Research Paper

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Acute toxicity of alpha-cypermethrin to oligochaete worm, Branchiura sowerbyi (Beddard, 1982) along with their behavioural responses.

Biology

KEYWORDS : Acute toxicity, alpha-cypermethrin, *Branchiura sowerbyi*, behavioural response.

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ABSTRACT

Acute toxicity of alpha-cypermethrin to Branchiura sowerbyi and their behavioural changes were evaluated in the present study. The 24, 48, 72 and 96 h LC50 values of alpha-cypermethrin to B. sowerbyi were 43.39, 39.75, 22.58 and 13.41 µg/l respectively. A significant variation ($p<0.05$) between mortality rate of worm and concentrations of the test chemical at all the exposure times (24, 48, 72 and 96 h) was observed. The mortality rate also significantly varies ($p<0.05$) with the exposure time at all the concentrations except 48h. In addition, a dose dependent change in the behavioural pattern of the worm was recorded. It was directly proportional to the increasing concentration of alpha-cypermethrin.

Introduction

Alpha-cypermethrin, an active isomer of cypermethrin, is used to control the common domestic insects and pests belonging to families like Lepidoptera and Coleoptera infesting vegetables, citrus fruits, cotton, rice, etc. (Yordanova et al., 2009). Cypermethrin has been reported to be used against lice invasion in fishery (Das & Mukherjee, 2003). Alpha-cypermethrin is a synthetic pyrethroid which acts as a contact and stomach poison. It is classified as a Schedule 6 poison in the Standard for the Uniform Scheduling of Drugs and Poisons (Sarikaya, 2009). Alpha-cypermethrin acts on pre-synaptic neurons of organisms where it prevents the transmission of nerve impulses through the axon by blocking the passage of sodium ions across the channels in nerve fibers (Yordanova et al., 2009). It is non-systemic in nature and is moderate to highly toxic to diverse aquatic and terrestrial life (Mokry & Hoagland, 1990; Chamber, 1994). Very little information is available on the acute toxicity of alpha-cypermethrin on aquatic invertebrates (Yordanova et al., 2009). It is highly toxic to fish. The 24 h LC_{50} values of alpha-cypermethrin to silver barb and common mirror were 20.0 and 4.50 µg/l respectively (Grayson et al., 1990). There is no specific report on the lethality of alpha-cypermethrin on aquatic annelids particularly on the benthic ones that play a vital role in detritus food chains. This sprouted the present need to investigate the acute toxic effects of alpha-cypermethrin to *Branchiura sowerbyi* and their behavioural responses.

Materials and Methods

Test organism used in the bioassay was the benthic oligochaete worm, *Branchiura sowerbyi* (Class: Oligochaeta, Family: Tubificidae). The test organisms were collected from local non-polluted sources. The worms were allowed to acclimatize in the test water for 3 days before the experiment.

Commercial grade alpha-cypermethrin (10% w/w, EC) was collected from the local market. Static replacement bioassays with the *B. sowerbyi* was conducted in 500 ml Borosil glass beakers each containing 250 ml unchlorinated tap water (Temperature 28.3 ± 0.4 °C, pH 7.1 ± 0.2, Free CO_2 9.9 ± 0.5 mg/l, Dissolved Oxygen 5.5 ± 0.3 mg/l, Total alkalinity 181 ± 8.6 mg/l as $CaCO_3$, Hardness 119 ± 6.2 mg/l as $CaCO_3$). A set of four beakers were exposed to each concentration of alpha-cypermethrin. Each set of tests was accompanied by four replicates of control.

Stock solution of the test chemical and its dilutions were made following the method of American Public Health Association (2012). Initially, rough range finding tests were conducted for the test organism to determine the dose range at which mortality occurs (data not shown). The selected test concentrations of alpha-cypermethrin were finally used for the determination of LC_{50} values for the *Branchiura sowerbyi*. Ten test organisms (mean length 18.5 ± 6.22 mm) were used in each replicate. The number of dead organisms was counted at every 24h during the experiment. The dead animals were removed immediately to avoid any organic decomposition. A constant amount of test medium was replaced every 24h by freshwater and the desired quantity of alpha-cypermethrin was immediately added to water to assure a fixed concentration of the toxicant in solution and also to avoid other interfering factors affecting the test animals' performance. Water chemical analysis and the bioassays were done following the methods outlined in American Public Health Association (2012). Similar technique was also followed by previous workers (Badanthadka & Mehendale, 2005; Mukherjee & Saha, 2012). The LC_{50} values for 24, 48, 72 and 96h along with 95% confidence limits were estimated by a computer program (US EPA, 1999).

The behavioural changes of the exposed worms at each concentration of alpha-cypermethrin were also recorded during the bioassay. The data on percent mortality was subjected to analysis of variance using the computer software provided by the R Development Core Team (2011) followed by Duncan's multiple range test to determine significant differences among means at different times of exposure and concentrations (Gomez & Gomez, 1984).

Results and Discussion

The lethal concentration of alpha-cypermethrin to *Branchiura sowerbyi* is summarized in Table 1. No mortality of *B. sowerbyi* was recorded in control during the experiment. The mortality rate (%) of the test animals was significantly increased ($p<0.05$) from control with increasing concentration of the test chemical except 0.8 µg/l at all the exposure times (24, 48, 72, 96h). On the other hand, the mortality rate also varied significantly ($p<0.05$) with the progress of exposure time except 48h at all the concentrations (Table 2).

With the increasing concentration of test chemical and the progress of exposure time, *B. sowerbyi* showed different irregular behaviors which are shown in Table 3. The control worms were active throughout the test period and showed clumping tendency

with normal movement. The clumping tendency of the exposed worms was gradually reduced with increasing concentrations and times of exposure. The worms showed rapid movement at all the higher doses. The rate of movement was increased gradually with the increasing concentrations and time of exposures. The wrinkling effect and mucous secretion of *B. sowerbyi* gradually increased at 60, 80, and 100 µg/l of alpha-cypermethrin at all the exposure times.

In the present study, the 96h LC_{50} value of alpha-cypermethrin to *B. sowerbyi* is 13.41 µg/l. The aquatic invertebrates and fish are most vulnerable to alpha-cypermethrin as its rate of metabolism and elimination are much slower in their body (WHO, 1992; Greulich & Pflugmacher, 2003). The 96h LC_{50} value of cypermethrin for *Tilapia nilotica, Cyprinus carpio, Salmo trutta, Salmo gairdneri* and *Scardinius erythrophthalmus* were 2.20, 0.90-1.10, 1.20, 0.50 and 0.40 µg/l respectively (Sarikaya, 2009).

The present investigation indicates that the 96h LC_{50} value of alpha-cypermethrin to *B. sowerbyi* is slightly higher than the other aquatic invertebrates and vertebrates. The 48h EC_{50} value of alpha-cypermethrin for *Daphnia magna* was 0.8 µg/l and for *Gammarus pulex* 24h LC_{50} value was 0.3 µg/l (Yordanova et al., 2009). Yilmaz et al. (2004) and Sarikaya (2009) reported that the 96h LC_{50} value of alpha-cypermethrin for guppy and nile tilapia were 9.43 µg/l and 5.99 µg/l respectively.

The LC_{50} value of alpha-cypermethrin to *Branchiura sowerbyi* may provide useful data in the determination of safe level for the agricultural effluents before their release to the natural water resources. The evaluation of the toxicity of alpha-cypermethrin is not sufficient for assuring safe level of the receiving water bodies. In addition, potential risk from alpha-cypermethrin metabolites should also be considered to get a more accurate picture in terms of toxicity.

Table 1: Median lethal concentration (LC_{50}) along with 95% confidence limits of alpha-cypermethrin to the *Branchiura sowerbyi* at different hours of exposure

Test organism	Concentration (µg/l)			
	24h	48h	72h	96h
Branchiura sowerbyi	43.39 (28.55-59.14)	39.75 (25.62-54.48)	22.58 (10.40-37.58)	13.41 (5.42-23.55)

Table 2: Mean values (± SD) of mortality rate (%) of *Branchiura sowerbyi* exposed to different concentrations of alpha-cypermethrin at different hours of exposure (24h, 48h, 72h, 96h). Mean values within columns indicated by different superscript letters (a-h) and mean values within rows indicated by different superscript letters (m-o) are significantly different (DMRT at 5% level)

Dose (µg/l)	Hours of exposure			
	24	48	72	96
0.0	00^{am} ± 0.00	00^{am} ± 0.00	00^{am} ± 0.00	00^{am}± 0.00
0.8	00^{am} ± 0.43	00^{am} ± 0.43	10^{an} ± 0.43	10^{an}± 0.00
8	10^{bm} ± 0.43	10^{bm} ± 0.00	20^{bn} ± 0.71	30^{bo}± 0.50
20	20^{cm} ± 0.71	20^{cm} ± 0.71	30^{cm} ± 0.83	50^{cn}± 0.50
40	40^{dm} ± 0.87	50^{dm} ± 0.50	50^{dm} ± 0.83	70^{dn}± 0.43
60	50^{em} ± 0.50	60^{emn} ± 0.83	70^{eno} ± 0.43	80^{eo}± 0.50
80	70^{fm} ± 0.43	70^{fm} ± 0.00	80^{fn}± 0.50	90^{fo}± 0.50
100	80^{gm} ± 0.50	80^{gm} ± 0.00	90^{gn}± 0.43	100^{go}± 0.43
120	90^{hm} ± 0.43	90^{hm} ± 0.00	100^{gn}± 0.43	100^{gn}± 0.00
150	100^{hm} ± 0.43	100^{im}± 0.00	100^{gm}± 0.00	100^{gm}± 0.00

Table 3: Impact of alpha-cypermethrin on behavioural responses of *Branchiura sowerbyi* (M: movement; CT: clumping tendency; MS: mucus secretion; WE: wrinkling effect; –: none; +: mild; ++: moderate; +++: strong) exposed to various concentrations during different hours of exposure

Behaviour of *Branchiura sowerbyi*							
Time of exposure	Behavioural parameter	Dose (µg/l)					
		8	20	40	60	80	100
24h	M	-	+	++	++	+++	+++
	CT	+++	+++	++	++	-	-
	MS	-	-	-	+	++	++
	WE	-	-	+	+	+++	+++
48h	M	-	+	++	++	+++	+++
	CT	+++	+++	++	+	-	-
	MS	-	-	+	+	++	++
	WE	-	-	+	++	+++	+++
72h	M	-	-	++	++	+++	+++
	CT	+++	+++	++	-	+	-
	MS	-	-	-	+	++	++
	WE	-	-	+	++	+++	+++
96h	M	-	+	++	+++	+++	+++
	CT	++	++	++	-	-	-
	MS	-	+	++	++	+++	+++
	WE	-	+	+	++	+++	+++

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