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ORIGINAL PAPER

Glyphosate Toxicity: Effects on the Behavioural and Growth Responses of Juvenile *Clarias gariepinus*

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Abstract

Glyphosate is a broad spectrum herbicide used primarily in agricultural applications for the control of variety of weeds in both terrestrial and aquatic environments. Pollution of aquatic habitat may result in mass fish mortality or affect the physiology and survival of fish. Thus, this study evaluated the effects of exposure of juveniles of the African catfish, *Clarias gariepinus*, to sub-lethal doses of glyphosate, to determine its effects on the behaviour and growth response. Eight fish samples were introduced into individual plastic tank containing 0.00 ml (control), 2 ml/L (Treatment 1), 4 ml/L (Treatment 2), 6 ml/L (Treatment 3) and 8 ml/L (Treatment 4) of glyphosate in triplicate for 28 days. The physico-chemical parameters of water were within the permissive limits for aquaculture. However, the presence of herbicides in the water threatened the fish's survival, which had detrimental impacts on the experimental fish that could be seen. According to this study, fish exposed to glyphosate displayed a variety of abnormal behaviors, including erratic swimming, loss of equilibrium, restlessness, respiratory distress, paleness/bleaching of the skin, and gasping for oxygen. In contrast, fish samples in the control group did not exhibit any discernible behavioral changes. Also, the weight of exposed fish did not increase significantly ($P < 0.05$) with period of exposure. This study concluded that glyphosate is highly toxic to *Clarias gariepinus* (because of the erratic behaviours and mortality that was directly proportional to concentration and exposure duration). Therefore, indiscriminate application of glyphosate (as well as other herbicides) should be totally discouraged.

Keywords Chemicals · Eco-toxicity · Environmental pollution · Herbicides · Mortality rate

1 Introduction

Pesticides are widely used around the world to prevent pests from wreaking havoc on agriculture and fish farms. Despite the positive impacts of pesticides in agriculture, their usage in the environment is often associated with negative environmental and public health consequences. After being applied, pesticides end up in many aquatic environments, where they have been discovered to be very hazardous to non-target animals, particularly aquatic life forms and their environment (Nwani et al. 2010). The herbicide glyphosate, in particular, is one of the most widely used herbicides in both urban and rural areas. Agricultural and household runoff account for the majority of glyphosate entering the aquatic environment (Green and Young 2006).

The consequences of Pesticide on fish are of great concern (Nwani et al. 2010; Bagheri and Nezami 2000). Despite being mildly harmful to aquatic animals, glyphosate is one of the most major water pollutants in rain, fresh, marine, and groundwater (Battaglin et al. 2003, 2008). It is well-known for its environmental tenacity and global concern, and it has negative consequences for both the environment and humanity (Jones and Kerswell 2003). Although research on the toxicity of glyphosate-based herbicides have been conducted, there is limited evidence on their toxicity and impacts on fish

Significance' Statement Fish exposed to glyphosate exhibited varying abnormal morphology and behavioural responses such as erratic swimming, loss of equilibrium, restlessness, respiratory distress, paleness/bleaching of skin and gasping for oxygen. Toxicity was concentration and time of exposure dependent, hence resulting in the deleterious effects, especially at higher concentrations. The weight of exposed fish did not increase significantly ($P < 0.05$) with period of exposure.

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and aquatic organisms, particularly in the case of sub-chronic exposure (Wilson et al. 1996; Orme and Kegley 2005; Phyu et al. 2006). Determination of toxicity is necessary for detecting the fish's susceptibility to toxicants, as well as assessing the degree of damage to the target organs and the resulting physiological, biochemical, and behavioral abnormalities. As a result, information on the herbicide glyphosate's toxicity and impacts on some native species like *C. gariepinus* is needed to compliment risk assessment studies.

Most studies on glyphosate toxicity on aquatic organisms focuses on haematology (Udume and Anyaele 2022), acute toxicity (Ali and Muhammad 2016), histological aspects, metabolic parameters and genotoxic potential (Moura et al. 2017), immunological and histopathological responses (Ma et al. 2015), genotoxic effects (Moreno et al. 2013) and neurotoxicity effects (Roy et al. 2016). In contrast, effects of contaminants on fish behaviour using glyphosate are less frequently studied. Since behaviour links physiological function with ecological processes, behavioural indicators of toxicity appear ideal for assessing the effects of aquatic pollutants on fish populations. Here, we considered toxicants disruption of fish complex behaviours, such as changes in body colouration, avoidance and social behaviours. Toxicant exposure often completely eliminates the performance of behaviours that are essential to fitness and survival in natural ecosystems, frequently after exposures of lesser magnitude than those causing significant mortality. Unfortunately, the behavioural toxicity of many xenobiotics is still unknown, warranting this study.

The African catfish (*Clarias gariepinus*) was chosen as the test organism because it is the most widely cultivated fish species in Nigeria and a good biological model for toxicological studies (Mhadhbi et al. 2010) due to a variety of characteristics, including high growth rates, efficiency in adapting to various diets, great resistance to diseases and handling practices, easy reproduction in captivity at a prolific rate, easy acclimatization to laboratory conditions and wide distribution in the freshwater. *Clarias gariepinus*, in particular, can withstand severe conditions that other cultivable fish species cannot (Ayoola 2008). As a result, this study looked into the behavioural and growth responses of *Clarias gariepinus* when it was exposed to the herbicide glyphosate. Juveniles were used in the experiments because they are more susceptible to environmental changes than older, more mature fish.

2 Methods

2.1 Experimental Set-up

One hundred and fourty juveniles of *Clarias gariepinus*, of mean weight 35.82 ± 0.02 g, aged 6 weeks were acclimatized in a well aerated plastic aquarium in the Department of Fisheries and Technology, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State for 14 days. The fish samples were fed twice daily with 2 mm commercial feeds at 5% body weight by 09:00 h and 17:00 h. During the acclimation period, there was no mortality. Also, fish were examined for pathogens and illnesses. The culture water was regularly replenished after every two days to prevent the build-up of metabolic wastes and to ensure that the water quality components were standardized. Moreover, water was monitored for temperature using mercury-in-glass thermometer; pH using pH meter; dissolved oxygen (DO) and conductivity according to American Public Health Association (2005).

After the acclimation period (14 days), preliminary examination of length and weight of each fish were done using a well graduated measuring board and weighing balance respectively prior to the application of herbicides. A preliminary acute toxicity test was performed to identify the appropriate concentration range for evaluating the herbicide glyphosate in order to obtain the value of the 96-h LC_{50} according to Owolabi et al. (2021). Therefore, concentrations in each test media were modestly maintained below the 96-h LC_{50} value of 20 ml/L for the sub-chronic toxicity experiment.

The experimental set-up lasted for 28 days, four different concentrations of the Glyphosate-based herbicides was prepared including the control stock which were all in triplicates in the order of 0.00 ml (control), 2 ml/L, 4 ml/L, 6 ml/L and 8 ml/L). 8 fully acclimatized test fish were exposed into each concentration dissolved in 60 L transparent tank of water according to Rahman et al. (2002). The test media was regularly renewed at intervals of every 24 h (Concentrations were maintained to keep potency strength and minimise the ammonia level). The fishes were being observed for any possible behavioural changes through their swimming activities as well as any physical change and even mortality (USEPA Ecotoxicology Database 2004; Adesina et al. 2020). Experimental fish were fed twice daily with 2 mm feed pellets.

2.2 Determination of Growth Performance Indices

The fishes in different concentrations of the Glyphosate-based herbicides including the control were weighed initially and at the end of the exposure using weighing balance. Growth in this study was expressed as weight gain, percentage weight gain, specific growth rate and Nitrogen metabolism according to Adesina et al. 2020) as follow:

$$\text{Weight Gain} = (\text{Final weight} - \text{Initial weight}) \tag{1}$$

$$\text{Percentage weight gain (PWG \%)} = \frac{\text{weight gain}}{\text{Initial weight}} \times 100 \tag{2}$$

$$\text{Specific Growth Rate (SGR)} = \frac{(\text{Ln final weight} - \text{Ln initial weight})}{\text{Time (experimental period in days)}} \times 100 \quad (3)$$

$$\text{Nitrogen metabolism NM} = \frac{0.549 \times (\text{Initial weight} + \text{Final weight})\,t}{2} \quad (4)$$

where: t = Experimental period in days.
0.549 = Metabolism factor.

2.3 Determination of fish Percentage Mortality and Survival rate

The percentage mortality rate was calculated according to Owolabi (2011) as follows:

$$\%\text{Mortality} = \frac{\text{number of dead fish}}{\text{Initial number of stocked fish}} \times 100 \quad (5)$$

2.4 Statistical Analysis

The results were subjected to analysis of variance (ANOVA) at $P = 0.05$. Duncan's multiple range test was used to separate the means in SPSS (20.0). Descriptive statistics was used to present mean ± Std.

3 Results and Discussion

The appearance and morphology of *C. gariepinus* exposed to varying concentration of glyphosate as presented in Table 1 revealed that the toxicity of the herbicide glyphosate was time and concentration specific because Treatment 1 (2 ml/L) and Treatment 2 (4 ml/L) exhibited normal appearance and morphology in the first week of exposure while Treatment 1 (2 ml/L) (which also exhibited normal appearance and morphology in week 2) was slightly bleached and had greyish-white colouration of the body in weeks 3 and 4. The table also revealed that glyphosate herbicide caused the fish to be seriously bleached including whitish colouration of the fin and yellowish colouration of operculum at high concentration and period of exposure (Fig. 2c). The appearance and morphology of *C. gariepinus* exposed to varying concentration of glyphosate in this study revealed that the toxicity of the herbicide glyphosate was dependent on time of exposure and concentration, since fishes exposed to 2 ml/L and 4 ml/L exhibited normal appearance and morphology in the first week of exposure while they progressively exhibited slightly bleached and greyish-white colouration of the body in the third and fourth week of exposure. This study also revealed that high concentration of glyphosate

Table 1 Appearance and Morphology of *C. gariepinus* exposed to varying concentration of glyphosate

Periods of exposure	Control (0.00)	Trt 1 (2 ml/L)	Trt 2 (4 ml/L)	Trt 3 (6 ml/L)	Trt 4 (8 ml/L)
Week 1	Normal	Normal	Normal	Slightly Bleached	Bleached and white colouration of the body.
Week 2	Normal	Normal	Slightly Bleached	Slight bleached and greyish-white colouration of the body (Fig. 1).	Bleached and white colouration of the body including whitish colouration of the fin and a light yellowish colouration of operculum (Figs. 2a and 3b).
Week 3	Normal	Slight bleached and greyish-white colouration of the body.	Slight bleached and greyish-white colouration of the body.	Bleached and white colouration of the body including whitish colouration of the fin and a light yellowish colouration of operculum.	Bleached and white colouration of the body including whitish colouration of the fin and a light yellowish colouration of operculum.
Week 4	Normal	Slight bleached and greyish-white colouration of the body.	Slight bleached and greyish-white colouration of the body.	Seriously bleached and white colouration of the body including whitish colouration of the fin and yellowish colouration of operculum (Fig. 3a and b)	Seriously bleached and white colouration of the body including whitish colouration of the fin and yellowish colouration of operculum (Figs. 3a and b)

Fig. 1 **A & B:** (Slightly bleached and whitish colouration of the body)

Fig. 2 **A, B and C:** (Bleached and whitish colouration of the body and fins)

herbicide caused the fish to be seriously bleached including whitish colouration of the fin and yellowish colouration of operculum (Fig. 2). Morphological changes of *C. gariepinus* observed in this study is similar to the findings of Ayanda et al. (2017) who observed more pronounced behavioural changes with increase in herbicide concentration. Fafioye et al. (2005) opined that these behavioural changes could results from nervous system disruption, depending on the toxicant concentration. Oladunjoye et al. (2022) made a similar submission that these behavioural changes may be as a result of change in the rearrangement of fish biochemical functions, such as the liver hepatic functions.

The behavioural responses of *C. gariepinus* exposed to varying concentration of glyphosate (as presented in Table 2) revealed that herbicide glyphosate affected the behaviour of the fish based on concentration and time of exposure. The table revealed that the behavioural characteristics of exposed fish ranged from hyperactiveness (Treatment 1) to restlessness, under-reactive, loss of appetite; loss of equilibrium, low rate of opercula movement, holding out of the pectoral and pelvic fins, gasping for oxygen indicated by raising the mouth towards the water surface, resting at the bottom and frequent surface to bottom movement (Weeks 3 and 4). These behavioral responses are akin to the observations of some earlier researchers. Akinsorotan et al. (2019) reported discolouration, restlessness, erratic swimming losss of equilibrium of *Oreochromis niloticus* exposed to paraquat. Restlessness, erratic swimming, sudden quick movement were also reported by Ladipo et al. (2011). This study showed that there were no signs of abnormal behaviour in the control fish. Therefore, the presence of the different concentrations of herbicide glyphosate was toxic to juveniles of *C. gariepinus*. The studied toxicity indices based on behaviour and morphology (erratic swimming, loss of reflex/equilibrium, discolouration, increased opercular activities, hyperventilation, incessant jumping, sudden quick movement/restlessness, under-reactive, resting at the bottom, loss of appetite, holding out of the pectoral and pelvic fins, gasping for oxygen indicated by raising the mouth towards the water surface and frequent surface to bottom movement) were mostly positive to varying degrees in all the concentrations of herbicide glyphosate. The results of this study particularly revealed that behavioural abnormalities of the juvenile fish increased with increasing concentrations of the herbicides. These observations were similar to the findings of Hussein et al. (1996); Chandra (2008); Pandey et al. (2009); Erhunmwunse et al. (2018); Owolabi et al. (2021).

The results of the growth responses of *C. gariepinus* exposed to varying concentration of glyphosate was presented in Table 3. This table revealed that the final weight (g) of exposed *C. gariepinus* decreased as the concentration of glyphosate increased. The table also revealed that the weight gain, percentage weight gain, specific growth rate and the nitrogen metabolism decreased significantly ($P<0.05$) in the exposed fish as concentration of glyphosate increased. The findings reveal that glyphosate toxicity in *C. gariepinus* was time and concentration dependent, accounting for variability in behavioural and growth responses reported at various doses and times of exposure. Chemical toxicity in aquatic organisms has been shown to be influenced by the species' age, size, and health (Abdul-Farah et al. 2004; Erhunmwunse et al. 2018). Physiological characteristics such as water quality, temperature, pH, dissolved oxygen, and turbidity, amount and kind of aquatic vegetation, chemical concentration and formulation, and exposure all have a significant impact on such investigations

Fig. 3 **A & B:** Seriously Bleached

Table 2 Behavioural responses of *C. gariepinus* exposed to varying concentration of glyphosate

Periods of exposure	Control (0.00)	Trt 1 (2 ml/L)	Trt 2 (4 ml/L)	Trt 3 (6 ml/L)	Trt 4 (8 ml/L)
Week 1	Normal	No loss of equilibrium	Loss of equilibrium.	Under-reactive and reduced of appetite, Loss of equilibrium.	Under-reactive and reduced of appetite; Loss of equilibrium, Low rate of opercular movement, holding out of the pectoral and pelvic fins.
Week 2	Normal	Under-reactive and reduced appetite; Loss of equilibrium.	Under-reactive and reduced appetite; Loss of equilibrium.	Under-reactive and reduced appetite; Loss of equilibrium, Low rate of opercular movement, holding out of the pectoral and pelvic fins.	Under-reactive and loss of appetite; Loss of equilibrium, Low rate of opercular movement, holding out of the pectoral and pelvic fins, gasping for oxygen indicated by raising the mouth towards the water surface, and resting at the bottom.
Week 3	Normal	Under-reactive and reduced appetite; Loss of equilibrium.	Under-reactive and reduced appetite; Loss of equilibrium.	Under-reactive, loss of appetite; Loss of equilibrium, Low rate of opercula movement, holding out of the pectoral and pelvic fins, gasping for oxygen indicated by raising the mouth towards the water surface, and resting at the bottom. Frequent surface to bottom movement.	Under-reactive, loss of appetite; Loss of equilibrium, Low rate of opercula movement, holding out of the pectoral and pelvic fins, gasping for oxygen indicated by raising the mouth towards the water surface, and resting at the bottom. Frequent surface to bottom movement.
Week 4	Normal	Under-reactive and reduced appetite; Loss of equilibrium.	Under-reactive and reduced appetite; Loss of equilibrium.	Under-reactive, loss of appetite; Loss of equilibrium, Low rate of opercula movement, holding out of the pectoral and pelvic fins, gasping for oxygen indicated by raising the mouth towards the water surface, and resting at the bottom. Frequent surface to bottom movement.	Under-reactive, loss of appetite; Loss of equilibrium, Low rate of opercula movement, holding out of the pectoral and pelvic fins, gasping for oxygen indicated by raising the mouth towards the water surface, and resting at the bottom. Frequent surface to bottom movement.

All observations of the control treatment from week 1 to week 4 are all normal. Also, Hyperactiveness, Erratic swimming and restlessness are generally observed across the treatment groups except control groups

Table 3 Growth responses of *C. gariepinus* exposed to varying concentration of glyphosate

Parameters	Control (0.00)	Trt 1 (2 ml/L)	Trt 2 (4 ml/L)	Trt 3 (6 ml/L)	Trt 4 (6 ml/L)
Initial Weight	35.82 ± 0.02^{a}	35.82 ± 0.02^{a}	35.82 ± 0.02^{a}	35.82 ± 0.02^{a}	35.82 ± 0.02^{a}
Final Weight	43.55 ± 1.14^{d}	42.33 ± 0.49^{cd}	41.73 ± 1.44^{c}	40.05 ± 0.94^{b}	38.41 ± 1.62^{a}
Weight Gain	7.73 ± 1.14^{d}	6.51 ± 0.49^{cd}	5.91 ± 1.44^{c}	4.23 ± 0.94^{b}	2.59 ± 1.62^{a}
%Weight Gain	21.59 ± 3.17^{d}	18.16 ± 1.36^{cd}	16.50 ± 4.01^{c}	11.82 ± 2.64^{b}	7.23 ± 4.53^{a}
Specific Growth Rate	0.70 ± 0.09^{d}	0.60 ± 0.04^{cd}	0.54 ± 0.13^{c}	0.40 ± 0.08^{b}	0.25 ± 0.15^{a}
Nitrogen Metabolism	610.07 ± 8.74^{d}	600.63 ± 3.75^{cd}	596.04 ± 11.05^{c}	583.16 ± 7.26^{b}	570.52 ± 12.47^{a}

Mean values in the same column with the same superscript do not differ significantly at $P^{>}0.05$

(Nwani et al. 2010). Langiano and Martinez (2008) found that behavioral changes varies based on the size, age, and condition of the test species, as well as experimental conditions, even in single species and single toxicants.

The LC_{50} value of glyphosate varies greatly, according to the World Health Organization (1994), and is dependent on fish species, test conditions, and the active chemicals present in the herbicide. The glyphosate herbicide's LC_{50} on juvenile catfish was found to be 20 ml/L in this investigation. This was in contrast to Nwani et al. (2010), who found a glyphosate LC_{50} of 32.54 mg/L in freshwater air-breathing fish *Channa punctatus*. Similarly, the LC_{50} values found in this investigation differed significantly from those published by Bathe et al. (1973); Neškovic et al. (1993; Hussein et al. (1996) for herbicide-exposed *Lepomis macrochirus* (Bluegill sunfish), *Cyprinus carpio*, and *Oreochromis niloticus*, respectively. The differences in LC_{50} values could be due to differences in fish species, weight, and glyphosate herbicide types used (Erhunmwunse et al. 2018).

Moreover, the mortality rate of *C. gariepinus* exposed to different concentrations of glyphosate (as presented in Table 4) revealed that 37.50 ± 0.00% mortality was recorded in Treatment 4 (8 ml/L) while 12.50 ± 0.00% and 16.67 ± 7.22% mortality was recorded in treatments 2 (4 ml/L) and 3 (6 ml/L) respectively. The table also revealed that no (0.00 ± 0.00%) mortality was recorded in the control (0 ml/L) and Treatment 1 (2 ml/L). Statistically, the mortality recorded in Treatment 4 (8 ml/L) was significantly different from the observed mortality in treatments 2 (4 ml/L) and 3 (6 ml/L). According to Warren (1997) and Pandey et al. (2009), introducing a toxicant into an aquatic environment can lower the dissolved oxygen concentration, impairing respiration and resulting to asphyxiation. The observed behavioural reactions of glyphosate-exposed fish demonstrated that they become increasingly anxious through time and concentration before dying as buttressed by the statistical representation in Table 4. The respiratory impairment caused by the toxic effect of glyphosate on the gills of *C. gariepinus* (increased opercular activities, hyperventilation, holding out of the pectoral and pelvic fins, gasping for oxygen indicated by raising the mouth towards the water surface) was similar to the reports of Abdul-Farah et al. (2004); De Mel and Pathiratne (2005); Tilak et al. (2007); Ayoola (2008); Nwani et al. (2010) and Erhunmwunse et al. (2018). As a result, death could have occurred either directly by poisoning or indirectly through making the medium unsuitable for the fish, or both.

The physico-chemical parameters of water during exposure of juvenile *C. gariepinus* to sub-lethal concentrations of glyphosate (Fig. 4) showed that dissolved oxygen (5.82 ± 0.06 mg/l), pH (6.80 ± 0.05), temperature (29.20 ± 0.26°C) and conductivity (36.36 ± 1.05μ/s) In this study, the physico-chemical parameters of water during exposure of juvenile *C. gariepinus* to sub-lethal concentrations of glyphosate showed that dissolved oxygen, pH, temperature and conductivity were within the permissible range recommended by FAO (2007) for freshwater fishes.

Table 4 Mortality Rate of *C. gariepinus* exposed to varying concentration of glyphosate

Conc. of Glyphosate (ml)	**Number of Exposed Fish**	**% Mortality**				Overall
		Week 1	Week 2	Week 3	Week 4	
Control (0.00)	24	0.00	0.00	0.00	0.00	$\mathbf{0.00 \pm 0.00^{a}}$
Trt 1 (2 ml/L)	24	0.00	0.00	0.00	0.00	$\mathbf{0.00 \pm 0.00^{a}}$
Trt 2 (4 ml/L)	24	0.00	0.00	4.17	8.33	$\mathbf{12.50 \pm 0.00^{b}}$
Trt 3 (6 ml/L)	24	0.00	0.00	8.33	8.33	$\mathbf{16.67 \pm 7.22^{b}}$
Trt 4 (8 ml/L)	24	0.00	4.17	12.50	20.83	$\mathbf{37.50 \pm 0.00^{c}}$

Mean values in the same row with the same superscript do not differ significantly at $P^{>}0.05$

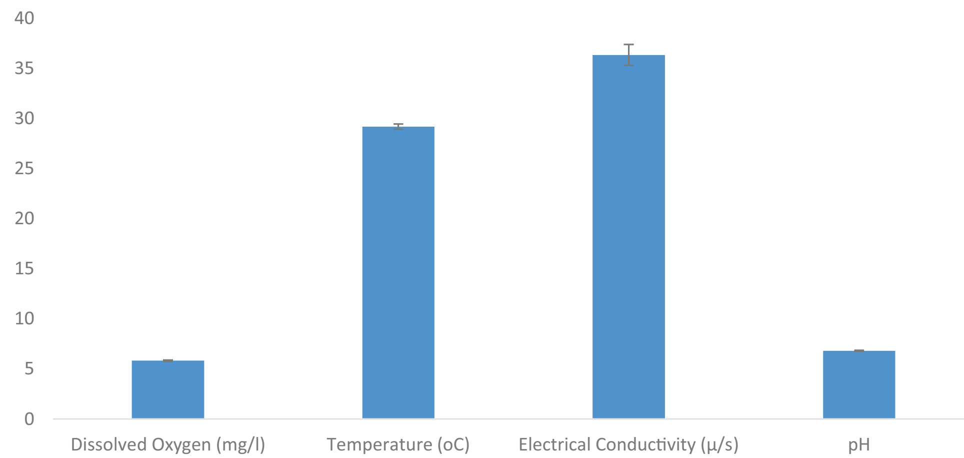

Fig. 4 Water Quality Parameters during sub-lethal exposure of *C. gariepinus* to varying concentration of glyphosate

4 Conclusions

The present investigation indicated that the herbicide glyphosate was toxic to the appearance, behaviour and growth of *Clarias gariepinus* juveniles and further substantiate that its toxicity was concentration and time of exposure dependent, hence resulting in the deleterious effects, especially at higher dose. Therefore, the indiscriminate use of herbicides in both terrestrial and aquatic habitats should be discouraged. Moreover, this study specifically recommends that the use of glyphosate herbicides in agricultural farms should be properly monitored to avoid continuous leaching into water bodies.

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Authors' Contributions All of the authors worked together to complete this project. AAO and APO conceptualised, designed and participated in the work. The protocol was written by AAO. The statistical analysis was carried out by AAO and APO. The study's analyses and literature searches were handled by AAO and APO. The original draft of the manuscript was written by AAO. The final manuscript was read, corrected and approved by both authors.

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Data Availability All data generated or analysed during this study are included in this article.

Declarations

Ethical Statement The authors confirm that all experiments were carried out following the approval of the appropriate ethical review committee of the Central Research Laboratory, Olusegun Agagu University of Science and Technology Okitipupa, Nigeria (reference number not applicable) and were in accordance with both the national and international safety regulations and ethical principles for animal welfare. The authors also confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Animal Welfare Statement The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval of the Central Research Laboratory, Olusegun Agagu University of Science and Technology Okitipupa, Nigeria has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Competing Interests The authors declare that they have no competing interests.

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