Global Veterinaria 9 (2): 215-219, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.2.63235

Evaluation of the Acute Toxicity of Cypermethrin and its Effect on Behavioral Responses of Caspian Roach (*Rutilus rutilus caspicus*) and Silver Carp (*Hypophthalmicthys molitrix*)

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Abstract: The acute toxicity of cypermethrin to Caspian roach (*Rutilus rutilus* caspicus) and silver carp (*Hypophthalmicthys molitrix*) was assessed in a static non-renewal bioassay method for 96 h. Groups of seven silver carp and Caspian roach in three replicates were exposed to the technical grade of cypermethrin (0, 0.01, 0.5, 2, 4, 8 and 16 µg L^{-1}). Data obtained from the cypermethrin acute toxicity tests were evaluated using the probit analysis statistical method. The 24, 48, 72 and 96 h LC_{50} values of cypermethrin for Caspian roach were estimated at 2.314, 1.023, 0.732 and 0.627 µg L^{-1}, respectively. The LC_{50} for 24, 48, 72 and 96 h for the silver carp were 2.962, 1.653, 1.030 and 0.917 µg L^{-1}, respectively. At various concentrations of cypermethrin (0.5-16 µg L^{-1}), fish showed abnormal behavioral responses (rapid gill movement, nervous manifestations, erratic swimming, loss of equilibrium and inability to remain upright). The abnormal swimming behavior increased with increasing concentration of Cypermethrin and exposure time. Results of the present study indicate that Cypermethrin is highly toxic to the studied fish.

Key words: Acute Toxicity · Cypermethrin · LC_{50} · Behavioral Changes

INTRODUCTION

With exploding population and increasing industrialization and urbanization, water pollution by agricultural run-off, municipal and industrial sources has become a great major concern for the welfare of humanity [1]. Pesticides are used in agriculture to control weeds (herbicides), pests including insects (insecticides) and plant diseases (fungicides). Cypermethrin (a-cyano-3-phenoxybenzyl ester of 2, 2-dimethyl-3-(2, 2-dichlorovinyl)-2-2-dimethyl cyclopropane carboxylate) is a synthetic pyrethroid insecticide extensively used for pest control programs in domestic, industrial and agricultural situations [2]. The low toxicity of pyrethroid to mammals and birds and their limited soil persistence has encouraged the widespread and increasing use of pyrethroids in agriculture, as potent agents against pests [3]. Application of these synthetic derivatives of pyrethrins is highly toxic to a number of non-target organisms such as bees, freshwater fish and other aquatic organisms even at very low concentration [4]. Among the aquatic animals fish are extremely sensitive to the pyrethroids pesticides due to their neurotoxic effects. Pyrethroids are 1000 times more toxic to fish than other groups of mammals and birds at comparable concentrations [5].

Bradbury and Coats [5] have reviewed and reported the toxic effects of pyrethroids in non-target organisms (mammals, birds, fish, amphibian and invertebrates) and cited 96-h LC_{50} of cypermethrin as 0.9-1.1 µg L^{-1} for common carp, *Cyprinus carpio*, 1.2 µg L^{-1} for brown trout, *Salmo trutta*, 0.5 µg L^{-1} for rainbow trout, *Salmo gairdneri*, 0.4 µg L^{-1} for common rudd, *Scardinius erythropthalmus* and 2.2 µg L^{-1} for Tilapia, *Tilapia nilotica*. Due to their lipophilicity, pyrethroids easily permeate through the gill even when present at very low concentrations in the water thereby rendering fish as most sensitive to the pesticides [6]. Moreover, the

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hypersensitivity of fish to pyrethroid toxicity is due to the low ability of fish to hydrolyze these compounds [7].

Silver carp, *Hypophthalmicthys molitrix*, is one of prime cultured freshwater fish in polyculture and of great economic importance. Caspian roach, *Rutilus rutilus caspicus,* is one of the valuable and important species of Caspian Sea. Hence, the present study was performed to determine acute toxicity of cypermethrin, a synthetic pyrethroid pesticide and evaluated behavioral changes of Caspian roach and silver carp exposed to different concentrations.

MATERIALS AND METHODS

Experimental Animals: Live specimens of the silver carp (40.68±5.87 g, 15.34±3.12 cm) and Caspian roach (3.5±0.32 g, 7.3±0.65 cm) were purchased from Bony Fish Propagation and Rearing Center of Sijeval (Bandar Torkaman, Gorgan, Iran). Fish were transferred to Aquaculture Research Center of Gorgan University of Agricultural Sciences and Natural Resources and acclimated to the laboratory conditions for two weeks. Fishes were parceled in 12 fiberglass tanks (500 L). Fish were fed commercial trout food (Biomare, 0.8 mm in diameter, 45% crude protein and 24% crude lipid) during acclimation period under continuous aeration condition and were fasted for 24 h before starting the experiments.

Water: Water (dechlorinated tap water) was renewed daily and the water quality parameters were measured twice a week. Water pH, temperature and dissolved oxygen were determined by Wagtech portable pH/temp meter and oxygen meter (Berkshire, UK). Water total hardness was determined using portable photometer with commercial kits provided by the manufacturer (Wagtecch Portable Photometer 7100, Berkshire, UK). Moreover, water quality parameters were monitored daily during experiments period with no substantial changes recorded throughout acclimation and experiments period (Table 1).

Toxicant: Technical grade Alpha-cypermethrin (95% pure) manufactured by Daga global chemicals, India and supplied by Gol Sam Co, Iran, was used for evaluation of its toxicity to fish.

Acute Toxicity Experiment: The stock solution of Cypermethrin was prepared by dissolving in acetone 0.95% (at a ratio of 1:10) and appropriate amount of tap water. Control group received acetone at the maximum acetone volume used in the dilution of the dosing concentrations. The acute toxicity test was conducted following the Organization for Economic Cooperation and Development guideline under static non-renewable test conditions (OECD 2002) [8]. Uniform sized rectangular aquaria (120 L capacity) were used for acute toxicity test. In each aquarium total volume of water was maintained at 100 L and was provided with non-stop aeration. Seven fish (for each species) were exposed to 0,.01, 0.5, 2, 4, 8 and 16 μg L^{-1} Cypermethrin in three replicates. Strong aeration was applied to the aquarium for 10 min to obtain a homogeneous concentration of the Cypermethrin in the experimental aquaria. Fish mortality was recorded at an interval of 24 h over a period of four days. Dead fish were immediately removed by dip net to avoid possible deterioration of the water quality. The behavior of the exposed fish, including their activities, loss of equilibrium, abnormal swimming of the fish was closely followed and recorded. For statistical analyses, the statistical software EPA Probit Analysis V. 1.5 was used. Data obtained from the Cypermethrin acute toxicity tests were evaluated using Finney's Probit analysis statistical method. All data were accepted if calculated Chi square for heterogeneity was lower than the tabular value at the 0.05 significant level [2].

RESULTS AND DISCUSSION

The physicochemical characteristics of the test water are given in Table 1. Water quality parameters throughout the acclimation and experiments period were similar and relatively stable.

No mortality was observed in the control group during the experiment. The mortality of studied fishes at different concentrations of Cypermethrin after exposure for 24, 48, 72 and 96 h were recorded as in Tables 2 and 3. Fish mortality increased significantly with increasing the toxicant concentration and time of exposure.

Results of the present study indicate that cypermethrin is highly toxic to Caspian roach and silver carp. Fish mortality was observed after 15 min exposure to 16 μg L^{-1} cypermethrin. 100% mortality of the Caspian roach and silver carp was observed after exposure to 16 μg L^{-1} Cypermethrin for 60 min. and 150 min. respectively. LC_{50} is the most widely accepted basis for acute toxicity test and it is the concentration of a test chemical which kills 50% of the test organisms after a particular length of exposure, usually 96 h [9]. The LC_{50} for 24, 48, 72 and 96 h for the silver carp were 2.962, 1.653, 1.030 and 0.917 2.2 μg L^{-1}, respectively (Table 4). The 24, 48, 72 and 96 h LC_{50} values of cypermethrin for Caspian roach were estimated

Table 1: Water quality parameters during the experimental period

	Temperature (°C)	Total hardness (mgL^{-1} as C_aCO_3)	pH	DO (mgL^{-1})
Acclimation period	19.2 ± 1	294.56 ± 10.85	7.69 ± 0.23	7.82 ± 0.5
Experiments period	19.95 ± 1.5	300 ±12.25	7.88±0.76	8.01±0.35

Table 2: Cumulative mortality of silver carp during acute exposure to Cypermethrin (n=21, in three replicates)

	No. of mortality			
Cypermethrin concentration ($\mu g\ L^{-1}$)	24 h	48 h	72 h	96 h
Control	-	-	-	-
0.01	-	-	-	-
0.5	-	3	7	7
2	8	11	14	15
4	12	16	16	18
8	19	21	ND	ND
16	21	ND	ND	ND

-No mortality. ND, No data because of 100% mortality

Table 3: Cumulative mortality of Caspian roach during acute exposure to Cypermethrin (n=21, in three replicates)

	No. of mortality			
Cypermethrin concentration ($\mu g\ L^{-1}$)	24 h	48 h	72 h	96 h
Control	-	-	-	-
0.01	-	-	-	-
0.5	2	8	11	11
2	9	13	13	13
4	14	15	19	21
8	18	20	21	ND
16	21	ND	ND	ND

-No mortality. ND, No data because of 100% mortality

Table 4: Lethal concentrations ($LC_{1\text{-}99}$) of Cypermethrin depending on time (24-96 h) for silver carp.

	Concentration ($\mu g\ L^{-1}$) (95 % of confidence limits)			
Point	24 h	48 h	72 h	96 h
LC 1.00	0.516 (0.177- 0.888)	0.185(0.051- 0.365)	0.055(0.006- 0.152)	0.213 (0.009- 0.163)
LC 5.00	0.861 (0.382- 1.318)	0.351(0.130- 0.602)	0.129(0.025- 0.290)	0.138(0.029- 0.294)
LC 10.00	1.132(0.573 - 1.635)	0.494(0.214- 0.790)	0.204(0.050- 0.411)	0.210(0.056- 0.405)
LC 15.00	1.360 (0.750 - 1.896)	0.623(0.297- 0.953)	0.278(0.081- 0.523)	0.278(0.086- 0.504)
LC 50.00	2.962 (2.192-3.807)	1.653(1.119- 7.060)	1.030(0.559- 1.546)	0.917(0.507- 1.357)
LC 85.00	6.452 (4.908- 9.975)	4.389(3.174- 24.063)	3.816(2.550- 6.940)	3.023(2.055- 5.296)
LC 90.00	7.756(5.747- 12.948)	5.530(3.894- 9.652)	5.201(3.364- 10.748)	4.009(2.655- 7.874)
LC 95.00	10.189 (7.189- 19.246)	7.787(5.197- 15.561)	8.230(4.936-21.110)	6.090(3.780- 14.555)
LC 99.00	16.996(10.757- 41.163)	14.795(8.713- 39.061)	19.464(9.713- 78.093)	13.343(7.029- 48.057)
Chi - Square (calculated)*	1.756	2.45	3.46	1.47
Intercept ± SE	3.55± 0.34	4.46 ± 0.22	4.97± 0.18	5.07± 0.19
Slope ± SE	3.06± 0.54	2.44 ± 0.40	1.82± 0.33	2.00± 0.37

*Chi - Square for Heterogeneity (tabular value at 0.05 level) = 9.48

Table 5: Lethal concentrations (LC_{1-99}) of Cypermethrin depending on time (24-96 h) for Caspian roach.

Point	Concentration (μg L^{-1}) (95 % of confidence limits)			
	24 h	48 h	72 h	96 h
LC 1.00	0.199(0.050- 0.413)	0.040(0.004- 0.124)	0.032(0.003- 0.104)	0.034(0.003- 0.106)
LC 5.00	0.408(0.143- 0.722)	0.103(0.016- 0.253)	0.081(0.011- 0.205)	0.079 (0.010- 0.198)
LC 10.00	0.599(0.248- 0.978)	0.172(0.036- 0.371)	0.132(0.024- 0.295)	0.125(0.022- 0.278)
LC 15.00	0.776(0.359 - 1.205)	0.241(0.060- 0.483)	0.183(0.041- 0.378)	0.171(0.036- 0.351)
LC 50.00	2.314(1.576-3.173)	1.023(0.524- 1.578)	0.732(0.343- 1.159)	0.627(0.284- 0.995)
LC 85.00	6.902(4.869- 11.858)	4.332(2.818- 8.316)	2.930(1.898- 5.424)	2.304(1.495- 4.189)
LC 90.00	8.939(6.079- 16.944)	6.096(3.814- 13.554)	4.069(2.572- 8.645)	3.135(2.002- 6.511)
LC 95.00	13.111(8.337- 29.127)	10.112(5.797- 28.794)	6.617(3.886-17.907)	4.947(2.964- 13.033)
LC 99.00	26.895(14.752- 82.238)	26.127(12.157- 123.748)	16.476(7.957- 74.313)	11.641(5.810- 50.982)
Chi - Square (calculated)*	1.104	5.40	4.61	8.86
Intercept ± SE	4.20± 0.23	4.98±0.18	5.23± 0.18	5.37± 0.18
Slope ± SE	2.18± 0.36	1.65±0.31	1.71± 0.33	1.83± 0.37

*Chi - Square for Heterogeneity (tabular value at 0.05 level) = 9.48

at 2.314, 1.023, 0.732 and 0.627 2.2 μg L^{-1}, respectively (Table 5). The acute toxicity results of the present work are in agreement with the results of other workers. Review of toxicity of pyrethroids by Bradbury and Coats [5] documents LC_{50} value of most freshwater fish to range from 0.4 to 2.2 μg L^{-1}. Saha and Kaviraj [10] observed Cypermethrin as highly toxic to freshwater catfish, *Heteropneustis fossilis,* (72 h LC_{50} value as 0.67 and 1.27 μg L^{-1} for water solubilized and acetone solubilized Cypermethrin). Yilmaz *et al*. [11] studied the acute toxicity of alpha-Cypermethrin to guppy, *Poecilia reticulate* and reported 96-h LC_{50} value as 9.43 μg L^{-1}. Yilmaz [12] found the 96-h LC_{50} value of alpha-cypermethrin for Tilapia, *Oreochromis niloticus,* as 3.42 μg L^{-1}. Examining cypermethrin toxicity to other aquatic organisms, the work of Clark *et al* [13] reported the Cypermethrin 96-h LC_{50} for grass shrimp, *Palaemonetes pugio*, as 0.016 μg L^{-1}. Wilis and Ling [14] observed EC_{50} values of Cypermethrin to the cyclopoid copepod, *Oithona similis,* to range from 0.14 to 0.24 μg L^{-1} for nauplii and adults, respectively. Comparative pyrethroid studies in the lobster, *Homarus americanas* and shrimp, *Crangon sptemspinosa*, also indicated Cypermethrin to be most toxic (96 h LC_{50} value about 0.01 μg L^{-1}) [5]. Saha and Kaviraj [15] observed Cypermethrin is highly toxic to aquatic insects (96 h LC_{50} 0.06 μg L^{-1}) and 96h LC_{50} values of aqueous Cypermethrin ranged from 0.03 μg L^{-1} for the crustacean to 9.0 μg L^{-1} for the tadpole larva [15]. The24, 48, 72 and 96 h LC_{50} values of Cypermethrin for Great sturgeon juveniles were estimated at 6.860, 4.751, 2.677 50 and 0.952 μg L^{-1}, respectively [2]. Behavioral changes are the most sensitive indicators of potential toxic effects. The results of different concentrations of Cypermethrin on behavior changes of silver carp and Caspian roach are presented in Tables 6 and 7. Fish in the control experiment appeared active and healthy throughout the test period. The changes in behavioral response started 5 min after exposure to 16 and 8 μg L^{-1} Cypermethrin.

The abnormal swimming behavior increased with increasing concentration and exposure time. Rapid gill movements increased initially but decreased later steadily in the lethal as compared to the control group. Finally the fish loss of equilibrium and inability to remain upright and then sank to the bottom of tanks with their least opercular movements and died with their mouths open. Similar behavioral responses determined in this study have been observed with the guppy [11], freshwater catfish [10], young mirror carp [16] and hamilton [17] exposed to various concentrations of the synthetic pyrethroids cypermethrin and deltamethrin. In the toxic environment, fish exhibited erratic and darting swimming movements and loss of equilibrium which is due to inhibition of Acetylcholinesterase (AChE) activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyperstimulation [18].

In conclusion, the results of the present investigation demonstrated that Cypermethrin is highly toxic to the studied fish and had a profound impact on behavior of these fish. A colossal increase in use of Cypermethrin and other pyrethroids has been recorded in last two decades and it is expected that this trend will continue. According to environmental quality standards, the maximum allowable concentration of Cypermethrin in aquatic environment is 1 ng L^{-1} [19]. Therefore, biological methods and development of target specific pesticides could be used for controlling pests and flies instead of Cypermethrin in order to protect the natural environment.

ACKNOWLEDGEMENT

We are grateful to the Gorgan University of Agriculture & Natural Resources for providing technical and financial facilities.

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