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Acute Toxicity of Synthetic Pyretroid Cypermethrin to the Young Rainbow Trout (*Oncorhychus mykiss* Walbaum, 1792)

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The acute toxicity of synthetic pyrethroid cypermethrin contaminating aquatic ecosystems as a potential toxic pollutant was examined in this study. Young rainbow trout (*Oncorhynchus mykiss*), 50-60 g in weight and 10-15 cm in length was used for the bioassay experiments. Cumulative mortality and 24, 48, 72 and 96 h LC_{50} values were determined for young rainbow trout. The behaviour of young rainbow trout at each cypermethrin concentration was also observed. Data obtained from this study were evaluated by use of probit analysis statistical method. The 24, 48, 72 and 96 h LC_{50} values (95 % confidence limit) for young rainbow trout were estimated to be 68.492 (35.704-101.280), 55.941 (27.616-84.266), 47.597 (43.344-51.715) and 41.786 (39.823-43.371) µg/L, respectively.

Key Words: Acute toxicity, Bioassay, Cypermethrin, *Oncorhynchus mykiss*, Behavioural changes.

INTRODUCTION

Cypermethrin is a synthetic pyrethroid insecticide used to control pests in domestic, industrial and agricultural situations[1]. There is no doubt that like all living organisms in water, fish are also affected by alterations in their environmental conditions[2]. The expanding use of synthetic chemical pesticides is causing worldwide contamination risk[3]. In spite of the fact that, pesticides by their nature are toxic and are formed to kill undesired organisms, when applied to the land, they may be washed to waters and kill or at least unfavourably affect the life of aquatic organisms[3-5]. Common use of these pesticides by methods like crop dusting, orchard and forest spraying or mosquito control means that some inevitable enter aquatic ecosystems. It is essential to know the influence of these broad spectrum pesticides on aquatic organisms[2,6]. Synthetic pyretroids are generally found to be highly toxic to fish[7,8] and zooplankton communities[9,10]. Effects of cypermethrin on biochemical[11], haematological[2,11] and enzyme system[12] of fish are reported. United States Environmental Protection Agency classified cypermethrin as in toxicity class II (moderately toxic) chemicals. According to environmental qualify standards the maximum allowable concentration[13] is 1 ng L^{-1}.

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In the area where this study was carried out rainbow trout produced at 3500-4000 tons/year capacity from 30 fish farm and cypermethrin is also commonly used for the control of insects in agriculture close to fish farms and lakes. Therefore, present study was aimed to investigate the acute toxicity of cypermethrin, especially toxic to young rainbow trout.

EXPERIMENTAL

In the present study, a static acute toxicity bioassay was carried out in 3 series according to standard methods[14] to detect the 24, 48, 72 and 96 h LC_{50} of cypermethrin for young rainbow trout. Experimental fish from 50-60 g and 10-15 cm in length were obtained form Fish Breeding Unit of Sürgü High School Malatya, Sürgü, Turkey. They were transferred to the laboratory at Sürgü High School and acclimatized for 4 d under standard laboratory conditions. Fish were fed with flour pellets during adaptation. However, they were not fed during the last 24 h of adaptation and throughout the duration of the trial. Before the beginning of the trail, all aquariums (200 L capacity) were cleaned and filled with 200 L of dechlorinated top water (temperature 11.2 ± 1 ºC; pH 7.2 ± 0.2). Four different concentrations (40, 45, 50, 55 μL^{-1}) of cypermethrin, with 3 replicate were used in this trail. Control units with 3 replicate were also prepared. Aeration was applied to the aquariums and then 15 fish were transferred in each aquarium. Mortality was assessed at 24, 48, 72 and 96 h following the trail. Dead fish were removed immediately. Behavioural changes of fish were closely followed and recorded. Toxicity was assigned based on LC_{50}, according to the guidelines given by Kamrin[15]. The results were expressed as mean ± standard deviation (SD) or range. All replicates were used for calculation of mean values. Statistical analysis was performed with the SPSS statistical software package (Version 10.1, SPSS Inc., Chicgo, Illinois, USA).The LC_{50} and 95 % confidence limits were calculated by the probit method. Duncan's multiple range tests was employed for comparing the significance level for time using a significance level of 0.05.

RESULTS AND DISCUSSION

The behavioural responses of the fish were observed at 1-10 h during the first day of exposure and then every 6 h during the last 3 days of trail. Normal behaviour was observed for the fish of control group. First changes in behaviour were observed 10 min after exposure to the highest cypermethrin concentrations (50 and 55 µg/L). Some abnormalities, such as less activity and loss of equilibrium were observed on fish exposed to 40 µg/L when compared with the control group fish. Loss of equilibrium and hanging vertically in the water were observed at all other concentrations of cypermethrin applied to the experimental fish. For the last two highest concentrations (50 and 55 µg/L), these relevant response symptoms at highest status could be observed beginning 10 min after start of the test. The other abnormal behavioural responses observed at all concentrations above 40 µg/L were rapid gill movement, erratic swimming, swimming at the water surface, air gulping from the surface or staying motionless on the tank bottom. Colour darkening was observed on fish exposed to the two highest concentrations (50 and 55 µg/L). Similar behavioural responses have previously observed on different fish species exposed to various concentrations of synthetic pyrethroids cypermethrin[1,2]. However, published experimental studies on cypermethrin toxicity for young rainbow trout (*Oncorhynchus mykiss*) are fairly limited.

The calculated 24, 48, 72 and 96 h LC_{50} values (95 % confidence limits) of cypermethrin, using a bioassay system to young Rainbow trout were found as 68.492 (35.704-101.280), 55.941 (27.616-84.266), 47.597 (43.344-51.715) and 41.786 (39.823-43.371) µg/L, respectively. No mortality was observed in the control group during the experiment (Table-1). There were significant differences between LC_{50} values calculated for 24, 48, 72 and 96 h (Duncan's multiple range test, $p < 0.05$). All fish exposed to 50 and 55 µg/L of cypermethrin died at the end of 96 h exposure (Table-1). The percentage of living animals in 45 µg/L concentrations was only 13.33 at the end of the trial.

TABLE-1
CUMULATIVE MORTALITY AND LC_{50} VALUES WITH 95 % CONFIDENCE LIMITS (IN PARENTHESES) OF CYPERMETHRIN FOR YOUNG RAINBOW TROUT (n =15 IN 3 REPLICATES)

Concentration (µg/L)	Exposure time (h)			
	24	48	72	96
55	2	6	10	15
50	2	5	11	15
45	1	5	7	13
40	-	-	2	4
Control	-	-	-	-
LC_{50} values (μL^{-1}) with 95 % confidence limits	68.492^{a} (35.704-01.280)	55.941^{b} (27.616-84.266)	47.597^{c} (43.344-51.715)	41.786^{d} (39.823-43.371)

Values in rows with different superscripts are significantly different ($p < 0.05$).

Factors affecting toxicity are species, size, age and sex of test animals, condition of the animal, water temperature, water quality, duration of exposure and pesticide formulation. Therefore, several LC_{50} values may exist for the same pesticide for even same species of fish.

When compared with previous studies[7,16] (Table-2), present findings for LC_{50} values for *Oncorhynchus mykiss* show differences. Most pesticides used in agriculture are known to be toxic to the non-target aquatic fauna. Cypermethrin belongs to this group. The toxic affects of cypermethrin to various fish species are summarized in Table-2. For example; Smith and Stratton[17] found LC_{50} values of cypermethrin 96 h 2 µg/L for *Salmo salar*, 96 h 6 µg/L for *Salmo gairdneri*, 24 h 9 µg/L for *Gambusia affinis*, 24 h 8 µg/L and 96 h 9 µg/L for *Cyprinoden macularius*. Bradbury and

TABLE-2
TOXICITY STUDIES FOR CYPERMETHRIN ON VARIOUS FISH SPECIES

Scientific name	Life stage	Exposure time (h)	LC_{50} (μg/L)	Chemical description	Experiment type	Acute toxicity rating*	References
Anguilla japonica	NR	24	7.5	NR	NR	VHT	19
	NR	48	5.3	NR	NR	VHT	19
Cyprinoden macularius	NR	24	10	NR	NR	VHT	17
		48	6.0	NR	NR	VHT	17
Cyprinus carpio	NR	96	0.9-1.1	NR	NR	VHT	18
	10 g	48	60	NR	Static	VHT	21
	15 g	48	62	5% EC Cypermethrin	NR	VHT	20
	NR	96	2.1	NR	Static	VHT	21
	NR	24	4.5	NR	NR	VHT	22
Gambusia affinis	NR	48	2.5	5% Cypermethrin	NR	VHT	20
	Susceptible species	48	5.5	NR	NR	VHT	25
	Resistant species	48	7.5	NR	NR	VHT	25
	NR	24	9.0	NR	NR	VHT	17
Labeo rohita	Fry (2.4 cm)	96	0.23	NR	Renewal	VHT	17
	10 g	96	5.24	NR	Static	VHT	26
Oncorhynchus mykiss	3 g, 6 cm fingerling	24	11.0	40 % EC Cypermethrin	Static	VHT	27
	1.1-2.5 g and 45-60 mm, Juvenile	12	20.52	95 % Cypermethrin	Flow through	VHT	7, 16
Poecilia reticulate	2.5-3.0 cm	24	92	25 % EC Cypermethrin	Static	VHT	10
	NR	48	21.4	99 % Beta-Cypermethrin	Static	VHT	24
	NR	96	9.43	98 % Alpha-cypermethrin	Static	VHT	1
Salmo gairdneri	NR	96	6	NR	NR	VHT	17
	NR	96	0.5	NR	NR	VHT	18
Salmo salar	NR	96	2	NR	NR	VHT	17
Salmo trutta	NR	96	1.2	NR	NR	VHT	18
Tilapia Mossambica	1.15 g, 4.26 cm	24	18.9	54 % Cypermethrin	NR	VHT	23
Tilapia nilotica	NR	96	2.2	NR	NR	VHT	18

*Narrative descriptions of toxicity were assigned based on LC_{50} according to the guidelines of Kamrin, (1997). VHT, Very Highly Toxic (LC_{50} < μg/L) NR, Not Reported; EC, Effective Concentrations.

Coats[18] calculated 96 h cypermethrin toxicity (LC_{50}) to *Cyprinus carpio* as 96 h 0.9-1.1 µg/L; *Salmo trutta* as 1.2 µg/L; *S. gairdneri* as 0.5 µg/L; *Tilapia nilotica* as 2.2 µg/L. For *Anguilla japonica* LC_{50} values of cypermethrin were found[19] to be 7.5 and 5.3 for 24 and 48 h, respectively. Although, Sun[20] reported toxicity of 5 % EC cypermethrin (LC_{50}) to 15 g *Cyprinus carpio* as 48 h 62 µg/L, Reddy and Bashamohideen[21] found 48 h 60 µg/L for 10 g of same species. Reddy and Bashamohideen[21] and Grayson *et al.*[22] also studied LC_{50} values of cypermethrin for *C. carpio* and found 96 h 2.1 µg/L and 24 h 4.5 µg/L, respectively. Reddy and Bashamohideen[22] reported 24 h LC_{50} value of cypermethrin for *Tilapia mossambica* as 0.2 µg/L but Ruparelia *et al.*[23] found 24 h 18.9 µg/L for same species. For *Poecilia reticulata* LC_{50} values of cypermethrin estimated as 24 h 92 µg/L (for 2.5-3.0 cm[10]; 48 h 21.4 µg/L[24] and 96 h 9.43 µg/L[1]. For *Gambusia affinis* LC_{50} values were found to be 48 h 2.5 µg/L[21]; 48 h 5.5 µg/L (for susceptible stage)[25] and 48 h 7.5 µg/L (for resistant stage)[25]; 48 h 8 µg/L and 96 h 9 µg/L[17]. Toxicity of cypermethrin (LC_{50}) to fry (2.4 cm) and 10 g *Labeo rohita* were recorded 96 h 0.23 µg/L[26] and 96 h 5.24 µg/L[27], respectively.

The results of present work showed that cypermethrin is indeed highly toxic to young Rainbow trout. The toxicity of cypermethrin on fish increased with increasing concentration and exposure time.

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