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Acute and sublethal effects of cypermethrin on fresh water fish *Cyprinus carpio*

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Abstract

The discharge of improvident and inconsiderable level of organophosphorous pesticide, especially cypermethrin into aquatic bodies have caused ecological problems to all classes of aquatic organisms including fish. The study was made in assessing the toxic effects of cypermithrin on behavioral and mortality parameters of mirror carp, *Cyprinus carpio*.The observed percentage mortality of fresh water fish *Cyprinus carpio* when exposed to the 10% emulsifiable concentration (E.C.) cypermethrin for time periods 24, 48, 72 and 96 h in static system toxicity is in the range of 0.26ppm, 0.23 ppm, 0.16 ppm, 0.14 ppm respectively. In the present study, the experimental fish exhibited different toxicity level for various time exposures. The mortality of the fish at different concentration of cypermethrin was determined at 24, 48, 72 and 96 h exposure. With the increase period of exposure (96 h), the fish showed mortality at less concentration and with decrease of duration of exposure the fish exhibited mortality at higher concentration. Several behavioral changes were observed when exposed to sublethal concentration of pesticides such as loss of schooling behavior, swimming near the water surface, hyper activity, erratic movements, seizures, loss of buoyancy, elevated cough, restlessness before death, darting movements and hitting against the walls of test tanks were noticed in all the species tested. A film of mucus was also observed all over the body and also on the gills. The study showed that use of cypermethrin is threat for the mirror carp and hence should be legitimated to use in agriculture.

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Introduction

Cypermethrin is an insecticide in the synthetic pyrethroid family. All of the insecticides in this family have chemical structures that are loosely based on pyrethrins, insecticidal compounds found in chrysanthemum flowers. Most synthetic pyrethroids are complex molecules; cypermethrin is no exception. Cypermethrin is a mixture of all eight isomers. The trade name of the pesticide is Relothrin 10 EC. The IUPAC name of cypermethrin is: (+/-) alpha-cyano-(3-phenoxyphenyl) methyl (+)-cis, Trans-3-(2, 2-dichloroethenyl)-2,2-dimethyl cyclopropane carboxylate(WHO, 1989). Cypermethrin is metabolized and eliminated significantly more by fish than by mammals or birds, which may explain this compound's higher toxicity in fish compared to other organisms (Stephenson, 1983). The work of Nicholson (1961) and several such other workers have clearly established that the pesticide residues are transported to the aquatic environment either through surface runoff or through precipitation into which they get in by evaporation from cropland.

The potential impact of the pollutants is more on the aquatic organisms than on the terrestrial organisms, (Murty, 1986) because pesticide and other substances are transported to a greater distance and affect the non-target organisms. The purpose of a toxicity test is to determine how toxic an agent is to the test species. The toxicity test is used to denote all types of test that are conducted to measure some adverse effect caused by pesticides. The domestic wastes and untreated or partially treated industrial effluents, supplemented with pollutants like heavy metals, pesticides and many organic compounds, have greatly contributed to massive fish death of aquatic ecosystems. These toxic chemicals and metals have changed the quality of water that affects the fish and other aquatic organisms. Fish are the most often tested aquatic organisms because they are the most conspicuous as predominant and are economically important to man because they are linked to the food chain. This has resulted in its discharge into the aquatic environment and consequently several laboratory studies have been performed, which evidenced that cypermethrin is extremely toxic to fish at very low concentrations and to aquatic invertebrates (Sarkar *et al.,* 2005).

Cypermethrin is one of the newly synthesized insecticides. Different types of toxicity tests serve different purposes. The 96 h toxicity test or the short term or acute toxicity test is one of the most commonly employed tests in the evaluation of toxicity. It is also recognized that its toxicity is reduced under field conditions in water bodies with abundant particulate material (Haider and Inbaraj, 1986).

Considering the toxic effects of Cypermethrin on fish and other aquatic organisms discussed above, it is essential to know the extent of damage in aquatic environment caused by pesticides and to find out the concentrations below which aquatic lives live in safe environment. Fish exposed to pesticides present in the ecosystem which receives it from agriculture runoff and industrial wastes, exhibited a variety of reproductive problems such as reducing number of sperm, abnormal gamete, reducing fecundity low hatching rate and survivability.The aim of this study is to determine the acute and chronic toxicity of Cypermethrin on mirror carp,*Cyprinus carpio* to determine the lethal concentration (LC_{50}) of cypermethrin pesticide on mirror carp andobserve behavioral changes in mirror carp due to Cypermethrin toxicity.

Materials and methods

Collection of species and pesticide

Live specimens of *C. carpio* (average weight for big fish 50.7g ± 0.46 and average weight for small fish 19.98g ± 0.25) were collected from Jashore, Bangladesh and acclimatized in the aquarium water for about 24 hr. Cypermethrin was selected as the test chemicals and collected from local market. The trade name of the pesticide is Relothrin 10 EC.

Experimental design

The experiment was conducted in 16 glass aquaria (size: 40×9×19 cm). The present experiment was designed with four treatments with three replications

which indicate as T_1, T_2, T_3 and T_4 and a control set was maintained, where no pesticides were used. Different concentrations of cypermethrin as 0.1, 0.2, 0.3 and 0.4 mg/l used in T_1, T_2, T_3 and T_4respectively. Since toxicity of the pesticide is dependent on fish density (Holden, 1970), a constant ratio of fish biomass to water volume was maintained by loading one fish per five liter of water as per the guidelines of APHA (1985).

Water quality parameter

Water parameter such as pH, DO and water temperature were measured two times daily, before and after application of pesticides, by pH meter (session 156, N/S made in India), DO meter (session YK22DO, made in Taiwan) and thermometer respectively.

Dose preparation and propagation

Four different doses 0.1, 0.2, 0.3, and 0.4 ppm of cypermethrin added to each group of fish consisting of 32 individuals after 24 hours of adaptation.

Determination of LC_{50}

Experiments were conducted to detect the mortality range from 12.5% to 100%for 24, 48, 72 and 96 in static system. The data on the mortality percent of fish was taken in to consideration to calculate LC_{50} values. The data were recorded from these tests at the end of each specific time period. Finney's (1971) probit analysis as reported in Roberts and Boyce (1972) was followed to calculate the LC_{50} values. The respective probit values for percent mortality were taken for the determination of LC_{50} values at 95% confidence limit. The mortality of the fish at different concentration of Cypermethrin was determined at 24, 48, 72 and 96 h exposure. For this experiment fish were divided in to batches of 8 each and were exposed to different concentrations of cypermethrin ranging from 0.1ppm to 0.4ppm of cypermethrin.

Behavioral changes

Monitoring of experimental fish, *Cyprinus carpio* was performed at regular time intervals to observe and record the behavioral responses at both the control (toxicant free medium) and cypermethrin treated fish as described by Murthy (1987) and Shivakumar and David (2008).

Data analysis

Collected data was analyzed through different software. Statistical and graphical analysis performed by Statistical package for Social Science (SPSS), version 16, 2015 and Microsoft Office Excel, version 10, 2015.

Results

Water quality parameters

Water parameters are one of the critical factor throughout the exposure period of Cypermethrin at two concentrations as well as in control (Table 1).

Temperature was almost uniform throughout the study period irrespective of the treatment. Though pH and dissolved oxygen values among treatments were not significantly different, but tended to decrease with increasing concentrations of the Cypermethrin (P> 0.05).

Table 1. Water quality parameters (Means ± SD) during the study period.

Parameters	Treatments				
	Control	T_1 (0.1ppm)	T_2 (0.2ppm)	T_2 (0.3ppm)	T_2 (0.4ppm)
Temperature (^{0}C)	28.00±0.20	28.00±0.15	28.00±0.21	28.00±0.25	28.00±0.17
pH	7.35±0.15	7.45±0.12	7.2±0.15	7.3±0.18	7.22±0.20
Dissolved oxygen (mg/l)	4.15±8.79	4.03±9.36	3.96±7.95	3.55±7.10	3.02±6.90

The LC_{50} value of Cypermethrin for Mirror Carp

In the present study the percentage of mortality and probit mortality of C*yprinus carpio* at different exposure time is presented in Table 2-5. The calculated LC_{50} values at different exposure time are presented in Table 6. C*yprinus carpio* has shown differential toxicity level to different exposure periods. By increasing exposure period (96 h), the fish

showed mortality at less concentration in table 6 and decreasing exposure period, fish exhibited mortality at higher concentration presented in Table 2-5. No mortality was taken place in control condition. The LC_{50} was calculated by probit analysis. The toxicity of insecticide cypermethrin to *C. carpio* was studied used static bioassay method. The 96 h LC_{50} was found to be 0.1434 ppm.

Table 2. The observed percentage of mortality and probit mortality of the fish C*yprinus carpio* exposed to cypermethrin (10%EC) for 24 hrs.

Hours of exposure	Concentration mg/l	Log 10 Concentration	Observed mortality	Probit values
24	0.1	-1	12.5%	3.8363
24	0.2	-0.69897	37.5%	4.6852
24	0.3	-0.52288	62.5%	5.3262
24	0.4	-0.39794	75%	5.6641

Behavioral changes in Mirror Carp

In the present investigation, when fish were exposed to sub lethal concentration of cypermethrin for 96 hours, several behavioral changes (Table 7) were observed which include loss of schooling behavior, swimming near the water surface, hyper activity, erratic movements, scizures, loss of buoyancy, elevated cough, restlessness before death, darting movements and hitting against the walls of test tanks.

Table 3. The observed percentage of mortality and probit mortality of the fish C*yprinus carpio* exposed to cypermethrin (10%EC) for 48 hrs.

Hours of exposure	Concentration mg/l	Log 10 Concentration	Observed mortality	Probit values
48	0.1	-1	25%	4.3215
48	0.2	-0.69897	50%	5.0321
48	0.3	-0.52288	62.5%	5.3262
48	0.4	-0.39794	75%	5.6641

Discussion

Water quality parameters

Among all the environmental parameters, the effect of temperature on the toxicity of pesticides has been studied a lot and it is very important because it directly influence factors like enzyme activity, metabolic rate, oxygen uptake etc. Fish that are exposed to high temperatures may suffer respiratory stress and nervous activity. According to Chubb (2001) increasing water temperatures also have an indirect effect on the carp fish's health by increasing the toxicity of certain pollutants, such as heavy metals.

pH has an excellent role in influencing various physicochemical properties of pesticides like hydrolysis, volatilization and inbalancing the dissociated and undissociated forms (Weber, 1972). However, in the present investigation the water quality parameters (Temperature, Dissolved oxygen and pH) monitored during the exposure period did not differ within various concentrations of pesticides as well as with the control. As the water quality parameter were not significantly changed, so it did not influenced the experiment.

LC_{50} of Cypermethrin for Cyprinus carpio

The present study assessed the toxicity of a widely used pesticide, cypermethrin with the evaluation of its effects on *Cyprinus carpio*. The 24h, 48h, 72h and 96 h LC_{50}values of cypermethrin for the fish were found as 0.26mg/l, 0.23 mg/l, 0.16 mg/l and 0.14ppm

respectively. Cypermethrin caused 100% mortality of *C. carpio* at 0.40 ppm and 50% mortality (96 hours) at 0.10 ppm. LC_{50} values of different pesticides to the fish for different periods of exposure reveals the occurrence of a wide differences between duration of exposure and types of fishes (Santhakumar and Balaji, 2000; Mathivanan, 2004 and Ramasamy *et al.*, 2007). Sabra and Mehana (2015) described that a Pesticides capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose rate, and persistence in the environment. With the increase of period exposure (96h), the fish showed mortality at less concentration and with decrease of duration of exposure the fish exhibited mortality at higher concentration.

Table 4. The observed percentage of mortality and probit mortality of the fish C*yprinus carpio* exposed to cypermethrin (10%EC) for 72 hrs.

Hours of exposure	Concentration mg/l	Log 10 Concentration	Observed mortality	Probit values
72	0.1	-1	37.5%	4.6852
72	0.2	-0.69897	62.5%	5.3262
72	0.3	-0.52288	75%	5.6641
72	0.4	-0.39794	87.5%	6.1437

The reported LC_{50} values of cypermethrin to the fish were found to be varied for different pyrethroids on different species of fish. Among them, LC_{50}values reported for cis-cypermethrin were 9.0 ppb 8.0 ppb for 24 and 48 h respectively for *Gambusia affinis,* 10.0 and 6.0 ppb for 24 48 h respectively in *Cyprinodon macularies (*Mulla *et al.*, 1978). However, very high LC_{50} value 55.0 ppb was reported by Coats and Jaffery (1979) in case of *Salmogairdneri* for 96 h. Aguigwo (2002) was recorded much higher 96h LC_{50}value of cypermethrin as 4.17 mg/l for *Clarias gariepinus* and also found that mortality increased with increase in concentration and also estimated the 48h and 96h LC_{50} value with 95% of confidence limits of cypermethrin for *Common carp* embryo and larvae as 0.909 and 8.09 µg/l respectively.Wang *et al.* (2006) determined the LC_{50} of cypermethrin at 96h for mirror carp as 12.6 µg/l. However, Collins and Gappello (2006) was recorded the LC_{50}'s of cypermethrin to freshwater prawn *Palaemonetes argentines* as 0.003 and 0.0020 µg/l for 24h and 96h respectively.

Table 5. The observed percentage of mortality and probit mortality of the fish C*yprinus carpio* exposed to cypermethrin (10%EC) for 96 hrs.

Hours of exposure	Concentration mg/l	Log 10 Concentration	Observed mortality	Probit values
96	0.1	-1	50%	5.0321
96	0.2	-0.69897	75%	5.6641
96	0.3	-0.52288	87.5%	6.1437
96	0.4	-0.39794	100%	8.0914

The present investigation is in agreement with the above findings that *Cyprinus carpio* showed highly susceptibility to cypermethrin for 96h exposure period and even very low concentration at all selected environmental variables.Samajdar and Mandal (2015) reported that the organophosphate pesticide, chlorpyrifos, is highly toxic to the Indian minor carp, *Labeo bata* as revealed from the acute toxicity test and estimated 24h, 48h, 72h and 96h LC_{50} valueas 257.03µg L-1, 208.92µg L-1, 177.82µg L-1 and 109.64µg L-1 respectively.Veni and Veeraiah (2014) observed that percentage mortality of species of fresh

water fish *Cirrhinus mrigala* when exposed to the 10% emulsifiable concentration (E.C.) of cypermethrin for time periods 24, 48, 72 and 96 h in static system toxicity is in the range of 2.69 ppb, 2.61 ppb, 2.41 ppb, and 2.28 ppb respectively.

Behavioral changes

The changes in body color in the present study have also been reported in severel studies like*Anabas testudineus* after exposure to monocrotophos (Santhakumar and Balaji, 2000), *C. punctatus* to organophosphorus (Sandhu, 1993) and *Cyprinus carpio* to ammonia stress (Israeli-weinstein and Kimmel, 1998).

Table 6. The LC50 values, 95% confidence limits and regression equation of cypermethrin 10% EC of fish *Cyprinus carpio* for 24, 48, 72 and 96 hours in static system.

Duratio (Hour)	Type of test	LC50 Value	Upper Confidence Limit	Lower Confidence Limit	Regression Equation
24	Static System	0.26	2.672	1.126	Y = 0.34 + 0.058X
48		0.23	2.238	0.784	Y = -0.66 + 0.074X
72		0.16	2.222	0.768	Y = -0.140 + 0.074X
96		0.14	2.153	0.682	Y = -0.214 + 0.073X

In the present investigation, when fish were exposed to sub lethal concentration of cypermethrin for 96 hour, several behavioral changes were observed which include loss schooling behavior, migration to the bottom, spread out and swimming independently, irregular, erratic and darting movement, escaping phenomenon, repeated opening and closing of the mouth and operculum, corkscrew pattern swimming behavior, color of the gill lamellae from reddish to light brown, and engulfed the air through mouth were noticed in all the species tested.

The migration of the fish to the bottom of the tank following the addition of cypermethrin clearly indicates the avoidance behavior of the fish, which was reported by (Murthy, 1986) in trout.

Table 7. Behavior of fish observed in different concentration of test chemical in respect of time.

Behavior of Fish	Hours at different concentration			
	0.1ppm	0.2ppm	0.3ppm	0.4ppm
Migration to the bottom	6th	4th	3rd	1st
Spread out and swimming independently	8th	5th	2nd	1st
Schooling behavior disrupted	16th	10th	5th	4th
Irregular, erratic and darting movements	14th	7th	3rd	2nd
Escaping phenomenon	24th	12th	8th	6th
Repeated opening and closing of the mouth and opercula	8th	4th	2nd	2nd
Hyperextension of all fins	5th	3rd	1st	1st
Corkscrew pattern swimming behavior	9th	5th	2nd	1st
Sudden, rapid, non-directed spurt of forward movement	5th	3rd	3rd	2nd
Signs of tiredness	36th	28th	18th	11th
Engulfed the air through mouth	8th	6th	3rd	1st
Mouth and operculum wide opened	5th	2nd	1st	1st
Color of the gill lamellae from reddish to light brown	78th	66th	42th	24th

The opercula movement of the fish ceases immediately following exposure to cyperrnethrin. The decrease in operculum movement and corresponding increase in frequency of surfacing of fish indicates that fish adaptively shifts towards aerial respiration and the fish tries to avoid contact with the pesticides through gill chamber (Shanthakumar *et al*, 2000). The rapid opercular movements may be due to accumulation of mucous over gill due to the toxicant (Sadhu, 1993; Sabita and

Yadav, 1995 and Jagadeesan and Vijayalakshimi, 1999). Similar findings were observed by Prasanth *et al. (*2005), when freshwater fish *C. mrigala* exposed to cypermenthrin. The fish *L. bata* exhibited irregular, erratic darting movements with imbalanced swimming activity. Occasionally the fish tried to jump out of the toxic medium, which shows the avoidance behaviour of the fish to the toxicant. Similar behavior patterns were observed in fish named trout and *L. rohita*when exposed to fenvelrate (Murthy, 1987) and in *L. rohita* to endosulfan (Shivakumar and David, 2004).The present study also similar with the above findings.

Conclusion

Cypermithrin are highly toxic for fishes and aquatic animals. It has found to be highly toxic in fishes even in very low concentration. The 96 h LC_{50} value recorded in this study falls well within the range reported in acute toxicity test. From the 96hr LC_{50} it can be stated that cypermithrin is highly toxic to *C. carpio*. The results clearly indicate that the usage of cypermithrin in the fields of aquaculture may be a threat to mirror carp. It is suggested that the use of these types of pyrethroid pesticides should be judicious and controlled so as to control agriculture pests.

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