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Lc50 assessment of cypermethrin in *Heteropneustes fossilis*: Probit analysis

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Abstract
Cypermethrin is one among the synthetic pyrethroids and a major pollutant present in agriculture and domestic runoff water that enter in aquatic environment and have harmful effect on aquatic organisms specially fishes. The present study was performed to investigate the toxicity of cypermethrin (25% EC) on fresh water fishes *Heteropneustes fossilis*. In acute toxicity bioassay LC_{50} values after 24, 48, 72 and 96 h were determined by direct interpolation method. LC_{50} values obtained by plotting a graph between % mortality and concentrations of toxicant were0.00064 ml/l, 0. 00050 ml/l, 0.00036 ml/l and 0.00025 ml/l after 24, 48, 72 and 96 h of cypermethrin intoxication. Data obtained from acute toxicity test were evaluated using the probit analysis statistical method. The LC_{50} values for different exposure periods were 0.00066, 0.00044, 0.00033, and 0.00022. The results revealed that a lower concentration of cypermethrin is found to be highly toxic to fishes.

Keywords: Cypermethrin, *Heteropneustes fossilis*, Acute Toxicity, Probit.

1. Introduction

The damage caused by any chemical substance in an organism is called toxicity. Toxicity tests are experiments designed to predict the concentrations of toxicant and its duration of exposure required to produce an effect [1]. Toxicity is species-specific because individuals have different levels of response to the same dose of a toxic substance [2]. The toxicity bioassays are used to detect and to calculate the potential toxicological effects of chemicals on organisms. These tests provide a data base that can be used to assess the risk associated with a situation in which the organisms live. A variety of methods have been developed to evaluate the hazard and potential toxicity of chemicals to organisms, such as acute toxicity test, sub-acute toxicity test or chronic toxicity test.

Acute toxicity is the severe effect suffered by an organism from short term exposure to toxic chemicals [3]. LC_{50} is the estimation of the dose/concentration necessary to kill 50% of a large population of the test species. Experimentally, this is done by administrating a chemical at different doses to a group of organisms and then observing the resulting mortalities in a set time periods like 24, 48 72 and 96 h. The acute toxicity data are important and beneficial in the fixation of sub lethal concentrations for chronic toxicity tests.

Generally pesticides are toxic to aquatic environment. The pesticides mostly used for the control of undesirable insects, pest, weeds and herbs for an increased yield finally find their way into the aquatic environment through water runoff from agricultural fields resulting in disturbance of the aquatic ecosystem [4]. The extensive use of different pesticides in agriculture leads to harmful effects on non- target species [5, 6]. Cypermethrin is a potent insecticidal pyrethroid widely used against pests to increase the production of food grains and other agricultural products. Cypermethrin is highly toxic to aquatic animals and fishes are particularly susceptible to cypermethrin [7].The toxic effect of Cypermethrin on histochemical, heamatological and biochemical parameters has been studied by several workers [8-11]. According to Bradbury and Coats, (1989) [12] fishes shows extreme sensitivity to pyrethroid than corresponding values for mammals and birds [12]. Due to high toxicity and lipophilic nature a pyrethroid pesticide causes serious threat to fish population [13].

Probit analysis is a type of regression used to analyze binomial response variables [14]. Probit analysis is commonly used in toxicology to determine the relative toxicity of chemicals to living animals. This is done by testing the response of an organism under various concentrations of chemicals and then comparing the concentrations at which a response

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occurs. Probit method is widely accepted and most accurate method for calculating LC_{50}. Therefore the present study aimed to evaluate the acute toxicity bioassay of cypermethrin in easiest way using Miller and Tainter (1994) method in fresh water fishes *Heteropneustes fossilis* of Bundelkhand region [15].

2. Materials and Methods
2.1. Collection and maintenance of fishes
Heteropneustes fossilis was selected as an experimental animal. The fishes (wt 80-90g) were collected from different water bodies in Jhansi district of Bundelkhand region during February and March. The fishes were checked against injury or infection by keeping in 0.2% of potassium permanganate solution for 2-4 min [16]. The fishes were acclimatized in laboratory conditions for 6-10 days. During acclimatization the fishes were fed with commercial diet, egg albumin and small insects. Fishes were not given feed 24h before experiment.

2.2. Chemical Used
Jackpot 25 (cypermethrin 25% EC) insecticide manufactured by Crystal Crop Protection Private Limited, Delhi (Chemical formula- $C_{22}H_{19}Cl_2NO_3$) was used in the toxicity studies.

2.3. Estimation of LC_{50} by direct interpolation method
Under acute toxicity study LC_{50} values after 24, 48, 72 and 96 h were determined by direct interpolation method, which includes two exploratory and a definitive test. The mortality was recorded after a period of 24, 48, 72 and 96 h and dead fishes were removed when observed. Stock solution of cypermethrin for I^{st} exploratory test was prepared by dissolving 1ml of Jackpot 25 in 1 litre distilled water and 2 concentration lower (0.001 ml/l) and higher (0.1 ml/l) were employed from the stock solution in rectangular glass aquaria (2'x1'x1') separately containing 5 fishes each to estimate mortality between 0% and 100%. For IInd exploratory test and definitive test stock solution was prepared by dissolving 0.1ml of Jackpot 25 in 1 litre distilled water so the concentration became 0.0001 ml/l. In IInd exploratory test 4 concentrations were taken to find out narrow range of concentrations. In IInd exploratory and definitive test 10 fishes were exposed to each concentrations. On the basis of 2 ranges finding test 7 concentrations were selected for definitive test. The mortality was recorded after a period of 24, 48, 72 and 96h. The concentrations from the definitive test were employed to determine the LC_{50} values by plotting a dose response curve between % mortality and concentrations of toxicant.

2.4. Conversion of percentage mortalities to probits
To calculate LC_{50} by probit analysis the concentrations obtained from definitive test were converted into log concentration and corrected % was obtained. The percentage dead for 0 and 100 are corrected before the determination of probit as under:
Corrected % formula for 0 and 100 % mortality [17].

For 0% dead: 100(0.25/n)
For 100% dead: 100(n-0.25/n)

Where 'n' is number of fishes, used in the experiment.
The probit values of correct % mortality were obtained from Finney's table given below [18]. Curve was plotted between the log concentrations and probit values. LC_{50} values for different time interval were obtained from the curves by

drawing a perpendicular line at 5 probit corresponding to the 50% mortality. The actual LC_{50} was determined by taking the inverse log of the concentrations associated with it.

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
—	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Finney's Table: Transformation of percentage mortalities to probits

2.5. Calculation of standard Error (SE) of LC_{50}
Formula for the calculation of the standard Error of LC_{50} was calculated by the following formula [17].

$$SE \text{ of } LC_{50} = \frac{(\text{Log } LC_{84} - \text{Log } LC_{16})}{\sqrt{2N}} \quad (N=10)$$

Where 'N' is number of fishes in each group
The probit of Log LC_{84} − Log LC_{16} were taken from the Finney table which is 5.99 and 4.01 respectively. These values are approximately equal to probit 6 and 4. The log concentrations of the probits 6 and 4 were obtained from the line on the graph plotted between probit and log concentrations of different time intervals (24, 48, 72 and 96h). Then log values were converted into antilog. Using these values in the above formula standard error of LC_{50} was calculated.

3. Results
In I^{st} exploratory test 100% mortality occurred after 24h at 0.1 ml/ltr concentration, whereas at 0.001 ml/l concentration 80 % and 100% mortality was observed after 24 and 48h respectively. In IInd exploratory test 4 range finding concentrations viz., 0.0001 ml/l, 0.0003 ml/l, 0.0006 ml/l and 0.0009 ml/l were taken and mortality rate was observed after 24, 48, 72 and 96 h exposure. Highest mortality was recorded at 0.0009 ml/l concentration and lowest mortality was at 0.0001 ml/l concentration. On the basis of IInd exploratory test 7 concentrations viz 0.00010 ml/l, 0.00025ml/l, 0.00040ml/l, 0.00055ml/l, 0.00070ml/l, 0.00085ml/l and 0.001ml/l were chosen for definitive test and mortality rate was recorded (Table-1). To determine the LC_{50} value graphs were plotted between % mortality and concentrations of toxicant. The concentrations obtained by drawing a perpendicular against 50% mortality were 0.00064 ml/l, 0.00050 ml/l, 0.00036 ml/l and 0.00025 ml/l after 24, 48, 72 and 96 h cypermethrin intoxication respectively (Fig. 1).
The concentrations taken in definitive test were converted into log concentrations. The correct % and their corresponding probit values are shown in Table-2 & 3. After plotting a graph between the log conc and probit, the values at 5 probit following different exposure were -3.18, -3. 35, -3.48, -3.65. By taking antilog of these values the actual LC_{50} were 0.00066, 0.00044, 0.00033, and 0.00022 after 24, 48, 72 and 96 h respectively.

Table 1: Definitive Test for Direct Interpolation method

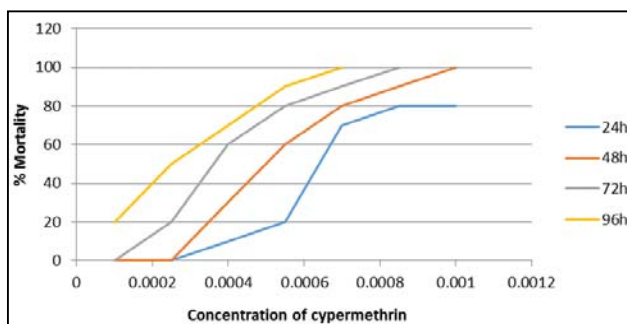| Conc. Of toxicant (ml/l)	No. of fishes	24hrs		48hrs		72hrs		96hrs	
		M	% M	M	% M	M	% M	M	% M
0.00010	10	0	0	0	0	0	0	2	20
0.00025	10	0	0	0	0	2	20	3	50
0.00040	10	1	10	2	30	3	60	1	70
0.00055	10	2	20	4	60	2	80	1	90
0.00070	10	7	70	1	80	1	90	1	100
0.00085	10	8	80	1	90	1	100		
0.001	10	8	80	2	100				

Fig 1: Estimation of LC_{50} at different exposure period

To calculate SE the log LC values for probit 6 and 4 were obtained from the line on the graph in fig. 2 - 5, which in the present case were -2.88 & -3.49 for 24h, -3.08 & -3.58 for 48h, -3.21 & -3.71 for 72h and -3.32 & -4 for 96 h respectively and their antilog values were 0.00131 & 0.00032 for 24h, 0.00083 & 0.00026 for 48h, 0.00060 & 0.00019 for 72h and 0.00047 & 0.0001 for 96h respectively. The standard errors of LC_{50} are –

24 h - 0.00066 ± 0.00022, with 95% confidence interval of 0.00044 – 00088.

48 h - 0.00044 ± 0.00013, with 95% confidence interval of 0.00031- 0.00057.

72 h- 0.00033 ± 0.00009, with 95% confidence interval of 0.00024 – 0.00042.

96 h- 0.00022 ± 0.00008, with 95% confidence interval of 0.00014 – 0.00030.

Some behavioural changes were also observed during the experiment. Random movement of fishes just after the addition of toxicant, mucus secretion through body surface, fishes came to surface due to low rate of oxygen uptake, the colour of fishes appeared little pale, restlessness before death and hitting against the walls of aquarium.

Table 2: Log concentrations and probit values when exposed to cypermethrin after 24 and 48 h.

S. No.	Conc. 24h	Log Conc.	No. of Fishes	24h			48h		
				% dead	Correct %	Probit	% dead	Correct %	Probit
1	0.00010	-4	10	0	2.5	3.04	0	2.5	3.04
2	0.00025	-3.60206	10	0	2.5	3.04	0	2.5	3.04
3	0.00040	-3.39794	10	10	10	3.72	30	30	4.48
4	0.00055	-3.25964	10	20	20	4.16	60	60	5.25
5	0.00070	-3.1549	10	70	70	5.52	80	80	5.84
6	0.00085	-3.07058	10	80	80	5.84	90	90	6.28
7	0.001	-3	10	80	80	5.84	100	97.5	6.96

Table 3: Log concentrations and probit values when exposed to cypermethrin after 72 and 96 h.

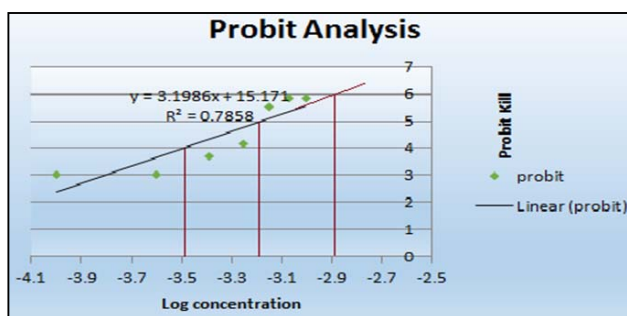| S. No.	Conc. 24h	Log Conc.	No. of Fishes	72h			96h		
				% dead	Correct %	Probit	% dead	Correct %	Probit
1	0.0001	-4	10	0	2.5	3.04	20	20	4.16
2	0.00025	-3.60206	10	20	20	4.16	50	50	5
3	0.0004	-3.39794	10	60	60	5.25	70	70	5.52
4	0.00055	-3.25964	10	80	80	5.84	90	90	6.28
5	0.0007	-3.1549	10	90	90	6.28	100	97.5	6.96
6	0.00085	-3.07058	10	100	97.5	6.96	100	97.5	6.96
7	0.001	-3	10	100	97.5	6.96	100	97.5	6.96

Fig 2: Plot of log conc. versus probit from table (2) for 24h
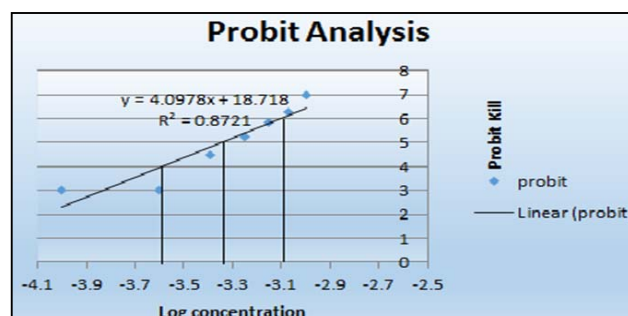

Fig 3: Plot of log conc. versus probit from table (2) for 48h
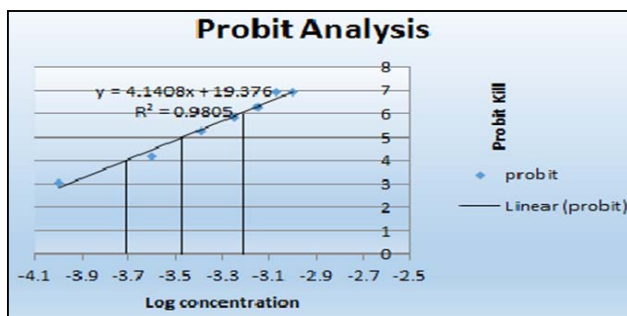
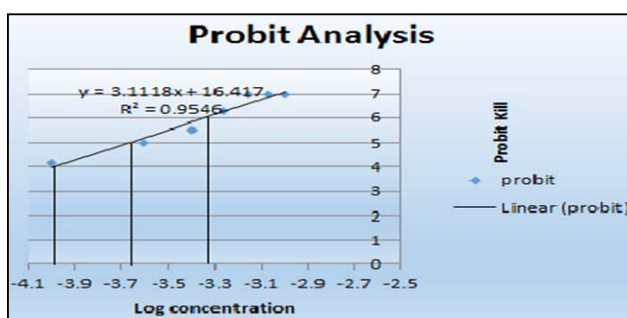**Fig 4:** Plot of log conc. versus probit from table (3) for 72h

Fig 5: Plot of log- conc. versus probit from table (3) for 96h

4. Discussion

Cypermethrin continuously pollute the water by its toxic effect on aquatic organisms. The present study was carried out to determine the toxic effect of cypermethrin on *Heteropneustes fossilis* that was assessed by the LC_{50} values calculated at different exposure period. The fishes showed mortality at low concentration and with decrease of duration of exposure the fishes exhibit mortality at higher concentration. It has been reported earlier that pesticide, chemicals and xenobiotic accumulated in natural waters, which results in toxicity to aquatic organisms [19]. The values of LC_{50} of cypermethrin obtained by other workers were found to be different for pyrethroid insecticides on different species of fishes [3, 20]. According to Finney's probit analysis as reported by Miller and Tainter (1944) [15], the LC_{50} values in *Heteropneustes fossilis* were found to be 0.00066, 0.00044, 0.00033, and 0.00022 in this investigation. This shows that cypermethrin is highly toxic to fishes. These results are in agreement with the results of other workers [21]. Tiwari *et al.* (2012) [22] reported the toxicity of cypermethrin in very low concentration in fingerlings of *Labeo rohita* [22]. The result obtained from acute toxicity of alpha cypermethrin on *Tilapia* at 96h LC_{50} value was 5.99 μg/L [5]. The toxic effects of cypermethrin on various fish species was also reported by Smith and Stratton (1986) [2] which were 2.0 μg/L (96h) for Atlantic salmon, 6.0 μg/L (96h) for rainbow trout, 9.0 μg/L (24h) and 8.0 μg/L (48h) for desert pupfish [2]. The acute toxicity of fresh water fish *Cirrhinus mrigala* when exposed to 10% cypermethrin was found to be in the range of 2.69–2.28 ppb after 24 to 96 h exposure [23].

Cypermethrin intoxicated behavioural alternations were reported in several fishes [22, 24, 25]. Yaji *et al.* (2011) [26] studied behavioural alternations in *Oreochromis niloticus* juveniles exposed to cypermethrin at different concentrations 0.0007, 0.0008, 0.0009, 0.010 and 0.11 mg/L for 96h [26]. Initially exposed fishes came to surface to engulf air frequently. Hyper excitability was noticed by the jerky and random movement of fishes just after the addition of toxicant [27].

Ojutiku *et al.*, (2014) [25] also reported restlessness, loss of balance, excessive accumulation of mucus on skin and jumping [25]. The experimental fishes in this study showed similar observations even at lower concentration of cypermethrin. The changes induced by cypermethrin can be attributed to an increase in physiological stress due to neuronal excitation, low rate of oxygen consumption and histological changes.

5. Conclusion

The present study was an attempt to find the toxicity of cypermethrin on *Heteropneustes fossilis* and the results conclusively showed that the cypermethrin is highly toxic to fishes even at very low concentration. The study on fishes will be very useful to provide a future understanding of ecological impact.

6. Acknowledgement

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