# Evaluation of Lignan as a Natural Hormonal Diluent for Enhancing Reproductive Performance of African Catfish, Clarias Gariepinus

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## ABSTRACT

This study evaluated the effectiveness of lignan extracted from watermelon (Citrullus lanatus) seeds as a hormonal diluent during the artificial propagation of C. gariepinus. A completely randomised design consisting of five treatments was employed: 0% (control), 25%, 50%, 75%, and 100% lignan inclusion levels, each replicated three times. Reproductive performance indices, hormone residue levels, and water quality parameters were assessed. Results showed that lignan significantly influenced reproductive performance ($P<0.05$). The highest fertilisation rate (92.78%), hatchability (66.42%), and larval survival (52.53%) were obtained at 25% lignan inclusion, compared with 78.52%, 43.57%, and 37.72%, respectively, in the control group. Fecundity and relative fecundity were highest at 75% inclusion, while complete reproductive inhibition was observed at 100% lignan concentration. Hormone residue analysis at 24 and 48 hours revealed no significant differences ($P>0.05$) among treatments, indicating that lignan did not interfere with hormone metabolism or clearance. Water quality parameters remained within acceptable ranges and were not significantly affected by lignan inclusion. Third-order polynomial regression analysis identified approximately 35% lignan inclusion as the optimum concentration for hatchability enhancement, with a strong model fit ($R^2 = 0.9596$). The study demonstrates that lignan possesses reproductive-enhancing properties and can effectively function as a hormonal diluent during artificial propagation of C. gariepinus. Therefore, lignan represents a safe, environmentally friendly, and cost-effective alternative for improving hatchery performance and promoting sustainable aquaculture production.

**Keywords:** Clarias gariepinus, Lignan, Hormonal diluent, Reproductive performance, Hatchability

## INTRODUCTION

The growing global demand for fish has intensified the need for sustainable aquaculture production systems capable of supplying high-quality animal protein (Mohd & Mushtaq, 2025). As capture fisheries continue to face pressure from overexploitation, habitat degradation, and environmental changes, aquaculture has emerged as a major contributor to food security and rural livelihoods (Saidu, 2025). The sector has experienced remarkable growth over the past decades, making reliable fish seed production a fundamental requirement for increasing aquaculture productivity and meeting market demands (FAO, 2024). In Nigeria, where fish constitutes an important component of dietary protein, the gap between fish demand and domestic production has further emphasised the importance of efficient hatchery technologies and reproductive management practices (Nwuba et al., 2022). Among freshwater aquaculture species, Clarias gariepinus is widely cultured due to its adaptability to diverse environmental conditions, rapid growth, high survival rate, and strong consumer preference (Klimuk et al., 2024). The continuous expansion of catfish farming has increased the demand for quality fingerlings, placing greater emphasis on improving breeding efficiency in hatcheries. Artificial propagation has become the primary means of ensuring year-round seed availability; however, its success depends largely on effective broodstock management, gamete quality, fertilisation efficiency, and subsequent embryo development (Cabrita et al., 2019). Challenges associated with reproductive performance often result in reduced hatchery output and increased production costs. The induction of spawning through

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hormonal intervention is a routine practice in catfish hatcheries because natural spawning rarely occurs under captive conditions. Synthetic hormonal preparations have proven effective in stimulating final gonadal maturation and ovulation, thereby facilitating controlled breeding (Zamri et al., 2022; Priyadarshi et al., 2021). Nevertheless, concerns regarding their cost, accessibility, and long-term sustainability have encouraged the search for alternative reproductive enhancers derived from natural sources. Increased attention has therefore been directed towards plant-derived bioactive compounds that possess physiological activities capable of supporting reproductive processes while reducing dependence on synthetic products (Arthur et al., 2022; Khatun et al., 2024). Natural phytochemicals have gained recognition for their diverse biological properties, including antioxidant, endocrine-modulating, and reproductive-enhancing effects. Such compounds may offer practical advantages in aquaculture by improving reproductive performance without introducing harmful residues into the culture environment (Sepehrfar et al., 2023). Their availability, affordability, and biodegradability also make them attractive options for sustainable hatchery management, particularly in developing countries where access to commercial reproductive products may be limited (Sedyaaw & Bhatkar, 2024; Boyd et al., 2020). Lignans are naturally occurring polyphenolic compounds widely distributed in seeds, grains, fruits, and vegetables. Watermelon (Citrullus lanatus) seeds contain appreciable quantities of lignans and other phytochemicals with antioxidant and phytoestrogenic properties (Oladipupo and Salami, 2020; Zamuz et al., 2021). As phytoestrogens, lignans can interact with endocrine pathways and influence reproductive processes through estrogen-like biological activities (Rotimi and Asaleye, 2023). In addition, their antioxidant properties help protect reproductive cells from oxidative stress, thereby improving gamete quality and viability (Saini et al., 2023). These characteristics suggest that lignans may function as natural hormonal diluents capable of enhancing reproductive efficiency during fish breeding operations. Although lignans have attracted attention in animal and human reproductive studies, information on their application in fish hatchery management remains limited. Their potential role in supporting hormonal activity, improving reproductive performance, and providing a natural alternative to synthetic reproductive agents has not been adequately investigated in C. gariepinus. Therefore, evaluating the effectiveness of lignan during artificial propagation is necessary to establish its suitability for aquaculture applications. This study was therefore conducted to evaluate lignan extracted from watermelon (Citrullus lanatus) seeds as a hormonal diluent during the artificial propagation of African catfish (Clarias gariepinus), and its effects on reproductive performance and hatchery success.

## MATERIALS AND METHODS

### Site of the Experiment

The experiment was conducted in the experimental section of the Teaching and Research Fish Farm, Federal University of Technology, Akure, located at Obakekere, Akure.

### Procurement and Identification of Watermelon (Citrullus Lanatus)

Fresh watermelons were purchased from Oja Oba Market in Akure, Ondo State. They were identified and authenticated at the Federal University of Technology, Akure (FUTA) Herbarium, where they were assigned the voucher number 0445.

### Extraction of Lignan from Watermelon Seed

Lignans were isolated from Citrullus lanatus seeds by combining physical processing and solvent extraction, adapting protocols described by Gupta and Kaur (2016) and Patyra et al. (2022). First, the seeds were separated by hand from the watermelon pulp, washed thoroughly to remove debris, and air-dried. The dry seeds were then pulverised in a laboratory mill to maximise surface area for extraction. To prevent lipids from interfering with lignan yield, a defatting process was conducted: the seed powder was placed in a Soxhlet apparatus and extracted with ethanol at a 10:1 (v/w) solvent-to-sample ratio for 8 hours, continuing until the siphoning liquid ran clear. The resulting defatted cake was filtered and allowed to dry completely in a fume hood. For the targeted lignan extraction, this defatted matrix was mixed with 80% ethanol at a 10:1 (v/w) ratio. The solution was continuously agitated on a mechanical shaker at room temperature for 48 hours. Afterwards, the mixture was passed through Whatman filter paper to isolate the liquid phase. This step was performed three

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times in total to ensure maximum recovery, and the resulting filtrates were pooled. The combined solution was evaporated using a water bath under gentle heating inside a fume hood. Finally, the concentrated lignan extract was transferred to amber vials and stored at roughly 4 °C in the dark. Before HPLC analysis, the sample was passed through a 0.45 µm membrane filter.

**Procurement and Selection of Broodstock**

Twenty-two healthy Clarias gariepinus broodstocks (comprising 15 females and 7 males) were sourced from a reliable fish farm in Akure. Standard physical examinations dictated selection: females exhibited swollen abdomens that readily yielded eggs under gentle pressure, while males displayed the characteristic reddish tint at the apex of the genital papilla. Following procurement, they were kept separately in 2.44 × 2.44 × 1.52 m concrete tanks for acclimation and aggression management. Over a five-day pre-experimental period, the fish received a commercial feed containing 40% crude protein to boost reproductive readiness. Feeding was suspended 24 hours before stripping to ensure empty digestive tracts. Depriving the fish of feed minimised the risk of faecal contamination during gamete collection, a necessary precaution to protect egg and milt quality for high fertilisation success and healthy larval development.

**Experimental Design**

A total of fifteen (15) trials were conducted, consisting of five treatments in triplicate. The treatments were based on varying inclusion levels of undiluted Ovaprim® and Lignan, a natural enzyme extracted from pineapple. The treatments were designated as follows:

Treatment A: 0% Ovaprim (control group)

Treatment B: 25% Lignan

Treatment C: 50% Lignan

Treatment D: 75% Lignan

Treatment E: 100% Lignan

**Hormone Injection**

To induce ovulation, female C. gariepinus broodstock were gently lifted onto a preparation slab and given an intramuscular injection of synthetic hormone (Ovaprim®; Syndel Laboratories Ltd., Nanaimo, Canada) at a dosage of 0.5 mL per kilogram. The needle was inserted above the lateral line at a 45° angle directed toward the gonads for targeted delivery of the solution. Depending on the allocated treatment group, the injection solutions incorporated different concentrations of lignan (0%, 25%, 50%, 75%, and 100%) to maintain a consistent and precise dosing regimen. Once injected, each female was moved into a separate recovery trough. Isolating the induced breeders protected them from aggressive encounters, minimising physical stress and injury that could otherwise compromise egg quality and overall spawning performance.

**Stripping and Fertilisation**

Following a 12-hour latency period, the induced female breeders were removed from their troughs and stripped into dry plastic bowls. Aliquots of one gram of eggs were partitioned into pre-labelled containers to ensure accurate sample identification. Simultaneously, milt was harvested by sacrificing and dissecting the male breeders; the testes were carefully lacerated with a clean razor blade to extrude the sperm. Fertilisation was executed using a ratio of 0.01 mL of milt per gram of eggs, in accordance with FAO (1996) guidelines. The gametes were subsequently mixed thoroughly to optimise fertilisation success across all experimental treatments.

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**Fecundity**

The fecundity of the female fish was determined for each treatment. Each fish was weighed before hormone injection, and reweighed after stripping to determine the approximate weight of the eggs. One gram of egg was counted and multiplied by the total egg mass to estimate fecundity. Both fecundity and relative fecundity were calculated following the method described by Adebayo and Fawole (2012).

$$\text{Fecundity} = \text{Weight of eggs} / \text{No. of eggs in 1g of egg mass}$$
$$\text{Relative Fecundity} = \text{Total no of egg} / \text{weight of fish} \times 100$$

**Determination of Percentage Fertility**

Fertility was evaluated after 20 minutes of incubation. Eggs that appeared translucent and showed visible embryonic eyes at the time of polar cap formation were classified as fertilised, while opaque eggs that turned whitish were recorded as unfertilized. The percentage of fertilized eggs and percentage hatchability were calculated using the procedure described by Adebayo (2006).

$$\% \text{ Fertility} = \text{Number of fertilized eggs} / \text{Total number of eggs counted} \times 100$$

**Determination of Egg Adhesiveness Percentage**

The percentage of non-adhesive (completely free) eggs in each experimental bowl was determined. The proportion of non-adhesive eggs was then calculated accordingly.

$$\text{Non} - \text{adhesive eggs } (\%) = \text{Number of non} - \text{adhesive egg} / \text{Initial number of eggs} \times 100$$

**Incubating Period**

The incubation period for each solution was determined by calculating the time difference between fertilisation and hatching.

**Hatching Rates Determination**

Incubation was monitored between 23 and 36 hours. The number of hatched larvae was counted to determine hatching rates.

$$\% \text{ Hatchability} = \text{Number of egg hatched} / \text{Total number of eggs counted} \times 100$$

**Deformity**

All deformed larvae in each treatment were counted, and their percentage was calculated.

$$\% \text{ Deformity} = \text{Number of deformed larvae} / \text{Total number of larvae} \times 100$$

**Survival**

After hatching and assessing hatching rates, unhatched eggs were siphoned out of the spawning bowls to improve larval survival. Partial water changes were carried out to enhance dissolved oxygen levels. The larvae were observed daily, and survival percentages were calculated following the method described by Adebayo (2006).

$$\% \text{ Survival} = \text{Number of hatchling} / \text{Total number of hatchling} \times 100$$

**Water Quality Parameters**

Water quality parameters, including temperature, pH, dissolved oxygen concentration, and electrical conductivity, were monitored throughout the study. Temperature was measured using a mercury-in-glass

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thermometer (YSI-DO 550, U.S.A.), pH levels were determined with a pH meter (HANNA H198106 model), and dissolved oxygen concentration was measured using a dissolved oxygen meter (JPP-607 model). Appropriate water quality measuring instruments were inserted into each treatment bowl, and readings for all parameters were recorded accordingly.

### The Determination of Hormone Residues in Clarias Gariepinus

Hormone residue analysis was conducted to evaluate the persistence of administered reproductive hormones in Clarias gariepinus following dilution with Lignan at varying concentrations. Fish specimens were sampled at 24 and 48 hours post-hormonal administration, corresponding to the hormone utilisation and metabolic clearance during artificial propagation. Blood samples were collected from the fish for detailed evaluation of hormone distribution and residual levels. Sample preparation and analytical procedures followed the protocol outlined by Monobind Inc. for the LH AccuLite CLIA Test System. Hormone quantification was carried out using a Luteinizing Hormone (LH) Test System, based on a chemiluminescence immunoenzymometric assay. The assay utilised streptavidin-coated 96-well microplates for immobilisation of the LH sandwich complex and enzyme-labelled, biotinylated monoclonal antibodies specific to LH epitopes. Calibration was achieved using six LH standards (0–200 mIU $ml^{-1}$) to generate a standard dose-response curve. Fish blood was collected into plain tubes without anticoagulants and centrifuged to separate serum from cellular components. Assays were performed at room temperature (20–27 °C). A volume of 50 µl of calibrators, controls, and serum samples was dispensed into designated wells, followed by 100 µl of LH tracer reagent. Plates were incubated for 45 minutes, washed thoroughly to remove unbound components, and treated with a luminol-based working signal reagent. Chemiluminescent signals were quantified as Relative Light Units (RLUs) using a microplate luminometer, and hormone concentrations were extrapolated from the calibration curve.

## RESULTS

### Effect of Lignan as a Hormonal Diluent on the Reproductive Performance of C. Gariepinus

Table 1 presents the effects of varying concentrations of lignan on reproductive performance and hatchery indices of Clarias gariepinus. The mean weight of female broodstock ranged from 1032.37 g in the control treatment group to 1034.61 g in the 25% lignan treatment group. The 50%, 75%, and 100% lignan treatment groups recorded 1032.58 g, 1034.54 g, and 1033.33 g, respectively. However, broodstock weight did not differ significantly ($P>0.05$) among treatment groups. The weight of eggs differed significantly ($P<0.05$), ranging from 141.23 g in the 50% lignan treatment group to 227.52 g in the 75% lignan treatment group. The 25% lignan treatment group recorded 209.24 g, while no egg production was recorded in the 100% lignan treatment group. Fecundity showed significant variation ($P<0.05$), ranging from 98,858.67 eggs in the 50% lignan treatment group to 159,266.33 eggs in the 75% lignan treatment group. The 25% lignan treatment group recorded 146,465.67 eggs, while the control group recorded 125,594.00 eggs. No fecundity was obtained in the 100% lignan treatment group due to spawning failure. Similarly, relative fecundity ranged from 9,595.47 eggs/kg in the 50% lignan treatment group to 15,306.20 eggs/kg in the 75% lignan treatment group, with the control and 25% lignan treatments recording 12,520.74 and 14,468.43 eggs/kg, respectively. The percentage fertilisation differed significantly ($P<0.05$), ranging from 78.52% in the control treatment group to 92.78% in the 25% lignan treatment group. The 50% and 75% lignan treatment groups recorded 89.16% and 88.28%, respectively. The percentage non-adhesive eggs ranged from 50.57% in the control treatment group to 80.92% in the 25% lignan treatment group, with values of 64.52% and 53.83% recorded at 50% and 75% lignan treatments, respectively. These differences were significant ($P<0.05$). The incubation period varied significantly ($P<0.05$), ranging from 1424.00 minutes in the control treatment group to 1400.20 minutes in the 75% lignan treatment group. The 25% and 50% lignan treatment groups recorded 1409.40 minutes and 1411.20 minutes, respectively, while no incubation period was recorded in the 100% lignan treatment group. The hatching time ranged from 23.33 h in the 75% lignan treatment group to 23.73 h in the control treatment group. However, the hatching commencement did not differ significantly ($P>0.05$) among treatment groups. The hatching period remained constant at 32.00 h across all treatments where hatching occurred, also showing no significant difference ($P>0.05$). Percentage hatchability differed significantly ($P<0.05$), ranging from 43.57% in the control treatment group to 66.42% in the 25% lignan treatment group. The 50% and 75% lignan treatment groups recorded 52.36% and 43.92%, respectively. Similarly, percentage survival showed significant

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variation (P<0.05), ranging from 37.72% in the control treatment group to 52.53% in the 25% lignan treatment group, while the 50% and 75% lignan treatment groups recorded 42.62% and 39.85%, respectively.

Table 1: Effect of lignan as a hormonal diluent on the reproductive performance of C. gariepinus

| Parameters | A (0%) | B (25%) | C (50%) | D (75%) | E (100%) |
|---|---|---|---|---|---|
| Weight of fish (g) | 1032.37±22.64 | 1034.61±26.94 | 1032.58±30.62 | 1034.54±33.60 | 1033.33±46.20 |
| Weight of eggs | 179.42±20.58$^{c}$ | 209.24±20.93$^{b}$ | 141.23±3.73$^{d}$ | 227.52±15.09$^{a}$ | |
| Fecundity | 125594.00±14406.11$^{c}$ | 146465.67±14650.04$^{b}$ | 98858.67±2614.71$^{d}$ | 159266.33±10565.61$^{a}$ | |
| Relative Fecundity | 12520.74±1744.88$^{c}$ | 14468.43±1473.07$^{b}$ | 9595.47±520.37$^{d}$ | 15306.20±1326.70$^{a}$ | |
| Fertilised (%) | 78.52±8.06$^{c}$ | 92.78±1.28$^{a}$ | 89.16±3.18$^{b}$ | 88.28±3.81$^{b}$ | |
| Incubation period (mins) | 1424.00±11.09$^{a}$ | 1409.40±0.69$^{ab}$ | 1411.20±1.59$^{ab}$ | 1400.20±2.12$^{b}$ | |
| Hatchability (%) | 43.57±2.23$^{c}$ | 66.42±2.34$^{a}$ | 52.36±0.79$^{b}$ | 43.92±3.07$^{c}$ | |
| Survival (%) | 37.72±5.501 | 52.53±4.68$^{a}$ | 42.62±5.20$^{b}$ | 39.85±4.44$^{c}$ | |
| Hatching time | 23.73±0.19$^{a}$ | 23.49±0.01$^{a}$ | 23.52±0.02$^{a}$ | 23.33±0.04$^{a}$ | |
| Hatching Period | 32.00±0.10$^{a}$ | 32.00±0.05$^{a}$ | 32.00±0.01$^{a}$ | 32.00±0.12$^{a}$ | |

The mean values in the same row with different superscripts are significantly different (P<0.05)

**The Determination of Hormone residues in Clarias Gariepinus when lignan was used as a Hormonal Diluent**

At 24 hours, hormone residue levels did not differ significantly (P > 0.05) among treatments, as shown in Table 3. The highest value, 1.08, was recorded in the control group, while the lowest value, 1.05, was recorded at 25% lignan, closely followed by 50% lignan, 1.05. The 75% and 100% treatments recorded 1.06 and 1.07, respectively, indicating minimal variation across concentrations. At 48 hours, residue levels also did not differ significantly (P > 0.05). The highest value, 1.06, was recorded in the control group, whereas the lowest value, 1.02, was recorded at 25% lignan. The 50%, 75%, and 100% treatments recorded 1.04, 1.04, and 1.05, respectively. A slight reduction in residue levels was recorded at 48 hours compared to 24 hours across all treatments, indicating normal metabolic clearance rather than concentration-related accumulation.

Table 2: Analysis for the determination of hormone residues in Clarias gariepinus when lignan was used as a hormonal diluent

| Lignan/Time | 24 hours | 48 hours |
|---|---|---|
| A (0%) | 1.08±0.00$^{a}$ | 1.06±0.00$^{a}$ |
| B (25%) | 1.05±0.00$^{a}$ | 1.02±0.00$^{a}$ |
| C (50%) | 1.05±0.02$^{a}$ | 1.04±0.15$^{a}$ |

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| | | |
|---|---|---|
| D (75%) | 1.06±0.02[a] | 1.04±0.01[a] |
| E (100%) | 1.07±0.00[a] | 1.05±0.01[a] |

Means in the same column with different superscripts are significantly different (P<0.05)

**Optimal Concentration of Lignan as a Hormonal Diluent**

At the end of the experimental period, the diluent that yielded the highest fertilisation, non-adhesive eggs, and the highest hatchability and survival rates was subjected to Third-order Polynomial Regression Analysis to ascertain the optimal concentration. The optimum concentration of lignan that serves as the best hormonal diluent and efficiently removes egg adhesiveness in C. gariepinus using lignan was recorded at a concentration of 35% in hatchability using 3rd order polynomial regression, as shown in Figure 1. The relationship between lignan concentration (x) and percentage hatchability (y) is described by the polynomial regression equation $y = 8E\text{-}06x^3 - 0.0158x^2 + 1.0644x + 44.765$. The coefficient of determination ($R^2 = 0.9596$) indicates that 95.96% of the variation in hatchability is explained by lignan concentration. This also reflects a strong goodness-of-fit of the model. The positive linear coefficient indicates that hatchability initially increases with lignan concentration, whereas the negative quadratic coefficient shows a decline after reaching an optimum level. The small positive cubic component further confirms the nonlinear nature of the relationship. Therefore, the model demonstrates a strong nonlinear relationship between lignan concentration and hatchability.

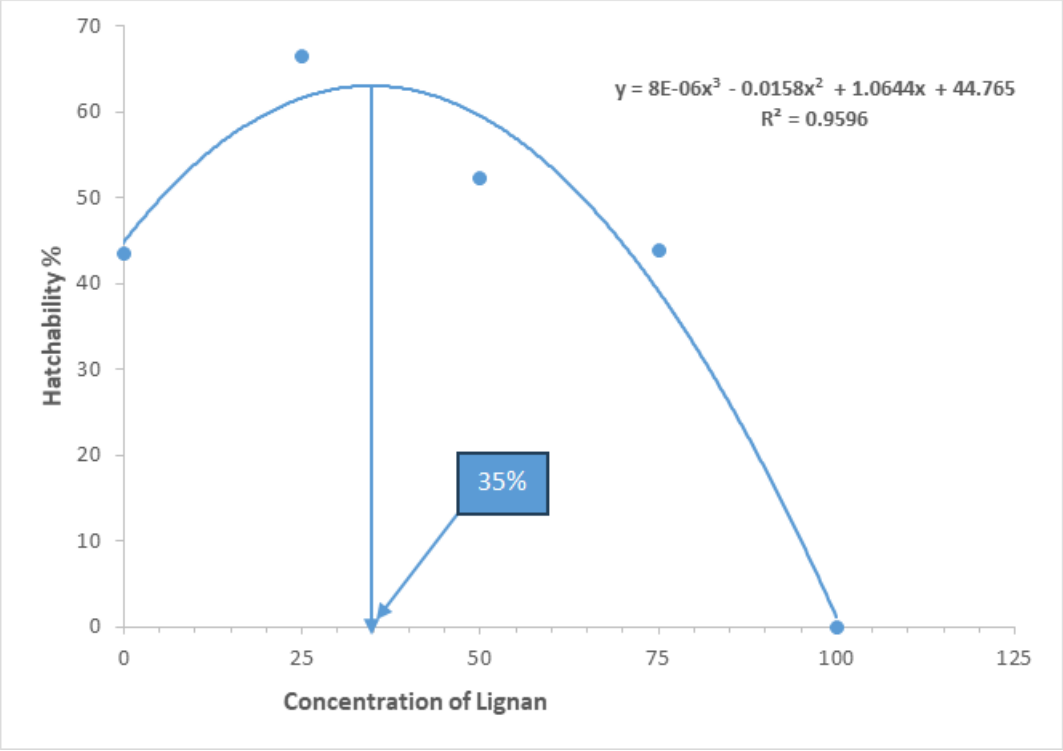


Figure 1: Third-order Polynomial Regression for optimum concentration of lignan used as a hormonal diluent and its effect on percentage hatchability during artificial propagation of C. gariepinus

**Water Quality Parameters**

As shown in Table 3, water quality parameters for lignan as a hormonal diluent revealed that the water temperature across the treatments showed no significant difference (P > 0.05), ranging from 25.29°C in the 25% lignan treatment group, which was the lowest, to 25.37°C recorded in both the control and 75% lignan treatment groups, which represented the highest values. The 50% lignan treatment recorded 25.33°C, indicating only slight variation among treatments. Similarly, the pH values remained relatively stable and did not differ significantly (P > 0.05) across the treatments. The values ranged from 6.95 in the 50% lignan treatment group, which was the lowest, to 6.98 in 75% lignan treatment group, which was the highest, while

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6.96 and 6.97 were recorded in the control group and 25% lignan treatment group, respectively. The dissolved oxygen concentration also showed no significant difference ($P > 0.05$) among treatments. The values ranged from 5.75 mg/L in the control group, representing the lowest value, to 5.87 mg/L in 25% lignan treatment group, which was the highest, while 5.77 mg/L and 5.82 mg/L were recorded at 75% and 50% lignan concentrations, respectively. These results indicate that lignan inclusion did not noticeably influence the water quality parameters during the experimental period.

Table 3: Water quality parameters for lignan as a hormonal diluent

| Parameters | Temperature | pH | Dissolved Oxygen |
|---|---|---|---|
| Control | $25.37\pm0.03^{a}$ | $6.96\pm0.01^{a}$ | $5.75\pm0.03^{a}$ |
| Lignan (25%) | $25.29\pm0.03^{a}$ | $6.97\pm0.03^{a}$ | $5.87\pm0.08^{a}$ |
| Lignan (50%) | $25.33\pm0.02^{a}$ | $6.95\pm0.01^{a}$ | $5.82\pm0.06^{a}$ |
| Lignan (75%) | $25.37\pm0.03^{a}$ | $6.98\pm0.02^{a}$ | $5.77\pm0.03^{a}$ |
| Lignan (100%) | | | |

The mean values in the same row with different superscripts are significantly different ($P<0.05$)

# DISCUSSION

### Effect of lignan as a Hormonal Diluent on the Reproductive Performance of C. Gariepinus

The results of hormonal diluent and egg de-adhesion agent during artificial propagation of Clarias gariepinus, exerted clear concentration-dependent effects on reproductive performance. The absence of significant differences in female broodstock weight confirms uniformity in broodstock selection and eliminates body mass as a confounding variable. Maternal size is a recognised determinant of fecundity and egg mass in African catfish. Arome Ataguba et al. (2013) and Ataguba et al. (2012) demonstrated a positive relationship between female body weight and egg output, while Bichi et al. (2014) reported that reproductive performance increases with broodstock condition factor. The variations observed in egg weight, fecundity, and spawning performance across lignan treatments can be attributed to treatment effects rather than broodstock heterogeneity (Esa et al., 2023). Although egg weight did not exhibit a linear pattern, the highest value recorded in 75% lignan indicates that this concentration may enhance ovarian hydration or promote more complete final oocyte maturation. Lignans are plant-derived polyphenolic compounds with weak phytoestrogenic and antioxidant properties. Shohreh et al. (2024) reported that phytoestrogens can bind to estrogen receptors in teleosts and modulate vitellogenesis, while Farooq et al. (2025) reported that moderate phytoestrogen exposure enhances oocyte maturation through estrogen-mediated pathways. The complete inhibition of spawning at 100% lignan indicates endocrine disruption at excessive concentration, likely through interference with gonadotropin signalling within the hypothalamic pituitary gonadal (HPG) axis. Fecundity exhibited a biphasic pattern, with the highest value recorded at 75% lignan, followed by reduced output at 50% and complete inhibition at 100%. Hamed et al. (2024) reported that moderate exposure to phytoestrogenic compounds can stimulate ovarian output in C. garipeinus by enhancing endocrine responsiveness during final oocyte maturation. Muhammad et al. (2023) and Adebayo et al. (2026) described such responses as hormetic, where low to moderate doses are stimulatory but higher doses produce inhibitory feedback effects. The antioxidant activity associated with lignan may have reduced ovarian oxidative stress, thereby facilitating ovulation at moderate inclusion levels, whereas excessive concentration likely disrupted endocrine balance and impaired ovulatory processes. Fertilisation improved significantly between 25% and 75% lignan, with the highest value recorded at 25%. Fertilisation in C. gariepinus depends on milt membrane integrity, ionic balance, and protection against oxidative damage. The antioxidant properties of lignan likely preserved milt membrane stability and enhanced fertilisation efficiency. These findings align with Maradun et al. (2019), who reported higher fertilisation at lower concentrations in C. anguillaris and C. gariepinus when ovulin was suspended in saline, and Otoh et al.

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(2024a), who reported a fertilisation percentage of 79.02% using varying concentrations of natural and synthetic hormones in C. gariepinus. This showed that the decline in fertilisation at higher lignan concentration suggests that excessive biochemical modification of egg membrane proteins may interfere with milt binding or micropyle access. Egg de-adhesion was most effective at 25% lignan and declined progressively at higher concentrations. Adhesiveness in C. gariepinus eggs is mediated by chorionic glycoproteins and mucopolysaccharides (Muchlisin et al., 2014). Controlled modification of these adhesive substances improves aeration and prevents egg clumping. Fawehinmi et al. (2019) opined that plant extracts reduce egg stickiness in C. gariepinus at optimal concentrations but may compromise chorion integrity when excessive. Similarly, Salisu et al. (2021) reported enhanced de-adhesion at low concentrations of watermelon juice and milk solution, while Ojebuola et al. (2025) reported maximum de-adhesion at the lowest Vernonia amygdalina extract concentration. Incubation period showed a slight reduction from 1424.00 minutes in the control to 1400.20 minutes at 75% lignan. Egwenomhe et al. (2022) reported that egg clumping restricts oxygen diffusion and prolongs embryonic development. Improved de-adhesion in this present study likely enhanced oxygen transfer, modestly accelerating development without inducing premature hatching. Hatchability and larval survival were highest at 25% lignan and declined at higher concentrations, reinforcing the existence of an optimal inclusion threshold. Improved performance at moderate levels may be attributed to enhanced fertilisation efficiency, improved oxygen diffusion, reduced egg clumping, and antioxidant protection during embryogenesis. Sepehrfar et al. (2023) reported that plant-derived bioactive compounds enhance reproductive performance in farmed fish when applied within physiological limits. Maradun et al. (2019) observed the highest hatchability and survival at moderate saline-diluted ovulin concentrations, while Ataguba et al. (2023) reported optimal hatchability and survival in C. gariepinus broodstock when diluted gonadotropin-releasing hormone analogue (GnRHa, Ovulin) was supplemented with Buserelin acetate (Suprecur), noting that higher doses reduced performance. El-Hawarry et al. (2012) similarly reported improved hatchability in Hypophthalmichthys molitrix when buserelin was combined with a dopamine antagonist, highlighting the importance of hormonal balance during induced spawning. The decline in hatchability at higher lignan concentrations may therefore reflect partial chorion destabilisation or mild endocrine interference during embryogenesis. However, the similarity in hatching end time across treatments indicates that lignan did not disrupt developmental synchrony, an important consideration in catfish hatchery management to minimise cannibalism and size heterogeneity.

**Analysis for the Determination of hormone residues in Clarias gariepinus when lignan was used as a Hormonal Diluent**

The present result shows that lignan, when used as a hormonal diluent during artificial propagation of C. gariepinus, did not significantly influence hormone residue levels in fish tissues at either 24 or 48 hours post-administration. The non-significant differences among treatments at both sampling intervals indicate that inclusion of lignan neither impaired hormone absorption nor altered metabolic clearance pathways. At 24 hours post-administration, residue values across treatments remained within a narrow range, indicating comparable systemic distribution and physiological utilisation of the administered hormones irrespective of lignan concentration. Reproductive hormones used in induced spawning are characterised by rapid circulation, receptor binding, and subsequent metabolic degradation following ovulatory stimulation. Okoye et al. (2020) reported that exogenous gonadotropins in C. gariepinus exhibit a rapid onset of action with limited tissue persistence; also, Shokr (2020) noted that induced ovulation is typically followed by prompt endocrine downregulation. The slightly lower numerical residue recorded at 50% lignan, but not statistically significant, may suggest marginally improved dispersion or receptor interaction; however, the lack of statistical separation confirms that lignan did not significantly modify early pharmacokinetic behaviour. At 48 hours, hormone residues remained statistically comparable across all treatments, confirming uniform metabolic elimination. In C. magur, exogenous gonadotropins and steroid analogues are primarily metabolised by hepatic enzymatic systems and subsequently excreted via renal and biliary routes (Laxmi, 2023). The similarity between control and lignan-treated groups indicates that lignan did not interfere with hepatic biotransformation enzymes, renal excretion, or endocrine feedback mechanisms within the hypothalamic–pituitary–gonadal (HPG) axis. Lignans are plant-derived phytoestrogenic compounds with relatively weak affinity for estrogen receptors. Li et al. (2023) reported that dietary lignans may modulate estrogen receptor activity without causing persistent endocrine accumulation, while Burgberger et al. (2025) emphasised that phytoestrogens at moderate

concentrations rarely induce systemic hormone retention in aquatic vertebrates. The present findings support these observations, demonstrating that lignan did not potentiate hormone persistence or bioaccumulation in C. gariepinus. Zamri et al. (2022) reported that effective induced breeding protocols in teleosts are characterised by transient hormone presence rather than prolonged tissue retention. It was also noted that the consistently low and stable hormone residue levels at 48 hours are a result of rapid metabolic clearance, which reduces the likelihood of hormone carry-over into edible tissues and limits ecological exposure through effluent discharge.

However, the comparable residue profiles across treatments confirm that lignan functions as a safe hormonal diluent that does not compromise hormone pharmacokinetics or promote residue accumulation in C. gariepinus. These results reinforce its suitability for hatchery application, provided that inclusion levels remain within physiologically acceptable limits.

**Optimal Concentration of Lignan as a Hormonal Diluent**

The third-order polynomial regression analysis demonstrated that lignan has strong potential as both a hormonal diluent and egg de-adhesion agent during the artificial propagation of C. gariepinus. The optimum concentration of 35% obtained for hatchability indicates that lower lignan inclusion enhanced reproductive performance more effectively than lower or higher concentrations. The high coefficient of determination values ($R^2 = 0.9602$ and 0.9596) further confirm that lignan concentration strongly influenced egg de-adhesion and hatchability. This finding agrees with Fawehinmi et al. (2019), who reported that lower concentrations of plant extracts improved egg hatching performance in C. gariepinus. The increase in performance at lower lignan concentrations may be linked to the bioactive properties of lignan, which possibly enhanced egg surface condition and fertilisation efficiency. However, the decline beyond the optimum level suggests that excessive lignan concentration may impair egg membrane integrity and embryonic development.

**Water Quality Parameters**

Water quality remains a primary determinant of fertilisation success, embryonic development, and larval survival in Clarias gariepinus, making it essential to confirm that experimental treatments do not introduce environmental bias. In this study, the use of lignan as a hormonal diluent did not significantly influence key physicochemical parameters. Water temperature ranged from 25.26 to 25.37 °C, with no significant variation among treatments. These values fall within the optimal reproductive range of 24–30 °C for African catfish, ensuring stable thermal conditions throughout the experiment (Awoke and Mkpuma, 2024). Similarly, pH values (6.93–6.98) remained consistent across treatments and within the acceptable range of 6.5–8.0 for freshwater fish reproduction, indicating that lignan inclusion did not disrupt acid–base balance (FAO, 2022; Bhatnagar and Devi, 2013). Dissolved oxygen levels (5.67–5.87 mg/L) were also stable and above the minimum threshold required for successful fertilisation and embryo development (4.5–8.0 mg/L), confirming adequate oxygen availability (Marimuthu et al., 2019). These values remain within established optimal limits for gamete activation, fertilisation, and early larval development in C. gariepinus.

## CONCLUSION AND RECOMMENDATIONS

The present study demonstrated that lignan extracted from watermelon (Citrullus lanatus) seeds has considerable potential as a hormonal diluent during the artificial propagation of Clarias gariepinus. Lignan inclusion significantly influenced reproductive performance, with moderate concentrations producing superior results compared with the control and higher inclusion levels. The 25% lignan treatment yielded the highest fertilisation rate, hatchability, and larval survival, indicating that low lignan inclusion effectively enhances reproductive efficiency and hatchery success. Although fecundity and relative fecundity were highest at 75% inclusion, reproductive performance declined at higher concentrations, while complete inhibition of spawning occurred at 100% lignan, suggesting possible endocrine disruption beyond physiological limits. The absence of significant differences in hormone residue levels among treatments confirmed that lignan did not interfere with hormone metabolism, clearance, or tissue accumulation, thereby supporting its safety for hatchery application. Furthermore, stable water quality parameters across all treatments demonstrated that lignan inclusion did not adversely affect the culture environment. Polynomial regression analysis established approximately 35% lignan inclusion as the optimum concentration for hatchability enhancement, confirming a strong

concentration-dependent response. Based on these findings, lignan is recommended as a natural and eco-friendly hormonal diluent for induced breeding of C. gariepinus, particularly at low to moderate inclusion levels. The use of approximately 25–35% lignan inclusion is suggested for optimum reproductive performance and hatchery productivity. Further studies should investigate the biochemical and endocrine mechanisms underlying lignan activity, evaluate its long-term effects on broodstock and offspring performance, and assess its applicability in other cultured fish species. Additional research on standardisation, extraction efficiency, and large-scale hatchery utilisation is also recommended to facilitate its adoption in commercial aquaculture operations.

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### Competing Interests

The authors declared that they have no competing interests

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