Volume-5, Issue-11, November - 2016 • ISSN No 2277 - 8160

Original Research Paper

Zoology

A Study on Acute Toxicity of An Insecticide Triazophos on Zooplankton *Cyclops Viridis* (Jurine, 1820) Along With The Changes in Their Behaviour

Chandan Sarkar Assistant Professor, P. G. Department of Zoology, Krishnagar Govt. College, Krishnagar, District- Nadia, PIN- 741109, West Bengal, India.

*** Nimai Chandra Saha** Professor of Zoology, Presently working as the Vice-Chancellor, The University of Burdwan, Rajbati, Burdwan-713104, West Bengal, India. * Corresponding Author

ABSTRACT *The objective of the present work was to find out the acute toxic effects of Triazophos to Zooplankton, Cyclops viridis along with the changes in their behaviour during the study. The 24, 48, 72 and 96 h LC50 values of Triazophos to Cyclops viridis were 0.57, 0.27, 0.16 and 0.13 ng/l respectively. The mortality rate of Cyclops viridis varied significantly (p<0.05) at all treatments irrespective of exposure times (24, 48, 72 and 96h). A significant variation (p<0.05) in the rate of mortality of C. viridis was also recorded at all exposure times at all the doses. The swimming rate, hopping frequency and angular turns and bends were initially increased with the increasing concentrations of the toxicant upto 48h of exposure but they were decreased at 72 and 96h.*

KEYWORDS : Triazophos, LC50, Cyclops viridis, behavioural

Introduction

Insecticides have a widespread impact on aquatic biotic community. The direct effects of insecticides have been found at every trophic level starting from plankton to fish (Bej et al. 2015; Sarkar et al, 2016). Insecticides are mostly used in crop protection and thereby transported to water bodies by many routes like surface run-off, point source or wind drifts (Hill et al.1994). It has been widely viewed that insecticide concentrations in the natural ecosystem are often highly potent to kill certain biota (Hatakeyama et al., 1991, 1994) and the structure and function of natural biological communities are also affected (Helgen et al., 1988; Hatakeyama et al., 1990). In ecotoxicological tests the zooplanktons are frequently used. Because they are one of the groups most susceptible to toxic chemicals and they belongs a central position in the lentic (static water) food chain (Mukherjee and Saha, 2012; Hanazato, 2000). Triazophos is an organophosphate pesticide introduced newly and used largely with the chemical name of *O*,*O*-diethyl- *O*-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate (Jayakumar et al, 2014). It is a broad-spectrum insecticide, acaricide and nematicide (Jayakumar et al, 2014). It is used throughout the world in huge quantities and applied to cotton and rice to eradicate aphids, fruit borers, leave hoppers and cutworms (Worthing and Hance, 1991). Primarily it is neurotoxic and accumulates neurotransmitter acetylcholine with effects on synaptic, cholinergic and neuromuscular junction (Kamanyire et al. 2004; Singh et al. 2005).The bioaccumulation of Triazophos affects severely to the organisms of higher trophic levels (Darling and Thomas, 2005) which is persistent in nature and undergoes biomagnifications through food chain and thereby causes serious threats to the human health (Naveed et al., 2011).

The earlier studies indicate that Triazophos is very toxic to zooplanktons. The 1h EC_{20} and EC_{90} values of Triazophos to zooplankton *Ceriodaphnia quadrangular* are 4.3 and 40.0 µg/l respectively (www.pesticideinfo.org). No earlier reports are found on acute toxicity of Triazophos to *Cyclops* especially in Indian context. The objective of the present investigation was to evaluate the acute toxic effects of Triazophos to the commonly found zooplankton *Cyclops viridis* and their behavioural changes.

Materials and methods

The test organisms used in the bioassay is freshwater Cyclopoid Copepod, *Cyclops viridis* (Class: Maxillopoda, Subclass: Copepoda, Family: Cyclopoidae). This zooplankton forms important link in many food chain. The zooplankton were collected from local unpolluted source. They were acclimatized to the test water in laboratory for minimum 48h. The commercial grade of Triazophos (40% EC) collected from the local market was used as test chemical. Static replacement bioassay with the Zooplankton was conducted in 500ml Borosil glass beakers each containing 250 ml unchlorinated tap water for determination of acute toxicity. The values of different physico-chemical parameters of test water were estimated (temperature 28.0 ± 0.5 °C, pH 7.7 ± 0.3, free CO_2 13.2 ± 0.7 mg/l, Dissolved Oxygen 4.8 ± 0.8 mg/l, total alkalinity 169 ± 5.9 mg/l as $CaCO_3$, hardness 119 ± 3.5 mg/l as $CaCO_3$) following the methods of APHA (2012). Each test was accompanied by four replicates with control. Each replicate was provided by ten zooplanktons (mean length 0.09 ± 0.02mm). Required amount of test chemical was added to the test medium and stirred with a magnetic stirrer for uniform mixing. Before the onset of final experiment, a rough range finding test was performed to find the mortality range. Finally, selected doses were used to estimate the LC_{50} value of Triazophos to *Cylops viridis* at 24, 48, 72 and 96h of exposure. The number of dead plankton were counted and removed quickly for preventing any bacterial decay causing depletion of dissolve oxygen. At every 24h 10% of test medium was replaced with freshwater and immediately add sufficient amount of Triazophos to maintain proper concentration and avoid other abiotic factors which may affect animals' performance. Similar technique was also followed by the earlier workers (Sarkar, et al., 2016; Bej et al., 2015 etc).The median lethal concentration (LC_{50}) for 24, 48, 72 and 96h along with 95% confidence limits were calculated with help of a statistical software programme (US EPA, 1999).The percent mortality data of the test animals was subjected to ANOVA using the R- software (R Development Core Team, 2011) followed by DMRT to determine significant variation (*p*-value) among the means of control and treatments at different times of exposure. Different behavioural responses like swimming rate, hopping frequency and angular turns and bends were observed by naked eye during the study at all exposure times.

Results and discussion

The median lethal concentration of Triazophos to copepod zooplankton *Cyclops viridis* are summarized in Table 1.The 24, 48, 72 and 96 h LC_{50} values of Triazophos to *Cyclops viridis* were 0.57, 0.27, 0.16 and 0.13 ng/l respectively. The *Cyclops viridis* is more sensitive to Triazophos than *Ceriodaphnia quadrangular*. The 1h EC_{20} and EC_{90} values of Triazophos to *Ceriodaphnia quadrangular* were found as 4.3 and 40.0 µg/l respectively (www.pesticideinfo.org).

Table 2 indicates that the mortality rate of *Cyclops viridis* varied significantly (p<0.05) at all treatments irrespective of exposure times (24, 48, 72 and 96h). A significant variation (p<0.05) in the rate of mortality of *C. viridis* was also recorded at all exposure times at all the doses.

The swimming rate, hopping frequency and angular turns and bends were initially increased with the increasing concentrations of the toxicant upto 48h of exposure but they were decreased at 72 and 96h (Table 3). Similar observations were also recorded at higher concentration of 2, 4, 6-TCP to *Cyclops viridis* (Mukherjee et al. 2012).

The present study showed that Triazophos caused different behavioural changes of *C. viridis*. The initial increase of swimming rate, hopping frequency and angular turns and bends can be taken as an index of stress felt by the organism. The LC_{50} values indicates that the Triazophos is highly toxic to zooplankton which may affect the food chain and community structure in the aquatic ecosystem even at very low concentration. The LC_{50} values from the current study might provide useful data for setting up national and local water quality criteria (WQC) for Triazophos before its disposal. This data can also be used in ecological risk assessment.

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

Test organism	Concentration (ng/l)			
	24h	48h	72h	96h
Cyclops viridis	**0.57** (0.28-1.18)	**0.27** (0.09-0.57)	**0.16** (0.05-0.30)	**0.13** (0.04-0.21)

Table 2: Mean values (±SD) of % mortality of *Cyclops viridis* 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 (ng/l)	% mortality of ***Cyclops viridis*** at different times of exposure (h)			
	24h	48h	72h	96h
0.1	00^{am}±0.00	20^{an}±0.50	30^{ao}±0.00	40^{ap}±0.43
0.13	20^{bm}±0.43	30^{bn}±0.43	40^{bo}±0.43	50^{bp}±0.00
0.17	40^{cm}±0.43	50^{cn}±0.43	60^{co}±0.50	60^{co}±0.43
0.2	50^{dm}±0.43	60^{dn}±0.43	70^{do}±0.43	70^{do}±0.43
0.5	60^{em}±0.43	70^{en}±0.43	70^{dn}±0.00	80^{eo}±0.43
1.0	60^{em}±0.43	70^{en}±0.43	80^{eo}±0.43	90^{fp}±0.43
5.0	70^{fm}±0.43	80^{fn}±0.43	90^{fo}±0.00	100^{gp}±0.00
10.0	90^{gm}±0.43	90^{gm}±0.43	100^{gn}±0.00	100^{gn}±0.00
50.0	100^{hm}±0.43	100^{hm}±0.00	100^{gm}±0.00	100^{gm}±0.00

Table 3: Behavioural responses of *Cyclops viridis* (SR= Swimming Rate, HF=Hopping Frequency, ATB= Angular Turns and Bends; –: absent, +: mild, ++: moderate, +++: high, X= not recorded due to death) exposed to different concentrations of Triazophos at different times of exposure

Dose (ng/l)	Behavioural responses of ***Cyclops viridis*** at different times of exposure											
	24h			48h			72h			96h		
	SR	HF	ATB	SR	HF	ATB	SR	HF	ATB	SR	HF	ATB
0.0	+	+	+	+	+	+	+	+	+	+	+	+
0.1	+	+	+	+	+	+	+	+	+	+	+	+
0.13	+	+	+	+	+	+	++	+	++	++	++	++
0.17	+	+	+	+	+	+	++	+	++	++	++	++
0.2	+	++	+	++	++	++	++	++	++	++	++	++
0.5	++	++	++	++	++	+++	++	+++	+++	++	+++	+++
1.0	++	++	++	++	+++	+++	+++	+++	+++	++	++	++
5.0	++	+++	+++	++	+++	+++	++	++	++	+	+	+
10.0	++	+++	+++	++	+++	+++	++	++	++	X	X	X
50.0	+++	+++	+++	+++	+++	+++	X	X	X	X	X	X

Acknowledgement

The authors are grateful to the Head, Department of Zoology, The University of Burdwan, and the Head, P.G. Department of Zoology, Krishnagar Govt. College for providing infrastructural facilities to carry out the work.

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