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Evaluation of Acute Toxicity Levels and Behavioral Changes to the Fry of Common Carp, *Cyprinus carpio* (Linnaeus, 1758) Under Exposure of a Pyrethroid Insecticide Cypermethrin

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Abstract
Toxicity of an insecticide Cypermethrin to the fry of common carp, Cyprinus carpio along with their behavioural changes was evaluated in the present study. The 24, 48, 72, and 96 h LC_{50} values of Cypermethrin to the fry of Cyprinus carpio were 1.04, 0.92, 0.89, 0.79 ng/l, respectively. The mortality rate of C. carpio showed significant variation ($p < .05$) with the increasing concentrations at all exposure times. On the other hand, the mortality rate of C. carpio also varied significantly ($p < .05$) at all the doses with increasing exposure times (24, 48 and 72, and 96 h). The excess mucous secretion with increasing concentration of toxicant and exposure time was recorded in the exposed fish. The treated fish showed faster movement at lower doses but it was gradually reduced at higher doses at all exposure times. Wide opening of mouth and gills was observed at higher concentrations. Opercular movement of fish initially increases with the increasing concentration but decreases at higher concentrations. Similarly the opercular movement increases significantly ($p < .05$) with increasing exposure time up to 72h but it decreases significantly ($p < .05$) at 96h of exposure at all the treatments.

Keywords: *Behavioral change, cypermethrin, Cyprinus carpio, LC_{50}, opercular movement*

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INTRODUCTION

The pollution of rivers, streams, and wetlands with chemical contaminants has become most serious problems of this century [1]. Among these harmful chemicals, insecticides occupy unique positions which are used regularly by man [2]. Insecticides applied directly to the field are washed away by flood and rainwater as runoff to adjacent wetlands and thereby alteration of physicochemical properties of water occurs [3]. Due to effectiveness and biodegradability, pyrethroids are very commonly used insecticides. Pyrethroids are synthetic analogues of pyrethrins produced naturally by *Chrysanthemum cinerariaefolium*, an ornamental plant [4]. Their 96 h LC_{50} for fish is below 10 μg/l. Thus, pyrethroids becomes highly toxic to fish and other aquatic organisms [4].

Cypermethrin (a-cyano-3-phenoxybenzyl ester of 2, 2-dimethyl-3-(2, 2-dichlorovinyl)-2-2-dimethyl cyclopropane carboxylate) is extremely effective nonsystemic Type II synthetic pyrethroid. It is used in animal husbandry to resist ectoparasites and a wide range of other pests in agricultural field [5]. It acts by blocking sodium channels and affecting the function of GABA-receptors of nerve filaments [6]. The relatively slow metabolism and less efficiency to eliminate the compounds may cause the fish sensitivity to pyrethroids [7]. A large portion of world's food is come from fish source, so it is important to ensure the health of fishes [8].

The earlier studies indicate that the Cypermethrin is very toxic to various groups of fishes. According to Bradbury and Coats, 1989 [7], the 96-h LC_{50} of Cypermethrin as 1.2 μg/L for brown trout (*Salmo trutta*), 0.5 μg/L for rainbow trout (*Salmo gairdneri*), 0.4 μg/L for *Scardinius erythropthalmus* and 2.2 μg/L for

Tilapia nilotica. The lethal concentration (LC_{50}) value of cypermethrin was 0.063 mg/l for 96h of exposure in *Clarias gariepinus* (Burchell 1822) juvenile [2]. The acute toxicity value of Cypermethrin was found to be 4.0 μg/l for *Labeo rohita* fingerlings for 96 h [1]. According to Jones [9], in Brown trout (96 h) LC_{50} is 2.0-2.8 ppb, in Rainbow Trout (96 h) LC_{50} is 0.82 ppb, in Bluegill Sunfish (96 h) LC_{50} is 1.78 ppm, in *Daphnia magna* LC_{50} is 0.26 ppb. In *Poecilia reticulata* (Peters) at 25^0C, the 24, 48, 72, and 96h LC_{50} values are 219.83, 202.72, 186.22, and 168.07 ppb, respectively [10]. According to Clark et al. (1989) [11], 96 h LC_{50} of Cypermethrin for grass shrimp (*Palaemonetes pugio*) is 0.016 μg/l. Collins et al (2006) [12] recorded the LC_{50} of Cypermethrin to freshwater prawn *Palaemonetes argentinus* as 0.0031 and 0.0020 μg/l for 24 and 96h, respectively. The reports on the toxicity of Cypermethrin to common carp (*Cyprinus carpio*) are scanty [7,13]. In Indian context, especially in West Bengal, the data of LC_{50}on fry of common carp during 24, 48, 72, and 96h, no earlier reports are found.

Therefore, the present study was aimed to determine the effect of acute toxicity of commonly used synthetic pyrethroid insecticide Cypermethrin on fry of common carp (*Cyprinus carpio*) and their behavioural changes.

MATERIALS AND METHODS

Fry of common carp (*Cyprinus carpio*) belonging to Order: Cypriniformes and family Cyprinidae (mean length 4.03 ± 0.70 cm, mean weight 2.35 ± 0.49 gm) was used as test animals in the bioassay. The specimens were obtained from local fish farm in early morning. They were transferred and stocked to laboratory immediately and allowed to acclimatize gradually to the test water for 72h inthere before experiment. The commercial grade of Cypermethrin (10% EC) was used as test chemical. To determine the acute toxicity of the test chemical, the static replacement bioassay method was conducted in 15 L glass aquarium each containing 10 L of unchlorinated tap water following the methods of earlier workers [14,15,16]. Fishes were randomly parcelled in glass aquaria (15 L) supplied with oxygenated unchlorinated tap water water maintaining constant dissolved oxygen at 7.6 ± 0.5 mg/L, temperature at 27 ± 1°C, pH at 7.13 ± 0.9, total hardness 195 ± 10 mg/L as $CaCO_3$ alkalinity 167 ± 11.89 mg/L and photoperiod at 14L/10D light: dark natural photoperiod. No food was supplied to the fishes for 24 h before and during the bioassay. Each experiment was conducted with four replicates with control. Each replicate was provided with ten fishes. Initially, rough range finding experiments were conducted to fix the dose range at which lethality of fish occurs. Finally, only the selected test concentrations of Cypermethrin were used to determine the LC_{50} measure to *Cyprinus carpio* at 24, 48, 72, and 96 h of exposure. Fishes were considered lifeless when gill opercula and body movement ceased. The number of dead fish was noted at every 24 h of experiment. The dead fish were eliminated quickly from the aquaria to avoid any organic decomposition causing variation of dissolved oxygen. The 10% of the test water was changed by stock water at every 24 h of exposure time and desired quantity of test chemical was added quickly to test water to maintain a fixed concentration. To evaluate the effects of Cypermethrin on respiratory rate of fish, the mean opercular movements (number of movement/min/fish) were noted at every 24 h during the experiment. The behavioural changes like excess mucous secretion, movement, wide opening of mouth and gills were also recorded [17].

For statistical analysis, a statistical software, Probit program version 1.5 [18] were used to calculate the LC_{50} values (95% confidence limit) using mean mortality of *Cyprinus carpio* after 24, 48, 72, and 96 h. The data of percentage of mortality and opercular movement were subjected to analysis of variance with the use of R-software [19] followed by Duncan's Multiple Range Test (DMRT) to find the significant variation among the mean values at different concentrations of Cypermethrin at different exposure times (24, 48, 72, and 96 h).

RESULTS AND DISCUSSION

The LC_{50} values (with 95% confidence interval) of Cypermethrin to *Cyprinus carpio* fry are shown in Table 1.

Table 1: *LC_{50} Values (With 95% Confidence Interval) of Cypermethrin to the Cyprinus Carpio Fry at Different Times of Exposure (24, 48, 72, and 96 h).*

Test organism	Concentration (ng/l)			
	24 h	48 h	72 h	96 h
Cyprinus carpio	1.04 [0.97, 1.11]	0.92 [0.84, 0.99]	0.89 [0.79, 0.97]	0.79 [0.68, 0.86]

The 24, 48, 72, and 96 h LC_{50} values of Cypermethrin to *Cyprinus carpio* are 1.04, 0.92, 0.89, 0.79 ng/l, respectively. The mortality rate of *C. carpio* showed significant variation ($p < .05$) with the increasing concentrations at all exposure times (Table 2).

On the other hand, the mortality rate of *C. carpio* also varied significantly ($p < .05$) at all the doses with increasing exposure times (24, 48 and 72, and 96h). The excess mucous secretion with increasing concentration of toxicant and exposure time was recorded in the exposed fish. The treated fish showed faster movement at lower doses but it was gradually reduced at higher doses at all exposure times. Wide opening of mouth and gills was observed at higher concentrations (Table 3).

Table 2: *Mean Values (±SD) of % Mortality of Cyprinus Carpio Exposed to Different Concentrations of Cypermethrin at Different Times of Exposure (24, 48, 72, and 96 h).*

Dose (ng/l)	Percentage of mortality of fish at different times of exposure (h)			
	24 h	48 h	72 h	96 h
0.6	00^{am} ± 0.00	00^{am} ± 0.00	10^{an} ± 0.83	20^{ao} ± 0.50
0.7	00^{am} ± 0.43	20^{bn} ± 0.43	30^{bo} ± 0.50	40^{bp} ± 0.71
0.8	20^{bm} ± 0.71	30^{cn} ± 0.87	40^{co} ± 1.12	60^{cp} ± 1.09
0.9	20^{bm} ± 0.43	50^{dn} ± 0.50	50^{dn} ± 0.50	60^{co} ± 0.43
1.0	40^{cm} ± 0.50	60^{en} ± 0.43	60^{en} ± 0.43	70^{do} ± 0.43
1.1	60^{dm} ± 0.87	70^{fn} ± 0.71	70^{fn} ± 0.43	80^{eo} ± 0.71
1.2	60^{dm} ± 0.83	80^{gn} ± 0.83	70^{fo} ± 0.71	90^{fp} ± 0.83
1.3	80^{em} ± 0.83	90^{hn} ± 0.87	90^{gn} ± 0.83	100^{go} ± 0.43
1.4	90^{fm} ± 0.83	100^{in} ± 0.00	100^{hn} ± 0.00	100^{gn} ± 0.00
1.5	100^{gm} ± 0.50	100^{im} ± 0.00	100^{hm} ± 0.00	100^{gm} ± 0.00

Note. 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).

Table 3: *Behavioural Responses of Cyprinus Carpio.*

Dose (ng/l)	Behavioural responses of fish at different times of exposure											
	24 h			48 h			72 h			96 h		
	M	MS	WOMG	M	MS	WOMG	M	MS	WOMG	M	MS	WOMG
0.0	+++	–	–	+++	–	–	+++	–	–	+++	–	–
0.6	+++	–	–	+++	–	–	+++	–	–	++	–	+
0.7	+++	–	–	++	–	–	++	+	–	++	+	+
0.8	++	–	–	++	+	–	++	+	+	+	+	+
0.9	++	+	–	++	+	++	+	+	++	+	++	++
1.0	++	+	+	+	+	++	+	+	++	+	++	++
1.1	++	+	++	+	+	++	+	++	++	+	++	++
1.2	+	+	++	+	+	++	+	++	++	+	++	++
1.3	+	+	++	+	++	++	+	++	+++	–	++	++
1.4	+	+	++	+	++	+++	-	++	+++	X	X	X
1.5	+	+	+++	X	X	X	X	X	X	X	X	X

Note. M = movement; MS = mucous secretion; WOMG = wide opening of mouth and gills; –: absent, +: mild, ++: moderate, +++: high, X = not recorded due to death (exposed to different concentrations of Cypermethrin at different times of exposure).

Table 4: *Mean Values (±SD) of Opercular Movement/Minute of Cyprinus Carpio Exposed to Different Concentrations of Cypermethrin at Different Times of Exposure (24, 48, 72, and 96 h).*

Dose ng/l	**Opercular movement/minute of fish at different times of exposure**			
	24 h	**48 h**	**72 h**	**96 h**
0.0	$85^{eo} \pm 0.50$	$85^{fo} \pm 0.43$	$82^{en} \pm 0.43$	$72^{gm} \pm 0.00$
0.6	$90^{fn} \pm 0.00$	$120^{ho} \pm 0.50$	$90^{fn} \pm 0.43$	$78^{hm} \pm 0.43$
0.7	$95^{gm} \pm 0.43$	$120^{hn} \pm 0.50$	$130^{ho} \pm 0.50$	$95^{im} \pm 0.50$
0.8	$100^{hn} \pm 0.50$	$110^{go} \pm 0.43$	$115^{gp} \pm 0.50$	$60^{fm} \pm 0.43$
0.9	$84^{eo} \pm 0.50$	$82^{en} \pm 0.50$	$90^{fp} \pm 0.50$	$55^{em} \pm 0.50$
1.0	$80^{do} \pm 0.43$	$77^{dn} \pm 0.43$	$80^{fo} \pm 0.43$	$54^{dm} \pm 0.43$
1.1	$65^{co} \pm 0.50$	$64^{cn} \pm 0.50$	$70^{cp} \pm 0.00$	$50^{cm} \pm 0.43$
1.2	$63^{bo} \pm 0.50$	$60^{bn} \pm 0.43$	$65^{bp} \pm 0.50$	$47^{bm} \pm 0.50$
1.3	$60^{ap} \pm 0.43$	$57^{ao} \pm 0.43$	$55^{an} \pm 0.50$	$45^{am} \pm 0.43$

Note. 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).

Opercular movement of fish initially increases with the increasing concentration but decreases at higher concentrations (Table 4).

Similarly, the opercular movement increases significantly ($p < .05$) with increasing exposure time upto 72 h but it decreases significantly ($p < .05$) at 96 h of exposure at all the treatments.

In the current study, the 24 and 96 h LC_{50} value (1.04 and 0.79 ng/l) corresponds to the LC_{50} of Cypermethrin to freshwater prawn *Palaemonetes argentinus* as 0.0031 and 0.0020μg/l for 24 and 96 h, respectively [12]. The 96h LC_{50} value of the present study (0.79 ng/l) is much lower than the 96h LC_{50} value 1.2 μg/L for brown trout (*Salmo trutta*), 0.5 μg/L for rainbow trout (*Salmo gairdneri*), 0.4 μg/L for *Scardinius erythropthalmus* and, 2.2 μg/L for *Tilapia nilotica* [7], 0.063 mg/l for *Clarias gariepinus* (Burchell 1822) juvenile [2], 1.78 ppm in Bluegill Sunfish [9]. The 24, 48, 72, and 96h LC_{50} value of the present study (1.04, 0.92, 0.89, 0.79 ng/l, respectively) are also much lower than 24, 48, 72, and 96h LC_{50} values of *Poecilia reticulata* (Peters) at 25˚C (219.83, 202.72, 186.22, and 168.07 ppb, respectively) [10]. It indicates that the fry stage of *Cyprinus carpio* was comparatively more sensitive to Cypermethrin than brown trout (*Salmo trutta*), rainbow trout (*Salmo gairdneri*), *Clarias gariepinus* (Burchell 1822) juvenile, bluegill sunfish, and *Poecilia reticulata* (Peters).

The abnormal behaviors like excess mucous secretion, erratic movement, wide opening of mouth and gills observed in the current study were probably due to the enzymatic as well as ionic alteration in blood and tissues [20]. The excess mucous secretion of fish was probably for the dysfunction of endocrine gland especially pituitary gland over the integument for xenobiotic stress [21]. The abnormal behaviors of the treated fish may be due to adjust and adapt a compensatory mechanism to obtain energy to escape from stress due to toxicity of insecticide [22].

The results indicate that such low amount of Cypermethrin (0.7 ng/l) in aquatic ecosystem may have a significant effect on fry of *Cyprinus carpio* populations. Moreover, according to Kumaragura and Beamish [23], acute toxicity of synthetic pyrethroids to fish population was negatively correlated to temperature. Thus, the presence of pyrethroids in the aquatic ecosystem when water temperature decreased may increase the toxical influence of Cypermethrin on fish [24].

CONCLUSION

The results of the present experiment may provide supplement to the current knowledge on toxicity of Cypermethrin. In conclusion, Cypermethrin contamination is seriously harmful to aquatic ecosystems and this information should be taken into thought when this insecticide is applied in agriculture.

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