



Acute toxicity, behavioural responses, and serum biochemical alterations in juvenile African catfish (*Clarias gariepinus*) exposed to African oil bean (*Pentaclethra macrophylla* Benth.) seed powder

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ABSTRACT

Plant-derived materials used in food processing may enter freshwater systems and expose non-target aquatic organisms to complex mixtures of bioactive constituents. This study determined the acute toxicity of African oil bean seed powder and evaluated associated behavioural and serum biochemical responses in juvenile African catfish. A 96-hour static, non-renewal bioassay was conducted using six nominal concentrations (0.00, 0.42, 0.50, 0.60, 0.70, and 0.80 mg/L), each in duplicate, with ten fish in 30 L of unchlorinated water per tank. Mortality and visible behavioural responses were monitored throughout exposure. At 96 hours, blood from three fish per tank was pooled, allowed to clot, and the serum was analysed for creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, and cholesterol. Regression of probit response against log10 concentration yielded an estimated 96-hour median lethal concentration (LC₅₀) of 0.756 mg/L. Creatinine generally declined across the exposure series and differed significantly from the control at 0.60-0.80 mg/L. AST activity increased markedly at 0.70 and 0.80 mg/L, whereas ALT and cholesterol did not differ significantly among treatments. Total protein showed a variable, non-monotonic response. Body colouration changes, air gulping, erratic swimming, altered barbel condition, and loss of reflex became more evident with increasing concentration and exposure duration. The combined mortality, behavioural, and biochemical findings show that unprocessed African oil bean seed powder can induce acute biological stress in juvenile African catfish under static laboratory exposure. The study provides baseline evidence for further characterisation of the aquatic effects of African oil bean processing residues.

HIGHLIGHTS

- African oil bean seed powder produced an estimated 96-hour LC₅₀ of 0.756 mg/L in juvenile African catfish.
- The higher exposure concentrations altered serum creatinine and AST activity.
- Behavioural disturbances increased with concentration and exposure duration.

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1. Introduction

Freshwater organisms are exposed to a wide range of natural and anthropogenic substances, including agricultural chemicals, industrial discharges, domestic effluents, and plant-derived processing residues. Acute toxicity tests provide a controlled means of establishing short-term concentration-response relationships and identifying visible signs of biological disturbance. In standard fish acute-toxicity designs, mortality is recorded over a 96-hour period and used to estimate the median lethal concentration, while abnormalities in swimming, respiration, pigmentation, posture, or reflex provide additional evidence of toxic stress (Organisation for Economic Co-operation and Development [OECD], 2025). Although mortality remains the principal endpoint, behavioural changes often appear before death and can reveal impairment of functions directly related to feeding, predator avoidance, respiration, and habitat use.

The African catfish, *Clarias gariepinus*, is widely used in aquatic toxicology because it is hardy under culture conditions yet responds measurably to waterborne contaminants. Its ecological and aquaculture importance in Africa also gives toxicological

findings direct regional relevance. Studies using pesticides and other waterborne stressors have documented changes in survival, swimming behaviour, blood-cell profiles, serum biochemistry, oxidative balance, and genetic integrity in this species (Alarape et al., 2024; Amaeze et al., 2020; Kanu et al., 2023). These responses demonstrate that the species can integrate lethal and sublethal effects across several levels of biological organisation.

Serum biochemical indices are particularly useful for assessing acute physiological disturbance because they reflect changes in metabolism, membrane integrity, nutrient utilisation, and excretory function. Aminotransferases such as AST and ALT may change following tissue stress or altered cellular permeability, but neither enzyme should be treated as an organ-specific marker in isolation. Creatinine can respond to changes in nitrogen metabolism, hydration, muscle activity, or renal handling, while total protein and cholesterol reflect broader shifts in protein and lipid homeostasis. The direction of these changes is not uniform across toxicants, species, or exposure conditions; interpretation is strongest when several indices are considered together with mortality and behaviour (Bojarski et al., 2025).

African oil bean (*Pentaclethra macrophylla* Benth.) is a leguminous tree native to the humid regions of West and Central

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Africa. Its oil-rich seeds are processed and fermented to produce *ugba*, a traditional food widely consumed in southern Nigeria. The seed is nutritionally valuable, with appreciable lipid and protein contents, and has attracted interest as an underutilised food and industrial raw material (Nwokocha et al., 2023). At the same time, chemical studies have identified saponins, terpenoids, cardiac glycosides, and other bioactive constituents in raw or processed seed preparations (Akinlabu et al., 2019; Ugbogu et al., 2020). Fermentation and other processing steps alter the chemical profile of the seed, indicating that unprocessed material may differ biologically from the finished food product.

Where seed preparation occurs close to drainage channels or surface waters, fragments, wash water, or unused residues may create a route of exposure for aquatic organisms. Plant-derived substances are not inherently environmentally benign: several botanical extracts used in traditional fishing, parasite control, or food processing have produced concentration-dependent mortality, behavioural abnormalities, and tissue injury in African catfish (Akinsanya et al., 2016; Ekpenyong et al., 2022). Despite the economic and cultural importance of African oil bean, evidence concerning the effects of its raw seed material on freshwater fish remains limited. Existing studies have concentrated mainly on food composition, fermentation, or mammalian biological activity rather than aquatic toxicity.

This study therefore examined the acute effects of *P. macrophylla* seed powder on juvenile *C. gariepinus*. The specific objectives were to: (i) estimate the 96-hour LC₅₀ of the seed powder; (ii) document concentration- and time-related behavioural responses; and (iii) determine changes in serum creatinine, AST, ALT, total protein, and cholesterol after acute exposure. It was anticipated that increasing exposure would be accompanied by greater mortality, more pronounced behavioural abnormalities, and measurable changes in selected biochemical indices.

2. Research Methodology

2.1. Experimental site and test fish

The experiment was conducted in the Fisheries and Aquaculture Laboratory of the Central Laboratory, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Two hundred and fifty healthy juvenile *C. gariepinus* of both sexes were obtained from the Research Farm of the Federal University of Technology, Akure, Ondo State. The fish weighed 6.6–13.4 g and measured 7.2–9.4 cm in total length. They were transported in plastic containers containing source water and acclimatised under laboratory conditions for 14 days. Fish showing visible injury, abnormal appearance, or deformity were excluded before the toxicity tests.

2.2. Collection, authentication, and preparation of seed powder

Mature African oil bean seeds were collected within Adekunle Ajasin University, Akungba-Akoko. The plant material was authenticated as *Pentaclethra macrophylla* Benth. by Dr Ezekiel Ajayi of the Department of Forestry and Wildlife, Adekunle Ajasin University. The hard seed coats were opened and removed, after which the kernels were air-dried for three weeks. The dried kernels were milled with an electric blender to obtain a fine, unsieved powder. The powder was stored in airtight containers at room temperature until required for the exposure tests.

2.3. Preliminary range-finding test

A preliminary test was conducted to identify concentrations suitable for the definitive 96-hour bioassay. Ten juvenile *C. gariepinus* were stocked in each of 12 tanks (75 × 45 × 45 cm), each containing 30 L of unchlorinated water. Six nominal concentrations of seed powder (0.333, 0.667, 0.833, 1.000, 1.333, and 1.667 mg/L) were evaluated in duplicate. Behaviour, visible body changes, and mortality were monitored, and dead fish were removed promptly to minimise deterioration of the exposure medium.

2.4. Definitive 96-hour acute exposure

The definitive test consisted of a control and five seed-powder concentrations: 0.00, 0.42, 0.50, 0.60, 0.70, and 0.80 mg/L. Each concentration was evaluated in duplicate, producing 12 experimental tanks. Ten juvenile *C. gariepinus* were stocked in each tank containing 30 L of unchlorinated water. The exposure system was static and non-renewal; the tanks were not aerated, and fish were not fed during the 96-hour test. Seed powder was added directly to the designated tanks at the start of exposure.

Mortality and visible responses were checked every 15 minutes during the first hour, hourly during the next four hours, at four-hour intervals during the remainder of the first 24 hours, and subsequently at 24-hour intervals until the end of the 96-hour exposure. Fish were considered dead when opercular movement ceased and no response occurred following gentle stimulation. Dead fish were removed immediately.

2.5. Behavioural observations

Behavioural and external responses were assessed qualitatively at tank level. The recorded endpoints were body colouration change, air gulping at the water surface, altered barbel condition, erratic swimming, and loss of equilibrium or reflex. A response was scored as present (+) when it was observed in a treatment tank and absent (-) when it was not observed. Control tanks were observed concurrently throughout the test.

2.6. Blood collection and serum biochemical assays

At the end of the 96-hour exposure, three fish were sampled from each tank. Blood was withdrawn through the caudal vasculature using a sterile needle and syringe. Blood from the three fish within each tank was pooled to obtain approximately 5 mL and allowed to clot. Serum was separated and used for the determination of creatinine, AST, ALT, total protein, and cholesterol with commercial diagnostic assay kits (Agape) according to the manufacturer's procedures. Each pooled tank sample was assayed in triplicate.

2.7. Statistical analysis

Biochemical data were analysed using one-way analysis of variance in IBM SPSS Statistics version 21. Treatment means were compared using Duncan's multiple range test at $p < 0.05$, and the results are presented as mean ± standard deviation. The 96-hour LC₅₀ was estimated from the fitted linear relationship between probit response and the base-10 logarithm of the nominal exposure concentration.

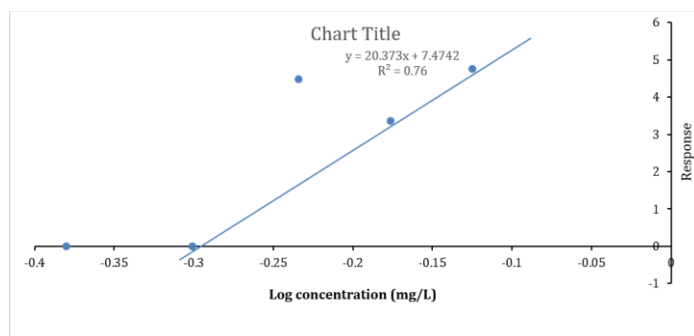


Figure 1. Log concentration-probit relationship for juvenile *Clarias gariepinus* exposed to *Pentaclethra macrophylla* seed powder for 96 hours

3. Results and Discussion

3.1. Acute toxicity and estimated LC₅₀

The mortality response obtained during the definitive exposure was used to derive the log concentration-probit relationship presented in Figure 1. The fitted equation was $y = 20.373x + 7.474$ ($R^2 = 0.760$), where x represents $\log_{10}$ concentration and y represents probit response. The concentration corresponding to a probit response of 5 produced an estimated 96-hour LC₅₀ of 0.756 mg/L for juvenile *C. gariepinus*.

The estimated LC₅₀ shows that raw *P. macrophylla* seed powder produced an acute lethal response in juvenile *C. gariepinus* under the static exposure conditions used. This estimate is specific to the seed material, fish size, exposure medium, and laboratory system employed in the study. Acute toxicity can vary substantially among plant-derived materials because their biological activity depends on chemical composition, preparation method, particle characteristics, and the susceptibility of the exposed species. The value obtained here should therefore be treated as a baseline estimate for raw African

oil bean seed powder rather than a universal threshold for all seed residues or processing effluents.

The whole-seed powder constituted a complex exposure rather than a purified chemical. African oil bean seeds contain substantial lipid and protein fractions as well as reported secondary constituents, including saponins, terpenoids, and cardiac glycosides (Akinlabu *et al.*, 2019; Nwokocha *et al.*, 2023). Such constituents may interact with biological membranes, respiratory surfaces, or metabolic pathways, either independently or in combination. Nevertheless, the individual constituents of the test powder were not quantified in this study. The acute lethal response should therefore be attributed to the unprocessed seed powder as a whole rather than to a specific compound. This distinction is important because fermentation and other processing methods can alter the chemical and biological properties of African oil bean products (Ugbogu *et al.*, 2020).

3.2. Types and Frequency of Access to In-Service Training Programme

No recorded behavioural abnormality occurred in the control tanks. In exposed tanks, behavioural responses became more widespread as concentration and exposure duration increased. At 24 hours, body colouration changes were observed from 0.50 mg/L, whereas air gulping was present from 0.42 mg/L. Erratic swimming and loss of reflex were first observed at 0.60 mg/L, while altered barbel condition was recorded at 0.70 and 0.80 mg/L. By 48 hours, all five responses were observed at every exposure concentration and remained present through 72 and 96 hours (Table 1).

The concentration- and time-related progression of these responses complements the acute lethality estimate. Air gulping appeared at the lowest exposure concentration within 24 hours, followed by changes in colouration, swimming, reflex, and barbel condition as exposure intensity increased. Similar signs, including surfacing, air gulping, erratic swimming, loss of balance, and reduced reflex, have been reported in African catfish exposed to pesticides and botanical preparations (Ekpenyong *et al.*, 2022; Nwani *et al.*, 2012). Although these responses do not identify a single toxic mechanism, they indicate disruption of normal respiratory, sensory, neuromuscular, or homeostatic function.

Table 1. Behavioural responses of juvenile *Clarias gariepinus* during 96-hour exposure to *Pentaclethra macrophylla* seed powder

Time	Response	0.00 mg/L	0.42 mg/L	0.50 mg/L	0.60 mg/L	0.70 mg/L	0.80 mg/L
24 h	Body colouration change	-	-	+	+	+	+
	Air gulping	-	+	+	+	+	+
	Altered barbel condition	-	-	-	-	+	+
	Erratic swimming	-	-	-	+	+	+
	Loss of reflex	-	-	-	+	+	+
48 h	Body colouration change	-	+	+	+	+	+
	Air gulping	-	+	+	+	+	+
	Altered barbel condition	-	+	+	+	+	+
	Erratic swimming	-	+	+	+	+	+
	Loss of reflex	-	+	+	+	+	+
72 h	Body colouration change	-	+	+	+	+	+
	Air gulping	-	+	+	+	+	+
	Altered barbel condition	-	+	+	+	+	+
	Erratic swimming	-	+	+	+	+	+
	Loss of reflex	-	+	+	+	+	+
96 h	Body colouration change	-	+	+	+	+	+
	Air gulping	-	+	+	+	+	+
	Altered barbel condition	-	+	+	+	+	+
	Erratic swimming	-	+	+	+	+	+
	Loss of reflex	-	+	+	+	+	+

Key: + = response observed at tank level; - = response not observed.

Table 2. Serum biochemical parameters of juvenile *Clarias gariepinus* after 96-hour exposure to *Pentaclethra macrophylla* seed powder

Parameter	Control	0.42 mg/L	0.50 mg/L	0.60 mg/L	0.70 mg/L	0.80 mg/L
Creatinine (mg/dL)	0.61 ± 0.13 ^d	0.53 ± 0.36 ^{cd}	0.50 ± 0.05 ^{bcd}	0.26 ± 0.10 ^{abc}	0.02 ± 0.00 ^a	0.18 ± 0.08 ^{ab}
AST (U/L)	44.78 ± 1.30 ^{abc}	20.86 ± 3.47 ^a	14.13 ± 6.30 ^a	33.91 ± 0.43 ^{ab}	81.52 ± 45.43 ^c	62.60 ± 8.26 ^{bc}
ALT (U/L)	78.57 ± 12.67 ^a	25.44 ± 6.16 ^a	6.60 ± 3.57 ^a	5.08 ± 1.16 ^a	6.51 ± 1.33 ^a	10.89 ± 0.17 ^a
Total protein (g/dL)	1.33 ± 0.12 ^{ab}	1.98 ± 0.26 ^{ab}	0.36 ± 0.21 ^a	3.64 ± 3.23 ^b	0.58 ± 0.23 ^a	0.87 ± 0.04 ^{ab}
Cholesterol	65.30 ± 40.30 ^a	45.04 ± 7.54 ^a	54.04 ± 39.00 ^a	35.12 ± 4.52 ^a	36.85 ± 10.99 ^a	45.09 ± 11.46 ^a

Values are mean ± SD. Within a row, means sharing at least one superscript letter are not significantly different ($p > 0.05$). Each treatment comprised two tanks; serum from three fish per tank was pooled and each pooled sample was assayed in triplicate. AST = aspartate aminotransferase; ALT = alanine aminotransferase.

From an ecological perspective, persistent air gulping, impaired swimming, altered reflexes, and changes in barbel condition could reduce the ability of fish to locate food, maintain position, escape predators, and respond to their surroundings. The persistence of all recorded responses from 48 to 96 hours suggests that the biological disturbance was sustained rather than transient under the exposure conditions. Behavioural observations therefore provided an important functional dimension to the mortality endpoint and showed that exposed fish experienced measurable impairment before or alongside lethal effects.

3.3. Serum biochemical responses

The serum biochemical values recorded after the 96-hour exposure are presented in Table 2.

3.3.1. Creatinine

Serum creatinine generally declined across the exposure series. Values at 0.60, 0.70, and 0.80 mg/L were significantly lower than the control ($p < 0.05$), and the lowest mean was recorded at 0.70 mg/L. This pattern indicates that acute exposure altered creatinine homeostasis at the higher concentrations. In fish, circulating creatinine may be affected by muscle metabolism, hydration, excretion, and renal handling; both increases and decreases have been observed following toxicant exposure. The decline recorded here should therefore not be interpreted in isolation as evidence of a particular renal lesion. A recent 96-hour study in *C. gariepinus* similarly reported non-linear changes in creatinine and related biochemical indices across glyphosate concentrations, illustrating the dependence of serum responses on dose and exposure conditions (Alarape et al., 2024).

3.3.2. Aminotransferase activities

AST activity differed significantly among treatments. The highest activity was recorded at 0.70 mg/L, followed by 0.80 mg/L, while the lowest activities occurred at 0.42 and 0.50 mg/L. In contrast, ALT was numerically lower in all exposed groups than in the control, but the differences were not significant ($p > 0.05$). The marked AST response at the two highest concentrations indicates an acute biochemical disturbance. Aminotransferases participate in amino-acid metabolism and may enter the circulation when cellular permeability changes or metabolic demand is altered. Because AST occurs in several tissues and cellular compartments, the observed increase cannot identify a single affected organ without complementary histological or tissue-specific measurements. The weaker ALT response also demonstrates why the enzymes should be interpreted collectively rather than as isolated diagnostic markers. Treatment-dependent changes in serum aminotransferases have likewise been reported in African catfish exposed to pesticide mixtures and glyphosate-based formulations (Alarape et al., 2024; Kanu et al., 2023).

3.3.3. Total protein and cholesterol

Total protein exhibited a non-monotonic response. The highest mean was recorded at 0.60 mg/L and differed significantly from the lower values at 0.50 and 0.70 mg/L. Acute variation in circulating protein may reflect changes in synthesis, redistribution, energy utilisation, immune-related protein production, hydration, or other compensatory processes. The lack of a progressive concentration-response pattern suggests that more than one physiological process contributed to the observed variation, and the high dispersion at 0.60 mg/L also warrants cautious interpretation. Cholesterol means were lower in exposed fish than in controls, but the differences were not statistically significant. The cholesterol values therefore represent descriptive variation and do not provide firm evidence of an exposure-related lipid response.

Considered collectively, the biochemical profile shows that the acute exposure affected selected components of serum homeostasis, particularly creatinine and AST, while ALT and cholesterol remained statistically unchanged. Short-term fish toxicology frequently produces non-linear biochemical patterns because exposure can simultaneously activate compensatory responses, alter activity and feeding, and affect the balance between tissue release, circulation, and clearance. The present biochemical findings are therefore most informative when considered together with the LC₅₀ estimate and the sustained behavioural disturbances rather than as independent evidence of specific organ damage (Bojarski et al., 2025).

3.4. Integrated significance and research implications

The acute lethality estimate, progressive behavioural abnormalities, and significant changes in creatinine and AST demonstrate that raw African oil bean seed powder is biologically active in juvenile *C. gariepinus* during short-term exposure. These findings expand the scientific understanding of *P. macrophylla* beyond its established nutritional and cultural importance by documenting an interaction with a non-target aquatic organism. They also show the value of combining mortality, behaviour, and serum biochemistry: mortality defines the acute concentration-response relationship, behaviour reveals functional impairment, and biochemical indices identify physiological disturbance among surviving fish.

Further work should characterise the soluble and particulate fractions of the seed powder, quantify major phytochemical groups, and compare raw, boiled, and fermented seed materials. Concurrent measurement of dissolved oxygen, pH, ammonia, and turbidity would help distinguish effects associated with the seed constituents from those linked to organic loading. Histopathological assessment of gill, liver, and kidney tissues, together with oxidative-stress and neurotoxicity biomarkers, would strengthen mechanistic interpretation. Longer-duration studies at lower concentrations would also help determine whether the acute

responses observed here translate into persistent effects on growth, health, or survival.

4. Conclusion

African oil bean seed powder caused acute mortality and concentration- and time-related behavioural disturbances in juvenile *C. gariepinus*. The estimated 96-hour LC₅₀ was 0.756 mg/L. Exposure also altered serum creatinine, AST, and total protein, while ALT and cholesterol did not differ significantly among treatments. The combined endpoints indicate acute physiological stress under the static laboratory conditions used. These findings provide baseline evidence that unprocessed *P. macrophylla* seed material can adversely affect a non-target freshwater fish and support careful management of seed residues and processing materials near aquatic environments. Chemical characterisation and longer-term exposure studies are required to define the active constituents and broader ecological significance of the response.

CRedit authorship contribution statement

ICA: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing. **OFO:** Software, Writing – Review & Editing. **TTO:** Conceptualization, Writing – Review & Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Akinlabu, D. K., Owoeye, T. F., Owolabi, F. E., Audu, O. Y., Ajanaku, C. O., Falope, F., & Ajani, O. O. (2019). Phytochemical and proximate analysis of African oil bean (*Pentaclethra macrophylla* Benth.) seed. *Journal of Physics: Conference Series*, 1378(3), 032057. <https://doi.org/10.1088/1742-6596/1378/3/032057>
- Akinsanya, B., Utoh, O. U., & Ukwa, U. D. (2016). Toxicological, phytochemical and anthelmintic properties of rich plant extracts on *Clarias gariepinus*. *The Journal of Basic & Applied Zoology*, 74, 75-86. <https://doi.org/10.1016/j.jobaz.2016.09.003>
- Alarape, S. A., Adeoye, D. D., Amusa, A. O., & Adeyemo, O. K. (2024). Haematological parameters and biochemical indices of African catfish (*Clarias gariepinus*) exposed to glyphosate-based herbicide (Force up®) for 96 hours. *Frontiers in Toxicology*, 6, 1448861. <https://doi.org/10.3389/ftox.2024.1448861>
- Amaze, N. H., Komolafe, B. O., Salako, A. F., Akagha, K. K., Briggs, T.-M. D., Olatinwo, O. O., & Femi, M. A. (2020). Comparative assessment of the acute toxicity, haematological and genotoxic effects of ten commonly used pesticides on the African catfish, *Clarias gariepinus* Burchell 1822. *Heliyon*, 6(8), e04768. <https://doi.org/10.1016/j.heliyon.2020.e04768>
- Bojarski, B., Witeska, M., & Kondera, E. (2025). Blood biochemical biomarkers in fish toxicology-A review. *Animals*, 15(7), 965. <https://doi.org/10.3390/ani15070965>
- Ekpenyong, J., Iheanacho, S., Asuquo, F., & Okey, I. (2022). Ichthyotoxicity of ethanolic extract of *Justicia extensa* on behavioral and histopathological changes in *Clarias gariepinus* (Burchell, 1822). *Environmental Chemistry and Ecotoxicology*, 4, 211-215. <https://doi.org/10.1016/j.enceco.2022.11.001>
- Kanu, K. C., Okoboshi, A. C., & Otitolaju, A. A. (2023). Haematological and biochemical toxicity in freshwater fish *Clarias gariepinus* and *Oreochromis niloticus* following pulse exposure to atrazine, mancozeb, chlorpyrifos, lambda-cyhalothrin, and their combination. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 270, 109643. <https://doi.org/10.1016/j.cbpc.2023.109643>
- Nwani, C. D., Okeke, O. C., Onyishi, G., Atama, C., Chinkwu, U., & Eneje, L. O. (2012). Toxicity and effects of diazinon on behaviour and some haematological parameters of African catfish *Clarias gariepinus*. *Zoology and Ecology*, 22(3-4), 246-253. <https://doi.org/10.1080/21658005.2012.733555>
- Nwokocha, B. C., Chatzifragkou, A., & Fagan, C. C. (2023). Impact of ultrasonication on African oil bean (*Pentaclethra macrophylla* Benth.) protein extraction and properties. *Foods*, 12(8), 1627. <https://doi.org/10.3390/foods12081627>
- Organisation for Economic Co-operation and Development. (2025). *Test No. 203: Fish, acute toxicity test*. OECD Publishing. <https://doi.org/10.1787/9789264069961-en>
- Ugbogu, E. A., Nwoku, C. D., Ude, V. C., & Emmanuel, O. (2020). Evaluating bioactive constituents and toxicological effects of aqueous extract of fermented *Pentaclethra macrophylla* seeds in rats. *Avicenna Journal of Phytomedicine*, 10(1), 101-113. <https://doi.org/10.22038/ajp.2019.13557>

