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ACUTE TOXICITY OF SYNTHETIC PYRETHROID CYPERMETHRIN TO AN INDIGENOUS MAJOR CARP, *Cirrhinus mrigala* (Ham.)

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ABSTRACT

Cypermethrin (trade name- super-killer) is a synthetic pyrethroid insecticide widely used in agriculture to control insect pests. Present study was a static boassay conducted to assess acute toxicity of cypermethrin for fingerlings of *Cirrhinus mrigala.* Fingerlings were exposed to cypermethrin for 96 h, mortality and behavioral alterations were recorded. Mortality data obtained were analyzed by LC_{50} software of US EPA, based on Finney's probit method to determine LC_{50} values for 24 h, 48 h, 72 h and 96 h which were found as 1.18, 1.10, 0.98 and 0.85 µg/l respectively. Behavioral alterations observed included erratic swimming, loss of balance, increased mucus secretion and change in body color. The comparative toxicity data presented in the table indicates that cypermethrin is highly toxic to fish and therefore, judicious use of cypermethrin is warranted.

KEYWORDS: Pyrethroid, Cypermethrin, Acute Toxicity, *Cirrhinus mrigala*, Behavioral Alterations.

Aquatic ecosystems are continuously being polluted with a variety of pollutants including synthetic pesticides. Among synthetic pesticides, pyrethroids are widely used as insecticides in houses, buildings, agriculture, orchards, veterinary and hygiene programmes, due to their less persistence but high efficacy, and low toxicity to mammals and aves. Wide spread use of pyrethroids results in exposure and toxicity to non-target organisms including fish fauna in aquatic ecosystems (Neelima et al. 2016; Singh et al. 2008).

The term pyrethroid is commonly used to designate synthetic insecticides that are structurally derived from natural pyrethrins (pyrethrin I & II, cinerin I & II, jasmolin I & II) present in the extract of pyrethrum, *Chrysanthemum cinerariaefolium*. Natural pyrethrins exhibit little photostability and are, therefore, of limited use in agriculture, whereas synthetic pyrethroids demonstrate greater photostability, in addition to high insecticidal activity and low mammalian toxicity (Soderlund et al. 2002).They work as nerve poisons by modifying the kinetics of voltage-sensitive sodium channels of insect nerves by increasing the sodium permeability of the neurilemma (Bloomquist, 1993). Formulation of pyrethroid insecticides frequently contains the insecticide synergist piperonyl butoxide [5-{2-(2-butoxyethoxy) ethoxymethyl}-6-propyl-1,3-benzodiaxole], which acts to increase the efficacy of the insecticide by inhibiting the cytochrome P450 enzymes responsible for the breakdown of the insecticide (Cope et al. 2004). Cypermethrin, (CAS no. 52315-07-8) is a very effective pyrethroid insecticide which kills the insects that eat or come into contact with it.

Fish show extreme sensitivity to pyrethroid neurotoxicants, and succumb to them at 10 -1000 times lower concentrations than corresponding values for mammals and birds (Bradbury and Coats, 1989). Therefore, pyrethroid pesticides pose serious threat to fish population especially to young fish which tend to be less tolerant to pesticides (Kumaraguru and Beamesh 1981). Due to high toxicity of Cypermethrin, [(R,S)-α-cyano- (3-phenoxyphenyl) methyl (1RS)-cis-trans-3-(2, 2dichloro-ethenyl) -2, 2-dimethyl cyclopropane carboxylate)], to non-target organisms like fish and honeybees, it is categorized as restricted use pesticide by US EPA. The bioconcentration factor for cypermethrin ranges from 444x to 446x (Saha and Kaviraj 2008). High toxicity to fish occurs due to lipophilic nature of cypermethrin which enhances absorption through gills, and deficiency of enzymes which hydrolyze pyrethroids (Polat. et al. 2002).

Among edible fishes, major carps are the most valued and popular. *Cirrhinus mrigala* is omnivorous indigenous major carp widely cultured along with *Labeo* and *Catla* in composite fish culture. Therefore, the present study was undertaken to assess acute toxicity of cypermethrin on *Cirrhinus mrigala*

MATERIALS AND METHODS

Fingerlings of *Cirrhinus mrigala* (Teleostei; Cypriniformes; cyprini) were collected from local government fish hatchery. They were brought to laboratory carefully in plastic bags to avoid any injury

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and disinfected by giving a bath for two minutes in 0.05% $KMnO_4$ solution. Thereafter, they were transferred to plastic pools of 500 L capacity filled with tap water, for one week acclimatization to laboratory conditions. During acclimatization fish were fed daily with rice bran mixed with mustard oil cake in the ratio of 2:1. Leftover food in the pool was removed daily when water of the pool was changed. Dead fishes, whenever located, were removed immediately to avoid fouling of the tank water.

After one week acclimatization, the fingerlings were starved for 24 hours prior to the toxicant exposure (Cypermethrin 25% EC, Northern Minerals Limited, Gurgaon). Stock solution of 1µg/ml cypermethrin was prepared in acetone. Variable quantities of stock solution were added to 10 L of water in different glass troughs to prepare desired toxicant concentrations (0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, and 3.0 µg/l) for determining range of acute toxicity. The pesticide was mixed thoroughly by stirring with a glass rod before adding fishes into glass troughs. Fishes of similar size (6.0 – 8.4 cm) and weight (9.0 – 12.5 gm) were sorted out and separated in to eight groups of four each, which were transferred in to the troughs.

After determining the range, fish were exposed to final concentration of 0.5, 0.7, 0.9, 1.10, 1.30 and 1.50 µg/l of cypermethrin to determine LC_{50} values for 24 h, 48 h, 72 h and 96 h. Four replicates were taken for each concentration. A control was run simultaneously containing 2 ml. of acetone in 10 L water. During the course of 96 h study no food was administered to fishes. Mortality in each group was recorded and dead fishes were immediately removed. Fish mortality data obtained with respect to time was analyzed for determination of LC_{50} values by EPA Probit Analysis, Version 1.5, statistical software based on Finney's Probit Analysis method. LC_{50} values, confidence limits, intercept and slope functions of mortality data are given in the table 1. Plot of adjusted probits and predicted regression line for 96 h exposure is given in figure 1. Behavioral responses of fishes were noted during first six hours and thereafter, at 24 h, 48 h, 72 h and 96 h of exposure.

Experiment was conducted under natural photoperiod and temperature in the month of September. Physicochemical characteristics of water were as follows: temperature 28 ± 2 ^{o} C, pH 7.4 ± 0.5, Dissolved oxygen 7.2 ± 0.5 mg/l and total hardness as $CaCO_3$, 115.24 ± 1.3 mg/l.

RESULTS AND DISCUSSION

At concentrations less than 0.60 µg/l, no mortality was found at any duration of exposure. However, at concentration 1.60 µg/l and above, 100% mortality was recorded at all duration of acute toxicity test. From mortality data, the LC_{50} values for 24 h, 48 h, 72 h and 96 h were calculated as 1.18, 1.10, 0.98 and 0.85 µg/l, respectively (Table 1).

The fish of control group and those exposed to 0.5 µg/l exhibited normal behavior pattern. Behavioral alterations such as erratic movements, hyperactivity and increased mucus secretion appeared within fifteen minutes of exposure at all lethal concentrations. At higher concentrations (above 1.40 µg/l), the fish appear stunned by the toxicant and aggregate near bottom of the trough within minutes of exposure. But, soon they become hyperactive, swim in excitement all around and frequently visit the surface. Gradually swimming becomes very fast and fishes dart from one point to another frequently colliding with the wall of the trough and try to jump out. With passage of time, however, swimming is slowed down and becomes jerky, fishes start losing balance, make somersaults and swim upside down. Gradually, they show signs of exhaustion, spend much time on bottom lying on flanks. Finally they lose strength for movement and succumb to poison with open mouth and body coated with mucus. Dead fish showed symptoms of heavy internal hemorrhage as redness appears at skin around pharyngeal opening. Body color of exposed fish becomes pale and dull.

Table 1: LC_{50} Values, 95% Confidence limits, Intercept and Slope functions for different duration of cypermethrin exposure on *Cirrhinus mrigala*

Duration (hours)	LC_{50} (µg/l)	95% lower confidence limits	95% upper confidence limits	Intercept	Slope
24	1.18	1.09	1.27	4.65±0.18	9.87±1.70
48	1.10	0.99	1.19	5.27±0.15	6.63±1.17
72	0.98	0.87	1.09	5.78±0.17	7.21±1.34
96	0.85	0.78	0.96	5.88±0.18	7.26±1.38

Figure 1: Plot of adjusted Probits and predicted regression line for 96 h Cypermethrin exposure to *Cirrhinus mrigala*

LC_{50} values of commonly used pyrethroid insecticides for fish are generally lower than 10 µg/l (Bradbury and Coats, 1989). In the present study LC_{50} values for cypermethrin for fingerlings of *Cirrhinus mrigala* were calculated as 1.18, 1.10, 0.98 and 0.85 µg/l for 24 h, 48 h, 72 h and 96 h respectively. Manjulasri veni and Veeraiah (2014) reported LC_{50} values for cypermethrin for fingerlings of *Cirrhinus mrigala as* 2.69, 2.61, 2.41 and 2.28 ppb respectively for 24, 48, 72 and 96 hours. Tandon *et al*. (2005) have observed the 96 h LC_{50} of cypermethrin for *Catla catla* as 4.0 µg/l and it was lowest when compared to other pyrethroids (cypermethrin > deltamethrin > fenvalerate). While Marigoudar *et al*. (2009) reported LC_{50} value of cypermethrin for another indigenous carp *Labeo rohita* as 4.0 µg/l. Thus in all indigenous major carps LC_{50} values reported by different workers are in agreement with Bradbury and Coats (1989).

Among exotic major carps many workers have reported LC_{50} value of cypermethrin in the common carp, *Cyprinus carpio*. Bradbury and Coats (1989) have reported 0.90 µg/l (at 10 ^{o}C) and 1.10 µg/l (at 20-25 ^{o}C) of cypermethrin as 96 h LC_{50} value for *Cyprinus carpio,* whereas, Saha and Kaviraj (2008) found 1.70 µg/l as 96 h LC_{50} value of cypermethrin for the same fish. However, much higher LC_{50} values of cypermethrin for 96 h in common carp were reported as 6.0 µg/l (Smith and Stratton 1986) and 50-70 µg/l (Malla-Reddy *et al*. 1995). At other extreme, very low 96 h LC_{50} value of cypermethrin is reported for common carp which is 0.5 µg/l (extoxnet 1996). Velisek *et al* (2006) determined the LC_{50} value of rainbow trout for alimethrine 10 as

31.4 µg/l (equal to 3.14 µg/l of cypermethrin formulation). Thus LC_{50} values may vary significantly for the same species and for same toxicant. These variations are in agreement with findings of Sprague (1969), who reported variations in LC_{50} values for the same species depending upon age, sex, weight, health of experimental group and also on physicochemical parameters of the test water. Kumaraguru and Beamesh (1981) also reported variation in lethal toxicity of permethrin in relation to body weight and water temperature. Temperature dependent variation in fish mortality after dimethoate exposure has been observed by Pandey *et al.* (2008). Variability in LC_{50} values may also be accounted by chemical composition of cypermethrin. Since most products of cypermethrin are mixtures of cis and trans isomers, their ratio decides the toxicity of formulations. Cis-cypermethrin has been found more toxic than its trans form, for mammals; but for trout, cis and trans isomers have been found equitoxic (Bradbury and Coats, 1989).

Cypermethrin exposure induces behavioral alterations in the fingerlings of *Cirrhinus mrigala*. Symptoms of hyperactivity, restlessness, erratic swimming and loss of balance are common to pyrethroid toxicity and have been reported after cypermethrin exposure in rainbow trout (Edwards *et al* 1986), and in *Labeo rohita* (Marigoudar *et al*. 2009). Bradbury *et al.* (1985) made similar observations in fathead minnows after fenvalerate exposure. Loss of equilibrium, and erratic swimming were also observed in fingerlings of European catfish *Silurus glanis* (Koprucu *et al*. 2006) and in *Cyprinus carpio* (Calta and Ural 2004) after deltamethrin exposure. The symptoms are supposed to appear due to deficiency in neural coordination, as pyrethroids are known to damage nervous system. Gradual weakening of movement and exhaustion are probably caused by impairment of nervous system and damage of gills which hampers oxygen uptake. Damage of gills has been reported after exposure to permethrin (Kumaraguru *et al.* 1982) and deltamethrin (Cengiz 2006). Increased mucus secretion is probably the result of stress caused by the neurotoxicants and to combat irritating effect of the poison. Increased mucus secretion has also been observed by Rao *et al.* (2005) and Pandey *et al.* (2008) after exposure to organophosphate pesticides. Body color change may be due to damage of chromatophores caused by impairment of pituitary functions (Ram *et al.* 2001, Pandey *et al* 2008).

The mortality data indicates that cypermethrin is highly toxic to this indigenous major carp which is an important food fish and therefore, judicious use of cypermethrin is warranted. More work with toxicity testing methods on juvenile fish will be useful in assessment of the possible ecological risk of these pesticides on non-target economically important organisms.

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