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Research Article

TOXICITY EFFECT OF CYPERMETHRIN (10% EC) TO THE FRESHWATER FISH *CIRRHINUS MRIGALA* (HAMILTON)

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ABSTRACT

The toxic effects may include both lethal and sublethal concentrations, which may change the growth rate, development, reproduction, histopathology, biochemistry, physiology and behavior on target organisms and undesirable perturbations in the environment. The result of the present work i.e., observed percentage mortality of species of fresh water fish *Cirrhinus mrigala* 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 2.69 ppb, 2.61 ppb, 2.41 ppb, 2.28 ppb Respectively. In the present investigatiion, the test species *C. mrigala* has shown differential toxicity level with a functin of period. With the increase period of exposure (96 h), the fish showed mortality at less concentration and with decrease of duration of exposrue the fish exhibited mortality at higher concentration. In the present investigation, when fish were exposed to sublethal concentration of cypermethrin for 4 days. Several behavioural changes were observed which include loss of schooling behaviour, 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.

Keywords: Cypermethrin, *Cirrhinus mrigala*, Lethal concentrations, Sublethal concentrations, Behavioral changes.

INTRODUCTION

All manmade chemicals eventually find their way into the aquatic environment, where they may prove to be toxic to many non-target organisms. 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. Moreover, in the aquatic environment the body of organism is bathed by the medium containing the toxicant. In the young Science of aquatic toxicology fish plays an important role in toxicity testing .

Unfortunately after use, pesticides do not stay in their place of application but move to the other parts of the environment and ultimately to the aquatic environment. This leads to the mass mortality of non-target organisms and undesirable perturbations of the environment (Ware, 1980). 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 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. Fish are the most often tested aquatic organisms because they are the most conspicuous as predominent and are

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economically important to man because they are linked to the food chain.

Synthetic pyrethroids are one of the wide varieties of pesticides contributing to these situations. Cypermethrin is one of the newly synthesised insecticide. Hence the thought of using plant extracts, namely pyrethroids received much attention. But these insecticides also tend to affect the biology of non-target species along with pests (Elliot and Janes, 1978; Reddy and Yellamm, 1991; Veeraiah and Durga Prasad, 1998). Some pesticides are extremely toxic to fish and to aquatic invertebrates at a very low concentrations. These pesticides also differ in toxicity to different aquatic organisms.

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.

The modern aquatic toxicity protocols in use are the result of a series of attempts at the standardization of the test methodology. The earliest and one of the most useful of these test methods is that of Doudoroff *et al.,* (1951) which forms the basis of all other attempts. In the standard methods of the American public health Association (APHA, 1985), bioassay and toxicity test procedures are described in detail. Sprague (1971) reviewed the state of the art upto 1970 and emphasized the need for having a clear concept of the different aspects of toxicity testing. The United states Environmental protection Agency (USEPA, 1973), committee on the methods for toxicity tests with aquatic organisms published a comprehensive review on the methods for conducting acute aquatic toxicity test. Later this Committee was replaced by the American Society for Testing and Materials (ASTM, 1992), committee on pesticides and the sub committe on safety to aquatic organisms. Hence, the acute mortality test is made mandatory in the tired systems of testing followed by the European Economic Community (EEC) and Organization of Economic Co-operation and Development (OECD) countries for any chemical produced on an industrial scale (Smeets, 1980).

The present study was aimed to investigate the toxicity and behavior of cypermethrin on freshwater fish, *Cirrhinus mrigala.*

MATERIALS AND METHODS

The test fish of size 5-6 cm and 3-4gm weight were brought from fish hatcheries of Nandivelugu, Tenali mandal, Guntur district, Andhra Pradesh, India which is 20 km away from the University. The fish were acclimated to laboratory conditions in large tubs with unchlorinated water for one week at a room temperature of 28 +/- 2^{o}c. During the period of acclimation, the fish were fed daily with fish feed on an average at 3% of their body weight. The fish were not given feed a day prior to the experimentation.

Preparation of stock solution

The cypermethrin 10% (EC) Insecticide supplied by Gujarat Agro Industries Corporation Limited, Ahmedabad, was used for the toxicity studies. Stock solution is prepared by dissolving the pesticide in acetone. Controls were also kept which recieved the solvent, acetone equal to concentration used in the test.

Selection of sub-lethal and lethal concentrations

The toxic substances are present in the aquatic system at concentrations too low to cause rapid death directly but may impair the other functions in organisms. Though pesticides may not be present in lethal concentration, accidental spillages may result in toxic concentration. Hence in the present investigation, 1/10 of 96 hrs LC_{50} and 96 hrs LC_{50} were selected as sub lethal and lethal concentrations respectively to study the effects.

Experimental set up

The ground water used for acclimation and conducting experiments was clear and unchlorinated. The physical and chemical properties of water were recorded.

Pilot test

Pilot tests were conducted to choose the concentration at which the fish were killed. For each test, 5 concentrations were taken and 10 fish were placed in container with a capacity of 10 litres.

Experiments were conducted to select the mortality range from 10% to 90% for 24, 48, 72and96 in static system. The data on the mortality percent of fish was taken in to

consideration to calculate LC_{50} values. The dead fish were removed immediately. The data were recorded from these tests at the end of each specific time period.

Finney's Probit analysis Finney (1971) 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 95% confidence limits of the LC_{50} values and for each test were calculated.

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 devided in to batches of 10 each and were exposed to different concentrations of cypermethrin ranging from 2.0 mg/l to 3.2 mg/l of cypermethrin 10% EC formulation.

This range was obtained on trial and error basis of toxicity evaluation was carried out in static water Doudoroff *et al.,* (1951) and mortality rate was observed at all concentrations of cypermethrin after 24, 48, 72 and 96 h exposure. A batch of fishes maintained in control chamber. The experiments were repeated thrice for accuracy. The mortality rate at each concentration was derived from the mean of the three replicates of percent mortality values.

RESULTS AND DISCUSSION

The result of the present work i.e., observed percentage mortality of species of fresh water fish *cirrhinus mrigala* when exposed to the 10% emulsifiable concentration (E.C.) cypermethrin for time periods 24, 48, 72 and 96 h (table 1-4) and the lethal concentration, i.e., LC_{50} Values 95% confidence limits and regression equations for 24, 48, 72 and 96 h (table 5).

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 are 9.0 and 8.0 ppb for 24 and 48 h respectively for *Gambusia affinis* (Mulla *et al.,* 1978b), 10.0 and 6.0 ppb for 24 and 48 h respectively in *Cyprinodon macularis* (desert pup fish) Mulla *et al*.,(1978), 55.0 ppb in *Salmo gairdneri* for 96 h, Coats and O'Donnell Jaffery (1979).

In the present study, as per the table, the toxicity is in the range of 2.69 ppb, 2.61 ppb, 2.41 ppb, 2.28 ppb, in static tests for 24, 48, 72 and 96 h respectively. In the investigatiion, the test species *C. mrigala* has shown differential toxicity level to different periods. With the increase of period exposure (96 h), the fish showed mortality at less concentration and with decrease of duration of exposrue the fish exhibited mortality at higher concentration.

The LC_{50} values increase with the increase in temperature. At 10^oC 0.9 and at 20-25^oC 1.1 ppb for the Carp, *Cyprinus carpio* (Stephenson 1982), 1.2, 0.5, 0.4 and 2.2 LC_{50} values were reported for the Brown trout (*Salmo trutta*), Rainbow trout (*Salmo gairdneri*), *Sardinius erythroptholmus* and *Tilapia nilotica* respecrtively by Stephenson (1982) reported 0.96, 0.84, 0.62 and 0.57 ppm for 24, 48, 72 and 96 hours repectively to the fish *Lepidocephalychthys thermalis.* The values are also in agreement with the findings of Stephenson (1982).

Earlier static tests with nominal or estimated toxicant concentrations indicated that E.C. formulations of several synthetic pyrethroids were about 2 to 10 time more toxic to Rainbow trout *Selma gairdneri,* (Coats and O'donnell Jeffrey, 1979) and Atlantic salman, *Satmo salar* (Zinko *et al.,* 1979) than technical formulation. This is probably due to the synergistic interaction between active ingredient and other components of the formulation. The selective toxicity of pyrethroids to vertebrates is in the order of Fish > Amphibians > Birds >Mammals (Haya, 1988).

The predominant determination of cypermethrin toxicity to fish appears to be the test species but toxicity may be influenced by exposure conditions, formulation source and size of fish and water quality. It was reported that the static values of LC_{50} are higher than the continuous flow through systems. The higher values are in agreement with the earlier reports (Anita Susan, 1994; Luther Das *et al.,* 2000, Koteswara Rao. 2003; Tilak *et al.,* 2001; Madhavi, 2006).

In the present investigation, when fish were exposed to sublethal concentration of cypermethrin for 4 days, several behavioural changes were observed which include loss of schooling behaviour, 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 were noticed in all the species tested. A film of mucus

was also observed all over the body and also on the gills. The secretion of mucus in the gills can be attributed to the histological changes of gill caused by the organophosphate pesticide chlorpyrifos (Tilak *et al.,* 2001).

The toxicity of insecticide cypermethrin to the freshwater fish *C. mrigala* was studied used static bioassay method. The 96 h LC_{50} was found to be 5.13 microg/l. Increase in opercular movement, loss of equilibrim, surface behaviour, change in body colour, increase secretion of mucous, irregular swimming, jerk movements, 'S' jerky, partial jerk and agressiveness were observed in fish exposed to concentration of cypermethrin. But in sublethal concentration these changes were observed initially and the approach of 14 and 21 day, fish attained the normalcy.

The symptoms induced by the cypermethrin insecticide in fishes can be attributed to an increase in physiological stress. Physiological stress has occured in the form of neuronal excitation, which apparently has resulted in the continuous synthesis and distruction of neuro transmitters and enzymes (Tilak *et al.,* 2001). The morphological and behavioural changes exhibited by the fish can be taken as useful paramater as a bio-indicator in assessing the extent of aquatic pollution in them.

Table 1. The observed percentage of Mortality and probit mortality of the fish *Cirrhinus mrigala* exposed to cypermethrin (10 % EC) for 24 hr.

Hours of exposure	Cone mg/l	Observed mortality	Probit values
24	2.4	30%	4.4756
24	2.6	40%	4.7467
24	2.8	50%	5.0000
24	3	70%	5.5244
24	3.2	90%	6.2816

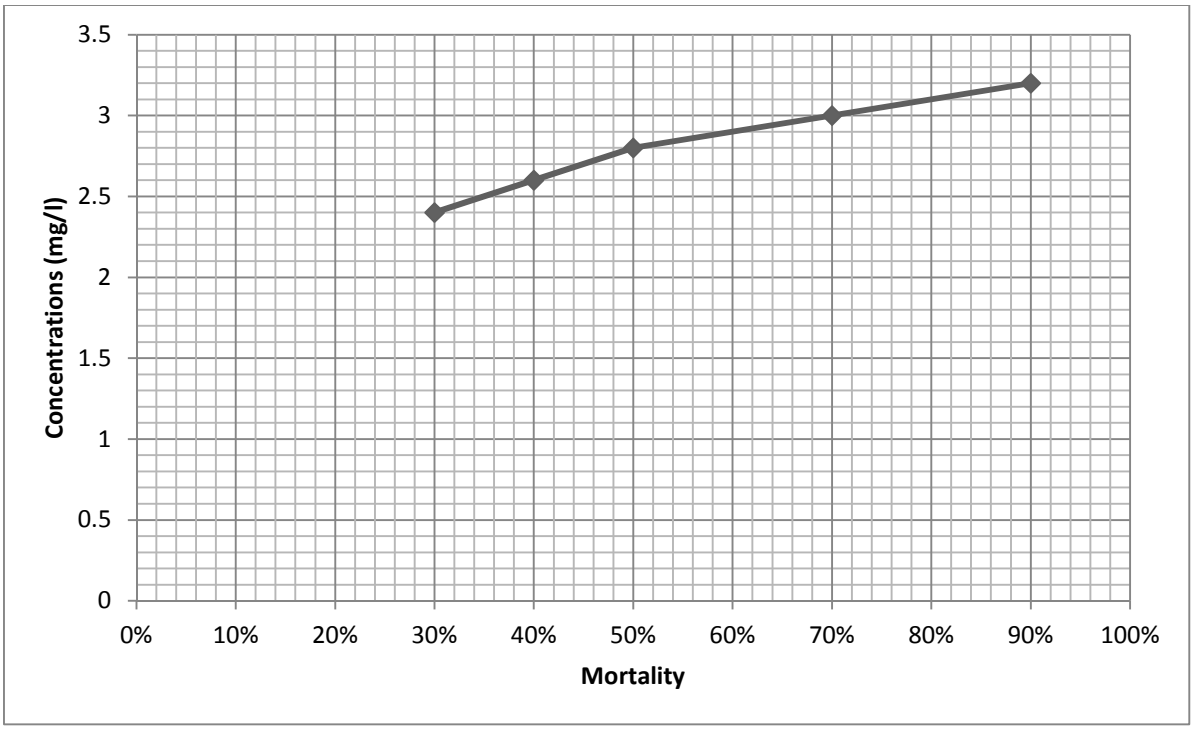

Figure 1. The graph showed percentage of mortality and probit mortality of *Cirrhinus mrigala* exposed to cypermethrin (10% EC) for 24 hrs.

Table 2. The observed percentage of Mortality and probit mortality of the fish *Cirrhinus mrigala* exposed to cypermethrin (10% EC) for 48 hrs.

Hours exposed	Cone mg/l	Observed mortality	Probit values
48	2.3	20%	4.1584
48	2.5	40%	4.7467
48	2.7	60%	5.2533
48	2.9	80%	5.8416
48	3.1	90%	6.2816

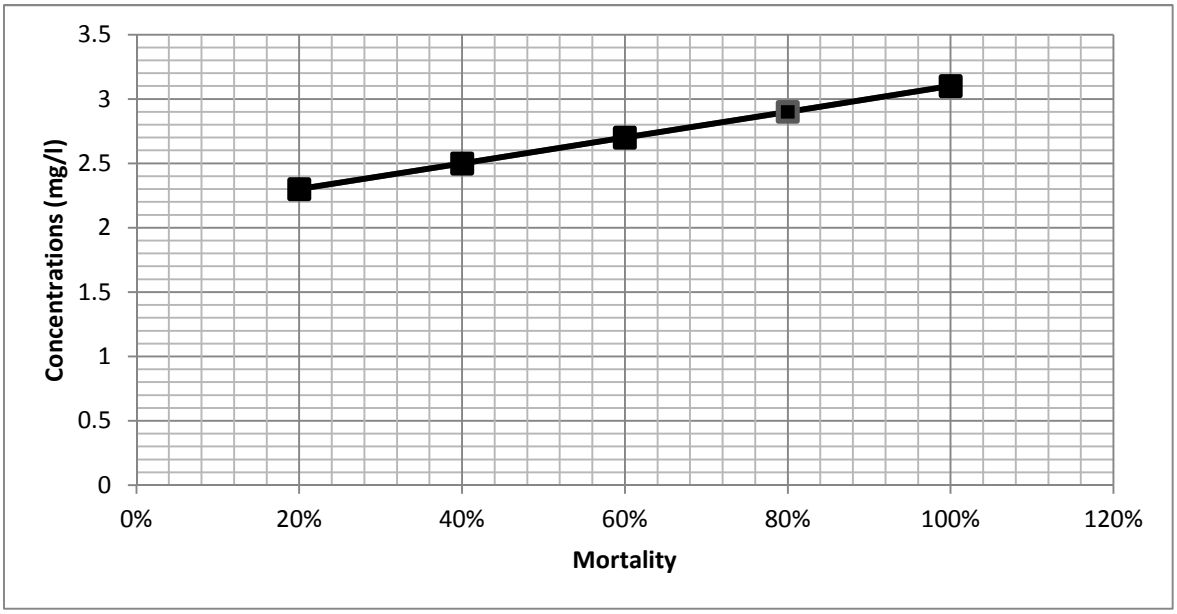

Figure 2. The graph showed percentage of Mortality and probit mortality of the fish *Cirrhinus mrigala* exposed to cypermethrin (10%EC) for 48 hrs.

Table 3. The observed percentage of Mortality and probit mortality of the fish *cirrhin mrigala* exposed to cypermethrin (10%EC) for 72 hrs.

Hours exposed	Cone mg/l	Observed mortality	Probit values
72	2.1	20%	4.1584
72	2.3	30%	4.4756
72	2.5	60%	5.2533
72	2.7	80%	5.8416
72	2.9	90%	6.2816

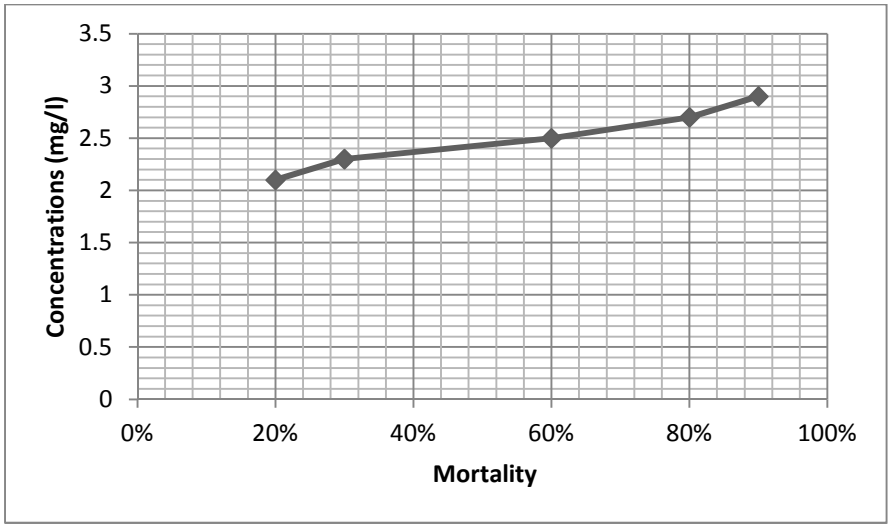

Figure 3. The graph showed percentage of Mortality and probit mortality of the fish *Cirrhinus mrigala* exposed to cypermethrin (10%EC) for 72 hrs.

Table 4. The observed percentage of Mortality and probit mortality of the fish C*irrhinus mrigala* exposed to cypermethrin (10%EC) for 96 hrs.

Hours exposed	Cone mg/l	Observed mortality	Probit values
96	2.0	20%	4.1584
96	2.2	40%	4.7467
96	2.4	60%	5.2533
96	2.6	80%	5.8416
96	2.8	90%	6.2816

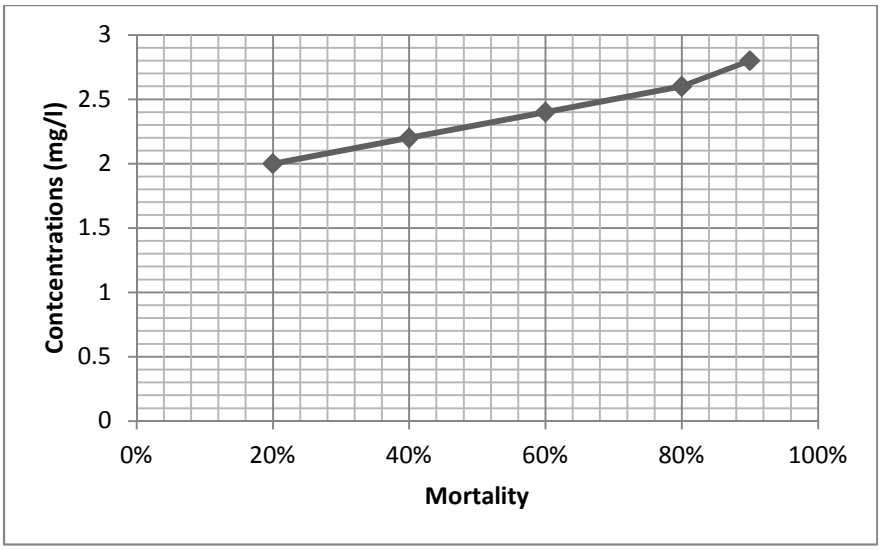

Figure 4. The graph showed percentage of mortality and probit mortality of the fish *Cirrhinus mrigala* exposed to cypermethrin (10% EC) for 96 hrs.

Table 5. The LC_{50} values , 95% confidence limits and Regression equation of cypermethrin 10% EC of fish *Cirrhinus mrigala* for 24, 48, 72 and 96 hours in static system.

Duration	Type of test	LC_{50} Value	Upper Confidence Limit	Lower Confidence Limit	Regression Equation
24 Hours	Static System	2.69	2.5321	2.3284	Y= -29.5771+14.2268X
48 Hours	Static System	2.61	2.7153	2.0341	Y= -38.4789+18.0273X
72 Hours	Static System	2.41	2.5184	2.2174	Y= -29.9136+14.6616X
96 Hours	Static System	2.28	2.4616	2.2579	Y= -30.0560+14.8551X

CONCLUSION

Toxicity experiments for cypermethrin 10% EC formulation conducted using static system for 24, 48, 72 and 96 h revealed that the percentage of mortality increased with the increase concentration of cypermethrin. With the increase of exposure time (96 h), the fish showed mortality at less concentration and with decrease of exposure time the fish exhibited mortality at higher concentration.

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