ACUTE EFFECT OF GLYPHOSATE ON WATER QUALITY PARAMETERS AND SOME ANTIOXIDANT RESPONSE OF HETEROCLARIAS FINGERLINGS

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Abstract: This study was carried out to determine the acute effect of glyphosate on water quality parameters and some antioxidant response of heteroclarias fingerlings. A range finding test was conducted six times in order to get reliable values that could be used for the definitive test. In the definitive test, the toxicant introduced at varying concentrations of 0.00 mg/l, 5.40 mg/l, 7.20 mg/l, 9.00 mg/l, 10.80 mg/l and 12.60 mg/l for 96 h in a renewal bioassay procedure showed that the 96 h LC_{50} was 6.838 mg/l. There was a sharp increase in some water quality parameters such as pH, temperature, total dissolved solids, electrical conductivity and total hardness after exposure of the toxicant for 96 h, while dissolved oxygen and alkalinity decreased. There was significant difference in gill catalase activity of heteroclarias fingerlings exposed to different concentration of glyphosate for 96 h (P<0.05), heteroclarias exposed to 5.40 mg/l of glyphosate had the highest gill catalase activity (1.323±0.00 U/mg protein) and 0.00 mg/l with the lowest gill catalase activity (0.003±0.00 U/mg protein), gill peroxidase activity also differed significantly (P<0.05), heteroclarias fingerlings exposed to 9.00 mg/l of glyphosate had the highest and 0.00 mg/l with the lowest gill peroxidase activity. Liver catalase and peroxidase activity of heteroclarias fingerlings also differed significantly at vary concentrations of glyphosate (P<0.05), heteroclarias exposed to 12.60 mg/l showed the highest liver peroxidase activity level, i.e. the antioxidant response of heteroclarias fingerlings was concentration dependent.

Keywords: Acute effect, glyphosate, heteroclarias fingerlings, acute toxicity and fish toxicology.

Introduction

Glyphosate a unique global herbicide under the trade name Round-up® was introduced to the market by Monsanto Company during the 1970s. The tonnage of glyphosate herbicide application has been constantly increasing since the introduction of this group of chemicals in 1971 (Dill *et al*., 2010). Many commercial herbicides have been formulated using glyphosate as active ingredient such as Rodeo, Round-up and Aquamaster (Mozdzer *et al*., 2008; Papchenkova *et al*., 2009). The continuous use of these herbicides has brought about some concern of the effects of these chemicals on the early life stages of fish (Skea *et al*., 1987). Since gill is the first site where these toxins gain access and the liver which is a detoxifying organ, there is need to check the oxidative stress of the fish. Oxidative stress develops as a consequence of disturbance between generation and elimination of Reactive Oxygen Species (ROS) with certain physiological consequences (Luschak, 2011) and some of this antioxidant enzymes catalase (CAT) and peroxidase (POD) acts to eliminate these ROS produced within the cell. Thus, the levels of these enzymes that were determined during this investigation reflect the stress level caused by glyphosate.

Materials and Methods

Experimental site

The experiment was conducted at the Fisheries laboratory Department of Biological Sciences Ahmadu Bello University Zaria, Kaduna, Nigeria.

Experimental fish

Fingerlings of Heteroclarias of mixed sexes and fairly uniform size (2.2±0.7 g weight and 6.7±0.7 cm standard length) were obtained from National Open University Nigeria (NOUN) Fisheries Unit Kaduna-Zaria express road, Kaduna and transported in plastic container to the laboratory in the Department of Biological Sciences, Ahmadu Bello University Zaria. They were acclimatized for two weeks in four oval/rectangular shaped bath tubs, separately containing water of about 150 L. The fish were been fed twice daily on a 35% crude protein diet.

Fingerlings of fairly equal weight (2.2 ±0.7 g), total length (6.7±0.7 cm) and standard length (5.9 ±0.6 cm) was selected randomly, weighed and distributed into 10 glass aquaria containing definitive concentration of the glyphosate and 2 controls with only distilled water without glyphosate. The bioassay test was carried out in 12 glass tanks each of size 30.5 x 30.5 x 92.5 cm into which approximate quantity of glyphosate were taken to give a final volume of 20.0 L. The fish were starved for 24 h before commencement of the experiment. The solutions was stirred for homogenous mixing before each aquarium were randomly stocked in duplicates with 10 fingerlings of fish while the test solution and control were renewed daily.

Determination of glyphosate concentrations

Pilot studies were carried out to determine the definitive concentration range for testing Round-up following the methods of Solbe (1995). This was done by introducing three nominal concentrations into three separate test tanks (using pipette) containing 20 litres of dechlorinated water in triplicate. Five fish per concentration of toxicant was used with 3 replicates each for 96 h. When the fish died in all the test tanks, lower range of concentrations of the toxicants were prepared until when 80 to 90% of fish died in the highest concentration test tank and 20 to 30% of fish died in the lowest concentration test tank. The five nominal concentrations were then range between the highest and the lowest concentrations geometrically (5.40, 7.20, 9.00, 10.80 and 12.60 mg/l). The methods of acute toxicity tests as described by Sprague (1973) and APHA (1995) was employed. The range of concentrations of glyphosate (5.40, 7.20, 9.00, 10.80 and 12.60 mg/l) obtained in the pilot tests were dispensed with a pipette into 20 litres of each test tank in duplicate. Ten fingerlings

were exposed to five different concentrations of the toxicant in each test glass tank in duplicate and the control.

Water quality parameter determination

Determination of pH, temperature, electrical conductivity and total dissolved solids were measured with Hanna instrument (Model- HI 98129. HI 98130). The pH mode on the instrument was selected with the set/Hold button. The electrode was submerged in the sample water for 60 s. The measurement was then taken when the stability symbol on the top left of the liquid crystal display (LCD) disappeared. The pH value automatically corresponding to the temperature was shown on the primary LCD while the secondary LCD shows the temperature of the sample. The Electrical Conductivity (EC) or TDS mode was selected with Set/Hold button. The probe was submerged in the sample. The measurement was taken when the stability symbol on the top left of the liquid crystal display (LCD) disappeared. The electrical conductivity (EC) or total dissolved solid (TDS) value automatically corresponding to the temperature was shown on the primary LCD while the secondary LCD shows the temperature of the sample.

The DO was determined by modified Winkler Azide method (Lind, 1979). Water samples were collected in 1litre glass bottles (Mayer flask). They were fixed with 2 ml manganese sulphate ($MnSO_4$) solution using a graduated syringe by inserting its tip below the water surface in the bottle; 2ml alkaline iodide azide reagent was added next in a similar manner as the first reagent followed by the addition of 2ml concentrated sulphuric acid (H_2SO_4). To a conical flask was poured 100 ml of measure fixed sample with addition of 2 drops of starch solution and which was titrated with 0.0125N sodium thiosulphate (Na_2SO_3) until it turns colorless as the end-point. The reading was then taken and recorded in mg/L. About 100ml of the water sample was poured into a conical flask after which 2 drops of methyl red solution and 2 drops of BromoCreasol green were added, a faint greenish color was observed after thorough agitation. This was then titrated against 0.02N standard sulphuric acid solution until a pink colour was observed which served as the end point APHA (1989).

Total alkalinity = mg/L = ml of titrant × 10

About 25 ml of the water sample and 25 ml of distilled water was poured into a beaker after which 2 ml of buffer solution of pH 10.4 was added and chips of Errochrome black T dye were added, this was then titrated against ethylenediaminetetracetic acid (EDTA) titrant (0.01M) until it changed to blue. The titrant value was multiplied by 40, as $CaCO_3$/L APHA (1989).

Antioxidant response determination

Catalase activity was determined by the method of Luck (1974). The tissue homogenate employed for the assay was prepared in phosphate buffer (0.067M, pH 7.0). The samples were read against a control without homogenate, but containing the H_2O_2 phosphate buffer (2 mM). To the experimental cuvette, 2.9 ml of H_2O_2 phosphate buffer was added, followed by the rapid addition of 0.1 ml enzyme extract and mixed thoroughly. The samples were read against a control containing 3 ml H_2O_2 phosphate buffer without enzyme extract. The time interval required for a decrease in absorbance by 0.05 units was recorded at 240 nm. The enzyme solution containing H_2O_2 free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

The Catalase activity was calculated as follows:

Catalase activity (units/ml enzyme) = (3.45)/(Min) (0.1)

Where:

3.45 correspond to the decomposition of 3.45 micromoles of hydrogen peroxide in a 2.0 ml of reaction mixture; 0.1= Volume of enzyme used (milliliters)

The activity of peroxidase was measured by the method of Reddy *et al*. (1995). The tissue samples used for the assay were prepared as homogenate in 0.1M phosphate buffer (pH 6.5). Exactly 2.9 ml of pyrogallol solution (0.05M in 0.1M phosphate buffer, pH 6.5) and 0.1 ml enzyme extract was placed in a cuvette using a pipette. The spectrophotometer was adjusted to read zero at 430 nm followed by the addition of 0.5 ml of H_2O_2 (1% in 0.1M phosphate buffer, pH 6.5) after which it was mixed. The change in absorbance was recorded every 30 seconds up to 3 min. One unit of peroxidase activity was then defined as the change in absorbance per minute at 430 nm.

Peroxidase activity (units/mg) =

$$\frac{\Delta A_{510} / \min}{6.58 \; x \; ml \; enzyme / ml \; rxn \; mixtur}$$

Where: A = Absorbance

Data analyses

Data was subjected to one-way analysis of variance (ANOVA) using SPSS IBM Version 20, 2011 statistical software package to test for the significant differences between means and Duncan's Multiple Range Test (DMRT) was used to separate mean where there were significant difference among treatments, Principal component analyses was used to establish the relationships between glyphosate concentrations, water quality parameters and antioxidants activities level of heteroclarias fingerlings exposed to various glyphosate concentration for 96 h using PAST (Paleontological Statistical Software).

Results and Discussion

Water quality parameters

Results of the water quality parameters used for dilution of the administered pesticide concentrations before and after 96 h of exposure in the laboratory are presented in Table 1. The parameters such as pH, Total Dissolved Solids, Electrical Conductivity, and Hardness before exposure have their range values as 5.95-6.28, 53-68 mg/L, 96-141 µS/m, and 136-164$CaCO_3$, respectively. However, after exposure of the toxicant for 96 h, there was a sharp increase in pH (6.30-6.67), Total Dissolved Solids (60-83 mg/L), electrical conductivity (128-163 µS/m), and Hardness as $CaCO_3$ (116-180).

Table 1: Mean(±SE) of water quality parameters before and after 96 h exposure of glyphosate

Parameters	Before Exposure		After Exposure	
	Range	Mean	Range	Mean±SE
Ph	5.95-6.28	6.09±0.08	6.30-6.67	6.47±0.04
Temperature (°C)	25.90-26.20	26.15±0.09	26.70-28.60	27.65±0.19
Total dissolved solids (mg/L)	53-68	57.34±2.21	60-83	71.67±1.18
Electrical conductivity (µS/m)	96-141	115.67±7.59	128-163	145±1.58
Dissolved oxygen (mg/L)	7.40-8.40	7.85±0.05	5.40-6.80	6.12±0.05
Alkalinity (mg/L.$CaCO_3$)	36-59	48.34±4.70	31-36	36.33±1.64
Hardness ($CaCO_3$)	136-164	153.34±6.83	116-180	154.67±12.25

Other parameters such as temperature, dissolved oxygen, alkalinity before exposure have their range values as 25.90-26.20^{o}C, 7.40-8.40 mg/L, and 36-59 mg/L.$CaCO_3$, respectively. There was however increase in the range value after exposure for 96 h such as Temperature (26.70-28.60^{o}C) and decrease in Dissolved Oxygen (5.40-6.80 mg/L), and Alkalinity (31-36 mg/L.$CaC0_3$). The results of the water quality parameters shows a significant increase after exposure for pH, temperature, total dissolved solids (TDS), electrical conductivity (EC), and hardness while dissolved oxygen and alkalinity decreased. There was a positive correlation between glyphosate concentration, temperature, total dissolve solid, electrical conductivity, alkalinity, gill catalase and peroxidase activity and liver catalase and peroxidase activity while negatively correlated with dissolve oxygen, pH and hardness as presented in Fig. 1 and 2. Studies have shown that when water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van Vuren, 1986). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology, since they live in close association with their environment and are sensitive to slight fluctuation that may occur within their surrounding water (Cassilas & Smith, 1977).

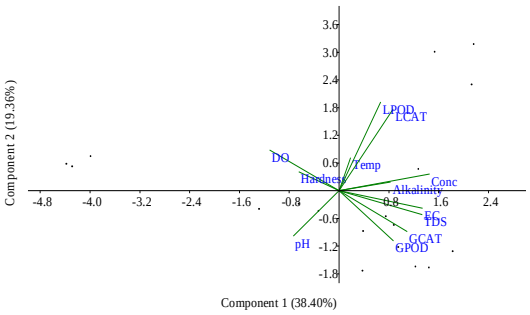

Fig. 1: Principal component analysis (PCA)biplot of glyphosate concentration, water quality parameter and antioxidants response levels of heteroclarias exposed to different glyphosate concentration

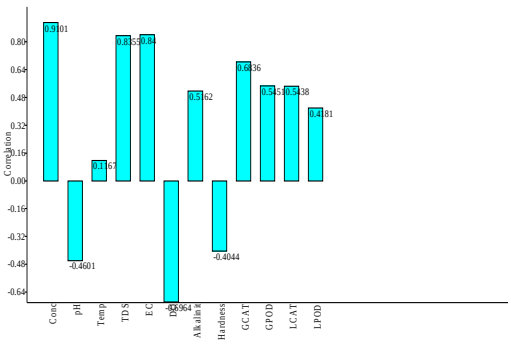

Fig. 2: Principal component analysis correlation plot of glyphosate concentration, water quality parameters and antioxidants response levels of heteroclarias exposed to different glyphosate concentration.

Antioxidant response

There was significant difference in gill catalase activity (P<0.05) as presented in Table 2, heteroclarias fingerlings exposed to 5.40 mg/l glyphosate had the highest gill catalase activity followed by 7.20, 9.00, 10.80, 12.60 and 0.00, respectively. This indicates that as the concentration increases the activity increases i.e. the activity is concentration dependent. However, there was also significant difference in gill peroxidase activity (P<0.05) heteroclarias fingerlings exposed to 9.00 mg/l having the highest followed by 7.20 mg/l, 12.60 mg/l, 10.80 mg/l, 5.40 mg/l and 0.00 mg/l.

There was significant difference in liver catalase activity (P<0.05). This indicates that as the concentration increases the activity also increases. However, there was also significant difference in liver peroxidase activity (P<0.05) heteroclarias fingerlings exposed to 12.60 mg/l having the highest activity followed by 7.20 mg/l, 9.00 mg/l, 5.40 mg/l, 10.80 mg/l and 0.00 mg/l as shown in Table 3.

Table 2: Table showing catalase and peroxidase activity (U/mg protein) for exposure of glyphosate concentration on Heteroclarias gills

Conc.(mg/l)	GCAT	GPOD
0.00	0.003±0.000^{f}	0.005±0.001^{f}
5.40	1.323±0.000^{a}	0.007±0.001^{e}
7.20	1.213±0.001^{b}	0.123±0.001^{b}
9.00	1.006±0.000^{c}	0.141±0.000^{a}
10.80	0.855±0.000^{d}	0.027±0.000^{d}
12.60	0.750±0.001^{e}	0.037±0.001^{c}

Means with different superscript along the columns are significantly different (P<0.05); GCAT = Gill Catalase Activity; GPOD = Gill Peroxidase Activity

Table 3: Table showing catalase and peroxidase activity (U/mg protein) for acute exposure of glyphosate concentration on Heteroclarias liver

Conc. (mg/l)	LCAT	LPOD
0.00	0.013±0.002^{f}	0.027±0.001^{f}
5.40	1.261±0.000^{b}	0.033±0.001^{d}
7.20	1.233±0.000^{c}	0.145±0.003^{b}
9.00	1.162±0.001^{d}	0.042±0.003^{c}
10.80	0.995±0.001^{e}	0.025±0.000^{e}
12.60	7.768±4.307^{a}	24.867±12.519^{a}

Means with different superscript along the columns are significantly different (P<0.05); LCAT = Liver catalase activity; LPOD = Liver peroxidase activity

The antioxidant activity for acute toxicity on gill shows that catalase and peroxidase activities increased at 5.40 mg/l and 7.20 mg/l but decreased at 9.00 mg/l, 10.80 mg/l and 12.60 mg/l while peroxidase activities increased from 5.40 mg/l to 7.20 mg/l but decreased from 9.00 mg/l to 10.80 mg/l further increase at 12.60 mg/l. The increase in activities indicates that the fish is stressed as a result of the toxicant levels. Different authors like Radovanovic *et al.* (2010), Kandemir *et al.* (2010), Doherty *et al.* (2010), Aysel *et al.* (2010), Nogueira *et al.* (2010), Obaiah and Usha (2012) variously supported the present result.

Conclusion

In conclusion, glyphosate has effect on water quality parameters and posed toxic metabolic stress to heteroclarias. It is therefore recommended that there should be regulations on the use of herbicide which are toxic to other untargeted species.

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Conflict of Interest

There is no conflict of interest.

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