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IMPACT OF GLYPHOSATE ON BIOCHEMICAL CONSTITUENTS OF THE FRESHWATER FISH, *CATLA CATLA*

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Abstract: The freshwater fish, *Catla catla* was exposed to the test toxicant glycil (SL 41%) (glyphosate), an organophosphate herbicide under static and continuous flow-through systems to determine the acute toxicity values for 24, 48, 72 and 96 h. The LC_{50} values obtained in static method were 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. After determination of LC_{50} values the fish were exposed to both lethal and sub-lethal concentrations of glyphosate to study the changes in biochemical constituents (glycogen and protein content) of the vital organs viz.; gill, brain, liver, kidney and muscle of the fish *Catla catla.* The 24, 48, 72 and 96 h LC_{50} value of glyphosate were found to be decreased in static and in continuous flow through system indicating a decrease with time of exposure and also the static LC_{50} values were found to be higher, compared to the continuous flow-through values. Depletion of total glycogen and protein contents were observed in all the tissues of the fish exposed to toxicant. The results obtained were discussed with the available literature.

Key words: LC_{50}; Glyphosate; Biochemical constituents; *Catla catla*

INTRODUCTION

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). These effects are amplified by the following phenomena: (a) high solubility of many classes of herbicides; (b) magnification of concentrations of herbicides upon entry into the food chain and successive concentration in lipids for higher species in that chain; and (c) persistence of many herbicides and transformation to other harmful metabolites upon residence in soil, water and biota.

N-(phosphonomethyl (glycine) is a nonselective, non-residual broad-spectrum, foliar applied herbicide used in the postharvest treatment of crops. It acts as a plant growth regulator, used in smaller quantities (RED Facts, 1993). It was one of the most widely used herbicides worldwide; its annual global use exceeded globally to 9, 07,000 tons in 2007 (Reuters, 2011). Glyphosate is classified as a Toxicity Category II (moderately toxic) herbicide by United States Environmental Protection Agency (USEPA). Commercial glyphosate formulations are more toxic than technical glyphosate (Peixito, 2005).

Herbicides reach the surface water through runoff or leach from treated plants and soil and cause biological impairment effecting fish and other aquatic organisms. Hence, the present study was undertaken to know the toxic effect of glyphosate on the total glycogen and protein content on freshwater fish, *Catla catla.*

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MATERIALS AND METHODS

The fish *Catla catla* with a size range of 6-8 ±1/2cm, irrespective of their sex, have been chosen as the test organism in the present study. The fresh water fish were brought from a local form and acclimatized to laboratory conditions for one week. Groundnut cake and rice bran used as feed to the fish during the period of acclimatization. In any batch during acclimatization, if 5% mortality is observed the total batch was discarded. All the precautions laid down by APHA (1998) were followed. Physico-chemical properties of water used for experiment had temperature 28 ± 2°C, pH at 28°C, 8.2, dissolved oxygen (mg/L) 8-10, total hardness (mg/l as $CaCO_3$) 320.

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 (Finney, 1971) as recorded by Roberts and Boyce (1972) 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 ($1/10^{th}$ of 96 h LC_{50}) of glyphosate for four exposure periods i.e., 24, 48, 96 h and 8 days.
In the present investigation, the level of glycogen and proteins were estimated in various tissues (gill, brain, liver, kidney and muscle) of fish exposed to lethal and sub-lethal concentrations of glyphosate besides the control fish. The glycogen was estimated by the method of Kemp *et al.,* (1954). Total protein content was estimated by the modified method of Lowry *et al.,* (1951). The mean, standard deviation (SD) and student's 't' test was calculated following the method of Pillai and Sinha (1968).

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 (41% SL) 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 Morgan and Kiceniuk, 1992 on *Oncorhynchus mykiss;* Neskovic *et al.,* (1996) on *Cyprinus carpio;* OPP 2000 on *Ictalurus punctatus, Lepomis macrochirus;* Jiraungkoorskul *et al.,* (2002) on *Oreochromis niloticus;* Relyea (2005) on *Oreochromis sp;* Glusczak *et al.,* (2007) on *Rhambdia quelen;* langiano and Martinez (2008) on *Prochilodus lineatus;* Cericato *et al.,* (2008) on *Rhambdia quelen;* Modesto and Martinez (2010) on *Prochilodus lineatus;* kreutz *et al.,* (2011) on *Rhambdia quelen.*

The toxicity may be influenced by exposure conditions, formulation, source and size of fish and water quality. Okayi *et al.,* (2010) reported the acute toxicity of glyphosate on fingerlings of *Claria gariepinus* and found 96 h LC_{50} value as 0.0018 ml/L. Ayanda and Egbamuno (2012) elucidated the toxic effect of glyphosate on *Clarias gariepinus* and noticed the LC_{50} value for 96 h as 1.05 mg/L. Nwani *et al.,* (2014) reported the LC_{50} value of Primextra on African catfish *Clarias gariepinus* for 96 h as 4.70mg/L (lethal concentration) (Kavitha and Binukumari, 2014) found the LC_{50} value for 96 h as 0.18.

Figure 1: Changes in the amount of Glycogen mg/g wet weight of the tissue in different tissue of the fish *Catla catla* exposed to glycil (glyphosate, 41% SL) for 96 h .

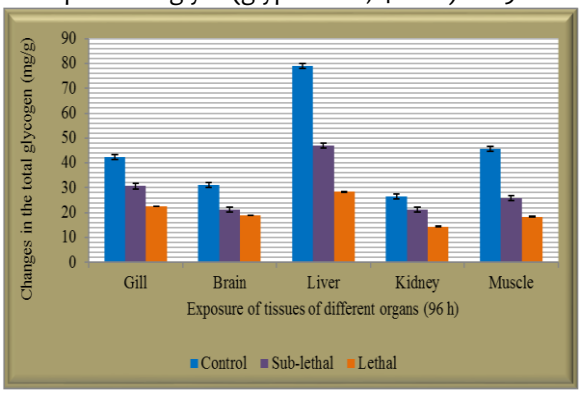
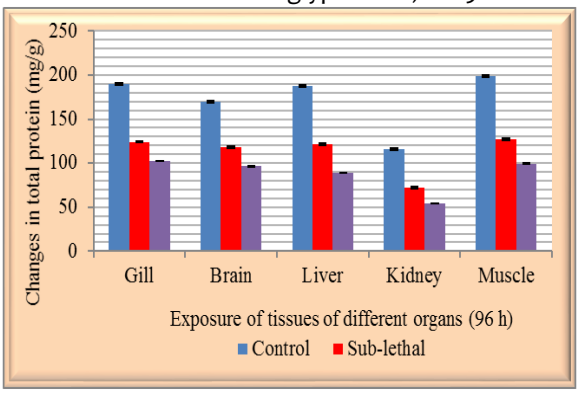


Figure 2: Change in the Protein content (mg/gram wet weight of the tissue) and % change over the control in different tissues of *Catla catla* on exposure to lethal and sub-lethal concentration of glyphosate, for 96 h.



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 results of the present study for glycogen in control and exposed fish were graphically represented in Figure 1.

Among the test tissues, higher glycogen content was observed in liver. Highest glycogen

content of liver is acceptable due to its involvement in glycogen synthesis and utilization. Glycogen is the major storage form of carbohydrate in animals which occurs mainly in liver and muscle. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of blood glucose. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself (Harper, 1985). Though brain tissue is metabolically active, lower glycogen content was observed, since it lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities (Lehninger, 1983).

The main storage form of polysaccharide in cells is glycogen. It is especially in liver of control fish the glycogen content was more and then follows muscle, gill, brain and kidney.

In the present study, the glycogen content was found to be highest in liver and lowest in the kidney after sub-lethal exposure and highest in liver and lowest in brain in lethal exposure. Decreased glycogen synthesis is attributed to the inhibition of the enzyme glycogen synthetase, which mediates glycogen synthesis (Stamp and Lesker, 1967). Pesticides are known to act on endocrine system (Edwards, 1973). Hence, it is assumed that decrease in glycogen content may be due to inhibition of hormones which contribute to glycogen synthesis. The depletion of glycogen may be due to utilization of stored carbohydrates in liver for energy production as a result of pesticide-induced hypoxia. A fall in total carbohydrate level clearly indicates its rapid utilization to meet the enhanced energy demands in pesticide treated animals through glycolysis or Hexose Monophosphate pathway (Cappon and Nicholas, 1975). Veeraiah, and Durga Prasad (1998), reported that the total glycogen levels of brain, liver, muscle, gill and kidney of *Labeo rohita* were decreased on exposure to sub-lethal concentration of cypermethrin. Abbas *et al.,* (2007) recorded a decline in the content of liver and muscle glycogen in *Oreochromis niloticus* treated with thiobencarb. Tilak *et al.,* (2009) noticed decrease in glycogen level in the tissues of fish *Channa punctatus* on exposure to alachlor. Gijare *et al.,* (2011) reported notable alteration in liver and intestine glycogen of *Ophiocephalus punctatus* exposed to sub-lethal concentration of cypermethrin. Significant decrease in liver glycogen level was reported by Ganeshwade (2011) when *Puntius ticto* was exposed to lethal and sublethal concentrations of dimethoate for 96h and 60 days duration. Israel Stalin and Sam Manohar Das (2012) reported Glycogen mobilization is maximum in the liver tissue under exposure to Lebaycid (Fenthion). The significant decrease of glycogen was observed in the liver, kidney and muscle tissues of *Oreochromis mossambicus* after exposure to dichlorvos (Lakshmanan *et al.,* 2013). Suneel kumar (2014) observed significant decrease in liver glycogen level in the fish *Channa punctatus* exposed to nuvan. In muscle tissue of *Labeo rohita*, carbohydrate level in control was 46.60 mg/g and it was decreased to 44.80, 30.92 and 27.11 mg/g in 0.398 ppm of Dimethoate 30% EC exposures for 24, 48 and 72 h respectively (Binukumari and Vasanthi, 2014). Giridhar *et al.,* (2015) noticed variations in liver and muscle glycogen nuvan exposed freshwater fish, *Labeo rohita.*

In the present study, it was observed that exposure to lethal and sub-lethal concentrations of glyphosate, caused changes in the total glycogen level which may be attributed to toxic stress, resulting in the disruption of enzymes associated with carbohydrate metabolism in the test fish *Catla catla,*.

The variation in distribution suggests gradual difference in metabolic calibers of various tissues. The present trend in the tissues is justifiable in the wake of mechanical tissue of muscle intended for mobility and it does not participate in metabolism. The liver is also much in proteins because of metabolic potential being oriented towards it and is the seat for the synthesis of various proteins, and also the regulating centre of metabolism. The decreased trend of the protein content in the fish tissues is due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose; or due to directing amino acids for the synthesis of necessary proteins (Tilak *et al.,* (2005).

Under sub-lethal and lethal concentrations of glyphosate, the total protein was found to decrease in most of the tissues (Figure 2). Exposure of *Oreochromis niloticus* to lethal and sub-lethal levels of thiobencarb resulted in significantly lower values of total protein compared with the control group (Abbas *et al*., 2007). Tilak *et al.,* (2009) elucidated the effect of alachlor in *Channa punctatus* on total proteins. The results indicate decrease in the level of total proteins and the percent decrease is more pronounced at lethal concentrations than in sub-lethal concentrations. Bose *et al.,* (2011) elucidated that various sublethal doses of thiamethoxam had significant impact on liver total protein in the exotic fish *Oreochromis niloticus*. Butachlor caused noticeable protein reduction in lethal and sublethal concentrations in liver and muscles of *Clarias batrachus* (Rajput *et al.*, 2012). Lakshmanan *et al.,* (2013) assessed the impact of sub lethal doses of Dichlorvos on tissue and total protein content in the liver, kidney and muscle of *Oreochromis mossambicus* and found significant decrease in the liver, kidney and muscle protein content. Sudhasaravanan and Binukumari (2014) found profound effect on protein

content in the tissues of liver, kidney, muscle and gill of atrazine treated fish, *Labeo rohita.* A decline in protein content was noticed in various tissues (gill, liver, kidney and muscle) of freshwater fish, *Cirrhinus mrigala* treated with glyphosate (Kavitha and Binukumari, 2014). Rajani *et al.,* (2014) observed marked changes were observed in the total protein content of the freshwater fish, *Clarias batrachus* during and after the cessation of the exposure to Alachlor 50% EC. Ramesh *et al.,* (2014) recorded decreased proteins levels in the liver of selenium treated fish *Labeo rohita.* Suneetha *et al.,* (2014) reported decrease in total proteins in brain, liver, gill, kidney and muscle of *Labeo rohita* exposed to endosulfan 35% EC and fenvelerate 20% EC.

In the present investigation, the decrease was more apparent in lethal concentrations than in sub-lethal concentrations. This may be due to the involvement of enzymes in detoxification, which is apparently slow. All these investigations support the present study of decreasing trend of proteins in the fish *Catla catla* exposed to lethal and sub-lethal concentrations of glyphosate.

Conclusion

The present study indicates that the glyphosate is toxic to fish *Catla catla* and the decline in the glycogen and protein level in various tissues (gill, brain, liver, kidney and muscle) of the treated fish may be due to high energy demands in order to detoxify and survive from the stressful conditions of the toxicant.

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