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Preliminary Evaluation of The Acute Toxicity of Cypermethrin and λ-Cyhalothrin to *Channa Punctatus*

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Abstract In the present study, the acute toxicity of the pyrethroid pesticides, cypermethrin and λ-cyhalothrin was conducted for a 96 h period using *Channa punctatus*. The LC_{50} values of cypermethrin and λ-cyhalothrin were found to be 0.4 mg/L and 7.92 μg/L, respectively. The λ-cyhalothrin was found to be about 50 times more toxic to the fish than cypermethrin. The behavioral pattern of *C. punctatus* got severely altered in each group due to pesticide treatment. The results suggested that even at low concentrations, these pyrethroid compounds may exert toxic effects, markedly modifying their behavioral pattern.

The recent development of a number of broad spectrum pesticides and their large scale outdoor and indoor applications has inevitably increased the probability of contaminating the aquatic bodies. The insecticides and pesticides used earlier are now getting replaced by the synthetic pyrethroids like cypermethrin and λ-cyhalothrin as they are more effective for the purpose. However, the recent reports have indicated that these compounds might be toxic to the other aquatic organisms in general and the fish in particular (Singh and Agarwal 1994; Moore and Waring 2001; Polat et al. 2002; Saha and Kaviraj 2003; Koprucu and Aydin 2004). The lipophilicity of pyrethroids facilitates their rapid access to the various tissues and thus leads to a high affinity of these pesticides to the CNS (Anadon et al. 1996). Since the fish have poor ability to metabolize and eliminate such xenobiotics as compared to those of higher vertebrates, these pesticides become relatively more toxic (about thousand times) to fish species (Demaute 1989) as compared to that of mammals and birds (Edwards et al. 1986; Bradbury and Coates 1989).

Pyrethroid toxicity is highly dependent on stereochemistry of the molecule. Each isomer has its own toxicity. Most formulations have a fixed isomeric ratio. Formulations made of a single isomer are likely to be much more toxic than those with four to eight isomers (Bradbury and Coats 1989). The difference in toxicity between formulations with the same ingredient may also depend upon carriers. Inert ingredients and contaminants can also affect the toxicity of a pyrethroid formulation (FMC Agricultural Chemicals Group 1989). Therefore, in light of earlier observations, it is significant to give emphasis on the calculation of LC_{50} values of each formula grade pesticides in addition to technical grade for target as well as non-target organisms so that the amount of a particular make may be fixed for various indoor and outdoor applications. Keeping this in mind, the present study was conducted to determine acute toxicity of formula grade pyrethroid pesticides such as cypermethrin and λ-cyhalothrin to a fresh water fish, *Channa punctatus.*

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Materials and Methods

The healthy freshwater teleosts, *C. punctatus* (average length 11–13 cm, weight 23 ± 2 g) were obtained from a local fish market and treated with potassium permanganate solution (0.5% w/v) for 1 min to remove any dermal

Table 1 Toxicity testing of cypermethrin on *Channa punctatus* for 96 h

Concentrations (mg/L)	No. of alive fish				% survival at 96 h	% Mortality at 96 h
	24 h	48 h	72 h	96 h		
0	12	12	12	12	100	–
0.1	12	12	12	12	100	–
0.2	12	12	12	12	100	–
0.3	12	12	11	9	75	25
0.4	12	12	9	6	50	50
0.5	9	6	4	3	25	75
0.6	7	3	1	0	–	100

adherent. Its binomial characterization was done as *C. punctatus* using the identification key (Day 1989). *C. punctatus* was chosen for this study because of its timely availability, survival for longer period under laboratory conditions and easy handling etc. The fish were acclimatized for 2 weeks in a rectangular glass aquarium (100 l) containing dechlorinated aerated tap water at room temperature (26 ± 1.0°C) with food ad libitum under standard laboratory conditions.

Two formula grade pesticides namely Colt®25 (25%EC cypermethrin procured from PI Industries Ltd., India) and REEVA-5 (5%EC λ-cyhalothrin received from Rallis India Ltd. India as a gift) were used in present study. Before exposure, quality of water was tested according to the APHA (1985) which showed the mean values as follows: temperature 27 ± 1°C, pH 6.8 ± 0.2, DO 6.9 ± 0.4 mg/L and total hardness 113.3 ± 2 mg/L. Twelve fish (22 ± 3 g each) were placed in each of the six glass aquaria containing dechlorinated aerated water. Thereafter, cypermethrin was added as per the following concentrations: 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/L. The water of aquaria was changed at an interval of 24 h and different concentrations of cypermethrin were restored afresh as mentioned above. The stock solution of cypermethrin used in this study was always freshly prepared when needed. The fish kept into the pesticide free medium served as a control. Equal volume of acetone was maintained in the control aquarium, as the pyrethroids used were solubilised in acetone. The same procedure was followed for λ-cyhalothrin exposure but with lower concentrations such as 2.5, 5, 7.5, 10, 12.5 and 15 μg/L.

The test was carried out for 96 h treatment period and dead fish were removed as the test proceeded. The number of dead fish per group was recorded against the time of their death in a tabular form as specified by Sprague (1969). The 96 h LC_{50} value of cypermethrin was calculated using arithmetic method of Karber as adopted by Dede and Kaglo (2001). The behavioral pattern of fish was monitored regularly under above treatment conditions as suggested by Kumari et al. (1997).

Results and Discussion

The fish in the control aquarium were observed to be healthy and normal and no mortality was recorded in it. In cypermethrin treated set no mortality was observed at 0.1 and 0.2 mg/L concentrations after 96 h exposure. However, at 0.3, 0.4, 0.5 and 0.6 mg/L concentrations, the percent mortality was found to be 25%, 50%, 75% and 100% respectively (Table 1). After 96 h treatment period, the LC_{50} of cypermethrin was calculated and it was found to be 0.4 mg/L for *C. punctatus* (Table 2). Similarly, the fish were exposed to λ-cyhalothrin for 96 h and observed carefully for their mortality rate. After 96 h treatment, λ-cyhalothrin caused 8.33%, 25%, 50%, 66.67%, 83.33%

Table 2 Determination of LC_{50}value of cypermethrin for 96 h based on arithmetic method of Karber (adapted by Dede and Kaglo 2001)

Concentration (mg/L)	Concentration difference	Number of alive fish	Number of dead fish	Mean death	Mean death × concentration difference
0 (Control)	–	12	0	0	0
0.1	0.1	12	0	0	0
0.2	0.1	12	0	0	0
0.3	0.1	9	3	1.5	0.15
0.4	0.1	6	6	4.5	0.45
0.5	0.1	3	9	7.5	0.75
0.6	0.1	0	12	10.5	1.05
					$\Sigma = 2.40$

Summation indicates sum of (Mean death × concentration difference).

LC_{50} for cypermethrin = $LC_{100} - \Sigma$(Mean death × concentration difference)/number of organisms per group = 0.6 – 2.40/12 = 0.6 – 0.2 = 0.4 mg/L

Table 3 Toxicity testing of λ-cyhalothrin on *Channa punctatus* for 96 h

Concentrations (μg/L)	Number of alive fish				% Survival after 96 h	% Mortality after 96 h
	24 h	48 h	72 h	96 h		
0	12	12	12	12	100.00	–
2.5	12	12	12	11	91.67	8.33
5.0	12	12	12	9	75.00	25.00
7.5	12	11	9	6	50.00	50.00
10.0	10	9	7	4	33.33	66.67
12.5	7	5	3	2	16.67	83.33
15.0	6	4	2	–	–	100.00

and 100% mortality at the concentrations of 2.5, 5, 7.5, 10, 12.5 and 15 μg/L, respectively (Table 3). The LC_{50} value for this pesticide was calculated and it was found to be 7.92 μg/L (Table 4). The higher LC_{50} value of the chosen formula grade cypermethrin for *C. punctatus* as compared to the earlier reports with its different formulations for different freshwater fish may be due to variations in its specific ingredients and formulation (FMC Agricultural Chemicals Group 1989). The data suggested that under the identical experimental conditions and treatment duration, formula grade λ-cyhalothrin used in this study was about 50 times more toxic to *C. punctatus* than that of cypermethrin.

Acute toxicity testing done by earlier workers and summarized by Smith and Stratton (1986) and Bradbury and Coats (1989) give a clear glimpse of comparative lethal values of these pyrethroids for different fish species. For the 96 h treatment period, LC_{50} values of cypermethrin in different fish species have been reported as follows: 2 and 6 μg/L for *Salmo salar* and *S. gairdneri*, respectively (Smith and Stratton 1986); 0.9–1.1, 1.2, 0.5, 0.4 and 2.2 μg/L for *C. carpio*, *Salmo trutta*, *S. gairdneri*, *Scardinius erythropthalmus*, and *Tilapia nilotica*, respectively (Bradbury and Coats 1989). Very recently for 72 h treatment period the LC_{50} value of cypermethrin has been reported to be 1.27 μg/L by Saha and Kaviraj (2003).

The LC_{50} values with different formula grades of λ-cyhalothrin for 96 h treatment period have been reported. Hill (1985a, b) found LC_{50} values of 5% EC λ-cyhalothrin for *S. gairdneri* and *C. carpio* to be 0.93 and 0.50 μg/L, respectively. However, with technical grade of λ-cyhalothrin the LC_{50} value for *Cyprinodon variegates* was found to be 0.81 μg/L. (Hill 1985c). Charles and Hance (1968) reported the 96 h LC_{50} value of λ-cyhalothrin for brown trout to be 2–2.8 μg/L.

Though we have not studied here the effect of varying degrees of incubation temperature on the determination of LC_{50} values for these pyrethroids, the reports available suggest that the temperature of the aquatic system plays an important role in the intensity of toxicity of pyrethroids. A negative correlation has been observed between temperature and acute toxicity of synthetic pyrethroids (Kumaragura and Beamish 1981). In another report it has been indicated that there may be an increase in the toxic impact of pyrethroids on reproduction during spawning season in the cold aquatic environment (Moore and Waring 2001).

The behavioral response to these pyrethroids started appearing only after 3 h of treatment. The alterations in behavior such as hyperactivity, loss of balance, rapid swimming, increased surfacing activity, enhanced rate of opercular activity and convulsions in treated fish were observed with the changing concentrations of these pyrethroids when compared to the control fish (Table 5). The effects of pesticides were found to be in dose and duration dependent manner. However, the alterations in fish behavioral patterns were more marked with λ-cyhalothrin treatment than cypermethrin at low concentrations. The

Table 4 Determination of LC_{50} value of λ cyhalothrin for 96 h based on arithmetic method of Karber (adapted by Dede and Kaglo 2001)

Concentration (μg/L)	Concentration difference	Number of alive fish	Number of dead fish	Mean death	Mean death × concentration difference
0 (Control)	–	12	–	–	–
2.5	2.5	11	1	0.5	1.25
5.0	2.5	9	3	2.0	5.00
7.5	2.5	6	6	4.5	11.25
10.0	2.5	4	8	7.0	17.50
12.5	2.5	2	10	9.0	22.50
15.0	2.5	0	12	11.0	27.50
					Σ = 85.00

Summation indicates sum of (Mean death × concentration difference).

LC_{50} for λ-cyhalothrin = LC_{100} – Σ(Mean death × concentration difference)/number of organisms per group = 15.0 – 85.0/12 = 15.0 – 7.08 = 7.92 μg/L

Table 5 Impact of cypermethrin and λ-cyhalothrin on the behavioral pattern of *Channa punctatus* exposed to the pesticides up to 96 h

Parameters	Control	Cypermethrin (mg/L)			λ-cyhalothrin (μg/L)		
		0.4	0.6	0.8	0.8	1.2	1.6
Hyperactivity	–	+	++	+++	++	+++	+++
Loss of balance	–	–	–	++	–	+	++
Rate of swimming	+	+	+	++	+	++	++
Surfacing activity	+	+	++	+++	+	++	+++
Rate of opercular activity	+	+	++	+++	++	+++	+++
Convulsions	–	–	–	++	–	–	+++

The increase or decrease in the level of behavioral parameters is shown by numbers of (+) sign. The (-) sign indicate normal behavioral conditions

results of the present study agree with similar observations made for some pyrethroid compounds by other workers (Edwards et al. 1986; Polat et al. 2002; Baser et al. 2003).

Although under field conditions, cypermethrin (as compared to λ-cyhalothrin) may pose relatively less risk due to its high adsorption to soil yet these data may be significant while assessing long term potential risks to the aquatic ecosystem. From our observations, it can be concluded that emphasis must be given on framing certain strict norms for each manufacturer compelling them to assess the toxicity range of each new pesticide formulation for both the target and the non-target organisms and thus to regulate/recommend their dosage for different purposes. This approach may reduce the potential health hazards of non-target organisms upon their exposure to these chemicals.

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