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Studies on acute toxicity to pesticide stress in a freshwater fish *Cirrhinus mrigala*

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Abstract
In the present study, the effects of quinolphos, an organophosphate pesticide on *Cirrhinus mrigala* have been analysed after exposing the fish to sub lethal concentrations. The LC_{50} values of *C. mrigala* for 12, 24, 48, 72, 96 and 120 hrs were 0.0140, 0.135, 0.132, 0.130, 0.128, and 0.124 ppm respectively. The results revealed that the percentage of mortality of *C. mrigala* increased with increase in concentration of pesticide, quiolphos as well as increase in exposure duration.

Keywords: Quinolphos, *Cirrhinus mrigala*, toxicity, mortality

1. Introduction
Pesticides have no doubt been a boon to the human civilization in sustaining agriculture revolution but at the same time bared its ravaging face on humanity pushing to a point of almost no return (Trivedi *et al.*, 1999) [23]. Modern agriculture practices, despite their remarkable contribution to the enhancement of crop production, have at the same time, also widely polluted the aquatic environment (Sadhu, 1993) [18].
Pesticide toxicity is a global problem with most of the poisoning occurring in developing nations [10]. In spite of its highly important use in agriculture, pesticides produce a number of pesticides residues in agriculture and dairy products, food water and in meat causing a great public health concern (Ram Narayanan Singh, 2013) [15]. Further, these compounds are related to environmental pollution and accidental poisoning Singh *et.al* (2000) [21]. However, the story is that less than 1% pesticides reach the target pest and remainder negatively affect human livestock and natural biota [13]. The ground water is also, being polluted by regular applications of chemical pesticides and fertilizers and thus, making the water unfit for consumption. Even banned pesticides are being frequently used in developing countries due to their socio economic conditions (Ghosh, 1987) [9].
Indiscriminate uses of pesticides are very common in India as well as in several Asian, American and African countries. Pesticides are used by farmers for spraying directly into the crops in the fields during storage etc. These chemicals are capable of killing pests and insects but on the other hand, they are highly toxic to animals as well as human beings (Budimir, 1998) [6]. As considerable amount of pesticides and their byproducts enter the fish body through the food chain, they are distributed and metabolised depending upon the detoxifying ability of the fish. This elicits some responses in fish depending on the nature and concentration of pesticides as well as on the duration of retainment of these pesticides in fish body. (Doudoroff, 1951) [7].
The route of pesticide transport to different aquatic eco system has been well documented. It is well known that various pollutants, including pesticides are carried to the freshwater by means of different processes like surface run-off, disposal through wastes, spray drift and atmospheric fall out, rain etc (Menzer *et.al.*, 1994) [12]. Natural water is the ultimate recipients of much of the chemical wastes. As far as aquatic ecosystem is concerned, it has been recognized as an important global problem, and thus, stimulated the eco-toxicologists to determine the lethal and sub-lethal impact on the fish and other aquatic organisms (Aswathi, 1984) [4].
When pesticides accumulate in aquatic system, it undergoes break down depending upon the physico-chemical and biological factors of the water eco-system.

As fish is considered as the most important and vital link in the food chain of ecosystem, a thorough understanding of pesticide effects on fishes would be really vital for fish conservation. The magnitude of pesticide pollution and the resultant contamination of food chain in India have been studied at different locations. Awasthi, (1998) [5].

Of the various groups of insecticides used in agriculture, veterinary and public health practices organophosphorus insecticides constitute the bulk. These compounds are preferred due to their high rapid degradation in animal body and ecosystem (Shailendra Kumar Singh, 2010) [20]. Majority of the studies concerning effects of pesticide have been confined to the acute toxicity tests with the death of fish as an end point. Acute toxicity tests aimed at estimating the effects of toxicants on organism in a short period of time have been conducted by authors like Doudroff *et al.* (1951), Menzer *et al.* (1994), Budimir *et al.* (1998) and Alam (2000) [7, 12, 6,1].

Several investigators have studied the acute toxicity of different pesticides on various fish species and other aquatic organisms Holcombe *et al.*, (1982) [11]. In acute toxicity testing, fishes are continuously exposed to progressively increasing concentrations of pesticides and death of test organisms are recorded at several selected times. Acute toxicity of quinolphos, phosphamidom and *monocil* to *Oreochromis mossambicus* and *Channa punctatus* are reported separately by Rama Krishnan and Sivakumar (1993) [14]. Short term and long term effects of quinolphos and padan on the *Labeo rohita* were studied by Amali (1995) [2]. Acute toxicity of nuvan and dimecron to freshwater murrel, *Channa orientalis* and *Channa punctatus* was determined by conducting bioassay experiments (Saxena *et al.*, 1996) [19]. Very recently, the acute toxic effect of Metacid-50 on *Channa punctatus* and curacron on *Cyprinus carpio* and *O. mossambicus* was reported by Alam (2000) [1].

Toxic responses are many, among which biochemical responses are the most subtle indicators to toxicity. Most toxicants exert their effect at a basic level of the organisms by reacting with metabolites and binding or interacting with all functional components of the cell. Such primary interaction between the toxic substances and various cell components may induce a sequence of structural and functional alternations. Biochemical changes in responses to pesticides usually lead to irreversible and detrimental disturbances of integrated functions (Singh *et al.*, 2000) [21]. Hence in the present study, the effects of quinolphos, an organophosphate pesticide on *Cirrhinus mrigala* have been analysed after exposing the fish to sub lethal concentrations.

2. Materials and methods

2.1 Collection and maintenance of candidate species

The period of study for the experiment is from March-July2014.The candidate species, *Cirrhinus mrigala* (Ham.) were collected from private fish farms, located 4 kms. from Nagercoil town**.** They were then transported to laboratory in polytene bags containing oxygenated water, and special care was taken to reduce hyperactivity and physical injuries to the fish. They were then stocked and maintained in large cement tanks containing chlorine free bore-well water. Before stocking, the tank was washed with 0.1% $KMnO_4$ to free the walls from fungal infections of dermal infection.

2.2 Experiment design for Toxicity studies

Well acclimatized 100 *C. mrigala* fishes, with an average weight of 7.5 gm. were selected from the stock and exposed to different concentrations of quinolphos individually for the state bioassay test. The experiments were conducted in 10 litre tanks with 10 fishes each, starved for 24 hrs. prior to the experiments for the maintenance of bio assay. The experimental medium was renewed daily till the end of the experiment. The mortality of fishes in different concentration was noted at 12, 24, 48, 72, 96 and 120 hrs, and the dead animals were removed immediately. LC_{50} values of quinolphos was computed using software by transforming mortalities (percentage values) into probit scale (Finney, 1971) [8]. Simultaneously ten fishes were reared in pesticide-free medium and are treated as control.

2.3 Statistical Analysis

Per cent mortality was calculated and the values were transferred into probit scale. Probit analysis was carried out as suggested by Finney (1971) [8]. Regression lines of probit against logarithmic transformations of concentrations were made. Confidential limits (upper and lower) of the regression line with chi-square test were calculated by a computerized programme for Finney's probit analysis.

3. Results and Discussion

The present study involved the acute toxic effects of mercury on freshwater fish, *C. mrigala*. The percentage of mortality was found to be increased with the increase in the concentration of pesticide quinolphos. The minimum mortality rate (20%) was recorded at lowest concentration (0.120 ppm) of quinolphos when *C. mrigala* was reared to 120 hrs exposure, whereas the highest concentration (0.136 ppm) of quinolphos produced 100% mortality at 24 hrs. exposure (Table 1)

The LC_{50} values of quinolphos to *C. mrigala* ranged from 0.124 (120 hrs) to 0.140 ppm (12 hrs) and they are summarised in Table 2. Based on the regression equation fitted for log dose and probit mortality "a" values (Y intercept) ranged between -0.005 (12hrs) and a 0.008 (48 hrs) While "b" values (regression coefficient) varied between 4.460 (24 hrs) and 4.541 (96 hrs). The lower and upper fiducial limits were obtained for each LC_{50} and the highest deviation was recorded for 120 hrs exposure (0.122 and 0.128 ppm). Using chi-square test, the deviation between empirical and expected probabilities was also tested and each value was compared with critical values at appropriate degree of freedom at 0.05 probability levels. In all the cases the chi-square value was statistically not significant. The LC_{50} values of quinolphos with respect to *C. mrigala* in 12, 24, 48, 72, 96, 120 hrs exposures are presented in fig.1.

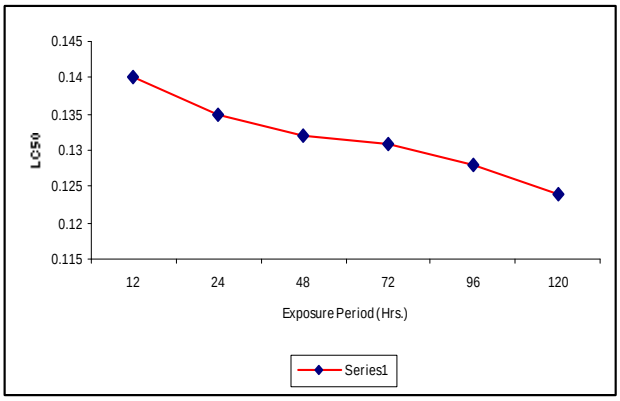

Fig 1: LC_{50} Values of Quinolphos to *C. mrigala*

Table 1. Percentage mortality of *C-mrigala* exposed to different concentrations of quinolphos for various exposure periods

Concentrations of quinolphos (ppm)	Exposure (hours)					
	12	24	48	72	96	120
Control	0	0	0	0	0	0
0.080	0	0	0	0	0	0
0.090	0	0	0	0	0	10
0.100	0	0	0	0	10	10
0.120	0	0	0	0	10	20
0.122	0	0	0	10	20	30
0.124	0	0	10	20	30	40
0.126	0	10	30	40	50	60
0.128	0	30	40	50	60	80
0.130	10	40	50	70	80	100
0.132	20	50	70	80	100	
0.134	30	70	90	100		
0.136	50	100				
0.138	60	100				

Table 2: Toxicity parameters of quinolphos to *C. mrigal* to log-dose/probit regression analysis

Exposure period (Hrs)	LC_{50} (ppm)	Regression equation	Regression co efficient	95% fiducial limits		Chi-square test	
				Lower (LFL)	Upper (UFL)	X^2	Critical value
12	0.140	Y: -0.005+4.226 X	4.225	0.138	0.142	0.08	7.81
24	0.135	Y: -0.008+4.460 X	4.460	0.133	0.137	3.25	11.07
48	0.132	Y: -0.008+4.482 X	4.482	0.130	0.134	0.70	9.49
72	0.130	Y:0.009+4.496 X	4.496	0.128	0.132	1.82	12.59
96	0.128	Y: 0.010 + 4.541 X	4.541	0.126	0.130	2.09	12.59
120	0.124	Y: 0.010+4.588	4.588	0.122	0.128	2.11	12.59

In the present study, the toxicity tests were conducted to evaluate the acute toxicity of quinolphos on the freshwater fish, *C. mrigala.* Quinolphos exerted lethal effect on *C. mrigala.* The mortality rates increased with the increase in exposure period with a decrease of LC_{50} value. The decrease in LC_{50} value suggested decreasing resistance of the fish with increasing exposure duration, as signified by the co-efficient of the fitted regression equation. Ravikrishnan *et al.* (1997) [17] have also reported exactly similar findings in toxicity studies involving organophosphorous pesticides with freshwater fishes.

The 96 hrs LC_{50} values of quinolphos to *C. mrigala* was 0.128 ppm. Organophosphate was found to be toxic for freshwater fishes as evident in early studies. The LC_{50} values for monocrotophos and dichlorvos to *Cirrhinus mrigala* were 10.1 ppm and 10.40 ppm respectively (Rajamannar and Manohar, 1992) [16]. For *Channa striatus* the LC_{50} value for methyl parathion was 330 ppb. Further, the acute toxicity of malathion to *Heteropneustes fossilis* was 8.50 ppm. for dimethyl parathion to *Labeo rohita* was 11.00 mg/l. The 96 hrs LC_{50} values recorded for *Gambusia affinis* on phosphamidon was 3.0 mg/l, for quinolphos and padan to *L. rohita* were 1.61 ppm and 0.70 ppm respectively Anatasi *et al* (1980) [3]. Similarly the 96 hrs LC_{50} values recorded for metasystox and glyphosate to *H. fossilis* were 127.0 mg/l and 50.0 mg/l Hemant and Gupta, (1997)[10], for nuvan and dimecron to *Channa orientalis* were 2.66 and 5.54 mg/1 (Saxena *et al.*, 1996)[11], for nuvan to *C. Batrachus* was 1.58 mg/12 (Trivedi and Saxena, 1999) [23], for profenofos to rainbow trout was 0.025 mg/l and blugill sunfish 0/3 ppm for sicocil to *Mystus vittatus* was 2.687 ppm and for curacron to *Cyprinus carpio* was 0.134 ppm (Sornaraj, R, 2000) [22].

It has been pointed out that the toxicity of a pesticide can be modified by various factors including the physico-chemical characteristics of the medium, and the biological behaviours and status of test animal (Holcomb *et.al.*, 1982) [11]. The status of test fishes includes size, weight, age, sex and life cycle stage. The physico-chemical factors such as temperature, pH, alkalinity and hardness also influence the toxicity (Veeraiah, *et al.*, 2013) [24].

The impact on fish may be passed on to other trophic levels inflicting a wider range of damages. Since quinolphos is more toxic it is advisable not to apply the pesticide on wet lands. The acute toxicity test provides rapid, cost efficient way to measure relative toxicity in different types of water. Toxicity is the characteristic of an individual organs response to a chemical at a particular concentration or dosage for a specific period of time. The application of LC_{50} values has gained acceptance among toxicologists and is generally the most highly related tests for assessing the potential advance effect of aquatic life. The LC_{50} values differ from species to species for the same pesticide and different pesticide due to their mode of action.

Toxicity curve is generally plotted to understand the mode of action of the pesticides. The shape of the curve denotes the nature of the pesticides action which has either a cumulative or a regular or irregular impact. The LC_{50} values are useful measure of acute toxicity of tested pesticide used under certain environmental conditions, but do not really represent concentration may be safe or harmless to fish habitats subjected to pollutant discharges. The concentration which was harmless to the fish within 96 hrs may be very toxic under condition of continuous exposure in a receiving water. Therefore the safe factors in an application factor should be used. (Muthukumaravel, 2013) [13].

4. Conclusion

The present work records the observations on the toxicity studies of quinolphos on freshwater fishes, *Cirrhinus mrigala* (Ham.) under control conditions in the laboratory. The important results obtained from the experiment is that the percentage of mortality of *C. mrigala* increased with increase in concentration of pesticide, quinolphos as well as increase in exposure duration. By applying to log-dose probit analysis,

the LC_{50} values for quinolphos was calculated in *C. mrigala*. The 12, 24, 48, 72, 96 and 120 hrs LC_{50} values ranged from 0.0140, 0.135, 0.132, 0.130, 0.128, and 0.124 ppm. The Chi-square values obtained for the selected exposure duration were not statistically significant ($P>0.05$). In the light of this study comprising bio-assay, it is clearly evident that quinolphos are toxic chemicals to fishes. To sum up, these findings recommend that farmers should avoid indiscriminate use of this pesticide, quinolphos and they should take great care in making the agricultural activities.

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