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Studies on acute toxicity and determination of lethal concentration (LC_{50}) of Cypermethrin (25% EC) to *Ophiocephalus striatus*

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Abstract - The present investigation was carried out to determine the acute toxicity of Cypermethrin 25% emulsifiable concentration (25% EC), a synthetic pyrethroid on snake headed fish *Ophiocephalus striatus* by Pilot bioassays test. For the experiment, 6 concentrations were prepared and ten fishes were kept in each aquarium having capacity of 20 lit. Average weight of fishes was 100 ±14.74 gm. Different concentrations of Cypermethrin (25% EC) prepared were 0.25µl Lit^{-1}, 0.50 µl Lit^{-1}, 0.75 µl Lit^{-1}, 1.00 µl Lit^{-1}, 1.25 µl Lit^{-1} and 1.50 µl Lit^{-1}. All the exposed fishes were observed daily and the mortality was observed daily upto 96 hours at different concentrations. Dead fishes were immediately removed from aquarium. Finney's Probit analysis method was used to calculate the LC_{50} value. The LC_{50} value at 96 hours was calculated to be 0.72± 0.007 µl Lit^{-1}. Physico-chemical parameters of water used in aquarium for experiment were carried out. The parameters tested were water temperature, pH, dissolved oxygen, dissolved carbondioxide, alkalinity, hardness, phosphate, Na^+ and K^+.

Keywords — ***Ophiocephalus, Cypermethrin, Probit analysis, Pyrethroid, LC_{50}, physico-chemical parameters***

I. INTRODUCTION

Increased use of pesticides in agriculture to prevent the damage of crop has caused serious threat to the non target organisms, due to indiscriminate killing [1]. It has resulted in the destruction of delicate balance between key species of the food chain and the ecosystem, thereby affecting the productivity of the ecosystem [2]. The organisms like zooplanktons and aquatic insects are very sensitive to these pesticides and thus significantly affected by these toxicants. The pesticides through surface water run off, get mix with river, lakes, ponds and other water bodies and thus change the aquatic environment and adversely affects the biotic components of water bodies [3]. These pesticides also have toxic effects on the economically important fishes. In fishes, these toxicants alter the normal behavior as well as cause the change in the histology of the vital organs like liver, kidney, gonads, gills etc.[4]. Sublethal dose of any insecticide also weakens the fish and make it susceptible to various diseases and thus adversely affects the economy of

the fisherman. Pyrethoids like Cypermetrin is among the widely used insecticides in the agriculture due to its effect against wide range of insect pest and these are chemically unstable which break down rapidly upon exposure to air and sunlight. These pyrethroids affects the neurotransmission by blocking GABA receptors and alters the voltage dependent sodium ion channels in nervous system leading to prolonged openings of these channels. Repetitive discharge of neurotransmitters and disturbance in synaptic membrane, results in hyperexcitatory symptoms of poisoning in animals [5, 6]. Thus main aim of this present study was to assess the LC_{50} dose for Cypermethrin (25% EC), in economically significant species of fish *Ophiocephalus striatus* by Pilot bioassays test and understanding the behavioral effects on this fish when exposed to the sublethal dose of the Cypermethrin.

II. MATERIALS AND METHODS

For experiment, *Ophiocephalus striatus* (average length 15 ± 1.5 cm, average weight 100 ± 14.75 g) were procured from the local fisherman of Sakkardara Market, Nagpur. All the specimens were brought to the laboratory and length and weight of each specimen was measured with the help of electronic balance. Fishes were acclimatized for one week to the laboratory conditions in aquarium of thirty liter capacity. In each aquarium 10 fishes were kept in 15 litres of water. The well water used for the experiment was clear and unchlorinated. During acclimatization fishes were fed with Red sea freeze-dried blood worms (containing 60 ± 5% crude protein, 8 ± 5% crude fat, 7 ± 5% crude fiber, 12 ± 5% Crude ash and 5 ± 2% moisture packed by- Insha Products, Mumbai- 16) in the morning and at evening, twice a day. The acclimatized fishes were not given feed a 24 hours prior to the start of experiment.

Preparation of stock solution: The Cypermethrin 25% (EC) insecticide Shoot supplied by Yawalkar Agro Industries Corporation Limited, Nagpur, was used for the present toxicity experiments. Stock solution is prepared by dissolving the pesticide in acetone. Acetone equal to the concentrations were mixed in the aquarium of the control fishes. In the present study, LC_{50} dose of Cypermethrin 25% EC for 96 hours was selected as a lethal dose to observe the histological changes in the vital organs like liver, kidney and spleen. The present study was conducted in controlled physico-chemical characteristics of water. The physico-

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chemical parameters analysed were temperature, pH, dissolved oxygen (Wrinkler's method), dissolved carbondioxide (Titration method) , alkalinity (Methyl Orange titration method), hardness (EDTA titration method) , Na^+ (Flame photometry) and K^+ (Flame photometry). The analysis of various physic-chemical parameters of water samples were carried out by following the standard methods [7, 8]. Triplicates of each analysis were performed and mean values were used for calculation.

Acute Toxicity Test: In the present investigation, ten fishes were kept in each aquarium. The Cypermethrin 25% emulsifiable concentration was increased gradually. The concentrations used during experiment were 0.25 µl Lit^-, 0.5 µl Lit^-, 0.75 µl Lit^-, 1.00 µl Lit^-, 1.25 µl Lit^-, 1.50 µl Lit^-. The range for doses was obtained by trial and error basis. Mortality of Fish was observed at 96 hours of exposure to different doses of Cypermethrin. Dead fishes were immediately removed from aquarium after the death. The experiments for toxicity were was carried out in triplicates for getting the accuracy. Mortality rate at each concentration was obtained by calculating the mean of the experiment which was carried out in triplicate for each concentration.

Statistical Analysis: Finney's Probit analysis method [9] was used to calculate the 96 hour LC_{50}. The probit mortality, log concentration, LC_{50}, Chi-square, slope, regression equation, 95% upper and lower confidence limit was calculated by using Statistical Package for Social Sciences (SPSS 10.0).

III. RESULTS

Physico-chemical characteristics of water used in aquarium for experimental purpose for determining LC_{50} in *Ophiocephalus striatus* was analyzed. The parameters like temperature (22 ± 1°C), pH (7.33), dissolved oxygen (6.4 mg Lit^{-1}), dissolved carbon dioxide (1.99 mg Lit^{-1}), total alkalinity (168 mg Lit^{-1}), total hardness (148 mg Lit^{-1}), Na^+ (5 ppm) and K^+ (1ppm) are presented in table 1.

Median lethal concentration (LC_{50}) :

The LC_{50} values for Cypermethrin toxicity to the *Ophiocephalus striatus* was calculated by Probit Analysis (Finney, 1971). The % mortality and probit mortality at different concentrations of cypermethrin 25% EC for 96 hours of exposure to *Ophiocephalus striatus* is shown table 2. The LC_{50} value , slope, regression equation, 95% confidence limits, i.e. upper confidence limit (UCL) and lower confidence limit (LCL) for LC_{50} value, ratio of confidence limits (R = UCL/LCL) which signify the test was computed for 96 hours of exposure and shown in Table 3. The LC_{50} values and regression equation for 96 hours was observed to be 0.72 ± 0.007µl lit^{-1} and Y = 3.86 X + 5.524 respectively. The observed ratio of confidence limits is -3.833 which is highly significant that indicates the good performance of the bioassay test. The Linear regression equation showing probit mortality of *Ophiocephalus striatus* against log concentration is presented in figure 1.

Table 1. Physico-chemical characteristics of water used in aquarium

Sr. No.	Physico-chemical parameters	Mean
1.	Temperature	22± 1 °C
2.	pH	7.33
3.	DO	6.4 mg Lit^{-1}
4.	CO_2	1.99 mg Lit^{-1}
5.	Alkalinity	
	Phenolphthalein Alkaliniy	16 mg Lit^{-1}
	Methyl Orange Alkalinity	152 mg Lit^{-1}
	Total Alkalinity	168 mg Lit^{-1}
6	Hardness	
	Calcium Hardness	92 mg Lit^{-1}
	Magnesium Hardness	56 mg Lit^{-1}
	Total Hardness	148 mg Lit^{-1}
7.	Na^+	5 ppm
8.	K^+	1ppm

Figure 1. Linear regression equation showing probit mortality of *Ophiocephalus striatus* against log concentration.

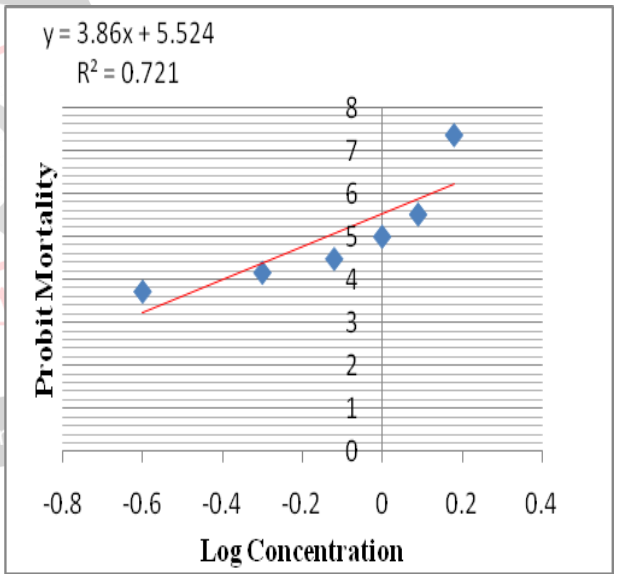

Figure 2. The graph showing percent mortality of *Ophiocephalus striatus* against log concentration of Cypermethrin 25% EC.

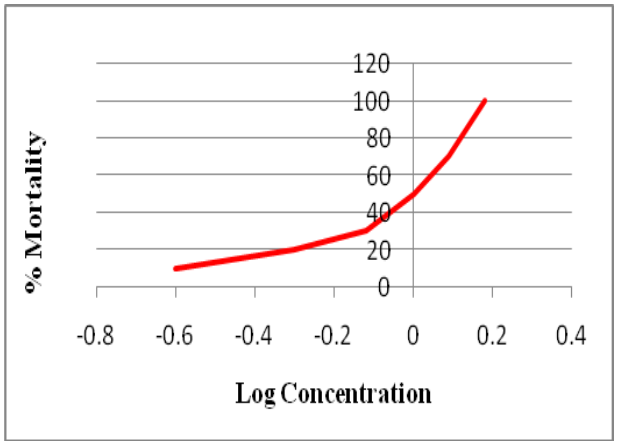

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Table 2: **% Mortality and Probit mortality of *Ophiocephalus striatus* at different concentration and Log concentration of Cypermethrin 25% EC exposed for 96 hours.**

Sr. No.	Conc. of Cypermetrin (25% EC) µl Lit^{-1}	Log Conc.	No. of Fish Exposed	No. of Fish alive	No. of Fish dead	% Mortality	Probit Mortality
1.	0.25	- 0.60	10	09	01	10	3.72
2.	0.50	-0.30	10	08	02	20	4.16
3.	0.75	-0.12	10	07	03	30	4.48
4.	1.00	00	10	05	05	50	5.00
5.	1.25	0.09	10	03	07	70	5.52
6.	1.50	0.18	10	00	10	100	7.37

Table 3: LC_{50}, slope, Regression equation and 95% confidence limits of Cypermethrin 25% EC in the *Ophiocephalus striatus* exposed to different concentrations for 96 hours.

Pesticide	96 hrs. LC_{50} ± SE	Slope	Regression Equation	95% Confidence Limit		
				Upper Limit	Lower limit	Ratio of confidence limits
Cypermetrin 25% EC	0.72 ± 0.007	r^2= 0.721	Y = 3.86 X + 5.524	0.023	- 0.006	-3.833

Table 4: Showing the Chi Square Probit Pearson goodness of fit test.

Chi Square Test		
Probit Pearson goodness of fit test		
Chi- Square	Df	Significance
0.186	4	0.996

The slope for the regression line r^2 is found to be 0.721. The % mortality against log concentration is shown in figure 2. Chi Square Probit Pearson goodness of fit test was also performed to observe the significance and is presented in table 4. Observed Chi-square for the bioassay test is 0.186 which is highly significant (0.996).

Behavioral Changes in *Ophiocephalus striatus* after exposure to Cypermethrin 25% EC:

When *Ophiocephalus striatus* were exposed to the sublethal doses of the Cypermethrin (25%EC) for 96 hours, several changes in the behavior of the fish were observed. As soon as the fishes were exposed to the sublethal dose of the Cypermethrin, these become restless, hyperactive and loss of balance and equilibrium was observed. Loss of buoyancy was also noted. Irregular and irratic swimming behavior, increase in opercular movements and fishes tried to gulp air at the surfaces. Increased secretion of mucous all over the body and decolorisation of the skin was reported. Fish settle down at the base of the aquarium and died with their mouth open and ventral surface facing in upward direction.

IV. DISCUSSION

For evaluation of acute toxicity, 96 hours toxicity test is commonly performed. Acute toxicity test is performed to observe the hazardous effects of toxicant on the histology of the vital organs and behavior of the animal [10]. Pyrethroids act on GABA receptors in the synapse and cause the neurotoxicity in organisms [11]. Pyrethroids are toxic to wide variety of pests but it also causes the indiscriminate killing of many of the beneficial and economically important organisms like fishes, crustaceans and honey bees [12,13,14]. Cypermethrin is not stable and thus it does not biomagnify in the ecosystem. Due to lipophilicity, Cypermethrin have high rate of absorption through gills. The 96 hours LC_{50} value for *Ophiocephalus striatus* was found to be 0.72 ± 0.007µl lit^{-1}. Suja and Williams [15] had observed the 4.521 ppm, LC_{50} of organophosphate chlorpyrifos to *C. striata* for 96 hours of exposure. The evaluation of 96 hours LC_{50} of Cypermethrin in different species of fish have been done by many workers [16, 17,18, 13, 19, 20, 21] and reported the dose of Cyprmethrin in the range of 5.13ng lit^{-1} to 9.0 µg lit^{-1}. Prashanth [21] has observed the 96 hours LC_{50} in *C. mrigala* as low as 5.13 ng lit^{-1} whereas Bhargava and Rawat [20] had reported the 96 hours LC_{50} in *Heteropneustes fossilis* was 0.274 µg lit^{-1}. Reddy and Yellamma [13] had observed the LC_{50} in *Tilapia mossambica* for 96 hours was 9.0 µg lit^{-1}. Synthetic pyrethroids are relatively safe for mammalian species. LD_{50} for mice and rats varies from 100 to 200 mg/kg body weight [22]. Oral dose of deltametrin 100mg/kg to rat showed no histopathological changes in the nervous system as well as in other tissues and vital organs. However, cyfluthrin at the dosage of 2000 mg/kg diet revealed the thymic atrophy, adrenal enlargement with vacuolization and incomplete spermatogenesis in rats [11, 23, 24, 25, 26, 27]. Pyrethroids

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mainly act on nervous system, but in mammals it also shows the effects on the respiratory tract like massive haemorrhages and oedema of the lungs due inhalation of Cypermethrin above or near lethal dose [28].

After exposure to sublethal dose of Cypermethrin, alteration in the behavior of the fish have been observed. Fish showed restlessness and lack of balance and erratic swimming movements. The changes in the behavior of the fish after exposure to sublethal dose of Cypermethrin may be due hyperexcitaion and physiological stress in the fish. Prolonged opening of sodium ion channel [29] and repetitive discharge and destruction of neurotransmitters causes the poisoning symptoms in the fish [5,6.30]. Increased secretion of mucous all over the body and on gills may be for avoiding the contact of Cypermethrin to gills which minimizes the absorption of Cypermethrin through gills [30, 31].

V. CONCLUSION

Pyrethroids are frequently used against pests because of their high insecticidal property for wide range of insects, low toxicity for mammals, less persistence and easy biodegradability due to unstable nature. On the basis of present investigation it can be concluded that the Cypermethrin is highly toxic at very low doses and causes physiological stress in *Ophiocephalus striatus***.** It has also effect on behavior of fish at lethal and sublethal concentrations. Inhibition of acetylcholine esterase due to exposure of Cypermethrin has resulted in abnormal swimming behavior in *Ophiocephalus striatus*. As Cypermethrin is a lipophilic chemical it is easily absorbed by the important vital organs like gills, liver, kidney, central nervous system and peripheral nervous system and causes histological abnormalities and adverse effects in them, resulting in mortality of fishes. Probit analysis indicates the mortality rate of fishes increased with increase in the concentration of Cypermethrin. However increase in concentration of Cypermethrin results in decrease in mortality time. Cypermithrin have been proved to be acute toxicant to *Ophiocephalus striatus*. The 96 hours LC_{50} value for Cypermethrin (25%EC) was found to be 0.72 ± 0.007 µl Lit^{-1}. Singh et al. (2010) has shown the adverse effect of sub-lethal doses of Cypermethrin in *Colisa fasciatus* after exposure of 96 hours. He has reported the significant alteration in the levels of total protein and total free amino acid in liver and muscles, level of DNA and RNA in gonadal tissues and the activity of enzyme acetylcholinesterase (AChE), lactic dehydrogenase (LDH) and succinic dehydrogenase (SDH) in nervous tissue of the fish [32].

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