



A study on acute toxicity, oxygen consumption and behavioural changes in the three major Carps, *Labeo rohita* (ham), *Catla catla* (ham) and *Cirrhinus mrigala* (ham) exposed to Fenvalerate

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Abstract

Acute toxicity experiments for Fenvalerate technical grade and 20% EC formulation were conducted using static renewal bioassay and continuous flow through systems for 24h, 48h and 96 h on the three major carps, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*. Although toxicity studies were conducted on both fry and fingerling stages, further experiments were carried out with fingerling stages only. After determining the LC₅₀ concentrations for the three fish individually, one-tenth of the 24 h LC₅₀ was taken as sublethal concentration for studies on oxygen consumption. The toxicant exposed fish showed anomalous behaviour like surfacing phenomenon, irregular, erratic and darting swimming movements, hyperexcitability, loss of equilibrium and hitting to the walls of the test tank before finally sinking to the bottom just before death. Oxygen consumption studies for a period of 12 hours, at intervals of 2 hours, in both sublethal and lethal concentrations indicated that lethal concentrations had profound effect than sublethal concentrations and 20% EC was found to be more deleterious than technical grade of fenvalerate. During experimentation, severe respiratory distress, rapid opercular movements leading to the higher amount of toxicant uptake, increased mucus secretion, higher ventilation volume, decrease in the oxygen uptake efficiency, laboured breathing and gulping of air at the surface were observed in all the three carps studied.

Keywords: Carps, Oxygen consumption, Behavioural changes, Toxicity, Fenvalerate, 20% Emulsifiable concentrate, Metabolic rate etc.

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INTRODUCTION

The pollution of aquatic environment with wide array of xenobiotic compounds has become a menace to the aquatic flora and fauna and is a problem of immediate concern. These contaminants are let out into the water bodies from industrial and agricultural areas and as most of them are highly persistent, their levels fast reach to life threatening in terms of both space and time (Brack *et al.* 2002; Diez *et al.* 2002). The recent development of biomarkers based on the study of the response of organisms to pollutants has provided essential tools for vigilant contamination monitoring (Korami *et al.* 2000). As pyrethroids are found to be less persistent and relatively safe as compared to organochlorines and organophosphates, a variety of them are widely used to control pests, such as moth pests attacking cotton, fruit and vegetable crops, including structural pest control and/or landscape maintenance (Marigoudar *et al.* 2009). Fish sensitivity to pyrethroids could be explained by their relatively slow metabolism and elimination of these compounds (David *et al.* 2003).

Depletion in oxygen content occurs in the medium when pesticides, chemicals, sewage and other effluents containing organic matter are discharged into water bodies. Pesticides in sublethal concentrations present in the aquatic environment are too low to cause rapid death directly but may affect the functioning of the organisms, disrupt normal behaviour and reduce the fitness of natural population. In the aquatic environment one of the most important manifestation of the toxic action of chemical is the over stimulation or depression of respiratory activity. The changes in the respiratory activity of fish have been used by several investigators as indicators of response to environmental stress.

The respiratory potential or oxygen consumption of an animal are the important physiological parameters to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general (Prosser and Brown, 1977).

Pesticides are indicated to cause respiratory distress or even failure by affecting respiratory centres of the brain or the tissue involved in breathing. The effect of toxicants on the respiration of fishes and invertebrates have received wide spread attention and were reviewed by Hughes (1976) and Wright (1978).

As aquatic organisms have their outer bodies and important organs such as gills almost entirely exposed to water, the effect of toxicants on the respiration is more pronounced. Pesticides enter into the fish mainly through gills and with the onset of symptoms of poisoning, the rate of oxygen consumption increases (Premdas and Anderson, 1963; Ferguson *et al.*, 1966a). Holden (1973) observed that one of the earliest symptoms of acute pesticide poisoning is respiratory distress. This serves not only as a tool in evaluating the susceptibility or resistance potentiality of the animal, but also useful to correlate the behaviour of the animal.

By cannulating the blood system of fishes, it is possible to measure the concentrations of oxygen, metabolites and pollutants and hence understand more fully the mode of action of toxic pollutants. Skidmore (1970) using cannulation techniques, found that zinc reduced the oxygen level of blood leaving the gills. It reduced the efficiency of oxygen transport across the gill membrane, so that fish die of hypoxia. Respiratory responses to lethal concentrations increase the ventilation volume and symptoms of pyrethroid intoxication suggesting that the effect on respiratory surface are lethal in fish. It is known that pyrethroids are less persistent and are effective substituents for organochlorine (OC) pesticides. Like OC compounds, the mechanism of pyrethroid interference is with nerve membrane function through the interaction with the sodium channels. The symptoms of fenvalerate intoxication suggest that, besides effect on the nervous system, effect on respiratory surfaces and renal ion regulation may be associated with the mechanism of lethality in fish (Bradbury *et al.*, 1987). The toxic effects of pyrethroids on the metabolism particularly oxygen consumption have been reviewed by Bradbury *et al.*, 1986; Kumaraguru and Beamish, 1983; Haya and Waiwood, 1983; Pandi baskaran, 1991.

Total oxygen consumption is one of the indicators of the general well being of the fish. It may also be useful to assess the physiological state of an organism, helps in evaluating the susceptibility or resistance potentiality and also useful to correlate the behaviour of the animal, which ultimately serve as predictors of functional disruptions of population. Hence the analysis of oxygen consumption can be used as a biodetector system to evaluate the basic damage inflicted on the animal which could either increase or decrease the



oxygen uptake. Therefore an attempt was made to study the effect of sublethal and lethal concentrations of fenvalerate, technical grade and 20% active ingredient emulsifiable concentrate (EC) on oxygen consumption for twelve hours to the three Indian major carps, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*.

MATERIALS AND METHODS

The fish were brought from a local fish farm and acclimatized to the laboratory conditions in well aerated water for one week. The water used for acclimatization and experimentation was the same as used in the toxicity experiments (Table 1). During this period the fish were regularly fed, but the feeding was stopped for two days prior to the experiment. The fish measuring 6 to 7 ± 0.5 cm in length and 6 to 8 ± 0.5 gm in weight were used in the experiment. Toxicity experiments for fenvalerate technical grade and 20% EC formulation were conducted on the fingerlings of the three carps, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*, using static and continuous flow through systems for 24h, 48h and 96h exposures. The experiments on the oxygen consumption of the fish *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* were carried out in a respiratory apparatus developed by Job (1955). The sublethal and lethal concentrations of the toxicants used for the three carps for oxygen consumption studies are presented in Table 2.

The respiratory chamber consists of a wide mouthed bottle fitted with a rubber stopper with three holes. A glass tube which serves as an inlet passes through one hole, through the other hole passes a glass tube with a regulator serving as an outlet. Another hole is fitted with a glass tube and regulator which serves as atmospheric vent.

The test fish in good condition were taken and introduced into respiratory chamber filled with water. The respiratory chamber was closed without air bubbles and sealed with paraffin wax. The respiratory chamber is connected to a 25 litre capacity reservoir through an inlet. The flow rate was so adjusted, that one litre of water flows per hour.

In each experiment, two respiratory chambers, one with fish and another without fish (i.e. control) were taken. The control serves to estimate initial amount of oxygen present. After one hour of acclimatization of the fish, desired concentration of the toxicant was added to the reservoir and flow rate was adjusted. After fifteen minutes, the water of the respiratory chambers was replaced by the test water containing the toxicant, and the experiments with fenvalerate technical grade and 20% active ingredient emulsifiable concentrate were conducted for twelve hours. At the end of each hour, samples (50 ml) were collected and the amount of oxygen present was estimated by Winkler's method (Golterman and Clymo, 1969). The difference in the rate of oxygen

Table 1: Chemical Analysis of Water used for the present study

S. No.	Water Characteristic/Parameter	Quantity
1	Turbidity	8 Silica Units
2	Electrical conductivity at 28°C	816 micro ohms/cm
3	pH at 28°C	8.1
4a.	Alkalinity: Phenolphthaleine	Nil
4b.	Alkalinity: Methyl Orange	472
5	Total Hardness (as CaCO ₃)	232
6	Carbonate Hardness (as CaCO ₃)	232
7	Non Carbonate hardness (as CaCO ₃)	Nil
8	Calcium Hardness (as CaCO ₃)	52
9	Nitrite Nitrogen (as N)	Nil
10	Sulphate (as SO ₄ ²⁻)	Trace
11	Chloride (as Cl ⁻)	40
12	Fluoride (as F ⁻)	1.8
13	Iron (Fe)	Nil
14	Dissolved Oxygen (DO)	8-10 ppm
15	Temperature	28 ± 2 ^o C

**Table 2: Sublethal and Lethal Concentrations of Fenvalerate technical grade and 20% active ingredient EC to fish *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala***

Fish	Insecticide	Sublethal Concentration (mg/L)	Lethal Concentration (mg/L)
<i>Labeo rohita</i>	Fenvalerate Technical grade	0.0014	0.014
	20% EC Fenvalerate	0.0024	0.024
<i>Catla catla</i>	Fenvalerate Technical grade	0.0016	0.016
	20% EC Fenvalerate	0.00203	0.0203
<i>Cirrhinus mrigala</i>	Fenvalerate Technical grade	0.0006	0.006
	20% EC Fenvalerate	0.0015	0.015

consumption between the control and the test fish denotes the effect of the toxicant on oxygen consumption.

Like wise, experiments were conducted in sublethal and lethal concentrations of fenvalerate technical grade and 20% active ingredient EC and the data was compared with that of control. The sublethal and lethal concentrations of the two toxicants tested throughout the study are given in Table 2.

The amount of oxygen consumption was calculated using the formula:

$$O_2 \text{ mg/L} = \frac{8 \times 1000 \times \text{Normality of Hypo} \times \text{Vol. of Hypo} \times \text{run down} \times \text{Vol of the respiratory chamber}}{\text{Vol. of sample taken} \times \text{Correction factor}}$$

RESULTS AND DISCUSSIONS

Results of the toxicity experiments revealed that 20% active ingredient EC is about 2 to 6 times more toxic than technical grade fenvalerate. Since pyrethroids are fast acting and produce mortality within 24 hours (mostly by around 8-10 hours), the static LC₅₀ values for 24 hours are same as for 48 hours and 96 hours. The range of concentrations producing mortality is narrow for 20% EC formulation, compared to technical grade fenvalerate. Higher mortality rate, almost double was recorded in winter (18⁰ C ± mean average temperature) than in summer (32⁰ C ± mean average temperature). Thus for pyrethroids, there appears to be an inverse relationship between temperature and toxicity.

When fish were exposed to sublethal and lethal concentration of fenvalerate, several behavioural changes were observed which include swimming at the surface of water. This surfacing

phenomenon was more in fish exposed to lethal concentration and sublethal concentration over the control fish. Hyperexcitation, loss of equilibrium, increased cough rate, flaring of gills, increase in production of mucus from the gills, darting movements, hitting against the walls of test tanks and curvature of spine were also noticed in all the three major carps. When exposed to lethal concentration, body surface acquired dark colour before their death which is one of the symptoms of toxicity. A film of mucus was observed all over the body and also on the gill. Low rates of fenvalerate elimination and metabolism seem to be contributing factors in piscicidal activity of fenvalerate (Bradbury et al., 1986). Patil and David (2008) in their study on behaviour and respiratory dysfunction as an index of malathion toxicity in *Labeo rohita* clearly reported that while the control fish were active with controlled and co-ordinated movements, the toxicant exposed fish exhibited irregular, erratic and darting movements and loss of equilibrium due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyperstimulation. These findings are in corroboration with those of Murshigeri and David, 2005 and others viz., Dube and Hosetti, 2010, Rao et al., 2003 and Parma de Croux et al., 2002. Recent studies on acute toxicity and behavioural responses of common carp, *Cyprinus carpio* (Linn.) to an organophosphate, Dimethoate reported erratic swimming of the test fish, their increased surfacing, decreased rate of opercular movement, copious mucus secretion, reduced agility and inability to maintain normal posture and balance with increasing exposure time (Pandey et al., 2009). In gist, the various behavioral anomalies in fish



exposed to different toxicants in general include initial increase in opercular movements followed by steady decrease with increased duration of exposure (Shiva kumar and David, 2004), gulping air at the surface, swimming at the water surface, disrupted shoaling behaviour and easy predation (Ural and Simsek, 2006). Gulping of air may help to avoid contact of toxic medium. Surfacing phenomenon might be a demand of higher oxygen level during the exposure period (Katja et al., 2005). Finally, fish sunk to the bottom with the least opercular movements and die with their mouth opened. In sublethal exposures, the fish body becomes lean towards abdomen position as compared to control owing to reduced amount of dietary protein consumed by the fish at pesticide stress, which was immediately utilized and was not stored in the body weight (Kalavathy et al., 2001).

The data on the whole animal oxygen consumption, calculated per gram body weight in sublethal and lethal concentrations of fenvalerate technical grade and 20% EC for *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* are given in the **Tables 3 and 4**. When a comparison is made between the sublethal concentrations of fenvalerate technical grade and 20% active ingredient EC among *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* on oxygen consumption, there was significant increase in oxygen consumption as compared to the controls in *Labeo rohita* and *Catla catla*, the respiratory rate being higher throughout the experimental period. On the contrary, in toxicant exposed *Cirrhinus mrigala*, oxygen consumption decreased than that of controls.

Similarly, when a comparison is made between the effects of lethal concentrations of fenvalerate technical grade and 20% active ingredient EC among *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*, highest oxygen consumption rates were attained during 2nd and 4th hours while in *Labeo rohita*, oxygen consumption reached to maximum during eighth and fourth hours in technical grade and 20% EC respectively. *Catla catla* proved to be more sensitive than *Labeo rohita* and *Cirrhinus mrigala* as is evidenced by its death during fifth hour in lethal concentrations of 20% active ingredient EC. From this it can be inferred that 20% EC is more toxic than technical grade fenvalerate. Lethal concentrations of the two toxicants had profound effect on the oxygen consumption than the sublethal concentrations for 12 hours during the exposure period (Tables 3 and 4).

During experimentation severe respiratory distress, rapid opercular movements, leading to the higher amount of toxicant uptake, increased mucus secretion, higher ventilation volume, decrease in the oxygen uptake efficiency, laboured breathing and engulfing of air through the mouth were observed in all the three major carps exposed to both the toxicants. However, the above said changes in the fish were more pronounced in EC than in technical grade fenvalerate.

The increased oxygen consumption in *Labeo rohita* and *Catla catla* under sublethal concentrations of both the toxicants is in corroboration with the increased consumption of oxygen in trout exposed to permethrin (Haya, 1989) and *P. pugio* larvae exposed to fenvalerate for twenty four hours (McKenney and Hamakar, 1984). Reddy et al. (1977) reported an elevation in oxygen uptake during first two hours of exposure followed by decrease in subsequent hours in *Channa striatus* exposed to cypermethrin. Similar trend was reported in *L. rohita* (Raju, 1991) and *C. punctatus* (Jeevaprada, 1990) exposed to cypermethrin. Bradbury et al. (1986) stated that the greater decrease in the rate of oxygen consumption in the fish *Cirrhinus mrigala* may be due to internal action of the pesticide as the toxicant alters the metabolic cycle at sub cellular level. Similar observations were also reported by Mushgeri and David (2003) and Jadhav and Sontakka (1977). The decrease in oxygen consumption at sub lethal concentrations of the toxicant indicates lowered energy requirements which in turn indicates pronounced haematological changes (Tilak and Satyavardhan, 2002). Similar reduction in oxygen consumption has been reported in *Channa striatus* exposed to organophosphate pesticide (Natarajan, 1981), *O. mossambicus* due to organochlorine intoxication (Vasanthi and Ramasamy, 1987), *M. cupanus* following carbamide treatment (Arunachalam and Palanichamy, 1982), and *C. punctata* exposed to carbaryl (Tilak, 1979).

Changes in the gill surfaces and increased mucus production is consistent with observed histological effects such as hyperplasia, necrosis and lamellar aneurysms in all the three fish exposed to sublethal concentration of technical grade fenvalerate. Kumaraguru et al. (1982) reported that the gill is the target organ for synthetic pyrethroid toxicity in fish. The technical as well as commercial formulations will pass through the gills, and interfere in the gill movements which is



Table 3: The amount of oxygen consumed in mg/g body weight of the fish *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* exposed to sublethal and lethal concentrations of fenvalerate technical grade.

Hours of Exposure	Oxygen mg/g body weight											
	<i>Labeo rohita</i>			<i>Catla catla</i>			<i>Cirrhinus mrigala</i>					
	Control	Sublethal concentration	Lethal Concentration	Control	Sublethal Concentration	Lethal Concentration	Control	Sublethal Concentration	Lethal Concentration	Control	Sublethal Concentration	Lethal Concentration
2	0.452	0.750	0.684	0.531	0.874	0.782	0.874	0.485	0.883	0.874	0.485	0.883
4	0.500	0.756	0.622	0.414	0.826	0.526	0.831	0.647	1.000	0.831	0.647	1.000
6	0.480	0.748	0.685	0.417	0.957	0.654	0.788	0.681	0.883	0.788	0.681	0.883
8	0.550	0.756	0.825	0.483	0.950	0.672	0.788	0.647	0.829	0.788	0.647	0.829
10	0.500	0.700	0.658	0.480	0.900	0.600	0.790	0.500	0.700	0.790	0.500	0.700
12	0.506	0.750	0.600	0.475	0.810	0.680	0.800	0.577	0.666	0.800	0.577	0.666

Table 4: The amount of oxygen consumed in mg/g body weight of the fish *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* exposed to sublethal and lethal concentrations of 20% active ingredient EC fenvalerate.

Hours of Exposure	Oxygen mg/g body weight											
	<i>Labeo rohita</i>			<i>Catla catla</i>			<i>Cirrhinus mrigala</i>					
	Control	Sublethal concentration	Lethal Concentration	Control	Sublethal Concentration	Lethal Concentration	Control	Sublethal concentration	Lethal Concentration	Control	Sublethal concentration	Lethal Concentration
2	0.686	0.510	0.758	0.220	0.346	0.265	0.570	0.412	0.456	0.570	0.412	0.456
4	0.675	0.696	0.862	0.241	0.279	0.221	0.600	0.338	0.750	0.600	0.338	0.750
6	0.675	0.810	0.758	0.209	0.279	-	0.584	0.341	0.448	0.584	0.341	0.448
8	0.625	0.557	0.706	0.241	0.301	-	0.590	0.553	0.425	0.590	0.553	0.425
10	0.580	0.460	0.500	0.275	0.298	-	0.550	0.490	0.547	0.550	0.490	0.547
12	0.575	0.464	0.502	0.304	0.325	-	0.584	0.483	0.547	0.584	0.483	0.547



directly proportional to the respiratory activity of the fish, primarily effecting the oxygen uptake. The respiratory metabolism was impaired and damage was also observed in the gill of fish exposed to pesticides (Hughes and Perry, 1976; Rama Murthy, 1988). In the freshwater fish, *Ctenopharyngodon idella* exposed to Nuvan[®], an organophosphate, the depletion of the oxygen consumption is due to the disorganization of the respiratory action caused by rupture in the respiratory epithelium of the gill tissue. The experimental data reveals that oxygen consumption decreases with the time of exposure to toxicant (Tilak and Swarna kumari, 2009).

Under toxic conditions, the oxygen supply becomes deficient and a number of poisons become more toxic increasing the amount of poison being exposed to the animal. The fish breathe more rapidly and the amplitude of respiratory movements will increase. Lloyd (1961) reported that the toxicity of several poisons to rainbow trout increased in direct proportion to decrease in oxygen concentration of water. In general, it is observed that the lack of oxygen increases the ventilation volume of fishes and the cardiac output is reduced. This reduces the rate of passage of blood through the gills, so allowing a longer period of time for uptake of oxygen, and also conserves oxygen by reducing muscular work. The zone of resistance is reached when the oxygen tension in the water is so low that homeostatic mechanisms of the fish are no longer able to maintain the oxygen tension in the afferent blood and the standard metabolism begins to fall.

Changes in the architecture of gill under fenvalerate stress would alter diffusing capacity of gill with consequent hypoxic/anoxic conditions and thus respiration may become problematic task for the fish. The results of the present study suggest that the altered rates of respiration of fresh water fish may serve as a rapid biological monitor of the pesticide exposure to important components of fresh water community.

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