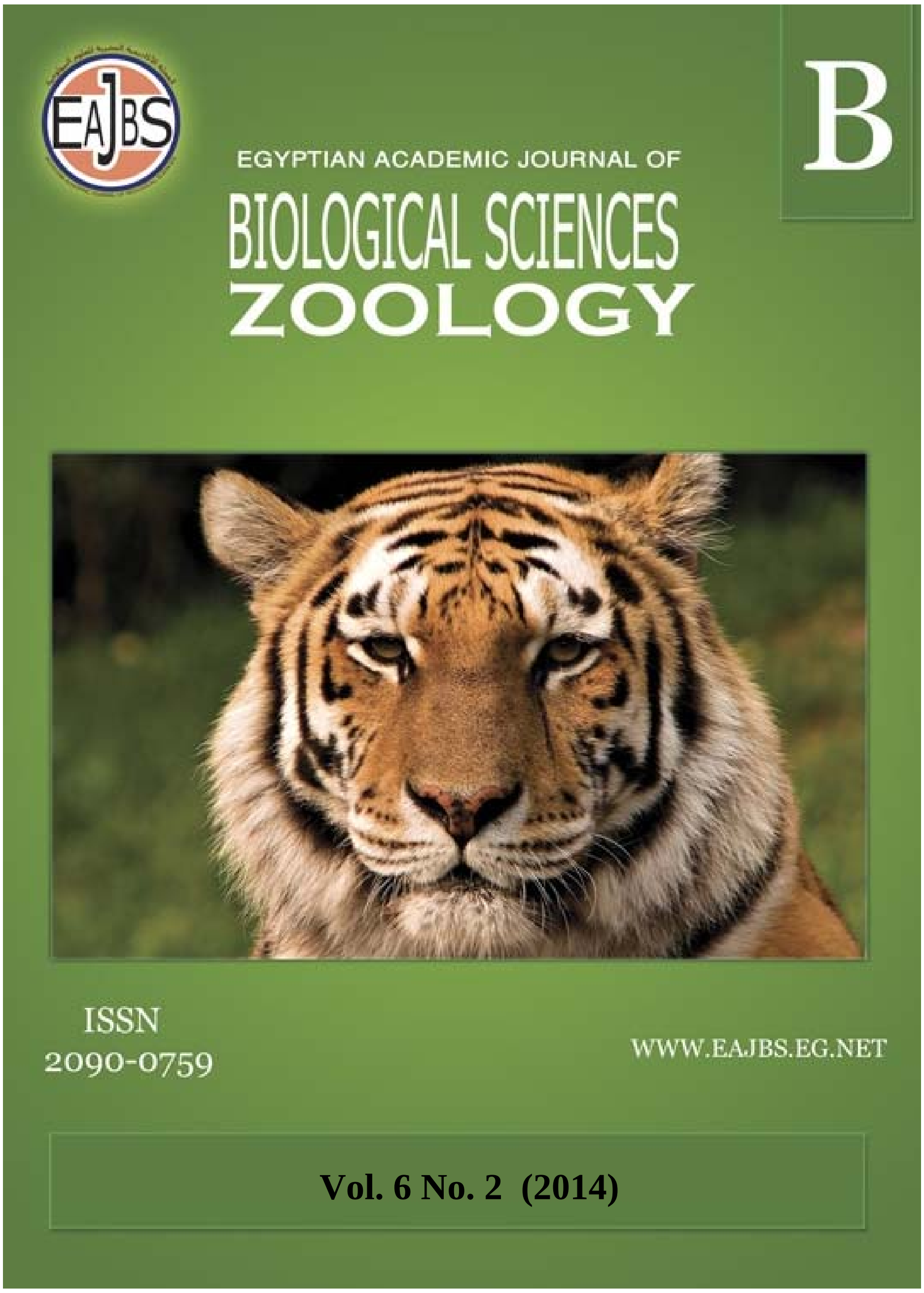

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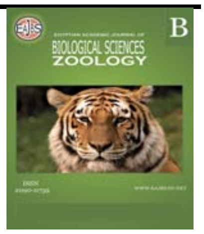

Effect of lethal and sub- lethal concentrations of glyphosate on some biochemical parameters and growth responses of African catfish (*Clarias gariepinus*)

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ABSTRACT

Background: Technology advancement in the agricultural production which prompted the use of herbicides to control weeds is found to be potentially harmful to the environment and human health.

Method: lethal and sub-lethal effect of glyphosate on some biochemical parameters and growth responses of African catfish, *Clarias gariepinus* were investigated using static renewal bioassays and continuous aeration for a period of 96 h and 28 days using varying concentrations (20.0 mg/l, 30.0 mg/l, 40.0 mg/l, 50.0 mg/l and 60.0 mg/l) and (1.0 mg/l, 2.0mg/l, 3.0mg/l, 4.0mg/l and 5.0mg/l) respectively. 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.

Result: During the exposure period, the fish showed different abnormal behaviours such as restlessness, loss of balance, respiratory distress, grouping together, darting movements, and loss of equilibrium, mucous secretion and erratic swimming. Compared with the control, the result showed a significant increase ($P < 0.05$) in the activities of alanine aminotransferase (ALT), and aspartate aminotransferase (AST), superoxide dismutase (SOD) and lactate dehydrogenase (LDH) in the gill, liver and blood of *Clarias gariepinus* exposed to glyphosate for 96 h while the activities of enzymes ALT, and AST in both the blood and the gill of fish exposed to glyphosate for 28 dsys, except in liver where it showed a significant reduction. However the values of SOD and LDH showed significant increase in the blood with a noticeable decrease in both the gill and liver of fish. Growth rate was insignificantly different ($p>0.05$) as the concentration increased compared to the control experiment. The highest percentage weight gain (12%) was observed in the control, while the lowest percentage weight gain (7%) was observed in the highest concentration. The specific growth rate of the fish reduced ($p>0.05$) insignificantly as the concentration increased.

Conclusion: The study showed that sub-lethal concentrations of glyphosate are 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, Biochemical, Conservation, public health, Growth.

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

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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). *Clarias 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 toxic 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; and 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 biochemical effect of glyphosate on *Clarias gariepinus*.

MATERIAL 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 96 h and 28 days. Five different concentrations (10.00 mg/l, 20.00mg/l, 30.00 mg/l, 40.00mg/l and 50.00mg/l) for acute test and (2.00 mg/l, 3.00 mg/l, 4.00 mg/l, 5.00 mg/l and 6.00 mg/l) for chronic test 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 4^oc 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 Reitman's 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

The water quality parameters during lethal and sub-lethal exposure of *Clarias gariepinus* to varying concentrations of glyphosate throughout the experimental periods remain constant. The mean value obtained for the experimental groups were (mean±SE): temperature, 31.00 ± 0.00^{o}C; pH, 6.52± 0.23; DO, 68.50±18.50 mgO_2 L^{-1}; COD, 20.40±13.60 mg/l; BOD, 48.50 ± 10.50 mg/l and Conductivity, 193.50± 49.50 µScm^{-1}. While that of control group were temperature, 30.00^{o}C;± 0.00; pH, 7.00 ± 0.02; DO, 97.00±10.0 mgO_2 L^{-1}2; COD, 16.55±12.95 mg/l; BOD, 49.00 ± 19.00 mg/l and conductivity, 154.50 ± 42.5 μ/s. 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. Figs 1 and 3 shows the activities of enzyme ALT and AST in the liver, gill and blood of *Clarias gariepinus* exposed to varying concentrations of glyphosate for a period of 96 h and 28days respectively. There was significant increase (P < 0.05) in the activities of ALT and AST in the gill, liver and blood of the fish after 96 h of exposure compared to control (Fig. 1). But at the end of 28 days of exposure, the activities of ALT in the liver significantly decreased (P < 0.05) and increased significantly (P < 0.05) in the gill and blood as the concentration increased.

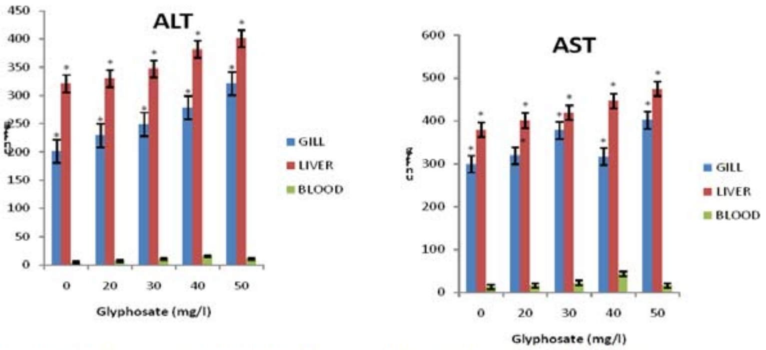

Figure 1: Activity of alanine amino-transferase (ALT) and aspartate amino-transferase (AST) in gill, liver and blood of *Clarias gariepinus* exposed to varying concentrations of glyphosate for 96 h. Values are means ±SE (n=3). *Significantly different from the control *(P < 0.05)*.

Compared to control, a significant increase (P < 0.05) was obtained in the concentration of AST in the liver and gill with increase in the concentration of glyphosate but showed a significant reduction (P<0.05) in the blood. (Fig.3). Activities of enzyme SOD in the liver, gill and blood of *Clarias gariepinus* exposed to varying concentrations of glyphosate for a period of 96 h and 28days is shown in Figs. 2 and 4. The data reveal a significant increase (P < 0.05) in the values of SOD and LDH in the liver, gill, and blood of exposed fish after 96 h compared with the control. But the values of SOD in both liver and gill of exposed fish showed a significant reduction (P < 0.05) with a significant increase in the blood after 28 days compared to control. And the value of LDH in the liver, gill and blood decreased significantly as the concentration increased compared to control (Figs. 2 and 4).

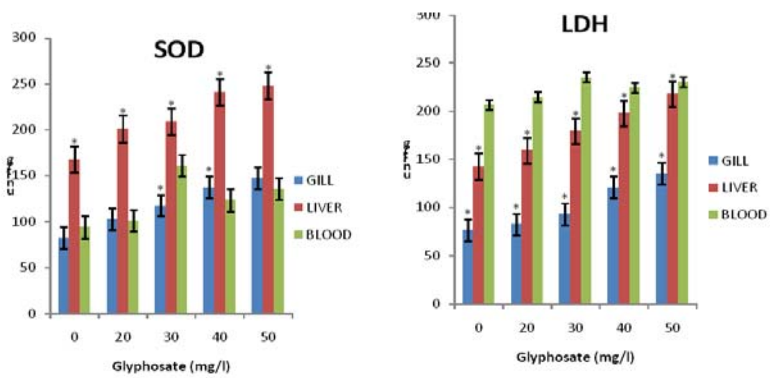

Figure 2: Activity of superoxide dismutase (SOD) and lactate dehydrogenase (LDH) in gill, liver and blood of Clarias gariepinus exposed to varying concentrations of glyphosate for 96 h. Values are means ±SE (n=3). *Significantly different from the control *(P < 0.05)*.

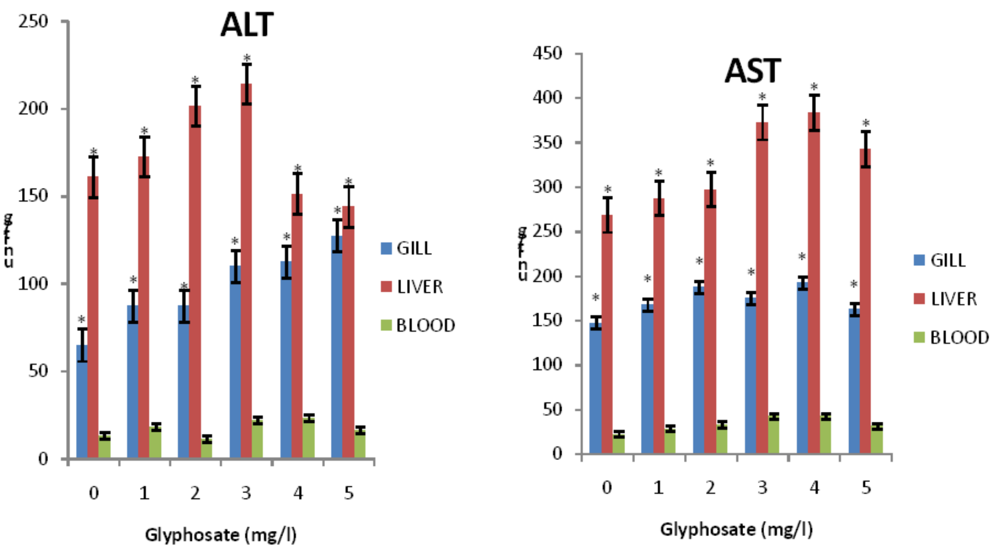

Figure 3: Activity of alanine amino-transferase (ALT) and aspartate amino-transferase (AST) in gill, liver and blood of Clarias gariepinus exposed to varying concentrations of glyphosate for 28 days. Values are means ±SE (n=3). *Significantly different from the control *(P < 0.05)*.

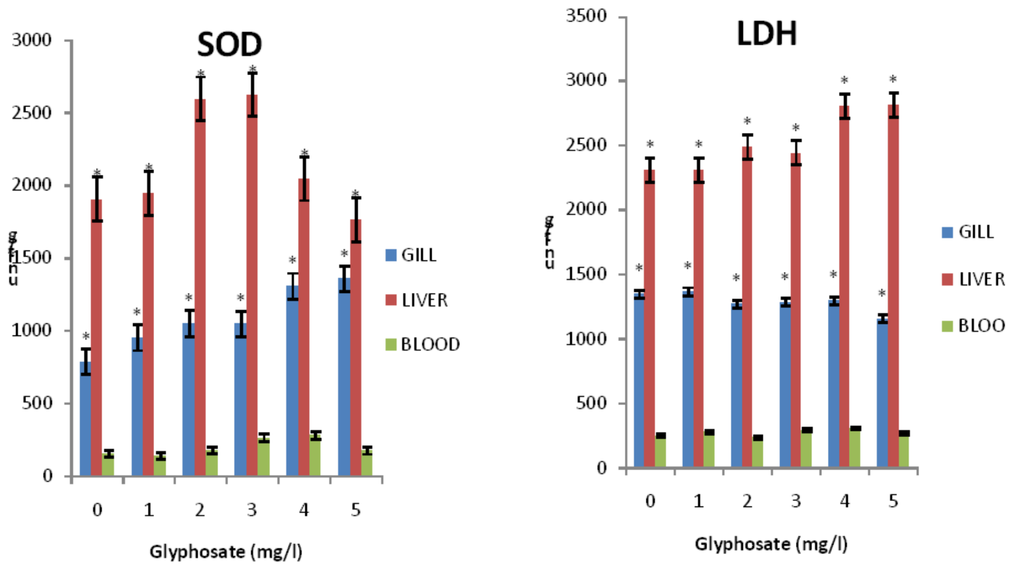

Figure 4: Activity of superoxide dismutase (SOD) and lactate dehydrogenase (LDH) in gill, liver and blood of Clarias gariepinus exposed to varying concentrations of glyphosate for 28 days. Values are means ±SE (n=3). *Significantly different from the control *(P < 0.05)*.

The specific growth rate of *Clarias gariepinus* exposed to different concentrations of glyphosate for 28 days are shown in Table 1, Figs 5 and 6. The percentage weight gain in the exposed fish decreased with increase in the concentrations of glyphosate. The least weight gain (2.15±0.45) was observed in fish exposed to highest concentration (5.0mg/L) of glyphosate, while the control experiment recorded the highest weight gain (2.75±1.15). The specific growth rate showed a general reduction in weight gain with increased concentrations. The lowest value of specific growth rate (0.07) was observed in the fish exposed to the highest concentration (5.0mg/L), while highest value was recorded in the control group (0.12). The lowest observable effect concentration (LOEC) and the no observable effect concentration (NOEC) were estimated to be 2.0mg/L and 1.0mg/L respectively. The maximum allowable toxicant concentration (MATC) was calculated to be 1.4mg/L.

Table 1: Mean weight of *Clarias gariepinus* exposed to six different concentrations each of Glyphosate for 28 days.

Concentration (mg/l)						
Parameters	0.00	1.0	2.0	3.0	4.0	5.0
Average initial weight (g)	24.00± 1.40^{a}	28.80± 1.20^{b}	24.85± 7.25^{c}	25.80± 5.80^{d}	32.65± 1.85^{e}	31.20± 2.30^{f}
Average final weight (g)	26.75± 1.15^{a}	31.20± 1.90^{b}	27.15± 8.15^{c}	28.10± 6.20^{d}	35.05± 1.75^{e}	33.35± 2.75^{f}
Weight gain (g)	2.75± 0.25^{a}	2.40± 0.70^{b}	2.30± 0.90^{c}	2.30± 0.40^{d}	2.40± 0.10^{e}	2.15± 0.45^{f}
(%) Weight gain	12	8	9	9	7	7
Specific growth rate btw day 1 and 28	0.12	0.08	0.09	0.09	0.07	0.07

Values are mean of two replicates ± SEM (standard error mean). Column values with different superscripts are significantly different ($P < 0.05$).

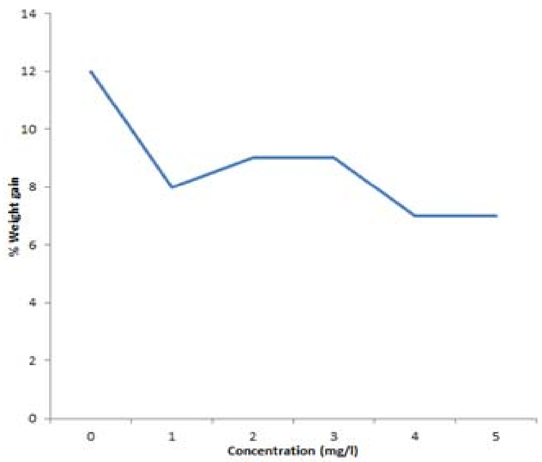

Fig. 5: Mean weight gain of *Clarias gariepinus* exposed to varying concentrations of glyphosate for 28 days.

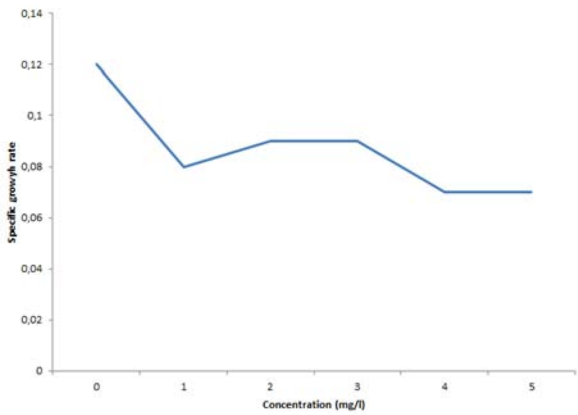

Fig. 6: Specific growth rate of *Clarias gariepinus* exposed to varying concentration of glyphosate for 28 days.

DISCUSSION

The abnormal behaviours such as restlessness, pronounced gasping for oxygen, uncoordinated movement, grouping, surfacing, and erratic swimming could be attributed to the interruption in the physiology and reaction of nervous system of the fish due to the irritating effect of glyphosate (Alkahem (1994). The behavioural responses observed in this study are in agreement with the report of Omoregie *et al*. (1998) and Aderolu *et al*. (2010).

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. The increase in the value of enzymes could be due to possible leakage of the enzymes across damaged liver cell in to the blood circulation and increased synthesis of enzymes by the liver to detoxify the herbicide (Mousa *et al*., 2008). It could also be as a result of extra metabolic activities of the fish involved in order to meet up with the oxidative stress and detoxification of the chemical (Gill *et al*., 1990). And the decrease in the values of enzymatic activities could be due the continue exposure of fish to toxicant which may caused complete damage to liver cell thereby reducing the production of the enzymes.

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); 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 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 on fish for food the agricultural community should therefore be conscious of the potentially adverse effects of pesticides. To protect water quality and safety of aquatic animals, Government should therefore have a constitution that guide against the use of dangerous herbicides in the control of terrestrial and aquatic weeds. And farming activities around 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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