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Effect of African Oil Bean Seed Powder on the Behaviour and Haematological Profile of Juveniles Catfish (*Clarias Gariepinus*)

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ABSTRACT

The effects of *Pentaclethra macrophylla* seed powder (African oil bean) at different concentrations on behavioural, physicochemical, and haematological parameters of juvenile *Clarias gariepinus* were evaluated under laboratory conditions. A 96-hour acute toxicity bioassay was conducted using six triplicated treatments: control (0.0 mg/L), 0.40 mg/L, 0.50 mg/L, 0.60 mg/L, 0.70 mg/L, and 0.80 mg/L. The water quality parameters were monitored and blood samples were collected from the experimental fish for haematological investigation. Changes in water quality parameters were not significant and were within levels tolerable by *C. gariepinus*. Catfish juveniles exposed to varying concentrations of the seed powder exhibited behavioural changes including loss of reflex, barbel deformation, erratic swimming, and air gulping, eventually leading to mortality. Haematological examination revealed significant increases in packed cell volume (PCV), red blood cells (RBC), mean corpuscular hemoglobin (MCH), hemoglobin (Hb), and mean corpuscular volume (MCV), while white blood cells (WBC), granulocytes and lymphocytes decreased. The increase in some haematological indices may represent a compensatory response to the toxic effects of *P. macrophylla* seed powder. These results indicate that *P. macrophylla* seed powder may cause both immediate and long-term adverse effects on the health of juvenile *C. gariepinus* and should be kept away from aquatic environments.

Keywords: Toxicity; *Pentaclethra macrophylla* seed powder; Haematology; Behaviour; *Clarias gariepinus*

Introduction

The African catfish, *Clarias gariepinus*, is an important freshwater fish used in aquaculture across Africa. It is highly preferred due to its rapid growth rate, tolerance to poor water quality, resistance to environmental stress and ease of artificial propagation (Ogwumba and Ugwumba, 2003; Ayoola, 2017). In addition, its high nutritional value makes it an important dietary

resource that contributes significantly to human health and economic development.

African Oil bean (*Pentaclethra macrophylla*) is a leguminous tree native to West Africa and valued for its oil-rich seeds, which are commonly used in traditional food and medicine (Ene-Obong, 2003; Ikegwonu *et al.*, 2014). The seeds contain high levels of protein, oil, and essential nutrients, but they also contain bioactive compounds such as alkaloids, tannins, saponins, flavonoids, and phytates which may

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be toxic to aquatic organisms when introduced into water bodies, despite some of their beneficial properties (Ajibade, *et al.* 2024). These substances can negatively affect fish physiology and disrupt aquatic ecosystem balance (Okwu and Ekeke, 2003; Nwachukwu *et al.*, 2020).

Haematological parameters, such as, red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Hct), and white blood cell count (WBC), are widely used as reliable indicators of fish health and physiological condition (Kori-Siakpere *et al.*, 2010). They help assess the effects of environmental stressors, including toxic substances, on fish. Changes in these haematological indices may indicate physiological stress, low oxygen transport, weakened immune response, and overall health deterioration, even before visible symptoms appear (Ovuru and Ekweozor, 2004; Shah and Altindag, 2004; Olafedehan *et al.*, 2010).

Plant-derived compounds have potential to induce physiological stress, alter haematological parameters, and reduce fish health and survival (Bulfon *et al.*, 2015, Ogueji *et al.*, 2020) and juvenile fish are particularly sensitive to toxic substances due to their rapid growth and developing immune systems, making them suitable bioindicators for toxicity studies. This study therefore evaluates the toxicity of *P. macrophylla* seed powder on juvenile *C. gariepinus* by assessing changes in haematological parameters, behavioural responses, and water quality properties. The findings will provide useful information for environmental monitoring, fish health management, and the sustainable use of plant-based materials in aquaculture.

Materials and Methods

The experiment was conducted at the Central Laboratory of the Department of Fisheries and Aquaculture, Adekunle Ajasin University, Akungba-

Akoko, Ondo State, Nigeria. The experimental unit consisted of eighteen (18) 50 L capacity plastic tanks which were filled with 30L of unchlorinated water. A total of 180 healthy juvenile *C. gariepinus*; with an average weight of 185g, were obtained from a reputable fish farm in Akure and transported to the laboratory in plastic containers. The fish were examined to ensure there were no physical deformities, and then evenly distributed into the experimental plastic tank where they were acclimatized for 14 days under laboratory conditions. They were fed regularly, maintained in clean water with daily renewal while uneaten feed and faecal matter were removed promptly to maintain good water quality.

P. macrophylla were identified at different locations within Akungba-Akoko and the fruits were collected. The seeds were then removed, air-dried, and milled into fine powder using a mechanical blender. The powdered samples were stored in clean, dry containers to prevent contamination. Water quality parameters, including pH, dissolved oxygen, conductivity, and total dissolved solids (TDS), were monitored before, during, and after the experiment using a HANNA multiparameter meter.

Static, non-renewable bioassays were conducted according to OECD (1992) guidelines. A range-finding test was conducted using varying concentrations of oil bean seed powder to determine appropriate exposure levels. Based on the results, a definitive 96-hour toxicity test was carried out using six concentrations: 0.0 (control), 0.4, 0.5, 0.6, 0.7, and 0.8 mg/L which were in triplicate. The experimental fish, *C. gariepinus* were not fed during this period, and tanks were covered with perforated lids. Mortality and behavioural changes were observed and recorded at intervals of 4, 12, 24, 48, 72, and 96 hours. Dead catfish were removed immediately.

Haematological Analysis

At the end of the exposure period, blood samples (3 mL) were collected from the caudal vein of each experimental fish using sterile hypodermic syringes. Samples were transferred into EDTA-treated bottles and preserved under refrigeration prior to analysis. Haematological parameters, including packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), and white blood cell count (WBC), were determined using standard laboratory procedures (Blaxhall & Daisley, 1973; Svobodova *et al.*, 1991). Derived indices, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), were calculated using standard formulas.

Statistical Analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) test in SPSS version 21. Differences between means were separated using Duncan’s New Multiple Range Test at a 5% significance level. Median lethal concentration (LC₅₀) values and confidence intervals were determined using probit and logit analyses in Minitab version 14.

Results

Behavioural responses of *C. gariepinus* exposed to Oil Bean Seed Powder

The behavioural responses of *C. gariepinus* to *P. macrophylla* seed powder are summarized in Table 1. Fish in the control group (0.0 mg/L) exhibited normal behaviour while exposure to increasing concentrations of the seed powder resulted in altered behaviours, including motionlessness, barbel deformation, loss of reflex, air gulping, excessive mucus secretion, hyperactivity, erratic swimming, and frequent surfacing. These responses intensified with higher concentrations and longer exposure periods. At the highest concentrations, fish exhibited loss of equilibrium, swimming backwards, vertical immobility, and ultimately death.

Acute Toxicity (LC₅₀) of *P. macrophylla* Seed Powder

Figure 1 presents the log concentration-probit relationship for juvenile *C. gariepinus*. The 96-h median lethal concentration (LC₅₀) of *P. macrophylla* seed powder was calculated as 756 mg/L, with a permissible concentration range of 7.56–75.6 mg/L,

Table 1: Behavioural responses of *C. gariepinus* exposed to oil bean seed powder, *P. Macrophylla*

Behaviour	24 hours						48 hours						72 hours						96 hours					
	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6
Colouration	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Air gulping	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Barbell deformation	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Erratic swimming	-	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Loss of reflex	-	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+

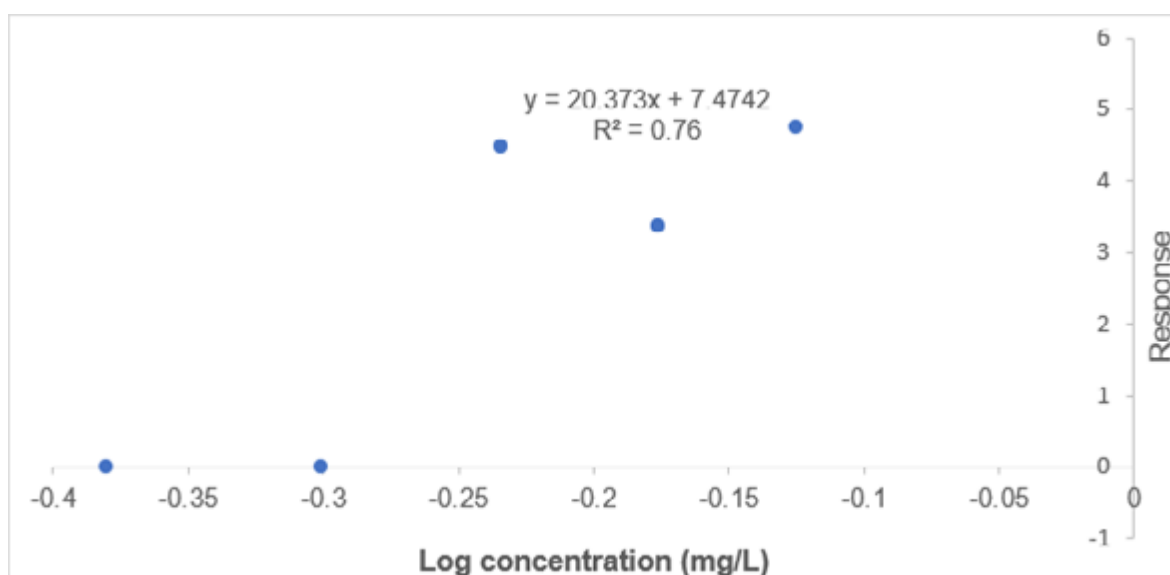


Figure 1: Log concentration of African oil bean and its probit value for juvenile *C. gariepinus*

based on the method of Koesoemadinata (2000). LC_{50} represents the concentration at which 50% of the exposed fish population is expected to die.

Haematological parameters of *C. gariepinus* exposed to *Pentaclethra macrophylla* seed powder

Haematological results are presented in Table 2. Packed cell volume (PCV) was highest in Treatment 6 ($27.99 \pm 0.00\%$) and lowest in Treatment 5 ($20.49 \pm 0.00\%$), showing a significant increase with concentration ($p < 0.05$). RBC values were highest in T6 ($1.40 \pm 0.05 \times 10^6/\mu\text{L}$) and lowest in T2 ($0.85 \pm 0.02 \times 10^6/\mu\text{L}$). MCHC values showed no significant difference ($p > 0.05$) across treatments. MCV was highest in T2 (264.59 ± 1.21 fL) and lowest in T5 (170.84 ± 2.39 fL). Hemoglobin concentration (Hb) peaked in T6 (9.34 ± 0.20 g/dL) and was lowest in T5 (6.84 ± 0.05 g/dL). WBC counts were highest in T6 ($1.10 \pm 0.05 \times 10^3/\mu\text{L}$) and lowest in T3 ($0.60 \pm 0.00 \times 10^3/\mu\text{L}$). Lymphocyte counts were highest in T6 ($0.78 \pm 0.20 \times 10^3/\mu\text{L}$) and lowest in T3 and T4 ($0.44 \pm 0.05 \times 10^3/$

μL). Significant differences ($p < 0.05$) were observed for all parameters compared to the control.

Water Quality Parameters

The result of the water quality parameters monitored is presented in Table 3. The temperature exhibited a slight downward trend over time; starting at 25.0°C in the control and progressively drops to 23.70°C by the 96 hours. The least temperature (23.0°C) was recorded in T4 (72 hours), T5 (48hrs and 72hrs) and T6 (48hrs). The pH values remain within a slightly acidic to neutral range (6.00 to 7.20) throughout the experiment across the various treatment levels. The conductivity value progressively increases in T1, T2, T3 and T4 from 168.0, 172.0, 171.5 and 171.5 mg/L respectively at 0 hour to 202.5, 199.0, 197.0 and 222.5 mg/l respectively at 96 hours. However, in T5 and T6, the value initially decreased between 0 hour and 4 hours and later increased progressively at 48 hours to 96 hours. It was also observed that the conductivity was stable between 12 to 24 hours across all the treatments. There was fluctuation in the dissolved oxygen (DO) levels across the treatments and test periods. Treatment

T1 (control), T2 and T3 had drastic decrease in the DO level at 96 hours when compared with the 0 hour, whereas, Treatments T4 and T5 had slight increase at the 96 hours.

Table 2: Haematological parameters of *C. gariepinus* exposed to *Pentaclethra macrophylla* seed powder

Parameters(mg/l)	T1(0.0)	T2(0.4)	T3(0.5)	T4(0.6)	T5(0.7)	T6(0.8)
PCV (%)	19.49 ±0.00a	22.48 ±0.01 ^b	21.48 ±0.01 ^{ab}	25.49 ±0.00 ^c	20.49 ±0.00 ^{ab}	27.99 ±00.00 ^d
RBC(x1012cell/μl)	1.00 ±0.11 ^{ab}	0.85 ±0.02 ^a	0.95 ±0.02 ^a	1.15 ±0.02 ^{bc}	1.20 ±0.00 ^c	1.40 ±0.05 ^d
MCHC (g/dl)	33.25 ±0.10 ^a	33.33 ±0.00 ^a	33.48 ±0.00 ^a	33.33 ±0.00 ^a	33.41 ±0.85 ^a	33.38 ±0.55 ^a
MCV (fl/cell)	202.09±20.43 ^{ab}	264.59±1.21 ^c	226.10±2.25 ^b	221.59±1.96 ^b	170.84±2.39 ^a	201.54±12.44 ^{ab}
Hb (g/dl)	6.50 ±0.11 ^a	7.50 ±0.28 ^b	7.18 ±0.28 ^{ab}	8.50 ±0.28 ^c	6.84 ±0.86 ^{ab}	9.34 ±0.20 ^d
WBC (x1012cell/μl)	1.30 ±0.23 ^c	0.95 ±0.14 ^{abc}	0.60 ±0.00 ^a	0.65 ±0.08 ^a	0.85 ±0.02 ^{ab}	1.10 ±0.05 ^{bc}
Granulocytes (cell/μl)	0.54 ±0.14 ^b	0.35 ±0.08 ^{ab}	0.15 ±0.02 ^a	0.20 ±0.05 ^a	0.25 ±0.02 ^a	0.31 ±0.05 ^{ab}
Lymphocytes (cell/μl)	0.75 ±0.08 ^b	0.59 ±0.05 ^{ab}	0.44 ±0.05 ^a	0.44 ±0.05 ^a	0.59 ±0.00 ^{ab}	0.78 ±0.20 ^b
MCH (pg/cell)	67.38 ±6.81 ^{ab}	88.19 ±0.40 ^c	75.39 ±0.75 ^b	73.85 ±0.66 ^b	56.94 ±0.77 ^a	67.19±4.15 ^{ab}

Mean values on the same row carrying similar superscripts are not significantly different (p>0.05). PCV, Packed cell volume; RBC, red blood cell; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Hb, hemoglobin; WBC, white blood cell; MCH, mean corpuscular hemoglobin.

Table 3: Water quality parameters of *C. gariepinus* exposed to oil bean seed powder (*Pentaclethra macrophylla*)

Time	Parameters	T1 (0.0mg/L)	T2 (0.42 mg/L)	T3 (0.5mg/L)	T4 (0.6mg/L)	T5 (0.7mg/L)	T6 (0.8mg/L)
Control	Temp	25.00 ± 0.00 ^a	24.00 ± 0.25 ^a	24.90 ± 0.20 ^a	25.00 ± 0.00 ^a	25.00 ± 0.00 ^a	24.50 ± 0.50 ^b
	pH	6.83 ± 1.30 ^a	6.99 ± 0.03 ^a	6.74 ± 0.21 ^a	6.63 ± 3.00 ^a	6.00 ± 0.13 ^a	6.73 ± 0.10 ^a
	DO	3.00 ± 0.10 ^c	2.80 ± 0.10 ^{bc}	2.65 ± 0.10 ^{bc}	2.10 ± 0.20 ^a	24.00 ± 0.10 ^b	2.40 ± 1.10 ^{ab}
	Cond	168.00 ± 1.00 ^a	172.00 ± 1.50 ^{ab}	171.50 ± 1.50 ^b	171.50 ± 1.50 ^{ab}	170.50 ± 0.50 ^{ab}	173.00 ± 0.00 ^b
4HR	Temp	24.40 ± 0.20 ^a	24.50 ± 0.00 ^{ab}	24.60 ± 0.10 ^{ab}	24.50 ± 0.00 ^{ab}	24.70 ± 0.00 ^b	24.90 ± 0.15 ^b
	pH	6.80 ± 0.709 ^a	6.40 ± 0.02 ^a	6.30 ± 0.31 ^a	6.12 ± 0.12 ^a	6.80 ± 0.10 ^a	6.80 ± 0.10 ^a
	DO	24.0 ± 0.20 ^a	3.40 ± 0.08 ^a	1.80 ± 0.40 ^b	2.50 ± 0.20 ^a	3.40 ± 0.15 ^a	2.70 ± 0.50 ^a
	Cond	172.00 ± 0.10 ^a	174.00 ± 11.00 ^a	166.00 ± 5.00 ^a	177.00 ± 8.00 ^a	165.00 ± 0.00 ^a	172.50 ± 1.50 ^a
12HR	Temp	24.70 ± 0.00 ^a	24.70 ± 0.00 ^a	24.60 ± 0.10 ^a	24.40 ± 0.12 ^a	24.50 ± 0.05 ^a	24.50 ± 0.00 ^a
	pH	6.50 ± 0.22 ^a	6.10 ± 0.10 ^a	6.50 ± 0.31 ^a	6.10 ± 0.10 ^a	6.70 ± 0.43 ^a	6.10 ± 0.10 ^a
	DO	2.45 ± 0.55 ^{ab}	2.50 ± 0.60 ^{ab}	1.80 ± 0.10 ^a	2.10 ± 0.10 ^{ab}	3.20 ± 0.40 ^b	2.80 ± 0.40 ^b
	Cond	175.50 ± 1.50 ^a	177.00 ± 6.00 ^a	174.00 ± 2.00 ^a	179.50 ± 5.50 ^a	167.50 ± 2.50 ^a	176.00 ± 1.00 ^a

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24HR	Temp	24.70 ± 0.00 ^a	24.70 ± 0.00 ^a	24.60 ± 0.10 ^a	24.40 ± 0.20 ^a	24.50 ± 0.30 ^a	24.50 ± 0.00 ^a
	pH	6.10 ± 0.10 ^a	6.10 ± 0.10 ^a	6.50 ± 0.31 ^a	6.10 ± 0.10 ^a	6.70 ± 0.43 ^a	6.10 ± 0.10 ^a
	DO	2.50 ± 0.60 ^{ab}	1.10 ± 0.20 ^{ab}	1.80 ± 0.10 ^a	2.10 ± 0.10 ^{ab}	3.20 ± 0.40 ^b	2.80 ± 0.40 ^b
	Cond	175.50 ± 1.50 ^a	177.00 ± 6.00 ^a	174.00 ± 2.00 ^a	179.50 ± 5.50 ^a	167.50 ± 2.50 ^a	176.00 ± 1.00 ^a
48HR	Temp	23.50 ± 0.10 ^c	23.20 ± 0.00 ^b	23.20 ± 0.00 ^b	23.20 ± 0.10 ^b	23.00 ± 0.00 ^a	23.00 ± 0.00 ^a
	pH	6.33 ± 0.30 ^a	6.20 ± 0.12 ^a	6.20 ± 0.10 ^a	6.60 ± 0.20 ^b	7.13 ± 0.12 ^b	7.01 ± 0.15 ^b
	DO	2.50 ± 0.50 ^{bc}	1.60 ± 0.10 ^a	1.60 ± 0.30 ^{ab}	2.00 ± 0.20 ^{ab}	3.30 ± 0.00 ^c	2.10 ± 0.15 ^{bc}
	Cond	186.00 ± 4.00 ^{ab}	181.00 ± 4.00 ^{ab}	178.00 ± 1.00 ^{ab}	196.00 ± 0.00 ^{ab}	172.50 ± 12.50 ^a	198.00 ± 1.00 ^b
72HR	Temp	23.30 ± 0.10 ^a	23.20 ± 0.10 ^a	23.40 ± 0.10 ^a	23.00 ± 0.00 ^a	23.00 ± 0.00 ^a	23.30 ± 0.10 ^a
	pH	6.10 ± 0.10 ^a	6.10 ± 0.19 ^a	6.62 ± 0.50 ^a	7.20 ± 0.50 ^a	6.91 ± 0.32 ^a	6.60 ± 0.04 ^a
	DO	2.80 ± 0.40 ^b	1.40 ± 0.10 ^a	23.0 ± 0.6 ^{ab}	2.20 ± 0.30 ^{ab}	2.90 ± 0.11 ^b	2.80 ± 0.10 ^b
	Cond	193.00 ± 3.00 ^a	188.00 ± 7.00 ^a	185.00 ± 1.00 ^a	200.50 ± 1.50 ^a	195.50 ± 21.00 ^a	218.00 ± 2.00 ^a
96HR	Temp	23.70 ± 0.10 ^a	23.70 ± 0.10 ^a	23.40 ± 0.00 ^a	23.50 ± 0.00 ^a	23.50 ± 0.10 ^a	23.70 ± 0.10 ^a
	pH	6.60 ± 0.30 ^a	6.53 ± 0.30 ^a	6.50 ± 0.60 ^a	6.50 ± 0.24 ^a	6.10 ± 0.31 ^a	6.62 ± 0.31 ^a
	DO	2.10 ± 0.70 ^a	1.90 ± 0.50 ^a	2.10 ± 0.80 ^a	2.20 ± 0.10 ^a	2.60 ± 0.60 ^a	2.40 ± 0.30 ^a
	Cond	202.50 ± 3.50 ^a	199.00 ± 9.00 ^a	197.00 ± 9.00 ^a	222.50 ± 5.50 ^a	214.50 ± 5.50 ^a	243.00 ± 1.00 ^a

a,b,c,d = mean on the same row but with different superscript are statistically significant (p<0.05). T1=0%; T2=0.42%; T3=0.5%; T4=0.6%; T5=0.70%; T6=0.8% inclusion of oil bean seed powder (*Pentaclethra macrophylla*).

Discussion

The behavioural alterations observed in *C. gariepinus* exposed to *Pentaclethra macrophylla* seed powder, such as loss of reflex, air gulping, erratic swimming, barbel deformation, and hyperactivity are consistent with the effects of neurotoxic compounds on fish. These behaviours likely result from disruption in neurotransmission, particularly the hydrolysis of acetylcholine in cholinergic pathways of both the central and peripheral nervous systems. Similar observations were reported in fish exposed to plant derived toxicants and pesticide compounds which

interfered with acetylcholinesterase activity, leading to accumulation of acetylcholine at synaptic junctions and prolonged neuronal stimulation (Ogueji et al., 2020; Svobodová et al., 2017). Impaired neurotransmission leads to reduced coordination, stress, and respiratory difficulty, causing the fish to settle at the bottom of tanks and eventually die. Swimming performance is considered a sensitive indicator of acute or sub-lethal toxicity, as observed in other studies on *Cyprinus carpio* and *C. gariepinus* exposed to pesticides (Alkahemal-Balawi et al., 2011; Adewumi et al., 2018).

Monitoring of LC_{50} and permissible concentrations is critical in establishing safe environmental and aquaculture practices. The 96-hour median lethal concentration (LC_{50}) of *P. macrophylla* seed powder for juvenile *C. gariepinus* calculated at 756 mg/L, with a permissible concentration range of 7.56-75.6 mg/L, indicated moderate toxicity. This value demonstrates that even at sub-lethal concentrations, prolonged exposure could adversely affect fish health, growth, and survival.

Water quality parameters, including pH, dissolved oxygen (DO), and conductivity, were significantly affected by increasing concentrations of the toxicant, while temperature remained stable. A negative correlation between DO and toxicant concentration suggests that agitation and abnormal behaviours of exposed fish may have caused the reduction in the available oxygen in the water. The observed increase in conductivity with rising concentrations may be attributed to elevated organic matter and electrolyte content in the water due to the decomposition of the seed powder and excretory by-products of stressed fish, and this is in agreement with findings of Akaahan *et al.* (2016) and Seiyaboh *et al.* (2013). Maintaining optimal water quality is essential for fish survival, and these changes indicate that *P. macrophylla* can disrupt aquatic homeostasis even at sub-lethal concentrations.

Haematological analyses revealed significant alterations in blood parameters, which serve as reliable indicators of physiological stress and toxic exposure. PCV, RBC, Hb, MCV, and MCH increased with increasing toxicant concentration, while WBC, lymphocyte, and granulocyte count decreased. The initial increase in the WBC may reflect a compensatory immune response to chemical stress, while the subsequent decrease at higher concentrations indicates immune-suppression. Elevated Hb and RBC levels suggest a physiological attempt to maintain oxygen transport under stress,

whereas changes in PCV reflect alterations in blood volume and erythrocyte production. These findings are consistent with previous studies on pesticide and plant-based toxicants in *C. gariepinus* (Amaeze *et al.*, 2020; Bala *et al.*, 2019; Adene *et al.*, 2017).

The observed haematological changes suggest that sub-lethal concentrations of *P. macrophylla* may cause long-term effects on fish physiology, including reduced immunity, increased susceptibility to disease, and impaired growth. From an ecological perspective, such toxic effects could disrupt food webs in freshwater ecosystems, as juvenile fish are critical both as predators of invertebrates and as prey for larger fish and birds. Therefore, the release of *P. macrophylla* seed powder into water bodies should be carefully monitored to prevent ecological disturbances.

Conclusion and Recommendations

This study revealed that sub-lethal and acute exposures to *P. macrophylla* seed powder caused significant behavioural and haematological changes in juvenile *C. gariepinus*, including alterations in RBC, WBC, Hb, PCV, MCV, and MCH. These changes indicate stress, reduced immunity, and impaired physiological functions, which may increase vulnerability to disease and reduce survival in aquaculture systems. The study also demonstrated that even non-lethal concentrations of *P. macrophylla* seed powder can adversely affect fish health and water quality. Consequently, this plant by-product should be avoided in or near aquaculture systems, ponds, or natural water bodies to prevent toxicity and maintain ecosystem balance. These findings provide baseline data for environmental monitoring, toxicological assessment, and sustainable management of freshwater ecosystems exposed to plant-derived compounds. It is recommended that: Proper disposal and management of *P. macrophylla* by-products be enforced to prevent accidental contamination of aquatic

habitats, Further studies investigate chronic exposure effects, including reproductive and growth impacts on fish. Alternative non-toxic feed supplements be explored in aquaculture to minimize reliance on plant byproducts with potential toxic effects.

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