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DETERMINATION OF LC50 OF AN ORGANOPHOSPHATE PESTICIDE IN A FRESHWATER CATFISH, *MYSTUS SEENGHALA*

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Abstract- In the present study Indian cat fish was exposed to the new generation pesticide for 96 h LC50 (Lethal concentration) value of Chlorpyrifos 20% EC (an organophosphate) was determined for a fresh water catfish, *Mystus seenghala*. The average length and average weight of the fishes were 9.2±1.5 cm and 12.4±2.0 g respectively. For determination of LC50 value, following 9 chlorpyrifos concentrations 0.5, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00 mg/l and a control were taken. A set of 30 acclimatized fishes were chosen randomly for toxicity determination and mortality rate was determined at the end of 24, 48, 72 and 96 h. According to Finney's probability analysis (1971), LC50 of Chlorpyrifos 20% EC to *Mystus seenghala* for 96 h of exposure was determined as 1.776 mg/l with lower and upper confidential limits (95%) as 1.583 mg/l and 1.977 mg/l respectively.

KEYWORD: - 96 h LC50, Chlorpyrifos, *Mystus seenghala*, probability analysis.

I. INTRODUCTION-

Pesticides are widely used in modern agriculture to aid in the production of high quality food. However, some pesticides have the potential to cause serious health and/or environmental damage. Repeated exposure to sub-lethal doses of some pesticides can cause physiological and behavioral changes in fish that reduce populations, such as abandonment of nests and broods, decreased immunity to disease, and increased failure to avoid predators. (Helfrich, et al., 1996)Pesticides can contaminate soil, water, turf, and other vegetation. In addition to killing insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants. Exposure to higher concentrations of persistent, bio accumulative, and toxic contaminants such as DDT (1,1,1-trichloro-2,2-bis[pchlorophenyl] ethane) and PCBs has been shown to elicit adverse effects on reproductive and immunological functions in captive or wild aquatic mammals (Helle et al., 1976; Reijnders, 1986; Martineau et al., 1987; Kannan et al., 1997 ; Ross et al., 1995; Ross et al., 1995 Colborn and Smolen, 1996). The undue persistence, high mammalian toxicity and developing resistance of the organo chlorine, organophosphate and carbonate insecticides led to a ban or restriction on their use in many developed and developing countries. Thus, attention was focused on the synthesis of less persistent, low mammalian toxicity new generation compounds like indoxacarb.

Pesticides are one of the most hazardous chemicals to the environment. Various types of pesticide are used extensively in agricultural fields to protect the crops from injuries or damages caused by different types of pest. These chemicals may reach other ecological sections like lakes, reservoirs, rivers and surrounding water areas through rains and winds affecting a number of organisms other than primary target. According to Livingstone (2001), the injuries of pesticides to aquatic environments are incontestable and the significant increase of this chemical discharge in the water bodies from surrounding area has led to deleterious effects for inhabiting aquatic organisms.

The fishes act as a bio-indicator because they respond very quickly against the changes in the aquatic environment and thus they play a major role in understanding different types of pollution of a water body. The 96 h LC50 tests are conducted to measure the susceptibility and survival potentials of organisms to toxic substances.

II. MATERIAL AND METHODS:-

Test chemical: Technical grade Chlorpyrifos (20% EC) [IUPAC name: 0,0–diethyl 0–3,5,6–trichloro–2–pyridylphosphorothioate] with trade name PYRIFEX (manufactured by – SAFEX chemicals, INDIA ltd.) was purchased from local market.

Test organism: *Mystus seenghala* one of the most important species of freshwater catfish cultured in India.

The fishes were collected from local market for experiments. The average length and average weight of the fishes (both sexes) were 9.2±1.5 cm and 12.4±2.0 g respectively. At first the fishes were given prophylactic treatment by bathing them twice in 0.05% $KMnO4$ solution for 4-5 minutes to avoid dermal infections. The fishes were then acclimatized for 15 days under laboratory conditions before exposure to pesticide.

Analysis of toxicity was conducted in the rectangular glass aquaria (30×60×30 cm). The supply of oxygen into the water of aquariums was done by electrical aerators. The fishes were fed with commercial pelleted food, 2-3 times per day during the acclimatization period and the feeding was stopped 24 h prior to acute toxicity test. The water was renewed daily and the faecal matter and other waste materials were siphoned off daily.

The acute toxicity bio assay to determine 96 h LC50 value of chlorpyrifos in *Mystus seenghala*. The stock solution of chlorpyrifos was prepared by dissolving the analytical grade of chlorpyrifos 20% EC in acetone. For determination of LC50 value, following a range finding test of nine chlorpyrifos concentrations 0.5, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00 mg/land a control were chosen for *Mystus seenghala*. The control group was used for comparison by using the tap water containing acetone (the volume of acetone used was the same as used for preparing the chlorpyrifos solution at different concentration). A set of 30 acclimatized fish specimens were chosen randomly and then exposed to different concentration grade of chlorpyrifos. Separate group of 30 fishes served as control. The mortality rate was determined at the end of 24, 48, 72 and 96 h. In this study the acute toxic effect of chlorpyrifos 20% EC on *Mystus seenghala* was determined by the use of Finney's probability analysis LC50 determination method (1971).

Confidential limits (upper and lower) were calculated and also used SPSS, version 17.0 for LC50 value of chlorpyrifos 20% EC with the help of Probit analysis. The physico-chemical characteristics of water were determined by standard methods of APHA (1995) and Trivedi and Goel (1984). The water temperature of different experimental tanks was ranged from 27.91 to 28.90 ºC; the pH of water ranged between 6.90 and 7.72; the dissolved oxygen values varied from 3.81 to 3.97 mg/l and that of alkalinity from 77.40 to 92.81 mg/l.

III. RESULT AND DISCUSSION:-

The relation between the organophosphate pesticide (Chlorpyrifos) concentration and mortality rate of the fresh water catfish *Mystus seenghala* according to SPSS analysis were shown in the table 1. The results indicated different mortality rate of fishes which increased with the corresponding increase in concentration of Chlorpyrifos. The mortality in control treatment and 0.50 mg/l concentration of Chlorpyrifos were virtually absent. According to Probability analysis by Finney (1971), the median lethal concentration (LC50) of Chlorpyrifos 20% EC to *Mystus seenghala* for 96 h of exposure was calculated as 1.776 mg/l. The lower and upper lethal confidence limits (95%) for chlorpyrifos indicate a range of 1.583 mg/l to 1.977 mg/l within which the concentration response for 96 h exposure (Table 2). So it is concluded that higher percentage of mortality occurred with the increase in concentration and exposure period. Susceptibility of *Mystus seenghala* to the different dose of Chlorpyrifos was duration and concentration dependent as mortality increased with an increase in its concentration. Almost same result was observed by Srivastavet. al., (2012) while experimenting the morpho-toxicology of Chlorpyrifos to a freshwater catfish, *Mystus seenghala*. The results of this study may help to understand the acute toxicity of the pesticide in the field and may works as early warning indicators of pesticide toxicity in the freshwater catfish, *Mystus seenghala*..

Table 1: Correlation between the chlorpyrifos concentration and the mortality rate of *Mystus seenghala* at 96 hours of exposure.

Concentration(mg/l)	Number of fishes	Observed responses	Expected responses	Residual	Probability
0.00	30	0	0.000	0.000	0.000
0.50	30	0	0.450	-0.450	0.015
0.75	30	1	2.100	-1.110	0.070
1.00	30	6	4.882	1.115	0.165
1.50	30	14	11.287	2.412	0.365
2.00	30	18	17.450	0.580	0.581
2.50	30	21	21.425	-0.625	0.720
3.00	30	24	24.650	-0.462	0.816
3.50	30	25	26.325	-1.320	0.875
4.00	30	28	27.535	0.467	0.916

Table 2: LC50 (96 h) value of Chlorpyrifos with lower and upper (95%) confidence limits

Point	Estimated LC values and confidence limits		
	Concentration (mg/l)	95% Confidence limits	
		Lower Bound	Upper Bound
LC 1.00	0.456	0.310	0.595
LC 5.00	0.679	0.506	0.832
LC10.00	0.840	0.656	0.997
LC 15.00	0.969	0.782	1.120
LC 50.00	1.776	1.583	1.980
LC 85.00	3.253	2.850	3.890
LC 90.00	3.754	3.232	4.615
LC 95.00	4.641	3.890	5.980
LC 99.00	6.910	5.455	9.767

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