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TOXICITY EVALUATION AND OXYGEN CONSUMPTION STUDIES ON THE FISH *CTENOPHARYNGODON IDELLA* EXPOSED TO λ-CYHALOTHRIN 5% EC

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Keywords:

λ-Cyhalothrin, *Ctenopharyngodon idella*, LC_{50}, Oxygen consumption, Lethal and Sublethal concentration

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ABSTRACT: The natural physiological functioning of an organism gets disturbed on exposure to toxicant stress. Toxicants affect first at cellular levels. It ultimately causes physiological, pathological, and biochemical alterations in an organism. Present study λ-Cyhalothrin of 5% EC formulation pyrethroid was used static renewal bioassay to evaluate 96 h LC_{50} in the freshwater fish *Ctenopharyngodon idella* and it was determined at 0.026 mg/l. The exposed fish *Ctenopharyngodon idella* showed irregular, erratic, and darting swimming movements, rapid opercular movements, gulping of air at the surface, hyperexcitability, increased mucus secretion, loss of equilibrium and hitting to the sidewalls of the test tank and finally sinking to the bottom. $1/10^{th}$ of the 96 h LC_{50} was taken as sub-lethal (0.0026 mg/l) concentration for oxygen consumption studies, and it was carried out for a period of 24 h, at regular intervals of 2 h. In the lethal and sub-lethal concentration, the rates of oxygen consumption were increased during the initial time of exposures, *i.e.*, 1 to 6 h after that it was suddenly falling gradually up to 24 h, and the control fish oxygen consumption also fell 0 h to 24 h.

INTRODUCTION: Water pollution is the contamination of water bodies, usually as a result of human activities and affects the entire biosphere. Plants and organisms living in or being exposed to polluted water bodies are impacted. The effect can damage individual species and impact the natural biological communities they are part of. The levels of pesticide water pollution can be ranked from highest to lowest concentration as cropland water > field ditch water > runoff > pond water > groundwater > river water > deep groundwater > seawater [1]. After uptake, the major factor in determining the fate of organic chemicals in the organisms is their enzymatic biotransformation.

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The natural physiological functioning of an organism gets disturbed on exposure to toxicant stress. Toxicants affect first at a cellular or molecular level but ultimately cause physiological, pathological, and biochemical alterations.

Fish are exposed to diverse anthropogenic water pollutants, originating from various point and non-point sources. Fish serves as a bioindicator species as it responds with great sensitivity to changes in the aquatic environment and, thus, has an important role in the monitoring of water pollution [2]. As fish is a major source of food for humans, any pesticide effect in an aquatic animal and ultimately affects human beings through the food chain. The toxicant accumulated is further increased by bio-magnification of the synthetic pesticides from water by aquatic organisms [3, 4]. The unrestricted, heavy use of synthetic pesticides results in a lethal effect on various non-target organisms in the aquatic environment and direct or indirect effect to users [5, 6, 7].

The high toxicity of synthetic pesticides has been found to aquatic, zooplankton, and mammalian species [8]. Dimethoate and Lambda-cyhalothrin showed to be lethal for *Labeo rohita* [9]. Therefore, review effects of synthetic pyrethroid insecticides on several toxicological end-points in fish is provided. Various toxic effects of pyrethroids to biomarkers in different fish species were studied [10].

The rate of oxygen consumption is considered as a reflection of the total metabolism and metabolic rate of the aquatic organisms. The variations in the lethal and sub-lethal concentration values depend upon various factors *viz.* sensitivity to the toxicant, its concentration, and duration of exposure [11.]

Many biological early warning systems monitor the abnormal movement of fish opercular and it indicates respiratory stress, a more direct measurement of stress in this sense necessitates the quantification of oxygen consumed by the fish [12].

MATERIALS AND METHODS: The cultured fish *Ctenopharyngodon idella* was obtained from the fish hatcheries of Kuchipudi, Guntur District, AP, India. It is 20 km away from the Acharya Nagarjuna University. The fish were acclimatized to the laboratory conditions in large plastic tanks with unchlorinated groundwater for two weeks at a room temperature of 28 ± 2 ºC. During the period of acclimatization, fish were fed daily with a fish meal on an average of 3% of their body weight. Feeding was stopped one day prior to the experimentation. All the precautions laid down by committee on toxicity tests to aquatic organisms [13] were followed. Such acclimated fish were exposed to Lethal and Sublethal concentrations of λ-cyhalothrin to evaluate toxicity and amount of oxygen consumption.

A. Determination of LC_{50}: Experiments were conducted to determine the toxicity of λ-cyhalothrin 5% EC, and the data on the mortality rate of fish were recorded. The dead fish were removed immediately. Cumulated mortality data were analyzed to determine 96 h LC_{50} values following the Finney's probit analysis method [14].

For the estimation of LC_{50}, dose concentrations were prepared by mixing different portions of pesticide formulations of λ-cyhalothrin.

B. Oxygen Consumption: The oxygen consumption of the fish *Ctenopharygodon idella* exposed to sub-lethal and lethal concentrations of Lambda-cyhalothrin 5% EC, and it was carried out in a respiratory apparatus developed [15]. Lethal and sublethal ($1/10^{th}$ of lethal) concentrations were taken for oxygen consumption studies, and it was carried out for a period of 24 h, at intervals of 2 h.

RESULTS AND DISCUSSION:

A. 96 h LC_{50}: Toxicity studies have played an important role in man's efforts to monitor and modify the effects of its activities on the biota [16]. In the present study, the observed percent mortality along with exposure concentration of λ-cyhalothrin 5% EC for 96 h to the fish, *Ctenopharyngodon idella* in static renewal bioassay are given in **Table 1**. The results of the LC_{50} of the present study at 96hr was found at 0.026 mgL^{-1} for λ-cyhalothrin 5% EC, according to Finney Probit analysis. The percent mortality and probit mortality increased with the increasing concentration of toxicant. The 96 h LC_{50} of λ-cyhalothrin 5% EC was obtained by taking the mean LC_{50} derived from the percent and probit mortality.

The pyrethroid pesticide λ-cyhalothrin is highly toxic to fishes as it is strongly absorbed by the gills even at very low concentrations in water due to its high lipophilicity [17.] The 96 h LC_{50} was 20-70 µg/L for shrimp and 0.98-7.55 µg/L for zebrafish. In drainage water ponds with Lambda-cyhalothrin concentrations ranging from 0.45 to 0.90 µg/L, the 96 h mortality was 100% for shrimp, but the drainage water showed no toxicity to shrimp on the fourth day after application of Lambda-cyhalothrin [18].

The 96 h LC_{50} value found at 11.008 mg/L for grass carp (*Ctenopharyngodon idella*) exposed to chlorantraniliprole pesticide [19]. The percent mortality rates are gradually increased by increasing pesticide concentration. The freshwater fish *Labeo rohita* were exposed to flubendiamide (Insecticide) and the LC_{50} values for 24 h (17 mg/lit), 48 h (15 mg/lit), 72 h (13 mg/lit) and 96 h (11 mg/lit) [20]. In the present study, when fish were exposed to a sub-lethal and lethal concentration of λ-cyhalothrin 5% EC, several behavioral changes were observed, which include swimming at the surface of the water.

This surfacing phenomenon was more in fish exposed to lethal concentration and sub-lethal concentration over the control fish. Pesticides disturb normal fish behavior, so decreased swimming behavior and increased respiration rate were found after exposure to pesticides [21].

When exposed to lethal concentration, body surface acquired dark color 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. Hyper excitation, 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 the spine. The same observations were also noticed in [22]. The toxicity evaluation and behavioral changes in fishes are very sensitive indicators under the toxicity of chemicals [23].

TABLE 1: OBSERVED PERCENT MORTALITY, PROBIT MORTALITY AND LC50 VALUE OF THE FISH *CTENOPHARYNGODON IDELLA* EXPOSED TO Λ-CYHALOTHRIN FOR STATIC 96 H

S. no.	Dose in mg/L	Log conc.	No. of fish exposed	No. of fish alive	No. of fish died	Percent of Mortality	Probit value (Y)
1	0.022	-1.6576	10	8	2	20%	4.1584
2	0.024	-1.6133	10	7	3	30%	4.4756
3	0.026	-1.5850	10	5	5	50%	5.0000
4	0.028	-1.5528	10	4	6	60%	5.2533
5	0.03	-1.5229	10	2	8	80%	5.8416

B. Oxygen consumption: Indiscriminate use of pesticides affects the aquatic environment, including fishes. Symptoms of acute pesticide poisoning are induced the alterations or failure of respiratory metabolism. Oxygen consumption measurements provide a robust indicator of whole animal stress and contaminant water quality. Oxygen consumption is one of the indicators of the general well being of the fish.

It might be useful to assess the physiological state of an organism, helps in evaluating the susceptibility and resistance potentiality, and also useful to correlate the behavior of the organism, which ultimately serves as an indicator of functional disruption of pollution. Hence, the differential oxygen consumption can be used as a bio-indicator to evaluate the basic damage inflicted on the animal, which could either increase or decrease the oxygen uptake.

The oxygen consumption is a very sensitive physiological process, and the change in respiratory activity has been used as an indicator of stress in animals exposed to toxicants. The physiological and behavioral alterations that occur in fishes in the absence of sufficient oxygen have been investigated. The rate of oxygen consumption is considered as a reflection of the total metabolism and metabolic rate of the aquatic organisms. The water current flowing around the gills; carries the toxicants directly, before all other internal organs.

In accordance with the fluctuations in the physico-chemical characteristics of the ambient waters, the air-breathing fishes are equipped with dual-mode gas exchange machinery, employing respiration using highly vascularised air-breathing organs and branchial integument exchange of gases with water. Oxygen uptake of fish is intimately connected with the extent of damage of gill. Changes in the architecture of gill stress, altered diffusion due to the capacity of gill with consequent hypoxic/anoxic conditions, and thus respiration might become problematic for the fish. Present study labored breathing was observed in the fish with concomitant gill damage, respiring through the mouth was observed as an indication of respiratory distress/or hypoxic condition. The decrease in oxygen uptake might cause the interference of the toxicant with Haemoglobin content. The decrease in the rate of respiration with increasing concentration to toxicants was recorded in fishes [24].

During experimentation, the control fish demanded more oxygen in the early hours later get settled and maintained a constant uptake of oxygen, while the exposed fish demanded more oxygen in the initial hours and later showed a decrease in the oxygen demand. In the present study, λ-cyhalothrin exposed to the fish *Ctenopharygodon idella* initial elevation in the rate of oxygen consumption could be explained in terms of acceleration of oxidative metabolism during the initial hours of exposure, as a result of a sudden response to the toxic stimulus of the pesticide. Decreased oxygen consumption can also be attributed to the induction of hypoxic conditions within the animal due to the intimate contact of the respiratory surface with toxic water resulting in the alteration of the normal respiratory area of the animal. In the present study, the comparative data on the whole animal oxygen consumption of control and experimental fish, calculated per gram body weight in sub-lethal and lethal concentrations of λ-cyhalothrin 5% EC for *Ctenopharygodon idella* was given in **Table 2,** and the results were graphically representing exposure time on X-axis, and the amount of oxygen consumed per gram body weight of test fish *Ctenopharygodon idella* on Y-axis was given in **Fig. 1**. In the present investigation oxygen consumption of the fish clearly showed that gradually decreases in the control group throughout the study period whereas in the lethal concentration the rates of oxygen consumption are initially increases and suddenly falls down, and in sub-lethal concentrations, also similar trend was observed in the test fish *Ctenopharygodon idella* for 24 h. Present study O2 consumption of fish *Ctenopharygodon idella* exposed to λ- cyhalothrin. 0hr in control (0.819), in sublethal (0.794) and lethal concentration (0.805) and 6hrs O2 consumption in control (0.788) and sublethal (0.995) lethal (1.093), *i.e.*, sudden changes in O_2 consumption levels were observed in both lethal and sublethal concentrations when compared with control values after remaining hrs the O_2 consumption levels were gradually decreased in all exposure of the fish *Ctenopharygodon idella*. It indicates severe respiratory distress and rapid molecular movement leads to more intake of toxicant, which results in increased mucus secretion and decrease in oxygen uptake efficiency. Alteration in oxygen consumption increased and thereafter decreased, which is a bio-indicator for assessing pesticide toxicity, which is correlated with the present study.

TABLE 2: THE AMOUNT OF OXYGEN CONSUMED IN MG/GR BODY WEIGHT OF THE FISH *CTENOPHARYNGODON IDELLA* (VALENCIENNES) EXPOSED TO LETHAL AND SUBLETHAL CONCENTRATION OF Λ- CYHALOTHRIN

Exposed period in hrs	Control	Lethal	% Change	Sub-lethal	%Change
0	0.819 ± 0.004	0.805 ± 0.003	1.70	0.794 ± 0.004	
2	0.787 ± 0.003	0.926 ± 0.002	-17.66	0.865 ± 0.003	-9.91
4	0.769 ± 0.002	1.016 ± 0.003	-32.12	0.922 ± 0.002	-19.89
6	0.788 ± 0.001	1.093 ± 0.003	-38.70	0.995 ± 0.002	-26.27
8	0.859 ± 0.003	0.904 ± 0.002	-5.24	0.843 ± 0.002	1.86
10	0.749 ± 0.002	0.778 ± 0.004	-3.87	0.711 ± 0.008	5.07
12	0.704 ± 0.003	0.645 ± 0.003	8.38	0.568 ± 0.003	19.32
14	0.662 ± 0.006	0.633 ± 0.003	4.38	0.619 ± 0.006	6.49
16	0.646 ± 0.004	0.554 ± 0.009	14.24	0.511 ± 0.002	20.89
18	0.621 ± 0.007	0.428 ± 0.004	31.08	0.448 ± 0.003	27.86
20	0.592 ± 0.005	0.394 ± 0.005	33.45	0.379 ± 0.003	35.98
22	0.559 ± 0.005	0.323 ± 0.017	42.22	0.360 ± 0.005	35.50
24	0.515 ± 0.004	0.291 ± 0.005	43.49	0.318 ± 0.004	38.25

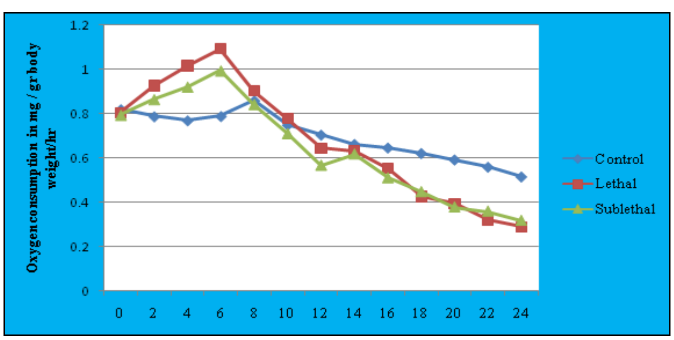

FIG. 1: THE AMOUNT OF OXYGEN CONSUMED IN MG/GR BODY WEIGHT OF THE FISH *CTENO-PHARYNGODON IDELLA* (VALENCIENNES) EXPOSED TO LETHAL AND SUBLETHAL CONCENTRATION OF Λ- CYHALOTHRIN

In the present study, the increased oxygen consumption at initial stages of exposure was observed that fish *Ctenopharyngodon idella* showed a tendency of increase in oxygen consumption during the initial time of exposures *i.e.*, 0 h to 6 h and a gradual decrease was observed during the subsequent period of study.

The presence of a sub-lethal concentration of toxicants is inevitable. In such a case, the fish *Ctenopharyngodon idella* was more sensitive to toxicant. The toxicant stress in oxygen consumption along with depletion in oxygen in aquaculture practices, makes them less fit and reduction in growth due to lack of proper metabolism. The fish was in more stress during the first hour, and later they are showing signs of recovery. The recovery was evident as the toxicant exposure increased during the 24 h experiment. The comparison was made between the effects of sub-lethal and lethal concentrations of λ-cyhalothrin on *Ctenopharygodon idella* was decreased in sub-lethal, under lethal concentration of λ- significant cyhalothrin increase in oxygen consumption was found in the initial stages of exposure, *i.e.*, 1-4 h in *Ctenopharygodon idella*. Hence, this fish was sensitive to λ- cyhalothrin toxic stress; as a result, more amount of oxygen is consumed.

Present study the fluctuated response in respiration might be attributed to respiratory distress because of the impairment of oxidative metabolism was reported earlier under cypermethrin toxicity [25]. The secretion of mucus over the gill curtails the diffusion of oxygen, which ultimately reduces the oxygen uptake by the animal or the membrane functions are disturbed by a changed permeability, oxygen uptake rate would rapidly be decreased. Fish in the sub-lethal concentration were found under stress, but that was not fatal [26]. In sub-lethal λ- cyhalothrin medium, the respiration rate of fish decreased in the subsequent period of exposure, which is due to the acclimatization of the fish in the toxicant environment, and fluctuations in the oxygen consumption were noticed. The consumption was more at initial periods later it was decreased. The reduced opercula activity has resulted in low oxygen consumption. These fluctuations were because of variations in gill ventilation rate coupled with the concentration of a pollutant in water and efficiency of assimilation of gills and also the length of time.

The test fish *Ctenopharygodon idella* absorbed pesticide λ- cyhalothrin across the gill and diffused into the bloodstream, resulting in toxic stress in the fish that was suffering from lack of O_2 consumption. A similar trend was observed on the exposure of monocrotophos during sub-lethal treatment that produces severe toxic effects on the respiratory organ of the estuarine fish *Mugil cephalus* [27]. The changes in oxygen consumption due to the time of exposure and concentration of pesticide and inhibition for metabolic activity disturb the gill at the cellular level. The rate of oxygen consumption is found to increase initially and then decreased up to the end of the experiment in fish *Rasbora daniconius* exposed to a lethal concentration of dimethoate [28]. It is supported that is lethal and sub-lethal concentration experimental fish *Cyprinus carpio* exposed to cypermethrin 25% EC showed an increased tendency in oxygen consumption during the initial time of exposures, *i.e.*, 2 to 4 h and fish showed a gradual decrease in oxygen consumption from the starting period of exposure to till the end of the experiment in lethal concentration [29, 30]. *Labeo rohita* has a sensitivity to toxic stress as a result of more oxygen consumption [31].

The present work coincides with the report of the other researchers. The increased rate of oxygen might increase ventilation volume in order to compensate for the drop in oxygen due to a reduction in permeability of gills [32]. Either increase or decrease in oxygen consumption pertaining to vital tissue damage caused by lethal and sub-lethal doses of cypermethrin on freshwater fish *Cirrhinus mrigala* [33]. Cypermethrin exposed to *Tilapia mossambica* altered rate of oxygen consumption under sublethal concentration of cypermethrin at 24, 48, 72 and 96 h (0.446, 0.094, 0.194 and 0.197 ml/g/h) compared to control (0.462 ml/g/h) [34]. The fish *Tilapia mossambica* exposed to dimethioate elevated levels of oxygen consumption at 24 h and a decline in oxygen consumption at 72 h of exposure [35]. Cypermethrin induced respiratory alterations in *Labeo rohita* [36]. Cypermethrin, a pyrethroid, highly toxic and affect the respiration significantly in *Cyprinus carpio* in both lethal and sublethal concentration [37]. Hence, synthetic pyrethroids induced oxidative stress leads to the different type of instant toxicities as well as toxicities and weak immunity at later stages in fish [10].

In the present study, it is clear than an oxygen disturbance causes metabolic disorders in fish, which ultimately leads to the deterioration of the general health of the fish. Unlike the terrestrial environment, in the aquatic environment, the body of the organism is bathed by the medium containing the toxicant on the respiration is more pronounced. The changes in the respiratory activity of fish and aquatic macro-invertebrates have been used by several investigators as indicators of aquatic organisms respond to environmental stress. Although these investigations have proved very useful in describing responses to sublethal exposure and have direct implications for the mode of action, the interpretation of the ecological significance of the numerous respiratory responses remains difficult. In the present study *Ctenopharyngodon idella* exposed to λ- cyhalothrin, the variation in oxygen consumption is an indicator of stress, which is frequently used to evaluate the changes in the metabolism under environmental deterioration. Oxygen consumption of *Ctenopharyngodon idella* under lethal and sublethal concentrations was compared with control. Since most of the fish breathe in the water in which they live, any changes in the chemical properties it might be reflected in the animal's ventilator activity, particularly if the environmental factors affect respiratory gas exchange.

Gills are the major respiratory organs, and all metabolic pathways depend upon the efficiency of the gills for their energy supply, and damage to these vital organs causes a chain of destructive events, which ultimately leads to respiratory distress. Pronounced secretion of the mucus layer over the gill lamellae has been observed during stress. The secretion of mucus over the gill curtails the diffusion of oxygen [38], which may ultimately reduce the oxygen uptake by the animal. The mechanism of toxicant, which was uptake by gills, probably occurs through pores by simple diffusion and is then absorbed through cell membranes.

In the course of oxygen consumption in lethal and, sublethal concentrations indicate the sequence of the type of compensatory mechanism, if any, which operates within the animal to overcome the load of toxic stress. Variation in oxygen consumption was due to impaired oxidative metabolism and pesticide-induced stress. A significant decline in catalase activity in zebrafish exposed to atrazine. Curiously, this same xenobiotic was assayed and noticed an elevation of liver catalase activity after the exposure to *Channa punctatus* [39, 40]. However, oxidative stress levels in fish from water contaminated by different concentrations of pesticides were entirely different, and in some cases, a clearly inhibitory effect was observed.

CONCLUSION: Pesticide reaches the aquatic environment, it might present there for several days or weeks, depending upon its solubility, producing mass mortality, morphological, physical and behavioral changes in the organisms. On the other hand, the accumulation of pesticides in the results of the organisms in delayed mortality, reduced reproductive capacity, altered growth rate, and reduced ability to withstand the changes in the environment. Since fish serves as a major food source of humans, any effect of pesticides in fish ultimately affects humans through the food chain. So farmers should know the toxicity of pesticides on non-target organisms to minimize the consumption of pesticides, and they should prefer to use natural ways to protect their crops and the environment also.

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