Respiratory Effects in a Fresh Water Fish Cyprinus carpio Exposed to Permethrin, Technical Grade Type I Synthetic Pyrethroid and 25% EC

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Abstract: Respiratory as oxygen consumption was studied in the fresh water *Cyprinus carpio* exposed to Permethrin, a synthetic Pyrethroid of class I type, technical grade and 25% EC, in the laboratory in lethal and sublethal concentrations. When compared with control values in sublethal and lethal concentrations of technical grade Permethrin, there is an increasing demand of O_2, percentage increase is for 2 h duration is 164.5%, 147.2%; 4 h duration is 199.5%, 127.0%; 6 h duration is 229.4%, 156.8%; 8 h duration is 198.13%, 139.13%; 10 h duration is 187.5%, 125%; 12 h duration is 170.5%, 143.1%and for its 25% EC has also similar demand for O_2 intake as percentage for 2 h duration is 174%,164.5%; 4 h duration is 228.5%, 227.8; 6h duration is 229.4%, 181.1%; 10h duration is 192.7%, 177.0%; 12 h duration is 184.2%, 173.6% in sublethal and lethal. The demand is more for increase in oxygen uptake in EC which has ingredients that are imparting more toxicity. The demand precludes the damage to the gill architecture in the carp.
Key words: oxygen consumption, Permethrin, 25% EC, lethal and sublethal, gill architecture

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INTRODUCTION

Heterotrophic, poikilothermic teleostean fish with air bladder are very dynamic in aquatic systems. Any pollutant whether is toxicant or not, even in sub lethal concentration renders the fish uncomfortable referred as contamination. In such instances make the fish unsustainable due to physiological disturbances of the non target organisms for pesticide affecting the oxygen consumption which is the first activity that lead to damage of gill architecture.

Fish bioassay experiments are indices to determine the acute toxicity and possible effect on oxygen consumption due to the stress[1]**.** First there is a warning sign of abnormal opercular movement as an indicator of respiratory stress and a more direct measurement of it in this sense necessitates the quantification of oxygen consumed by the fish. Studies on oxygen consumption from a suitable tool in the assessment of toxicity stress in aquatic organisms and give an energy expenditure mechanism for environment variation [2-5]**.**

Measurement of such physiological parameters of oxygen to assess the strenuous stress is important because it is a valuable indicator of energy expenditure in particular metabolism in general.

The metabolic response to changes in oxygen availability may vary depending on the physiological state of animal level activity and temperature[6]**.** Hence differential oxygen consumption can be used as a bioindicator of toxicity stress in biological early warny system.

The total oxygen consumption of fish reflects its basal metabolic status and is one of the indicators of the general health or/and well being of the fish. It is far either susceptibility or resistance potentiality to correlate the ultimate effect as toxic resulting toxicity which ultimately serve as predicators of functional disruption of population.

According to[7] O_2 in its molecular state is essential for many metabolic processes that are vital to aerobic life. Like all aerobic organisms fish are susceptible to the effects of reactive oxygen and have internal and effective of different biotic and abiotic factors as antioxidant defenses in fish. Similar thing was also opined by[8] as variation in respiration rate is an indicator of stress due to pesticide intoxication and is frequently used to evaluate changes under environment deterioration. Earlier by [9, 10] reported that pesticides are indicated to cause respiratory distress or even failure by affecting respiratory centers of the brain or the tissue involved O_2 requirement.

The toxic effects of Pyrethroids on the metabolism particularly oxygen consumption have been reviewed by[11, 12, 13]**.** The reports that are in pesticide toxicology had variation either decrease or increase in different Pyrethroids and fishes[15-28]. The carps are oxygen regulators meaning that they maintain their oxygen consumption as a constant level along a gradient of environmental oxygen concentrations along a critical oxygen concentration can be used as biodectory system to evaluate the basic damage inflicted on the animal which could either increase or decrease the oxygen uptake. Hence the present study was undertaken to evaluate at the toxic level of lethal and sublethal concentration of Permethrin technical grade as well as 25% EC in oxygen consumption of the fresh water exotic teleost fish *Cyprinus carpio*.

Materials and Methods

Experiment on the oxygen consumption of the fish *Cyprinus carpio* was carried out in a respiratory apparatus developed by [29]**.** The fish were brought from a local fish farm at Nandivelugu, Guntur district (Andhra Pradesh). They were acclimatized to the laboratory conditions in well aerated water for 10 days. The water used for fish acclimatization and experimentation was the same as used in the toxicity experiments. During the experimental period, the fish were regularly fed, but the feeding was stopped for two days prior to the experiment. The fish measuring 3 to 4 cm in length and 4 to 6 g in weight were used in the experiment. All the precautions laid down by [30] are followed, for maintaining the fish. The fish were exposed to 96h LC_{50} lethal, sublethal ($1/10^{th}$ of 96 h LC_{50} i.e., 0.27µg/L), concentrations of synthetic pyrethroid pesticide Permethrin technical grade and (25% EC). The samples for estimation were taken from the respiratory chamber, at alternate hours of intervals for 24 hours.

DESCRIPTION OF RESPIRATORY CHAMBER

The apparatus used for the measurement of whole animal oxygen consumption is a wide mouthed bottle which is called a respiratory chamber. Its mouth was fitted with a four holed rubber stopper and through one of the holes a thermometer was passed to know the temperature of the medium in the respiratory chamber. From the remaining three holes three glass tubes were passed whose outer ends were fitted with rubber tubes. These three tubes served as delivery tubes are designated as T1, T2, and T3 respectively. They were fitted with pinch locks P1, P2 and P3. T1 was connected with the reservoir and through this water could be drawn (inlet) into the respiratory chamber. T2 was atmospheric tube; useful for testing the air tightness of the respiratory chamber. Through the outlet (T3 tube) water samples from the respiratory chamber were collected for estimation of dissolved oxygen. The respiratory chamber was coated black to avoid photochemical reactions and to keep the animal activity at normal condition during the experiment.

SETTING UP OF THE RESPIRATORY APPARTUS

Only one fish was introduced into each respiratory chamber and was fitted with water drawn through T1 from the reservoir. After checking the air tightness pinch lock P2was closed and pinch lock P3 was opened slightly so that a very gentle and even flow of water was maintained through the respiratory chamber. This was continued for 15 minutes to facilitate the animal in returning to a state normalcy from the state of excitement, if any, due to the handling and also to allow the animal to adjust to the darkness in the chamber (acclimatization).

COLLECTION OF THE INITIAL AND FINAL SAMPLES

After allowing the animal to settle in the chamber, the initial sample was collected from the respiratory chamber through T3. After the collection of initial sample, the respiratory chamber was closed by closing P3 first and then P1 after one hour. The next sample was collected from the respiratory chamber. Likewise, other samples were also collected at the end of each alternate hour for total 24 hours period of the experiment. Along with four experimental fish chambers and control, one respiratory chamber without fish was maintained to estimate the initial amount of oxygen. The experiments were conducted with sublethal and lethal concentrations of technical grade and Permethrin (25% EC) to experimental fish *Cyprinus carpio*. The amount of dissolved oxygen consumption was calculated per gram body weight per hour

O_2 consumed by fish / gram body weight/ hour=

$$\frac{\alpha - \beta \times \text{N of hypo} \times 8 \times 1000}{\text{Vol. of the sample} \times \text{Correction factor} \times \text{Wt. of the fish} \times \text{Time interval for sample}}$$

Whereas α = Hypo rundown before exposure

β = Hypo rundown after exposure

Student's t-test was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant[31] **(Fisher, 1950).**

RESULTS

Comparative data on the whole animal oxygen consumption of control and experimental fish calculated per gram body weight in lethal and sublethal concentration of Permethrin technical grade and 25% EC for *Cyprinus carpio* was given in the table 3.1 and

3.2 .The results of the experiment and control values are graphically represented in figure 3.1; by taking time on X- axis and the amount of O_2 consumed per gram body weight on Y –axis.

Table: 1 Comparative data on the whole animal oxygen consumption of control and experimental fish in lethal and sublethal concentration of Permethrin technical grade

S.No	Hours of Exposure	Control	Technical grade Sublethal	Technical grade Lethal
1.	2h	0.531	0.874 (164.5)	0.782 (147.2)
2.	4h	0.414	0.826 (199.5)	0.526 (127.0)
3.	6h	0.417	0.957 (229.4)	0.654 (156.4)
4.	8h	0.483	0.950 (198.1)	0.672 (139.13)
5.	10h	0.480	0.900 (187.5)	0.600 (125)
6.	12h	0.475	0.810 (170.5)	0.680 (143.1)

Table: 2 Comparative data on the whole animal oxygen consumption of control and experimental fish in lethal and sublethal concentration of Permethrin 25% EC

S.No	Hours of Exposure	Control	25% EC Sublethal	25% EC Lethal
1.	2h	0.531	0.924 (174.0)	0.874 (164.5)
2.	4h	0.414	0.946 (228.5)	0.905 (218.5)
3.	6h	0.417	0.957 (229.4)	0.950 (227.5)
4.	8h	0.483	0.975 (201.8)	0.8750 (181.1)
5.	10h	0.480	0.925 (192.7)	0.850 (177.0)
6.	12h	0.475	0.875 (184.2)	0.825 (173.6)

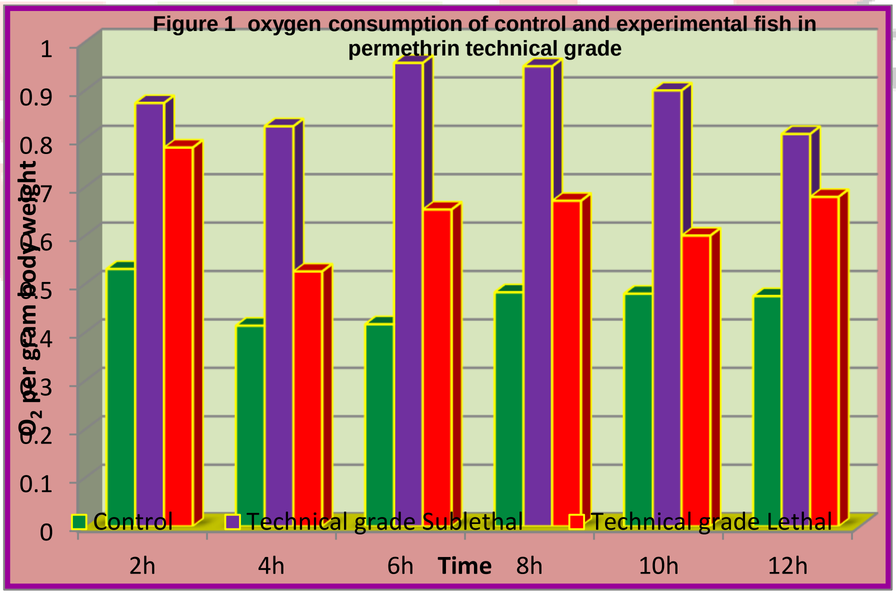

Figure: 1 Comparative data on the whole animal oxygen consumption of control and experimental fish in lethal and sublethal concentration of Permethrin technical grade

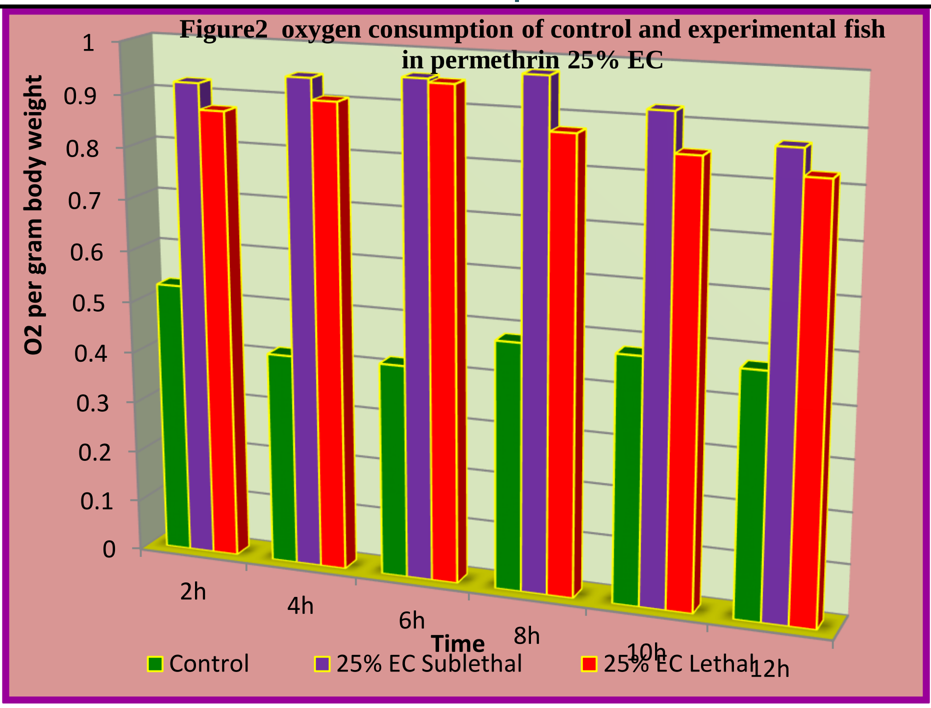

Figure: 2 Comparative data on the whole animal oxygen consumption of control and experimental fish in lethal and sublethal concentration of Permethrin 25 % EC

DISCUSSION

According to earlier reports [12]**Bradbury et al and** [32]**Tilak and Satyavardhan** the commercial formulations are more toxic due to synergistic effects of the ingredients that are formulated and marketed.

The fish showed differential oxygen consumption in different duration of exposure in the test media. Initial period of 2-4-6-hrs more demand for oxygen consumption (table1 and figure 1). During the initial period the more oxygen intake is due to stress and later period a lot of variation which is more so pronounced in 25% EC.

Anitha et al[33], reported on a study of oxygen consumption in the three major carps *Labio rohita* (Ham), *Catla catla* (Ham), Cirrhinus mrigala (Ham) exposed to Fenvelerate a synthetic Pyrethroid of class II, sublethal concentration more profound effect than lethal concentration. They also reported severe respiratory stress rapid opercular movements leading to the higher amount of toxicant uptake increased mucus secretion, higher ventilation . Volume decrease in the oxygen uptake efficiency labored breathing and gulping of air at the surface. Of the three fishes only *Cirrhinus mrigala* behaved differently being bottom/ benthic fish. The present work is similar in oxygen consumption with the other two carps *Labio rohita* and *Catla catla*.

Haya[34]**,** reported for Permethrin with increased consumption of oxygen in trout exposed. **Bradbury et al**[12] **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. **Mushigeri and David**[26]opined the same. The decrease in oxygen consumption at sublethal concentration of the toxicant –indicates a lowered energy requirement which in turn indicates pronounce hematological changes **Tilak and Satyavardhan**[32]**.** The other members of class II Pyrethroids – Cypermethrin and Deltamethrin, on different fishes behaved in a similar manner. **Neelima et al**[35]**, Deshpandae et al**[36], the work and reported values in *Channa striatus* exposed to organophosphate pesticide. **Natarajan**[37]**,** Dimethoate by **Joythinarendran**[38]too emphasized the same trend. Even other pesticides like organochlorines and Carbamates behaved the same. **Shivakumar and David**[39] **and Tilak**[40] respectively, the toxicants resulting changing in the gill surfaces and increased mucus production in consistent with observed histological effects such as hyperplasia, necrosis and lamellar aneurysms that lead to the changes in the oxygen consumption.

Swarnakumari and Tilak[41]**,** under toxic conditions the oxygen supply becomes deficient and a number of poisons become more toxic (cumulative effect) increasing the amount of poison being exposed to the animal. The fish breathe more rapidly and amplitude of respiratory movements will increase.

Llyold[42] reported that the toxicity of several poisons to rainbow trout increased in direct proportion to decrease in oxygen concentration of water. In general, he opined lack of oxygen increase ventilation volume of fishes and cardiac output of the two chambered bronchial heart is reduced. This reduces the rate of passage of blood through the gills allowing 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 function in the water is so low that homeostatic mechanism of fish are no longer able to maintain the oxygen function in the afferent blood and the standard metabolism begins to fall. Changes in the architecture of gill under Fenvelerate stress would alter diffusing capacity of gill with consequent hypotoxic / anoxic conditions and then respiration becomes problematic task for the fish.

Quisar and Sadhu[43] reported in the Muryel fish *Channa gachua* due to Malacid 50 an organophosphate; Dithane M-45, a carbamate and Kelthane organochlorine, 46% and 54% more oxygen respectively in aquatic and aerial respiration route. It has brought significant decrease in aquatic as well as total oxygen uptake, while increases in oxygen consumption through aerial route. They too opined that the action of pesticide on acetyl cholinesterase enzyme respiratory muscle paralysis and respiratory failure causing finally death.

According to report of **Madanmohan**[44] oxygen consumption of rainbow trout Salmo gairdneri there is an impairment of food consumption due to which metabolism is impaired.

According to **Canadian Water Quality guidelines**[45]**,** Permethrin has emphasized on flailing gills full and rapid contractions and loss of equilibrium and lethal action mechanism in fish involves numerous physiological systems including respiratory surfaces of the fish which lead to the death of the fish. The report on the effect of Chlorantraniliplole on the oxygen consumption of the fresh water fish *Labeo rohita* (Hamiltan), the gill damage and severe respiratory distress and rapid opercular movements are the causes for decrease in oxygen consumption.

The report of **Maria Cristiana Ponepal et al**[46]; reveals that clinical symptoms during Pyrethroid exposures, the direct contact between the aquatic environment and gill epithelium may cause these surfaces to become more sensitive to environmental alterations in presence of toxic materials and other irritants. The use of respiratory stress to monitor sub lethal effects of intoxication was previously applied to a variety of toxicants and subjects. Respiratory irregularities are thought to be caused by mucus precipitation on the gill epithelium in response to the toxicant.

According to **Padmanabhan et al**[47]**,** who studied and reported on chloropyriphos on oxygen consumption and food consumption of fresh water fish *Orechromis mossamtricus* (peters) that at lethal and sub lethal concentration of LC_{50} values of 48 h significant effect on functional activity of the experimental fish by altering respiration rate and impairing feeding behavior. They also stated that the highest oxygen consumption rate was attained in lethal concentration during 12th hour than in sub lethal concentration. A decrease in respiratory rate in both lethal and sub lethal concentrations due to toxicant induced stress avoidance and biotransformation. If gills or membrane functions are destroyed due to Xenobiotic chemicals or the membrane functions are disturbed by a change in permeability of the oxygen uptake rate would rapidly decreased.

Deshponde et al[36]**,** reported due to Pyrethroid Fenvalerate 20% EC and Cypermethrin 25% EC induced respiratory change in the rate of O_2 consumption in *Labeo rohita* and in general there was increase in the rate of oxygen consumption due to the effect of both the Pyrethroids, in sub lethal concentration.

Priyanka and Ansari[48]**,** reported on a comparative study of acute toxicities of Endosulphan, Chloropyriphos and Permethrin in zebra fish *Danio rerio* (Cyprinidae) and mentioned the fish exhibited respiratory distress such as gasping of air, loss of balance and erratic swimming prior to death.

Several reports by different people like Jispa et al[15]; Mukaddham and Kulkarani[49]; Murthy et al[17]; Anita Susan et al; Sivakumar[25]; Omitoyin et al[50]; Vutukuru[51]; Patil et al[52]; Prasanth et al[53]; Saxena and Chanhau[54]; Rao et al[55]; David et al[56]; Dharmalatha and Namitha Joshi [57]; Aguigwo[58]; David et al[59]; Hartl et al[60] ; Magare and Patil[61] ; Cornell et al [62]; Rao[63], Khillare and Wash[64]; Rajamannar and Manohar [65]; Malla Reddy[66]; Ali [67]; Nagarathamma and Rama murthy[68]; Kumaraguru et al [69]; Rath and Mishra[70]; Verma and Dalela[71]; Thomson[72]; O' Brien [73] and Ferguson[74] reported using different toxicants and fish and other aquatic organisms reported differently and there are many variations.

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