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Acute Toxicity of Chlorpyriphos 50% + Cypermethrin 5% EC to the Guppy, *Poecilia reticulata* (Peters, 1859)

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Abstract: The present study was aimed to investigate the acute toxicity of Chlorpyriphos 50% +Cypermethrin 5% EC to the juveniles, males, females and mixed population of a freshwater fish, *Poecilia reticulata* (Peters). The experimental water of hardness 560±5 mg/l and pH 7.4±0.3 were used in bioassay tests. The LC_{50} values and the 95 percent confidence limits were calculated statistically at different concentrations and time intervals (24, 48, 72 and 96 hrs) to juveniles, males, females and mixed population of *Poecilia reticulata* by Probit analysis method. Behavioural changes in the studied fish were also observed carefully after subjected to the various concentrations of this pesticide. The order of sensitivity of selected pesticide to different specimens of *Poecilia reticulata* was recorded as: females < mixed population <males< juveniles. The presumable safe or harmless concentration and safe dischargeable concentrations of Chlorpyriphos 50% + Cypermethrin 5% EC were ranged in between 4.381to 32.216 and 1.044 to1.069 ppb respectively.

Key words: Toxicity · Chlorpyriphos · Cypermethrin · LC_{50} Values · *Poecilia reticulata*

INTRODUCTION

Chlorpyriphos is a broad-spectrum organophosphorus compound, used as a contact and stomach poison for the control of pest such as cockroaches, mosquitoes, flies, bedbugs and ants on a wide variety of crops [1]. It is extremely toxic to aquatic invertebrates, freshwater fish and marine organisms [2]. Chlorpyriphos inhibit the cholinesterase enzyme causes accumulation of acetylcholine in the synaptic cleft which leads to neurotoxicity and eventually death [3, 4]. Cholinesterase inhibition was observed at very low concentrations of this insecticide during acute toxicity tests in fish [5]. Acute toxicity of Chlorpyriphos is due to production its active metabolite Chlorpyriphosoxon [6].

Cypermethrin is a synthetic, broad-spectrum pyrethroid based insecticide, extensively used in households, industrial and agriculture fields [7] for controlling many insect pests [8]. Cypermethrin enters into aquatic system through agriculture run-off water and affects the non-target organisms like fishes and thus alter the metabolism [9], haematology [10] and population of fish [11]. Therefore, an attempt has been made to screen the short-term toxicity of Chlorpyriphos 50% + Cypermethrin 5% EC to juveniles, males, females and mixed population of a freshwater fish, *Poecilia reticulata* (Peters, 1859) to estimate the LC_{50} values, the 95 percent confidence limits, presumable safe and safe dischargeable concentrations for better management of aquaculture.

MATERIALS AND METHODS

The experimental fish, *Poecilia reticulata* were collected from local ditches of Udaipur and separated as juveniles, males and females for further rearing and acclimatization. The juveniles, males and females were acclimatized separately in plastic tank of 250 litres capacity for ten days prior starting the experiment and were fed rice bran and oil cake (1:1). During the bioassay tests, the fishes were not provided any food supplement to avoid excretory waste products and change in metabolic rate, which may influence the toxicity of the test solution. Healthy, juveniles (1.0±0.2cm), males (2.8±0.2cm) and females (3.9±0.3 cm) were selected for the bioassay

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tests. For the preparation of common stock solution for Chlorpyriphos 50% + Cypermethrin 5% EC, following formula was used: $N_1V_1 = N_2V_2$, Where, N_1 = Concentration of selected pesticide, V_1 = Volume of selected pesticide, N_2 = Required concentration of pesticide to be prepared, V_2 = Volume of solution required for application. The series of different concentrations (in 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 logarithmic scales [12]. The experimental routine static bioassay for the evaluation of short-term toxicity (96 hrs) for Chlorpyriphos 50 % + Cypermethrin 5 % EC 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.

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 tested 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 tested fishes died or survived within a specified period of exposure, i.e. 24, 48, 72 and 96 hrs.

After preliminary exploratory tests, elaborate experiments were conducted to evaluate the toxicity of Chlorpyriphos 50% + Cypermethrin 5% EC. The toxicities of Chlorpyriphos 50 % + Cypermethrin 5 % 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 tested 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 percent confidence limits were estimated statistically at different concentrations and time intervals (24, 48, 72 and 96 hrs) for selected pesticides by Probit Analysis [13]. Presumable safe and dischargeable concentrations of Chlorpyriphos 50% + Cypermethrin 5% EC for juveniles, males, females and mixed population of *Poecilia* were calculated by the formula of Hart *et al*. [14]. 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 Chlorpyriphos 50% + Cypermethrin 5% EC.

RESULTS AND DISCUSSION

The obtained results revealed that these pesticides are highly toxic to the tested fishes, as evident from the LC_{50} values recorded in ppb. The 24, 48, 72 and 96 hrs LC_{50}'s for Chlorpyriphos 50% + Cypermethrin 5% EC to juveniles, males and females were observed as: 18.898, 16.510, 15.208 and 13.396 ppb; 24.130, 21.737, 19.874 and 18.845 ppb and 326.392, 298.955, 281.160 and 261.866 ppb respectively. Whereas, the LC_{50} values of Chlorpyriphos 50% + Cypermethrin 5% EC for the mixed population of *Poecilia reticulata* for 24, 48, 72 and 96 hrs were observed as 131.697, 118.879, 110.196 and 106.255 ppb respectively (Table 1 and Fig. 1).

Table 1: Median lethal concentrations (LC_{50}'s) of Chlorpyriphos 50% + Cypermethrin 5% EC (in ppb) for 24, 48, 72 and 96 hrs for juveniles, males, females and mixed population of *Poecilia reticulata*

	LC_{50}'s of Chlorpyriphos 50% + Cypermethrin 5% EC (ppb)			
Duration (hrs)	Juveniles	Males	Females	Mixed population
24	18.898	24.130	326.392	131.697
48	16.510	21.737	298.955	118.879

72	15.208	19.874	281.160	110.196
96	13.396	18.845	261.866	106.255

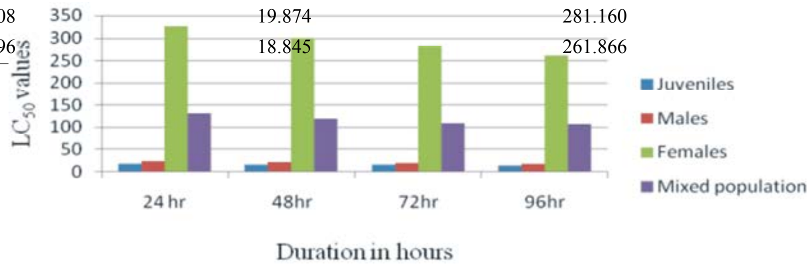

Fig. 1: Median lethal concentrations (LC_{50}'s in ppb) of Chlorpyriphos 50% + Cypermethrin 5% EC for 24, 48, 72 and 96 hrs to juveniles, males, females and mixed population of *Poecilia reticulata*.

Table 2: 95% confidence limits for 24, 48, 72 and 96 hrs LC_{50}'s of Chlorpyriphos 50% + Cypermethrin 5% EC for the juveniles, males, females and mixed population of *Poecilia reticulata*

Duration (hrs)	Juveniles			Males			Females			Mixed population		
	LCL	UCL	R	LCL	UCL	R	LCL	UCL	R	LCL	UCL	R
24	16.702	27.040	1.618	21.114	32.901	1.558	283.702	380.833	1.342	114.872	175.251	1.525
48	14.784	19.721	1.333	18.263	27.488	1.505	253.856	334.228	1.316	100.646	139.878	1.389
72	12.186	18.117	1.486	16.057	23.280	1.449	240.618	308.931	1.283	91.162	124.525	1.365
96	11.463	14.851	1.295	15.607	21.258	1.362	195.955	290.881	1.484	10.319	102.822	9.964

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

Table 3: Safe or harmless and safe dischargeable concentrations of Chlorpyriphos 50% + Cypermethrin 5% EC for juveniles, males, females and mixed population of *Poecilia reticulata*

Concentrations (ppb)	Juveniles	Males	Females	Mixed population
Safe or harmless	4.381	5.874	82.205	32.216
Safe dischargeable	1.069	1.053	1.044	1.052

The upper and lower confidence limits and their ratios for Chlorpyriphos 50% + Cypermethrin 5% EC to juveniles, males females and mixed population for the durations of 24, 48, 72 and 96 hrs have been summarized in Table 2. There is only one insignificant ratio of confidence limits, i.e. 9.964 for mixed population for the duration of 96 hr may indicates variation in sensitivity to Chlorpyriphos 50% + Cypermethrin 5% EC.

The presumable safe or harmless concentrations for Chlorpyriphos 50% + Cypermethrin 5% EC were noticed as: 4.381, 5.874, 82.205 and 32.216 ppb, Whereas, the safe dischargeable concentrations of Chlorpyriphos 50% + Cypermethrin 5% EC were recorded as: 1.069, 1.053, 1.044 and 1.052 ppb for the juveniles, males, females and mixed population respectively (Table 3).

DISCUSSION

In the present investigation, the 96 hrs LC_{50} values for Chlorpyriphos 50% + Cypermethrin 5% EC to juveniles, males, females, mixed population of *Poecilia* were noticed as: 13.396, 18.845, 261.866 and 106.255 ppb respectively (Table 1 and Fig. 1). However, the range of safe dischargeable concentrations (1.044-1.069 ppb) were estimated too low in comparison of safe or harmless concentrations (4.381-82.205) (Table 3). Polat *et al.* [9] have studied the acute toxicity of beta-Cypermethrin to a guppy fish (*Poecilia reticulata*) and estimated 48 hrs LC_{50} as 21.4 µg/l at a temperature of 22±1ºC and found that beta-Cypermethrin is highly toxic to fish. However, the 96 hr LC_{50} value of Cypermethrin was noted as 4.17 mg/l for C*larias gariepinus* and also found that mortality increased with increasing concentrations [15]. The 48 hrs LC_{50}values and 95% confidence limits; and 96 hr LC_{50} values and 95 % confidence limits of Cypermethrin for common carp (*C. carpio*) embryos and larvae were estimated as: 0.909 and 0.256-5.074; and 0.809 and 0.530-1.308µg/l respectively [16]. Whereas, the 96 hrs LC_{50} of Cypermethrin for carp were observed as 12.6 µg/l and found 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 [17].

According to Jaensson *et al.* [18] high concentrations of Cypermethrin changes the reproductive behaviour of brown trout, *Salmo trutta.* However, the range of safe dischargeable concentration and safe or harmless concentrations of Cypermethrin were assessed as: 1.04 to 1.09 ppb and 45.18 to 75.25 ppb respectively for the juveniles of *Poecilia reticulata* at selected levels of environmental condition such as temperature, pH, hardness and salinity [19]. Whereas, Marigouder *et al.* [20] have recorded the 96 hrs LC_{50} for Cypermethrin to *L. rohita* as 4.0 µg/l and also noticed behavioural patterns of *L. rohita* in both lethal and sublethal concentrations of Cypermethrin such as, erratic and darting movements with imbalanced swimming activity, hyper and hypo opercular activity, loss of equilibrium and mucous secretion all over the body. However, changes in behavioural patterns like spontaneous movement, hatching as well as non-lethal malformation such as curved body axis or edema in embryo (or larva) of zebrafish in bifenthrin toxicity were observed by Jin *et al.* [21]. Dangi and Gupta [22] have recorded the 96 hours LC_{50} values of Cypermethrin for the males of *Poecilia reticulata* as 52.43 and 38.38 µg/l; 64.34 and 62.56 µg/l at water hardness of 680±5 and 290±1 mg/l and pH of 9.5 and 5.0 respectively. However, for females these values were noticed as 229.87 and 177.64 µg/l and 132.97 and 116.97 µg/l at water hardness of 680±5 and 290±1 and pH of 9.5 and 5.0 respectively. They also found that presumable safe concentrations of Cypermethrin ranged in between 13.98-77.39 µg/l for males and females of *Poecilia*; and safe dischargeable concentrations ranged in between 1.06-1.14 µg/l at selected level of hardness and pH. The 24, 48, 72 and 96 h LC_{50} values of Cypermethrin for Caspian roach were noted as 2.314, 1.023, 0.732 and 0.627 µg/l respectively. However, these values were recorded for the silver carp as 2.962, 1.653, 1.030 and 0.917 µg/l, respectively [23]. Whereas, Jahanbakhshi *et al.* [24] assessed the acute toxicity of Cypermethrin to Great sturgeon (*Huso huso*) juveniles and estimated the 24, 48, 72 and 96 h LC_{50} values as 6.860, 4.751, 2.677 and 0.952µg/l, respectively.

Chlorpyriphos is very highly toxic to freshwater fish, aquatic invertebrates and other estuarine and marine organisms [25]. De Silva and Samayawardhena [26] observed the effects of Chlorpyriphos on reproductive performances in Guppy (*Poecilia reticulata*) and also found that low soluble concentrations of Chlorpyriphos affect mating behaviour, number of offspring and offspring survival of guppy. Gul [27] has assessed the acute toxicity of Chlorpyriphos-methyl on larvae of Nile tilapia, *Oreochromis niloticus* and also noted the behavioural changes at each Chlorpyriphos-methyl concentration of the individual fish. The 96 hr LC_{50} value for Nile tilapia larvae was calculated as 1.57 mg/L whereas, the 96 hr LC_{50} value and 95% confidence limit of Chlorpyriphos-methyl for Guppy (*Poecilia reticulata*) were estimated as: 1.79 mg/l and 1.47-2.10 respectively [28] by using static bioassay method. Selvi *et al*. [28] also observed the behavioural changes in fish exposed to Chlorpyriphos-methyl in the form of neurotoxin toxicity like less general activity than control group, loss of equilibrium, erratic swimming and staying motionless at a certain location generally at mid-water level for prolonged periods. Tomlin [29] has noticed the 96-hr LC_{50} as: 0.007-0.051 mg/l for rainbow trout (*Oncorhynchus mykiss*), 0.002-0.010 mg/l for bluegill sunfish (*Lepomis macrochirus*) and 0.12-0.54 mg/l for fathead minnows (*Pimephales promelas*) and the 48-hr LC_{50} for *Daphnia* as 1.7 µg/l. Ramesh and Saravanan [30] have also studied the acute toxicity of Chlorpyriphos considering haematological and biochemical parameters for the fish, *Cyprinus carpio* under static condition and at the end of 21 day and also determined their LC_{50}'s as 5.28 ppm. The LC_{50} values for Cypermethrin to *L. irrorata* for 24, 48, 72 and 96 hrs were reported as 0.00092, 0.00068, 0.00035 and 0.00034 ppm respectively, whereas, LC_{50} values for Chlorpyriphos for same species were noticed as: 0.01251, 0.00549 and 0.00510 ppm for 24, 48 and 72 hrs respectively [31]. Vidyarani *et al*. [31] also noticed behavioural pattern of *L. irrorata* such as slow swimming, lying the body by the side at the bottom of the aquarium, sluggish and imbalance movement prior to death of the fish, reddish colour of the gills, which might be due to hemorrhage in the gills. Sharbidre *et al*. [32] have performed acute toxicity tests for methyl parathion (MP) and Chlorpyriphos (CPF) on guppies (*Poecilia reticulata*) and recorded the 96 hr LC_{50} values of MP and CPF for *Poecilia* as 8.48 ppm/l (5.98-10.89) and 0.176 ppm/l (0.313-0.224) respectively.

The LC_{50} values and behavioural changes in *Poecilia* exposed to Chlorpyriphos + Cypermethrin were recorded in the present study such as unbalanced swimming activity, reddish colour of the gills might be due to haemorrhage, hatching at high concentration, non-directional, whirling movements with open operculum at lower concentration in juveniles, whereas, jerky movements at higher concentration in both males and females, are also in agreement with the findings of previous investigators. However, the safe concentrations have been recorded in present study are supported the view of Rahmi *et al*. [16], Gautam and Gupta [19], Ural and Saglam [33] and Koprucu *et al*. [34]. Results of the present study indicate that Chlorpyriphos 50% + Cypermethrin

5% EC are highly toxic to freshwater fishes *Poecilia reticulata.* Therefore, users selecting both Chlorpyriphos and Cypermethrin from aquaculture point of view, they should take consideration of both safe or harmless and safe dischargeable concentrations for better management of aquaculture.

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