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Acute Toxicity of Synthetic Pyrethroid Cypermethrin to Some Freshwater Organisms

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Abstract Ninety-six hours static bioassays were made in the laboratory to determine acute toxicity of cypermethrin to five non-target freshwater organisms belonging to different taxa and niche. Susceptibility of the organisms to cypermethrin was in the order: the crustacean *Diaptomus forbesi* > the aquatic insect *Ranatra filiformis* > the freshwater carp *Cyprinus carpio* > the tadpole larva of the toad *Bufo melanostictus* > the oligochaet worm *Branchiura sowerbyi*. Ninety-six hours LC50 values of aqueous cypermethrin ranged from 0.03 μg/L for the crustacean to 9.0 μg/L for the tadpole larva. The value was very high (71.12 μg/L) for the oligochaet worm. LC50 values changed with hours of exposure till 72 h after which cypermethrin became inactive in both aqueous and acetone solution. Acetone solution of cypermethrin was more toxic to *B. sowerbyi*, *C. carpio* and the tadpole larva. There was no significant difference in susceptibility of any other test organism between aqueous and acetone solution of cypermethrin.

Keywords Pyrethroid · Cypermethrin · Toxicity · LC50 · Non-target · Environment

Synthetic pyrethroids have been introduced over the past two decades for agricultural and domestic use as replacements for more toxic pesticides, such as chlorinated hydrocarbons, organophosphates and carbamates (Moore and Waring 2001). The ecological implications of this large-scale shift in pesticide application have not yet been fully explored. Several studies have indicated that pyrethroids are highly toxic to a number of non-target organisms such as honeybees, freshwater fish and aquatic arthropods even at very low concentrations (Smith and Straton 1986; Oudou et al. 2004). Cypermethrin is a type of cyanophenoxybenzyl pyrethroid and is categorized as restricted use pesticide (RUP) by US EPA because of its high toxicity to fish (ETN 1996). Many invertebrates are even more sensitive to cypermethrin (Wilis and Ling 2004). But, lethality of cypermethrin to freshwater invertebrates is poorly documented.

In India, cypermethrin is registered for use on a wide array of crops including cotton, cabage, okra, brinjal, sugarcane, wheat and sunflower. Almost 70% of all sprays used on cotton in Andhra Pradesh in India are pyrethroids, which consist mostly the cypermethrin (Jayswal 1989). Cypermethrin is considered as immobile and not expected to biomagnify through food chain. Bioconcentration factor of cypermethrin for whole fish has been found to vary between 444X to 446X (URL1). Due to its lipophilicity, pyrethroids have a high rate of gill absorption thereby rendering fish as most sensitive to the pesticides. Yet toxicity of pyrethroids to aquatic organisms varies widely depending upon its stereochemical structure (Milam et al. 2000) and its solubility in the diluent medium (Datta and Kaviraj 2003). Therefore, it is pertinent to evaluate the susceptibility of the aquatic resources to cypermethrin. The main objective of this study was to determine the acute toxicity of cypermethrin to five selected freshwater organisms belonging to five different taxa and different ecological niches to assess potential risk of the pesticide to the aquatic ecosystem.

Materials and Methods

Three non-target freshwater invertebrate species (Crustacean *Diaptomus forbesi*; aquatic insect *Rantra filiformis*

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Table 1 Range of concentrations of cypermethrin used in different bioassays

Test specimens	n	Cypermethrin (μg/L)	
		Dissolved in water	Dissolved in acetone
Diaptomus forbesi	3	0.009–0.088	0.011–0.088
Ranatra filiformis	3	0.011–2.00	0.055–2.00
Branchiura sowerbyi	3	9–1000	9–1000
Cyprinus carpio	3	0.3–10.0	0.3–10.0
Bufo melanostictus larva	3	1–50	1–50

and the oligochaet *Branchiura sowerbyi*), one species of freshwater fish (*Cyprinus carpio*) and a one-week-old tadpole larva of toad (*Bufo melanostictus*) were used as the test organisms. Specimens of the fish were procured from a local hatchery. The other organisms were captured from local ponds. All the organisms were acclimatized to the test conditions for 96 h before use.

Static bioassays following the method of the APHA (1995) were conducted in two different test vials. Bioassays for *C. carpio* (L = 4.2 ± 0.2 cm; W = 1.7 ± 0.1 g), *Ranatra filiformis* (L = 1.14 ± 0.03 cm; W = 0.09 ± 0.02 g) and the tadpole larvae (L = 1.21 ± 0.04 cm; W = 1.32 ± 0.02 g) were made in 15 L glass aquaria each holding 3 L of water and three test organisms, while those for *Diaptomus forbesi* (L = 0.08 ± 0.02 cm; W = 0.02 ± 0.01 g) and *Branchiura sowerbyi* (L = 3.00 ± 0.01 cm; W = 0.84 ± 0.04 g) were made in 500 mL beakers each containing 300 mL of water and 10 test organisms. Deep tube-well water stored in an overhead tank was used as diluent (temperature 20°C, pH 8.23 ± 0.04; free CO_2 5.1 ± 0.3 mg/L; dissolved oxygen 5.9 ± 0.2 mg/L; total alkalinity 70.75 ± 3.77 mg/L as $CaCO_3$; hardness 249.33 ± 11.01 mg/L as $CaCO_3$). Two different stock solutions of cypermethrin (Ustad 10% EC, United Phosphorus Ltd., Worli, Mumbai) were prepared, one dissolved in water and another in 0.5% acetone. The concentrations of cypermethrin used for both solvents are given in Table 1. For each concentration of cypermethrin tested, a control (water or 0.5% acetone) with three replicates was conducted simultaneously. Mortality and reaction of the test animals were recorded every 24 h and dead animals were removed. No food was provided during the bioassay to avoid interference of excretory products of the test organisms with the test chemical. Lethal concentration of cypermethrin at which 50% mortality of the test organism occurred (LC_{50}) and its 95% confidence limit were estimated for 24, 48, 72 and 96 h by probit analysis (Finney 1971). Duncan's multiple range test (DMRT) was employed for comparing mean mortality values, after estimating the residual variance by repeated measures ANOVA (Winner 1971) for arc sine transformed mortality data (dead individuals/initial number of individuals). Time of exposure was the repeated measure factor while treatment (concentrations and controls) was the second factor. In addition, LC_{50} values were compared by the method of APHA (1995).

Results and Discussion

LC_{50} values of cypermethrin for the invertebrate specimens have been summarized in Table 2 while those for fish and the amphibians (tadpole larva) have been summarized in Table 3. The crustacean *Diaptomus forbesi* was found most susceptible to cypermethrin and the oligochaet worm *Branchiura sowerbyi* most resistant to cypermethrin. LC_{50} values changed with hours of exposure and solvent condition. Twenty-four hours LC_{50} values of both aqueous and acetone solubilized cypermethrin were significantly higher than those of 48 and 72 h for all test organisms. After 72 h, no change occurred in the LC_{50} values for any test organism.

Comparing LC_{50} values between different hours of exposure, it was observed that 24, 48 and 72 h LC_{50} values of both water solubilized and acetone solubilized cypermethrin significantly varied from each other for all species tested except *Diaptomus forbesi*, which showed similarity in LC_{50} values between 48 h and 72 h exposure (Table 2).

Table 2 LC_{50} values, expressed in μg/L (95% CL), of cypermethrin under water and acetone soluble condition for three invertebrate species

Solvent	24 h	48 h	72 h	96 h
Diaptomus forbesi				
Water	0.04^{a} (0.03–0.05)	0.03^{b} (0.02–0.04)	0.03^{b} (0.02–0.04)	0.03^{b} (0.02–0.04)
Acetone	0.04^{a} (0.03–0.05)	0.03^{b} (0.02–0.04)	0.03^{b} (0.02–0.04)	0.03^{b} (0.02–0.04)
Ranatra filiformis				
Water	0.12^{a} (0.11–0.13)	0.09^{b} (0.08–0.10)	0.065^{c} (0.06–0.07)	0.065^{c} (0.06–0.07)
Acetone	0.12^{a} (0.11–0.13)	0.09^{b} (0.08–0.10)	0.065^{c} (0.06–0.07)	0.065^{c} (0.06–0.07)
Branchiura sowerbyi				
Water	218.7^{a} (194.0–247.0)	128.7^{b} (116.0–143.1)	71.12^{c} (64.0–78.0)	71.12^{c} (64.0–78.0)
Acetone	218.7^{a} (194.0–247.0)	128.7^{b} (116.0–143.1)	43.65^{c} (34.3–55.4)	43.65^{c} (34.3–55.4)

Different superscript letters in a row indicate significant difference of LC_{50} values between hours of exposure

Table 3 LC_{50} values, expressed in µg/L (95% CL), of cypermethrin under water and acetone soluble condition for fish *Cyprinus carpio* and the tadpole larva of toad *Bufo melanostictus*

Solvent	24 h	48 h	72 h	96 h
C. carpio				
Water	5.2[a] (4.9–5.4)	3.8[b] (3.6–4.1)	2.6[c] (2.3–2.8)	2.6[c] (2.3–2.8)
Acetone	5.2[a] (4.9–5.4)	3.8[b] (3.6–4.1)	1.7[c] (1.5–1.8)	1.7[c] (1.5–1.8)
Tadpole larva of *B. melanostictus*				
Water	18.2[a] (16.9–19.5)	12.0[b] (10.2–20.6)	9.0[c] (3.4–20.8)	9.0[c] (3.4–20.8)
Acetone	18.2[a] (16.9–19.5)	12.0[b] (10.2–20.6)	6.0[c] (4.9–7.0)	6.0[c] (4.9–7.0)

Different superscript letters in a row indicate significant difference of LC_{50} values between hours of exposure

A comparison was also made between the LC_{50} values of water solubilized (WS) and acetone solubilized (AS) cypermethrin for different hours of exposure for each species. Up to 48 h, there was no significant difference in LC_{50} values between AS and WS for any species of the test organisms. At 72 h, LC_{50} values of WS cypermethrin for *B. sowerbyi* (Table 2), *C. carpio* and the tadpole larva of toad (Table 3) were significantly higher than the AS cypermethrin. Duncan's multiple range test carried to compare mean mortality between concentrations of cypermethrin also showed that minimum effective concentration that produced significant mortality from control was 0.02 µg/L (both WS and AS) for *Diaptomus forbesi*, 1.0 µg/L (both WS and AS) for *Ranatra filiformis* 3.0 µg/L (WS) and 2.0 µg/L (AS) for *Cyprinus carpio*, 7.0 µg/L (WS) and 5.0 µg/L (AS) for tadpole larva and 50.0 µg/L (WS) and 30.0 µg/L (AS) for *Branchiura sowerbyi*.

All the organisms showed hyperactivity when exposed to high concentrations of cypermethrin. Fish started vigorous movement and tried to come out of water and continued to exhibit such hyperactivity for 10–18 h. Worms showed brisk movements, the aquatic insect started jumping and the tadpole larva started moving in a circular fashion.

Results of the present study indicate that cypermethrin is highly toxic to freshwater aquatic organisms irrespective of taxa and solubility condition. The order of toxicity of cypermethrin for the freshwater organisms tested are as follows: crustacean > aquatic insect > fish > tadpole larva of toad > oligochaet worm. Comparing the present results with previous studies revealed that 96 h LC_{50} value of cypermethrin for *Diaptomus forbesi* (0.03 µg/L) observed in the present investigation is close to 96 h LC 50 value of cypermethrin (less than 0.01 µg/L) for several marine invertebrates (Clark et al. 1989). Wilis and Ling (2004) observed EC_{50} values of cypermethrin to the cyclopoid copepod, *Oithona similis* to range from 0.14 to 0.24 µg/L for nauplii and adults, respectively. Comparative pyrethroid studies in the lobster (*Homarus americanas*) and shrimp (*Crangon sptemspinosa*) also indicated cypermethrin to be most toxic (96 h LC_{50} value about 0.01 µg/L; Bradbury and Coats 1989).

The present results indicate that cypermethrin is also highly toxic to aquatic insects (96 h LC_{50} 0.06 µg/L). High sensitivity of crustaceans and insects to pyrethroids is not surprising because these compounds are designed to control arthropod pests. We did not find any report of sensitivity of oligochaet worms to pyrethroids. But it has been found that these organisms are relatively more tolerant than crustaceans and fish to organic pollutants (Kaviraj et al. 2004). Results of the present study also confirm it. However, wide variety of data are available for sensitivity of fish to pyrethroids. Review of toxicity of pyrethroids by Bradbury and Coats (1989) documents LC_{50} value of most freshwater fish to range from 0.4 to 2.2 µg/L. We (Saha and Kaviraj 2003) also observed cypermethrin as highly toxic to freshwater catfish *Heteropneustes fossilis* (72 h LC_{50} value as 0.67 and 1.27 µg/L for WS and AS cypermethrin). Ninety-six hours LC_{50} values of cypermethrin to *Cyprinus carpio* (1.7 µg/L as AS cypermethrin and 2.60 µg/L as WS cypermethrin) as observed in the present investigation are close to 96 h LC_{50} value of cypermethrin recorded for *Cyprinus carpio* (0.9 µg/L) in a flow through bioassay (Bradbury and Coats 1989). However, a few freshwater fish showed increased tolerance to cypermethrin e.g., rainbow trout *Salmo gairdneri*: 96 h – 6.0 µg/L; *Gambusia affinis*: 24 h – 9.0 µg/L and 48 h – 8.0 µg/L as *cis*-cypermethrin; desert puff fish *Cyprinodon macularis*: 24 h – 10.0 µg/L and 48 h – 6.0 µg/L as *cis*-cypermethrin (Smith and Stratton 1986). Even higher tolerance to cypermethrin was found for guppy *Poecilla reticulata* (48 h LC_{50} 21.4 µg/L, Polat et al. 2002). Toxicity of pyrethroids to fish is influenced by several factors. Most products of cypermethrin are mixtures of its isomers. Toxicity of cypermethrin depends on the composition of mixture as well as on the ratio of *cis* and *trans* isomers in the mixture. *Cis*-cypermethrin has been found more toxic than its *trans* form to mammals; but for trout the *cis* and *trans* isomers have been found equitoxic (Bradbury and Coats 1989). Technical grade (99%) beta-cypermethrin [(1R*cis*)S + (1S*cis*)R and (1R*trans*)S + (1S*trans*)R enantiomer pair of cypermethrin in 4:6 ratio] used by Polat et al. (2002) yielded less toxicity to guppy as compared to toxicity rendered by 10% EC of cypermethrin to *H. fossilis* (Saha and Kaviraj 2003). Solubility is another important factor, which determines the toxicity of pesticides in water. All pyrethroids are not equally soluble in water. Datta and Kaviraj (2003) determined acute toxicity of K-obiol®, a formulation of deltamethrin, for *Clarias gariepinus*. This formulation was least soluble in water and was almost non-toxic to *C. gariepinus* in aqueous solution in contrast to being highly toxic in acetone solution. Results of the present study indicate that aqueous cypermethrin is significantly less toxic to *B. sowerbyi*, *C. carpio*, and the tadpole larva than its acetone solution. This is in contrast to the result obtained for toxicity of cypermethrin to *H. fossilis* (Saha and Kaviraj 2003). In both the cases the difference in LC_{50} values between the two solutions of cypermethrin is narrow indicating that the formulation of cypermethrin used in the present study is toxic irrespective of the solvent and medium and thus pose a greater threats to aquatic ecosystem than many other pyrethroids.

In summary, cypermethrin was found highly toxic to freshwater non-target organisms. The crustacean *D. forbesi* and the aquatic insect *R. filiformis* were most sensitive to this pyrethroid followed by the fish *C. carpio* and the tadpole larva of toad *B. melanostictus*. Ninety-six hours LC_{50} value of aqueous cypermethrin ranged from 0.03 μg/L for the crustacean to 9.0 μg/L for the tadpole larva. The oligochaet worm *B. sowerbyi* was relatively tolerant to cypermethrin. Susceptibility of *B. sowerbyi* to cypermethrin varied widely between acetone solution (96 h LC_{50}, 43.65 μg/L) and aqueous solution (96 h LC_{50}, 71.12 μg/L) of the pyrethroid. However, there was little difference in toxicity between the acetone and aqueous solution of cypermethrin for the sensitive species. The study revealed that cypermethrin remained active in water for a maximum period of 72 h.

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