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Lethal Toxicity of Glyphosate Herbicide on Koi Carp, *Cyprinus carpio* (Linnaeus, 1758) Fingerlings

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Abstract

Bioassay experiments were conducted to determine the lethal toxicity of glyphosate herbicide on Koi carp, *Cyprinus carpio* fingerlings. Koi carp fingerlings with mean length 8.06 ± 0.99 cm were obtained from a freshwater fish seed hatchery of the university. The fishes were exposed to different concentrations of glyphosate and mortality was recorded after every 6 h for a period of 96 h. The 96 h LC_{50} concentration for glyphosate on Koi carp fingerlings was found to be 33.2 mgL^{-1}. The results indicated that glyphosate had impacts on exposed fish, hence, the need for regulation of usage to protect non-targeted species and the environment.

Keywords: Bioassay, Glyphosate, Herbicide, Koi Carp, Lethal Toxicity

1. Introduction

The beginning of the green revolution brought with it the use of herbicides to protect crops and enhance agricultural yields to feed the ever growing population. The first selective herbicide to be introduced was 2,4-D (2,4-dichlorophenoxyacetic acid) during the year 1940. Following its introduction, a number of effective herbicides have been manufactured which have allowed agricultural producers in developed countries to increase yield of agricultural crops. [1]

Glyphosate, an isopropyl amine salt of N–(phosphonomethyl) glycine is one of the most important post-emergent herbicides used in agriculture and industry today. Numerous commercial formulations containing glyphosate as the active ingredient have become popular around the world due to their effective action and low toxicity to mammals. It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation in susceptible plants.[2]

Asia-origin Koi carp are currently listed among the most important ornamental species as they can be reared in all countries throughout the world.[3] They are a high value ornamental fish which fetch a good price in the market. The fish is also used as food fish when caught from the wild. In the wild, males mature after 2 years and females mature after 3 years. Breeding of the species is induced by temperature and rising of water level during rainfall or flooding event.

Toxicological study on the effects of glyphosate on the aquatic environment are needed to ensure sustainability of Koi carp since mostly growth is conducted in ponds and brood stock for breeding are captured from the wild.

The current study was carried out to determine the lethal toxicity (LC_{50}) of glyphosate herbicide on Koi carp, *Cyprinus carpio* fingerlings.

2. Material and methods

Koi carp fingerlings with mean length 8.06 ± 0.99 cm were collected from a freshwater fish seed hatchery of the university at Sindhudurg district. The fishes were acclimatized for a period of two weeks prior to the start of the bioassay experiments. Water temperature and pH

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were recorded daily and ranged from 28 to 29 °C and 6.5 to 7.0 respectively. Feeding was done twice a day during the acclimatization period using commercial pelleted feed.

Glyphosate 41% SL herbicide (Trade name: CEDAAR) was procured from a local agro-dealer in Ratnagiri market. Its chemical name is (N-(phosphonomethyl)-glycine). The test liquid was measured using a volumetric pipette for making stock solution. The test solution was prepared immediately prior to commencement of each experiment

Range finding tests were conducted to determine concentration range in which the LC_{50} for glyphosate on Koi carp fingerlings was expected to be found. The concentrations were chosen using the geometric scale which were 0.0001, 0.001 0.1, 1.0, 10 and 100, etc (mgL^{-1}) for toxicants such as heavy metals and for liquids such as pesticides, herbicides and fungicides.[4] Controls i.e. without the herbicide were also set for comparison purposes.

Ten healthy Koi carp fingerlings were randomly selected and introduced in tanks (40 L capacity) with different concentrations. Mortality was recorded every 6 h from the start of the experiment up to 96h. Mortality was calculated as follows;

$$\text{Mortality of fish (\%)} = \frac{\text{No.of fish dead in aquaria}}{\text{Total No.of fish in aquaria}} \times 100 \qquad (1)$$

The lethal toxicity bioassay was conducted based on the range obtained in the range finding tests. Controls i.e. without herbicide were also set during each experiment for comparison. The median lethal concentration (96 h LC_{50}) was calculated using probit analysis.

3. Results and discussion

In 10 and 100 mgL^{-1} concentrations, 30 and 100% mortalities respectively were observed. Based on the geometric scale experiment results, the second range finding bioassay experiment using, 20, 30, 40, 50, 60, 70, 80 and 90 mgL^{-1} concentrations. Cent-per-cent mortality was observed from 50 to 90 mgL^{-1} concentration with no mortality from 20 to 40 mgL^{-1}. Third bioassay test involved, 40.0, 42.5, 45.0, 47.5 and 50 mgL^{-1} concentrations. Cent-per-cent mortality was observed in all concentrations within 48 h. In the fourth experiment, 20, 22.5, 25.0, 27.5, 30.0, 32.5, 35.0 and 40 mgL^{-1} concentrations were tried. There was no mortality in concentrations of 20 to 30 mgL^{-1}. In the concentrations of, 32.5, 35.0 and 40.0 mgL^{-1}, mortalities of 30%, 70% and 100% respectively were observed.

The subsequent lethal toxicity bioassay was conducted using, 32, 33, 34, 35, 36 mgL^{-1} with 10 numbers of fish stocked in each tank in triplicate. In the concentrations of, 0, 32, 33, 34, 35, 36 mgL^{-1} mortalities of, 0, 30, 46.66, 63.33, 93.33 and 100% respectively were observed. (Table 1). After

Table 1. Mortality of Koi carp fingerlings during lethal toxicity bioassay

Time	Ctr	Ctr	Ctrl	32	32	32	33	33	33	34	34	34	35	35	35	36	36	36	
0:00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 hrs
6:00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
12:00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
6:00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
12:00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24 hrs
6:00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
12:00	-	-	-	-	1	-	2	-	-	-	2	-	-	1	-	-	-	1	
6:00	-	-	-	-	-	-	2	1	-	1	-	-	-	-	2	-	1	6	
12:00	-	-	-	1	2	1	2	1	-	1	-	-	1	-	-	1	1	3	48 hrs
6:00	-	-	-	2	-	1	-	-	-	-	-	-	-	2	6	2	3	-	
12:00	-	-	-	-	-	-	-	-	-	1	-	1	4	4	2	4	1	-	
6:00	-	-	-	-	-	-	-	-	2	-	2	-	3	-	-	2	2	-	
12:00	-	-	-	-	1	-	1	-	-	2	1	2	1	-	-	1	-	-	72 hrs
6:00	-	-	-	-	-	-	-	-	1	-	-	2	1	1	-	-	-	-	
12:00	-	-	-	-	-	-	-	1	-	2	1	-	-	-	-	-	-	-	
6:00	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	2	-	
12:00	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	96 hrs

probit analysis calculations, a concentration of 33.2 mgL^{-1} was determined as the LC_{50} value of glyphosate for Koi Carp fingerlings (Figure 1).

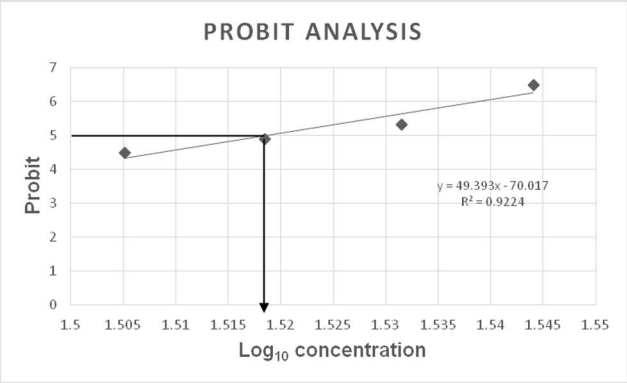


Figure 1. 96 hrs LC_{50} of fingerlings of Koi carp exposed to glyphosate herbicide**.**

Studies on the 96–h LC_{50} of glyphosate for *Huso huso*, *Acipenser stellatus* and *Acipenser persicus* reported that were 26.4, 23.2 and 27.5 mgL^{-1} respectively[5] are in line with the present study. The LC_{50} for Atlantic salmon was found to be 13.69 mgL^{-1} [6] and the 96 h- LC_{50} values of Roundup for rainbow trout (*Oncorhynchus mykiss*) of 28 mgL^{-1} and 42 mgL^{-1} for Atlantic salmon (*Salmo salar*).[7] However, it has to be noted that values vary depending on the test conditions, species and size of the fish and the general health of the test organisms.

4. Conclusion

From the forgoing account, it has indicated that glyphosate had effects on Koi carp fingerlings. Erratic swimming, abnormal swimming patterns and death were the effects observed within 96 h of exposure. Due to its impacts on fish, use of glyphosate should be regulated to protect fish and other non-targeted organisms. The herbicide can be classified as moderately toxic if used at low concentrations. The results also indicated that toxicological impacts of glyphosate are dose dependent with high mortalities being observed at high concentration.

5. References

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