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# Histopathological Effects of Glyphosate on Juvenile African Catfish (*Clarias gariepinus*)

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**Abstract:** Most aquatic herbicides have undergone some toxicity testing for effects on non-target aquatic organisms, little of this testing has been conducted on early life stages of *Clarias gariepinus*. African catfish *C. gariepinus* were exposed to acute concentrations of glyphosate for 96hrs. The lethal concentration ($LC_{5O}$) value of glyphosate was 0.295 mg $l^{-1}$ for 96h of exposure. The mean mortality percentages were 0, 0, 43, 73, 93 and 96% in the order of concentration of 0, 19, 42, 94, 207 and 455 mg $l^{-1}$, respectively. Glyphosate concentration corresponding to the 96h $LC_{5O}$ value for juvenile *C. gariepinus* was used to study the effects of glyphosate exposures in inducing histopathological changes of gills, liver, kidney and brain. In the gills, cellular infiltrations were observed. In the liver there was fatty degeneration, severe fat vacuolation, diffuse hepatic necrosis and darkly stained specks of necrotic nuclei and infiltration of leukocytes. In kidney there was haematopoietic necrosis and severe pyknotic nuclei. The brain showed mononuclear infiltration, neuronal degeneration and spongiosis. These changes occurred predominantly in the 96h exposure. Respiratory stress, erratic swimming and death of fish were observed in exposed fish which varies with the concentration of the toxicant and its showed that mortality increased with increasing in concentration. Glyphosate is toxic to juvenile fish. *C. gariepinus* are more susceptible to herbicide, therefore their use on/near fish farm or in area close to aquatic environment should be discouraged.

**Key words:** *Clarias gariepinus* · Glyphosate · Histopathological · Acute toxicity

## INTRODUCTION

Most aquatic herbicides have undergone some toxicity testing to evaluate effects on non-target organisms [1]. Unfortunately, these tests are rarely conducted on the early life stages of fish commonly found in water bodies in Nigeria being treated for "weed control". The continual use of these herbicides has prompted some concern of the effects of these chemicals on the early life stages of fish [2]. If an herbicide that is toxic to the early life stages of fish is used annually, the herbicide could contribute to a decline in the fishery of water. The toxicity study of these chemicals to early life stages is essential to understand the environmental impacts of herbicides.

Glyphosate, the active ingredient which is the 48% acid equivalent of the 180 propylamine salt of glyphosate (N-phosphonomethyl glycine), is used as a non-selective herbicide and for control of a great variety of annual, biennial and perennial grasses, sedges, broad-leaved weeds and woody shrubs, used in fruits orchards, vineyards, conifer plantations and many plantation crops. It is perhaps the most important herbicide ever developed [3]. Because of its low persistence, repeated applications of this herbicide are practiced for the control of weeds in agricultural fields and thereby, large quantities find their ways into the water bodies. 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 terms effects in the environment.

*C. gariepinus* respires bimodally. It is very hardy since it tolerates both well and poorly oxygenated waters. It is widely cultivated and found in water bodies in Nigeria hence used as biological indicators in ecotoxicological studies [4]. Thus, the objective of this study is to investigate the acute toxic effects of glyphosate on African catfish (*C. gariepinus*) and to determine the lethal concentration with emphasis in the histopathological changes in the gills, livers, kidney and brains.

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## MATERIALS AND METHODS

Five hundred species of *C. gariepinus* with the mean weight of 10.0±0.3g and standard length mean length of 6.0±0.1cm were used for the experiment. This is due to the more sensitive nature of juveniles than adult for toxicity test. [5-7]. They were purchased from a reputable fish farm in Oyo State, Nigeria. The fish were acclimatized in laboratory conditions for four weeks during which they were fed with commercial floating pellets at 10% of their body weight.

Unconsumed feed and faecal were removed and water replenished regularly as recommended by Oyelese and Faturoti [8].

Dechlorinated tap water of temperature = 26.0 ±0.8°C, pH = 7.0 and dissolved oxygen 6.3±0.1 mg $l^{-1}$ were used.

**Acute Toxicity Tests:** A static renewal bioassay technique (ASTM 729-90) [9] was adopted in which the test media was renewed at the same concentration once every 24hours. Preliminary screening was carried out to determine the appropriate concentration range for testing chemical as describe by Solbe [5]. The following concentration in weight per volume of glyphosate were used 0.0,0.01, 0.1, 1, 10, 100 and 1000 mg $l^{-1}$. Seven *C. gariepinus* juvenile per concentration of toxicant were used with 3 replicates each for 96h. Based on this, five concentrations (0.0, 1.9, 4.1, 9, 21 and 45 mg $l^{-1}$) of the insecticide were prepared and tested on the *C. gariepinus* juveniles for the definitive test. Ten acclimated fish were used in each aquarium containing different concentrations of glyphosate as well as in the control as describe by Solbe [5] and Rahman *et al.* [10].

At the beginning of the tests and every 30 minutes behavioural changes and the number of dead fish were recorded. Other external changes in the body of the fish were observed accordingly. Dead fishes were promptly removed and preserved in 10% formaldehyde. The organs (gills, livers, kidneys and brains) were removed and prepared for histopathological observation. They were fixed in Bouin's fluid for 24hours and washed with 70% ethanol and dehydrated through a graded series of ethanol Schalm *et al.* [11], Kelly [12]. They were embedded in paraffin, sectioned at 4-5 µm; thickness stained with hematoxylin and eosin and examined using light microscope and photomicrography [13]. The mean physico-chemical parameters of the test concentrations (glyphosate) on *Clarias gariepinus* were observed. The median lethal concentration ($LC_{50}$) at 96 h was computed using the probit, logit analysis and ANOVA.

## RESULTS

The mean physico- chemical parameters of the test concentrations (glyphosate) on *C. gariepinus* are presented in Table 1 while the effect of different concentrations and exposure time of glyphosate on *C. gariepinus* a juveniles are presented in Table 2. There were significant relationship ($p<0.05$) between the temp, pH and dissolved oxygen with glyphosate concentration.

**Toxicity of Glyphosate:** The $LC_{50}$ value based on probit analysis was found to be 0.063 mg $l^{-1}$ for 96h and is presented in Fig. 1. No adverse behavioral changes or any mortality was recorded in the control fish throughout the period of the bioassay. The behaviour of the control fishes and their colour were normal. Symptoms of toxicosis observed in fish behaviour with cypermethrin include lack of balance, agitated or erratic swimming, air gulping, restlessness, sudden quick movement, excessive section of mucus, rolling movement and swimming on the back were observed. The fish became very weak, settled at the bottom and died. The colour of the skin of *C. gariepinus* was changed from normal darkly pigmentation in the dorsal and lateral parts to very light pigmentation in the dorsal and lateral part.

**Histopathological Studies:** Summary of histopathological changes observed in the gill, liver, kidney and brain of *C. gariepinus* subjected to different concentration of glyphosate for 96h is presented in Table 3.

**Gills:** No recognisable changes were observed in the gills of the control fish. Each gill consisted of a primary filament and secondary lamellae. At different concentrations of glyphosate there were cellular infiltration, swollen tip of the gill filament, congestion, severe gill damaged and hererophilic infiltration.

Table 1: Mean physico-chemical parameters of the test concentrations (glyphosate) on *Clarias gariepinus*

| Concentration (mg $l^{-1}$) | Physico-chemical parameters | | |
|---|---|---|---|
| | Do (mg $l^{-1}$) | pH | Temp (°C) |
| 0.0 | 6.3±0.1$^{d}$ | 7.0±0.1$^{b}$ | 26.0±0.8$^{a}$ |
| 19 | 5.9±0.3$^{c}$ | 6.9±0.3$^{a}$ | 26.9±0.6$^{b}$ |
| 42 | 5.8±0.3$^{b}$ | 6.9±0.3$^{a}$ | 27.0±0.1$^{b}$ |
| 94 | 5.8±0.4$^{b}$ | 6.9±0.2$^{a}$ | 27.0±0.3$^{b}$ |
| 207 | 5.8±0.1$^{b}$ | 6.9±0.2$^{a}$ | 27.8±0.1$^{c}$ |
| 455 | 5.7±0.3$^{a}$ | 6.9±0.1$^{a}$ | 27.8±0.3$^{d}$ |

*Mean values followed by the superscript in each column are not significant different ($p<0.05$)

Table 2: Rate of mortality of *Clarias gariepinus* juvenile on exposure to glyphosate per treatment

| Treatment/hr | 1 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 42 | 45 | 48 | 51 | 54 | 57 | 60 | 63 | 66 | 69 | 72 | 75 | 78 | 81 | 84 | 87 | 90 | 93 | 96 | Total Mortality | % mortality |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_{0A}$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 00 | 00 |
| $T_{0B}$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 00 | 00 |
| $T_{0C}$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 00 | 00 |
| $T_{1A}$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 00 | 00 | 00 |
| $T_{1B}$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 00 | 00 | 00 |
| $T_{1C}$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 00 | 00 | 00 |
| $T_{2A}$ | | | | | | | | | | | | | | | | | | | | 01 | | | | 01 | | | | | | | | 02 | 04 | 40 |
| $T_{2B}$ | | | | | | | | | | | | | | | | | | | | 00 | | | | 02 | | | | | | | | 03 | 05 | 50 |
| $T_{2C}$ | | | | | | | | | | | | | | | | | | | | 01 | | | | 02 | | | | | | | | 01 | 04 | 40 |
| $T_{3A}$ | | | | | | | | | | | | | | | | 02 | 01 | | | 01 | | | | | 01 | | | 01 | | | | 01 | 07 | 70 |
| $T_{3B}$ | | | | | | | | | | | | | | | | 00 | 02 | | | 02 | | | | | 01 | | | 02 | 01 | | | 00 | 08 | 80 |
| $T_{3C}$ | | | | | | | | | | | | | | | | 00 | 01 | | | 02 | | | | | 01 | | | 02 | 02 | | | 01 | 09 | 70 |
| $T_{4A}$ | | | | | | | | | | 01 | | | | | | 01 | | | | | | | | 01 | 01 | | | 02 | | | 01 | 02 | 09 | 90 |
| $T_{4B}$ | | | | | | | | | | 02 | | | | | | 01 | | | | | | | | 01 | 01 | | | 01 | | | 02 | 02 | 10 | 100 |
| $T_{4C}$ | | | | | | | | | | 01 | | | | | | 02 | | | | | | | | 02 | 01 | | | 01 | | | 01 | 01 | 09 | 90 |
| $T_{5A}$ | | | | | | 01 | | | 01 | | | | 01 | | | | 02 | | | | | | | | 02 | | | | | | | 03 | 10 | 100 |
| $T_{5B}$ | | | | | | 01 | | | 02 | | | | 01 | | | | 01 | | | | | | | | 02 | | | | | | | 02 | 9 | 90 |
| $T_{5C}$ | | | | | | 01 | | | 02 | | | | 01 | | | | 01 | | | | | | | | 01 | | | | | | | 04 | 10 | 100 |

$T_0$= Control treatment without toxicant, $T_1$=19 mg $l^{-1}$ of toxicant $T_2$=42 mg $l^{-1}$ of toxicant, $T_3$=94 mg $l^{-1}$ of toxicant, $T_4$=207 mg $l^{-1}$ of toxicant, $T_5$=455 mg $l^{-1}$ of toxicant,

Table 3: Summary of histopathological changes observed in the brain, gill, liver and kidney of *Clarias gariepinus* juveniles subjected to different concentrations of glyphosate (force-up) for 96h

| Treatment Concentration | Hour of Merit | Organs | Congestion | Necrosis | Cellular Infiltration | Spongiosis | Pyknosis | Hemorrhage |
|---|---|---|---|---|---|---|---|---|
| O (Control) mg $l^{-1}$ | 96 | B | - | - | - | - | - | - |
| | | G | - | - | - | - | - | - |
| | | L | - | - | - | - | - | - |
| | | K | - | - | - | - | - | - |
| 19 | 96 | B | + | - | - | - | - | - |
| | | G | - | - | ½ | - | - | - |
| | | L | - | ½ | - | - | - | - |
| | | K | - | - | - | - | ½ | - |
| 42 | 96 | B | + | - | - | ½ | - | ½ |
| | | G | + | - | + | - | - | + |
| | | L | - | + | - | - | - | ½ |
| | | K | - | - | - | - | + | ½ |
| 94 | 96 | B | + | + | - | + | - | + |
| | | G | + | - | + | - | - | + |
| | | L | - | ++ | - | - | - | + |
| | | K | + | + | - | - | + | + |
| 207 | 96 | B | + | + | - | ++ | - | + |
| | | G | ++ | + | ++ | - | - | + |
| | | L | - | ++ | + | - | - | + |
| | | K | + | + | + | - | ++ | + |
| 455 | 96 | B | ++ | ++ | ++ | ++ | + | ++ |
| | | G | ++ | + | ++ | ½ | - | ++ |
| | | L | ½ | ++ | + | - | + | ++ |
| | | K | + | ++ | + | - | ++ | ++ |

Key: B = Brain, G = Gill, L = Liver, K = Kidney, - = completely absence, + = Present, ½ = Mild, ++ = Severe.

Note: Treatment with negative signs indicated no histopathological changes were observed

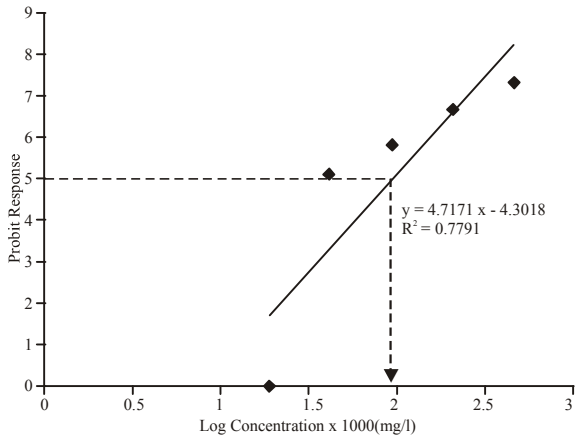


Fig. 1: Linear relationship between probit response and log concentration of Glyphosate on juveniles of Clarias gariepinus

**Liver:** The histology of control fish liver revealed normal typical paranchymatous appearance. The liver was made up of hepatocytes that were polygonal cells with a central spherical nucleus and a densely stained nucleolus. There were glycogen vacuolation, fatty infiltration, hemosiderosis and congested central vein at the concentration of 1.9 to 9 mg $l^{-1}$, severe infiltration of leukocytes, pyknotic and hepatic necrosis were also observed at 21 and 45 mg $l^{-1}$ concentration severe necrotic, haemrrhage and vacuolation were observed accordingly as described in Table 3.

**Kidney:** No recognisable changes were observed in the kidney of the control fish. At the light microscopic level, the renal corpuscle was composed of the glomerulus and Bowman's capsule. The kidney tissue from *C. gariepinus* exposed to different concentrations of glyphosate showed necrosis, degenerated kidney tubules pyknosis, exfoliated and swollen with pyknotic nuclei.

**Brain:** No significant lesion was seen in the brain of the control fish and the morphological structure is normal. The histopathological observation on the brain showed that there was discolouration on the brain of fish exposed to different concentration of glyphosate. Fish exposed to concentration of 1.9 to 45 mg $l^{-1}$ showed mononuclear infiltration neuronal degeneration, infiltration and severe spongiosis.

## DISCUSSION

Glyphosate is one of the widely used herbicides and it is considered to be persistent and mobile in soil and water and is known to be one of the most common terrestrial and aquatic contaminants [14] The $LC_{50}$ values of glyphosate vary widely from 2- 5ppm have been reported depending on the species of fish and test conditions [3]. The present study showed that the 96-h $LC_{50}$ value of glyphosate (herbicide) was 0.04 mg $l^{-1}$. The corresponding $LC_{50}$ values obtained from the present study fell with in the concentration ranges reported in the previous studies. Glyphosate toxicity is shown to increase with increasing concentration. The observation is in consonance with earlier reports [3,14,15]. Neibor and Richardson [16] reported that the level of toxicity of any pesticide depends on its bioaccumulation, the different chemistries of the compound forming the pesticide and the reactions of the organisms receiving the toxicant. The three physico-chemical parameters of the test were fluctuated slightly during the toxicity test. The values were normal for toxicity test [17]. The water quality parameters may have probably contributed to the variation in behavioural pattern and the mortality of the test fish during the study period. There was a significant negative correlation between pH and dissolved oxygen values. In case of dissolved oxygen, the treatments did not only show a dose dependent decline in concentration, but also rapid depletion of dissolved oxygen with time. Warren [18] had earlier reported that the introduction of a toxicant into an aquatic system might decrease the dissolve oxygen concentration, which will impair respiration leading to asphyxiation.

*C. gariepinus* juveniles were stressed progressively with time before death. The stressful behaviour of respiratory impairment due to the toxic effect of glyphosate on the gills was similar with the report of Omitoyin *et al.* [19] and Aguigwo [20] that herbicide impair respiratory organ. Death could therefore have occurred either by direct poisoning or indirectly by making the medium unconductive or even by both, which ever is the case, the source of death was glyphosate. Several abnormal behaviour such as incessant jumping and gulping of air, restlessness, surface to bottom movement, sudden quick movement, resting at the bottom were similar to the observations of Omoniyi *et al.* [21], Rahman *et al.* [10] and Aguigwo [20]. The stressful and erratic behaviour of C. *gariepinus* juvenile in the experiment indicates respiratory impairment, probably due to the effect of the toxicant glyphosate on the gills. The fishes became inactive at higher concentrations with increasing time of exposure to toxicant. According to Kulakkatolickal and Kramer [22], this is a normal observation in acute and chronic toxicity test.

It was also observed that the highest concentration of the toxicant resulted in highest mortality rate. This demonstrates the observation of Fryer [23], that in all toxicant threshold is reached above which there is no drastic survival of animal. Below the threshold, animal is in a tolerance zone, above the tolerance zone is the zone of resistance. The time of toxicity disappearance and mortality were observed from the record of the relative mortality time in different concentrations of glyphosate for 96hours. The histopathological examination of the brain, liver, gill and kidney of the exposed fish indicated that the liver and kidney were the most affected organs.

Damages of the gills indicated that the lethal concentrations of herbicide caused impairment in gaseous exchange efficiency of the gills.

The literature on histopathological effects of glyphosate on fish is extremely sparse. Neskovic *et al.* [24] conducted sub acute toxicity test (14days) of sub lethal glyphosate concentrations on histopathological changes of carp organs such as gills, livers and kidneys. In fish exposed to glyphosate concentration (96-h $LC_{50}$) in the present study, the major changes of the gills were Oedama, epithelial lifting and thickening of the primary lamellar epithelium and fusion of secondary lamellae. Histopathological changes of gill such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenols and heavy metals [25].

In the present study, the liver of *C. gariepinus* exposed to glyphosate concentration showed an infiltration of leukocytes, increasing hepatocyte size with pykrotic nuclei and presence of vacuoles. In the study of Risbourg and Bastide [26], the exposure of fish to atrazine herbicide increased in the size of lipid droplets, vacuolization in the liver. The most frequent encountered types of degenerative changes are those of hydropic degeneration, cloudy swelling, vacuolization and focal necrosis. The liver of the exposed fish had slightly vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue that were observed probably resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis. In the present study, the kidney of *O. niloticus* exposed to glyphosate concentrations showed dilation of bowman's space and accumulation of hyaline droplets in the tubular epithelial cells of the first proximal tubule. Oulmi *et al.* [27] studied the effects of linuron herbicide on the rainbow trout (*Oncorhynchus mykiss*). Their results showed small cytoplasmic vacuoles, nuclear deformation in the epithelium of the first and second segments of the proximal tubule. The kidney cells were observed to have been massively destroyed. The renal corpuscles of the kidney were scattered resulting in their disorganization and consequently obstruction to their physiological functions.

Some of the kidney cells were found clogging together while they were disintegrated in some tissues of the organ. This also agrees with the findings of Omoniyi *et al.* [21] and Rahman *et al.* [10]. The brain also indicated severe congestion and generalized spongiosis that indicate severe brain damage. This agreed with the findings of Omitoyin *et al.* [19].

## CONCLUSION

This study revealed that glyphosate (herbicide) is toxic to fish and causes histopathological changes in fish organs. *C. gariepinus* are more susceptible to herbicide; therefore their use on/near fish farm or in area close to aquatic environment should be discouraged.

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