

Evaluation of Acute Toxicity and Lethal Concentration (LC₅₀) of Cypermethrin (25% EC) in Adults of *Cyprinus carpio* (Linnaeus 1758)

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ABSTRACT

The present study attempted to determine the acute toxicity and medial lethal concentrations of Cypermethrin (25% EC) for common carp *Cyprinus carpio* using Finney's static bioassay (1971), probit analysis method. The LC₅₀ values for 24, 48, 72 and 96 hr were found to be 3.962 µl Lit⁻¹, 3.767 µl Lit⁻¹, 3.349 µl Lit⁻¹ and 2.924 µl Lit⁻¹ respectively. Fish (weight 90–100 g, length up to 18±2 cm) were exposed to different concentrations of cypermethrin which were 1.5 µl Lit⁻¹, 2.0 µl Lit⁻¹, 2.5 µl Lit⁻¹, 3.0 µl Lit⁻¹, 3.5 µl Lit⁻¹, 4.0 µl Lit⁻¹ and 4.5 µl Lit⁻¹ up to 96 hr. LC₅₀ value decreases with an increase in exposure time. During this exposure period, abnormal behaviors like restlessness, hyperactivity, erratic movements, increased opercular movements, and loss of equilibrium and response were noticed in carp. Over secretion of mucous all over the body surface along with respiratory stress and mortality was also observed.

Keywords Probit analysis, *Cyprinus carpio*, Cypermethrin, LC₅₀, Finney's bioassay.

INTRODUCTION

Excess pesticide infestation of the environment can induce a threat to terrestrial and aquatic fauna. In contemporary agricultural practices, synthetic pyrethroids have replaced many insecticides such as carbamates and organophosphates. These synthetic insecticides come with enhanced effectiveness and stability. Cypermethrin is a type II synthetic pyrethroid that has been derived from the flower of *Chrysanthemum* species having pyrethrin as an active ingredient. In ancient practices, an extract of pyrethrin was used directly against insects but later its chemical analogs were produced artificially. Pyrethrin had remained in practice for decades to counter insects in a wide range as they were safe and had a short half-life therefore was not persistent enough to reach water on normal application (Di Giulio and Hinton 2008). Despite being a pyrethrin derivative, synthetic pyrethroids production involves many chemical modifications that add enhanced toxicity and stability to them which make them more persistent in the environment (Thatheyus and Selvam 2013). On application, to encounter the insects of households or insects that act as pests for crops, synthetic pyrethroids can percolate into the soil to ultimately reach freshwater resources. On reaching the rivers or streams aquatic animals get exposed to them which

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may interfere with their behavior or physiology. In context with fish, pyrethroid may come in contact via gills, an organ of chief importance that makes direct contact with the surrounding water (Wendelaar 1997). The lipophilic nature of pyrethroid promotes their solubility in non-polar constituents of cell and act as an important contributing factor to make pyrethroids more potent to fish even at shallow levels (Mishra *et al.* 2005). Acute toxicity studies inspect the behavioral and cellular alterations caused by these pyrethroids on administration in fish. In understanding the relative toxicities induced by insecticides, LC_{50} value determination can play a crucial role.

MATERIALS AND METHODS

Collection and maintenance of fish

The experimental fish *C. carpio* (weight 90–100 g, length up to 18 ± 2 cm) were collected from the Deoli fish farm Ghaghus, district Bilaspur, Himachal Pradesh in well-oxygenated polythene bags and acclimatized in the aquarium of laboratory for 10 days at Himachal Pradesh University. Fish were kept in 0.2% potassium permanganate solution for 2-4 minutes to treat unwanted infection or injury. All the fish were kept in an aquarium of capacity approximately 180 liters. The water was dechlorinated and free from any other pollutants.

Analysis of water chemical parameters

The average values for tap water used in both acclimation and experiments were pH 7.85 ± 0.510 , dissolved oxygen 8.06 ± 0.014 mg/l, and water temperature was maintained at $24 \pm 2^\circ\text{C}$. The aquariums were aerated with air stones attached to an air compressor to saturate with oxygen. The water quality parameters mentioned above were assessed in the experimental period. Throughout the acclimation period and experiment periods, fish were held under a photoperiod of 8 h of light and 16 h of darkness.

Feeding

During the acclimation period, the fish were fed on commercial pellet feed based on rice and wheat flour composition (minimum 29% protein, 3% fat, 8%

fiber, 9% ash along with vitamin and mineral premix).

Test chemical

The test chemical cypermethrin of technical grade (25%) was purchased from the local market of Shimla under the trade name Cyperkill. The stock solution of the test chemical was prepared by dissolving 100 μl of cypermethrin in 1 liter of distilled water. All experimental procedures were conducted after the approval of the Department of Fisheries, Himachal Pradesh.

Acute toxicity test

Fish were starved for 24 hrs and feeding was stopped during the acute toxicity evaluation. Fish were exposed to different concentrations of cypermethrin stock solution which were 1.5 $\mu\text{l Lit}^{-1}$, 2.0 $\mu\text{l Lit}^{-1}$, 2.5 $\mu\text{l Lit}^{-1}$, 3.0 $\mu\text{l Lit}^{-1}$, 3.5 $\mu\text{l Lit}^{-1}$, 4.0 $\mu\text{l Lit}^{-1}$, and 4.5 $\mu\text{l Lit}^{-1}$ up to 96 hr in glass aquarium of capacity around 180 liters. Test water was renewed every 24 hrs intervals to keep insecticide concentration maintained. Fish mortality was observed from 24 to 96 hrs and dead fish were removed immediately to minimize the risk of contamination or infection. LC_{50} was calculated by Finney's probit bioassay test (Finney 1971).

RESULTS AND DISCUSSION

Behavioral response

In the control group, no mortality was observed while in the treated group, an extreme shift in behavior was noticed accompanied by mortality. The mortality rate increased with an increase in concentration and exposure period. Compared to the control group the

Table 1. Behavioral response in control and treated group.

Group→ Behavior↓	Control	Treated
Schooling behavior	++	-
Opercular movement	++	+
Darting behavior	-	++
Response and equilibrium	++	-
Bottom migration	-	++
Surfacing phenomena	-	++
Mortality	-	++

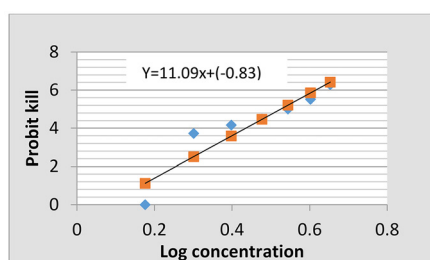


Figure. 1

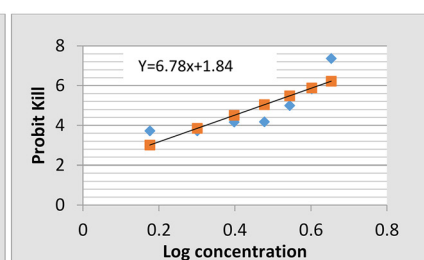


Figure. 2

Figs. 1-2. Linear regression equation showing probity mortality of *Cyprinus carpio* against log concentration of cypermethrin for 72 hr and 96 hr.

fish from the treated group showed some abnormal behavioral activities like distortion of schooling behavior, impaired response, and equilibrium on external disturbances such as tapping the aquarium or passing the light. The frequency of bottom migration and random surfacing phenomena was prominent in the exposed group to deal with a hypoxic ambience.

The darting movement which shows some fighting behavior among fish of treated groups due to sudden contaminant rush was also observed. The frequency of opercular movements which is important to ensure proper gaseous exchange at the gill surface increased with time and dose which was also reported in *Channa punctatus* (Chilke and Bacher 2014) and in *Cirrhinus mrigala* (Cheema *et al.* 2018) when exposed to chlorpyrifos and cadmium chloride. In support of the present investigation, similar results such as erratic swimming and avoiding behavior were mentioned by Dhara and Karmakar (2016) and Kumari *et al.* (2017) in *Channa gachua* and *Labeo rohita* respectively. In juveniles of *Oreochromis niloticus* very minute concentrations of cypermethrin

caused similar behavioral alterations which involved surfacing phenomena and enhanced air gulping activity and escaping phenomenon (Yaji *et al.* 2011). Restlessness, distorted balance, and mucus accumulation on the skin were caused by increased physiological stress (Ojutiku *et al.* 2013). All observed behavioral alterations observed during this experiment are listed in Table 1.

Median lethal concentration (LC₅₀)

Several LC₅₀ evaluation studies have reported cypermethrin as a very potent chemical to cause behavioral and morphological disturbances sometimes accompanied by death at very low concentrations which is reported in *H. fossilis*, *Oreochromis niloticus*, *Catla catla* and *Channa punctatus* by Bhutia *et al.* (2013), Sarikaya (2009), Jindal and Sharma (2019) and Kumar *et al.* (2007) which is found to be 3.783 µg/L, 5.99 µg/L, 1.24 µg/L and 0.4 mg/L respectively for 96 hr. Finney's probity bioassay median lethal concentration (LC₅₀) test was performed to determine the LC₅₀ values to *Cyprinus carpio* at different exposure

Table 2. Mortality (M), percent mortality (%M) and probit mortality (PM) of *Cyprinus carpio* at different concentrations of cypermethrin from 24 h to 96 h.

Sl. No.	Cyp conc (µg/L)	Log conc	Total	24 h			48 h			72 h			96 h		
				M	%M	PM	M	%M	PM	M	%M	PM	M	%M	PM
1	1.5	-0.176	10	0	0	-	0	0	-	0	0	-	1	10	3.72
2	2.0	-0.301	10	0	0	-	0	0	-	1	10	3.72	1	10	3.72
3	2.5	-0.397	10	1	10	3.72	1	10	3.72	2	20	4.16	2	20	4.16
4	3.0	-0.477	10	1	10	3.72	2	20	4.16	3	30	4.48	3	30	4.48
5	3.5	-0.544	10	3	30	4.48	4	40	4.75	5	50	5.00	5	50	5.00
6	4.0	-0.602	10	5	50	5.00	6	60	5.25	7	70	5.52	8	80	5.84
7	4.5	-0.653	10	6	60	5.25	7	70	5.52	9	90	6.28	10	100	7.37

periods to synthetic pyrethroid, cypermethrin. The mortality, percent mortality and probity mortality of fishes for 24, 48, 72 and 96 h of cypermethrin exposure at 1.5 $\mu\text{L Lit}^{-1}$, 2.0 $\mu\text{L Lit}^{-1}$, 2.5 $\mu\text{L Lit}^{-1}$, 3.0 $\mu\text{L Lit}^{-1}$, 3.5 $\mu\text{L Lit}^{-1}$, 4.0 $\mu\text{L Lit}^{-1}$, and 4.5 $\mu\text{L Lit}^{-1}$ concentrations are presented in Table 2. No mortality was observed at 1.5 $\mu\text{L Lit}^{-1}$ and 2.0 $\mu\text{L Lit}^{-1}$ at 24 hr while 100% mortality was at 96 hr at a concentration 4.5 $\mu\text{L Lit}^{-1}$. The regression equations for the 24, 48 and 72 and 96 hr was $Y=12.36X+(-2.40)$, $Y=13.11X+(-2.56)$, $Y=11.09X+(-0.83)$ and $Y=6.78X+1.84$ respectively (Figs. 1– 2). The LC_{50} of cypermethrin for common carp was found to be 3.962 $\mu\text{L Lit}^{-1}$, 3.767 $\mu\text{L Lit}^{-1}$, 3.349 $\mu\text{L Lit}^{-1}$ and 2.924 $\mu\text{L Lit}^{-1}$ for 24, 48, 72 and 96 hr respectively. The sub-lethal concentrations of 96 hr LC_{50} further can be used to study chronic toxicity studies.

CONCLUSION

From the current investigation, a conclusion can be drawn that cypermethrin, being lipophilic in nature can easily cross the gills lining and may severely affect the conduct and morphological characteristics of *Cyprinus carpio*. Present and previous studies strongly support the fact that cypermethrin is a very strong fourth-generation insecticide to promote structural and physiological disturbances in roughly all fish species. In the present study observed LC_{50} value of cypermethrin (25% EC) for *C. carpio* is very low to cause toxicity however it does, which can be attributed to the enhanced effectiveness and stability of cypermethrin in the environment.

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