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Sublethal effect of pesticide (Cyclaniliprole) on Indian major carp *Labeo rohita* (Hamilton,1822)

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Abstract
The effect of the pesticide Cyclaniliprole on fingerlings *Labeo rohita* was assessed in captivity. A control group (no pesticide) and three concentrations of Cyclaniliprole (0.050, 0.150, and 0.750 ppm) were managed to live, healthy rohu (average initial weight of 8 g) in two replications for each handling. Based on the LC50 value for the 96-hour measurement made for this experimentation, these values were recognized. In the investigational tanks, the average temperature was 28.44±0.31ºC, and the average pH was 8.44±0.016 ppt. Both deadly 0.050, 0.150 ppm, and sublethal concentrations of 0.750 ppm showed behavioural and mortality effects. Following hyper and hypo opercula activity, loss of equilibrium, and mucus secretion throughout the body, *L. rohita* in toxic conditions displayed unpredictable and darting motions with dysregulated swimming activity, which may be caused by neurotransmitter malfunction. According to the current study's findings, the morality and behaviour of the commercially significant fish *Labeo rohita* are significantly altered by Cyclaniliprole contamination.

Keywords: Cyclaniliprole, *labeo rohita*, pesticides, Toxic, mortality

Introduction

The extensive and careless use of pesticides in farming causes environmental pollution and contaminates water physiques. Fish, mostly rohu *Labeo rohita*, are valued bio-indicators for measuring the impact of toxicants, providing crucial intuitions into safety estimation and environmental eminence monitoring (Hong *et al*, 2018) [9]. Pesticides and insecticides are determinedly and broadly applied in agro invention practices to improve crop yield and control of various pests, animals, insects, and vector-borne infections. Recurrent and continuous usage of pesticides and insecticides may induce adverse toxic impacts on both animals and public strength due to their toxicity (Hussain *et al*,2020) [8]. In the published literature, studies have indicated that different types of insecticides, herbicides, and pesticides cause teratogenicity organ toxicity and classified as endocrine disrupting substances in animals, including humans (Shahid *et al*, 2019; Ghaffar *et al*, 2021) [20, 5]. Numerous insecticides and synthetic chemicals are not biodegraded easily and tend to remain in soil and water for years (Ahmad *et al*, 2021; Namratha *et al*, 2021) [1,16]. Accidental exposure to these chemicals in marine, freshwater, and terrestrial environments not only cause adverse effects but greatly decreases the average expectancy of life in numerous exposed organisms (Baralic *et al*, 2020; Merdana *et al*, 2021; Tahir *et al*, 2021) [3, 14, 22]. However, many researchers have been reported alteration in the blood parameters in different fish species due to pyrethroid and organophosphate pesticides exposure in Bangladesh (Jasmin *et al*, 2017; Khatun *et al*,2014; Mostakim *et al*, 2015) [10, 12, 15] but, the researches on the effects of pyrethroid and organophosphate pesticides on the hematology of minor carps are so scares. Cyclaniliprole is metabolized and eliminated significantly more slowly by fish than by mammals or birds, which may explain this compound's higher toxicity in fish compared to other organisms (Stephenson, 1983) [21]. Cyclaniliprole is widely used and common pesticides in Bangladesh. The effect of Cyclaniliprole in fish is very much hazardous. The present experiment was directed to regulate the median lethal absorption (LC50) of Cyclaniliprole pesticide on juvenile rohu (*L. rohita*) and to investigate the collaborative change of fish under laboratory disorder with different concentration of Cyclaniliprole

Materials methods

1. Experimental site

Fresh water fish *Labeo rohita* weighing 8.02 ± 0.02g were collected from the Suriya Fish Farm, Kallidaikuruchi, Tirunelveli. The fishes were thereafter brought to the lab in polythene bags with low-temperature, oxygenated water with least disturbance. Then they were acclimatized to the ambient laboratory room temperature range (28 ± 1.0°C) in (FRP) 20 liters of water and providing aeration facilities. Fine meshed nets were used to cover the aquarium to avoid jumping of fish.

2. Maintenance of test Fish

The fish were transferred to the laboratory and released in the rectangular glass aquaria for acclimatization (12 days). During the study period, the fish were fed with a commercial pellet feed twice daily to satiety for avoiding the effects of starvation. Tap water was used throughout the course of the experimental period and was aerated continuously through aerator. The experimental water was renewed every 24 hours to remove fecal matters and to maintain the better environment for the experimental fish.

3. Feed

The fish were not fed while they were acclimated. The fish were fed a commercial diet two days later. They were given a certain amount of food to determine how much they could consume each day. A suitable quantity of food is provided to the fish, and no food is left untouched.

4. Experimental design

To determine the LC50, batches of 20 healthy fish were subjected to varying concentrations of the insecticide cyclaniliprole 10% DC. Another group of fish is kept in the tap water as a control. Using a minimum of 10 fish per concentration and a suitable narrow range of concentrations, the median fatal concentration was determined. Mortality was recorded every 24 hours for a total of 72 hours. In 1000 ppm out of 10 fishes 10 are died at 72 hours. Thus 1000 ppm is selected as LC50. Four groups of fishes were exposed in 1000 ppm concentration of the pesticide cyclaniliprole for 24,48 and 72 hours respectively. Another group was maintained as control. At the end of each exposure period, Present study was accomplished in two replicates in order to ensure the reproducibility of the results. Randomly divided 20 fish were equally divided into three treatment groups as follows: In Treatment 1, control group had no pesticide exposure, whereas fishes in treatment 2, 3, and 4 had exposure of cyclaniliprole at a concentration of 0.050, 0.150 and 0.750 ppm, respectively.

5. Behavioral study

Fish exposed to an acute dose of cyclaniliprole displayed a variety of behavioral abnormalities, including decreased feed intake, abrupt trough movement, excessive mucus spreading over the body, nervousness, and skin color changes. Mortality was reported at 24, 48, 72, and 96 hours; at 96 hours, cumulative mortality (50%) was found. Dead fish were found to be pale and to have slippery skins. Fish treated with cyclaniliprole exhibited behavioral alterations comparable to those shown in acute studies during the sub-lethal toxicity research, albeit these changes happened in a slower phase. The experimental fish behaved like the control fish at the conclusion of the third day after treatment, with the exception of a modest skin colour change. The longer the exposure time, the paler the fish's skin colour grew. In conclusion, fish displayed excessive mucus secretion throughout their bodies and floated on the water's surface to absorb oxygen from the atmosphere

6. Biological data collection

Each group of fish consisting of twenty (20) individuals were selected at random and placed into aerated test chambers. After 24 hours of adaptation, different concentrations of cyclaniliprole were added to the experimental aquaria. Mortality was assessed every day and continuing at the end of the experiment. Dead individuals were removed immediately. Behavioral changes were followed closely. The dead fish was removed and used for further experiment for observed of gill, liver and kidney conditions

Results

The present experiment was conducted to determine the median lethal concentration (LC50) of Cyclaniliprole pesticide on juvenile *Labeo rohita* under laboratory condition with different concentration and exposure duration.

Batches of 5healthy fishes

Cyclaniliprole exposed to fishes

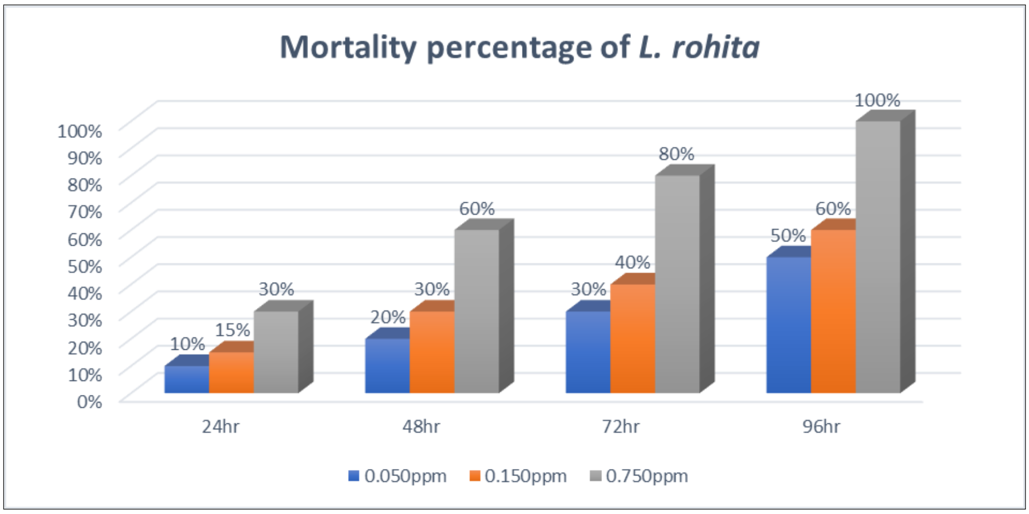
LC50 assay group of fishes

Determination of LC50

LC50 is a measure of pesticide acute toxicity by concentration. It is the concentration of a chemical at which 50% of the test group dies. The cumulative percentages mortality of rohu was recorded at 24 hours interval against 0.050, 0.150, and 0.750 ppm concentration of cyclaniliprole and rohu in the control tanks (no pesticides). There was no mortality found at 0.050 ppm concentration up to 72 hours. At three days 50 % mortality was found in the 0.050 ppm concentration aquarium. At three days 60% mortality was found in the 0.150 ppm and the mortality was found in second days at 0.150 ppm concentration to 48 hours. The maximum mortality was recorded at 100% with 0.150 ppm and 24 hours 0.750 ppm in the first day. In this concentration fish showed some peculiar behavior and all the fish are died before twelve hours. Within the 96 hours

4days the maximum mortality at 100 % was found in 4th day at 0.045 ppm concentration. After 4th days no fish are seen to be died in Table 1. The LC50 value was measured separately for 0.050, 0.150, 0.750 ppm concentration by plotting the mortality data against the days. In the sub-lethal concentration, the maximum mortality was recorded in 4th days.

Table 1: Cumulative mortality percentage of *L. rohita* in different concentration of Cyclaniliprole at different exposer time.



Behavior observed

Generally, fish showed very normal behavior. In these experiment different doses of pesticide used to understand the behavioral change of fish when they were exposed in those doses. The behavioral changes of fish in different concentration of pesticides were determined in different expose time of the experiment were recorded and presented in Table 2.

Table 2: Behavior of fish observed in different concentration of test chemical in respect of time

Behavior of fish	Day at different concentration		
	0.050	0.150	0.750
Migration to the bottom	-	48hr	48hr
Schooling behavior disrupted -	-	48hr	48hr
Spread out and swimming independently -	-	48hr	48hr
Irregular, erratic and darting movements -	72hr	48hr	48hr
Escaping phenomenon	-	48hr	48hr
Repeated opening and closing of the mouth and opercula		48hr	48hr
Hyperextension of all fins -		48hr	48hr
Excitement -		48hr	48hr
Corkscrew pattern swimming behavior -		48hr	48hr
Sudden, rapid, non-directed spurt of forward movement		48hr	48hr
Signs of tiredness		48hr	48hr
Barrel-rolled or spiraled at intervals -	72hr	48hr	48hr
Engulfed the air through mouth -		48hr	48hr
Mouth and operculum wide opened -	48hr	48hr	48hr
Colour of the gill lamellae from reddish to light brown		48hr	48hr

When the fish were exposed to the lethal concentration of Cyclaniliprole, they migrated immediately to the bottom of the tank. The schooling behavior was observed to be disrupted in the first day at (24hr) and the fish occupied about twice the area than that of the control group. They spread out and appeared to be swimming independently. Irregular, erratic and darting movements followed this with imbalanced swimming activity. The fish exhibited peculiar behavior of trying to leap out from the pesticide medium, which could be revealed by their escaping phenomenon. The frequency of surfacing phenomenon was greater on the (48hr) second day of exposure wherein the fish frequently come to the water surface. Respiratory disruption was observed in the normal ventilating cycle (cough, yawn) with a more rapid, repeated opening and closing of the mouth and operculum. Partially extended fins and singlewide opening of the mouth and opercula coverings accompanied by hyperextension of all fins were found and the fish was in a state of excitement on the (72hr) third day. The swimming behavior was in a corkscrew pattern rotating along horizontal axis and followed by sudden, rapid, non-directed spurt of forward movement (burst swimming). The fish progressively showed signs of tiredness and lost positive rheotaxis characterized by weakness and apathy. On the (96hr) 4th day they lost their equilibrium and response, to external stimuli such as touch and light followed by drowning to the bottom. They often barrel rolled or spiraled at intervals and engulfed the air through mouth before respiration ceased. The fish eventually died with their mouth and operculum wide opened. A change in color of the gill lamellae from reddish to light brown with coagulation of mucus on gill lamellae was seen in dead fishes. In sub lethal treatment, 0.150 ppm the schooling behavior of the fish was slowly disrupted during the(24hr) first day. The ventilation rate was increased, but hyperactivity, excitement, hyperventilation etc., were not influenced on exposure to the sub lethal concentration of cypermethrin at 48hr and 72hr. In the (96hr) 4days of the experiment the mortality observed. However, in the control tank the fish showed mortality to the pesticide tank. The movement of the fish was also normal and this group in compare to the pesticide group.

Discussion

The determined and unfortunate use of many synthetic compounds, chemicals, including pesticides and insecticides on vegetables and other cereal crops, can be a considerable risk of infertility and abnormal sperm counts in exposed animals, including humans. Moreover, many studies have investigated that exposure to pesticides and insecticides at low levels is mainly related to the induction of adverse effects like immunosuppression, cancer, endocrine disruption, and reproductive disorders. For toxicological estimation, various physical parameters such as clinical ailments, body weight, and weight of different visceral tissues are known as reliable toxicity biomarkers. Furthermore, frequent use of pesticides causes the presence of residues in the food chain contamination of the environment and water resources (Yang *et al*, 2021) [23]. The acute test for a long time has been a major component in toxicity testing in which acute chemical toxicity is determined as a 96 hr LC50 value. However, the environmental significance of death of individuals after short term exposure to high concentration is questionable. In the present experiment the LC50 value was 0.050 ppm at 48hr, 0.150 ppm at 24hr, different from this experiment. Lethality in the present study is comparable to the few previously published studies that exist, but that LC50’s for all species exceeded this concentration. This can be attributed to the inability of the *L. rohita* to withstand and metabolize the Cyclaniliprole intoxication. The varying degree of mortality reported in this study is consistent with the report of (David *et al*, 2000) [4]. The opercular movement of the fish ceases immediately following exposure to Cyclaniliprole. The decrease in opercular movement and corresponding increase in frequency of surfacing of fish clearly indicates that fish adaptively shifts towards aerial respiration and the fish tries to avoid contact with the pesticide through gill chamber (Santhakumar, *et al*,2000) [19]. The increased ventilation rate by rapid, repeated opening and closing of mouth and opercular coverings accompanied by partially extended fins was observed in the present study. The surfacing phenomenon of fish observed under cypermethrin exposure might be due to hypoxic condition of the fish as reported by (Radhaiah and Jayantha, 1988) [18]. The increased surfacing during the initial periods of exposure to cypermethrin concentrations suggests an elevated rate of metabolism. Variations in aeriation rate and surfacing incidences are the wide-ranging suggestions perceived in the fish after acquaintance to the pesticide and these activities help the fish to avoid contact with poison and fight against stress. Mortality of hurtful catfish Heteropneustes fossils rose at problematic concentrations of Envoy 50 (Akter *et al*, 2020) [2] and with exposure to sublethal concentration of Pb toxicant (Hussain *et al*., 2021). Chlorpyrifos has been shown to have significant effects on growth of O. niloticus (Majumder d Kaviraj, 2019) [13]. The fish behavior indicates that the fish has adapted to a compensatory mechanism to derive energy during pyrethroid toxicosis as suggested by (Philip *et al*, 1988). Fish species are sensitive to enzymic and hormone disruptors. Doses of pesticides that are not high enough to kill fish are associated with subtle changes in behavior and physiology that impair both survival and reproduction (Kegley *et al*, 1999) [11]. Similar changes in behavior are also observed in several fishes exposed to different pesticides (Haider, 1986) [6]. The insecticide Cyclaniliprole and fish *L. rohita* were selected for study because the former is used often in field and the latter is an important paddy fish of Indian capture fishery.

Conclusion

The maximum mortality was found in the 0.050 and 0.150 ppm concentration of Cyclaniliprole. The growth of fish also hampers when they are exposed in Cyclaniliprole medium. The gill, kidney, brain also get stress. If the fish are exposed for long time in the pesticide medium, then different types of biochemical compounds are decreased from the body. So, it requires minimum six months to minimize pesticide effect on fish. Furthermore, the results of our experimental study indicate that investigation and monitoring of toxic effects of various environments is of vital importance to develop control strategies regarding pollutants and to mitigate harmful effects in exposed organisms, including public health.

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