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“Investigate The LC_{50} Values And Toxic Effect Of Glyphosate On The Fresh Water Fish, Labeo Rohita In In Sarju River Of Gonda, Uttar Pradesh”

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Abstract:

The present study was undertaken to evaluate the toxicity and effects of a commercial formulation of the herbicide, Glyphosate on liver enzyme system in the freshwater air breathing fish Labeo rohita. The 24, 48, 72 and 96 h LC50 of Glyphosate, calculated by Direct interpolation Method, were determined to be 0.018ml/liter, 0.015ml/liter, 0.012 ml/liter and 0.009 ml/liter respectively. In addition to concentration and timedependent decrease in mortality rate, stress signs in the form of behavioural changes were also observed in response to the test chemical.

Keywords: Glyphosate, toxicity, Labeo rohita, oxidative stress, environmental pollution, LC50.

Introduction

The application of environmental toxicological studies on aquatic ecosystems is rapidly expanding, and fish are becoming indicators for the evaluation of the effects of toxic substances. Pesticides are one of the essential factors for increasing agricultural productivity, especially the herbicides the use of which is rapidly increasing every year round the world. Roundup (Glyphosate) is one herbicide that has been recently become too easily contaminate the aquatic ecosystem. This problem has attracted the attention of researchers all over the world (WHO, 2005) and research in this area has increased in the last few decades due to their extensive use of them in agricultural and non-agricultural settings and as a result, this herbicide is becoming a threat to the living organisms. Glyphosate, a widely used herbicide **(Ali Sani and Muhammad, 2016; and Adedeji and Okocha, 2012)**., Some surfactants that are present in the formulation of glyphosate are toxic to aquatic organisms and hence are unsuitable for aquatic use **(Okayi et al., 2010).** Glyphosate (Roundup ® 41% SL) on fingerlings of Cyprinus carpio and the calculated LC50 was 3.260 ppm and evaluated the histological and biochemical changes in liver of exposed fishes. **(Bawa et al., (2017))** has been studied for its potential effects on the enzyme activity of freshwater fish. When glyphosate enters freshwater ecosystems through agricultural runoff, it can impact fish in various ways, including altering enzyme activities. Here's an overview of its effects on enzyme function in fish. Cholinesterase is an enzyme involved in the transmission of nerve impulses. Glyphosate has been found to affect the nervous system of fish by inhibiting acetylcholinesterase activity. This inhibition can interfere with neurotransmission, potentially leading to neurological impairments or even death in more severe cases. The liver plays a crucial role in detoxification, and glyphosate's effects on liver enzyme activity can disrupt fish health. Research has shown that glyphosate exposure can affect liver enzymes like **SGOT, SGPT, ALP and ACP (Gould et al., 1976; Shahsavani et al., 2010).** Abnormal activities in these enzymes may indicate liver damage or metabolic disruptions caused by the herbicide.

Materials and Methods

The aquatic toxicity tests are frequently known as bioassay. These tests are used to detect and evaluate the potential toxicological effects of chemicals on organisms. Acute toxicity can be defined as the severe effect suffered by organisms from short – term exposure to toxic chemicals. These tests are designed to determine the dose or concentration of a particular test chemical that will produced a specific response/effect on a group of test organisms during a short-term exposure, under laboratory conditions. Experimentally, this is achieved by administering a chemical at different doses to a group of organisms and then observing the resulting mortalities in a set time periods like 24, 48, 72 and 96 hrs.

Collection of fishes and Acclimatization

During the whole toxicological and biochemical investigations, the fishes Labeo rohita were used. The fishes (wt. 124 ± 8g and size 19-28 cm) were collected from local fish market. The collected fishes were first treated with 0.2% some drops of potassium permanganate ($KMnO4$) Solution for 20-30 seconds (Herwig 1978) to check injury, diseases or infection. The fishes were acclimatized in the ordinary tap water in the plastic tank for 8- 10 days at temp. 29±5°C at P^H 7.2. The fishes were fed once a day on standard fish food. The feeding was stopped 24 hours before being used for bioassay test. For the toxicological experiments stalk solution was prepared by dissolving toxicants in distilled water. LC_{50} values were determined by the **Direct interpolation method: -** In acute toxicity test, an approximate LC_{50} can be initially determined as a pilot study by a so called 'staircase method' using a small number of fishes and increasing the dose of toxicant. No food was offered before 24 hrs of bioassay and during the experimental period. After acclimatization LC_{50} values were calculated by two exploratory tests and one definitive test.

Ist Exploratory test:- In first exploratory test, two concentrations (lower and higher) of toxicants were introduced in two separate aquarium , containing five fishes each to get supposed mortality between 0% to 100%.

IInd Exploratory test: - In second exploratory test or range finding test, four concentrations of toxicants were selected between the lower and higher concentrations of the first exploratory test and five fishes were exposed to each concentration for a period of 24, 48, 72 and 96 hrs.

Definitive test: - From the derivation of range finding examination seven different concentrations of the toxicants were selected for definitive test and ten fishes were exposed to each concentration and mortality data were observed after a period of 24, 48, 72 and 96 hrs. And dead fishes were removed when observed. Finally LC_{50} values were estimated by plotting a curve between percent mortality and concentrations of toxicants obtained from definitive test. A line was drawn between the point represent the percent mortality and concentrations. The concentration at which this line crosses the 50 % lethality line was the actual lethal concentration of toxicant.

Results

The LC_{50} values of Glyphosate were calculated by Direct Interpulation Method. In firstexploratory test no mortality occurred in 0.001 ml/liter of Glyphosate while 100% mortality wasobserved in 0.03 ml/liter within 24 hrs. exposure (Table 1). Results of second exploratory test are presented in Table 2. In this test 20% & 40% mortality occurred at 0.007ml/l after 72 &96 hrs, 20% , 40%, 60% and 80% mortality observed at 0.012ml/l concentration after 24, 48, 72 and 96 hrs respectively, where as 60%, 80% and 100% mortalities recorded at 0.019ml/l concentration after 24, 48, 72 and 96 hrs respectively but all the fishes died at 0.025ml/l after 24hrs. exposure. For Definitive test the mortality due to various concentrations between 0.008 ml/l to 0.022 ml/l is presented in Table 3. This table shows that 10%, 20% & 40% mortality was observed at 0.008 ml/l after 48, 72 and 96 hrs. Respectively. 20%, 40% and 60% mortality was observed at 0.010 ml/l after 48, 72 and 96 hrs. Respectively. 10%, 30%, 50% and 70% mortality was observed at 0.012 ml/l after 24, 48, 72 and 96 hrs. Respectively. 20%, 40%, 60% and 90% mortality was observed at 0.014 ml/l after 24, 48, 72 and 96 hrs. Respectively. 30%, 60%, 90% and 100% mortality was observed at 0.016 ml/l after 24, 48, 72 and 96 hrs. Respectively. 50%, 80%, 90% and 100% mortality was observed at 0.018 ml/l after 24, 48, 72 and 96 hrs. Respectively. 70% and 90% mortality was observed at 0.020 ml/l after 24 and 48 hrs. Respectively. 80% and 100% mortality was observed at 0.022 ml/l after 24 and 48 hrs. Respectively. but The curve was plotted between percent mortality and concentrations of toxicant. The lethal concentrations obtained at 50% mortality level were 0.018ml/liter, 0.015 ml/liter, 0.012 ml/liter and 0.009 ml/liter at 24, 48, 72 and 96 hrs (Fig.1&2).

Determination of LC_{50} values for Glyphosate on Labeo rohita

Table 1: First exploratory test

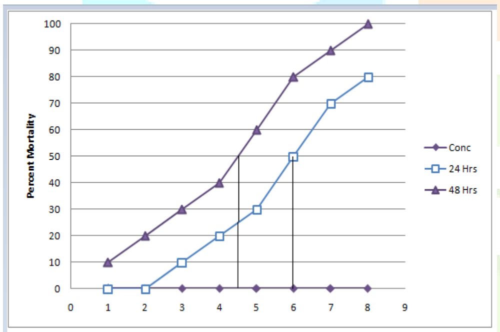
S. No.	Concentration ml/Liter	No. of fishes	24 hours		48 hours		72 hours		96 hours	
			M	%M	M	%M	M	%M	M	%M
1	0.001	5	0	0	0	0	0	0	0	0
2	0.030	5	5	100	-	-	-	-	-	-

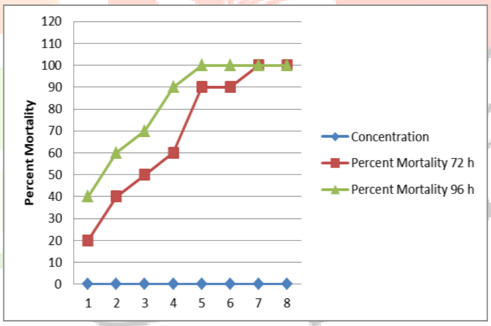
Table 2: Second exploratory test

S. No.	Concentration ml/Liter	No. of fishes	24 hours		48 hours		72 hours		96 hours	
			M	%M	M	%M	M	%M	M	%M
1	0.007	5	0	0	0	0	1	20	1	40
2	0.013	5	1	20	1	40	1	60	1	80
3	0.019	5	3	60	1	80	2	100	-	-
4	0.025	5	5	100	-	-	-	-	-	-

Table 3: Definitive test

S. No.	ncentration ml/Liter	No. of fishes	24 hours		48 hours		72 hours		96 hours	
			M	%M	M	%M	M	%M	M	%M
1	0.008	10	0	0	1	10	1	20	2	40
2	0.010	10	0	0	2	20	2	40	2	60
3	0.012	10	1	10	2	30	2	50	2	70
4	0.014	10	2	20	2	40	2	60	3	90
5	0.016	10	3	30	3	60	3	90	1	100
6	0.018	10	5	50	3	80	1	90	1	100
7	0.020	10	7	70	2	90	1	100	-	-
8	0.022	10	8	80	2	100	-	-	-	-





(B) Behavioral Response

The behavioral response of the fishes, Labeo rohita were recorded by exposing the teleost to acute concentrations of Glyphosate for 24,48, 72 and 96 hrs. The colours of the tested fishes appeared little pale as compared to the normal fishes. Hyper excitability followed by sluggishness was observed by the jerky and random movement of fishes just after the addition of toxicants. Excessive secretion of mucus through general body surface was also observed. Due to the low rate of oxygen uptake, fishes came to the surface to engulf air frequently.

Dicussion:

The results of the present work with reference to observed percent mortality of freshwater fish Labeo rohita when exposed to the concentration of 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 - 3). The results of static 24, 48, 72 and 96 h, Percent Mortality were graphically represented in Figure 1 - 2. The present LC_{50} values of glyphosate to the fish Labeo rohita are compared to the degree of harmfulness given by **Kannan (1997)** it was observed that the pesticide glyphosate is extremely toxic to the fish. The observed percentage mortality of fish under exposure to glyphosate for 24, 48, 72 and 96 h to freshwater fish, Labeo rohita in static system were noted. In the present study the toxicity is in the range of 0.018ml/liter, 0.015 ml/liter, 0.012 ml/liter and 0.009 ml/liter in static tests for 24, 48, 72 and 96 h respectively. During the investigation, the test species Labeo rohita 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, thefish exhibited mortality at higher concentration. 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**) 0.96, 0.84, 0.62 and 0.57 ppm for 24, 48, 72 and 96 hours respectively to the fish Lepidocephalychthys thermalis. The48 h LC_{50} for technical grade glyphosate to freshwater invertebrates is ranging from 55 ppm to 780 ppm. 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) . 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)** . 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).** Alterations in the chemical composition of the natural environment usually leadto cause effects on behavioral and physiological systems of the inhabitants, particularly of the fish.

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