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Toxic Effect of Alphamethrin 10 EC on Freshwater Fish, *Poecilia reticulata* (Peters, 1859)

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Abstract: An attempt has been made to study the short-term toxicity of Alphamethrin 10 EC to juveniles, males, females and mixed population of *Poecilia reticulata* (Peters, 1859) using water of hardness 560±5 mg/l and pH 7.4±0.3. The LC_{50} values and the 95% confidence limits of Alphamethrin 10 EC for different concentrations and time intervals (24, 48, 72 and 96 hrs) to juveniles, males, females and mixed population of *Poecilia* were estimated by Probit analysis statistical method. The LC_{50} values for Alphamethrin 10 EC were increasing considerably from juveniles, males, mixed population and females of *Poecilia reticulata.* Results indicate that Alphamethrin is highly toxic to all the specimen of *Poecilia reticulata* as their LC_{50} values were noticed in ppb. Presumable safe and dischargeable concentrations of Alphamethrin were ranged between 1.799 to 10.241 and 1.051 to 1.076 ppb respectively.

Key words: Toxicity · Alphamethrin 10 EC · LC_{50} Values · *Poecilia reticulata*

INTRODUCTION

Alphamethrin (Alpha-Cypermethrin) is a synthetic pyrethroid insecticide widely used in agricultural crop production and public health program. It is used for the control of ectoparasites such as fleas, lice, ticks and blowflies in veterinary medicine [1]. It is nontoxic to birds but highly toxic to fish and aquatic invertebrates [2]. Since, it is metabolized and eliminated more slowly by the fish than mammals [3]. Non-target organisms like aquatic invertebrates and fish are extremely sensitive to the neurotoxic effects of these insecticides [4, 5].Therefore, an attempt has been made to investigate the short-term toxicity of Alphamethrin 10 EC to juveniles, males, females and mixed population of *Poecilia reticulata* (Peters, 1859) for evaluation of LC_{50} values, presumable safe and safe dischargeable concentrations.

MATERIALS AND METHODS

The experimental fish, *Poecilia reticulata* were collected from local sources. The juveniles, males and females of *Poecilia* were acclimatized separately in plastic tank of 250 litres capacity for ten days and were fed rice bran and oil cake (1:1). During the course of bioassay tests, the fishes were not provided any food to avoid excretory waste products and change in metabolic rate, which may influence the toxicity of the test solution. Healthy juveniles, males and females of equal sizes with different length, 1.0±0.2, 2.8±0.2 and 3.9±0.3 cm respectively were selected for the bioassay tests. For the preparation of common stock solution for Alphamethrin, following formula was used: $N_1V_1=N_2V_2$, Where, N_1= Concentration of available pesticide, V_1=Volume of available pesticide, N_2= Required concentration of pesticide to be prepared, V_2=Volume of solution required for application. The series of different concentrations (ppb) of selected pesticides were prepared by adding the common stock solution into the measured diluents water with the help of micropipette. The series of different concentrations of selected pesticides used in the full-scale static bioassay tests were based on the progressive bisection of intervals on a logarithmic scale [6]. The experimental routine static bioassay for the evaluation of short-term toxicity (96 hrs) for Alphamethrin to the juveniles, males, females and mixed population of *Poecilia* were conducted in 1 litre glass jar containing experimental water of hardness 560±5 mg/l and pH 7.4±0.3.

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The preliminary or screening tests with different concentrations of toxicant were made by maintaining higher concentration of toxicant in the beginning and later lower concentrations were tested to discover the critical concentration range for each test fish. The test range for each pesticide for the full-scale bioassay was taken between the highest and lowest concentrations at which most, if not all, of the test fishes died or survived within a specified period of exposure (24, 48, 72 and 96 hrs).

After preliminary exploratory tests, elaborate experiments were conducted to evaluate the toxicity of Alphamethrin 10 EC. The toxicities of Alphamethrin 10 EC were measured by testing various concentrations in the range known by preliminary exploratory test. The test containers of 1 litre glass jars filled with one litre toxicant solution were placed in three rows and each container was labelled with the details of the experiment such as concentration, replicate number, date and time of the experiment. The acclimatized juveniles, males and females of *Poecilia* were transferred to these jars after about 20 minutes of the preparation of test solutions. The bioassays for juveniles, males, females and mixed population of *Poecilia* were conducted for selected pesticides. Ten acclimatized tests specimens of fish were placed in each experimental glass jars. Proper controls were run simultaneously. The test solutions were renewed after each 24 hrs by fresh toxicant solutions. The experiments were continued for a period of 96 hrs. The number of test fishes died in each concentration of toxicant solution were observed carefully and recorded at the time intervals of 24, 48, 72 and 96 hrs. The dead fishes were removed from the test solution after knowing the exact mortality, which was observed by their body movements. The LC_{50}s and the 95% confidence limits were estimated statistically at different concentrations and time intervals (24, 48, 72 and 96 hrs) for selected pesticides by Probit Analysis [7]. Presumable safe and dischargeable concentrations of Alphamethrin for juveniles, males, females and mixed population of *Poecilia* were calculated by using the formula of Hart *et al.* [8]. Behavioural changes if any in the exposed juveniles, males and females of *Poecilia* were also observed carefully after introduction in to the various concentrations of Alphamethrin.

RESULTS

The LC_{50} values for the time intervals of 24, 48, 72 and 96 hrs for Alphamethrin 10 EC to the juveniles, males, females and mixed population of *Poecilia* have been summarized in Table 1 and compared in figure 1. The 24, 48, 72 and 96 hrs LC_{50}s for juveniles, males and females for Alphamethrin 10 EC were 7.344, 6.635, 6.200 and 5.531 ppb; 12.167, 10.490, 9.153 and 8.495 ppb; and 42.857, 38.235, 34.615 and 32.439 ppb respectively. However, the 24, 48, 72 and 96 hrs LC_{50}s for Alphamethrin 10 EC to the mixed population of *Poecilia* were estimated as 20.539, 18.152, 16.905 and 15.202 ppb respectively. The LC_{50} values for Alphamethrin 10 EC were increasing considerably from juveniles, males, mixed population and females of *Poecilia.* The order of sensitivity for the test fishes to Alphamethrin 10 EC was recorded as females < mixed population < males < juveniles.

The safe concentrations for juveniles, males, females and mixed population of *Poecilia* for Alphamethrin 10 EC were recorded as 1.799, 2.715, 10.241 and 4.814 ppb. Whereas, the safe dischargeable concentrations of Alphamethrin 10 EC were 1.051, 1.076, 1.058 and 1.063 ppb for the juveniles, males, females and mixed population of *Poecilia* respectively (Table 2). The safe dischargeable concentrations have been reported too low as compared to safe or harmless concentrations.

Table 1: Median lethal concentrations (LC_{50}'s) of Alphamethrin 10 EC (in ppb) for 24, 48, 72 and 96 hrs to juveniles, males, females and mixed population of *Poecilia reticulata*

	LC_{50}'s of Alphamethrin 10 EC (ppb)			
Duration (hrs)	Juveniles	Males	Females	Mixed population
24	7.344	12.167	42.857	20.539
48	6.635	10.490	38.235	18.152
72	6.200	9.153	34.615	16.905
96	5.531	8.495	32.439	15.202

Table 2: Safe and safe dischargeable concentrations of Alphamethrin 10 EC for juveniles, males, females and mixed population of *Poecilia reticulata*

Concentrations (as ppb)	Juveniles	Males	Females	Mixed population
Safe or harmless	1.799	2.715	10.233	4.814
Safe dischargeable	1.051	1.076	1.058	1.063

Table 3: 95 % confidence limits for 24, 48, 72 and 96 hrs LC_{50}s of Alphamethrin 10 EC for the juveniles, males, females and mixed population of *Poecilia reticulata*

	Juveniles			Males			Females			Mixed population		
duration (hrs)	LCL	UCL	R	LCL	UCL	R	LCL	UCL	R	LCL	UCL	R
24	6.477	9.397	1.450	10.624	18.895	1.778	38.253	55.030	1.438	18.083	28.031	1.550
48	5.916	7.480	1.264	8.866	13.253	1.494	34.151	43.540	1.274	15.760	21.255	1.348
72	5.501	6.852	1.245	6.348	10.758	1.694	30.372	38.230	1.258	14.352	19.053	1.327
96	4.448	6.153	1.383	5.998	9.681	1.614	27.878	35.574	1.276	12.311	16.861	1.369

UCL = Upper Confidence Limits; LCL = Lower Confidence limits; R = Confidence Ratio (UCL/LCL)

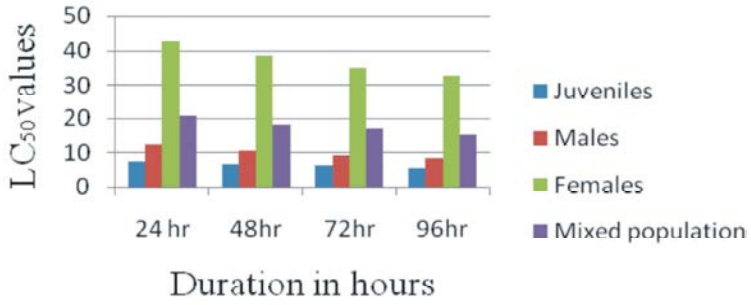

Fig. 1: Median lethal concentrations (LC_{50}s in ppb) of Alphamethrin 10 EC for 24, 48, 72 and 96 hrs to for juveniles, males, females and mixed population of *Poecilia reticulata*.

After introduction in toxicant solution, fish showed excitement with increasing opercular movement and try to jump out of container, jerky and abrupt swimming, lack of buoyancy and loss of equilibrium. Matured females sometimes hatch at high concentration. At the time of death, fish settled down on bottom with exposed belly upside. Deposition of whitish slimy substances on the bottom was seen after death. The reddish color gill might be due to hemorrhage in the gills.

The upper and lower confidence limits for median lethal concentrations (LC_{50}s) of the Alphamethrin 10 EC for juveniles, males, females and mixed population of *Poecilia* were 6.153 and 4.448; 9.681 and 5.998; 35.574 and 27.878; and 16.861 and 12.311 respectively for 96 hours. However, the significant ratios of confidence limit, i.e. upper and lower confidence limits for 96 hours LC_{50}s of Alphamethrin 10 EC for juveniles, males, females and mixed population of *Poecilia* were recorded as: 1.383, 1.614, 1.276 and 1.369 respectively (Table 3).

DISCUSSION

Results of the present investigation indicate that Alpha-Cypermethrin 10 EC is also highly toxic for the *Poecilia reticulata.* The 96 hrs LC_{50} values of Alphamethrin 10 EC for juveniles, males, females and mixed population of *Poecilia* were recorded as 5.531, 8.495, 32.439 and 15.202 ppb respectively. Grayson *et al.* [9] have suggested that Alpha-Cypermethrin is highly toxic to a number of aquatic arthropod taxa, but is lesser toxic to molluscs. According to these authors, short-term toxicity of this compound can be reduced by formulation of the product as an oil-enhanced suspension. Further, Grayson *et al.* [9] have also suggested that Alpha-Cypermethrin is highly toxic to fish and 96 hr LC_{50}s ranged in between 0.7 and 350 µg/l (URL 1).

Sarikaya [10] has reported 96 hr LC_{50} value of Alphamethrin for Nile tilapia as 5.99 µg/l and also noticed the behavioral changes for the individual fish. Acute Cypermethrin toxicity in rainbow trout such as gill failing, hyperactivity, loss of buoyancy and inability to remain upright were reported [11]. Bradbury and Coats [12] have studied the toxicity of pyrethroids in mammals, birds, fish, amphibian and invertebrates (Terrestrial and aquatic) and cited 96 hr LC_{50} for Cypermethrin toxicity as 2.2 µg/l for *Tilapia nilotica*, 0.9-1.1 µg/l for carp (*Cyprinus carpio*), 1.2 µg/l for brown trout (*Salmo trutta*), 0.5 µg/l for rainbow trout (*Salmo gairdneri*) and 0.4 µg/l for *Scardinius erythropthalmus*. Whereas, Polat *et al.* [13] have also investigated the acute toxicity of beta-Cypermethrin using guppy fish (*Poecilia reticulata*) and also estimated 48 hrs LC_{50} as 21.4 µg/l at a temperature of 22±1ºC and reported that beta-Cypermethrin is highly toxic to fish. Baºer *et al*. 14] studied the acute toxic effects of Permethrin on guppies and reported 48 hr LC_{50} value as 245.7 µg/l.

Lethal concentrations of Cypermethrin (Dissolved either in water or in acetone) for the freshwater catfish, *Heteropneustes fossilis* were studied at different hrs of exposure by static bioassays [15]. There was no difference between LC_{50} values of aqueous and acetone solubilized Cypermethrin up to 48 hrs. Whereas, 72 hr LC_{50} values of aqueous Cypermethrin and acetone-solubilized Cypermethrin to *Heteropneustes fossilis* were recorded as 0.67 and 1.27 µg/l, respectively. However, lethal values for Cypermethrin were remaining unchanged beyond 72 hrs. According to these authors [15], the fish exposed to even lower concentration of Cypermethrin (0.5 µg/l) showed symptoms of hyperactivity. The uptake and effects of environmentally relevant concentrations of the pyrethroid insecticide Cypermethrin on two different amphibian species, *Bombina variegata* and *Rana arvalis* were studied [16]. After 24 hrs of exposure, 153.9 ng Cypermethrin/g fresh weight were found in embryos, indicating that the jelly mass of the eggs does not act as a sufficient physical barrier to protect embryos from exposure to this compound. Uptake of Cypermethrin in tadpoles of both species caused dose-dependent deformities and behavioral abnormalities such as twisting, whirling and co-ordinate swimming and mortality. The observed physical and behavioral abnormalities due to environmentally relevant concentrations of Cypermethrin indicate that despite detoxification of the chemical via GST-system contamination of ponds by Cypermethrin causes adverse effects on the development of amphibian embryos and tadpoles [16]. Wang *et al.* [17] determined the 96 hrs LC_{50} of Cypermethrin for carp as 12.6 µg/l. Further, these authors [17] also noticed that the growth of carps restrained at a dose of 1.14 µg/l and hyperplasia in gill epithelia and twist of branchial lobule at doses of 0. 58 and 1.14 µg/l of Cypermethrin.

Tripathi and Bandooni [18] have suggested that both activity and specific activity of catalase declined (32-60 %) in different tissues (Liver, heart, kidney and intestine) of *C. batrachus* exposed to a sublethal concentration of Alphamethrin (0.018 ppm) for 14 days. Decrease in protein content in response to Alphamethrin may be due to the extensive proteolysis as evidenced by increased protease activity [19].

Ansari and Ansari [20] investigated the toxic effects of Alphamethrin to adult Zebra fish, their embryos and fingerlings and recorded 24 hr LC_{50} value as 1.29 µg/l and 96 hr LC_{50} value as: 0.17 µg/l for adults. The 72 hr LC_{50} for the embryo was 0.024 µg/l and for fingerlings, the 96 hr LC_{50} value of Alphamethrin was 0.020 µg/l. Ansari and Ansari [20] also aimed to investigate the changes in the activities of enzymes acid phosphatase (ACPase) and alkaline phosphatase (ALPase) in the gill and liver of Zebra fish after exposure to 20, 40, 60 and 80 % of the 24 hr LC_{50} values of Alphamethrin. They also found that the activities of ACPase and ALPase in treated fishes were significantly reduced ($p<0.05$) in response to treatment of pesticide as compared to control. The LC_{50} values and behavioral responses observed in the present study are also in agreement with the findings of Grayson *et al.* [9], Sarikaya [10], Polat *et al.* [13], Saha and Kaviraj [15], Greulich and Pflugmacher [16], Ansari and Ansari [20] and Marigouder *et al.* [21]. In the present experiment, the range of safe dischargeable concentrations of Alphamethrin 10 EC for juveniles, males, females and mixed population of *Poecilia* were noticed as: 1.051, 1.076, 1.058 and 1.063 ppb respectively (Table 2). However, the harmless or safe concentrations of Alphamethrin 10 EC for juveniles, males, females and mixed population of *Poecilia* were calculated as 1.799, 2.715, 10.241 and 4.814 ppb respectively. More or less similar pattern of safe concentrations have been reported by Srivastava *et al.* [22], Rahmi *et al.* [23], Ural and Saglam [24], Koprucu *et al.* [25] and Gautam and Gupta [26]. Result indicates that Alphamethrin are highly toxic to juveniles, males, females and mixed population of *Poecilia reticulata* (Peters).Therefore, it is suggested to the user, selecting Alphamethrin 10 EC from aquaculture point of view, both safe or harmless and safe dischargeable concentrations should be considered for better fishery management.

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