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Glyphosate Induced Nucleic Acid Alterations in Freshwater Fish, Catla Catla

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ABSTRACT

The freshwater fish, *Catla catla* were exposed to lethal and sub-lethal concentrations of glyphosate (glycil SL 41%) to study the alterations in the nucleic acid (DNA and RNA) content of various tissues (gill, brain, liver, kidney and muscle) after exposure to 24, 48, 72 and 96 h LC_{50}. In static method the LC_{50} values were found to be 6.622, 6.546, 6.05 and 5.798 mg/L respectively for 24, 48, 72 and 96 h and in continuous flow-through system the LC_{50} values were 5.759, 5.374, 5.249 and 5.191 mg/L respectively for 24, 48, 72 and 96 h. In both the methods the LC_{50} values showed decreased trend with time of exposure and the decrease is more in continuous flow through method than in static method. A decline in the DNA and RNA content was observed and the percentage decrease is more apparent at lethal concentrations than at sub-lethal concentrations.

Key words:

Glyphosate; $LC_{50,}$ Nucleic Acids and *Catla catla*;

Academic discipline:

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INTRODUCTION

Her biocides are applied to water bodies to control aquatic weeds that impede irrigation withdrawals or interfere with recreational and industrial uses of water **[16]**. The potential effects of herbicides are strongly influenced by their toxic mode of action and their method of application. The molecular site of action is challenging to predict because structural associations have not been identified **[14],** but modes of action are well-established. Herbicides can act by inhibiting cell division, photosynthesis, or amino acid production or by mimicking natural auxin hormones, which regulate plant growth, and causing deformities in new growth **[40]**.

Glyphosate, a nonselective, systemic and post emergence herbicide used to control unwanted weeds, grasses in agricultural field, urban area, forestry and aquatic systems **[10].** Polyethoxylene amine (POEA), a non-ionic surfactant, is added to increase the efficiency of glyphosate **[9].** Commercial glyphosate formulations are more toxic than glyphosate alone **[3]; [34].** A review on toxicological data says that POEA is more toxic to fish than glyphosate alone **[19].**

Glyphosate may be introduced directly by spraying on aquatic weeds or indirectly into the aquatic environment through spillage, accidental discharge, wind erosion of treated fields, or waste disposal during production, storage, and use. In addition to killing weeds, glyphosate can be toxic to non-target organism like fish. According to **[1],** the biochemical analysis of DNA and RNA are considered as markers in the toxicity study. Considering the role of above biomarkers in the field of eco-toxicology, the present study has been undertaken to understand the nucleic acid changes induced by glyphosate on exposure to sub-lethal and lethal concentrations to fish *Catla catla* in different tissues exposed.

MATERIALS AND METHODS

The test fishes *Catla catla* were brought from the fish market, Guntur, Andhra Pradesh, India. The size of the fishes were 6-8 ±1/2 cm. The fish were acclimatized at room temperature (28+2^{o}C) in the laboratory conditions for two weeks. All the precautions laid down by **[4]** were followed. Physico-chemical properties of water used for experiment had temperature 28 ± 2^{0}C, pH at 28^{o}C 8.2, dissolved oxygen (mg/L) 8-10, total hardness (mg/l as $CaCO_3$) 320. Batches of 10 healthy fishes were exposed to lethal and sub-lethal concentrations of glyphosate. A batch of untreated fish served as controls.

Pilot experiments were conducted with 1 L capacity glass chambers, to choose the concentrations at which the fish are killed. For continuous flow through system, reservoirs of 90 liters capacity were used. The test water was let into test containers at a rate of 4 liters per hour using polyethylene drip nets with regulators and for every 12 h fresh test solutions were prepared in reservoirs.

Experiments were conducted to determine the toxicity of glyphosate in various concentrations within static and continuous flow through systems. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The toxic tests were conducted to choose the mortality range from 10% to 90% for 24, 48, 72 and 96 h in static and continuous flow through systems.

Finney's probit analysis **[17]** as recorded by **[39]** was followed to calculate the LC_{50} values. For the determination of the 95% confidence limits, LC_{50} values and a normal variant of 1.96 were taken into consideration. After the determination of $LC_{50,}$ the fish were exposed to sub lethal concentration (1/10th of 96 h LC_{50}) of glyphosate for four exposure periods i.e., 24, 48, 96 h and 8 days.

After the expiry of the exposure periods, the tissues like gill, brain, liver, kidney and muscle were taken out from treated and control fish and processed for the estimation of nucleic acids. The deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were estimated by the methods of **[41][42].**

Statistical analysis

The data obtained in the present work were expressed as means ± SD and were statistically analyzed using student t-test, **[35]** to compare means of treated data against their controls and the result were considered significant at (P<0.05) level.

RESULTS AND DISCUSSION

The LC_{50} values and 95 % Confidence limits of glycil (41% SL) for 24, 48, 72 and 96 h to *Catla catla* in static and continuous flow through systems were given in Table 1 and 2.

Table 1. Calculated LC_{50} values for 24, 48, 72 and 96 h for glycil (41% SL) in static and Continuous flow through methods to the fish *Catla catla.*

S. No.	Exposure Period	Static mg/L	C.F.M mg/L
1	24	6.622	5.759
2	48	6.546	5.374

3	72	6.05	5.249
4	96	5.798	5.191

Table. 2. 95 % Confidence limits of Glycil (SL 41%) exposed to *Catla catla* at different exposure periods in Static and continuous flow-through methods.

S. No.	Exposure Periods	95 % Confidence levels			
		Static method		Flow through method	
		Lower	Upper	Lower	Upper
1	24	33.71	86.29	18.47	77.53
2	48	24.55	87.45	24.55	87.45
3	72	26.14	81.86	13.99	85.99
4	96	16.29	75.71	16.29	75.71

In general, *Catla catla* is sensitive towards the test toxicant. These findings are in agreement with **[29]** on *Oncorhynchus mykiss;* **[30]** on *Cyprinus carpio;* **[32]** on *Ictalurus punctatus, Lepomis macrochirus;* **[22]** on *Oreochromis niloticus;* **[38]** on *Oreochromis sp;* **[20]** on *Rhambdia quelen;* **[27]** on *Prochilodus lineatus;* **[11]** on *Rhambdia quelen;* **[28]** *Prochilodus lineatus;* **[24]** on *Rhambdia quelen.*

The toxicity may be influenced by exposure conditions, formulation, source and size of fish and water quality. **[33]** reported the acute toxicity of glyphosate on fingerlings of *Claria gariepinus* and found 96 h LC_{50} value as 0.0018 ml/L. **[6]** elucidated the toxic effect of glyphosate on *Clarias gariepinus* and noticed the LC_{50} value for 96 h as 1.05 mg/L. **[31]** reported the LC_{50} value of Primextra on African catfish *Clarias gariepinus* for 96 h as 4.70 mg/L (lethal concentration). **[23]** found the LC_{50} value for 96 h as 0.18.

The continuous flow-through system LC_{50} values are low when compared to the static values. This is due to the constant maintenance of concentration in flow-through system and fluctuations in static system due to bioaccumulation, herbicide absorption to toxicant chamber walls and degradation of toxic effect of the compound.

The data obtained during the present work on the DNA and RNA content in different tissues like gill, brain, liver, kidney and muscle in both control and glyphosate exposed fish, *Catla catla* were represented graphically in fig. 1 and 2.

In the control fish, the DNA content was highest in the gill followed by muscle, kidney, brain and liver. Whereas, in exposed fish tissues the level of DNA was found to be highest in muscle followed by liver, kidney, gill and brain at sub-lethal exposure and highest in the muscle followed by brain, kidney, liver and gill at lethal exposure. The results indicated reduction in the DNA content in all the tissues of test fish compared to controls.

The RNA content in control fish was found to be highest in liver followed by gill, brain, kidney and muscle. In contrast to control fish, test tissues showed highest level of RNA in kidney followed by brain, muscle, liver and gill exposed to sub-lethal concentration and highest in the brain, muscle, kidney, liver and gill exposed to lethal concentration. Compared to controls the RNA level also showed decreased trend in all the test tissues.

In both DNA and RNA levels the decreasing trend was more pronounced in lethal concentrations than in sub-lethal concentrations.

The Nucleic acids play a vital role in maintaining the physiological configuration of the fish. Nucleic acid and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating different activities of cells. Nucleic acid content forms an index of capacity of an organism for protein synthesis. The decrease of RNA may be due to interference in the incorporation of precursor in the nucleic acid synthesis or inhibiting the function of RNA polymerase. **[13]** and **[7]** have suggested that the decrement of RNA may also be due to the noncoding for the process of protein synthesis, thereby decrease in the RNA content, which in turn would have reduced the concentration of RNA.

The decrease in nucleic acid content in the present study was in accordance with **[5]** in *Clarias batrachus* exposed to endosulfan. **[6]** in *Oreochromis mossambicus* exposed to quinolphos who also found decrease in the nucleic acid content. **[18]** reported histo-chemical changes in nucleic acids the test tissues of *Channa punctatus* after exposure to endosulfan and diazinon pesticides.

[44] observed significant reduction in the level of DNA and RNA in *Channa punctatus* exposed to butachlor (technical grade) and machete (50% EC) (commercial grade). A decline in the DNA content in liver of the fresh water fish, *Ctenopharyngodon idella* treated with biopesticide "Triology®" was observed by **[21]**. **[45]** noticed decreased level of DNA and RNA content in alachlor treated freshwater fish, *Channa punctatus* (Bloch). Reduced DNA and RNA content was

noticed in the liver of Freshwater fish, *Tor putitora* caught from polluted portion of River Kabul compared to control fish caught from non polluted Warsak Dam **[46]** of sub-lethal concentration of cypermethrin exposed to fresh water fish Channa striata on DNA and RNA level in the gill was studied by **[36]**. He found decline in the nucleic acid level of treated fish. **[43]** reported that, the freshwater fish *Channa striatus* exposed to sub-lethal concentration of cypermethrin showed a significant decrease in the level of DNA and RNA. **[2]** showed decreased level of DNA and RNA in gill, liver, and ovary after lambda-cyhalothrin and Neemgold exposure to zebrafish, *Danio rerio* (Hamilton). **[37]** found variations in freshwater fish, *Labeo rohita* in the nucleic acid content when exposed to quinalphos technical and 25% EC and found that the degree of variability in quinalphos technical was less compared to 25% EC and also dose dependent.**[26]** reported decreased level of DNA and RNA in copper sulphate exposed freshwater fish, *Clarias batrachus.*

The results of the present work disagree with Das and Mukherjee (2000), who found increased levels of DNA in the tissues of freshwater fish*, Labeo rohita* exposed to quinalphos*.* Kumar *et al.* (2007) observed elevation in the level of DNA and RNA in *Channa punctatus* treated with cypermethrin*.* The changes in DNA levels may be attributed to the disturbances caused in the normal synthesis and turnover rate of DNA besides degenerative changes.

The results of the present study suggest that, the exposed glyphosate compound is a potent inhibitor of DNA synthesis, which in turn results in the reduction of RNA level. The effects of sub-lethal and lethal concentrations of glyphosate on DNA and RNA contents show moderate toxicity on the main biochemical machinery of the freshwater fish *Catla catla.*

The above results indicate that the sub-lethal and lethal exposures of glyphosate affected the DNA and RNA contents in the tissues of the test fish *Catla catla*.

Conclusion

From the present study it can be concluded that the decline in the nucleic acid content is more pronounced in lethal exposure of the glyphosate than in sub-lethal exposure and also the alterations caused during herbicide exposure may be due to the decreased catabolism of the biomolecules to meet the energy demand of test organism under stress or their reduced synthesis due to impaired tissue function**.**

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Fig.1. Change in the amount of Deoxy ribonuclic acid (DNA) (mg/gram wet weight of the tissue) and % change over the control in different tissue of *Catla catla* on exposure to sub-lethal and lethal concentration of glyphosate (41% SL) for 96 h.

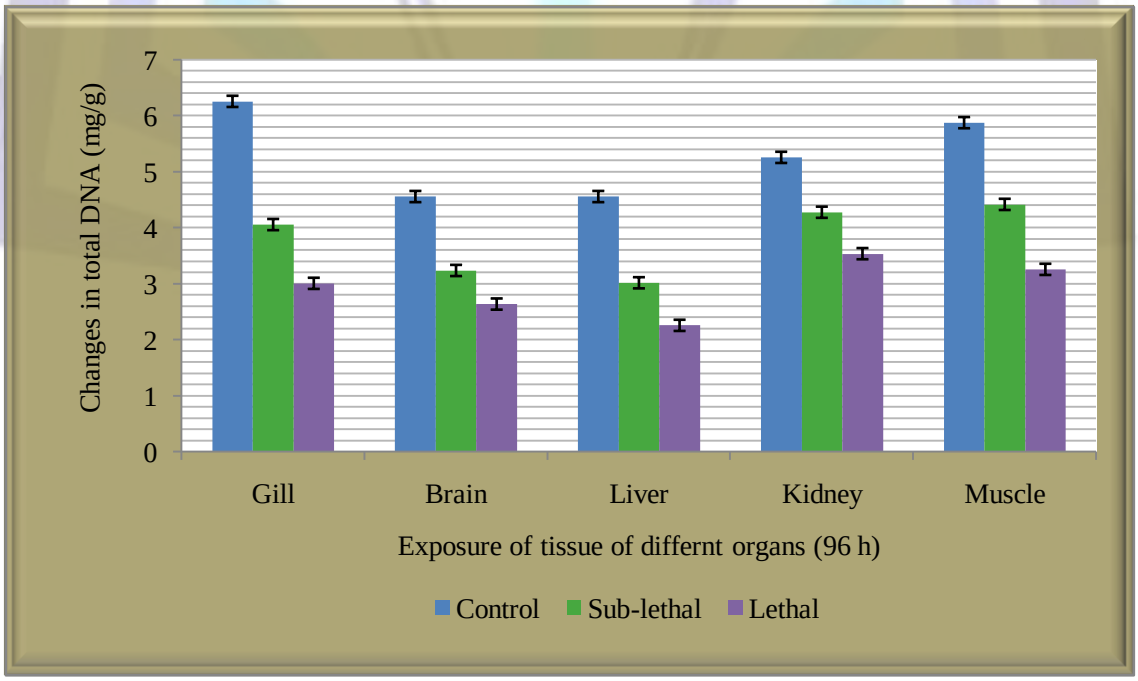

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Fig.2. Change in the Ribonucleic acid (RNA) (mg/gram body wet weight of the tissue) and % change over the control in different tissue of *Catla catla* on exposure to sub-lethal and lethal concentration of glyphosate (41 % SL) for 96 h.