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The antifertility effect of *Alchornea cordifolia* root powder on the prolific breeding of red tilapia (*Oreochromis mossambicus* × *O. niloticus*) broodfish

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Abstract
Background and Objective: The use of synthetic chemicals in the production of food for human consumption has been condemned by many nations, due to their potential health and environmental hazards. The study aimed to evaluate the impact of *Alchornea cordifolia* (A.c) powder root on the reproductive of red tilapia (*Oreochromis mossambicus* × *O. niloticus*) through gonadal sterilization.
Materials and Methods: During 60 days at the Minkama fish farm, 432 females' juveniles of *Oreochromis spp*, with an average weight of 18 ± 2g, were randomly divided into four treatments, each with three replicates. The treatments included different dietary levels of A.c root powder: T_1 (0% A.c), T_2 (5% A.c), T_3 (10% A.c), and T_4 (15% A.c). A 35.35% iso protein feed was formulated using an Excel spreadsheet, and fish were housed in hapas within a 400 m² earthen pond. Key parameters such as water quality, fish growth, reproductive metrics, and histopathological changes were monitored throughout the study.
Results: Results showed that water quality, growth, reproduction, and histopathology were monitored. Water parameters remained within acceptable ranges. A.c powder did not significantly (p>0.05) affect fish weight, growth rate, survival rate, or condition factor. A strong negative correlation (R = -0.97; p≤0.05) was found between ovary and individual weight. Tilapia fed A.c had smaller and lighter eggs, lower spawning percentages, and fewer larvae. Ovarian histology showed vacuoles and pyknotic nuclei with increased A.c dosages.
Conclusion: dietary supplementation of A.c seed powder affected the growth, reproductive performance, and ovarian histology of red tilapia, likely due to the presence of alkaloids and flavonoids. Based on the findings, it is recommended that farmers considering A.c for breeding control limit its inclusion to a maximum of 15% A.c powder per kg of feed.

Keywords: Control, anti-fertility, *Alchornea cordifolia*, growth, histology, Kola acuminata, red tilapia, reproductive parameters

1. Introduction
Aquaculture has become a vital global agricultural practice, contributing significantly to food security, poverty alleviation, income generation, and sustainable resource management [1, 2]. In Africa, the fishing sector engages 16% of the workforce, with aquaculture employing 10%, highlighting its potential in achieving food self-sufficiency [3]. Among the various aquaculture practices, tilapia farming ranks as the second most consumed fish species worldwide, following carp. The Food and Agriculture Organization (FAO) reports a steady increase in global tilapia production, from 3.1 million tonnes in 2010 to 5.6 million tonnes in 2018 [4]. This growth is attributed to tilapia's favorable aquaculture traits, including ease of breeding, prolific reproduction, adaptability to diverse feeds, and commercial viability.
Despite these advantages, tilapia farming faces challenges, particularly over-reproduction, which leads to unequal growth rates between males and females. Traditional methods to control reproduction, such as manual sexing, thermal shock, genetic manipulation, and hormone-induced sex reversal, can be costly and often present environmental and health risks, especially in developing countries [5, 6, 7]. As a result, plant-based alternatives have gained attention as potential solutions. *Alchornea cordifolia*, a plant known for its anti-estrogenic

properties due to compounds like isoflavones and alkaloids, has shown promise in regulating reproduction in other species [8]. However, research on its effects in fish reproduction remains limited. A study by [9] revealed adverse effects in female rats treated with A. cordifolia extracts, indicating the need for further investigation, particularly in teleost fish.

This study aims to evaluate the effects of *Alchornea cordifolia* root powder on growth performance, reproduction parameters, and ovarian histology in female red tilapia (*Oreochromis mossambicus* × *O. niloticus*), contributing to the exploration of plant extracts as natural alternatives for regulating fish reproduction.

2. Materials and Methods

2.1 Ethical statement

The experimental protocols followed in this study adhered to internationally accepted ethical guidelines for the use and care of laboratory animals, as specified by the European Community guidelines [10].

2.2 Study locations

This study was conducted at the Hatchery Farm of Minkama, located in the Central Region, Cameroon. It is on the latitude 4°10'00" N and longitude 11°32'00" E.

2.3 Acquisition of Fish

Red Tilapia (*Oreochromis mossambicus* × *O. niloticus*) for this research work were obtained from the Hatchery Farm of Minkama, central region, Cameroon.

2.4 Plants collection and preparation

A. cordifolia roots were collected from Obala village in the Central Region, based on ethnobotanical knowledge and visual identification by a botanist. The roots were thoroughly washed and shade-dried for two weeks in a ventilated room. After drying, they were ground into a fine powder using a Lab Mill (Christy Hunt Engineering, UK, Serial number 19, 911) with a 1.0 mm screen. The resulting powder was stored in dry containers at room temperature until required for use.

2.5 Phytochemical analysis

The phytocompounds were analysed by using cold-water extractions according to standard procedures [11, 12]. Total alkaloids were determined by spectrophotometric method as described by [13] whereas total flavonoids were evaluated by methods described by [14, 15]. The content of total alkaloids, triterpenoid and flavonoids were expressed as percentage of caffeine and catechin equivalent, respectively, per gram of dry powder of *A. cordifolia* roots.

Table 1: Phytochemical constituents of dry powder of *A.cordifolia roots*

Constituents	(+) present; (++) present in large quantities, (-) absent
Alkaloids	++
Triterpenoid	+
flavonoids	++
Anthocyanins	+

2.6 Experimental diets preparation

The control diet was formulated using Pearson's square method, incorporating fishmeal and maize bran. The control and experimental diets, which included 0%, 5%, 10%, and 15% A. cordifolia (T_1, T_2, T_3, T_4), were prepared based on the procedure described by [16]. The proximate compositions of the control diet and plant additives are shown in Table 2.

Table 2: Ingredients and proximate composition of experimental diets (g/kg of feed)

Ingredients (g/kg)	Treatements			
	T_1 (0%)	T_2(5%)	T_3(10%)	T_4(15%)
Fish meal	30	30	30	30
Soybean meal	15	15	15	15
Granut meal	13	13	13	13
Corn starch	25	25	25	25
Wheat bran	8	8	8	8
Crude palm oil	1	1	1	1
CMAV	5	5	5	5
Shell meal	1	1	1	1
Vitamin Mix	2	2	2	2
Alchornea cordifolia Powder	0	5	10	15
Proximate composition				
Dry matter (%)	41.00	41.12	41.24	41.36
Crude protein (%)	35.91	35.87	35.65	35.40
Crude lipids (%)	12.28	12.20	12.15	12.10
Ash (%)	10.3	10.7	11.2	11.7
Fiber (%)	4.1	4.54	4.98	5.02

Key: T_1 (0% roots powder of *Alchornea cordifolia)*, T_2 (05% roots powder of *Alchornea cordifolia*), T_3 (10% roots powder of *Alchornea cordifolia*), T_4 (15% roots powder of *Alchornea cordifolia*).

CMAV: Formulation (g/kg): α-tocopheryl acetate, 2; inositol, 5; choline bitartrate, 136.06; niacin, 4.5; riboflavin, 1; pyridoxine·HCl, 1; thiamin·HCl 0.92; D calcium pantothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.083; menadione 1.67; D-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135; and cellulose, 834.167.

Vitamin Mix: Calcium phosphate monobasic, 135.5; calcium L-lactate hydrate, 327.0; ferric citrate, 29.7; magnesium sulfate·$7H_2O$, 132.0; potassium phosphate dibasic, 239.8; sodium phosphate monobasic·H_2O, 87.2; sodium chloride, 43.5; potassium iodide, 0.15; cuprous chloride, 0.2; manganous sulfate·H_2O, 0.8; cobalt chloride·$6H_2O$, 1.0; zinc sulfate·$7H_2O$, 3.0; and sodium selenite, 0.011

2.7 Experimental fish and their management

The experiment was conducted in a 400 m² earthen pond, with 12 fine-mesh nylon hapas (cages) measuring 2.5 × 2.5 × 1.5 meters each. After acclimatization, a total of 432 sexually immature O. niloticus juveniles (36 females and 12 males per hapa) with an average weight of 18 ± 2 g and aged 3 months were stocked in triplicate. Fish were fed manually twice daily (10:00 AM and 5:00 PM) at 3% of their body weight for 60 days. Gonadal characteristics and histology were assessed at the end of the study. Water quality parameters were maintained within optimal ranges for tilapia survival and growth, with dissolved oxygen levels between 6.0 and 7.6 mg/L, water temperature ranging from 26.7 °C to 27.2 °C, and pH between 8.0 and 8.4.

2.8 Data collection

2.8.1. Growth parameters

Fish growth was monitored weekly for 8 weeks. Survival rate (%) was calculated as:

Survival rate (%) = (final count / initial count) × 100.

Growth and feed efficiency were determined using the following formulas:

- Weight gain (WG, g) = FABW – IABW (where FABW is the final average body weight, and IABW is the initial average body weight)
- Specific Growth Rate (SGR,%/day) = 100 × (ln FABW – ln IABW) / ($T_2 – T_1$)

- Feed Conversion Ratio (FCR) = FI (g) / WG (g) (FI = total individual feed intake)
- Protein Efficiency Ratio (PER) = WG / (FI × feed crude protein)

2.8.2. Reproductive traits assessments.
Reproductive traits were assessed by monitoring spawning behavior daily over 8 weeks. Eggs were collected from the buccal cavity of brooding females, and their weight and spawning dates recorded. Seed per spawner was calculated as the ratio of total seed to the number of females that spawned. A subsample of 100 eggs was selected from each spawning cycle, weighed, and preserved for biometric assessment, including egg diameter and volume. Eggs were incubated in plastic bottles at 28 ± 0.5°C, and larvae survival rate was calculated after 20 hours.
Gonadosomatic Index (GSI) was calculated after 25 weeks, using the formula:
GSI = (gonad weight / total body weight) × 100.

2.9. Gonad Histological Examination.
At the end of the trial, ovaries from ten fish per group were collected, preserved in 10% neutral formalin, and processed for histology. After dehydration, cleaning, and embedding in paraffin, 5 µm sections were stained with hematoxylin and eosin, following [17]. Histological development and structure were analyzed as described by [18].

2.10. Statistical Analysis
Data were analyzed using one-way analysis of variance (ANOVA) at $p< 0.05$. Significant differences between means were identified using Duncan's test. Statistical analyses were performed using SPSS 21.0 software.

3. Results

3.1. Effects of *Alchornea cordifolia* root powder on some growth parameters and survival rate in *Oreochromis spp*.
The effect of *Alchornea cordifolia* root powder on some growth parameters and survival rate in *Oreochromis spp* is summarized in Table 3.

Table 3: Effects of *Alchornea cordifolia* root powder on some growth parameters and survival rate in *Oreochromis spp*

Growth parameters	Treatments				p
	T_1(0%) n=108	T_2(5%) n=108	T_3(10%) n=108	T_4(15%) n=108	
Initial weight(g)	18.39±0.47	18.47±0.12	18.39±0.21	18.39±0.17	0.91
Final weight (g)	31.91±0.85	32.83±0.62	33.17±0.74	33.97±0.92	0.49
Average daily gain (g)	13.52±0.98	14.36±0.51	14.78±0.80	15.58±0.83	0.45
Food consumption (g)	18.24±0.83	16.82±0.65	16,44±0,70	17.93±0.75	0.12
Food conversion rate	1.34±0.15	1.17±0.14	1.11±0.1	1.22±0.08	0.33
Specific growth rate (%)	0.22±0.10	0.25±0.009	0.26±0.01	0.25±0.01	0.47
Condition factor K	1.09±0.09	1.10±0.01	1.18±0.03	1.09±0.03	0.77
Survival rate (%)	91.33±3.04	93.66±1.66	95.66±3.77	95.33±1.58	0.61

Key: T_1 (0% of *Alchornea cordifolia root powder*), T_2 (5% of *Alchornea cordifolia root powder*), T_3 (10% of *Alchornea cordifolia root powder*), T_4 (15% of *Alchornea cordifolia root powder*). n: Number of fish samples

From this table, it appears that no significant difference was observed ($p>0.05$) among the various growth characteristics evaluated. However, the highest value for these characteristics overall was observed in the subjects that received the dose from batch T_3.

3.2 Effects of *Alchornea cordifolia* root powder on reproduction parameters of *Oreochromis spp*
The effect of *Alchornea cordifolia* root powder on some reproduction parameters in *Oreochromis spp* is summarized in Table 4.

Table 4: Effects of *A. cordifolia* root powder on reproductive parameters of *Oreochromis spp*

Reproductive parameters	Treatements				p
	T_1(0%) n=108	T_2(5%) n=108	T_3(10%) n=108	T_4 (15%) n=108	
Ovarian weight	0.84±0.21	0.73±0.17	0.49±0.15	0.50±0.10	0.17
Gonado-somatic index	2.69±0.87^a	2.22±0.62^a	1.47±0.47^b	1.51±0.38^b	0.05
Spawning%/treatement	74.07±10.89^a	68.23±6.89^a	48.51±2.19^b	39.89±9.89^b	0.04
Number of seed/spawner	840.07±20.7^a	780.7±30.3^a	380.32±24.7^b	364.27±35.37^b	0.04
larvae survival rate%	76.27±12.0^a	69.07±20.07^a	21.17±16.37^b	21.10±12.07^b	0.03

a, b: The means of each row marked with different letters are significantly different ($p<0.05$).
Legend: 0% *A. cordifolia* root powder, T_2 (5% *A. cordifolia*), T_3 (10% *A. cordifolia*), T_4 (15% *A. cordifolia*). n: Number of fish

From this table, it appears that *Alchornea cordifolia* root powder significantly influenced ($p<0.05$) only the GSI, the number of females that ovulated, the number of eggs obtained, and the number of larvae obtained. However, no significant difference was observed for ovary weight; the lowest value was observed in fish from the batches that received the 10% powder.

3.3. Effects of *Alchornea cordifolia* root powder on ovarian histology
The effect of *A. cordifolia* root on ovarian histology is summarized in table 5 and figure 1.

Table 5: effect of *A. cordifolia* root powder on ovarian histology of *Oreochromis Spp.*

Gonad structure	Incorporation levels (%)			
	0 (n=10)	5(n=10)	10(n=10)	15(n=10)
Normal architecture of gonadal tissues with the presence of forming follicles	10(100%)	2(20%)	1(10%)	00 (00%)
Oocyte degeneration and mild necrosis of oocyte tissues	0(0%)	8(80%)	9(100%)	10(100%)
Degeneration of oocyte tissues, decrease in oocytes and ovaries devoid of oocytes	0 (0%)	5(50%)	9(90%)	9(90%)

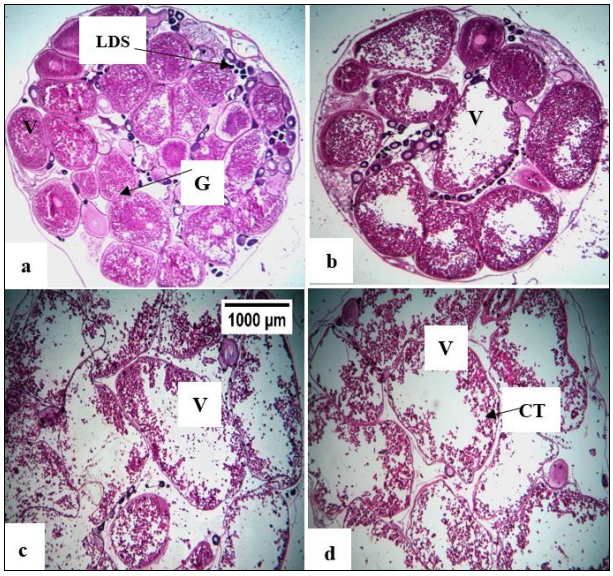

Fig 2: Representative cross - sections of ovary tissues from *Oreochromis Spp.* showing changes after feeding experimental doses of A. cordifolia: (Fig 2a) Ovary from control fish indicating ooplasm of vitellogenic follicle surrounded by a single layer of granulose cells (G), evenly distributed lipid droplets (LDs) and a few vacuoles (V); (Fig 2b) ovaries from T_2 (5% Ac) fish indicating atretic follicles with few dispersed lipid droplets (LDs) and coalescence of vacuoles (V) in the ooplasm; (Fig 2 c and d) ovaries from T_3 (10% and 15% Ac) fish, respectively showing atretic follicles with thin thecal layers and increased thickness of connective tissue fibres (CT), absence of lipid droplets (LDs) and coalescence of vacuoles (V) in the ooplasm.

The ovary of the control fish contained healthy follicles at different stages of development that is, primordial, previtellogenic and vitellogenic follicles (Figure 2a). In vitellogenic follicles, the oocyte contained ooplasmic vesicles filed with vitellogenic globules, few vacuoles and lipid droplets were observed on the peripheral regions. Single layer of granulosa cells surrounded the ooplasm, the cells contained single nucleus located centrally and thecal layer was clearly separated from theca interna and theca externa. No histological changes were observed in the ovarian follicles of Oreochromis Spp. at doses of 0.0%/kg for *A. cordifolia.* However, at doses of 5%, 10% and 15%/kg (Figure 2b, c and d), the ovaries of fish fed on the powder roots had numerous vacuoles in the ooplasm and few pyknotic nuclei were observed in granulosa cells. Thecal layers were also thin characterized with increased thickness of connective tissue fibres (Figure 2d).

4. Discussion

During the trial period, variations in the physicochemical properties of the water, including temperature, pH, and dissolved oxygen, were observed. The water temperature ranged from 26.87°C to 29.94°C, which is within the optimal range for *Oreochromis* species [19, 20]. The pH levels fluctuated between 6.11 and 7.67, remaining within the recommended range of 5 to 11 for the survival and growth of *Oreochromis* spp. [21] The optimal pH for tilapia growth is between 6 and 9, and deviations outside of this range can negatively affect physiological functions and hinder growth, potentially leading to mortality, particularly during developmental stages [22]. Dissolved oxygen levels were also within the recommended range, varying from 4.06 mg/L to 6.13 mg/L. Unfavorable environmental factors such as temperature fluctuations and low oxygen levels can adversely affect gonad development, gonadosomatic index, and overall reproductive processes.

The study findings indicated that the inclusion of *Alchornea cordifolia* powder at any dose over a 60-day period did not result in significant changes in fish weight ($p > 0.05$). This result contrasts with findings from [23], who reported weight changes in rainbow trout fed with *Tribulus terrestris* extract at 100 mg/kg for 90 days. The discrepancy may be due to differences in dosage and trial duration. Although not statistically significant, the highest average weight was recorded in batch T_3 (33.97 ± 0.92), which was higher than that of the control group (31.91 ± 0.85). The increased weight in fish fed with 10% *A. cordifolia* powder could be attributed to the presence of bioactive alkaloid compounds in the plant. Alkaloids, as [24] have reported, can influence hormone synthesis, potentially leading to sterility. Furthermore, compounds such as flavonoids, saponins, and alkaloids in *A. cordifolia* powder may disrupt reproduction and thereby promote growth. A strong negative correlation was observed between fish weight and gonadosomatic index (GSI) ($R = -0.97$; $p < 0.05$), suggesting that as ovarian weight decreased, the fish's body weight increased. This shift in energy metabolism likely reflects the redirection of resources from reproductive processes to growth. This phenomenon explains the significant weight gain observed in batch T_3 (15.58 ± 0.83) compared to the control group (T_1: 13.0 ± 0.98). Additionally, the specific growth rate was higher in batch T_3 (0.25 ± 0.01) compared to T_1 (0.22 ± 0.1), likely due to reproductive inhibition by bioactive compounds, a finding consistent with [23], who observed increased growth rates in rainbow trout exposed to *Tribulus terrestris* extract.

The survival rate did not show significant differences ($p > 0.05$) between treatment groups, suggesting that *A. cordifolia* extract did not exhibit toxicity to the treated fish. While not statistically significant, the condition factor (K), a measure of fish health, was highest in the 10% *A. cordifolia* group, which may indicate improved overall health. These enhanced growth parameters could be attributed to a redirection of metabolic energy, possibly due to the inhibition of reproductive processes. Similar effects have been reported in previous studies, such as the increased liver protein synthesis observed in Atlantic salmon (Salmo salar) and rainbow trout during the vitellogenic phase [25, 26, 27, 28]. This redirection of amino acids for hepatic protein synthesis likely results from the breakdown of proteins in the viscera and muscle tissues for reproductive purposes [29, 26, 30].

In addition, *A. cordifolia* root powder significantly reduced ($p \leq 0.05$) the number of ovulated females, the number of eggs obtained, and the number of larvae, as well as the morphometric characteristics of the eggs. While no significant differences were observed in the gonadosomatic index or ovary weight, the lowest values were found in the 15% powder group. These findings are consistent with those of [31, 32, 33], who observed similar reductions in reproductive parameters following the administration of *Hibiscus rosa-sinensis* leaf powder, *A. indica* leaf extract, and bitter kola seeds, respectively. The observed reductions in reproductive traits may be due to the presence of bioactive compounds like flavonoids and alkaloids in *A. cordifolia* powder, which could inhibit aromatase, an enzyme involved in the conversion of androgens to estrogens, leading to reproductive failure [34].

Histological examination of ovaries from females fed 10% and 15% *A. cordifolia* powder revealed numerous vacuoles in the ooplasm of vitellogenic follicles, a few pyknotic nuclei in granulosa cells, and thin thecal layers with increased

connective tissue fiber thickness. Similar histological changes were reported by [35, 31] in *Oreochromis niloticus* and *Tilapia zillii* fed with diets supplemented with *A. indica* powder. [9] also reported ovarian alterations and abortion in female rats treated with aqueous *A. cordifolia* extract at a dose of 400 mg/kg. This study suggests that *A. cordifolia* root powder can reduce gonadal indices and alter gonadal histology in *Oreochromis* species. Such changes may be due to the high content of phytoestrogens in *A. cordifolia*, which can bind to estrogen receptors and steroid-binding proteins, mimicking endogenous hormones and disrupting the normal functioning of female gonads. This disruption may lead to the cessation of vitellogenin accumulation and subsequent oocyte atresia [36]. Phytoestrogens have also been shown to interfere with steroid metabolism and estrogen receptor activity, contributing to endocrine disruption [37].

5. Conclusion

The present study highlights *Alchornea cordifolia* root powder as an effective, cost-efficient, and accessible natural alternative for regulating reproduction in Nile tilapia (*Oreochromis niloticus*) and mitigating early maturation issues without the need for expensive chemical hormones. The results demonstrate that a diet incorporating 15%/kg of *A. cordifolia* powder significantly influences reproductive parameters and ovarian development. Based on these findings, *A. cordifolia* root powder can be considered a promising feed additive for managing reproductive processes in red tilapia, offering a sustainable approach to tilapia breeding management.

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