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Effect of sub-lethal concentrations of glyphosate on behaviour and some biochemical parameters of African catfish (*Clarias gariepinus)*

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Abstract

The sub-lethal effect of glyphosate on behavior and some biochemical parameters of African catfish, Clarias gariepinus were investigated using static renewal bioassays and continuous aeration for a period of 28 days using varying concentrations (100ppm, 200ppm, 300ppm, 400ppm and 500ppm) . During the exposure period, the fish showed different abnormal behaviours such as restlessness, lost of balance, respiratory distress, grouping together, darting movements, loss of equilibrium, mucous secretion and erratic swimming. At the end of the experiments, the fish were sacrificed and the blood samples were collected. The gill and liver of the fish were removed for biochemical bioassay. Compared with the control, the result showed a significant increase ($P < 0.05$) in the activities of alanine aminotransferase (ALT), and aspartate aminotransferase (AST), in both the blood and the gill of exposed fish except in liver where it showed a significant reduction. However the values of lactate dehydrogenase (LDH) and superoxide dismutase (SOD), showed significant increase in the blood with a noticeable decrease in both the gill and liver of exposed fish. The study showed that sub-lethal concentrations of glyphosate are harmful to Clarias gariepinus. This study shows that sub-lethal concentrations of glyphosate is harmful to Clarias gariepinus The implication of these results in rational exploitation and conservation of fishery resources and the public health risk of consuming glyphosate-exposed fish are highlighted.

Keywords: Glyphosate, sub-lethal, biochemical, conservation, public health.

Introduction

The trend in the growing human populations, destruction of farm produce and technology advancement in the agricultural production prompted the use of herbicides to control weeds with less energy loss, low cost of production with high quality and quantity of farm products. Despite these benefits, herbicides are found to be potentially harmful to the environment and human health. Their residues often sink to the bottom of the water body where they exert effect on aquatic lives, particularly fish. There, they constitute a major scourge because of their toxicity, persistency and tendency to accumulate in the organisms (Joseph and Raj, 2010). Glyphosate is one of the common herbicides used as non-selective herbicide and for aquatic weed control in fish ponds, lakes, canals, slow running water, which is said to be slightly toxic to mammals and fish (USDA 1984). *C. gariepinus* is the most common cultured fish in Nigeria; it is an omnivore fresh water fish, and popular delicacy throughout Africa, because of its hardness and fast growth (Bruton, 1979; Clay, 1979). The realization of the polluting and potential public health effects of herbicides has therefore prompted a number of studies on the toxicity of various pesticides on fishes (Zikic et al., 2001; Zang et al., 2003; Simonato et al., 2006 and Ayoola, 2008). Amongst these, reports on toxicological study of glyphosate on Clarias gariepinus are insufficient particularly in Nigeria where this herbicide is mostly used to control weeds on farm land and in fish pond. To bridge this gap, this study is aimed to access the effect of glyphosate on Clarias gariepinus.

Materials and Methods

Two-hundred and sixty fishes were acclimatized to laboratory condition for 14 days. Ten fish were randomly grouped into six of three replicates and were exposed to glyphosate in a 10L of borehole water for 28 days. A stock solution was prepared by diluting 100ml of glyphosate with five litres of distilled water. Five different concentrations (100ppm, 200ppm, 300ppm,

400ppm and 500ppm) were made from the stock solution for the experiment. Juvenile of Clarias gariepinus (average weight = 20.50 ± 0.5g, average length 14.40 ± 1.5cm) were used for the experiment. Parallel control groups of fish were kept in 10L of borehole water only. Both experimental and control fish were fed twice a day throughout the period of experiment. Each concentrations and the control was renewed every 3 days (Meyer et al., 1993) to maintain a continuous exposure. Five fish from experimental as well as control groups were sacrificed. Thereafter, biochemical analyses were carried out. The tissues of fish were homogenized and the homogenates were centrifuged at 4oc for 20 minutes to obtained supernatant. The supernatants were used for the enzyme assays.The activities of ALT and AST were analyzed using methods from Tietz (1982) and Reitmans and Frankel (1957) respectively. LDH activity was analyzed at 365nm (DGKC, 1970)] and SOD activity was assayed using (Woolliams et al., 1983). Analysis of variance (ANOVA) and Duncan multiple range tests were used to test for differences between different levels of treatments and to separate means respectively. Test of significance were at 95% ($P < 0.05$) probability (Duncan, 1955). SPSS version 16 and Microsoft Excel (2007) were used.

Results

During the exposure period, the fish showed different abnormal behaviours such as restlessness, lost of balance, respiratory distress, grouping, surfacing, darting movements' loss of equilibrium, mucous secretion and erratic swimming. Table 1 and figure 1 present the enzymes activities in the blood of *Clarias gariepinus* exposed to varying concentrations of glyphosate for a period of 28days. The values of ALT, AST and SOD increased significantly, while that of LDH decreased significantly in the blood of exposed fish compared with the control.

Table 2 and figure 2 present enzymes activities in the gill of *Clarias gariepinus* exposed to varying concentrations of glyphosate for a period of 28days. The values of ALT and AST in the gills also increased significantly with increase in concentration but the values of LDH and SOD reduced in the gills of exposed fish compared to control.

Table 3 and figure 3 present enzymes activities in the liver of *Clarias gariepinus* exposed to varying concentrations of glyphosate for a period of 28day. The values of ALT, AST and LDH in the liver of exposed fish decreased significantly with increase in the values of SOD compared with the control.

Table 1: Enzymes activities in the blood of *C.gariepinus* exposed to varying concentrations of glyphosate for 28 days.

Conc. (mg/l)	Blood			
	ALT	AST	SOD	LDH
0.0	13.50 + 0.50^b	28.50 + 0.50^b	155.00 + 5.00	252.50 + 2.50^b
1.0	18.50 + 0.50^c	33.00 + 1.00^c	177.50 + 0.50	278.50 + 1.50^c
2.0	11.50 + 0.50^a	22.50 + 0.50^a	137.50 + 2.50^a	237.50 + 2.50^a
3.0	22.00 + 1.00^e	42.50 + 1.50^d	265.00 + 5.00^c	295.00 + 5.00^e
4.0	23.50 + 1.50^d	42.50 + 0.50^d	279.50 + 9.50^c	307.50 + 2.50^f
5.0	16.50 + 1.50^b	31.50 + 1.50^b	175.00 + 5.00^c	268.50 + 1.50^c

Table 2: Enzymes activities in the gill of *C. gariepinus* exposed to varying concentrations of glyphosate for 28 days.

Conc. (mg/L)	Gill			
	ALT	AST	SOD	LDH
0.0	87.50 + 2.50^b	167.50 + 2.50^b	1357.50 + 2.50^c	1347.50 + 2.50^d
1.0	87.50 + 2.50^a	187.50 + 2.50^d	1307.50 + 2.50^c	1367.00 + 1.00^e
2.0	65.00 + 5.00^a	147.50 + 2.50^a	1047.50 + 2.50^b	1272.50 + 2.50^b
3.0	110.00 + 10.00^c	175.00 + 5.00^c	1050.00 + 50.00^b	1284.50 + 15.50^b
4.0	112.50 + 2.50^c	192.50 + 2.50^c	952.50 + 47.50^b	1297.50 + 2.50^c
5.0	127.50 ± 2.50c	162.50 ± 2.50b	787.50 ± 12.50a	1157.50 ± 2.50a

Table 3: Enzymes activities in the liver of *C. gariepinus* exposed to varying concentrations of glyphosate for a period of 28 days.

Conc. (ml/L)	Liver			
	ALT	AST	SOD	LDH
0.0	214.00 + 1.00	372.50 + 2.50	2595.00 + 5.00	2805.00 + 5.00^b
1.0	201.50 + 1.50	383.50 + 3.50	2625.00 + 25.00	2812.50 + 2.50^a
2.0	172.50 + 2.50	268.50 + 1.50	2047.50 + 2.50	2490.00 + 10.00^d
3.0	161.00 + 1.00	287.50 + 2.50	1945.00 + 5.00^e	2440.00 + 10.00^b
4.0	151.50 + 1.50	297.50 + 2.50	1905.00 + 5.00^d	2310.00 + 10.00^a
5.0	144.00 + 1.00	342.50 + 2.50	1762.50 + 12.50	2305.00 + 5.00^a

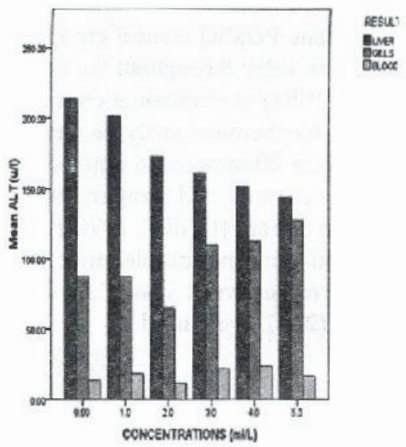


Fig.I: chart showing the result of ALT (u/l) of Clariasgariepenus exposed to sub lethal concentrations of glyphosate for 28d ays

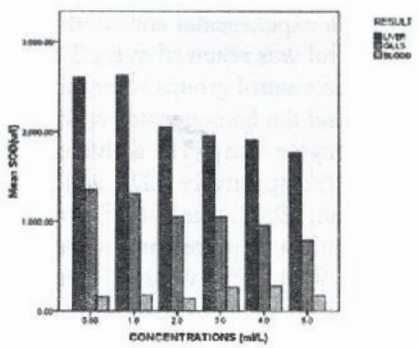


Fig.II: chart showing the result of AST (u/l) of Clariasgariepenus exposed to sub lethal concentrations of glyphosate for 28 days.

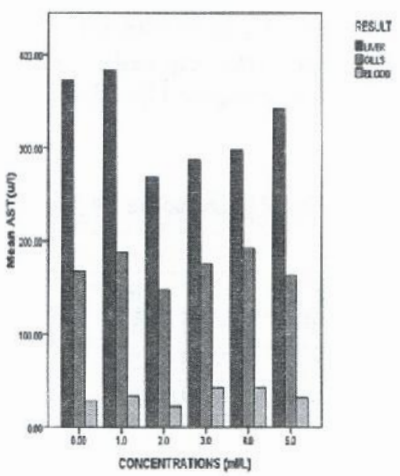


Fig.III: chart showing the result of SOD (WI) of Clariasgariepenus exposed to sub lethal concentrations of glyphosate for 28 days.

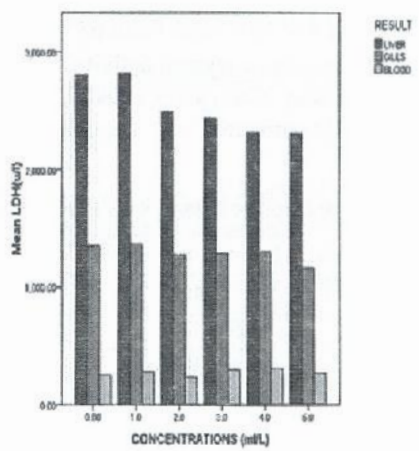


Fig.IV: chart showing the result of LDH (u/l) of Clariasgariepenus exposed to sub lethal concentrations of glyphosate for 28 days.

Discussion

The abnormal behaviours such as restlessness, lost of balance, respiratory distress, grouping, surfacing, and erratic swimming and alterations in the enzymes activities are indications that glyphosate causes a stress inducing effect on *Clarias gariepinus* during exposure period. Significant increase in the values ALT and AST activities observed in the blood and decrease in the liver of fish exposed to glyphosate are similar to the reports of Zikic et al. (2001), Rao (2006) and Soufy (2007). This could be due to possible leakage of enzymes across damaged liver cell and increased synthesis of enzymes by the liver to detoxify the herbicide.

The increased LDH activity in blood and reduction in both gill and liver of exposed fish can be considered as an adaptive process that help the organism with increased energy demand during exposure to stress factors (Martinez and Colus, 2002) and could be as a result of conversion of lactate to pyruvate and then to glucose. This result is similar to the findings of Saha *et al.* (1999) and Olua (2005).

Oxidative stress was induced in our experimental fish. Increase in the activity of this enzyme indicated an adaptive response to protect the fish from the toxicity of free radicals induced by the herbicides. Increase in the activities of SOD leads to increase oxygen production. This is in agreement with the results of Achuba and Osakwe (2003); Zang, et al. (2003) and Simonato et al. (2006).

Conclusion

Despite the benefit derived from pesticides, the result of this work has revealed that herbicides can potentially harm aquatic environment and our own health. These herbicides or their residues eventually sink to the bottom of the the water body which exerts effect on aquatic organisms. Fish are harvested from the polluted water for human consumption, since most of the world's population depend upon aquatic animals for food the agricultural community should therefore be constantly reminded

of potentially adverse effects of pesticides and other farm chemicals. To protect water quality and safety of aquatic animals, Government should therefore adopt guidelines that aim at reducing the use of dangerous herbicides in the control of terrestrial and aquatic weeds. Therefore, farming activities close to water body used for fish production should be discouraged. This is to prevent the water body from the residue of herbicides that would have washed down to the water.

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