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EFFECTS OF A PYRETHROID INSECTICIDE CYPERMETHRIN ON BENTHIC OLIGOCHAETE WORM, *Branchiura sowerbyi* (BEDDARD, 1982)

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ABSTRACT The present study was done to evaluate the acute toxic effects of Cypermethrin to benthic oligochaete worm, *Branchiura sowerbyi* along with the changes in their behaviour during the study. The 24, 48, 72 and 96 h LC_{50} values of Cypermethrin to *B. sowerbyi* were 3.18, 2.30, 1.70 and 0.75 mg/l respectively. The rate of mortality of *B. sowerbyi* varied significantly ($p<0.05$) at 24, 48, 72 and 96h of exposure time. The mortality rate of the worm also varied significantly ($p<0.05$) with all exposed concentrations of the toxicant except 3.2-4.0 mg/l at 72 and 96h. The worm showed different behavioural responses like abnormal clumping tendency, less movement and excessive mucous secretion which were recorded during the study at all exposure times.

KEYWORDS : Cypermethrin, LC_{50}, *Branchiura sowerbyi*, behavioural response.

Introduction

Indiscriminate use of various insecticides in agricultural field to protect the crop from harmful pest has increased many folds in the developing countries in last few decades (Santhakumar and Balaji, 2000). The insecticides, even when applied to agricultural land are washed away by rainwater and floods to adjacent water bodies like ponds and rivers causing changes in physicochemical properties of the water (Bhalchandra et al., 2001). They may be harmful to many non-target species including fishes and other aquatic animals (Madhab et al., 2002). The insecticide Cypermethrin is a non-systemic Type-II pyrethroid which acts by blocking Na channels and affecting the function of GABA-receptors of nerve filaments (Dobsikova et al., 2006). Beside pests, it also affects the non-target species. Cypermethrin is a potent toxic agent to fish and many other aquatic invertebrates, with 96h LC_{50}s ranging from 0.01-5.0 µg/l (Sarkar et al., 2005). The 96h median lethal concentration of Cypermethrin on grass shrimp (*Palaemonetes pugio*) is 0.016 µg/l (Clark et al., 1989). The 24 and 96 h LC_{50} of Cypermethrin to *Palaemonetes argentines* are 0.0031 and 0.0020 µg/l respectively (Collins et al., 2006). EC values of cypermethrin to the cyclopoid copepod, *Oithona similis* ranges from 0.14 to 0.24 µg/l for nauplii and adults (Wilis and Ling, 2004). There are very scanty reports on the acute toxicity of Cypermethrin to benthic oligochaetes (Saha and Kaviraj, 2008). So, the current study was aimed to evaluate the acute toxicity of Cypermethrin to a benthic Oligochaete worm, *Branchiura sowerbyi* (Beddard, 1982) along with their behavioural responses.

Materials and methods

Test organisms used in the bioassay was the bottom dwelling Oligochaete worm, *Branchiura sowerbyi* (Class: Oligochaeta, Family: Tubificidae; mean length 2.01±0.78 cm; mean weight 1.98±1.17mg). The commercial grade Cypermethrin [(±)-α-Cyano-(3-phenoxyphenyl) methyl (±)-*cis/trans*-3-(2,2-dichlorovinyl)-2,2 dimethylcyclopropanecarboxylate] (10% EC) was used as test chemical.

Static replacement bioassays were conducted in 500 ml Borosil glass beakers having 250 ml un-chlorinated tap water following the methods of APHA (2012). The values of different physicochemical parameters (temperature 25.6 ± 0.5 °C, pH 7.7 ± 0.5, free CO_2 13.2 ± 0.8 mg/l, Dissolved Oxygen 5.1 ± 0.7 mg/l, total alkalinity 171 ± 5.5 mg/l as $CaCO_3$, hardness 115 ± 3.9 mg/l as $CaCO_3$) of test water during the experiment were also estimated following the methods of APHA (2012). Each concentration of Cypermethrin along with control used during acute toxicity test was accompanied by four replicates. Each replicate was provided with ten test organisms (*Branchiura sowerbyi*).

Initially the rough range finding tests were executed. The number of dead worms were counted at every 24h of experiment and removed immediately to avoid any organic decomposition. A certain amount of test medium was replaced every 24h by un-chlorinated stock water with a specific quantity of test chemical to make the desired concentration. The LC_{50}values for 24, 48, 72 and 96h with 95% confidence limits were calculated with help of a statistical software programme (Finney 1971, US EPA, 1999). The behavioural responses of the worm due to acute toxicity of Cypermethrin were also recorded during the bioassay.

The data of percent mortality of the test organisms was subjected to ANOVA using the R- software (R Development Core Team, 2011) followed by DMRT to determine significant variation among the means of control and treatments at different times of exposure.

The behavioural changes of the worm exposed to the insecticide were recorded thoroughly by naked eye observation following the method of Rand (1985).

Results and Discussion

The 24, 48, 72 and 96h LC_{50} values (with 95% confidence limits) of Cypermethrin to *Branchiura sowerbyi* are given in Table 1. No mortality was recorded in the control at the time of experiment. The mortality rate of the worm varied significantly ($p<0.05$) at 24, 48, 72 and 96h of exposure time. A significant variation ($p<0.05$) in the mortality rate of worm was also recorded with increasing concentrations of the toxicant except 3.2-4.0 mg/l at 72 and 96h (Table 2). The behavioural responses observed in the worm exposed to Cypermethrin are shown in Table 3. The clumping tendency and movement of *B. sowerbyi* were decreased with the increasing concentration of the test chemical and exposure time in comparison to control. On the other hand, mucous secretion of the worm was increased with the increasing concentration and the time of exposure. The clumping tendency was totally absent at 3.60 mg/l and above test concentrations at all exposure times. But the rate of mucous secretion was high at these concentrations. Excessive mucous secretion along with loss in balance and movement during 72 and 96h of exposure was probably an indication of their avoidance reaction from the insecticide. The immobility of the worm was followed by disintegration and faster decomposition of the body and death at higher doses of toxicant. Similar changes for tubificid worms exposed to metals have also been reported by Khangarot (1991).

The present study shows that *Branchiura sowerbyi* is comparatively hardy to Cypermethrin than other aquatic invertebrates and fish. In the present study, the 24, 48, 72 and 96 h LC_{50} values of Cypermethrin

to *Branchiura sowerbyi* (3.18, 2.30, 1.70 and 0.75 mg/l respectively) are comparatively much higher than the 96h LC_{50} values of Cypermethrin on grass shrimp (*Palaemonetes pugio*) (0.016 µg/l), *Palaemonetes argentines* (0.0031 and 0.0020 µg/l), *Daphnia magna* (0.26 ppb) and the cyclopoid copepod, *Oithona similis* for nauplii and adults, (0.14 to 0.24 µg/l respectively) (Wilis and Ling, 2004, Clark et al., 1989, Collins et al., 2006). The 96 h LC_{50} values of Cypermethrin is much higher than *Clarias* sp. (0.063mg/l), *Labeo rohita* (4.0 µg/l), rainbow trout (0.82 ppb), brown trout (2.0-2.8 ppb) (Ayoola et al. 2008, Marigoudar et al. 2009, Keith and Walker,1992).

The behavioural changes due to Cypermethrin toxicity may be used as indicator for determination of neurotoxicity as potential toxic effect.The values of LC_{50} of Cypermethrin to *Branchiura sowerbyi* will provide useful data for safe release of agricultural run-off containing Cypermethrin to the natural water bodies.

Table 1: LC_{50} values (with 95% confidence limits) of **Cypermethrin** to the ***Branchiura sowerbyi*** at different times of exposure (24, 48, 72 and 96h)

Test organism	Concentration (mg/l)			
	24h	***48h***	***72h***	***96h***
Branchiura sowerbyi	3.18 (2.94-3.39)	2.30 (1.90-2.65)	1.70 (1.33-2.01)	0.75 (0.39-1.09)

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

Dose (mg/l)	% mortality of worm at different times of exposure (h)			
	24h	***48h***	***72h***	***96h***
0.2	00^{am}± 0.00	00^{am}± 0.00	00^{am}± 0.00	30^{an}± 0.50
0.8	00^{am}± 0.00	10^{bn}± 0.50	20^{bo}± 0.43	30^{ap}± 0.00
1.4	00^{am}± 0.00	10^{bn}± 0.43	30^{co}± 0.43	50^{bp}± 0.43
1.8	00^{am}± 0.00	30^{cn}± 0.50	40^{do}± 0.50	60^{cp}± 0.83
2.4	10^{bm}± 0.43	30^{cn}± 0.43	50^{eo}± 0.43	90^{dp}± 0.50
2.8	20^{cm}± 0.43	50^{dn}± 0.83	80^{fo}± 0.50	90^{dp}± 0.00
3.2	40^{dm}± 0.83	70^{en}± 0.50	100^{go}± 0.50	100^{eo}± 0.00
3.6	70^{em}± 0.50	90^{fn} ± 0.50	100^{go}± 0.00	100^{eo}± 0.00
3.8	90^{fm}± 0.50	100^{gn} ± 0.43	100^{gn}± 0.00	100^{en}± 0.00
4.0	100^{gm}± 0.43	100^{gm}± 0.00	100^{gm}± 0.00	100^{em}± 0.00

Table 3: Behavioural response of ***Branchiura sowerbyi*** (CT: clumping tendency; M: movement; MS: mucous secretion; –: absent, +: mild, ++: moderate, +++: high, X: not recorded due to death) exposed to different concentrations of **Cypermethrin** at different times of exposure

Dose (mg/l)	Behavioural response of worm at different times of exposure											
	24h			***48h***			***72h***			***96h***		
	CT	**M**	**MS**	**CT**	**M**	**MS**	**CT**	**M**	**MS**	**CT**	**M**	**MS**
0.0	+++	+++	-	+++	+++	-	+++	+++	-	++	+++	-
0.2	++	++	-	++	++	-	++	++	+	+	+	+
0.8	++	++	-	+	+	-	+	+	+	-	+	+
1.4	++	+	-	+	+	-	+	+	+	-	+	+
1.8	++	+	-	+	+	+	+	+	++	-	-	++
2.4	++	+	+	+	+	++	-	+	++	-	-	++
2.8	+	+	++	+	+	++	-	+	++	-	-	+++
3.2	+	+	+++	+	+	++	-	+	++	-	-	+++
3.6	-	+	+++	-	+	++	-	-	++	X	X	X
3.8	-	+	++	-	+	++	X	X	X	X	X	X
4.0	-	+	++	X	X	X	X	X	X	X	X	X

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References

1. APHA, 2012.Standard methods for the examination of water and wastewater. (Eds. Rice, Baird, Eaton, & Clesceri.)
2. Ayoola and Anjani, 2008, Histopathological effects of Cypermethrin on Juvenile African Catfish, WJoBR.
3. Bhalchandra & Lomte (2001). Acute toxicity of pesticides carbaryl and endosulfan to freshwater Bivalves, Parreysia cyclindrica. Pollut.Res. 20:25-29.
4. Clark, J.R., Goodman,. Bortwick, Patrick, Cripe, Moody, J.C. More and E.M.Lores,1989. Toxicity of pyrethroid to marine invertebrates and fish: a literature review and results with sediment-sorbed chemicals.EnvironToxicolChem., 8: 393-401.
5. Collins P and Capello S, Cypermethrin toxicity to aquatic life: Bioassay for freshwater prawn, Palaemonetes argentines, Arch Environ ContamToxicol, 2006, 18, 10-14.
6. Dobsikova R., Velisek J., Wlasow T., Gomulka P., svobodova Z., Novotny L (2006). Effects of Cypermethrin on some haematological, biochemical and Histopathological parameters of common carp (Cyprinus carpio L.), NeuroEndocrinolLett 2006; 27 (suppl.2):101-105.
7. Finney, D.J. 1971. Probit analysis. London: Cambridge University Press.
8. Khangarot, B.S. 1991. Toxicity of metals to a freshwater tubificid worm, Tubifex tubifex (Muller). Bulletin of Environmental Contamination and Toxicology 46: 906–912.
9. Keith L. H., Walker M. 1992. EPA'S Pesticide Fact Sheet Database, CRC Press.
10. Madhab P, Bandyopadhaya, Ajit,2002. Xenobiotic impact on sensitivity in Anabas testudineus(Bloch). J.Ecobiol. 14:117-124.
11. Marigoudar S R, Ahmed & David, 2009, Impact of Cypermethrin on behavioural responses in the freshwater teleost, Labeo rohita, WJofZoology 4(1): 19-23, 2009.
12. R Development Core Team, 2011. R: A language and environment for statistical computing.
13. Rand, G.M. 1985. Behavior. In Fundamentals of aquatic toxicology methods and applications, 221. New York: Hemisphere Publishing Corporation.
14. Saha, S. and A. Kaviraj, 2008. Acute toxicity of synthetic pyrethroid cypermethrin to some freshwater organisms. Bull.EnvironContamToxicol., 80: 49-52.
15. Santhakumar M, Balaji M (2000). Acute toxicity of an organophours insecticide monocrotophos and its effects on behaviour of an air-breathing fish, Anabas testudineus(Bloch). J.Environ.Biol. 21:121-123.
16. Sarkar B, Chatterjee A, Adhikari S, Ayyappan S. (2005). Carbofuran and Cypermethrin induced histopathological alteration in the liver of Labeo rohita(Hamilton) and its recovery. JApplIchthyol.2005; 21(2):131-5.
17. US EPA, 1999. Probit program version 1.5.
18. Wilis, K.J. and N. Ling, 2004. The toxicity of the aquatic pesticide cypermethrin to planktonic marine copepods. AquaRes., 35: 263-270.