticide and several workers here extensively study is toxicological property (Lee *et al*., 1985, Shaikh and Yeragi (2004).

The medium lethal concentrations of fenvalerate on the fingerlings of *Cirrhinus mrigala* were calculated to be 0.025 ppm. However, Tenden *et al.*, 2005, reported ½ of LC50 value as 0.003 ppm in the fish *Catlacatla*. It is found to be too little as compare to 0.025 ppm in the cause of *Cirrhinus mrigala*. The variation may be due to the environmental problem and the size of the fishes. Tanden *et al*., 2005 used the fingerlings of *Catla* sp. to the tune of 1.5 gm and temperature 28 $\pm$ 2° C. In the present investigation, the fish used were of 4.5gms and the temperature was 30 $\pm$ 1° C. This may be the reasons for variation of LC50. Shaikh and Yeragi 2004 also reported sub lethal concentration of fenvalerate on the freshwater fish *L.thermalis*. They have also studied the respiratory metabolism exposed to different sub lethal concentration of fenvalerate. The sub lethal concentration selected by Shaikh and Yeragi 2004, were 0.002, 0.003, 0.004, which is very close to the 1/10 LC50 value of the present experiment (0.025ppm). It conforms that the LC_{50} value of the present experiment is very closed to the value obtain in the present investigation.

The oxygen consumption is one of the most important energetic physiological phenomenons, which control the metabolic activities. It is an indicator of metabolic activities. It is an indicator of metabolic rate and status of stress condition of animal. Many investigators are Srinivasan, 1965; Nagabhusnam, 1966; Deshmarkh, 1972; Mane and Talikhedker, 1976; *etc*, studied the effect of heavy metals or respiration of several fishes. The fishes *Cirrhinus mrigala* (Hamilton)were exposed, to 0.01, 0.15 and 0.20 ppm of fenvalerate independently. The rate of oxygen consumption was determined under each concentration. The values of oxygen consumption were determined under respective concentrations of pesticide were compared with the control maintained simultaneously. It is evident that the rate of oxygen consumption shoots us in 0.00083 and 0.00125 ppm concentration of fenvalarate respectively, when compared with control. Further increase in concentration lead to depletion of oxygen consumption by the fish. Showing the effect of pesticide on the metabolic pathway due to the stress. The initial increase in the rate of the oxygen the oxygen consumption by the fish could be attributed, to the sudden stress caused to the fish leading to increased frequency of the gill opening. However, further increase in concentration showed more stress when activity of the organisms goes down resulting into the depletion in the oxygen consumption.

Substantial information on effect of toxicants resulting into the stress and affecting the metabolic pathway of different freshwater fishes has been dealt by Nagaratnamma and Ramamurthi (1982) and Koundinya and Ramamurthi (1979). Reddy*et al.,* (1987-1988) reported an increase in endosulfan concentration leads to depletion of oxygen consumption in a fresh water fish, *Netopterus notopterus*. Baby and Menon (1976), Kapoor and Lomte (1987), Prakasam *et al.,* (1989), Rao *et al*., (1991), Muley (1991), Masarrat sultana and Lomte (1998), Kulkarni *et al.,* (1983) and Savant and Amte (1992) also observed a reduction in oxygen consumption in their respective animals at different sub-lethal concentrations of heavy metals and/or pesticides. Reish *et al.,* (1978) have suggested that exposing animal to support enhanced physiological activity in metabolizing and eliminating the pollutant utilizes part of increased oxygen. Velmurugan *et al.,* (2007) reported that sub-lethal concentrations of fenvalerate damaged the *Cirrhinus mrigala* gills, particularly lamellae, epithelial hyperplasia and epithelial necrosis. Anita Susan *et al.,* (2012). Studied that the effect fenvalerate on Indian major carps. The fenvalerate persuaded extraordinary changes in energetic tissues like Gill, Liver and Kidney. The concentration of pesticides is increased, into the environment due to the various industrial activities. The potential ecological effects of rising level of heavy metal concentrations in the environmental and animal tissues have evoked increasing concern (Baby and Menon 1976). Parvaiz Ahmad Ganie, *et al.* (2018) Investigated the acute toxicity and behavioral response of Indian major carp *Cirrhinus mrigala.* Short-term exposure of cypermethrin altered behavior of experimental fish *Cirrhinus mrigala* such as, fast swimming, Imbalance and Hyperactivity were noticed. Vani *et al.,* (2020) observed that the *Cirrhinus mrigala* tissue protein, glycogen level was changed due to the short term exposure of Carbamate Insecticide.

Table1. Mortality value of *Cirrhinus mrigala* exposed to different concentration of fenvalerate at different hours.

Concentration [ppm]	No.of fish	Time [12hrs]	Time [24hrs]	Time [48hrs]	Time [72hrs]	Time[96hrs]
0.01	12	0	8.34	25	33.34	41.67
0.05	12	8.34	25	41.67	80	58.34
0.10	12	25	41.67	50	58.34	75
0.15	12	41.67	58.34	75	83.34	100
0.20	12	66.67	75	91.67	100	100

Table 2. Lethal concentration (LC_{50}) of *Cirrhinus mrigala* exposed to fenvalerate at different hours.

Exposure duration [hrs]	X^2	Regression equation [y=a+bx]	Lc50 [%]	Fiducial limits		Variances	Relative toxicity
				Lower limit	Upper limit		
12	0.29	Y=4.083X + 0.19	0.151	0.9566	1.3993	0.0128	1.000
24	0.47	Y=1.502X + 3.45	0.108	0.7158	1.3482	0.0260	1.398
48	1.70	Y=1.368X + 4.03	0.051	0.3101	1.1038	0.0410	2.960
72	2.86	Y=1.681X + 4.03	0.038	0.1160	1.0342	0.0549	3.974
96	3.19	Y=1.979X + 4.20	0.025	-0.0943	0.0646	0.0646	6.04

Table 3. The effect of PO_2 (mm/ hg) on the rate of metabolism of *Cirrhinus mrigala* response to three different sub lethal concentration of fenvalerate.

	PO_2							
Concentration	70	75	80	85	90	95	100	105
0.00083	0.26	0.31	0.44	0.5	0.56	0.62	0.67	-
0.00125	-	-	0.03	0.11	0.18	0.24	0.29	0.33
0.0025	-	0.068	0.08	0.086	0.089	0.092	0.095	-

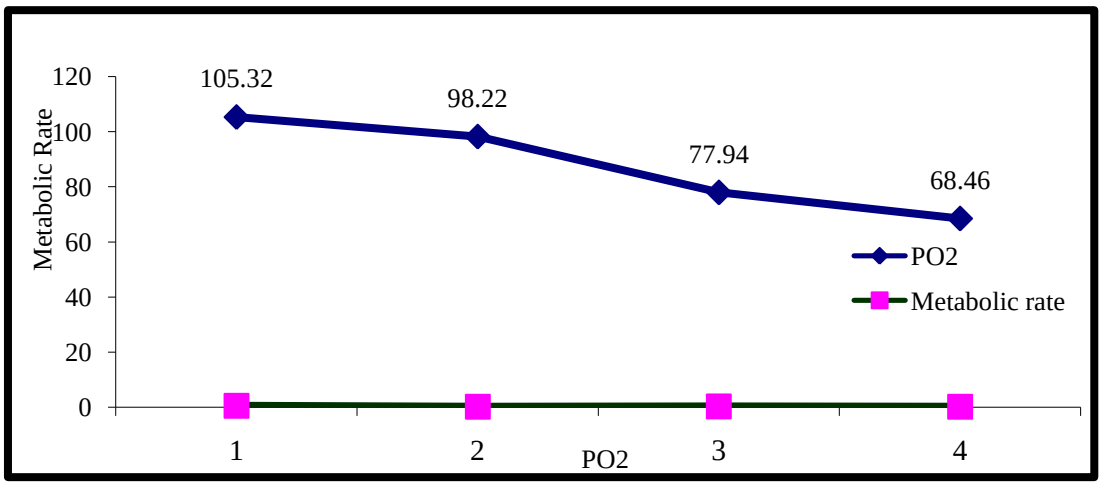

Figure1. Metabolic rate of *Cirrhinus mrigala* exposed to sun-lethal concentration 0.01ppm.

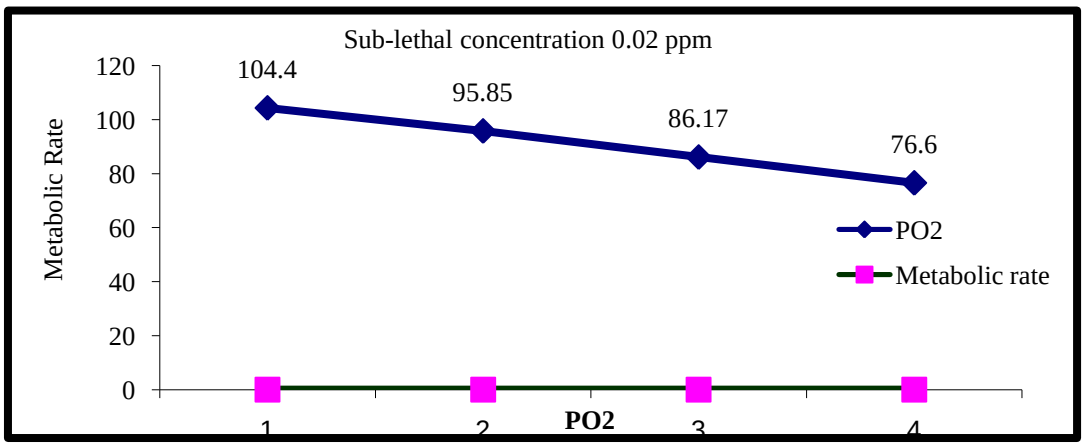

Figure 2. Metabolic rate of *Cirrhinus mrigala* exposed to sun-lethal concentration 0.02ppm.

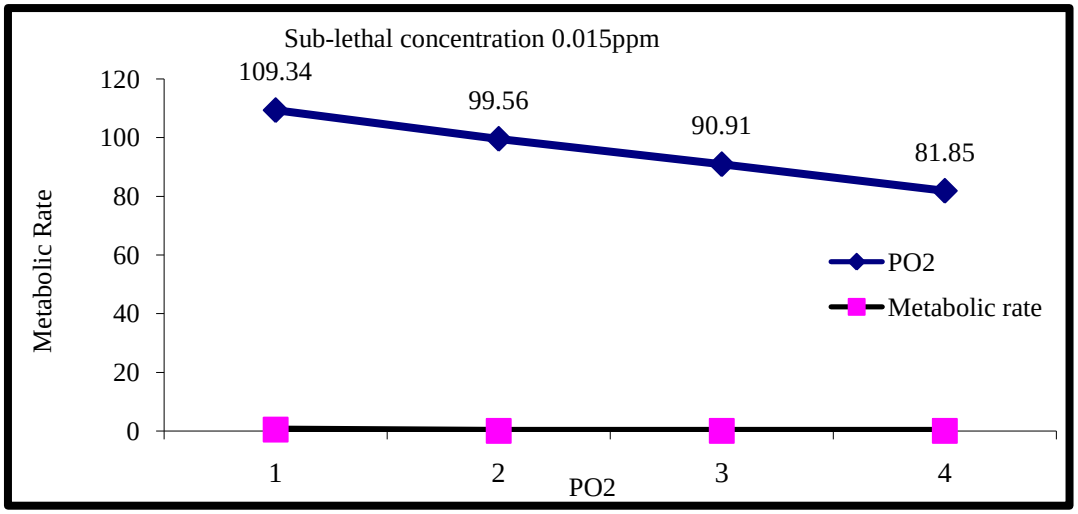

Figure 3. Metabolic rate of *Cirrhinus mrigala* exposed to sun-lethal concentration 0.15ppm.

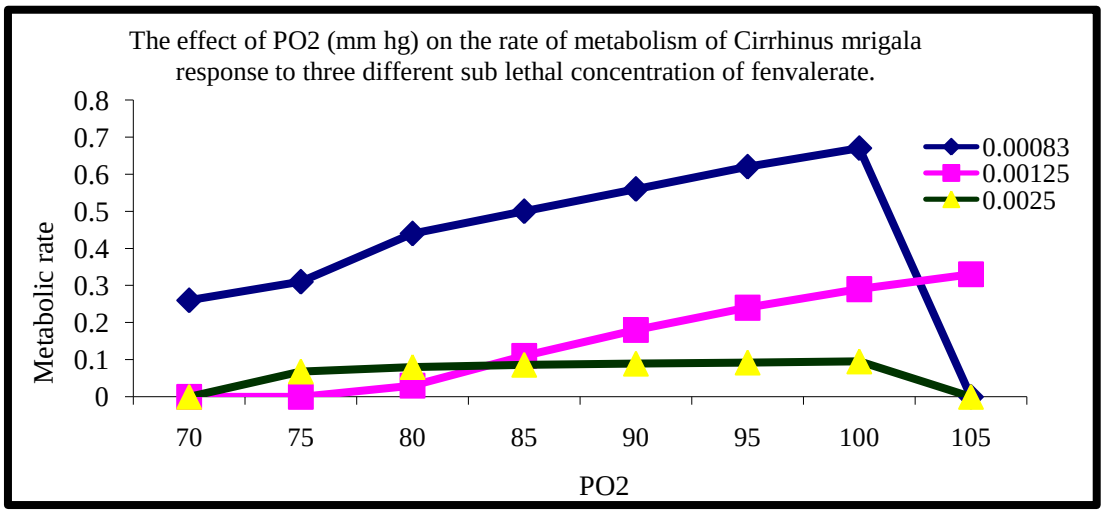

Figure 4. The effect of PO_2 (mm hg) on the rate of metabolism of *Cirrhinus mrigala* response to three different sub lethal concentration of fenvalerate.

From the 96 hrs LC_{50} value of 0.025 ppm. Three subs lethal concentrations were chose to find out the effect of three sub lethal concentration on the respiratory metabolism of the fingerlings of *Cirrhinus mrigala* (Figure 1-3). The sub lethal concentration were chosen as 1/10, 1/20 and 1/30 concentration of 96 hrs LC_{50}.The sub lethal concentration of fenvalerate for experiment chosen was 0.0025, 0.00125 and 0.00083 ppm. The experiment was carried out to study the various sub lethal concentration of fenvalerate on rate of oxygen consumption of *Cirrhinus mrigala*. It is interesting they point out the irrespective of concentration of pesticide, rate of oxygen consumption increased from 0.44 mg/g/hrs to 0.67 mg/g/hrs. When the partial pressure increase from 80 to 100 mm/hg (Table 3) in the exposed to 0.00083 ppm (Table 3; Figure 4). Similar result was also found in the fish exposed to 0.00125 and 0.0025 ppm of fenvalerate. In the present investigation, the decline of oxygen consumption was observed in the subsequence exposure. It can be due to the onset of poisoning and gills damage formation of mucus film over the gills and on the body surface. This may reduce the efficiency of oxygen uptake due to the morbid condition of gastropods. Fenvelerate may have adverse effects of on gills structure and could cause disruption in oxygen uptake and decrease in oxygen uptake efficiency.

CONCLUSION

In this study the experimental fish *Cirrhinus mrigala* fingerlings exposed to a sub-lethal concentration of

Fenvelerate was studied. The results of present study revealed that Fenvelerateis really extremely toxic to fingerlings of *Cirrhinus mrigala*. The toxicity of fenvelerate on fish increased with increasing concentration and exposure time. The decline of oxygen consumption was observed in the subsequence exposure of Fenvelerate.

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