Original Research Article

Evaluation of glyphosate Roundup Turbo (450 g/l) on haematobiochemical indices of fresh water African catfish *Clarias gariepinus*

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Abstract

The escalating use of glyphosate-based herbicides in agricultural practices has sparked concerns regarding their potential ecological impact. This study undertakes a comprehensive investigation into the acute ecotoxicological effects of a brand new glyphosate (Roundup Turbo 450 g/l) on haematology and biochemical indices of *Clarias gariepinus* a commercially vital fish species in Nigeria. Fish were exposed to 0.0 as control, 0.5, 0.7, 0.9 and 1.2 mg/l glyphosate for 96 hours in the labouratory. Water quality parameters monitored were temperature, pH, dissolved oxygen electrical conductivity, nitrate and nitrite. The blood, liver and gills were extracted and analyzed. Haematological and biochemical analyses revealed significant changes ($P < 0.05$) as concentration increases. The decrease in red blood cell, pack cell volume, haemaglobin and white blood cell was indicative of glyphosate-induced hematotoxicity. Biochemical analyses disclosed alterations in enzymatic activities within liver and gill tissues, including significant increase in alanine aminotransaminases, aspartate aminotransferase and alkaline phosphatase, suggesting hepatic and branchial dysfunction and decrease in total protein, globulin and albumin,

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showing enhanced disruption in protein synthesis. Glucose and albumin in liver were not detected. Behavioral observations indicated the fish heightened stress response, characterized by erratic swimming patterns, increased agitation, equilibrium loss, hyperactivity and skin erosion. The 96-hour LC_{50} value for this study was 2.50 mg/l and it highlights glyphosate roundup turbo's potential to exert detrimental effects on *Clarias gariepinus* populations. These findings underscore the imperative for sustainable agricultural practices, judicious pesticide management, and rigorous environmental monitoring to mitigate the ecological risks associated with glyphosate use.

Keywords: Acute study; aquatic toxicology; biochemistry; blood indices; *Clarias gariepinus*; water quality

1. INTRODUCTION

The increase in aquatic pollution caused by industrialization, urbanization and agricultural activities is a serious threat to human and environmental health (Amaeze *et al.*, 2020). Chemicals used by agriculture and industries that find their way to the aquatic ecosystem has lately gained more attentions on the agendas of regulating bodies (Mensah *et al.*, 2014; Brack *et al*., 2022; Groh *et al*., 2022). Despite the widespread use of numerous chemical, insufficient information and understanding exist about these chemical with respect to human toxicity and environmental health risk thus hindering effective management and the establishment of environmental safety guidelines.

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Pesticides represent a crucial group of chemicals that can be subject to regulation, and a more precise evaluation of their risk nature could result to major improvements in environmental safety. Pesticides are mixtures of substances used to regulate, prevent or eliminate pests, including weed, nematodes, insects and fungi. Its usage in agricultural fields to control pests is extremely toxic to non-target organisms like fish and affect fish health through impairment of metabolism, sometimes leading to mortality (Shankar *et al*., 2013). The increased use of pesticides and other agrochemical to boost crop yield and quality is closely linked to the

advancement of agricultural mechanization. Thus, pesticides have become an indispensable tool in large-scale agricultural development in Nigeria (Opute and Oboh, 2021). In addition, drainage and irrigation systems may become polluted through the application of pesticides during agricultural and pest control activities, and this may negatively impact the living and non-living members of the contaminated water (Mohamed *et al*., 2012). Pesticides includes fungicides, insecticides and herbicides.

The widespread adoption of herbicides in agricultural sectors worldwide serves as a primary mechanism for controlling unwanted plant growth. However, the uncontrolled use of herbicides intended to increase agricultural output can have a far reaching consequences for aquatic ecosystems and organisms that inhabit them. As such, it may become hazardous to the health of man when these aquatic organisms are harvested and consumed (Williams, 2011). There is a growing public concern about the amount of herbicides that have been introduced into the environment by leachate and runoff, not to mention that the contaminations of the aquatic environments generally occur by a mixture of these compounds and not by isolated substances (He *et al.,* 2012). Herbicide employed in this study is glyphosate Roundup turbo. Glyphosate (N–phosphonomethyl glycine) is the active ingredient in *Roundup turbo*, a broad-spectrum, post-emergence, non-selective herbicides used for controlling annual and perennial grasses, broad-based leafed weeds, trees and other species (Okayi *et al.,* 2010). Glyphosate is considered a probable human carcinogen based on scientific evaluation in humans and other laboratory animals (Portier, 2016).

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In aquatic organisms, including fish, any kind of waterborne pollution is easily reflected in the circulatory system Ismail *et al*. (2017); therefore, blood parameters are one of the commonest and most important biomarkers for diagnosing the structural and functional status of fish exposed to effluents and pollutants (Faggio *et al*., 2014; Burgos Aceves *et al.*, 2019). Changes in hematological parameters depend on the magnitude of the impact of contaminant (concentration), the duration of exposure, fish species, age and health status (Alimba *et al*., 2019). Alterations in white blood cell (WBC) numbers might be regarded as a prognostic tool or an early-warning signal of disturbance in homeostatic defense abilities of fish (Oladokun *et al*. 2020). Udume *et al*. (2022a) noted that increase in ammonia (NH_3) levels was principally responsible for the haematological modification of fish. Alterations in blood biochemical parameters as important diagnostic tool can be used for the detection of abnormalities in the liver and other tissues both in the labouratory and field studies (Banaee *et al*., 2011; Javed *et al.*, 2016; Adam *et al.*, 2019; Shah and Parveen, 2020)). According to Prabesh *et al*. (2021) Biochemical parameters of golden mahseer were affected by sub-lethal exposure of chlorpyrifos and dichlorvos.

The toxicity of herbicides to fish rely on size and species (Noga, 2012). This study employed African catfish, *Clarias gariepinus* as the test species becuase of its significant economic importance and wisespread cultivation in Nigeria and other developing country. *Clarias gariepinus* also referred to as mudfish, is very hardy and tasty, tolerate adverse aquatic conditions where other cultivable fish species cannot survive (NASS, 2010). The choice of fish as a model in ecotoxicological research could be valued as fish serves as a totally sensitive bio-indicator of aquatic infection in tropical regions (Mdegela *et al*., 2010). The potentiality of application of the findings from those researches on humans and different environmental health problems has made fish a greater appealing model organism in toxicology research (Govind, 2011).

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Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to environment and human (OECD, 2021). The study of these chemical is essential especially in the early life stages of fish because the continuous use could contribute to a decline in the fishery of water. Aquatic Bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals (USEPA, 2012; Omoniyi, 2018).

In this study, glyphosate a common herbicide with a brand name "*Roundup Turbo"* used by farmers especially in the South-Eastern part of Nigeria is evaluated for its effect of acute exposure in *Clarias gariepinus.*

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted in wet laboratory Fisheries Department, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture Umudike, Abia State in South Eastern Nigeria. The institute is situated in the tropical rainforest zone on Latitude $05^{0}26^{1}$ – $5^{0}25^{1}$N and Longitude $07^{0}34^{1}$ – $7^{0}36^{1}$E with total annual rainfall ranging from 1800 to 2100mm and at altitude of 122 m above sea level (NRCRI, 2010). The major activities carried out in this location is farming and pesticides commonly used by farmers is organophosphate chemical such as glyphosate hence the choice of the study area. The primary route of glyphosate entry into the research area is *via* human induced activities specifically indiscriminate use of agrochemical by farmers and other users consequently resulting in soil contamination that eventually drains into nearby water bodies.

2.2 Fish sampling and Acclimation

For this study, 150 fingerlings of *Clarias gariepinus* fingerlings with an average weight 2.9±1.90 g, and measuring 6.08±0.07 cm in total length and 5.40±0.20 cm in standard length were sourced from Treasure fish farm in Umuahia, Nigeria and conveyed to the experimental site in plastic containers. Prior the experiment specimen were treated with a prophylactic dose of 0.02% potassium permanganate solution to ensure disease free specimens. They were then acclimated to a 500 litres tank at temperature 26.5^{0}C, pH 7.4 and DO 5.9 for 14 days. The tank was filled with continuously aerated tap water and maintained in a static system. During acclimation period, fish were fed twice with 35% crude protein commercial feed.

2.3 Ethical Approval

Approval was granted by Michael Okpara University of Agriculture Umudike Ethical Committee at the College of Veterinary Medicine to conduct research on pesticides using animal model (MOUAU/CVM/REC/202418). All procedures involving test fishes were carried out with utmost care, adhering to the established ethical principles for animal use in scientific research, as outlined in the EU directive 2010/63/EU.

2.4 Assay chemical

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Comment [ms10]: For this study, 150 fingerlings of Clarias gariepinus were sourced from Treasure Fish Farm in Umuahia, Nigeria. The fingerlings had an average weight of 2.9 ± 1.90 g, a total length of 6.08 ± 0.07 cm, and a standard length of 5.40 ± 0.20 cm. They were transported to the experimental site in plastic containers.
Prior to the experiment, the specimens were treated with a prophylactic dose of 0.02% potassium permanganate solution to ensure they were disease-free. The fish were then acclimated in a 500-liter tank at a temperature of 26.5°C, with a pH of 7.4 and dissolved oxygen (DO) of 5.9, for a duration of 14 days. The tank was filled with continuously aerated tap water and maintained in a static system. During the acclimation period, the fish were fed twice daily with a commercial feed that contained 35% crude protein.

Glyphosate with the trade name "*Roundup turbo*" was used as a test chemical for the study. It was procured as a commercial formulated herbicide at a concentration of 450g/L in a one liter container in Agro-based shop within Umuahia, Abia State, Nigeria with a production date, 9th January, 2024 and expired date, 10th February, 2027.

2.5 Range finding test (LC_{50}) and Preparation of stock solution

This study used a static renewal bioassay, following ASTM standards (1990) to evaluate acute toxicity. Preliminary studies were carried out to evaluate the definitive concentration range for testing chemical. This was determined following the methods of Sogbanmu *et al.* (2018). Thirty (30) fishes divided into triplicate groups of ten (10), were subjected to varying levels of glyphosate treatment, consisting of a control group (0.0 mg/l) and four treated groups (0.5, 0.7, 0.9 and 1.2 mg/l) dissolved in 40-liter water volume. The fish were not fed during the 96-hour duration (Krishna and Hayashi 2000). Fish mortality were monitored at 24-hour time intervals (24, 48, 72 and 96 hours) and dead fish removed to maintain water quality. The test solutions were replaced every 48 hours with freshly prepared glyphosate solutions to ensure optimal exposure conditions. The median lethal concentration LC_{50} at 96 hours was calculated and recorded.

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2.6 Behavioral responses

Behavioral studies were carried out in the order of U.S.EPA, (2008) and guideline No. 249 of OECD, (2021). Behavioral and formative changes observed were equilibrium status, swimming rate, fin movement and hyperactivity. Experimental tanks were closely monitored for 10 to 12 minutes to allow enough time to accurately assess each specimen.

2.7 Water quality analysis

Water quality monitoring was performed daily (every 24 hours) throughout the study period in the aquaria. Key parameters including dissolved oxygen, pH, temperature, total dissolved solids and electrical conductivity were assessed using a HI-769828 multi-parameter probe. Ammonia, nitrate and nitrite analysis were conducted using NT LABS test kit.

2.8 Haematological Analysis

2.8.1 Blood sampling

Blood samples were extracted from the caudal peduncle of the test organism using 2.5ml syringes and hypodermal needle treated with an anticoagulant ethylenediaminetetraacetic acid (Lewbert 2001; Ada *et al.*, 2012). Composite samples of 3-5 fishes were taken from each replicate in order to obtain sufficient blood for haematological analysis. All samples were properly labeled. The haematological indices were evaluated to determine changes and parameters assessed include: red blood cell (RBC), packed cell volume (PCV), haemoglobin content (HBC), white blood cell (WBC). These haematological indices were assessed simultaneously for each blood specimen using an Automated Haematology Analyser BC-2800 following standard procedures outlined by the manufacturers Shenzen MINDRAY Bio-Medical Electronics Co., Ltd, China.

The derived hematological indices consisting mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were obtained using the calculation method described by Dacie and Lewis (1984).

$$MCV\ (fl) = \frac{\text{PCV (\%) X 10}}{\text{RBC Count in Millions/mm3}}$$ equation 1

$$MCH\ (pg) = \frac{\text{Hb X}\frac{\text{g}}{\text{dl}}\text{x 10}}{\text{RBC Count in Millions/mm3}}$$ equation 2

$$MCHC\ (g/dl) = \frac{\text{Hb X}\left(\frac{\text{g}}{\text{dl}}\right)\text{x 10}}{\text{PCV (\%)}}$$ equation 3

2.9 Determination of serum biochemical indices

The serum was isolated from the fish blood samples via centrifugation at 3500 rpm for 15 minutes, after which the extracted serum was preserved at -20 °C. After 96-hour exposure to glyphosate, serum biochemical parameters were evaluated in control and test fish. Liver and gill tissues were assessed for alanine amino transferase (ALT) and aspartate amino transferase (AST) activity using standardized kits from Randox Laboratories United Kingdom following recommended procedures. Alkaline phosphatase (ALP) were measured according to the method proposed by Bergmeyer *et al*. (1980). This underlying principle depend on the enzymatic action of serum alkaline phosphatase which hydrolyzes phenolphthalein monophosphate to produce phosphoric acid and phenolphthalein. The phenolphthalein then undergoes a colorimetric change to pink at alkaline pH values enabling photometric determination. The glucose levels in liver and gills were measured according to the protocols of Schmidt (1961). Serum total protein was determined according to the protocols of Lowry *et al*. (1951) as modified by Spector (1978), taking Bovine serum albumin (BSA) as a standard. A colorimeter was used to measure the absorbance of the sample relative to a standard at 540 nm. The globulin concentration was determined through subtraction of albumin values from the total protein values. Activities of ALT, AST and ALP in the serum were expressed in international unit per litre (IU/L).

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2.10 Data analysis

Data were analyzed using Statistical Packages for Social Sciences (SPSS) version 23 (IBM Corporation, Armonk, NY, USA). The study employed Analysis of Variance (ANOVA) to examine the mean values of hematological and biochemical parameters aiming to detect significances among various chemical exposures and concentrations. To further investigate significant differences, Least Square Difference (LSD) and Duncan test was used. The threshold for statistical significance was set at $p<0.05$ following the guidelines established by (Steel and Torries, 2003).

3. RESULTS

3.1. Acute toxicity effect of glyphosate Roundup Turbo on the experimental fish

Table 1 presented the result of an experiment where fish were exposed to different concentrations of glyphosate in separate tanks. The result revealed a clear dose-response relationship between mortality in *Clarias gariepinus* and exposure of test chemical. The control experiment yielded no mortality during the exposure duration (24-96 hours) demonstrating no toxic effect. However, in 24 hours 2% mortality was observed and at 96 hours 20% mortality occurred at concentration of 0.5 mg/l whereas at concentration of 1.2 mg/l, 22% mortality were observed under 24 hours and 100% mortality occurred under 96 hours. The highest percentage mortality (100%) were seen at concentration 1.2 mg/l of glyphosate followed by 0.9 mg/l which

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had 80% at 96 hour exposure time. This table therefore suggest that as the concentration of glyphosate increases mortality rate also increases with time.

Table 1: Total mortality of *Clarias gariepinus* exposed to varying glyphosate doses

		Exposure Duration (hours)							
Conc. (Mg/l)	**No of Fish**	**24**	**Mortality (%)**	**48**	**Mortality (%)**	**72**	**Mortality (%)**	**96**	**Mortality (%)**
0.0	30	0	0	0	0	0	0	0	0
0.5	30	2	6	2	6	4	10	6	20
0.7	30	5	16	6	20	8	26	10	30
0.9	30	12	40	15	53	20	66	25	80
1.2	30	22	73	26	86	30	100	30	100

3.2 Mean physicochemical parameters of the test concentrations glyphosate Roundup Turbo on *Clarias gariepinus*

Table 2 presented the result of water quality parameters at different concentrations of glyphosate. The temperature of the study remained relatively constant across all concentrations with a slight increase at 0.7 mg/l. The temperature ranged between 25.51±0.51 and 25.85±0.52 showing that the temperature was closely monitored and kept within a range, providing a stable environment for the research. pH decreases gradually as concentration increases but remained within a narrow range (5.24-6.57). Electrical conductivity increases as concentration increases indicating potential increase in ionic substances. The level of Total dissolved solids, ammonia, nitrate and nitrite remained relatively constant cross all concentrations. The result also showed that Dissolved oxygen decreased significantly as concentration increases indicating potential toxic effect to the fish. However, all other parameters showed no significant difference and were within the range of standard quality water (APHA, 2005).

Table 2: Mean water quality parameters of culture medium of *Clarias gariepinus* observed during 96-hour exposure

	Concentrations (Mg/l)				
Parameters	**0.0**	**0.5**	**0.7**	**0.9**	**1.2**
Temp (^{0}C)	25.51±0.51	25.60±0.19	25.85±0.52	25.62±0.10	25.49±0.18
pH	6.57±0.18^a	6.29±0.15^a	6.22±0.12^a	5.50±0.20^b	5.24±0.40^b
DO (mg/l)	5.11±0.21^c	4.08±0.18^d	3.95±0.26^c	3.98±0.20^b	3.85±0.35^a
TDS	95.83±1.10^a	96.00±1.60^a	95.54±2.31^a	95.17±1.70^a	95.08±1.90^a
EC (µs)	45.7±0.58^c	46.3±0.58^c	47.0±2.00^c	49.7±0.58^b	56.0±1.00^a
NH_3 (mg/l)	0.5±0.17	0.34±0.16	0.32±0.20	0.36±0.18	0.40±0.16
NO_3^- (mg/l)	7.10±2.62	7.50±2.47	6.68±2.46	7.50±2.47	7.08±2.58
NO_2^- (mg/l)	1.41±0.61	1.41±0.47	1.45±0.41	1.35±0.50	1.41±0.61

Remark: Mean values with different alphabetical superscripts are significantly different (P<0.05)
Temp – Temperature, pH – Potential Hydrogen, DO – Dissolved oxygen, TDS – Total dissolved solids, EC – Electrical conductivity, NH_3 – Ammonia, NO_3^- – Nitrate, NO_2^- - Nitrite

3.3 Behavioural changes of test specimen to glyphosate Roundup Turbo exposure

Table 3 presented the behavioural toxicity of glyphosate Roundup to *Clarias gariepinus* at various concentrations based on a 96-hour exposure time. The observed aberrant behavior demonstrated a strong temporal and dose-dependent relationship glyphosate exposure. In the control group (0.0 mg/l), no anomaly were observed from 24-96 hour of exposure. The fish in this group exhibited normal swimming patterns, body and fin movement and normal equilibrium status whereas fish in exposed groups exhibited a range of abnormal behaviours when exposed to test chemical ranging from swimming rapidly and erratically indicating agitation to increased mouth and opercula movement, decline in swimming rate, suggesting respiratory impairment and loss of equilibrium. As exposure progressed, fish produced excess mucus and became increasingly lethargic, settling at the bottom of the container. The severity of these anomalies were high in 0.9 and 1.2 mg/l during the 72 and 96-hour exposure to glyphosate.

Table 3: Behavioural changes of *Clarias gariepinus* subjected to glyphosate Roundup turbo at varying levels

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Duration/ Concentration (mg/l)	Swimming rate	Fin movement	Hyperactivity	Skin erosion	Equilibrium status	Uncoordinated movement
24 hour						
0.0	-	-	-	-	-	-
0.5	+	+	++	-	-	+
0.7	+	+	++	-	-	+
0.9	++	++	+++	-	+	+
1.2	++	++	++	+	++	+
48 hour						
0.0	-	-	-	-	-	-
0.5	+	++	+++	+	+	+
0.7	++	++	+++	+	+	++
0.9	++	++	++	+	++	++
1.2	++	++	+	++	++	++
72 hour			-			-
0.0	-	-	-	-	-	-
0.5	++	++	++	+	++	++
0.7	++	++	++	+	+++	++
0.9	+++	+	+	++	+++	++
1.2	+++	-	-	+++	+++	+++
96 hour						
0.0	-	-	-	-	-	
0.5	++	+++	++	+	+++	++
0.7	++	++	++	+	+++	++
0.9	+++	+++	+	+++	+++	+++
1.2	-	-	-	+	-	-

Key: None = (-), Low = (+), Medium = (++), High = (+++)

3.4 Haematological parameters of *Clarias gariepinus* during 96-hour exposure to glyphosate Roundup Turbo

Table 4 showed mean value of the haematology of the test fish exposed to acute concentrations of glyphosate for 96-hour. Packed cell volumes (PCV) of the control group (0.0 mg/l) were significantly higher (48.35±049) than the exposed group. Toxicant exposure in PCV decrease with increase in concentration of glyphosate except for 0.5 mg/l which was slightly higher than 0.7 mg/l but showed no significant differences. The white blood cell (WBC) of the exposed group were significantly lower than the control. There were no significant difference between 0.5 mg/l and 0.7 mg/l. Also, no significant difference between 0.9 mg/l and 1.2 mg/l. The haemoglobin was highest (25.84) in the control group while it was least (4.54) in the group exposed to highest (1.2 mg/l) concentration of glyphosate roundup turbo. The treated group showed gradual decrease in response to increased level of the toxicant while the ungroup group had the highest red blood cell count (RBC). The platelet (PLT) count exhibited significant difference between the control group and those exposed to glyphosate roundup turbo. A notable

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uptrend in platelet values was observed when comparing the control to the toxicant-exposed groups. In contrast, significant difference was observed in the erythrocyte indices, specifically mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Notably, MCV values at concentrations of 0.9 and 1.2 mg/l were markedly higher compared to the control group with a statistical significance at ($P < 0.05$). The highest mean corpuscular haemoglobin value was recorded at concentration of 1.2 mg/l while the control group exhibited higher mean corpuscular haemoglobin concentration (53.32±1.05) compared to the group exposed to glyphosate. The highest value of neutrophils (26.67±0.48) was recorded in the control group followed by 0.5 mg/l (24.54±0.57) and least value (16.45±0.45) at 1.2 mg/l of glyphosate. Lymphocyte values showed a dose dependent increase with increase in concentration. The highest value (89.40±1.02) were notable at 1.2 mg/l and the least value (75.00±0.10) was recorded at 0.0 mg/l (control). The result also showed that there were no detectable presence of monocytes, basophils and eosonophils in the haematological studies. Generally, there were notable differences in the values between the control group and other concentrations indicating a distinct effect of glyphosate on the parameters measured. The haematological profile showed reduction in mean values of PCV, RBC, HB and neutrophils with increasing concentrations while the mean values of WBC, MCV, PLT, MCH, MCHC and Lymphocyte demonstrated an increase suggesting a notable change in the blood parameters.

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Table 4: Mean haematological parameters of *Clarias gariepinus* exposed to varying concentrations of glyphosate Roundup Turbo during 96-hour

	Concentrations Mg/l				
Parameters	**0.0**	**0.5**	**0.7**	**0.9**	**1.2**
PCV (%V)	48.35±049^{e}	22.59±1.20^{d}	19.10±0.37^{c}	17.20±0.91^{b}	12.02±0.60^{a}
WBC (x10^{3}mm^{3})	2489.00±26.47^{c}	1816.68±75.39^{b}	1779.32±28.86^{b}	1450.00±68.27^{a}	1398.67±76.10^{a}
HB (g/dl)	25.84±0.40^{d}	7.95±0.52^{c}	7.38±0.07^{c}	6.32±0.12^{b}	4.54±0.72^{a}
RBC (x10^{7} /µl)	6.30±0.08^{e}	2.96±0.18^{d}	2.49±0.12^{c}	1.61±0.05^{b}	1.04±0.02^{a}
MCV (fl/cell)	77.00±0.01^{a}	76.60±0.48^{a}	76.97±0.01^{a}	128.08±2.59^{c}	123.50±2.90^{b}

MCH (pg/cell)	40.83±0.78^{b}	27.80±1.03^{a}	26.24±0.68^{a}	37.80±3.49^{b}	47.51±8.26^{c}
MCHC (g/dl)	53.32±1.05^{cd}	36.08±1.29^{bc}	33.82±1.24^{ab}	29.75±2.20^{a}	39.71±6.25^{c}
PLT	101.4±5.04^{d}	105.2±6.10^{c}	109.5±7.04^{b}	112.4±8.20^{a}	113.4±5.04^{a}
Neutrophils	26.67±0.48^{a}	24.54±0.57^{b}	22.24±0.67^{c}	19.45±0.50^{d}	16.45±0.45^{e}
Lymphocyte	75.00±0.10^{d}	76.40±0.50^{d}	79.20±0.85^{c}	84.10±1.00^{b}	89.40±1.02^{a}

Remark: Mean values with different alphabetical superscripts along a column for a parameter are significantly different ($P< 0.05$)
PCV - Pack Cell Volume, WBC - White Blood Cell, HB - Haemoglobin, RBC - Red Blood Cell, MCV - Mean Corpuscular Volume, MCH - Mean Corpuscular Haemoglobin, MCHC - Mean Corpuscular Haemoglobin Concentration, PLT – Platelet

3.5 Liver and gill biochemical changes in *Clarias gariepinus* exposed to varying concentration of glyphosate Roundup turbo

Table 5 presents the result of glyphosate exposure on various biochemical parameters in liver and gill tissues of fish. The result of the biochemical properties indicated a significant increase in all group exposed to the chemical compared to the control. The highest value (31.53±1.07) of Aspartate amino transferase (AST) enzyme in liver and in gill (23.10±1.72) was recorded at concentration 1.2 mg/l with lowest value in liver (22.33±2.89) and gill (18.50±1.45) observed in the control group. Alanine amino transferase (ALT) also recorded an increase with the highest value in the liver (53.85±0.30) and gills (40.85±1.30) at 1.2mg/l. Alkaline phosphatase (ALP) also showed a significant increase in both gill and tissue of the fish with an increase in glyphosate concentration. The highest value in liver (87.20±0.38) and gill (52.00±0.28) was recorded at 1.2 mg/l and lowest value in liver (57.20±0.08) and gill (44.00±1.20) was recorded at

0.0 mg/l (control group). Total protein, globulin and albumin decreased in values with increase in the levels of glyphosate roundup turbo. Non-significant difference (P>0.05) were observed in gills of glucose and albumin as it was not detected in the study. In general, this results showed significant differences in all the biochemical parameters except glucose and albumin in gills among different concentrations of glyphosate (P< 0.05).

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Table 5: Mean liver and gill biochemical changes in *Clarias gariepinus* exposed to varying concentration of glyphosate Roundup during 96-hour

		Concentration of glyphosate Mg/l				
Parameters	**Tissue**	**0.0**	**0.5**	**0.7**	**0.9**	**1.2**
AST (u/l)	Liver	22.33 ± 2.89^{d}	25.23 ± 1.50^{c}	25.90 ± 0.28^{c}	28.03 ± 1.89^{b}	31.53 ± 1.07^{a}
	Gill	18.50 ± 1.45^{d}	19.05 ± 1.20^{d}	21.50 ± 2.18^{c}	25.50 ± 1.90^{a}	23.10 ± 1.72^{b}
ALT (u/l)	Liver	40.20 ± 0.45^{c}	44.51 ± 0.30^{b}	49.90 ± 1.00^{a}	52.10 ± 0.85^{a}	53.85 ± 0.30^{a}
	Gill	25.00 ± 1.50^{b}	38.55 ± 2.04^{a}	39.40 ± 1.05^{a}	40.10 ± 2.15^{a}	40.85 ± 1.30^{a}
ALP (u/l)	Liver	57.20 ± 0.08^{b}	85.50 ± 0.40^{a}	86.20 ± 0.08^{a}	86.90 ± 0.36^{a}	87.20 ± 0.38^{a}
	Gill	44.00 ± 1.20^{b}	50.60 ± 1.31^{a}	51.01 ± 1.24^{a}	51.82 ± 1.47^{a}	52.00 ± 0.28^{a}
Total protein	Liver	25.14 ± 0.02^{a}	18.20 ± 0.02^{b}	16.14 ± 0.02^{b}	15.41 ± 0.04^{c}	11.55 ± 0.05^{d}
	Gill	$4.90.\pm0.45^{a}$	3.00 ± 0.90^{b}	2.45 ± 0.04^{c}	1.80 ± 0.30^{d}	1.05 ± 0.43^{d}
Globulin (g/dl)	Liver	5.00 ± 0.02^{a}	3.51 ± 1.22^{b}	2.1 ± 0.02^{c}	2.20 ± 0.05^{c}	1.00 ± 0.09^{d}
	Gill	4.20 ± 1.12^{a}	2.40 ± 1.02^{b}	2.10 ± 1.14^{b}	1.20 ± 1.50^{c}	1.00 ± 1.40^{c}
Glucose (ug/l)	Liver	28.10 ± 0.59^{c}	30.70 ± 0.28^{c}	35.50 ± 0.28^{a}	34.00 ± 0.28^{b}	36.70 ± 0.28^{a}
	Gill	0.00	0.00	0.00	0.00	0.00
Albumin (g/dl)	Liver	21.01 ± 0.03^{a}	19.10 ± 0.02^{b}	18.40 ± 0.09^{b}	15.09 ± 0.12^{c}	12.52 ± 0.20^{d}
	Gill	0.00	0.00	0.00	0.00	0.00

Remark: Mean values with different alphabetical superscripts along a column for a parameter are significantly different (P< 0.05) AST – Aspartate amino transferase, ALT – Alanine amino transferase, ALP – Alkaline phosphatase

4. Discussion

Evaluation of acute toxicity provides preliminary understanding of the adverse effects of emerging contaminants, facilitating the establishment of concentration limits for further research into sub-lethal effects (Majumder *et al.*, 2019). Based on our findings the 96-hour LC_{50} of glyphosate Roundup turbo recorded in this study was 2.50 mg/l. However, the 96-hour LC_{50} of glyphosate "Forceup" was 1.5 mg/l in *Clarias* species (Ifeoma *et al.*, 2023). The observed differences could be as a result of formulation of the toxicants and age of the fish used. The calculated 96-hour LC_{50} value reported in this study was higher than the value previously documented for 0.76 mg /l for *Lates calcarifer* Thanomsit *et al.* (2016) and 0.0025 mg/L in zebra fish Wang *et al.* (2023).

In contrast, LC_{50} of the present study was lower when compared to the findings of Nwani *et al.* (2013) who reported 96-hour LC_{50} for *Tilapia zilli* at 211.80 mg/l of glyphosate, *Cyprinus carpio*

at 86 mg/l (Deivasigamani, 2015) and *C. punctatus* at 5.93 mg/l Kumar *et al.* (2009). The observed variations in the recorded values may be influenced by a range of factors, ambient water temperature, species-specific traits and the developmental stage and size of the fish (Somdare, 2016). Furthermore, Findik *et al*. (2001) and Osioma *et al.* (2021) observed that differences in LC_{50} values can be attributed to variations in fish physiology, environmental parameters, water chemistry and the testing methodologies employed.

Acute toxicity data provides valuable insights into understanding the mechanism of action of substance and its potential harm to non-target species. This information is particularly useful for comparing the effects of different chemicals at various doses (Santos *et al.,* 2010). During this study, herbicide dosage increase resulted to corresponding increase in mortality. Our findings is also in agreement with Fishel *et al.* (2013) and Ali and Muhammad (2016) who reported that percentage mortality of *Clarias gariepinus* juvenile increases with increase in concentration of glyphosate. The introduction of pollutants into aquatic environments alters various water parameters such as temperature, pH, alkalinity, and dissolved oxygen (Fagbenro, 2002; Olufayo, 2009). Water temperature of the study ranged from 25.49 to 25.60^0C. This is in line with the report from Britz and Hecht (1987) for *Clarias gariepinus* juveniles with optimum temperature range 25^0C to 33^0C. In this study, pH level and dissolved oxygen in the treated groups test media were significantly reduced ($P < 0.05$), possibly due to glyphosate's oxidative effects on oxygen molecules. This result is constant with the findings of Ololade and Oginni (2010) who investigated the impact of nickel on *Clarias gariepinus*. Ammonia (NH_4), nitrate (NO_3) and nitrite (NO_2) measured during the study could be as a result of uneaten feed, faecal matter and the test chemical used. This findings is in agreement with (keremi *et al.*, 2014).

Observations of fish behaviour following exposure to toxic substances serve as a good indicator for their internal physiological condition, making it a valuable tool for assessing the ecological effects of aquatic pollutant (Simakani *et al.*, 2018). In this study, *Clarias gariepinus* exposed to acute concentrations at 0.5, 0.7, 0.9 and 1.2 mg/l of glyphosate for 96-hours showed increased anomalous behaviours like loss of equilibrium, abnormal swimming, uncoordinated movement, skin erosion and hyperactivity with decreased aggressive behavior when compared to the control group. These signs manifested before death occurred because of physiological reaction from acute glyphosate toxicity. Similar observations have been found in Nile tilapia Fouad *et al.* (2022) and common carp Fallah (2022) after acute exposure to fungicides. Also Ayanda *et al.* (2017) and Lanzarin *et al.* (2020) who exposed *Clarias gariepinus* and Zebra fish models respectively to acute concentration of glyphosate. Fish exhibit an increased secretion of mucus which is a characteristics response to stress whether induced by environmental factor or diseases. The production of excess mucus in this study was possibly due to the toxicant introduced in the aquatic media as the skin is one of the primary organs to come into contact with aquatic contaminants. The increased mucus secretion can be seen as a natural defensive mechanism. This protective layer forms a barrier between the fish's body and the contaminated environment, mitigating the effects and ultimately leading to the removal of excess epidermal mucus (Ibrahem 2012). More so, the abnormal behaviours caused by glyphosate exposure could be related to the release of its toxic metabolites aminomethylphosphonic acide (AMPA) which may contribute to disrupting brain functions causing neurotoxicity (Chung and Wong, 2022; Campanale *et al.*, 2023). In this regard, loss of equilibrium and lateral swimming are most likely caused by nervous system dysfunction (Sharma, 2019). This investigation also noted fin erosion in fish exposed to elevated acute levels of glyphosate-based Roundup turbo. This phenomenon may be attributed to COX-1 inhibition, triggering the release of endothelin-1, a strong vasoconstrictor potentially

Comment [ms29]: This finding

Comment [ms30]: Also,

causing epithelial cell degeneration and erosion. Studies by various researchers Matozzo *et al.* (2020) and Peillex and Pelletier (2020) reported similar findings in fish models exposed to xenobiotics.

The analysis of blood serves as a vital tool for evaluating animal health, as haematological markers reflect the body's response to environmental change, influencing energy level, respiratory function and immune defense (Iheanacho and Odo, 2020; Melefa, 2020, Onah, 2020). The presence of toxic chemicals in aquatic environments results in significant hematopoietic disturbances (Ahmed and Zakiya, 2022). In this present study, RBC, HB, PCV and WBC decreased significantly $P < 0.05$ during acute exposures to glyphosate roundup when compared to the control. This decline suggests that glyphosate exposure in water may have triggered anaemia in the experimental fish. The observed decrease in PCV may be linked to gill dysfunction and osmoregulatory imbalance, and haemodilution caused by toxic effects. Our findings are similar with previous studies by Udume *et al*. (2022b) who reported anemic condition on sub-lethal dose of glyphosate 360 g/l exposed to African catfish, Maurya *et al.* (2019) in *Heteropneustes fossilis* juveniles exposed to industrial wastewater pesticides and Udume *et al.*, (2022a) *Clarias gariepinus* exposed to varying levels of acute ammonia (NH_3). Furthermore, the decrease in haemoglobin concentration implies impaired oxygen delivery to tissues leading to respiratory stress and reduced physical performance (Amaeze *et al*., 2020). Research suggests that pesticide exposure can disrupt erythropoiesis, leading to decreased red blood cell production and haemoglobin synthesis, ultimately resulting in lower red blood cell counts and Hb levels (Mostakim *et al.,* 2015). More so, pesticide-derived reactive oxygen species (ROS) can cause oxidative damage to red blood cells and haemoglobin, impairing oxygen (Lutnicka *et al.,* 2016). The observed decrease in white blood cell count among treatment groups aligns with Adeyemo *et al*. (2007) findings, where *Clarias gariepinus* exposure yielded similar result. This phenomenon was further supported by Olanike (2007) who attributed the decline to stress-induced ephinephrine release, leading to leucocyte depletion and impaired immune function. Blood cell indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean copuscular haemoglobin concentration (MCHC) values provides useful information on the cause of anemic condition. Hence the fluctuations in MCV, MCH and MCHC are highly dependent on the values from RBC count, haemoglobin concentration and Pack Cell Volume. This study showed fluctuations in MCV, MCH and MCHC. The observed effect is linked to glyphosate exposure in fish as the data does not exhibit a specific pattern. These fluctuations could be due to deficiency in iron, B_{12}, and folic acid. Our findings is in agreement with Rao (2010), Hossain *et al.* (2015) and Salam *et al.* (2015) who reported that the values of blood cell indices were fluctuated in common carp and some other freshwater fish when they were exposed to acute toxic level of pesticides. Similar findings have been reported in studies involving Nile tilapia and *Clarias gariepinus*, where exposure to mancozeb and atrazine yielded comparable result (Ibrahim *et al.,* 2023; Kanu *et al.,* 2023)

Comment [ms31]: are

Amino transferases (AST and ALT) play a major role in protein metabolism due to their capacity to facilitate transamination reactions between amino acids and keto acids, ultimately contributing to protein synthesis (Ugbomeh *et al.,* 2019). Moreover, AST and ALT integrate protein, carbohydrate, and fat metabolism with the tricarboxylic acid cycle, particularly in response to physiological, pathological, or environmentally induced stress (Ronda *et al.,* 2019). Alterations in AST and ALT activities have been widely employed as biomarkers for detecting aquatic contamination and toxicant exposure in fish (Hedayati *et al.,* 2010; Ibrahim, 2019; Nayak *et al.,* 2019). Research by Tchounwou *et al.* (2015) and Mostakim *et al*. (2015) highlights the

significance of AST and ALT enzymes as sensitive indicators of hepatotoxicity and tissue damage in fish. Specifically, these enzymes are crucial markers for liver, kidney, muscle, and gill damage. The present study evaluated ALT, AST, and ALP enzymatic activities (liver and gills) and noted an increase in response to acute glyphosate roundup turbo exposure. Our findings also suggested that exposure to the toxicant severely impacted these tissues by its reactive oxygen system (ROS) resulting in cellular dysfunction and the subsequent release of biomarkers into the circulatory system. This finding is consistent with previous research (Pesce *et al.,* 2008), which demonstrated a direct relationship between toxicant concentration and enzymatic variation in fish. Similar findings by Sakr and Al-amoudi (2012); Sakr *et al.* (2013) were observed following melathionin exposure in albino mice and rats. The gills, essential for fish respiration, exhibit early signs of aquatic toxicity, including respiratory distress (Ortiz *et al.*, 2003). In this study, *Clarias gariepinus* exposed to glyphosate roundup turbo showed AST and ALT activities increased significantly in their gills compared to controls. This increase likely results from stress caused by the absorption of anthropogenic substances through epithelial cells on the gill surface (Monferran *et al.,* 2005). Additionally, the increase in liver during the study could suggest the generation of keto acids such as keto glutarate and oxaloacetate for energy production necessary to meet the energy demand in response to the stress induced by the herbicide (Varadharajan, 2010). This investigation revealed a marked increase in alkaline phosphatase (ALP) activity in both hepatic and branchial tissues of fish exposed to glyphosate roundup turbo, relative to the control group. These findings align with previous research Shaalan *et al*. (2024); Miri and Khandan (2017), which similarly documented increased ALP activity. The observed upsurge in ALP activity may be attributed to cellular damage or alterations (Khattab, 2007). Furthermore, the heightened ALP levels in the liver could be a response to stress triggered by hepatobiliary cells during detoxification processes. The primary energy source for organisms is carbohydrates, which are converted into glucose to fuel brain and muscle function (Holcombe *et al.,* 1982). However, stress exposure disrupts carbohydrate metabolism to accommodate shifting energy requirements (Shivaraj and Asiya, 2018). This study's findings suggest that exposure to glyphosate roundup turbo impairs carbohydrate metabolism in *Clarias gariepinus.* Similar to other animals, fish store glucose as glycogen in various tissues, including the liver, skeletal muscle, myocardium, and brain (Raje, 2018). Notably, this study observed a significant elevation in hepatic glucose levels (28.10-36.70 µg/l), potentially indicating stress triggered by glyphosate exposure. This glucose increase aligns with established responses of fish to acute and sub-lethal pollutant effects, characterized by increased glucose levels (Luskova *et al.,* 2002). However, glucose was not detected in the gill. An organism's physiological state can be inferred from its protein metabolic status (Magar and Shaikh, 2012). This study observed a significant decline in total protein levels (25.14 – 11.55) in both gill and liver tissues. This finding aligns with previous research documenting protein depletion in *Clarias gariepinus* exposed to primextra (Nwani *et al.,* 2014) and diazepam (Ogueji *et al.*, 2017). The decreased protein content suggests disrupted protein synthesis or enhanced degradation into amino acids, potentially triggered by glyphosate roundup turbo-induced stress. Stress-induced protein catabolism may funnel amino acids into the tricarboxylic acid cycle for energy production (Naveed *et al.,* 2010; Ganeshwade, 2012). Hepatic impairment due to toxicant stress can also lead to protein depletion (Dogan and Can, 2011).

Comment [ms32]: increases

Furthermore, cellular necrosis may have contributed to the decline in protein levels, disrupting protein synthesis mechanisms (Rajput *et al.,* 2012).

Conclusion

The study used a brand new chemical glyphosate *Roundup* turbo 450g/l. The 96-hour LC_{50} of the study was 2.50 mg/l. Our findings showed increased mortality rate of *Clarias gariepinus was* dose-dependent. It revealed that the chemical affected the liver and gill biochemistry, behaviour and haematological parameters of *Clarias gariepinus.* Blood indices showed significant decline in in erythrocyte count, hematocrit, hemoglobin levels, and leukocyte count suggesting that the chemical was toxic to the fish. Biochemical analyses indicated alterations in enzymatic activities within liver and gill tissues, including elevations in transaminases (ALT and AST) and alkaline phosphatase (ALP), suggestive of branchial and hepatic malfunction. The current investigation revealed a range of morphological and behavioural alterations in exposed specimens, characterized by erratic swimming, loss of balance, mucus secretions, hanging on the surface and inactivity. Water quality was affected due to the increase in the concentration of the toxicant. Generally, the result suggested that the presence of glyphosate roundup turbo is toxic to the health of *Clarias gariepinus*. Hence, we recommend integration of eco-friendly agricultural practices to minimize pesticide application and development of effective pesticide remediation technologies such as glyphosate remediation using bacteria and enzymes.

Comment [ms33]: brand-new

Ethical Approval

The authors hereby declare that the experimental procedures were approved by Michael Okpara University of Agriculture Umudike Ethical Committee (MOUAU/CVM/REC/202418). All procedures involving test fishes were carried out with utmost care, adhering to the established ethical principles for animal use in scientific research, as outlined in the EU directive 2010/63/EU on the protection of animals against cruelty.

Declaration of Competing Interest

The authors declare no competing interest.

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