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Studies on acute toxicity of glyphosate (Glycil 41%SL) impact on freshwater fish *Cirrhinus mrigala*

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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 non target organisms and undesirable perturbations in the environment. The result of the present work i.e., observed percentage mortality of species of freshwater fish *Cirrhinus mrigala* when exposed to the Glyphosate (41%SL.) time periods 24, 48, 72 and 96 h in static system toxicity is in the range of 3.757 mg/L, 3.461 mg/L, 3.15 mg/L and 3.043 mg/L**,** respectively. In the present investigation, the test species *Cirrhinus mrigala* has shown differential toxicity level with a function of period. 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. When fish were exposed to sublethal concentration of Glyphosate for 96 h. several behavioural changes were observed which include loss of schooling behaviour, swimming near the water surface, hyper activity, erratic movements, elevated cough, restlessness before death, darting movements and hitting against the walls of test tanks were noticed in the fish tested. A film of mucus was also observed all over the body and also on the gills.

Keywords: Glyphosate, *Cirrhinus mrigala*, lethal concentrations, sublethal concentrations, behavioral changes

1. Introduction
Glyphosate is the most widely used agrochemical products which is an herbicide. Glyphosate, a non-selective post emergent herbicide with systemic action. The herbicides are intended for certain plant organisms, the net result of their use is accumulation in large amounts in the environment (for example, the soil, aquatic, biotic and atmospheric systems). The potential effects of herbicides are strongly influenced by their toxic mode of action and killing a wide variety of plants, growth regulator, used in smaller quantities.
In turn, have been reported for adults in various observational studies of direct poisoning [1] and in the single study that analyze in a very specific setting, the causal impact of exposure to glyphosate on health outcomes[2]. So glyphosate was considered an advantageous herbicide until its use led to the evolution of glyphosate-resistant weeds [3] and studies suggesting effects of glyphosate-based formulations in humans and wildlife.
Nowadays, the most used herbicide in the world, glyphosate was discovered by Dr. John E. Franz of Monsanto in 1971 and first commercialized in 1974, under the name RoundUp. It is a systemic, post-emergence, non-selective, foliar applied herbicide. This means that it is used after the emergence of weeds, it is absorbed by the exposed plant and translocated through the whole plant, thereby it can affect any kind of plant [4].
The commercial product contains in addition to glyphosate, a cationic surfactant called polyoxyethylene (POEA), to increase the efficiency of glyphosate [5]. Commercial formulations are more toxic than glyphosate alone [6] a non-ionic surfactant, is added which gives the product toxicological properties different from those of glyphosate. It controls mites, thrips and sucking pests effectively on all crops. It is primarily an irritant to skin, eyes and respiratory tract and also affects the normal metabolism. Fishes are very sensitive to a wide variety of toxicants in water, various species of fish shows uptake and accumulation of many contaminants or toxicants such as herbicides [7].
In the Present study acute toxicity tests for 24, 48, 72 and 96 h to the fish, *Cirrhinus mrigala* were conducted with a Glyphosate (41% SL). The static 1/10th of 96 h LC_{50} value was taken as sublethal concentration to study the behavioral alterations and physiological alterations

(as per the recommendations of committee on toxicity studies).

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 predominant and are freshwater fish, *Cirrhinus mrigala.* The present study was aimed to investigate the toxicity and behavioural impact due to glyphosate on freshwater fish, *Cirrhinus mrigala.*

2. Materials and Methods

The freshwater fish *Cirrhinus mrigala* (Hamilton) is an edible and commercially valuable fish. Live fish of size 6-7± ½ cm and 6-8± ½ g weight were brought from a local fish farm and acclimatized at 28 ± 2°C in the laboratory for one week. During acclimatization period, if 5% mortality is observed the total batch was discarded.

2.1 Preparation of stock solution

The Stock solution is prepared by dissolving the pesticide in acetone. Controls were also kept which received the solvent, acetone equal to concentration used in the test.

2.2 Selection of sublethal 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, 1/10th of 96 h LC_{50} and 96 h LC_{50} were selected as sub lethal and lethal concentrations respectively to study the effects.

2.3 Experimental set up

The ground water used for acclimation and conducted experiments in clear and un-chlorinated water. The physical and chemical properties of water were recorded.

2.4 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, 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 dead fish were removed immediately. The data were recorded from these tests at the end of each specific time period. Finney's Probit analysis [8] as reported by Roberts and Boyce [9] 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 glyphosate was determined at 24, 48, 72 and 96 h exposure. For this experiment fish were divided into batches of 10 each and were exposed to different concentrations of 2.6 mg/L to 3.2 mg/L of glyphosate (41% SL) formulation.

This range was obtained on trial and error basis of toxicity evaluation was carried out in static water [10] and mortality rate was observed at all concentrations of glyphosate 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.

3. Results and Discussion

The results of the present work with reference to observed percent mortality of freshwater fish *Cirrhinus mrigala* when exposed to the concentration Glyphosate for time periods 24, 48, 72 and 96 h and the lethal concentration, i.e., LC_{50} Values for 24, 48, 72 and 96 h (Table 1 - 4). The results of static 24, 48, 72 and 96 h, Percent Mortality and Probit Mortality were graphically represented in Figure 1 - 4.

The present LC_{50} values of glyphosate to the fish *Cirrhinus mrigala* are compared to the degree of harmfulness given by Kannan (1997) [11], it was observed that the pesticide glyphosate is extremely toxic to the fish. The results of the present work i.e., the observed percentage mortality of fish under exposure to glyphosate 41% SL for 24, 48, 72 and 96 h to freshwater fish, *Cirrhinus mrigala* in static system were noted.

In the present study, as per the Table 5, the toxicity is in the range of 3.757 mg/L, 3.461 mg/L, 3.15 mg/L and 3.043 mg/L, in static tests for 24, 48, 72 and 96 h respectively. During the investigation, the test species *Cirrhinus mrigala* has shown differential toxicity level to different periods. With the increase of period of exposure i.e., 96 h, the fish showed mortality at less concentration and with decrease of period of exposure, 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* [12]. The LC_{50} values 1.2, 0.5, 0.4 and 2.2 ppm were reported for the Brown trout (*Salmo trutta*), Rainbow trout (*Salmo gairdneri*), Sardinius erythroptholmus and *Tilapia nilotica* respectively reported by Stephenson (1982) [12]; 0.96, 0.84, 0.62 and 0.57 ppm for 24, 48, 72 and 96 hours respectively to the fish *Lepidocephalychthys thermalis*.

The 48 h LC_{50} for technical grade glyphosate to freshwater invertebrates is ranging from 55 ppm to 780 ppm [13]. The 48 h LC_{50} for Daphnids as 3.0 mg/L and for mid-larvae as 16 mg/L when exposed for formulated product according to Folmar (1979) [14].

The determination of Glyphosate toxicity may be influenced by exposure conditions, formulation and size of fish and water quality. It was reported that the static values of LC_{50} higher than the continuous flow-through systems. The higher values are in the earlier reports of Anita Susan (1994), Luther Das *et al.* (2000) and Tilak *et al.* (2001) [15, 16, 17].

In the present investigation, when fish were exposed to sublethal concentration of glyphosate for 96 hours, several behavioral changes were observed which include erratic swimming movements and they appeared to be in distress. During the first four days opercular movements were quicker and the fish surfaced more frequently gasping for air but subsequently this breathing difficulty seemed to have subsidized. Hyper excitation, loss of equilibrium, increased cough rate, flaring of gills, increase in production of mucus from the gills, 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 chloropyrifos (Tilak *et al*., 2001) [17].

The symptoms induced by the synthetic pyrethroid insecticides in fish can be attributed to an increase in physiological stress. Physiological stress has occurred in the form of neuronal excitation, which apparently has resulted in the continuous synthesis and destruction of neurotransmitters and enzymes.
The morphological and behavioural changes exhibited by the fish can be taken as useful parameter as a bio-indicator in assessing the extent of aquatic pollution by them. The behavioral changes of the organism are an index of its physiological, biochemical motivational and environmentally influenced organism. The fish behavior in laboratory can be sensate marker of toxicant-induced stress (Atchison *et al*., 1987; Little *et al*., 1985; West Lake, 1984) [18, 19, 20]. Alterations in the chemical composition of the natural environment usually lead to cause effects on behavioral and physiological systems of the inhabitants, particularly of the fish.

Table 1: The observed percentage of mortality and probit mortality of the fish *Cirrhinus mrigala* exposed to Glyphosate (41% SL) for 24 hour

Hours of Exposure	Concentration	Mortality	Probit Mortality
24	3.2	10	3.7184
24	3.4	20	4.1584
24	3.6	40	4.7467
24	3.8	60	5.2533
24	4.0	70	5.5244

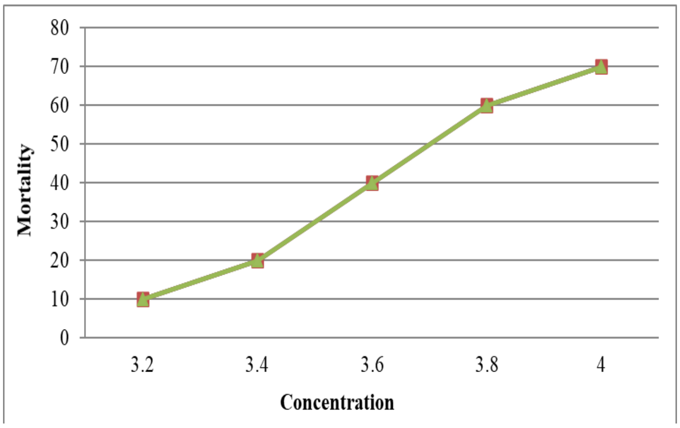

Fig 1: The graph showed percentage of mortality and probit mortality of *Cirrhinus mrigala* exposed to Glyphosate (41%SL) for 24 hour

Table 2: The observed percentage of mortality and probit mortality of the fish *Cirrhinus mrigala* exposed to Glyphosate (41%SL) for 48 hour

Hours of Exposure	Concentration	Mortality	Probit Mortality
48	3.0	10	3.7184
48	3.2	20	4.1584
48	3.4	40	4.7467
48	3.6	70	5.5244
48	3.8	80	5.8416

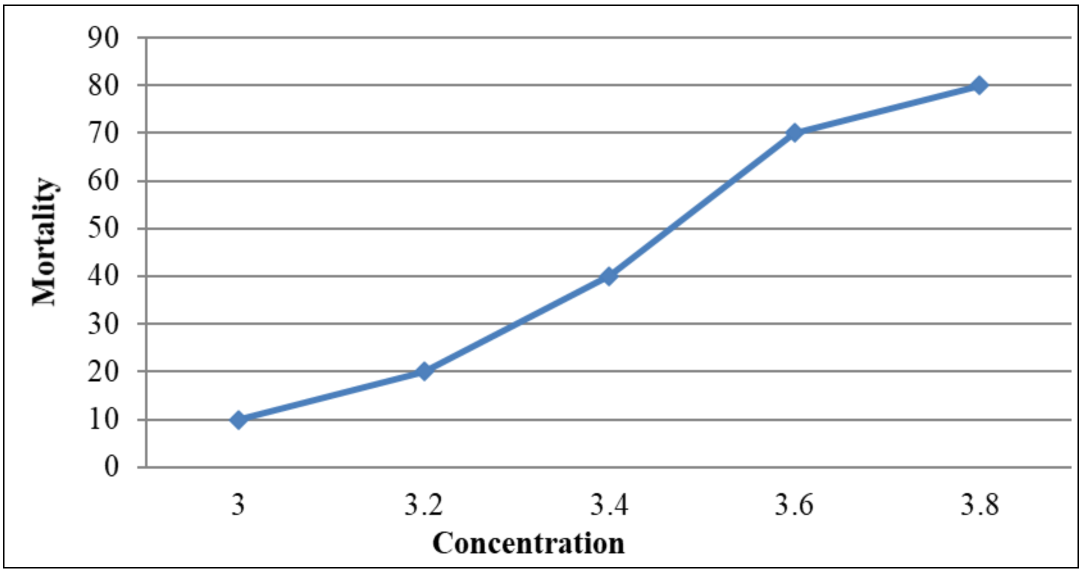

Fig 2: The graph showed percentage of mortality and probit mortality of *Cirrhinus mrigala* exposed to Glyphosate (41%SL) for 48 hour

Table 3: The observed percentage of mortality and probit mortality of the fish *Cirrhinus mrigala* exposed to Glyphosate (41%SL) for 72 hour

Hours of Exposure	Concentration	Mortality	Probit Mortality
72	2.8	10	3.7184
72	3.0	30	4.4756
72	3.2	50	5.0000
72	3.4	60	5.2533
72	3.6	80	6.2816

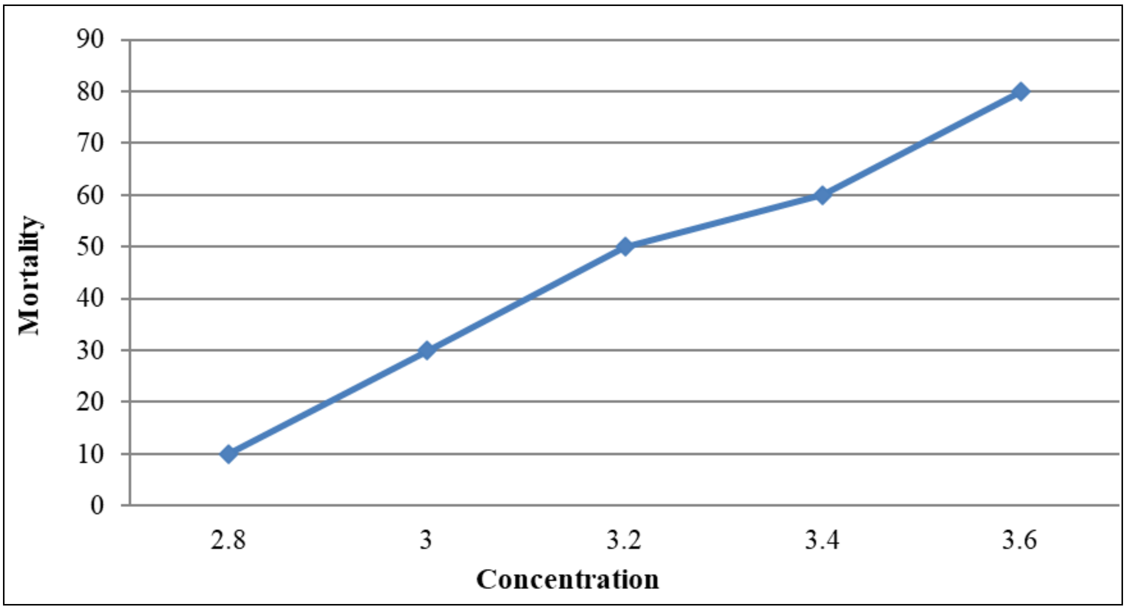

Fig 3: The graph showed percentage of mortality and probit mortality of *Cirrhinus mrigala* exposed to Glyphosate (41%SL) for 72 hour

Table 4: The observed percentage of mortality and probit mortality of the fish *Cirrhinus mrigala* exposed to Glyphosate (41%SL) for 96 hour

Hours of Exposure	Concentration	Mortality	Probit Mortality
96	2.6	10	3.7184
96	2.8	30	4.4756
96	3.0	50	5.0000
96	3.2	60	5.2533
96	3.4	80	5.8416

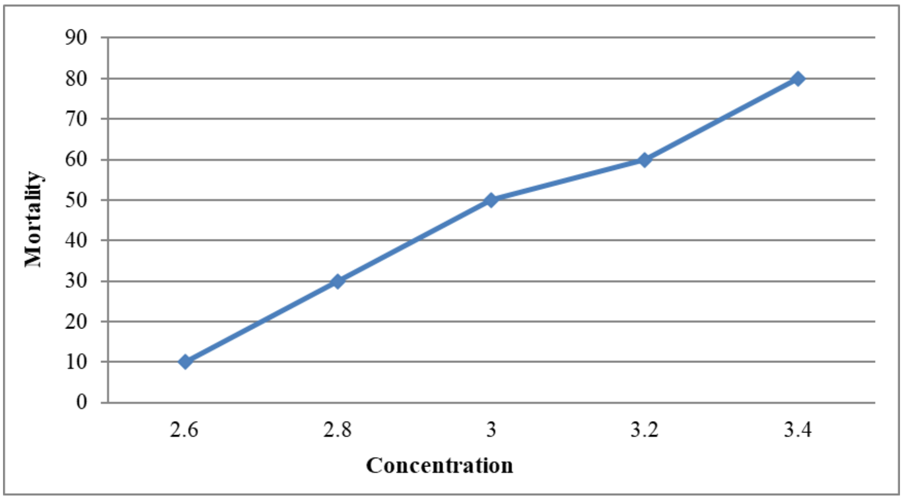

Fig 4: The graph showed percentage of mortality and probit mortality of *Cirrhinus mrigala* exposed to Glyphosate (41%SL) for 96 hour

Table 5: The 95% Confidence Levels of Glyphosate (Glycil 41%SL) 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
24 Hours	Static System	3.757	3.993	3.207
48 Hours	Static System	3.461	3.793	3.007
72 Hours	Static System	3.150	3.593	2.807
96 Hours	Static System	3.043	3.393	2.607

4. Conclusion

Toxicity experiments for Glyphosate (Glycil 41% SL) formulation conducted using static system for 24, 48, 72 and 96 h. That the percentage of mortality increased with the increase concentration of glyphosate. 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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