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Effect of Insecticide, Lesenta on Rate of Oxygen Consumption in Common Carp (*Cyprinus carpio* L.)

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ABSTRACT

For the toxicity investigation, the study of the rate of oxygen consumption is one of the indications of an organism's general health. The aim of this study was to see how Lesenta insecticide affected rate of oxygen consumption in the freshwater fish C. carpio. The rate of oxygen consumption in *C. carpio* after exposure to lesenta between 1 mg/L (1/10th of LC_{50}) and 2 mg/L (1/5th of LC_{50}) at sublethal concentrations on days 7, 14, 21, and 28 revealed a significant decrease in the rate of oxygen consumption. After 28 days of exposure, the rate of oxygen uptake in *C. carpio* was considerably lower than in the control group of fish. When compared to control, percentage of the rate of O_2 consumption decreased 20% and 38% on the 7th day, 30% and 53% on the 14th day, 46% and 68% on the 21st day, and 64% and 81% on the 28th day at 1 and 2 mg/L sublethal concentrations, respectively. The O_2 consumption rate of fish impacted by Lesenta. The reduction was greater at higher concentrations, which could be due to a slower metabolism caused by toxicant stress.

Key words - Toxicity, Oxygen, *Cyprinus carpio*, Lesenta, Insecticide, Sublethal, Metabolism, Stress

INTRODUCTION

Because fish are one of the biological indicators of water quality, any change in fish physiology indicates a decrease of water quality parameters. Toxicants in the environment mostly enter the respiratory system of fish. When hazardous contaminants are present in the aquatic environment, one of the most common physiological responses to toxicants is a change in respiration rate, which is easily detected through changes in oxygen consumption rate, which is commonly used to evaluate changes in metabolism under environmental deterioration.

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Total oxygen consumption is one of the indications of a fish's general health or well-being, as it indicates its baseline metabolic status. It may also be beneficial to assess an organism's physiological status, assist in determining sensitivity or resistance potentiality, and correlate animal behavior, all of which act as predictors of population functional disturbances.

Carps, like other fish, are oxygen regulators, which means they keep their oxygen intake constant along a gradient of environmental oxygen concentrations until they reach a critical oxygen concentration, beyond which oxygen consumption begins to plummet. This crucial oxygen level is expected to rise in stressed fish, reflecting the animal's reduced ability to cope with environmental changes. Analysis of oxygen intake can be utilized as a biodetectory system to assess the animal's basic harm, which can either raise or reduce oxygen uptake. As a result, the goal of this study was to see how hazardous sublethal concentrations of lesenta were on the oxygen consumption of the freshwater teleost fish *Cyprinus carpio.*

MATERIAL AND METHODS

Test Chemical

For the toxicity investigation on physiological changes in *C. carpio*, the insecticide Lesenta (Imidacloprid 40% + Fipronil 40%) was bought from a local market. This study used a sub-lethal concentration of lesenta (i.e., 2 mg/L) based on the toxicity study. Lesenta was made at sub-lethal doses 1 mg/L (1/10th of LC_{50}) and 2 mg/L (1/5th of LC_{50}).

Test Animal

Major carps, such as the common carp *Cyprinus carpio* (Linnaeus), are commercially important food fishes with high commercial value that may be found in abundance in fresh water tanks across the Adilabad district. These major carps are known to be adaptable to laboratory conditions and appear to be suitable test animals in toxicity investigations. As a result, these fish were chosen as the best experimental material for this study. About 200 fingerlings of Cyprinus carpio were obtained from a fish farm in Adilabad District. The average weight and length were 13.18±0.38 grams and 9.09 ±0.29 centimeters, respectively.

The fish were brought to the lab in oxygenated plastic bags with as little stress as possible. The fish were kept in glass tanks that were well aerated. The fish were housed in eight tanks, each with a size of 100 gallons. The fish were raised in tap water that had been dechlorinated and disinfected with a 0.1 percent potassium permanganate solution. Aeration was delivered by compressed air pumps via air stones, and daily water changes were performed manually in all of the tanks. Water was replaced daily during the acclimation phase, and fish were fed once a day with artificial food pellets purchased from the local market. The fish were acclimated for 15 days before being placed into seven groups, each of which contained ten fish.

Exposure to Sublethal Doses of the Lesenta

Probit analysis was used to calculate the median lethal concentration (LC_{50} 10 mg/L) of lesenta after 96 hours (Finney, 1971). In test chambers, fish were subjected to sublethal doses of lesenta of 1 and 2 mg/L (1/10th and 1/5th of 96 h LC_{50} value, respectively) for 28 days. In group I, parallel controls were maintained. For 28 days, Group II fishes were given 1/10th of the 96-hour LC_{50} value, i.e. 1 mg/L of lesenta, while Group III fishes were given 1/5th of the 96-hour LC_{50} value, i.e. 2 mg/L. In each test chamber (40 L capacity all glass aquarium tank), ten fish were stocked in 30 L test solution per concentration in triplicate. Every 24 hours, the test medium was replaced. At intervals of 7, 14, 21, and 28 days, both control and exposed samples (Group II and III) were obtained to estimate O_2 consumption.

Respiratory Chamber

The respiration chamber was made locally in a laboratory, with certain modifications, as described by (Job, 1957). The thermocol cap was designed to fit snugly over the neck of a 2 L conical flask. To keep ambient air out, paraffin wax was placed around the thermocol cover. A small hole was drilled in the thermocol cap, and an aeration tube was placed through it to collect water samples at regular intervals and to fill the chamber.

During the experiment, one end of the aeration tube was inside the chamber while the other was closed. After 7 days of exposure, 5 fish were transferred from the test chamber, a glass tank with a capacity of 40 L, to a respiratory chamber with a capacity of 2 L and the same concentration as the test chamber. After allowing the fish to settle for half an hour, the experiment was conducted for three hours. Oxygen was measured using the Winkler method (Golterman et al., 1969). (Initially and after completion of 3 h duration). Simultaneously, a control was performed to acquire information on fish in a normal state. The test fish were weighed and placed in their appropriate test chambers after the experiment. After 14, 21, and 28 days of sample intervals, the same procedure was performed. For O_2 estimation, initial and final samples were taken (Winkler's method, Golterman et al., 1969).

Estimation of O_2 Consumption

Winkler's method was used to determine the amount of dissolved oxygen (APHA, 2005).

Principle: The method first proposed by Winkler (1888) and improved by Strickland and Parsons is used to determine oxygen concentrations in saltwater (1968). Iodide ion (I-) is quantitatively oxidized to iodine (I2) by oxygen in the water sample. Titration with a standard thiosulfate (S2O3-2) solution determines the amount of iodine produced. Starch is used as a visual cue to determine the endpoint. One mole of oxygen reacts with four moles of thiosulfate, therefore the amount of oxygen may be calculated from the titer.

Dissolved oxygen is fixed by adding Mn (II) under basic conditions at the time of sampling, resulting in a brown precipitate, manganic hydroxide (MnO(OH)2). The material is acidified to a pH of 1.0-2.5 before analysis. As a result, the precipitated hydroxides dissolve and Mn(III) ions are released. Iodide ions introduced previously are oxidized to iodine by Mn(III) ions. With excess iodide ions, iodine forms a compound (I3-). Iodine and the complex are in equilibrium, therefore I3- acts as an I2 reservoir. The iodine is then titrated with thiosulfate, which reduces the iodine to iodide and oxidizes the thiosulfate to tetrathionate. Because the thiosulfate solution is unstable, it must be standardized using a primary standard, such as potassium iodate (KIO_3).

The co-proportionation reaction of iodide with iodate, which produces iodine, is used to standardize. The excess iodide attaches to the iodine, and the complex is titrated using thiosulfate, as stated previously.

Methodology

- Fill a 300-mL glass stoppered Biological Oxygen Demand (BOD) bottle halfway with sample water.
- By putting the calibrated pipette just below the surface of the liquid, 2mL of manganese sulfate was immediately added to the collection bottle. Squeezed the pipette slowly to avoid introducing bubbles through the pipette.
- In the same way, add 2 mL of alkali-iodide-azide reagent.
- Carefully close the bottle to ensure that no air gets in. Invert the sample multiple times to mix it. Check for air bubbles; if any are found, reject the sample and start over. A brownish-orange cloud of precipitate or floc will form if oxygen is present. When the precipitate has settled to the bottom, turn the sample upside down several times and let it settle again.
- Using a pipette placed just over the surface of the sample, add 2 mL of concentrated sulfuric acid. To dissolve the floc, carefully cork and invert numerous times. The sample is now "fixed," and it can be stored in a cool, dark place for up to 8 hours. Squirt distilled

Table-1: Changes in oxygen consumption rate in *Cyprinus carpio* when exposed to Sub- lethal concentration of lesenta

Experimental period (Days)	Oxygen consumption rate (mg/g/h)				
	Control (Group I)	(Group-II) Lesenta 1 mg/L ($1/10^{th}LC_{50}$)	Change (%)	(Group-III) Lesenta 2 mg/L ($1/5^{th}LC_{50}$)	Change (%)
7	0.22±0.004^a	0.17±0.006^b	-20	0.13±0.004^c	-38
14	0.21±0.004^a	0.14±0.004^b	-30	0.11±0.004^c	-53
21	0.22±0.006^a	0.11±0.004^b	-46	0.06±0.002^c	-68
28	0.22±0.006^a	0.07±0.004^b	-64	0.03±0.004^c	-81

Values are the mean of ten observations
Standard Deviation is indicated as (±).
Values in ( ) indicates percent variation over the respective control:
Values are significant at a,b,c = $p<0.05$

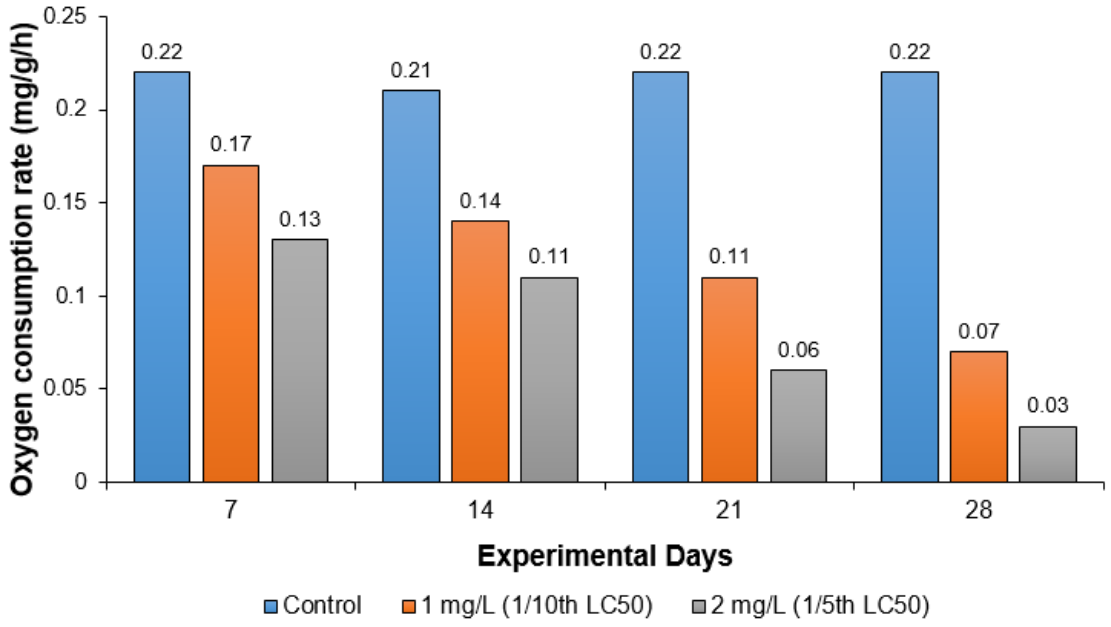

Figure-1: Changes in the oxygen consumption rate (mg/g/h) of *C. carpio* when exposed to different sub-lethal concentrations of Lesenta

water along the stopper as an extra precaution, then cover the bottle with aluminum foil and a rubber band for storage.

- Titrate 201 mL of the sample with sodium thiosulfate to a pale straw color in a glass flask. Titrate by slowly pouring titrant solution into the flask using a calibrated pipette while stirring or swirling the sample water.
- To make a blue color, add 2 mL of starch solution.
- Continue titrating carefully until the sample becomes clear. As the experiment progresses, only one drop of titrant will be required to erase the blue color. Make sure each drop is well blended into the sample before adding the next. Holding the flask up to a white sheet of paper to check for the disappearance of the blue tint can be useful.
- The number of milliliters of titrant used is equal to the concentration of dissolved oxygen in the sample. In steps 6 and 8, each mL of sodium thiosulfate added equals 1 mg/L dissolved oxygen.

Statistical Analysis

The fish's average O_2 consumption rate was determined using one-way ANOVA and Duncan's Multiple Range Test ($p<0.05$).

RESULTS AND DISCUSSION

Oxygen Consumption:

As shown in Table-6, the rate of oxygen consumption in *C. carpio* after exposure to lesenta on days 7, 14, 21, and 28 revealed a significant decrease in the rate of oxygen consumption. After 28 days of exposure, the rate of oxygen uptake in *C. carpio* was considerably lower than in the control group of fish. On acute treatment with lesenta, fishes demonstrated a steady decrease in the rate of oxygen consumption.

The O_2 consumption rate of fish impacted by Lesenta ranges between 1 and 2 mg/L at sublethal concentrations (Table-1 and Figure-1). When compared to control, the O_2 consumption rate decreased by 20% and 38% on the 7th day, 30% and 53% on the 14th day, 46 percent and 68 percent on the 21st day, and 64 percent and 81 percent on the 28th day at 1 and 2 mg/L concentrations, respectively.

Under both sub-lethal dosages, a decrease in oxygen consumption rate was detected, which could be due to toxicant-induced stress and oxidative metabolism impairment. If the gills or layer capacities are pulverized by xenobiotic synthetic compounds, or the film capacities are disrupted by a change in penetrability, the oxygen take-up rate will drop swiftly (Hartl et al., 2001; Poojari et al, 2014). In the current investigation, reduced O_2 consumption was found in C. Carpio as the concentration of lesenta and exposure length increased. Previously, a similar tendency was observed in profenofos toxicity in Catla catla (Maharajan et al., 2013) and nuvan toxicity in Ctenopharyngodon idellaon (Maharajan et al., 2013). (Tilak and Kumari, 2009). According to Kalavathy et al., the fluctuating reaction in respiration could be ascribed to a decrease in gill permeability, which causes a decline in oxygen intake, which the fish compensates for by increasing ventilation volume.

Respiratory distress as a result of oxidative metabolism impairment could explain the variable response in respiration. Because the gills are the primary respiratory organ, water constantly passes over them, using oxygen in the process. As a result, if toxicants occur in the water, the gills will be the first to be impacted. Respiration is a key phenomena of life, according to Dharmalata and Namitha Joshi (2002), and the rate of oxygen consumption affects metabolic activity. Changes in respiratory rates have been utilized as a stress indicator in pollutant-exposed species. Stressed fish were discovered in the sublethal concentration, however this was not fatal (Murthy et al., 2013). The respiration rate of the fish in the sublethal medium decreased over time, possibly due to adaptation of the fish to the chemical environment. The close contact of toxicants with the gills can cause defection of the normal respiratory region, resulting in gill tissue injury, which can impair the gill's diffusion ability, resulting in a reduction in oxygen intake.

Aquatic animals must pass a considerable amount of water over their respiratory surface and are therefore at a higher risk of toxic material exposure (Shelke and Wani, 2005). Anita Susan (2010); Shereena et al. (2009), Logaswamy and Remia (2009), Patil and David (2008), Vutukuru (2005), Shivakumar and David (2004), Rao et al. (2003), and David et al., (2003) discovered that disturbances in oxidative metabolism lead to changes in completely animal oxygen consumption in various species of fish exposed to pesticides. In the study of Logaswamy et al, the rate of oxygen consumption decreased during all of the exposure times (2009). The current work corresponds to the same report. Logaswamy and Remia (2009) investigated the effect of sublethal cypermethrin concentrations on Tilapia mossambica respiratory activity during time periods of 24, 48, 72, and 96 hours. The decrease in oxygen consumption in the fishes following exposure to lesenta may be related to lower gill respiration, according to the current study. The reduction was greater at higher concentrations, which could be due to a slower metabolism caused by toxicant stress. Mucus development on the respiratory organs can potentially cause respiratory inefficiency and severe respiratory collapse.

CONCLUSION

The experimental fish's entire metabolic rate, and thus its energy output, is reflected in the amount of oxygen it

consumes. Under Lesenta stress, changes in gill architecture would modify the diffusing capacity of the gill, resulting in hypoxic or anoxic conditions, making breathing a difficult chore for the fish. These findings suggest that changes in *Cyprinus carpio* respiration rates could be utilized as a rapid biological monitor to control Lesenta's impact on other biotic communities in the water body.

Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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