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SHORT COMMUNICATION KISA MAKALE

ACUTE EFFECTS OF GLYPHOSATE ON THE BEHAVIOURAL AND HEMATOLOGAL CHARACTERISTICS OF HETEROCLARIAS (HYBRID) FINGERLINGS

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Abstract:

The toxicity effect of glyphosate on fingerlings of Heteroclarias after series of range finding tests, the fishes were exposed to lethal concentration 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 hours in a renewal bioassay procedure showed that the 96 hours LC_{50} was 6.838 mg/L. Respiratory disturbance, erratic swimming, loss of equilibrium, lethargies and sudden fish death were observed in the exposed fish and these varied greatly with differences in concentration of the toxicant and this shows that mortality increases with an increase in concentration. Also, as the concentration of glyphosate increased the beats of the tail and operculum increased in 12 and 24 hours. Also the toxicant led to significant changes ($P<0.05$) in hematological parameters as the toxicant concentration increased. Mean Red Blood Cells (RBC), Hemoglobin content (Hb), Packed Cell Volume (PCV), reduced as the concentration of toxicant increased while other parameters increased proportional with the toxicant concentration. Others, such as Basophils, Eosinophils and Monocytes were tested but not detected.

Keywords: Glyphosate, Heteroclarias, Round-up, Toxicology

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Introduction

The major formulation is Round-up, in which glyphosate is formulated as isopropyl amine salt and a surfactant, polyethoxylene amine (POEA), is added to improve the quality of the herbicide (Tsui and Chu, 2004; Releya, 2005). The indiscriminate use of herbicides, careless handling, accidental spillage, or discharge of untreated effluents into natural water-ways have harmful effects on the fish population and other forms of aquatic life and may contribute long term effects in the environment (Ayoola, 2008). Most aquatic herbicides have undergone some toxicity testing to evaluate effects on non-target organisms (Urban and Cook, 1986). Okomoda *et al*. (2013) conducted research on hematological response of *Clarias gariepinus* fingerlings exposed to acute concentrations of sunsate®. This is because tests are rarely conducted on the early life stages of fish commonly found in water bodies in Nigeria being treated for 'weed control'.

Materials and Methods

The experiment was conducted at the Fisheries Laboratory Department of Biological Sciences Ahmadu Bello University Zaria, Kaduna, Nigeria. Fingerlings of Heteroclarias of mixed sexes and fairly uniform size (2.2 ±0.7g weight and 6.7±0.7cm 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 150L. The fish were being fed twice daily at 5% body weight with 35% crude protein diet. 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 liters of dechlorinated water in triplicate. Five fish per concentration of toxicant was used with 3 replicates each for 96 hours. 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 12.60mg/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 12.60mg/L) obtained in the pilot tests were dispensed with a pipette into 20 liters of each test tank in duplicate. Ten fingerlings fish were exposed to five different concentrations of the toxicant in each test glass tank in duplicate and the control.

Fingerlings of fairly equal weight (2.2 ±0.7g), total length (6.7 ±0.7cm) and standard length (5.9 ±0.6cm) 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 92.5cm into which approximate quantity of glyphosate were taken and to give a final volume of 20.0L. The fish were starved for 24 hours before commencement of the experiment. The solutions were 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. The investigation of opercula ventilation counts and tail fin movement rate was carried out for 96 hours which were counted using stop watch at 12, 24, 48, 72 and 96 hours per minutes. Three fish were used for the counting per tank and the average.

Data was subjected to one-way analysis of variance (ANOVA) using SPSS software to test for the significant differences between means and where significant differences are found, the Duncan's Multiple Range Test (DMRT) was used to separate the significantly different means. Mini Tab 17 statistical software was used to determine LC_{50}.

The blood was sampled as described by Blaxhall and Diasely (1973) for the assessment of the various blood parameters and was collected by severance of caudal peduncle from the caudal artery at 2cm away from the caudal peduncle. This process was done on the surviving fish tanks. Hemoglobin concentration was estimated as cyanmethemoglobin (Brown, 1980), Packed Cell Volume (PCV) was determined using microhaematocrit. The Red Blood Cell (RBC) were counted using haemocytometer (Improved Neubauer Weber Scientific Ltd), according to Wintrobe (1978).

Also the total white Blood Cell Counts (WBC) was estimated with an improved Neubauer. The RBC indices that include Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated by using the formula mentioned by Dacie and Lewis (1968).

Results and Discussion

Heteroclarias exposed to glyphosate showed increased tail fin beat and opercular ventilation with increase in the concentration of the toxicant (glyphosate) for 5.40, 7.20, 9.00, 10.80, and 12.60 mg/L as present (Figure 1 and 2 respectively). The activity of the opercula was observed and counted, especially during the first 48 hrs. The result of opercula ventilation as presented in figure 1 showed that the opercula beats of the exposed fish to the toxicant at 12 and 24 hours were higher than the one in the control fish. Increase in toxicant concentration resulted in an increase in opercula beats rate at 12 and 24 hours, and at 48 hour beats rate decreased. Further duration of exposure led to more decrease in the opercula ventilation beat of the fish. By the 96 hours the opercula ventilation rates of the exposed fish were significantly lower than those of the control group.

The activity of tail fin beat was observed and counted, in particular during the first 48 hours of exposure, the result of tail fin beat as presented in figure 2 showed that the tail fin beat of the exposed fish to the toxicant at 12 and 24 hours were higher than the one in the control fish. The tail fin beat increased initially and started decreasing at the 48 hours. As the duration of exposure progresses, there was a continuous decrease in tail fin beat of the fish. There was significant difference between the tail fin beat of the treated fish as seen in the figure (P<0.05). The values were dose dependent.

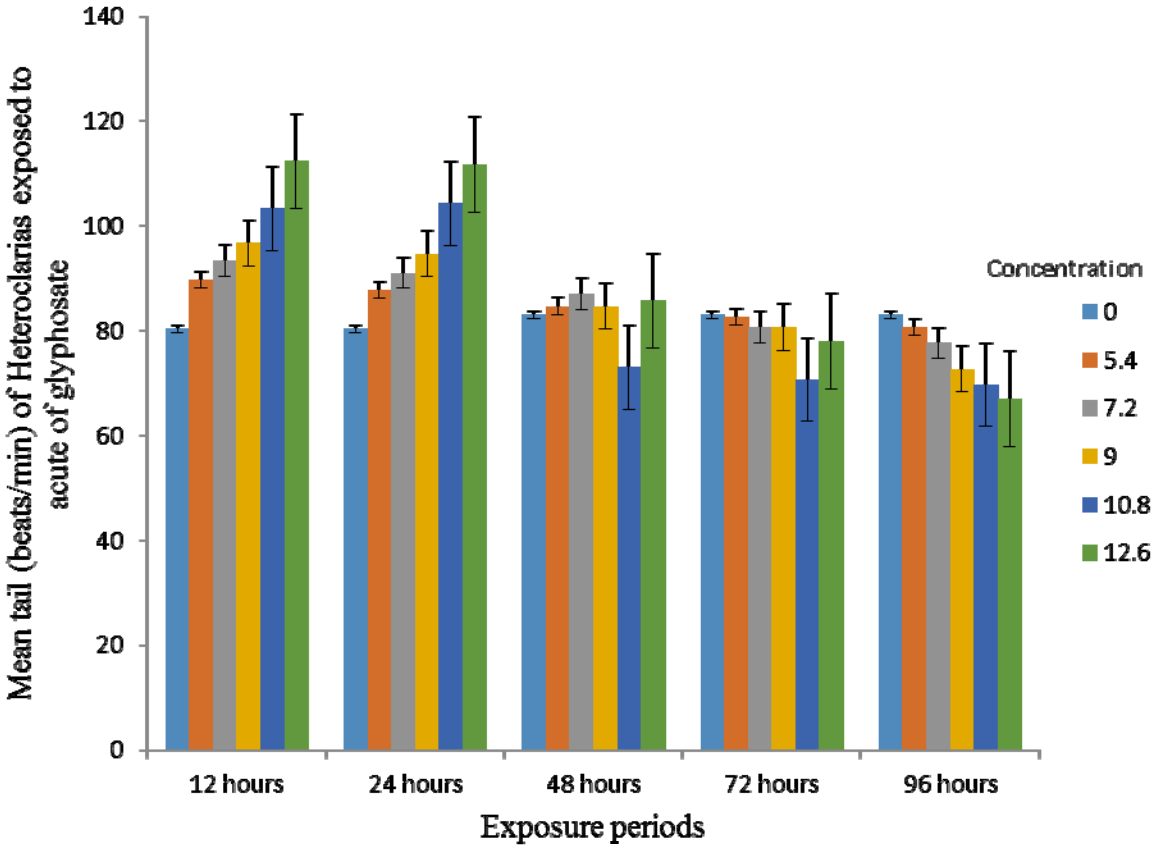

Figure 1. Mean (±SE) mean tail beat rate of Heteroclarias exposed to acute concentration of glyphosate

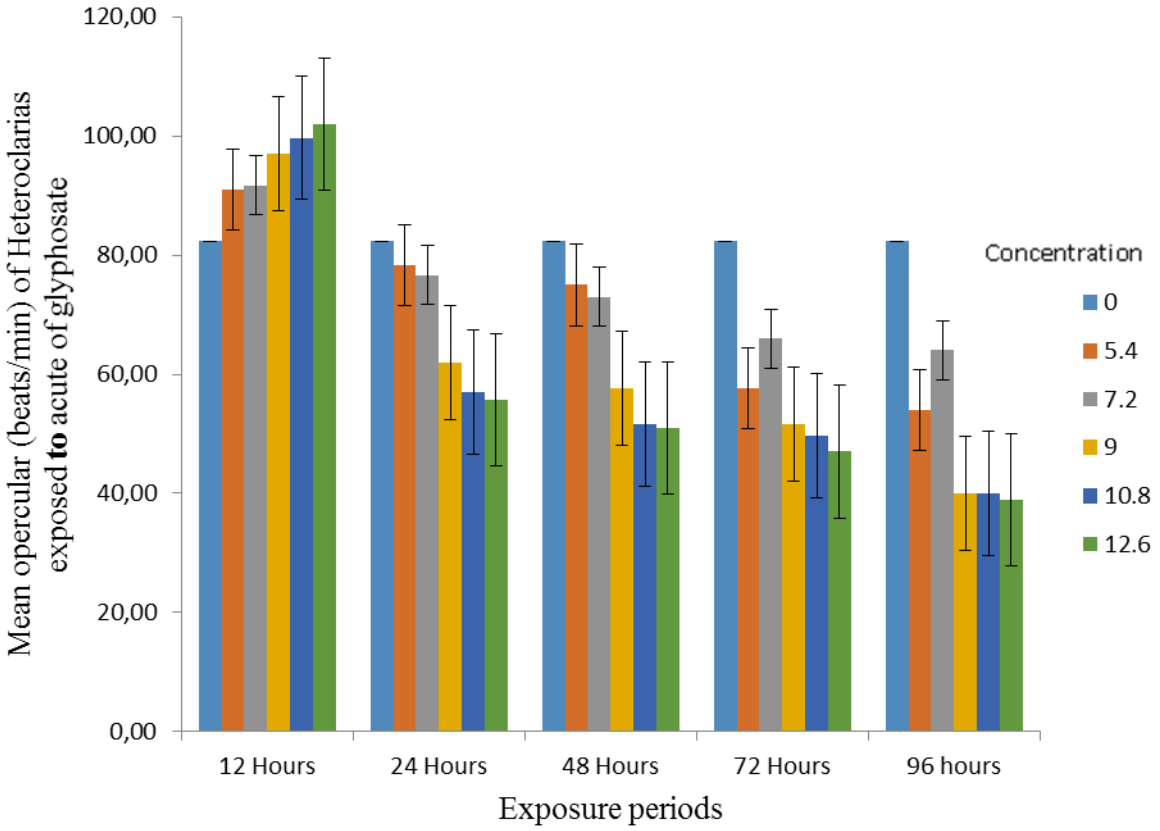

Figure 2. Mean (±SE) opercula beat rate of Heteroclarias exposed to acute concentration of glyphosate

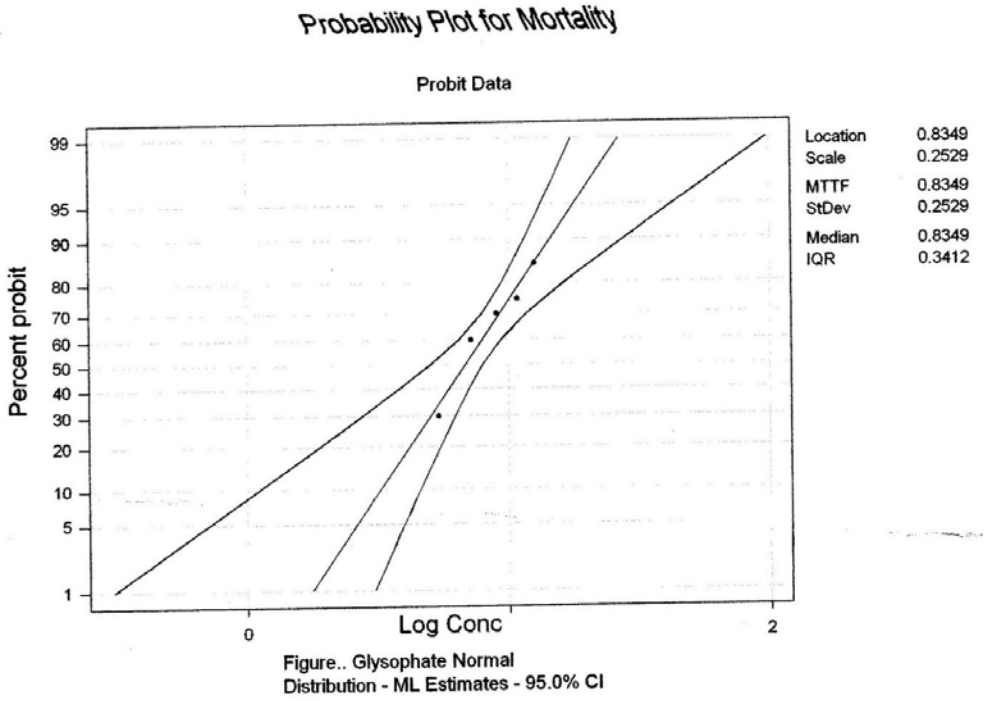

Figure 3. Probit Plot of LC_{50} at 96 hours' glyphosate herbicide exposure on fingerlings of Heteroclarias

The result of this study revealed that Heteroclarias exposed to various concentrations of glyphosate recorded decrease packed cell volume (mm^3), total red blood cell (RBC) and hemoglobin (Hb) but an increase in total white blood cells (WBC) as presented (Table 1). Neutrophil decreased with increase in glyphosate concentration while lymphocyte of test fish increased with increase in glyphosate concentration as presented (Table 2). The acute toxicity test showed hematological changes which is an indication of severity in the treated fish. The anemia effect could be due to an inhibition in erythrocyte production and haemodilution. Erythropenia (deficiency in the number of red blood cells) was reflected by the reduced hemoglobin content and hematocrit value as well as erythrocyte sedimentation rate (ESR) (Eisler, 1967). The findings were similar with anemia associated with erythropenia that was reported by Srivatava and Mishra (1979) in *Colisa fasciatus* after acute exposure to lead. Similar results have been reported for several freshwater fishes (Khalaf Allah, 1999; Balathakur and Bais, 2000; Rehulka, 2000; Gbem *et al*., 2003; Aderolu *et al*., 2010). The increase in white blood cell in acute bioassay studies could be associated with an increase in antibody production which help in survival and recovery in the fish exposed to sub-lethal concentration of glyphosate. Similar trend was also reported by Joshi *et al*. (2002) and Ekrem *et al*. (2013). The fluctuation in the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in the present study, clearly indicates that the concentration of hemoglobin in the red blood cells were much lower in the exposed fish than in the control fish, depicting anemic condition. Bhagwant and Bhikajee (2000) observed similar fluctuations. Figure 3 show mini tab LC_{50} probit plot at 96 hours, indicates the median value which gave an anti-log value of 6.838 mg/L which is the LC_{50} value at 96 hours.

Conclusion

In conclusion, acute concentrations of glyphosate are harmful and posed toxic metabolic stress to Heteroclarias and it is concentration and time dependent.

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Table 1. Effect of differential concentration of glyphosate on hematological parameters of Heteroclarias after 96 hours of exposure.

Conc. (mg/L)	RBCC ($x10^6mm^3$)	WBCC (x500mm3)	Hb (g/100mL)	PCV (%)	MCV ($x10^6$Pgcel)	MCH ($x10^6$Pgcel)	MHCH (g/100mL)
0	227.67±1.45^a	7552.00±73.90^e	10.50±0.12^a	31.67±0.33^a	1.39±0.01^c	0.46±0.03^c	32.49±0.66^b
5.4	222.67±1.45^b	6272.00±73.90^f	9.87±0.09^b	29.67±0.33^b	1.33±0.01^c	0.44±0.00^c	33.26±0.13^{ab}
7.2	187.67±1.45^c	8064.00±73.90^d	9.17±0.09^c	27.67±0.33^c	1.47±0.01^b	0.49±0.00^b	33.13±0.14^{ab}
9	137.67±1.45^d	9856.00±73.90^c	7.50±0.12^d	22.67±0.33^d	1.65±0.01^a	0.54±0.00^a	33.09±0.26^{ab}
10.8	122.67±1.45^e	10363.67±74.03^b	5.67±0.20^e	17.00±0.58^e	1.38±0.03^c	0.46±0.01^c	33.33±0.12^{ab}
12.6	112.67±1.45^f	10880.00±73.90^a	4.37±0.20^f	13.00±0.58^f	1.15±0.04^d	0.39±0.01^d	33.58±0.15^a

Means with the same superscript along the columns are not significantly different (P>0.05)

Table 2. Mean (±SE) of Heteroclarias exposed to acute concentration of glyphosate after 96 hours on some leucocytes differential count.

Conc.(mg/L)	Neutrophils (%)	Lymphocytes (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)
0.00	19.00±0.58^b	45.00±0.58^f	Nd	Nd	Nd
5.40	15.00±0.58^d	51.00±0.58^e	Nd	Nd	Nd
7.20	22.00±0.58^a	61.67±0.88^d	Nd	Nd	Nd
9.00	20.00±0.58^b	66.00±0.58^c	Nd	Nd	Nd
10.80	17.00±0.58^c	70.00±0.58^b	Nd	Nd	Nd
12.60	13.67±0.58^d	75.00±0.58^a	Nd	Nd	Nd

Means with the same superscript along the columns are not significantly different (P>0.05).
Nd = Not detected

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