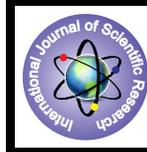


A Study On The Acute Toxicity of Triazophos To *Branchiura sowerbyi* (Beddard, 1982) and Their Behavioural Changes



Biology

KEYWORDS: Triazophos, LC₅₀, *Branchiura sowerbyi*, behavioural response.

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ABSTRACT

The study was made to evaluate the acute toxic effects of Triazophos to benthic oligochaete worm, *Branchiura sowerbyi* along with the changes in their behaviour during the experiment. The 24, 48, 72 and 96 h LC₅₀ values of Triazophos to *Branchiura sowerbyi* were 8.17, 7.07, 5.81 and 5.04mg/l respectively. The mortality rate of *Branchiura sowerbyi* varied significantly ($p < 0.05$) with the increasing concentrations of the toxicant from 5.92, 5.60, 4.80 and 4.32 mg/l at 24, 48, 72 and 96h of exposure respectively. A significant variation ($p < 0.05$) on the mortality rate was also recorded at each concentration (from 5.60 mg/l and above) with the increasing exposure time (24, 48, 72 and 96h). The clumping tendency and movement of *B. sowerbyi* were decreased with the increasing concentration of the test chemical and exposure time. The mucous secretion of the worm was increased with the increasing concentration and the time of exposure.

Introduction

The pesticides are used in agricultural field to eradicate different pest populations for increasing food production (Naveed et al., 2011). When an insecticide is used in the field, it affects its target pests only by 0.1%, but the rest of the insecticide spreads and contaminates the natural ecosystem (Hart and Pimentel, 2002; Mahboob et al., 2011). Triazophos, a newly introduced non-systemic, broad spectrum organophosphate insecticide is used to control different types of pest populations in the field of rice and cotton of all parts of the world (Worthing and Hanrce, 1991; Mingzing et al., 2003). It is neurotoxic in nature and leads to accumulate the neurotransmitter acetylcholine in synapse resulting continuous flow of neuromuscular signals causing paralysis and death of the insect (Kamanyire and karalliedde, 2004; Singh and Rishi, 2005). Triazophos is the most indiscriminately used insecticide which affect the different non-target organisms like crabs, fish etc (Reddy et al., 1983). The bioaccumulation of this toxicant in earthworm affects seriously to the animals of its higher trophic levels (Darling and Thomas, 2005; Hobbelen et al., 2006; Van Gestel et al., 2011). It undergoes biomagnifications through food chain due to its persistence nature and causes serious problem to the human health (Naveed et al., 2011).

The earlier study indicates that the Triazophos is very toxic to earthworm *Eisenia fetida*. The median lethal concentration with contact filter paper test of Triazophos to the *Eisenia fetida* is 14.21 μ g cm⁻² (Wang et al., 2011). The 1h EC₂₀ and EC₅₀ values of Triazophos to zooplankton *Ceriodaphnia quadrangula* are 4.3 and 40.0 μ g/l respectively. The 24, 48 and 72h LC₅₀ values of Triazophos to *Cirrhinus mrigala* are 1.05, 0.87 and 0.75 mg/l respectively (Mahboob et al., 2015). The acute toxicity values of Triazophos to Crucian carp and common carp are 8.4 and 1.00 mg/l respectively (Ismail et al., 2009; Jin et al., 2010). The 96h LC₅₀ value of Triazophos to *Anabas testudineus* is 0.270 mg/l (Jayakumar et al., 2014). There are no earlier reports on acute toxicity of Triazophos to benthic Oligochaetes. Therefore, the present study was aimed at evaluating the acute toxic effects of Triazophos to *B. sowerbyi* and their behavioural changes.

Materials and Methods

Test animal used in the bioassay was the benthic Oligochaete worm, *Branchiura sowerbyi* (Class: Oligochaeta, Family: Tubificidae). They were collected from local market. The test organisms were allowed to acclimatize in the test water for 72 hours before

the commencement of experiment. The commercial grade test chemical Triazophos (40% EC) was collected from the local market.

The Static replacement bioassays with the *Branchiura sowerbyi* was conducted in 500 ml Borosil glass beakers having 250 ml non-chlorinated tap water (APHA, 2012). The values of different physico-chemical parameters (temperature 25.0 \pm 0.5 °C, pH 7.6 \pm 0.4, free CO₂ 14.2 \pm 0.8 mg/l, Dissolved Oxygen 4.9 \pm 0.6 mg/l, total alkalinity 172 \pm 5.6 mg/l as CaCO₃, hardness 117 \pm 3.7 mg/l as CaCO₃) of test water during the experiment were estimated following the methods of APHA (2012). Each concentration of Triazophos along with control used during acute toxicity test was accompanied by four replicates. Each replicate was provided with ten test organisms (*Branchiura sowerbyi*) with mean length of 11.6 \pm 0.5 mm.

The rough range finding tests were initially performed to select the final test concentrations of Triazophos which were used to determine the median lethal concentration to *Branchiura sowerbyi*. The number of dead organisms was counted at every 24h of experiment. The dead organisms were removed immediately to avoid any organic decomposition. A certain amount of test water was replaced every 24h by non-chlorinated stock water with a specific quantity of test chemical to make the desired concentration. Similar technique was also followed by the earlier workers (Mukherjee & Saha, 2012; Bej et al., 2015). The median lethal concentration for 24, 48, 72 and 96h along with 95% confidence limits were calculated with help of a statistical software programme (US EPA, 1999). The behavioural changes of the test organisms due to acute toxicity of Triazophos 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.

Results and Discussion

No mortality of *Branchiura sowerbyi* was recorded in control during the experiment. The 24, 48, 72 and 96h LC₅₀ values of Triazophos to *Branchiura sowerbyi* are given in Table1. The mortality rate of *Branchiura sowerbyi* varied significantly ($p < 0.05$) with

the increasing concentrations of the toxicant from 5.92, 5.60, 4.80 and 4.32 mg/l at 24, 48, 72 and 96h of exposure respectively (Table 2). A significant variation ($p < 0.05$) on the mortality rate was also recorded at each concentration (from 5.60 mg/l and above) with the increasing exposure time (24, 48, 72 and 96h). 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 (Table 3). 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 7.20 mg/l and above test concentrations at all exposure times. But the rate of mucous secretion was very high at these concentrations.

In the present study, the 24, 48, 72 and 96 h LC_{50} values of Triazophos to *Branchiura sowerbyi* (8.17, 7.07, 5.81 and 5.04mg/l respectively) are comparatively much higher than the acute toxic value of Triazophos to common carp (1.00 mg/l), *Anabas testudineus* (0.270 mg/l) and *Cirrhinus mrigala* (0.43-0.84 mg/l) (Ismail et al., 2009; Jayakumar et al., 2014; Mahboob et al., 2015). The 24 h LC_{50} value (8.17 mg/l) of Triazophos to *B. sowerbyi* corresponds with the LC_{50} value of crucian carp (8.4 mg/l).

The present experiment shows that the *Branchiura sowerbyi* is less susceptible to Triazophos than other aquatic animals. But it plays an important role in detritus food chain. The toxicant may be bioaccumulated in *Branchiura sowerbyi* which in turn may affect seriously to the animals of its higher trophic levels through food chain as was found in earthworms by the earlier workers (Darling and Thomas, 2005; Hobbelen et al., 2006; Van Gestel et al., 2011).

The values of median lethal concentrations of Triazophos to *Branchiura sowerbyi* will provide useful data for safe release of agricultural run-off containing Triazophos to the natural water bodies.

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

Test organ-ism	Concentration (mg/l)			
	24h	48h	72h	96h
<i>Branchiura sowerbyi</i>	8.17 (7.50-8.87)	7.07 (6.42-7.70)	5.81 (5.24-6.37)	5.04 (4.29-5.60)

Table 2: Mean values (\pm SD) of % mortality of *Branchiura sowerbyi* exposed to different concentrations of Triazophos at different times of exposure (24, 48, 72 and 96h). Mean values within columns indicated by different superscript letters (a-h) 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)			
	24	48	72	96
00	00 ^{am} \pm 0.00	00 ^{am} \pm 0.00	00 ^{am} \pm 0.00	00 ^{am} \pm 0.00
4.0	00 ^{am} \pm 0.00	00 ^{am} \pm 0.00	10 ^{am} \pm 0.43	20 ^{ao} \pm 0.43
4.32	00 ^{am} \pm 0.00	00 ^{am} \pm 0.00	20 ^{bn} \pm 0.43	40 ^{bo} \pm 0.50
4.80	00 ^{am} \pm 0.00	10 ^{am} \pm 0.50	30 ^{bn} \pm 0.83	50 ^{bo} \pm 0.71
5.60	00 ^{am} \pm 0.00	20 ^{bn} \pm 0.71	50 ^{bn} \pm 0.83	60 ^{do} \pm 1.12
5.92	10 ^{bm} \pm 0.00	30 ^{cp} \pm 0.50	50 ^{cp} \pm 1.12	70 ^{dep} \pm 0.83
6.40	20 ^{cm} \pm 0.00	40 ^{dn} \pm 0.50	60 ^{do} \pm 0.83	70 ^{deo} \pm 0.43
7.20	30 ^{dm} \pm 0.00	50 ^{ep} \pm 0.50	70 ^{deo} \pm 0.71	80 ^{fo} \pm 0.71
8.00	50 ^{em} \pm 0.83	70 ^{fn} \pm 0.71	80 ^{fo} \pm 0.71	90 ^{go} \pm 1.12
8.80	50 ^{em} \pm 0.43	70 ^{fn} \pm 0.83	90 ^{go} \pm 0.43	90 ^{go} \pm 0.43
9.60	70 ^{fm} \pm 0.43	80 ^{gn} \pm 0.71	100 ^{go} \pm 0.43	100 ^{go} \pm 0.00
10.40	80 ^{gm} \pm 0.43	90 ^{hn} \pm 0.43	100 ^{go} \pm 0.00	100 ^{go} \pm 0.00
11.20	100 ^{hm} \pm 0.00	100 ^{hn} \pm 0.00	100 ^{gm} \pm 0.00	100 ^{gm} \pm 0.00

Table 3: Behavioural response of *Branchiura sowerbyi* (CT: clumping tendency; M: movement; MS: mucus secretion), -: absent, +: mild, ++: moderate, +++: high) exposed to different concentrations of Triazophos 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	+++	++	-	+++	++	-	++	++	-	++	++	+
4.32	++	++	-	++	++	-	+	++	+	+	+	+
4.80	++	++	-	+	++	-	+	+	+	-	+	+
5.60	+	+++	-	+	+	-	+	+	+	-	+	+
5.92	+	+++	-	+	+	-	+	++	-	+	++	+
6.40	+	+++	+	-	+	++	-	+	++	-	+	++
7.20	+	+++	++	-	+	++	-	+	++	-	-	+++
8.00	+	+++	++	-	+	+++	-	-	+++	-	-	+++
8.80	-	+++	+++	-	+	+++	-	-	+++	-	-	+++
9.60	-	+++	+++	-	+	+++	-	-	+++	-	-	+++

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