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RESEARCH ARTICLE

VARIATION IN ACUTE TOXICITY BETWEEN TECHNICAL GRADE AND COMMERCIAL FORMULATION OF CYPERMETHRIN TO SOME NON-TARGET FRESHWATER ORGANISMS

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ABSTRACT

Static bioassays were conducted in laboratory to evaluate variation in acute toxicity between technical grade (92 % a.i) and commercial formulation (10 % EC) of cypermethrin to four freshwater organisms *viz.* the crustacean *Cyclops viridis*, the oligochaete worm *Branchiura sowerbyi*, the gastropod *Pila globosa*, and one week old tadpole larva of the toad *Duttaphrynus melanostictus*. Commercial formulation (F) of cypermethrin was found more toxic than the technical (T) grade cypermethrin. Based on 96h LC_{50} values of T and F cypermethrin *C. viridis* was found most sensitive (0.08 and 0.04 µg/L) and *P. globosa* as most tolerant (1416 and 545 µg/L) to cypermethrin. Toxicity of T cypermethrin in water existed up to 48h except for *C. viridis* and *B. sowerbyi*, in which toxicity existed till 72h exposure. Toxicity of F cypermethrin existed till 96h in all species except *C. viridis* in which F cypermethrin became non-toxic after 72h. It was concluded from this study that acute toxicity of cypermethrin to aquatic organisms varied with the formulation and 10% EC of cypermethrin remained viable in freshwater aquatic ecosystem for longer period.

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INTRODUCTION

Cypermethrin belongs to type II (α-cyano) pyrethroids, which are extensively used in pest management all over the world because of their relatively low toxicity to birds and mammals and less environmental persistence (Kaviraj and Gupta, 2014). However, there is a growing concern over toxicity of these pesticides to non-target aquatic organisms and their eco-toxicological implications to aquatic environment. Cypermethrin is registered in India to control pests of cotton, cabbage, okra, brinjal, sugarcane, wheat and sunflower. Freshwater bodies adjacent to these crop fields are prone to be contaminated by cypermethrin from runoffs or spray drifts. Like other Type-II pyrethroids cypermethrin is a potent neurotoxicant (Reddy and Yellamna, 1991; Philip *et al.*, 1995) and has pronounced toxic effects on aquatic invertebrates (Saha and Kaviraj, 2008; David *et al.*, 2012; Demetrio *et al.*, 2014).

In the crop fields, farmers use various types of commercial formulations of pesticides, which contain a number of other chemicals or inert ingredients besides active ingredient of the pesticide. Information regarding the inert ingredients used in commercial formulations are not disclosed due to trade secrecy of the pesticide manufacturers (Cox and Surgan, 2006). Most of the pesticide related toxicity studies are based on active ingredient of the pesticide (De silva *et al.*, 2010). But documents on toxicity of active ingredients alone are not adequate to evaluate risk of the pesticides without evaluating toxicity of their commercial formulations (Abbassy and Mossa, 2012). Agostini *et al.* (2010) and Demetrio *et al.* (2014) reported that commercial formulation of cypermethrin render greater toxicity on organisms than active ingredient (a.i) due to inert ingredient added to the formulation.

Therefore, it is necessary to evaluate toxicity of both technical grade (containing more than 90 % of a.i.) and commercial formulation of the pesticide to obtain more relevant information regarding toxic potential of the pesticide on sensitive non-target organisms (Puglis and Boone, 2011; De Silva *et al.*, 2010). The objective of the present study was to evaluate differences in acute toxicity between technical grade and emulsified concentrate of cypermethrin to one freshwater crustacean zooplankton *Cyclops viridis*, tadpole of the toad *Duttaphrynus melanostictus*, oligochate worm *Branchiura sowerbyi* and the gastropod mollusk *Pila globosa*. These data will provide an idea of the eco-toxicological risk of the aquatic organisms in freshwater ecosystems susceptible to be contaminated by runoffs of the pesticide formulations frequently used by the farmers in agricultural fields.

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MATERIALS AND METHODS

The Zooplankton *Cyclops viridis* (Class: Crustacea; Order Copepoda; L= 0.09 ± 0.03 mm; W= 0.38 ± 0.22 mg), worm *Branchiura sowerbyi* (Class: Oligochaeta; Order: Archioligochaeta; L=2.93 ± 0.35 cm; W= 3.07 ± 0.42 mg), snail *Pila globosa* (Class Gastropoda; Order: Architaenioglossa; L=0.75 ± 0.13 cm; W= 92.4 ± 3.76 mg) and one week old tadpoles of the toad *Duttaphrynus melanostictus* (Class : Amphibia; Order: Anura; L= 1.23 ± 0.15 cm; W= 34.75 ± 8.14 mg) were procured from local ponds. These organisms were also acclimatized to the test conditions for 96h before use. Technical grade (92 % a.i.) Cypermethrin ((*RS*)- α-cyano-3-phenoxybenzyl (1*RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) was obtained from Krishi Rasayan Group of Companies, Kolkata-700 020 (India) and the emulsified concentrate (10 % EC) of cypermethrin was procured under the brand name Ustaad® from the United Phosphorus Ltd., Vapi-396195, Gujarat. A stock solution of 100 mg/L of technical (T) or formulation (F) of cypermethrin was prepared by dissolving appropriate amount of the pesticide in 10 ml of water or acetone. For each treatment, appropriate amount of stock solution was pipetted out and diluted with the test water. Active ingredient of cypermethrin was mixed with acetone due to its low solubility before adding to the test medium.

Bioassays were made according to static bioassay procedures of APHA (1995). Deep (300 feet underground) tube well water stored in an over head tank (Temperature 27-30^{0}C , pH 7.2 ± 0.1; free CO_2 3.37± 0.31 mg/L; dissolved oxygen 6.5 ± 0.2 mg/L; total alkalinity 70.75 ± 3.37 mg/L as $CaCO_3$; total hardness 240.33 ±11.01 mg/L as $CaCO_3$) was used as test medium for all tests. Bioassays for the crustaceans and the worms were carried in 500 ml glass beaker each containing 300 ml water. Bioassays for the gastropod were carried out in 15 liter glass aquarium each containing 2 liters of water. Two separate bioassays were made for each test organism: one with the technical grade cypermethrin and the other with the formulation (emulsified concentrate). In each category there was a control with equal number of replicates. Negative controls were done with and without solvent. For acetone control, 0.1ml/L acetone was added to the test water because maximum amount of acetone present in the highest concentration of this category of cypermethrin tested was less than 0.1 ml/L. No food was provided during the bioassay to avoid interference of excretory products of the test organisms with the test chemical.

Lethal concentrations of cypermethrin at which 50% mortality of the test organisms occurred (LC_{50}) and its 95% confidence limits were estimated for 24, 48, 72 and 96/h from the mortality data using EPA-Probit analysis version 1.5 statistical software based on probit analysis method of Finney (1971). LC_{50} values between the active ingredient and formulation were compared following the criteria of Mayer *et al.* (1986), Schmuck *et al.* (1994) and Demetrio *et al.* (2014).

RESULTS

96h lethal concentrations (µg/L) of technical grade (92% a.i) and commercial formulation (10% EC) of cypermethrin, in which 50 % mortality (LC_{50}) of the crustacean zooplankton (*Cyclops viridis*), worm (*Branchiura sowerbyi*), gastropod mollusk (*Pila globosa*) and the one week old tadpole larva of toad (*Duttaphrynus melanostictus*) occurred, have been presented in Table 1. The crustacean *Cyclops viridis* was found most sensitive and the gastropod *Pila globosa* most tolerant to both technical grade (T) and commercial formulation (F) of cypermethrin. Susceptibility of the test organisms to cypermethrin was in the order: *Cyclops viridis* > Tadpole of toad >*Branchiura sowerbyi* >*Pila globosa.* Changes in LC_{50} values of both T and F cypermethrin in respect of exposure hour have been presented in Fig. 1. LC_{50} values decreased with the increase of exposure period indicating increase in susceptibility of the organisms with the exposure period. However, there was no change in LC_{50} values of T cypermethrin beyond 72h in *C. viridis* and *B. sowerbyi* and beyond 48h in all other species indicating that T cypermethrin was degraded in water within 48 to 72h. On the contrary, LC_{50} values of F cypermethrin continued to decrease till 96h in all species except in *C. viridis* and *B. sowerbyi* in which there was no change in LC_{50} values beyond 72h. The results indicated that viability of F cypermethrin persisted in water for longer period than T cypermethrin.

Remarkable changes in the behaviour were noted in the exposed test organisms. *Cyclops viridis* exhibited irratic, uncoordinated lethargic movements which increased with the increase in concentration of cypermethrin. Finally they were found creeping at the bottom of the glass beaker before the death. The worm *B. sowerbyi* were coiled and wrinkled. At higher concentrations the worms showed fragmentation and degeneration of the body parts. The gastropod *P. globosa* showed avoidance reaction at higher concentration and tried to creep out of the glass jars. Profuse secretion of mucus and retraction of mantle were also observed at higher concentration

Table 1. 96h LC_{50} value with 95% confidence limit in parentheses of technical and formulation of Cypermethrin to different test organisms

Test Organism	Technical (T) (92 % a.i)	Formulation (F) (10 % EC)	Quotient (LC_{50} T / LC_{50} F)
Cyclops viridis	0.08 (0.07-0.10)	0.04 (0.04-0.06)	2.0
Tadpole of *Duttaphrynus melanostictus*	31.77 (22.92-42.97)	8.97 (6.08-12.59)	3.54
Branchiura sowerbyi	299 (250-352)	75 (61-88)	3.98
Pila globosa	1416 (992-1831)	545 (460-647)	2.59

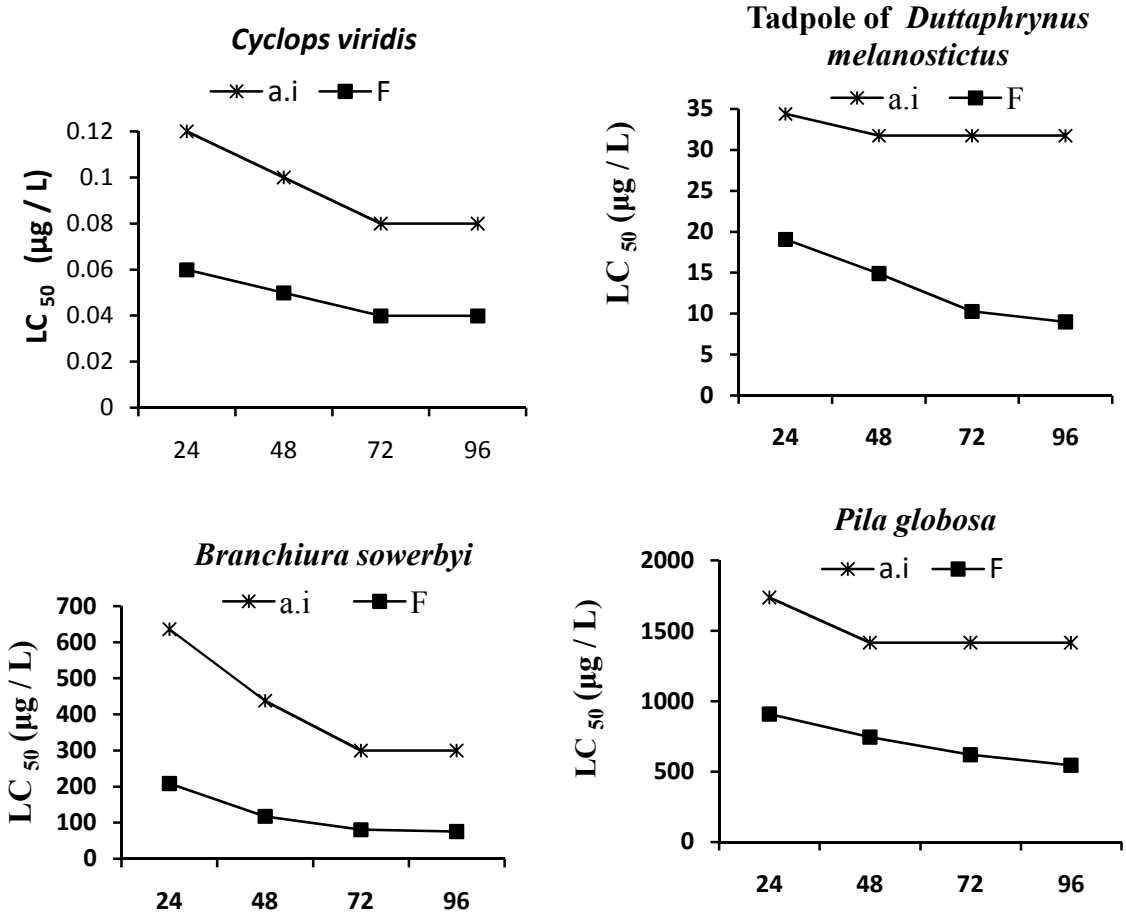
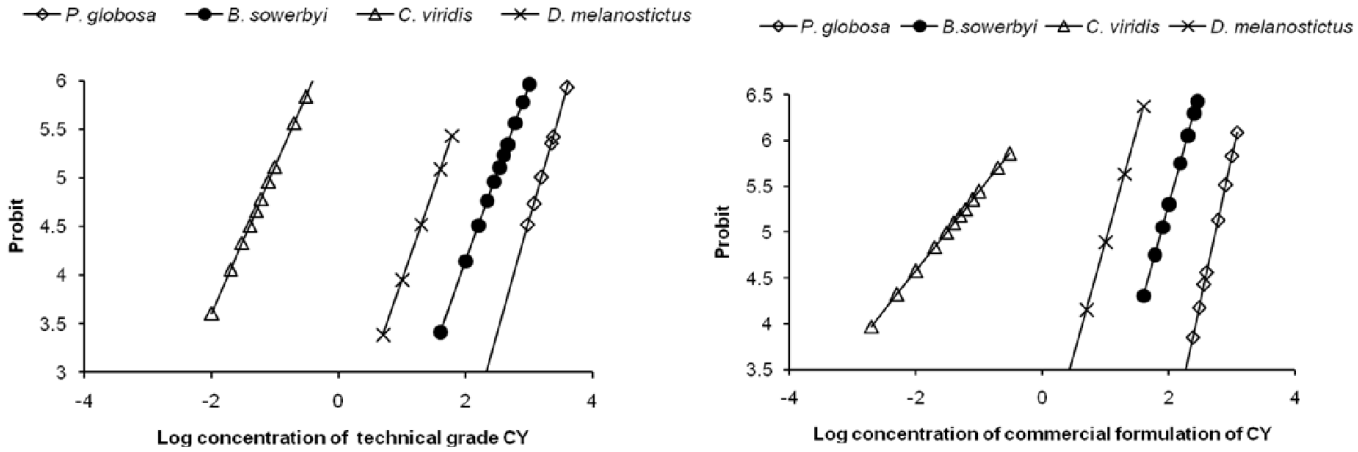


Fig. 1. Changes in LC_{50} values of cypermethrin to different aquatic organisms with respect to exposure period



of cypermethrin. The tadpoles, irrespective of cypermethrin concentrations, tried to escape from the exposed water. In higher concentrations, the tadpoles showed erratic movement and loss of balance.

DISCUSSION

The results of the present study clearly indicated that commercial formulation (10% EC) was more toxic than the technical grade (92 % a.i) cypermethrin for all species tested. These findings are in accordance with the reports of acute toxicity of cypermethrin to amphibian larvae *Hypsiboas pulchellus* (Agostini *et al.*, 2010) and *Rhinella arenarum* (Svartz and Perez-Coll, 2013) and the crustacea *Daphnia magna* (Demetrio *et al.*, 2014). Addition of inert ingredient renders commercial formulations of pesticides more toxic than their technical grade form (Kitulagodage *et al.*, 2008; Puglis and Boone, 2011). However, the magnitude of difference in toxicity between T and F cypermethrin varied between species. Quotient of LC_{50} values (LC_{50} T / LC_{50} F) for the crustacea

was 2.0, while those for other species it ranged from 2.59 to 3.98. Mayer *et al.* (1986) assumed formulation as more toxic when quotient was more than 1, while Schmuck *et al.* (1994) observed that there was a natural variability of quotient between 0.5 and 2.0 and considered formulation as more toxic when quotient was more than 2. Evaluating the results of the present study it was revealed that formulation was more toxic than the technical grade cypermethrin for all test organisms as per the criteria of Mayer *et al.* (1986), while there was no variation in toxicity between T and F cypermethrin for the crustacean as per Schmuck *et al.* (1994). More sensitive was a species to cypermethrin less was the quotient of its LC_{50} between the T and F. However, comparison of sensitivity based on quotient is not accepted universally. Since only one single point (LC_{50}) for the concentration–effect function was considered under criteria proposed by Schmuck *et al.* (1994) and Mayer *et al.* (1986). Demetrio *et al.* (2014) proposed to accept the criteria as valid only when the concentration effect lines were parallel. Slopes of regression for the log concentration - probit mortality line for 96h mortality data for all test organisms have been presented in Fig 2.

The slopes were found more or less parallel between T and F except that of crustacean. In the present study *C. viridis* was found as most sensitive to cypermethrin, 96h LC_{50} values being 0.04 and 0.08 µg/L respectively for 10 % EC and technical grade cypermethrin. Saha and Kaviraj (2008) observed susceptibility of another copepod *Diaptomus forbesi* to 10 % EC of cypermethrin (96h LC_{50} 0.03 µg/L) close to that observed in the present study. Crustaceans being taxonomically close to insects probably lacked the mechanism to metabolize pyrethroids and were thus as sensitive as insects, the target organisms of most pyrethroids. Tadpoles of toad appeared as more tolerant than crustaceans to cypermethrin. Only a few reports are available for cypermethrin toxicity to amphibian species. 96 h LC_{50} value of cypermethrin (10% EC) to tadpole of toad *Duttaphrynus melanostictus* (8.97 µg/L) as observed in the present investigation is close to that (9.0 µg/L) observed by Saha and Kaviraj (2008). However, 48h LC_{50} value of cypermethrin to tadpole larva of *Rana temporaria* (6.5 µg/L; Paulov, 1990) and *Duttaphrynus melanostictus* (3.34µg/L; David *et al.*, 2012) is lower than both 48h and 96h LC_{50} values observed in the present investigation. Susceptibility of amphibians to pesticide depends on developmental stage of the exposed individuals (Pauli *et al.*, 1999; Greulich *et al.*, 2002).

Even 1.0 µg/L of α-cypermethrin could reduce hatching success of egg of moor frog *Rana arvalis* (Greulich and Pflugmacher, 2003). Successfully hatched out larvae of *R. arvalis* showed abnormalities in behavior like twisting, abnormal swimming resembling abnormalities in behavior observed in the present study. Greulich and Pflugmacher (2003) also observed that even lower concentration of α-cypermethrin (0.1mg/L) could prolong metamorphosis. The oligochaete worm, *Branchiura sowerbyi* was found even more tolerant to cypermethrin than amphibian tadpoles. 96h-LC_{50} value of commercial cypermethrin to *Branchiura sowerbyi* (75 µg/L) as observed in the present study resemble that reported by Saha and Kaviraj (2008) for the same specimen (71.12 µg/L). The gastropod *Pila globosa* was found least sensitive to cypermethrin in the present study, 96h-LC_{50} value of commercial cypermethrin being 545µg/L. Release of excess mucus and shell closure probably acted as protection against cypermethrin stress (Ayad *et al.*, 2011). Only a few reports are available on cypermethrin toxicity to mollusks. 96h LC_{50} value of cypermethrin to *Katelysia opima*, an estuarine clam and *Lamellidens marginalis*, a freshwater mussel, the same is 2.75 mg/L (Mukadam and Kulkarni, 2014) and 20.30 mg/L (Kumar *et al.*, 2014) respectively. It is concluded from the present study that toxicity of cypermethrin to freshwater organisms depends on formulation. Emulsified concentrate of cypermethrin (10 % EC) is more toxic than the active ingredient of cypermethrin. The emulsified concentrate also remained viable in freshwater aquatic ecosystem for longer period and the inert ingredients contained in it probably rendered it more toxic than the technical grade cypermethrin.

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