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Toxicity of cypermethrin on fingerlings of rohu: *Labeo rohita* (ham)

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Abstract
The aim of the present investigation was to standardize the lethal concentration (LC_{50}) and safe concentration for the selected biocide cypermethrin on fingerlings of *Labeo rohita* and by following acute toxicity test. The effect was assessed on the basis of results obtained in the acute toxicity test. The static bioassay methodology was followed to find out the 24h, 48h, 72h and 96 h LC_{50} values for cypermethrin. Experiment was carried out with three replicates under a treatment. The 24h, 48h, 72h and 96 h LC_{50} values for cypermethrin was calculated to be be 3.84 (3.68 to 3.86) µg/l; 3.66 (3.63 to 3.71) µg/l; 3.53 (3.50 to 3.55) µg/l and 3.48 (3.32 to 3.63) µg/l respectively. The safe application factors for cypermethrin were 1.049 µg/l and presumable harmless concentration were 0.997 µg/l.

Keywords: Toxicity, lethal concentration, cypermethrin, *Labeo rohita*

1. Introduction
During the last decade of the twentieth century and the beginning of the twenty-first century the dependency of man on the earth's natural resources has become increasingly apparent. As society has become more technologically advanced, pollution has evolved from being primarily biohazards in our water to containing an ever expanding mixture of dissolved manufactured chemicals. Pollution is the important global limiting factor for man. The growing population together with the rapid industrialization has made the problem more serious. Widespread use of various pesticides and their impact on environment are now a worldwide phenomenon (Omitoyin *et al.,* 2006) [15]. Only about 1% of applied pesticides land on the target organisms and the rest contaminate the environment (Lawson *et al.,* 2011) [7]. Fishes, the most diverse group of vertebrate fauna are important component of the food chain and any effect of toxicant may have adverse influence on the nutritional value of fish and on human being through their consumption (Gupta and Srivastava, 2006) [4]. Due to injudicious and indiscriminate use of these pesticides which lead some serious problems to the non-target organisms such as fishes, mammals and birds (Kumari and Subisha, 2010) [6]. Pesticides belonging to different major classes such as organochlorines, organophosphates, carbamates and synthetic pyrethroids are known to result in widespread contamination of freshwater ecosystems and cause harm to aquatic biota. Cypermethrin is a highly potent synthetic pyrethroid insecticide that virtually used to control insects in agriculture, home and garden (Jee *et al.,* 2005) [5]. Cypermethrin is considered as immobile and not expected to bio-magnify through food chain. Due to its lipophilicity, have a high rate of absorption thereby rendering fish as most sensitive to pesticides (Saha and Kaviraj, 2009) [12]. Cypermethrin is extremely toxic to fish at very low concentrations and to aquatic invertebrates, in aquaculture cypermethrin are applied to control ectoparasites, especially lice, as well as insects in nursery and grow-out systems. However, fish are hypersensitive to pyrethroids due to the sensitivity of their nervous systems to these pesticides. In fact fish are generally regarded as sentinels of bioindicators for aquatic pollution and indispensable experimental models in eco toxicological studies. With this view *Labeo rohita* (Hamilton) was considered and selected for the present investigation. Therefore the present investigation was undertaken to standardize the lethal concentration (LC_{50}) for cypermethrin on fingerlings of *Labeo rohita* and find out the safe concentration of cypermethrin depending upon the LC_{50} values therefore the present investigation was undertaken to test for lethal toxicity of cypermethrin and its concentration standardisation.

2. Materials and method

Healthy and active *Labeo rohita* fingerlings Weighing 7.80±0.40 g, measuring 6.5 ± 0.25 cm were procured from the Instructional farm of College of Fisheries, Rangeilunda. and acclimatized in FRP tank under laboratory condition after providing a dip treatment in 0.1% potassium permanganate solution to prevent infection. The acclimatization process continued for 20 days under laboratory condition prior to initiation of the actual experiment. They were fed commercial pelleted feed twice daily during acclimatization period with exchange of rearing media in every 24 hours. The test medium for the above study against fingerlings of *Labeo rohita* at 24, 48, 72 and 96 h under acute toxicity and chronic exposures up to 30 days containing synthetic pyrethroid i,e cypermethrin. To commence with assays of cypermethrin, common stock solution was prepared by dissolving in one litre of distilled water. For the preparation of common stock solution following formula was used

$N_1V_1=N_2V_2$

Where N_1=concentration of availability percentage.

V_1= volume of available pesticide.

N_2= Required concentration of pesticide to be prepared.

V_2 = Volume of solution required

Series of different concentration of cypermethrin prepare as microgram per litre which were prepared by adding the common stock solution into the measured distilled water with the help of pipette.

The freshwater used for the bioassay studies conducted at College of Fisheries, Rangeilunda was collected from the fish pond located in the instructional farm. The collected pond water was filtered and stored in 500L FRP tanks for 24 h with aeration. The acclimatisation was done in FRP tank of 200L capacity and Glass aquariums of 25L capacity were used as test container. After cleaning with appropriate detergents, rinsed with acetone and properly washed with tap water prior to initiation of the acute toxicity test. After each test the containers were cleaned properly. Each experimental containers were covered with nylon screen to prevent fishes from escaping.

Range finding static bioassay for the fingerlings of *Labeo rohita* was conducted as per APHA, 1985 with test organisms exposed to different range of concentrations. Before initiation of the range finding tests, the animals are starved for 24 hours, no feed and aeration were given during the experiment. The percentage of mortality was recorded at an interval of 24, 48, 72 and 96 h. It is worth to mention here the concentration between 3.4 µg/l and 3.8 µg/l were selected for the present static bioassay study 0f cypermethrin. For determination of LC_{50} Cypermethrin, a series of five test concentrations 3.4, 3.5, 3.6, 3.7 and 3.8 µg/l a. Each concentration was run in triplicate with a control. The percentage of mortality at the end of every hour was recorded and then pulled to 24, 48, 72 and 96 hours. Test medium was renewed for every 24 h with their respective test solution and dead fishes were removed immediately after experiment period. of the toxicant without aeration. Behavioural signs and symptoms of the fingerlings in lethal toxicity. Lethal concentrations were determined by adopting short term static bio-assay technique recommended by Sprague (1969, 1970 and 1971) [18, 19, 13]. The data gathered during the present investigation were analysed by probit regression analysis (Finney, 1971) [3] for determination of LC_{50} values for cypermethrin. The percentage mortality against log concentration was plotted in probability paper to get LC_{50} values graphically

The Safe application factor was calculated as per Hert *et al*., 1948 using the formula

$C = 48\ h\ LC_{50} \times 0.3 / S^2$

Where, C = presumable harmless concentration

$S = 24\ h\ LC_{50} / 48\ h\ LC_{50}$

3. Results

The result pertaining to the lethal toxicity of cypermethrin exposed to different concentrations from3.4 µg/l to 3.8 µg/l indicated that Death of the fingerlings was not observed for first twelve hours in any of the concentration. and, no mortality was observed till the termination of the experiment in control. 50% mortality was observed at a cypermethrin concentration of 3.5 µg/l at the end of 96 h whereas, mortality of 50% test animals were observed at a cypermethrin concentration of 3.6 µg/l and 3.7 µg/l at the end of 72h and 48h respectively. The test animals exposed to the highest concentration of 3.8 µg/l of cypermethrin registered 50% mortality just after 24 h. (Table-1)

Table 1: Test for lethal toxicity of *Labeo rohita* fingerlings exposed to cypermethrin

Concentration (µg/l)	Concentration × 10	Log concentration	Total number of fish	Percentage mortality				Response			
				24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
3.40	34.0	1.5314	10	10	20	20	30	0.1	0.2	0.2	0.3
3.50	35.0	1.5440	10	20	30	30	50	0.2	0.3	0.3	0.5
3.60	36.0	1.5563	10	30	40	50	60	0.3	0.4	0.5	0.6
3.70	37.0	1.5682	10	30	50	70	90	0.3	0.5	0.7	0.9
3.80	38.0	1.5797	10	40	80	90	100	0.4	0.8	0.9	1.0

The mortality of fish increased with the increase in the concentration of the toxicant, depicting a direct correlation between the mortality and the concentration. The LC_{50} values for 24 h, 48 h, 72 h and 96 h along with its 95% lower and upper confidence limits (ML and MU) for synthetic pyrethroid Cypermethrin is presented in Table-2.

The probit regression analysis indicates the 24 h, 48 h, 72 h and 96 h LC_{50} value for *L. rohita* to be 3.84 (3.68 to 3.86) µg/l; 3.66 (3.63 to 3.71) µg/l; 3.53 (3.50 to 3.55) µg/l and 3.48 (3.32 to 3.63) µg/l respectively.

Table 2: Lethal toxicity (LC_{50}) values for *Labeo rohita* fingerlings exposed to different concentrations of cypermethrin

Duration	24 h		48 h		72 h		96 h	
	LC_{50} (µg/l)	Slope 'b'	LC_{50} (µg/l)	Slope 'b'	LC_{50} (µg/l)	Slope 'b'	LC_{50} (µg/l)	Slope 'b'
Fingerlings	3.84 (3.68 – 3.86)	2.07	3.66 (3.63 – 3.71)	2.87	3.53 (3.50 – 3.55)	2.92	3.48 (3.32 – 3.63)	2.98

Values in parenthesis represent 95% confidence limit

The safe application factor of the treated synthetic pyrethroids cypermethrin to fingerlings of rohu is presented in the Table-3. The safe application factors for cypermethrin were 1.049 µg/l and presumable harmless con centration were 0.997 µg/l.

Table 3: Safe application factor of the treated synthetic pyrethroids to fingerlings of *Labeo rohita*

Name of the Pyrethroids	S value = 24 h LC_{50} / 48 h LC_{50} (µg/l)	C = 48 h LC_{50} × 0.3 (µg/l) / S^2
Cypermethrin	1.049	0.997

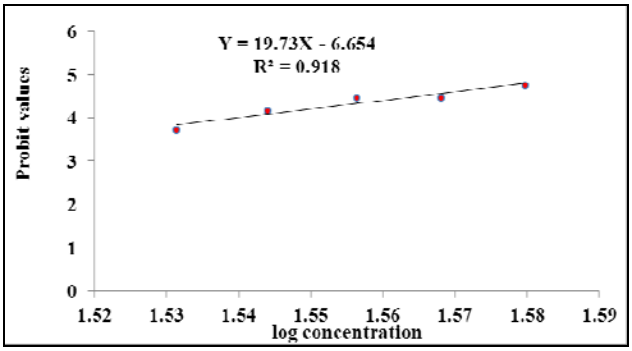

Fig 1: Linear curve between probit mortality against log concentration on 24 h exposure to cypermethrin in fingerlings of *Labeo rohita*.

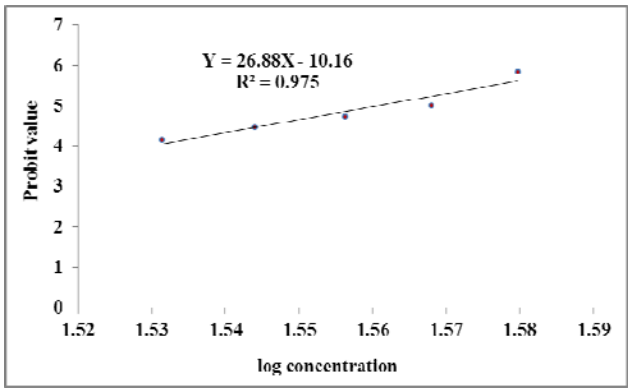

Fig 2: Linear curve between probit mortality against log concentration on 48 h exposure to cypermethrin in fingerlings of *Labeo rohita*.

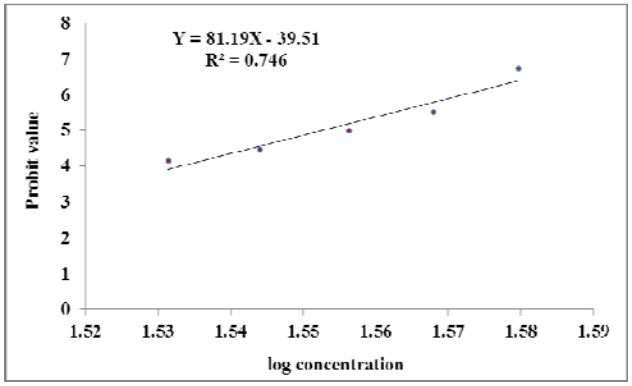

Fig 3: Linear curve between probit mortality against log concentration on 72 h exposure to cypermethrin in fingerlings of *Labeo rohita*.

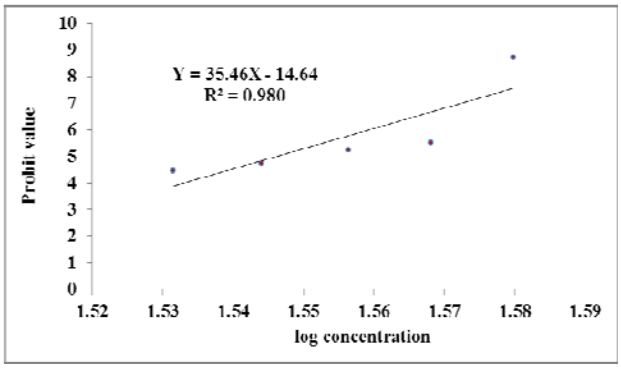

Fig 4: Linear curve between probit mortality against log concentration on 96 h exposure to cypermethrin in fingerlings of *Labeo rohita*.

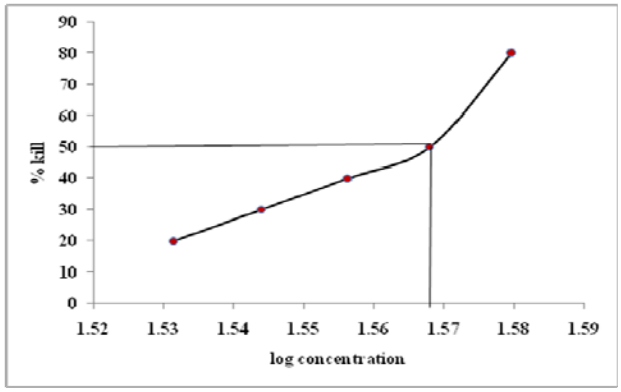

Fig 5: The percentage mortality against log concentration on 48h exposure to cypermethrin in fingerlings of *Labeo rohita*.

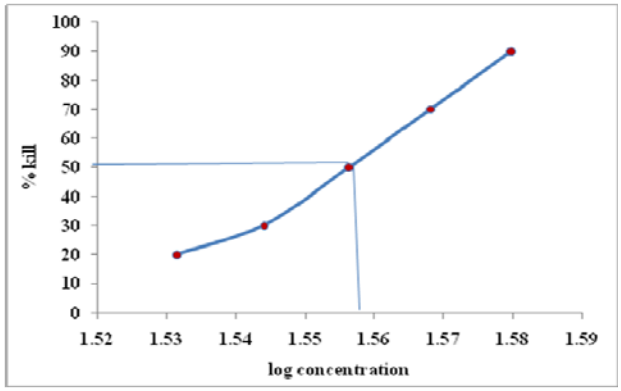

Fig 6: The percentage mortality against log concentration on 72h exposure to cypermethrin in fingerlings of *Labeo rohita*.

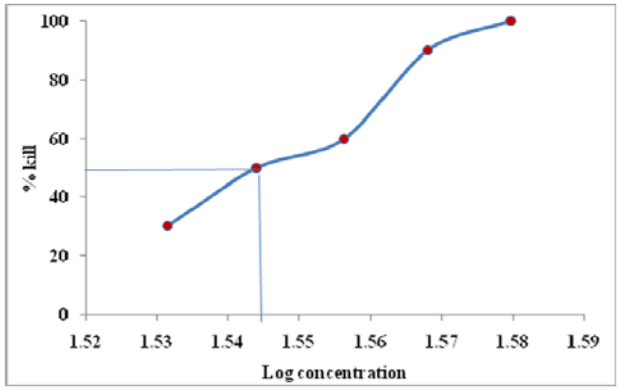

Fig 7: The percentage mortality against log concentration on 96h exposure to cypermethrin in fingerlings of *Labeo rohita*.

4. Discussion

Result relating to the test for lethal toxicity and standardisation of lethal concentration of Rohu fingerlings of cypermethrin is in consonance with the result of polat *et al*. 2002 [10]; Rahmi *et al*. 2005 [11], who reported cypermethrin could affect even early stages of fish more potentially. Bradbury and Coats (1989a) [2] gave the same type of results regarding the toxicology of pyrethroids in mammals, birds, fish, amphibia and invertebrates and cited 96-h LC_{50} cypermethrin toxicity as 2.2 µg/L for *Tilapia nilotica*, 0.9–1.1 µg/L for carp (*Cyprinus carpio*). In the present investigation the LC_{50} of fingerlings of rohu exposed to different concentrations of cypermethrin after 48, 72, and 96h was 3.66 µg/L, 3.53 µg/L, and 3.48 µg/L Respectively. Which is agreement with the result of Marigouder *et al.,* (2009) [8] who reported no 96 hr LC_{50} of cypermethrin for *L. rohita* as 4.0 µg/l. In the present investigation the safe concentration and presumable harmless concentration of *L. rohita* fingerlings against cypermethrin were 1.049 µg/l and 0.997 µg/l respectively, which confirm the result of Gautam and Gupta (2008) [17] who recorded range of safe dischargeable and presumable safe concentrations of cypermethrin as 1.04 to 1.09 µg/l and 45.18 to 75.25 µg/l respectively for the juveniles of *Poecilia* at selected levels of temperature, hardness, pH and salinity. Verma and Gupta (2008) [14] evaluated the safe dischargeable concentration of cypermethrin for the zooplankters in the range of 1.043 to 1.220 µg/l. Velisek *et al.,* (2011) [16] noticed similar result of, 96 h LC_{50} of common carp were 2.91 µg/l. However a slight variations in the result obtained in the present investigation may be attributed due to the differences in test conditions, species specificity of test chemicals, external factors influencing pesticide toxicity like dissolved oxygen, ambient temperature of water, pH, hardness of water etc. However, the present investigation are in agreement with the earlier findings (Marigouder *et al.,* 2009; Gautam and Gupta, 2008; Verma and Gupta, 2008; Velisek *et al.,* 2011) [9, 17, 14, 16].

5. Conclusion

The pesticides selected for the present investigation are used in the aquaculture for controlling of crustacean fish parasites, predatory fishes and predatory insects. Hence it is an important aspect to understand the impact of various doses of these pesticides on aquatic animals like fish. The LC_{50} value provides a base, upon which the technically valid and cost effective programmes can be designed for the benefit of farmers. Safe concentration of the different pesticides gives an idea on the non-hazardous impact on aquatic ecosystem and aquatic animals like fish A series of pesticide or chemical products are now used in the aquacultural practice. Hence, the farmer needs to be provided with the abreast knowledge on the toxicity of these products prior to their use.

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