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Acute Toxicity of Triazophos To Common Carp (Cyprinus Carpio) Fry and Their Behavioural Changes

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ABSTRACT

The study was made to assess the acute toxic effects of Triazophos to common carp (Cyprinus carpio) fry along with the changes in their behaviour during the test. The 24, 48, 72 and 96 h LC50 values of Triazophos to Cyprinus carpio are 3.26, 2.79, 2.59 and 2.04 mg/l respectively. The mortality rate of Cyprinus carpio varied significantly ($p<0.05$) with the increasing concentrations irrespective of exposure time. On the other hand mortality rate of the fish also varied significantly ($p<0.05$) irrespective of exposure times (24, 48 and 72 and 96h) at all the doses. The treated Cyprinus carpio showed different irregular behaviours like excess mucous secretion, erratic movement, gulping of air followed by sudden death syndrome (flaring of operculae, convulsion, loss of equilibrium and death) with the increasing concentrations of toxicant and progress of time of exposure. The opercular movement increased significantly ($p<0.05$) at the lower doses but decreased significantly ($p<0.05$) at the higher doses at all the exposure times. At a particular concentration the opercular movements increased significantly ($p<0.05$) at 48 and 72h but with the passage of exposure time, the opercular movement decreased significantly ($p<0.05$) at 96h.

KEYWORDS Triazophos, LC50, Cyprinus carpio, behavioural change, opercular movement.

INTRODUCTION

The freshwater ecosystems with adjoining agricultural fields have greater chance of being contaminated with agricultural runoff (Naveed et al., 2010). Many pesticides are used in agricultural field to eradicate various pest populationsfor enhancingthe production of food (Naveed et al., 2011). The most of these man-made chemicals find their way to natural water bodies through agricultural runoff and affect not only the target species but also many other non-target animal species in different degrees (Sanchez-Bayo F, 2011). In India almost 70% agricultural chemicals are believed to affect non-target species (Naveed et al, 2012). Fish are very sensitive to environmental contamination of water (Naveed et al., 2011). A report indicates that a significant part of the world food comes from fish source, so it needs to secure the fish health from pesticide pollution (Tripathy et al, 2002). Among all pesticides, the organophosphorous pesticides are widely used for their quick action and rapid biodegradation (Mahboob et al, 2002). In India, Triazophos (an organophosphate pesticide) is extensively applied for protection of food & cereal crops (jain et al, 2010; Smita et al, 2011). Triazophos is a neurotoxic in nature which leads to accumulate the neurotransmitter acetylcholine in synapse results in continuous flow of neuromuscular signals leads to paralysis and death of the insect. (Kamanyire and karalliedde, 2004; Singh and Rishi, 2005). The bioaccumulation of this toxicant in earthworm affects seriously to its higher trophic level animals (Darling and Thomas, 2005; Hobbelen et al., 2006; Van Gestel et al., 2011).

The earlier study indicates that the 24, 48 and 72h LC50 values of Triazophos to Cirrhinus mrigala were 1.05, 0.87 and 0.75mg/l respectively (Mahboob et al., 2015). Ghazala et al. (2014) reported that the24, 48, 72 & 96h LC50 values of Triazophos to Catla catla were 6.64, 5.83, 5.64, & 4.84 respectively. It was also reported that the 96h LC50 values of Triazophos to Anabus testudineas and Tilapia nilotica were 0.270 and 0.035 mg/l. respectively (Li Shaonan, 1998; Jayakumar et al, 2014). In Tilapia nilotica, the 48h LC50 value for Triazophos was 0.04 mg/l (www.pesticideinfo.org). The 72h LC50 value for Triazophos to the embryo and larvae of Gobiocypris rarus were 7.44 and 2.52 mg/l respectively (Zhu B, 2014). The reports on the toxicity of Triazophos to common carp (Cyprinus carpio) are scanty (Chen et al. 2014; Wang, 2015).

The present study was undertaken to evaluate the acute toxicity of commonly used organophosphorous pesticide Triazophos on fry of Cyprinus carpio and their behavioural changes.

MATERIALS AND METHODS

The common carp fry Cyprinus carpio belonging to Order: Cypriniformes and family Cyprinidae (mean length 4.01 ± 0.67 cm, mean weight 2.33 ±0.48 gm) was used as test animals in the bioassay. The fish were collected from local fish farm. They were allowed to acclimatize gradually to the test water for 72h in the laboratory before experiment. The commercial grade of Triazophos (40% EC) was used as test chemical. The static replacement bioassay method was conducted in 15l glass aquarium each containing 10l of water to determine the acute toxicity of the test chemical following the methods of earlier workers (Saha et al, 1999; American Public Health Association, 2012; Mukherjee and Saha 2013; Saha et al, 2016). Unchlorinated tap water (temperature 26.1 ± 0.12 0C, pH 7.6 ± 0.23, free CO_2 11.0 ± 0.42 mg/l, DO 5.28 ± 0.39 mg/l, alkalinity 168 ± 11.21 mg/l as $CaCO_3$, hardness 117 ± 4.30 mg/l as $CaCO_3$) was used as a diluent medium during the experiment. The fishes were kept in starvation for

24h before and during the bioassay. Each test was accompanied by four replicates with sufficient control. Each replicate was provided with ten test organisms. Primarily, rough range finding tests were performed to determine dose range at which mortality of fish occurs. Finally, the selected test concentrations were used to estimate the LC50 values of Triazophos to Cyprinus carpio at 24, 48, 72 and 96h of exposure. The number of dead fish was counted at every 24 h of experiment. The dead fish were removed quickly from the test water to avoid any bacterial decomposition causing depletion of dissolved oxygen. The 10% of the test medium was replaced by non-chlorinated stock water at every 24h of exposure time and required quantity of test chemical was added immediately to test medium to maintain a fixed concentration. The mean opercular movements of fish (Number of movement/min/fish) were noted at every 24h during the experiment to evaluate the effects of Triazophos on respiratory rate of fish. The behavioural changes like excess mucous secretion, erratic movement, gulping of air followed by sudden death syndrome (flaring of operculae, convulsion, loss of equilibrium and death) were also recorded (Rand, 1985; Mukherjee and Saha 2013).

Mean mortality of Cyprinus carpio after 24, 48, 72 and 96h was used to calculate LC50 values (95% confidence limit) using a statistical software, Probit program version 1.5 (US EPA 1999). The values of percent mortality and opercular movement of fish were subjected to analysis of variance (ANOVA) with the help of R-software (R Development Core Team, 2011) followed by Duncan's Multiple Range Test (DMRT) to determine the significant variation among the mean values at different concentrations of Triazophos at different times of exposure (24, 48, 72 and 96h).

RESULTS AND DISCUSSION

The LC50 values of Triazophos to Cyprinus carpio are shown in Table 1. The 24, 48, 72& 96 h LC50 values of Triazophos to Cyprinus carpio are 3.26, 2.79, 2.59 & 2.04 mg/l respectively. The mortality rate of Cyprinus carpio varied significantly ($p<0.05$) with the increasing concentrations irrespective of exposure time. On the other hand mortality rate of the fish also varied significantly ($p<0.05$) at all the exposure times (24, 48 and 72 and 96h) at all the doses (Table 2). The treated Cyprinus carpio showed different irregular behaviours like excess mucous secretion, erratic movement, gulping of air followed by sudden death syndrome (flaring of operculae, convulsion, loss of equilibrium and death) with the increasing concentrations of toxicant and progress of exposure time (Table 3). The opercular movement increased significantly ($p<0.05$) at the lower doses but decreased significantly ($p<0.05$) at the higher doses at all the exposure times (Table 4). At a particular concentration the opercular movements increased significantly ($p<0.05$) at 48 and 72h but with the progress of exposure time, the opercular movement decreased significantly ($p<0.05$) at 96h.

The 72h LC50 value (2.59 mg/l) of the present study corresponds to the 72h LC50 value (2.52 mg/l) of Triazophos to the larvae of Gobiocypris rarus (Zhu B, 2014). The present 96h LC50 value (2.04 mg/l) is lower than the 96h LC50 value (4.84 mg/l) of Triazophos to Catla catla (Ghazala et al., 2014). On the other hand this value is much higher than the 96h LC50 values of Triazophos to Tilapia nilotica (0.035 mg/l), Anabas testudineus (0.27 mg/l) and Cirrhinus mrigala (1.05 mg/l) (Shaonan, 1998; Jayakumar et al., 2014; Mahboob et al., 2015). It indicates that the fry of Cyprinus carpio was comparatively less sensitive to Triazophos than Tilapia nilotica, Cirrhinus mrigala and Anabas testudineus but was more sensitive than Catla catla.

The abnormal behaviours like excess mucous secretion, erratic movement, gulping of air followed by sudden death syndrome (flaring of operculae, convulsion, loss of equilibrium and death) observed in the present study were probably due to the ionic as well as enzymatic alteration in blood and tissues (Larsson et al. 1981). The excess mucous secretion of fish was probably for the dysfunction of pituitary gland over the integument for toxic stress (Pandey et al. 1990). The abnormal behaviours of the exposed fish may be due to adapt a compensatory mechanism to derive energy to avoid stress due to toxicity of chemical (Joshi 2011).

The results of the present study may provide supplement to the current knowledge on toxicity of Triazophos and also help in the effective management of natural water resources in respect to the input of this organophosphate from agricultural field.

Table 1: LC50 values (with 95% confidence limits) of Triazophos to the Cyprinus carpio at different times of exposure (24, 48, 72 and 96h)

Test organism	Concentration (mg/l)			
	24h	48h	72h	96h
Cyprinus carpio	3.26 (2.85-3.65)	**2.79** (2.34-3.22)	**2.59** (2.15-2.98)	**2.04** (1.62-2.39)

Table 2: Mean values (±SD) of % mortality of Cyprinus carpio 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-i) and mean values within rows indicated by different superscript letters (m-p) are significantly different (DMRT at 5% level)

Dose (mg/l)	Mean values (±SD) of % mortality of fish at different times of exposure (h)			
	24h	48h	72h	96h
0.0	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$
1.2	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$00^{am} \pm 0.00$	$20^{bn} \pm 0.43$
1.6	$00^{am} \pm 0.00$	$10^{bn} \pm 0.43$	$20^{bo} \pm 0.43$	$30^{bp} \pm 0.00$
2.0	$10^{bm} \pm 0.00$	$30^{cn} \pm 0.50$	$30^{cn} \pm 0.83$	$50^{co} \pm 1.30$
2.4	$20^{cm} \pm 0.50$	$40^{dn} \pm 0.50$	$40^{dn} \pm 0.43$	$60^{cdo} \pm 1.00$
2.8	$40^{dm} \pm 0.50$	$50^{emn} \pm 0.50$	$60^{eno} \pm 0.83$	$70^{deo} \pm 0.83$
3.2	$50^{em} \pm 0.43$	$60^{fn} \pm 0.43$	$60^{en} \pm 0.43$	$80^{efo} \pm 0.71$
3.6	$60^{fm} \pm 0.43$	$70^{gn} \pm 0.43$	$70^{fn} \pm 0.43$	$80^{efo} \pm 0.43$
4.0	$60^{fm} \pm 0.43$	$70^{gn} \pm 0.43$	$80^{go} \pm 0.50$	$90^{fgp} \pm 0.00$
4.8	$80^{gm} \pm 0.43$	$80^{hm} \pm 0.87$	$90^{hmn} \pm 0.43$	$100^{gn} \pm 0.43$
5.2	$90^{hm} \pm 0.71$	$100^{in} \pm 0.43$	$100^{in} \pm 0.00$	$100^{gn} \pm 0.00$
5.6	$100^{im} \pm 0.50$	$100^{im} \pm 0.00$	$100^{im} \pm 0.00$	$100^{gm} \pm 0.00$

Table 3: Behavioural responses of Cyprinus carpio (MS=Mucous Secretion, SDS=Sudden Death Syndrome, EM=Erratic Movement), –: absent, +: mild, ++: moderate, +++: high) exposed to different concentrations of Triazophos at different times of exposure

Dose (mg/l)	Behavioural responses of fish at different times of exposure											
	24h			48h			72h			96h		
	MS	SDS	EM	MS	SDS	EM	MS	SDS	EM	MS	SDS	EM
0.0	-	-	-	-	-	-	-	-	-	-	-	-
1.2	-	-	-	-	-	-	-	-	-	-	-	+
1.6	-	-	-	-	-	+	-	-	+	-	-	+
2.0	-	-	+	+	-	+	+	-	+	+	-	++
2.4	-	-	+	+	-	+	+	-	+	++	-	++
2.8	-	-	+	+	-	++	+	-	++	++	-	++
3.2	+	-	+	+	-	++	++	-	++	++	+	++
3.6	+	-	++	++	-	+++	++	-	++	++	-	++
4.0	+	+	+++	++	-	+++	++	-	+++	++	-	++
4.8	+	++	+++	++	-	+++	++	-	+++	++	-	++
5.2	+	+++	+++	++	-	+++	++	-	+++	++	-	++

Dose mg/l	Opercular movement/minuteof fish at different times of exposure			
	24h	48h	72h	96h
0.0	95^{cr}±1.49	92^{abn}±0.71	90^{ao}±1.48	93^{bcp}±0.83
1.6	100^{as}±0.71	122^{ct}±0.83	124^{dr}±0.83	120^{br}±0.71
2.0	100^{as}±1.12	110^{bs}±0.83	130^{ds}±1.58	120^{cr}±1.58
2.4	85^{aq}±0.71	100^{bp}±1.12	130^{ds}±1.22	119^{cr}±0.83
2.8	74^{ap}±0.83	96^{bo}±0.83	100^{cq}±1.5	98^{bcq}±1.22
3.2	72^{ao}±0.71	105^{dq}±1.22	96^{cp}±0.83	80^{bo}±1.92
3.6	68^{an}±0.83	107^{dr}±0.71	96^{cp}±1.00	78^{bno}±1.48
4.0	60^{am}±0.83	100^{dp}±1.58	82^{cn}±0.83	77^{bn}±1.48
4.8	61^{am}±1.12	77^{cm}±1.58	70^{bm}±0.83	63^{am}±0.83

Table 4: Mean values (±SD) of opercular movement/minute of Cyprinus carpio 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 (m-t) and mean values within rows indicated by different superscript letters (a-d) are significantly different (DMRT at 5% level)

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