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Acute Toxicity of Paraquat Herbicide (Dichloride) on Survival and Behaviors of African Catfish (*Clarias gariepinus* Burchell, 1822) Juveniles

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ABSTRACT **Original Research Article**

This study assessed the 96-hours acute toxicity of paraquat herbicide on juvenile African catfish (*Clarias gariepinus*). A static bioassay was conducted using a completely randomized design with six concentrations of paraquat (0.00, 0.80, 1.10, 1.40, 1.70, and 2.00 mg/L). A total of 180 juveniles were acclimatized for 14 days and exposed to the test solutions, with ten fish per treatment in triplicate. Mortality, survival, and behavioral responses were monitored at regular intervals. Exposed fish exhibited concentration-dependent behavioural abnormalities, including frequent air gulping, weakness, agitated swimming, vertical positioning, excessive mucus secretion, and loss of equilibrium, indicating respiratory distress and neurotoxicity. Mortality increased with both concentration and exposure duration, ranging from 20% at 0.80 mg/L to 90% at 2.00 mg/L, while no mortality was observed in the control group. Probit analysis revealed a 96-hours median lethal concentration (LC_{50}) of 1.41 mg/L, confirming the high toxicity of paraquat to juvenile *C. gariepinus*. The findings demonstrate that paraquat poses a significant threat to freshwater fish and may adversely affect aquatic ecosystems, highlighting the need for strict regulation of its use near water bodies.

Keywords: Paraquat Herbicide, Acute Toxicity, LC_{50}, *Clarias gariepinus.*

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Introduction

The extensive used of herbicides and other pesticidal chemicals in agriculture has contributed to increased crop production but has also resulted in contamination of aquatic ecosystems, particularly in developing countries such as Nigeria (Ezekiel *et al.*, 2025). Herbicides frequently enter water bodies through runoff, leaching, and improper disposal, posing serious risks to non-target aquatic organisms, especially fish (Adewoye and Fawole, 2019; FAO, 2022).

Paraquat (1, 1′-dimethyl-4, 4′-bipyridinium dichloride) is a fast-acting, non-selective contact herbicide widely used in sub-Saharan Africa due to its low cost and effectiveness. Despite being banned or restricted in many countries, paraquat remains commonly applied in Adamawa State, Nigeria. Previous studies have reported that paraquat induces oxidative stress, tissue damage, behavioral abnormalities, and mortality in freshwater fish (Akinrotimi *et al.*, 2020; WHO, 2021).

The African catfish (*Clarias gariepinus*) is one of the most important freshwater fish species cultured in Nigeria. Juvenile stages are particularly sensitive to environmental pollutants, making them suitable for acute toxicity studies (Ogueji *et al.*, 2018; Nwani *et al.*, 2021). Acute toxicity tests, expressed as median lethal concentration (LC_{50}), are essential for environmental risk assessment. However, data on the acute toxicity of paraquat to juvenile *C. gariepinus* under tropical Nigerian conditions remain limited. This study therefore assessed the 96-hour acute toxicity of paraquat herbicide on juveniles of *C. gariepinus*.

Materials and Methods

Total of 180 *Clarias gariepinus* juveniles were purchased from El-magnifico fish farm in Girei, Adamawa state, Nigeria. The fishes were acclimatized for 14 days in the laboratory in a plastic tank of the Department of Fisheries, Modibbo Adama University, Yola. Water quality parameters setups were monitored using standard methods (APHA, 2005; OECD, 2025).

Experimental Design

Ten (10) juvenile *C. gariepinus* were introduced individually into 18 aquaria tanks of 12 cm x 10 cm x 12 cm dimension. Each treatment and control had three replicates and lasted for 96-hours period. The tanks were covered with netted materials and supported with pegs to prevent the fish from escaping. Each aquarium size of 50 L capacity at 30 L water level contained ten (10) fishes.

Range Finding and Definitive Test for Paraquat (Herbicide) on *Clarias gariepinus* Juvenile (Pilot Study)

Series of preliminary investigation was conducted to obtain LC_{50} (Median lethal concentration that cause 50 % mortality) of the exposed animals within 96-hours period (Babatunde and Idris, 2017). The test was conducted on a single exposure system by using several concentrations of the toxicant. Ten (10) fishes of *Clarias gariepinus* juveniles were exposed to these various concentrations to observe the rate of mortality.

Exposure of Fish to Acute Concentrations of Paraquat to Determine Fish Behavioural Parameters and 96hr LC_{50} values

Static bioassay was conducted in the Department of Fisheries research farm following OECD (2025) guidelines to determine the toxicity of paraquat on *C. gariepinus.* From freshly prepared stock solutions of the LC_{50}, five concentrations of 0.00 mg/L, 0.8 mg/L, 1.1 mg/L, 1.4 mg/L, 1.7 mg/L, 2.0 mg/L, for paraquat was dispensed with a 100mL measuring cylinder and 5 mL syringe respectively into 50 L tanks (for each concentration) containing dechlorinated water and the control. Ten fishes were randomly distributed into each test tank in 3 replicates. The physicochemical parameters of the diluting water (temperature, pH, dissolved oxygen, total hardness, total alkalinity and conductivity) during the acute test was measured (Ezekiel *et al*., 2025). Air gulping index (AGI) was determined as the frequency of air gulping activity of fish per tank per minute, divided by the number of fish or surviving fish in the exposed groups. The AGI, Weakness, Agitated Swimming, Vertical positioning, Mucus Secretion and Imbalance were observed and recorded. Survival and mortality were observed during this period. Fish was considered dead when the opercular movement ceased and there was no response to gentle probing; this was used as a measure of mortality (Bala *et al*., 2023). The LC_{50} was determined by plotting a graph of the different concentrations of the toxicants against the number of dead fishes using the probit method.

Statistical Analysis

Regression coefficient between the probit value and log concentration of the Paraquat herbicide was determined after the acute toxicity study.

Results

The results of the behavioral studies of *Clarias gariepinus* Juveniles exposed to Paraquat herbicide are presented in Table1. The study indicated that the fish exhibited various behavioral abnormalities, including air gulping, weakness, agitated swimming, vertical positioning, mucus secretion, and imbalance, which increased in intensity with higher concentrations of Paraquat.

Air gulping was mild at 0.80 mg/L, became moderate at 1.10 mg/L and higher concentrations, and was strongly pronounced at 2.00 mg/L. Weakness was observed only at concentrations of 1.40 mg/L and above. Agitated swimming was mild at 0.80 mg/L, absent at 1.10 mg/L, and reappeared at higher concentrations. Vertical positioning was moderate at 0.80 mg/L, mild at 1.10 mg/L, absent at 1.40 mg/L, and reappeared at higher doses. Mucus secretion was mild at 0.80 mg/L and 1.10 mg/L, became moderate at 1.40 mg/L and 2.00 mg/L, and remained mild at 1.70 mg/L. Imbalance was mild starting from 0.80 mg/L, moderate at 1.70 mg/L, and strong at 2.00 mg/L.

Table 1. Behavioural Study of Juvenile *Clarias gariepinus* Exposed to Acute Concentration of Paraquat Herbicide for 96 hours

Behavioral Symptoms	0	0.80 (mg/L)	1.10 (mg/L)	1.40 (mg/L)	1.70 (mg/L)	2.00 (mg/L)
Air Gulping	-	+	++	++	++	+++
Weakness	-	-	-	+	+	+
Agitated Swimming	-	+	-	+	+	+
Vertical positioning	-	++	+	-	+	+
Mucus Secretion	-	+	+	++	+	++
Imbalance	-	+	+	+	++	+++

Key

− = None
+ (<10%) = Mild
++ (<50%) = Moderate
+++ (>50%) = Strong

The results of mortality and percentage mortality of juvenile *Clarias gariepinus* exposed to acute concentrations of Paraquat herbicide showed concentration and time-dependent lethality (Table 2). Mortality occurred in all treated groups, with no mortality observed in the control (0 mg/L). The mortality rate increased with higher concentrations, ranging from 20% at 0.80 mg/L to 90% at 2.00 mg/L. The calculated 96-hour LC_{50} value was 1.41, derived from probit analysis (Figure 1).

Mortality was low in the early exposure period and at lower concentrations (0.80–1.10 mg/L), where only 20–40% of fish died. However, at higher concentrations (1.40–2.00 mg/L), mortality escalated sharply, reaching 70–90% by 96 hours. The probit values (2.95–6.28) confirmed a significant dose-response relationship, with the steepest mortality increase occurring between 1.40 mg/L (70%, probit 5.52) and 2.00 mg/L (90%, probit 6.28).

Table 2. Probit Values of *C. gariepinus* Juveniles Exposed to Paraquat Herbicide at 96 hours

Conc (Mg/L)	Log conc	Total fish exposed	No. of Mortality	% Mortality	Probit Value
0	0.00	30	0	0	0.00
0.80	-0.10	30	2	20	4.16
1.10	0.04	30	12	40	4.75
1.40	0.15	30	21	70	5.52
1.70	0.23	30	24	80	5.84
2.00	0.30	30	27	90	6.28

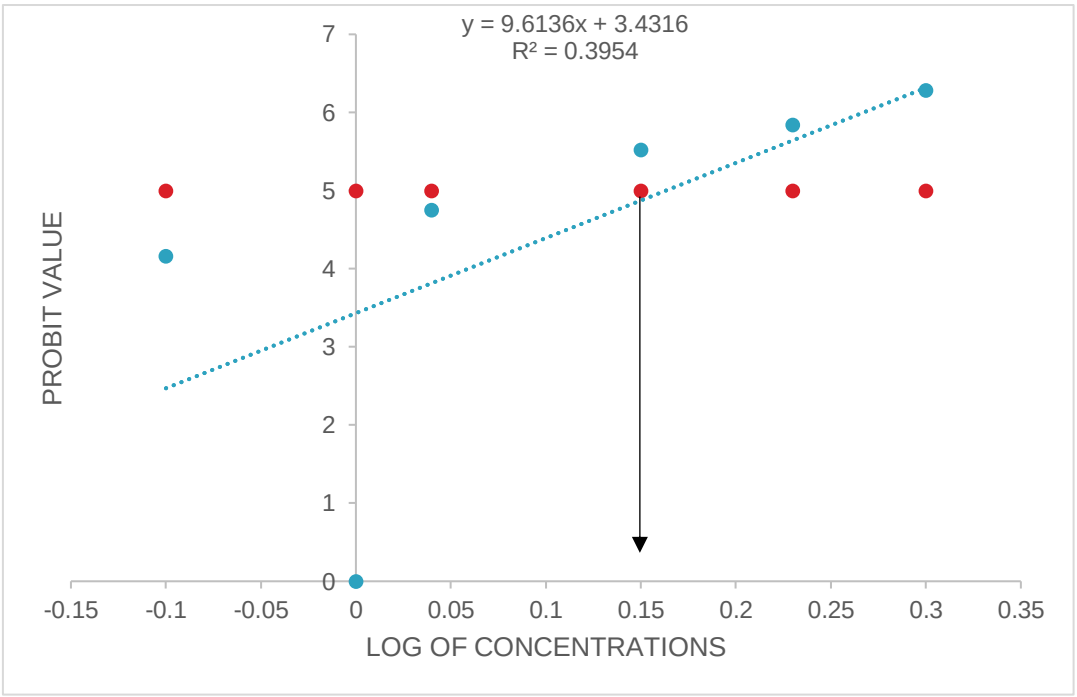

Figure 1. LC_{50} Value for *Clarias gariepinus* Juveniles Exposed to Acute Concentrations of Paraquat Herbicides for *96 Hours*

Discussions

The behavioural responses of juvenile *Clarias gariepinus* exposed to acute concentrations of Paraquat *Herbicides* revealed significant stress indicators, including air gulping, weakness, agitated swimming, vertical positioning, mucus secretion, and imbalance, with severity escalating at higher concentrations (Table 1). These abnormalities suggest neurotoxicity, respiratory distress, and osmoregulatory dysfunction induced by Paraquat. Air gulping, strongest at 2.00 mg/L, indicates hypoxia likely due to gill damage impairing oxygen uptake, which is consistent with the findings of Amaeze *et al.* (2020) who worked on the organophosphate pesticide effects on *Clarias gariepinus*. Weakness and imbalance at ≥1.40 mg/L align with reports by Adedeji *et al.* (2018), who linked Paraquat exposure to muscle paralysis and neurological impairment on liver and gill of *Clarias gariepinus* via oxidative stress. The non-linear pattern of agitated swimming absent at 1.10 mg/L but recurring at higher doses mirrors observations by Ayoola (2008), who documented alternating phases of hyperactivity and exhaustion in fish exposed to herbicides.

The dose-dependent behavioral disruptions underscore Paraquat's acute toxicity to aquatic organisms. In natural ecosystems, such effects could impair predator avoidance, feeding efficiency, and reproductive behaviors, threatening population stability, similar to patterns reported by Ullah *et al*. (2023). In aquaculture, even sublethal concentrations (e.g., 0.80 mg/L) may reduce growth and survival by elevating metabolic stress, as also noted by Esenowo *et al.* (2021). Mucus secretion, peaking at 1.40–2.00 mg/L, signals gill irritation and could increase susceptibility to secondary infections, a finding consistent with Oluah *et al*. (2020).

Similarly, behavioral patterns were also observed by Olagunju *et al*. (2019) in *C. gariepinus* exposed to glyphosate, including air gulping and erratic swimming, attributed to acetylcholinesterase inhibition. However, Paraquat induced more pronounced vertical positioning (moderate at 0.80 mg/L), whereas glyphosate-exposed fish showed surface orientation, as reported by Kori-Siakpere *et al*. (2022). This difference may be due to Paraquat's unique disruption of dopaminergic neurons, affecting buoyancy control, which agrees with the findings of Babatunde *et al*. (2021). Conversely, mucus secretion observed in the present study is comparable to that reported by Adeyemo (2005) in tilapia exposed to atrazine, suggesting a generalized stress response to herbicides.

The results demonstrated a clear concentration-dependent increase in mortality among *Clarias gariepinus* exposed to Paraquat herbicide, with the highest mortality rate recorded at the maximum concentration of 2.00 mg/L. The calculated 96-hour LC_{50} value of 1.41, derived from probit analysis, confirms the high toxicity of Paraquat to this species. This finding is consistent with Kori-Siakpere *et al*. (2022), who reported that Paraquat induces faster mortality than many other agricultural chemicals, including glyphosate and atrazine, due to its unique oxidative stress mechanisms.

The mortality patterns observed in this study can be attributed to Paraquat's well-documented ability to generate reactive oxygen species that cause systemic oxidative damage. This agrees with Adah *et al*. (2022), who documented similar lethal effects in fish exposed to redox-cycling herbicides. The herbicide's damaging effects on respiratory surfaces, particularly the gill epithelia, impair oxygen uptake and cause asphyxiation. In addition, the liver and nervous system suffer from lipid peroxidation and neurotransmitter disruption, contributing to the rapid mortality at higher concentrations.

The steep mortality curve between 1.40–2.00 mg/L highlighted Paraquat'ss extreme hazard to aquatic life, a pattern also noted by Ullah *et al*. (2023) in benthic fish species inhabiting shallow waters prone to herbicide runoff. The concentration-dependent lethality observed here supports previous findings that even short-term exposure to moderate levels of Paraquat can decimate local fish populations, potentially destabilizing entire aquatic food webs.

Conclusion

The behavioral responses of *Clarias gariepinus* exposed to Paraquat herbicide during acute toxicity tests revealed significant stress indicators, including air gulping, vertical positioning, mucus secretion, and loss of equilibrium, which intensified with increasing concentrations. The highest exposure levels (2.00 mg/L) induced severe neurological and respiratory distress, leading to mortality. The 96-hours LC_{50} of 1.41 confirmed Paraquat *Herbicides* high toxicity, with mortality rates escalating sharply at concentrations above 1.40mg/L.

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