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# Sublethal toxicity of glyphosate and propanil on some biochemical parameters in juveniles of freshwater catfish, *Clarias gariepinus* (Teugels, 1986)

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**Abstract**
Contamination serve as the primary danger to the environment and its aquatic biota. Recently, the increase in human population, industrialization, intensive agriculture and the practice of herbicides usage in progressing nations to control various weeds is a constant development. However, the purpose of the current study is to assess the prolonged effects of glyphosate and propanil on some serum biochemical parameters in the *Clarias gariepinus* juveniles. The study was conducted in an enclosed apartment for 56 days. Juveniles of *C. gariepinus* were randomly exposed to long term toxicants of glyphosate 0.11, 0.14, 0.21 mg/L and 0.00 mg/L as control, while 0.23, 0.31, 0.46 mg/L and 0.00mg/L as control for propanil respectively. However, samples of blood were collected for the determination of blood some biochemical parameters after 56 days. Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) revealed major increases in stimulating liver enzymes activities in all investigational groups related with control group. However, non-antioxidant enzymes metabolites activities such as glucose, triglyceride were detected to elevate expressively, while protein and cholestetrol decreased significantly in the expose group compare with the control.
These increases were either dose dependent, time dependent, or both. The rise in these enzymes actions were a little more in glyphosate-treated than in propanil-treated fishes indicating that glyphosate is more toxic to *C. gariepinus* juveniles. The study therefore, revealed that exposing juveniles of *C. gariepinus* herbicides toxicant such as glyphosate and propanil for a long term, upset biochemical parameters and implies poor environmental fitness for fish exposed to herbicides in natural environments.

**Keywords:** Biochemical parameters, glyphosate, propanil, *Clarias gariepinus*, sub lethal concentrations, herbicides

## 1. Introduction
Increasing human population in the recent times, intensive agriculture, industrialization and urbanization and the practice of pesticides/herbicides in undeveloped nations for the suppression of diverse weeds has been on the growing development. Herbicides are chemicals that are used for killing plant and are also used generally for industrial and agricultural purposes. They are known to cause the death of people, vegetation and water creatures such as fish due to their harmfulness [1, 2]. Environmental impact of herbicides can be observed largely in the topsoil, water and live habitat. These herbicides are found to be toxic to fish at very insignificant concentration, thereby causing hepatic and renal dysfunction in fish [3]. However, the death of fishes can be linked to pesticides and herbicides [1]. Equally, special effects of herbicides are centered on herbicides dissolution and toxicant access into soil, water and biota [3]. In Nigeria, glyphosate and propanil are among the common herbicides that interferes with the feeding relationship of an organism in its environment like; soil, water and biota [3].
In Nigeria, glyphosate and propanil are among the common herbicides being continually applied on farmlands for high crop yield. In 1974, the herbicide glyphosate, which is a biocide with a broad-spectrum action was discover for the control of weeds during the process of crops cultivation in the open fields [4]. The intensive and extensive used of herbicide such glyphosate worldwide has contributed to its prevalent contamination where it effects in plants, microorganisms, animals, and many constituents of the nutrients cycle of different ecosystem [5]. In Nigeria, farmers mostly use glyphosate as a popular herbicide due to its efficiency in eradicating weeds devoid of upsetting the crop. However, it is generally among the recognized herbicides used globally and it is also applicable for annual and perennial weeds controlled and

other species like broad-based leafed weeds and trees, these has attributed it as a main contaminant of rivers and surface water [6]. Additionally, because of its low persistence, it has been measured as the greatest significant herbicide ever established. The presence of some surfactants in the preparation of glyphosate are poisonous and are not appropriate to aquatic creatures [7]. Propanil (dichloropropionamide) is a common and widely used herbicide in agriculture, agro-forestry and domestic homes to control weeds [8]. It is mainly used in rice farms to control weeds. However, due to its accessibility in various forms, individuals including farmers indiscriminately apply this herbicide in farm lands and other facilities without recourse to its adverse impact on the immediate environment and aquatic health by extension. Presence of propanil in aquatic ecosystem and fish although in trace amount has been reported [9, 10]. According to Ensibi *et al.* [11], fish growth ultimately is affected by the presence of toxic substances in aquatic ecosystem, by reducing food accessibility and by altering their metabolism. However, toxic substances present even in small concentrations can cause the death of fish in aquatic environment [12]. Virtually 50% of the world fish harvests accounted for globally in aquaculture production in 2016 is destined for food [13]. The nutritional and digestibility value of fish accounted for it demand as rich source of animal protein and also serve as food to man. However, it nutritional value be determined by fish species, age, gender, health, nutritional status, biochemical composition also period of the year, which is affected by water pollution [14, 15]. Also, in Nigeria *C. gariepinus* is widely accepted as a popular choice as test organism due to it cheapness and serve as elementary component of animal protein, resistant, institute in all fresh waters cradles and widely cultured in Nigeria. Furthermore, it has been regarded as ideal organism in carrying out tentative studies such as toxicological and pharmacological investigation [16]. Based on the scientific evaluation on animals, glyphosate and propanil are considered probably human carcinogen [17]. This stimulates the necessity to study the harmfulness of glyphosate and propanil herbicides on *C. gariepinus* juveniles through; the biochemical properties determination of glyphosate and propanil on *C. gariepinus* juveniles effect in this fish after its introduction for 56 days to different sub lethal concentrations of glyphosate and propanil herbicides respectively. However, the data generated would provide more evidence to the possibilities of glyphosate and propanil to cause contrary effects on humans and other vertebrates. Plasma biomarkers such as glucose, triglyceride and total protein generally are used to ascertain well-being of animals. Serum enzymes such as alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) are vital serum indicators to study the fitness of animal species in request. The objective of the study was to determine the harmful effects of glyphosate and propanil to juveniles of the *C. gariepinus*.

## 2. Materials and Methods

### 2.1 Experimental Fish

*Clarias gariepinus* Juveniles of different sexes and fairly uniform sizes were gotten from a private fish farm in Zaria, Kaduna State, Nigeria. The fish were averaging in the range of 11.99 ± 1.56cm normal measurement and body weight of 4.25 ± 1.17g were used for the study. The fish were then transported using oxygenated polythene bags to the Fisheries Laboratory, Department of Biology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. However, the fish were fed on a marketable pellet food of 3% of body weight daily, then acclimatized for three weeks in 800 L four-sided tanks holding dechlorinated tap water (conductivity 2000 µs/cm; pH ≈ 7.5; Oxygen 90-95% saturation; temperature 25 ºC; photoperiod 12:12 Light: Dark). The feeding stopped 24 hours prior to the start of the investigate.

### 2.3 Preparation of Metal Test Solutions

Two herbicides were used in the examination. Both herbicides were bought from a marketable outlet in Kaduna. Out of 360g/L glyphosate, a stock solution 5mg/L toxicant was set through the addition of 1mL of the toxicant to 999mls of water [18]. Similarly, out of 276g/L propanil, stock solution of 1mg/L was prepared in the same way as glyphosate. The stock solutions were then used to formulate several concentrations of the toxicant through mixing appropriate capacities (i.e. 0.36mL in 999.64mL of dechlorinated tap water for 0.36mg/L concentration). Dechlorinated tap water was used as the control solutions.

### 2.4 Experimental Design

After the result of the acute bioassay, fractions, 1/5, 1/10 and 1/20 of the 96h $LC_{50}$ were used to choose sub-lethal concentrations for the herbicides as suggested by Oladimeji and Ologunmeta [27], using a static experimentation where the water in the tanks were changed every 24h. Applicable measurements of standard solution were dispersed by means of syringe and measuring cylinder in a 25L tank holding 20L of dechlorinated tap water in independently of the tanks excluding the blank solution. The fish were dispersed to small concentrations of contaminants for 56 days. The concentrations used for prolonged study of the two toxicants, 0.11, 0.14, 21 mg/L and control (0.00mg/L) for glyphosate and 0.23, 0.31, 0.46 mg/L and the blank (0.00mg/L) for propanil. Individually, concentration used was in triplicate. This means four treatments for each herbicide i.e. 12 experimental set-ups or tanks for each herbicide. The fishes were randomly assigned to give 10 fish per tank, given over-all of 240 fish were used for both testing with glyphosate and propanil. Fishes were fed 3% body mass with 35% crude protein equal pelleted food. The normal 12:12 day/night photoperiod was maintained. Test solution was changed after each 24 hours.

### 2.5 Procedures for Biochemical Studies of *Clarias gariepinus* Juveniles Exposed to Sub Lethal Concentrations of Glyphosate and Propanil

#### 2.5.1 Fish blood collection and analysis

At the termination of the investigate, blood samples were collected by means of a 2 mm needle and syringe by arbitrarily picking five fish in each replicate. The blood was placed in ethylene-diamine-tetra-acetic acid (EDTA) bottles to stop clotting. The blood samples were conveyed in ice packed to Chemical and Pathology Department, Ahmadu Bello University (ABU) Teaching Hospital for serum removal and enquiry.

#### 2.5.2 Serum Collection for Determination of Biochemical Parameters

To get the serum, the blood was positioned in micro centrifuge tubes, and instantly centrifuged at 1500 rpm (revolution per minute) for 10 minutes. Serum was at that juncture removed by pipetting and kept at 40 (ºC) prior to instant determination of biochemical parameters, and this was carried out using UV spectrophotometer [21].

### 2.6 Statistical Analysis

Data collected from the study was analyzed for descriptive statistics (mean and standard error of means) using Minitab 17. The data was further subjected to one-way Analysis of Variance (ANOVA). Duncan Multiple Range Test (DMRT), according to Duncan [22], was used to test for significant difference between treatments when at ($p$<0.05).

## 3. Results

Uniform temperature values were obtained in the tanks for the two herbicides, which ranged between 26.30-26.60 in the glyphosate tanks and 24.10 – 25.60 ºC for propanil. The highest mean value of temperature was recorded in the glyphosate exposed fish as 26.45±0.11 ºC, while the lowest mean was recorded in the propanil as 24.85±0.58 ºC. The highest mean value of Hydrogen Ion Concentration (pH) was recorded in the propanil exposed fish as 8.05±0.16, while the lowest mean was recorded in the glyphosate exposed fish as 7.85±0.14. The highest mean value of Electrical Conductivity (EC) was recorded in the propanil exposed fish as 263.00±12.19 µS/cm, while the lowest mean was recorded in the glyphosate as 204.42±5.41µS/cm. The Total Dissolved Solids (TDS) ranged from 76 –128 mg/L for glyphosate and 61 – 202 mg/L for propanil. The highest mean value of Total Dissolved Solids (TDS) was recorded in the propanil exposed fish as131.17±06.94 mg/L, while the lowest mean was recorded in the glyphosate as 102.22±5.22 mg/L. The dissolved oxygen concentration ranged from 4.43 – 5.30 mg/L for glyphosate and 5.00 – 5.10 mg/L for propanil. However, the highest mean value was 5.05±0.12 for propanil exposed fish, while the lowest was recorded in the glyphosate exposed fish as 4.86±0.09 mg/L respective.

**Table 1:** Physico-chemical parameters of water during the sub lethal exposure of *C. gariepinus* juveniles to glyphosate for 56 days

| Parameters | Range | Mean± S.E |
|---|---|---|
| Temperature (T) (ºC) | 26.30 – 26.60 | 26.45±0.11 |
| Hydrogen ion Concentration (pH) | 7.80 – 7.90 | 7.85±0.14 |
| Electrical Conductivity (EC) (µS/cm) | 152– 256 | 204.42±5.41 |
| Total Dissolved Solids (TDS) (mg/L) | 76 –128 | 102.22±5.22 |
| Dissolved Oxygen (DO) (mg/L) | 4.43 – 5.30 | 4.86±0.09 |

**Table 2:** Physico-chemical parameters of water during the sub lethal exposure of juveniles of *C. gariepinus* to propanil for 56 days

| Parameters | Range | Mean± S.E |
|---|---|---|
| Temperature (T) (ºC) | 24.10 – 25.60 | 24.85±0.58 |
| Hydrogen ion Concentration (pH) | 7.60 – 8.50 | 8.05±0.16 |
| Electrical Conductivity (EC) (µS/cm) | 122– 404 | 263.00±12.19 |
| Total Dissolved Solids (TDS) (mg/L) | 61 – 202 | 131.17±06.94 |
| Dissolved Oxygen (DO) (mg/L) | 5.00 – 5.10 | 5.05±0.12 |

The effects of biochemical parameters of *C. gariepinus* juveniles exposed to sub-lethal nominal concentrations of glyphosate after 56 days are presented in Table 3. Within the exposed group of fish, the levels of glucose, triglyceride and GPT increased while ALP and protein levels decreased with increasing concentration. Fluctuations of cholesterol and GOT levels occurred with increasing concentrations. When the concentration of exposure was increased from 0.11 to 0.14, blood cholesterol elevated expressively. However, further increased in the herbicide concentration to 0.21mg/L, shows a rapid drop in this parameter was recorded. The effects, within the sub-lethal concentrations on GOT level were comparable.

**Table 3:** The effect of sub-lethal doses of glyphosate on some biochemical parameters of *C. gariepinus* juveniles after 56 days of exposure

| Treatment (mg/L) | 0.00 | 0.11 | 0.14 | 0.21 |
|---|---|---|---|---|
| Parameters | | | | |
| Glucose(mg/dl) | 53.83±0.83[c] | 52.83±1.42[c] | 57.33±0.67[b] | 60.33±0.88[a] |
| Protein (mg/dl) | 3.75±0.18[ab] | 4.30±0.51[a] | 3.30±0.26[ab] | 2.95±0.28[b] |
| Cholesterol (mg/dl) | 130.50±0.92[c] | 146.50±24.98[b] | 162.83±22.45[a] | 141.00±27.44[b] |
| Triglyceride (mgl/dl) | 144.00±1.53[ab] | 139.67±2.91[b] | 145.67±5.12[ab] | 151.17±3.12[a] |
| GOT/AST (iu/L) | 49.17±0.70[a] | 47.17±1.08[ab] | 43.17±1.49[b] | 47.67±3.01[ab] |
| GPT/ALT (iu/L) | 58.67±0.56[a] | 46.17±1.25[b] | 51.67±1.05[b] | 59.17±3.84[a] |
| ALP (iu/L) | 16.67±0.49[a] | 20.17±3.09[a] | 17.17±2.65[a] | 5.17±2.65[a] |

Means with the same superscript along rows are not significantly different (P≤0.05) (Mean values ±SE) n=3
GOT = Glutamic Oxalo-acetic Transminase, (GPT) = Glutamate Pyruvate Transaminase and ALP = Alkaline Phosphatase

Consequences of biochemical parameters of *C. gariepinus* juveniles exposed to sub-lethal nominal concentrations of propanil after 56 days are presented in Table 4. In contrast, there was a significant ($p$≤0.05) concentration dependent decline in GOT, GPT, ALP and protein in the exposed fish. The levels of these parameters decreased with increasing concentrations of propanil. GOT, GPT, ALP and protein levels recorded for the exposed fish were lower than those recorded for the control group. significant increases ($p$≤0.05) concentration dependent for the group of fish exposed to sub lethal concentrations of the toxicant. Blood glucose and GPT levels were observed to increase with increasing concentrations of exposure of *C. gariepinus* to propanil. However, protein, cholesterol, triglyceride, GOT and ALP levels all increased with increasing concentrations of sub lethal exposure of the fish to the toxicant.

**Table 4:** The effect of sub-lethal doses of $Cd^{2+}$ on some biochemical parameters of juveniles in *C. gariepinus* after 56 days of exposure

| Treatment (mg/L) | 0.00 | 0.23 | 0.31 | 0.46 |
|---|---|---|---|---|
| **Parameters** | | | | |
| Glucose(mg/dl) | 56.17±0.60$^{c}$ | 57.67±3.76$^{bc}$ | 64.17±2.21$^{b}$ | 77.33±1.84$^{a}$ |
| Protein (mg/dl) | 3.59±0.18$^{b}$ | 5.17±0.30$^{a}$ | 3.93±0.19$^{b}$ | 3.66±0.19$^{b}$ |
| Cholesterol (mg/dl) | 127.00±0.97$^{a}$ | 137.17±2.67$^{a}$ | 119.83±24.20$^{a}$ | 79.00±5.40$^{b}$ |
| Triglyceride (mgl/dl) | 141.83±1.05$^{a}$ | 149.00±2.35$^{a}$ | 116.50±11.43$^{b}$ | 101.00±12.54$^{b}$ |
| GOT/AST (iu/L) | 50.67±0.56$^{a}$ | 44.17±2.80$^{ab}$ | 41.17±2.80$^{bc}$ | 35.67±2.14$^{c}$ |
| GPT/ALT (iu/L) | 57.50±0.56$^{a}$ | 32.00±0.93$^{d}$ | 40.50±2.84$^{c}$ | 50.00±2.59$^{b}$ |
| ALP (iu/L) | 18.02±0.57$^{a}$ | 17.72±1.03$^{a}$ | 15.53±0.98$^{b}$ | 13.08±0.78$^{b}$ |

Means with the same superscript along rows are not significantly different ($P \leq 0.05$) (Mean values ±SE) n=3
GOT = Glutamic Oxalo-acetic Transminase, (GPT) = Glutamate Pyruvate Transaminase and ALP = Alkaline Phosphatase.

## 4. Discussion

The increased or decreased of glucose level in the blood is a delicate as well as dependable sign of contaminants triggering biological stress in fish [23]. The substantial raise in the level of glucose observed in the exposed fish with reference to the control could be due to prolonged pressure triggered by continuous introduction of glyphosate and propanil respectively. These resulted in stirred, which then cause the discharge of amino acids, glycerol and fatty acids present in the blood and increased the synthesis of enzymes in the liver, which converted amino acids and glycerol into glucose (Gluconeogenesis). The raise in glucose levels (hyperglycaemia) was dose and duration dependent in the sublethal exposures of *C. gariepinus* to both herbicides glyphosate and propanil. The stimulation of plasma cortisol is accompanied by stress condition whose function is to preserve allostasis and recruit reaction to anxiety by means of regulation [24]. Canli [25], in his results revealed that glucose levels in fish in ecological pressure could be minimal which served as a consequence of superfluous energy response in the metabolism which could possibly mirror elevated levels of glucose in the serum. On the conflicting, Ogueji *et al*. [26], stated important reduction in glucose level in *C. gariepinus* exposed to diazepam. Protein breakdown could be seen as one of the vital factors essential for the biotic apparatuses of harmfulness [27]. Proteins are direct sources of vigor for the duration of pressure in several organisms, while decrease in plasma protein levels might be owing to weakened protein combination or digestion. Nwani *et al*. [8], reported significant drop in protein levels in pesticide paraquat exposed fish and suggested that the reduction could be concomitant to liver and kidney damage triggered by paraquat induced trauma. Disagreeing to the findings of Oner *et al*. [28], that observed increases in serum protein of fish exposed to herbicides and attributed it to, liver damage, loss of protein and reduced absorption. Cholesterol concentrations are vital organizational constituent of membranes and the pioneer of all steroid hormones [29]. Dose dependent reduction in cholesterol levels observed after exposure of *C. gariepinus* to acute and sublethal concentrations of the herbicides glyphosate and propanil may designate liver damage caused by the toxicants. Changes in cholesterol levels in fish were attributed to liver and kidney impairment caused by different toxicants [26]. Higher levels of triglyceride reported in in the higher concentrations of both herbicides in the exposure of *C. gariepinus* to glyphosate and propanil compared with the control in the present study indicate impairment in glycogen storage caused by glyphosate and propanil invasion, thus leading to the discharge of triglyceride in the blood system. Yaji *et al*. [10], stated significant increase in triglyceride levels of *O. niloticus* exposed to sub lethal concentrations of aronil (Propanil) herbicide. Serum enzymes (GOT/AST, GPT/ALT, ALP) are important biomarkers for evaluating liver condition and function in animals [30]. GOT and GPT are liver function enzymes which also served as biomarkers for evaluating well-being standing of the liver. Significant elevation in GOT and GPT was observed in *C. gariepinu*s juveniles after exposure to the maximum concentration of glyphosate and propanil correspondingly. These rises connote fish's reaction to the contaminant conditioned by manufacturing additional of these metabolic enzymes in an attempt to adjust to the fresh state. Atamaniuk *et al*. [31] and Gholami-Seyedkolaei [32], also reported a similar rise in the activities of these enzymes in goldfish and common Carp exposed to 2,4-dichlorophenoxyacetic acid and glyphosate respectively. The integration of keto acids into tricarboxylic acid (TCA) cycle through generation of glutamate via tissue transamination could have led to the significant elevation in the activities of AST, ALT in liver of fish exposed to both herbicides in the present study. However, these assertions might have followed by their transformation to α-ketoglutarate through oxidative deamination to yield energy via diverse energy-producing pathways [33]. Transaminases such as GOT and GPT signify valuable biomarkers for biomonitoring of chemical contaminants in aquatic organisms, and transformed levels of transaminases designate compensatory mechanisms against impaired metabolism [34, 35, 36]. Fluctuations in the activities of these enzymes also indicate damage of hepatic cells and tissues which is tantamount to deteriorating variations and dysfunction of the liver causing the release of serum enzymes into the blood serum [37]. Proliferations in ALT and AST activities in glyphosate and propanil exposed fish during the exposure suggest necrotic damage of liver caused by invasion of glyphosate and propanil pesticide. The gill, liver and blood of *Clarias gariepinus* exposed to glyphosate for 96 h shows a significant increase in the activities of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) as reported by Abdulkareem *et al.* [38], except in liver where the authors reported a significant reduction, while a significance increase in the activities of the enzymes ALT, and AST in both the blood and the gill of fish exposed to glyphosate for 28 days respectively. Following tissue impairment and dysfunction, transaminases similar to ALT and AST play important roles in amino acid and protein metabolism and they may be released into the plasma [39]. More energy was required at higher sublethal concentrations to attain the positive survival value due to significant inhibition in ALP. Since ALP is primarily localized at the cell membrane any harm in hepatic cells may result in alteration in ALP activity. However, ALP activity may be influence by different factors such as; water quality, life history, exposure duration and toxicants concentration. [40]. The decrease in ALP activity in the present study might be a result of disorder of the membrane transport system, however the increase in the activity might be related to tissue damage. The behavior of both stages of fish with respect to action of enzyme ALP after exposure to sub lethal concentrations of the herbicides glyphosate and Propanil followed comparable pattern. Fluctuations in the activities of these enzymes designate destruction of hepatic cells and tissues which is equivalent to deteriorating changes and dysfunction of the liver triggering the discharge of serum enzymes into the blood serum [37]. These results advocate that extensive contact to the contaminant might lead to modifications in the serum enzymes and also, cellular actions of vital organs such as liver, hence prompting changes in the physiological and metabolic activities of the fish.

## 5. Conclusion

The results of the biochemical parameters assayed also showed, alterations in biochemical parameters; Blood glucose, serum protein, cholesterol, triglyceride, GOT/AST, GPT/ALT and ALP observed during the sublthal glyphosate and propanil treated fish denote liver damage and stress elicited by the toxicant. The current study records that glyphosate and propanil rigorously damages numerous biochemical parameters, hence, would help as a valuable means for additional monitoring and ecological assessment of these aquatic organisms, which is measured to be a rich source of animal protein as well as significant food source for human beings.

## 6. Recommendation

Therefore, the effects of glyphosate, propanil and other herbicides should be scrutinized in the field hence, the use of herbicides should also be regulated for the improvement of the environment and protection of aquatic habitat.

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