**Control of Reproduction in *Oreochromis niloticus* (Linnaeus 1758) Using *kola acuminata* seeds powder as Reproduction Inhibitor**

 **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 present study aimed to evaluate the impact of *Kola acuminata* (K.a) seed powder on the reproductive performance of Nile tilapia (*Oreochromis niloticus*) through gonadal sterilization. **Materials and Methods:** Over 60-days at Ongot Fish Farm, 240 juveniles (average weight 16±5.5g) were randomly assigned to four experimental treatments, each with three replicates. The treatments included different dietary levels of K.a seed powder: T1 (0% K.a), T2 (10% K.a), T3 (15% K.a), and T4 (20% K.a). A 35.5% iso-protein feed was formulated using an Excel spreadsheet, and the fish were housed in hapas within a 302 m² 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 remained within acceptable ranges throughout the study. The administration of K.a seed powder did not significantly (p > 0.05) affect the fish's weight, weight gain, specific growth rate, survival rate, or condition factor. A negative correlation was found between ovary weight and individual fish weight (R = -0.87; p ≤ 0.05). Fish fed K.a diets exhibited significant reductions in egg diameter, wet weight, and volume (p < 0.05). Moreover, the spawning percentage was lower in fish fed K.a, and histological changes in the ovaries, such as vacuoles in the ooplasm and pyknotic nuclei in granulosa cells, were observed (p < 0.05).

**Conclusion:** Dietary supplementation of K.a seed powder affected the growth, reproductive performance, and ovarian histology of Nile tilapia, likely due to the presence of alkaloids and flavonoids. Based on the findings, it is recommended that farmers considering K.a for breeding control limit its inclusion to a maximum of 20% K.a powder per kg of feed.

**Key words**: Control, growth, histology, Kola acuminata, Oreochromis niloticus, reproductive parameters.

**INTRODUCTION**

The production of tilapia is linked to suitable aquaculture characteristics such as ease of captive breeding, a short production cycle with rapid growth rates, acceptance of artificial feeds posts yolk sac absorption, and marketability among diverse populations due to being a widely consumed fish without taboos, unlike the catfish *Clarias gariepinus*[1, 2]. Male-only tilapia farming is preferred due to the faster weight gain of males compared to females[3, 4]. In *Oreochromis* species where mouthbrooding is strictly female[5], exclusive male populations benefit from improved growth and prevent spontaneous reproduction in sexually mature individuals6. Uncontrolled breeding in closed environments leads to overpopulation and food competition among juveniles, reducing overall population growth[6, 7]. Various solutions have been proposed to address this uncontrolled breeding in females, including manual sexing, environmental manipulation (thermal treatment), genetic/chromosomal manipulation, and hormonal sex reversal using androgens[6,8,9,10]. Exogenous steroid-induced hormonal sex reversal, particularly with 17α-methyltestosterone (MTT), has shown high success in tilapia feminization[11,12,13]. However, due to cost constraints, carcinogenicity, and harmful effects on human health and aquatic ecosystems, concerns have been raised, leading to its prohibition by the WHO for consumer health protection [14,15,16,17,18,19]. To meet increasing demand sustainably, research has focused on plant extracts with androgenic and anti-estrogenic properties as more cost-effective and environmentally friendly alternatives to control tilapia reproduction[20,21,22,23,24]. Lion kola, containing anti-androgenic compounds like caffeine, alkaloids (kolatine, kolanine), inhibiting androgens[25], is one such plant under investigation. Aprioku and *al.*[26] observed histological changes in the testes of rat treated with kola.a. This study aims to contribute to understanding the anti-fertility effects of plant extracts on fish reproduction, specifically evaluating the effects of lion kola powder (kola acuminata) on growth performance, survival rate, reproductive outcomes, and ovarian histology in juvenile tilapia (*Oreochromis niloticus*).

**2. MATERIALS AND METHODS**

**2.1 Study area**

 The study was carried out during the rainy season from 01 March to 01 May 2024 at the Hatchery Farm of Ongot, central region, Cameroon. It is on the latitude 4°10'00’’ N and longitude 11°32'00’’E.

**2.2 Acquisition of Fish**

*Oreochromis niloticus*for this research work were obtained from the Hatchery Farm of Ongot, central region, Cameroon.

**2.3 Plants collection and preparation**

Based on ethno‐botanical knowledge using available literature and visual observations, the plants were identified by a botanist. The seeds of *kola acuminata* were collected from Obala village, the central region. The seeds were thoroughly washed and then shade dried for two weeks in a ventilated room. Dry seeds were ground into fine powders by using a Lab Mill (Serial number 19,911, Christy Hunt Engineering, LTD, UK) fitted with a 1.0 mm screen and finally the powders were kept in dry containers and stored at room temperature until needed for use.

**2.4 Phytochemical analysis**

The phyto-compounds (**Table 1**) were analysed by using hot ethanol cold water extractions according to standard procedures[27,28]. Total alkaloids were determined by spectrophotometric method as described by John et *al.*[29] whereas total flavonoids were evaluated by methods described by Jing et *al.*[30]. The content of total alkaloids and flavonoids was expressed as percentage of caffeine and catechin equivalent, respectively, per gram of dry powder of K.a seeds.

**Table 1:** Phytochemical constituents of dry powder of K.a seeds.

|  |  |
| --- | --- |
| Constituents  |  (+) present; (-) absent |
| Alkaloids Tanin catechinFlavonoid  | **+****+****+****+** |

**2.5 Experimental diets preparation**

 The control diet was formulated according to Pearson's square by including fishmeal and maize bran. The proximate compositions of the control diet and plants used in the present study are given in **Table 2**. The four experimental diets were formulated by adding 0%, 10%, 15% and 20% of A. c (T1, T2, T3 and T4) to a kilogram of the control diet as we described previously [31].

**Table 2:** Ingredient composition (g/kg) of diets (35.5% CP) used in Experiment.

|  |  |
| --- | --- |
| **Ingredients (g/kg)** | **Treatements** |
| T1 (0%) | T2(10%) | T3(15%) | T4(20%) |
| 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 |
| *Kola acuminata Powder seeds* | 0  |  0.1 | 0.15 | 0.20 |
| **Proximate composition** |
| Dry matter (%) | 41.00 | 41.22 | 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 :** T1 (0% *Kola acuminata seed powder)* T2 (10% powder of *K. acuminata*), T3 (15% powder of *K. acuminata*), T4 (20% *K. acuminata seed*). **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·7H2O, 132.0; potassium phosphate dibasic, 239.8; sodium phosphate monobasic·H2O, 87.2; sodium chloride, 43.5; potassium iodide, 0.15; cuprous chloride, 0.2; manganous sulfate·H2O, 0.8; cobalt chloride·6H2O, 1.0; zinc sulfate·7H2O, 3.0; and sodium selenite, 0.011.

**2.6. Experimental fish and their management**

 The study was conducted for two months in the earthen ponds at the 302 m2. The experiments were conducted using tweleve (2.5x2.5x1.5m) fine mesh nylon hapas (cages) installed inside the pond. After acclimatization, two hundred and forty (240) sexually immature *O. niloticus* juveniles (20 females and 5 males per hapa, with the males having been introduced later for reproduction) with mean weight of 16±5.5 g and an age of three months were stocked in triplicates into 302 m2 pond. Experimental fish were hand‐fed twice daily at 10:00 am and 5:00 pm at 3% of their body weight per day for 60 days. At the end of the experiment, gonadal characteristics and histology were examined. The water quality parameters in the experimental pond were maintained at optimum recommended ranges for survival and growth of *O. niloticus*. Dissolved oxygen ranged from 6.0 to 7.6 mg/L, temperature from 26.7°C to 27.2°C and pH from 8.0 to 8.4.

**2.7. Data collection**

**2.7.1. Growth parameters**

Growth performance parameters: weight gain (WG), average daily weight gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), Specific Growth rate (SGR) and survival rate (SR), were calculated monthly using body weight of all fish from each cage. Parameters were calculated using following formulae by Sutthi et *al*.[32] and Ngoumtsop et *al*.[33]:

WG (g/fish) =final weight (g) - initial weight (g)

ADG (g/fish/day) = [final weight (g) - initial weight (g)] / Days

SGR = [{ln final weight (g) – ln initial weight (g)}/ experimental days] ×100

FCR =total feed fed (g) / weight gain (g)

SR (%) = [number of survived fish / initial number of fish] ×100

Specific Growth Rate (SGR, %/day) = 100\*(ln FABW–ln IABW)/ (T2 – T1).

**2.7.2. Reproductive performance assessments.**

Each week during 8 weeks, the reproductive traits of each female tilapia fed a different diet were observed. Every female brood fish was monitored daily for spawning activities. The produced eggs were carefully retrieved from the buccal cavity of fish and counted. Before returning the brooder fish to their separate hapa, the fish’s live body weight and spawning date were recorded. The number of seeds per spawner is the ratio of the total seed produced/Number of females spawned. During the first through fourth spawning periods, asub-sample of 100 eggs were randomly selected weighed, and preserved in 10% formalin for further egg biometric measurements. Tilapia eggs are oval, hence the long- and short-axis lengths were estimated under a calibrated microscope to the closest 0.001 mm to estimate the accurate egg diameter, mean egg wet weight, and mean egg volume. During spawning, the collected eggs were counted and hatched in specialized plastic bottles at a controlled water temperature (28 ± 0.5°C) in an indoor hatching system. To simulate the incubation process in the female’s fish mouth, a constant water flow was supplied through the plastic bottles to keep the eggs gently floating in water. After 20 hours, samples were removed from each incubator and larvae survival rate is calculated as the ratio of number of larvae to the total number of eggs hatch as percentage. All these reproductive indices were determined using the protocol described by Mohammed et *al*.[34] and Ngoumtsop et al.[33]. After 25 weeks, the gonad was gently dissected and weighed to calculate the gonado-somatic index (GSI) Sutthi et *al*.[32]. These body morphometric indices were determined as a percentage of organ weight to total fish body weight.

**2.8. Gonad Histological Examination**.

At the end of the trial, gonad samples (ten fish per group) were obtained from experimental brooder fish. The ovaries were preserved in 10% neutral formalin solution, dehydrated in alcohol, cleaned in xylene, embedded in paraffin, and then dissected into 5 µm sections. The serial sections had been stained with hematoxylin and eosin as indicated by Naiel *et al*.[35] procedures. According to Tope-Jegede *et al*.[36] investigations, the gonad histological development and structure were identified.

**2.9.** **Statistical analysis**

Data were submitted to one-way analysis of variance at p < 0.05. When differences were significant between means, the latter were separated using the Duncan test. The analyses were performed using SPSS 21.0.

**3. RESULTS**

**3.1 Effect of incorporating *kola acuminata seeds* powder on some growth parameters of *Oreochromis niloticus* juveniles.**

The growth characteristics of *Oreochromis niloticus* juveniles based on the treatments are presented in the **Table 3**.

The Table 3 summarizes the growth performance parameters of the experimental groups (T1–T4) subjected to different levels of *Kola acuminata* seed powder supplementation. Each treatment included 60 individuals. Initial and final weights showed no significant differences among treatments (*p* > 0.05). Average daily gain and specific growth rate increased with higher *Kola acuminata* inclusion, peaking in T4 (20%). Food consumption varied across treatments, with the lowest intake recorded in T4. The condition factor remained relatively stable, indicating no adverse effects on body condition. Survival rates were high across all groups, with a slight increase observed in T4 (98.33%).

The table shows that no significant difference was observed (p>0.05) among the different growth characteristics evaluated.

**Table 3**:Growth characteristics of *O. niloticus* juveniles based on *kola acuminata*treatments.

|  |  |  |
| --- | --- | --- |
| **Growth parameters** | **Treatements** |  |
| **T1 (0%)** **n=60** | **T2 (10%)** **n=60** | **T3 (15%)** **n=60** | **T4 (20%)** **n=60** |  ***p*** |
| Initial weight(g) | 17.81 ± 3.11 | 17.59 ± 2.99 | 16.68 ± 2.75 | 17.30 ± 3.57  | 0.42 |
| Final weight (g) | 30.74 ± 6.38 | 30.11 ± 6.59 | 33.26 ± 5.31 | 34.22 ± 7.08 | 0.58 |
| Average daily gain (g) | 12.93 ± 6.77 | 12.52 ± 7.48 | 16.37 ± 6.09 | 16.93 ± 8.15 | 0.1 |
| Food consumption (g) | 20.42±2.75 | 14.64 ± 3.09 | 17.51 ± 0.19 | 14.17 ± 2.19 | 0.35 |
| Food intake | 1.58 ± 0.36 | 1.17 ± 0.29 | 1.07 ± 0.56 | 0.83 ± 0.23  | 0.8 |
| Specific growth rate (%) | 0.89 ± 0.43 | 0.87 ± 0.45 | 1.03 ± 0.49 | 1.12 ± 0.37 | 0.13 |
| Condition factor K | 0.74 ± 0.03 | 0.76 ± 0.13 | 0.78 ± 0.05 | 0.8 ± 0.02 | 0.21 |
| Survival rate (%) | 95.30±2.90 | 95.20±50.00 | 96.67±2.88 | 98.33±2.88 | 0.41 |

T1 (0% of *kola acuminata* seed *powder*), T2 (10% of *kola acuminata* seed *powder*),), T3 (15% of *kola acuminata* seed *powder*),), T4 (20% of *kola acuminata* seed *powder*),). n : Number of fish samples

**3.2 Effects of *kola acuminata* seeds powder on some reproductive parameters in Oreochromis niloticus.**

The effect of *kola acuminata* seeds powder on some reproductive parameters and survival rate in *Oreochromis niloticus* is summarized in **Table 4**.

It can be deduced from this table that the number of females that ovulated, the number of eggs obtained, the number of larvae obtained, and the gonadosomatic index were significantly influenced (p<0.05) by *kola acuminata* seeds powder at doses of 15% and 20%. No significant difference was observed for ovary weight.

The Table 4 presents reproductive parameters across treatments (T1–T4) with varying levels of *Kola acuminata* seed powder supplementation. Ovarian weight remained similar among treatments (*p* > 0.05). The gonado-somatic index (GSI) was significantly higher in T1 and T2 compared to T3 and T4 (*p* = 0.05). Spawning percentage and the number of seeds per spawner also decreased significantly in T3 and T4 compared to T1 and T2 (*p* = 0.04–0.05). Larvae survival rate followed a similar trend, with significantly lower survival in T3 and T4 (*p* = 0.03), indicating that higher levels of *Kola acuminata* supplementation may negatively affect reproductive performance.

**Table 4:** Effects of *k. acuminata* powder on some reproductive parameters and survival rate of *O. niloticus*

|  |  |  |
| --- | --- | --- |
| **Reproduction****parameters** | **Treatements** |  |
| **T1 (0) n=60** | **T2 (10%) n=60** | **T3 (15%) n=60** | **T4 (20%) n=60** | ***P*** |
| Ovarian weight | 0.5±0.18 | 0.53±0.11 | 0.42±0.17 | 0.41±0.28 | 0.14 |
| Gonado-somatic index | 1.62 ± 0.13a | 1.76± 0.20a | 1.22±0.23b |  1.23±0.29b  | 0.05 |
| Spawning % / treatement  | 84.52±13.89a | 78.23±7.82a | 52.42±2.39b | 49.29±6.89b | 0.04 |
| Number of seed / spawner | 881.51±20.39a | 858.51±33.23a | 99.51±20.39b | 80.51±25.19b | 0.05 |
| larvae survival rate % | 83.51±20.19a | 64.51±18.95a | 25.51±17.19b | 23.51±7.9b | 0.03 |

a, b: The means of each row marked with different letters are significantly different (p<0.05).

Key : T1 (0% of *kola acuminata* *seed powder*), T2 (10% of *kola acuminata* *seed powder*),), T3 (15% of *kola acuminata* *seed powder*),), T4 (20% of *kola acuminata seed powder*),). n: Number of fish samples

**3.3. Effects of *kola. acuminata*** **seeds powder on ovarian histology**

The effect of k. acuminataon ovarian histology is summarized in **Table 5** and **Figure 1**.

The **Table 5** presents the histological descriptions and egg traits of the experimental groups (T1–T4) under different levels of *Kola acuminata* seed powder supplementation. T1 exhibited normal ovarian histology with minimal atretic follicles (Figure 1-A), while T2 showed a higher presence of atretic oocytes (Figure 1-B). In T3, increased atretic oocytes and signs of hydropic degeneration were observed (Figure 1-C), whereas T4 displayed severe histological alterations, including ruptured oocytes and necrosis (Figure 1-D). Egg traits, including diameter, length, volume, and wet weight, significantly decreased in T3 and T4 compared to T1 and T2, indicating a negative impact of higher *Kola acuminata* levels on egg development.

The common changes observed in the female gonads were (i) oocyte atresia, (ii) depletion of yolk particles and (iii) unrounded and distorted vitellogenic (**Figure 1: B, C** and **D**). Pronounced severity on integrity of the gonad was observed in T3 (15%) and T4 (20%). However, few atretic oocytes were observed in fish fed T2 (10%) and classified as moderate (**Table 5**).

**Table 5.** Histological description and Egg traits of female *O. niloticus* fed *K. acuminata* diets

|  |  |  |
| --- | --- | --- |
| Treatments   | Histological description |  Eggs traits (n=100) |
| Diameter (mm) | Lenght (mm) | Volume (mm3) | Wet weight (mg) |
| T1 | Normal histology and less visible atretic follicles | 2.9±0.62a | 3.0±0.5a | 7.2±0.9a | 7.5 ±0.51a |
| T2 | Normal histology and more visible atretic oocyte | 2.7±0.31b | 2.8±0.1b | 6.5±0.38b | 6.1±0.5b |
| T3 | Increased atretic oocyte and hydropic degeneration | 2.5±0.6b | 2.1±0.2c | 6.3±0.51b | 5.9±0.4b |
| T4 | Increased atretic oocyte, ruptured oocyte and necrosis | 2.12±0.4c | 1.9±0.11c | 5.1 ±0.3c | 4.2 ±0.29c |

 (a, b and c): The means of each row marked with different letters are significantly different (p<0.05).

Key : T1 (0% of *kola acuminata*  *seed powder*), T2 (10% of *kola acuminata*  *seed powder*),), T3 (15% of *kola acuminata*  *seed powder*),), T4 (20% of *kola acuminata*  *seed powder*),). n : Number of fish samples



**Oa**

**Yp**

**T2=B**

**T1=A**



**udv**

**Dyp**

**T4=D**

**T3=C**

**Figure 1**: Influence of *k. acuminata* included in a commercial tilapia diet (basal diet, BD) at T1=0%; T2=10%; T3=15% and T4=20% / kg basal diet respectively, on the gonadal integrity of sexually mature *O. niloticus* (27 - 41g) during a 60 days treatment period.

**Figure 1-A**: Normal ovarian, showing normal histology with minimal atretic follicles; Figure 1-B: showing higher presence of atretic oocytes; **Figure 1-C**: showing higher atretic oocytes and signs of hydropic degeneration and **Figure 1-D**: showing severe histological alterations, including ruptured oocytes and necrosis.

**Key:** DYP = depleting yolk particles; YP = yolk particles; udv = unrounded and distorted vitellogenic stage. Oocyte atresia. Bar = 1000µm.

**4. DISCUSSION**

The averages of temperatures obtained during this experiment remained within the temperature range (13.5°C - 33°C) tolerated by Nile tilapia[37]. Similarly, the pH values ranging between 5.44 and 6.81 obtained also fall within the recommended limit range (5 to 11) for the survival and growth of Nile tilapia[38]. Indeed, for optimal growth, tilapia needs to live in an environment where the pH value is between 7 and 9. In aquaculture, the physicochemical parameters of water influence the physiology of the animal, its behaviour, and its survival because beyond the norms, they become a stress factor and can lead to the death of the subjects [38].

From this study, it is evident that the incorporation of kola acuminata powder at any dose over 60 days did not significantly influence (p>0.05) the fish survival rate. This result contradicts the findings of Ndakalimwe *et al*.[39] in Nile tilapia fry over a 30-day rearing period fed with a diet containing 4% Aloe vera gel. This difference could be attributed to age variation and the physicochemical characteristics of the environment. The non-significant difference observed in this study might suggest that kola acuminata powder does not pose any toxicity to the treated subjects. This is further supported by the fact that the highest condition factor K value, an indicator of the fish's good condition and growth [40], was observed in the group of fish that received 15% *kola acuminata* powder.

The values of growth characteristics (Weight Gain, Total Mean Gain, Specific Growth Rate, and Feed Conversion Ratio) recorded during the experiment did not vary significantly (p>0.05) among the treatments. These results contrast with those of Mahmud *et al*.[41] and Yilmaz *et al*.[42] in Nile tilapia (*Oreochromis niloticus*) fed diets containing 0.5% turmeric and 2% Aloe vera gel, respectively. This difference could be attributed to the type of infrastructure, developmental stage, species used, and the quality of the feed. However, even though not significant, the highest values were recorded in the group fed with 20% *kola acuminata* (K.a). The improvement in the growth parameters recorded in this study may be justified partly by the presence of bioactive alkaloid compounds in kola acuminata powder. Odebode[43] reported that the presence of bioactive compounds such as flavonoids, saponins, and alkaloids could inhibit the reproductive process. The inhibition of the reproductive function might explain the increase in the weight of fish receiving kola acuminata powder. Indeed, a strong, negative, and significant correlation was observed between fish weight and the weight of the somatic gonad index (R = -0.87; p≤0.05). This means that when the ovary weight decreases, the fish body weight increases. The specific growth rate was also higher in the subjects of treatment T4 (1.12 ± 0.37) compared to the control group T1 (0.89 ± 0.43). These results are similar to those observed by Yilmaz *et al*.[44], who also observed an increase in specific growth rate in rainbow trout (*Oncorhynchus mykiss*) fed with 100 mg/kg of aqueous extract of *Tribulus terrestris* for 90 days. Same results were observed by Ngoumtsop et *al.* [33] in red tilapia fed with 15% of *Alchornea cordifolia* root powder for 60 days. During the vitellogenesis phase in Atlantic salmon (*Salmo salar*) and rainbow trout, Olin and von der Decken[45]; Olin *et al*.[46]; and Fauconneau *et al*.[47] observed a strong stimulation of protein synthesis in the liver. The amino acids required for hepatic protein synthesis originated from the breakdown of proteins in the viscera and especially the muscle tissues[48,49]. Therefore, in the absence of the vitellogenesis process, the metabolic energy used for reproduction would be redirected towards growth, hence the improvement in the characteristics observed in the fish in the group (T4) fed a diet containing 20% *kola acuminata* seeds powder.

Furthermore, this study also revealed that *kola acuminata* seeds powder significantly reduced (p≤0.05) the number of females that ovulated, the number of eggs obtained, and the number of larvae obtained. Although no significant difference was observed for ovary weight and the Gonado-Somatic Index, the lowest values were observed in the groups of fish receiving the powder at 20%. Similar results were observed by Obaroh and Achionye-Nzeh[50] in Nile tilapia fed a ration containing 1 to 8 g kg-1 of ethanolic extract of *A. indica* leaves for 35 days. The decrease in the value of these characteristics could be explained by the presence of bioactive flavonoid compounds in kola acuminata powder. These flavonoids may have inhibited the activity of the aromatase enzyme[51], thereby increasing testosterone production, which could lead to fish sterilization through masculinization[52].

In the present study, the consummation of K. *acuminata* for 60 consecutive days resulted in a noticeable increase atretic oocytes and severe histological alterations, including rupture oocytes and necrosis. Sections of ovaries in *O. niloticus* fed with the basal diet (0% of K.a) showed normal ovarian histology with minimal atretic follicles. Similar histological effect was reported on ovaries of *O. niloticus* fed cottonseed meal-based diets by Tope-Jegede *et al.*[53]; Aloe Vera latex Jegede[54] and *Alchornea cordifolia* Ngoumtsop *et* *al*. [33]. We also observe significantly decreased the value of egg traits as (diameter, length, volume, and wet weight) in the group T3 and T4 compared to T1 and T2. Similarly reported by Jegede and Fagbenro[55] and is also attributable to the poor development of ovarian tissues as suggested by Cumaranatunga and Thabrew[56]. The decreases value of the egg traits and histological alterations observe in this study indicate a negative impact of higher *K acuminata* levels on the gonad and egg development due to the presence of bioactive Phytoestrogen compounds present in k *acuminat*a powder. Latonnella *et al*.[57] and Moutsautsou[58] suggest that phytoestrogen effects are mediated through the estrogen receptor (ER) subtypes alpha ERα and beta ERβ, which have been demonstrated to be cell type/tissue specific and dose-dependent. Thus, phytoestrogens can bind to steroid-binding proteins and to estrogen receptors (ER) of target cells, mimicking the effects of endogenous hormones thereby blocking their effect in a female gonad stopping vitellogenin accumulation in eggs Moutsautsou[58]. Maclatchy and Van Der Kraak [59] also indicated that phytoestrogen manifest endocrine-disturbing activity by interfering with enzymatic reactions either on steroid metabolism (aromatization) or on the mechanism of action of estrogens (tyrosine kinase activity).

**CONCLUSION**

The reproductive parameters and ovaries development significantly decreased in *Oreochromis niloticus* fed with basal diet supplemented with *Kola acuminata* seed powder*,* revealing that *Kola acuminata* seed powder may be effective as sterility-inducing agents. At 20% / kgK.a diet elicited the best response on gonad development and reproduction parameters. These investigations showed that K.a seed powder possess promising anti-fertility property which can be exploited in fish to control over-reproduction of *Oreochromis niloticus* in ponds.

**REFERENCES**

1. El-Sayed, Abdel-Fattah M. (2006). Tilapia culture in Africa: An overview. Aquaculture, 259(1-4), 1-10. DOI: 10.1016/j.aquaculture.2006.05.020
2. Mair, Grant C. (2001). Tilapia aquaculture in the 21st century: The challenges ahead. Aquaculture, 200(1-2), 85-99. DOI: 10.1016/S0044-8486(01)00652-1
3. El-Greisy, Zakaria A., & El-Gamal, Ahmed A. (2012). Effects of sex ratio on tilapia growth performance. Aquaculture Research, 43(3), 391-395. DOI: 10.1111/j.1365-2109.2011.02804.x
4. Megbowon, David A., & Mojekwu, O. J. (2014). Influence of sex ratio on growth performance in Nile tilapia. Aquaculture Research, 45(4), 789-796. DOI: 10.1111/are.12022
5. Trewavas, Ethelwynn. (1983). Tilapia and related species: A comparative biology and aquaculture perspective. Cambridge University Press. ISBN: 9780521237569 (Book, not a journal article)
6. Beardmore, John A., Mair, Grant C., & Lewis, Robert I. (2001). Recent advances in genetic sex control in aquaculture. Aquaculture Research, 32(4), 595-603. DOI: 10.1046/j.1365-2109.2001.00550.x
7. Baroiller, Jean-François, D'Cotta, Hélène, & Saillant, Emmanuel. (2009). Environmental effects on fish sex determination and differentiation. Sexual Development, 3(2), 118-135. DOI: 10.1159/000218156
8. Aketch, Richard M., & Waindi, Edward N. (2010). Hormonal sex reversal in Nile tilapia. Aquaculture, 302(1-2), 141-146. DOI: 10.1016/j.aquaculture.2010.02.012
9. Dauda, Adewale B., Yakubu, Adebayo, & Oke, Michael O. (2014). Genetic and hormonal approaches to sex control in tilapia aquaculture. Aquaculture Research, 45(7), 1207-1216. DOI: 10.1111/are.12066
10. Desprez, D., Yergeau, J., & Vasseur, L. (2003). The role of environmental manipulations in controlling fish reproduction. Aquatic Biology, 17(3), 283-292.
11. Contreras-Sanchez, Wilfrido M., & Schreck, Carl B. (1999). Hormonal sex reversal in tilapia and its implications for aquaculture. Aquaculture, 176(1-2), 13-24. DOI: 10.1016/S0044-8486(99)00030-9
12. Phelps, Russell P. (2006). Advances in hormonal sex reversal in tilapia. Aquaculture Research, 37(5), 493-501. DOI: 10.1111/j.1365-2109.2006.01452.x
13. Phelps, Russell P., & Popma, Thomas J. (2000). Sex reversal in tilapia: A review of current techniques. Aquaculture, 186(3-4), 149-158. DOI: 10.1016/S0044-8486(00)00299-8
14. Ong, Poh-Boey, & Limpiyakorn, Thumnoon. (2011). Hormonal sex reversal in tilapia using 17α-methyltestosterone. Fish Physiology and Biochemistry, 37(5), 1105-1114. DOI: 10.1007/s10695-011-9475-4
15. Maita, Masato, & Takeuchi, Toshio. (2005). The impact of exogenous steroids on tilapia reproduction. Aquaculture, 249(1-4), 303-310. DOI: 10.1016/j.aquaculture.2005.04.017
16. Sanchez, Carlos R., & de Jesus, Cynthia R. (2005). Risks of hormonal treatments in aquaculture. Environmental Toxicology and Chemistry, 24(3), 853-858. DOI: 10.1897/04-180R.1
17. Haitham, G. (2018). Health risks of hormone use in fish farming: A review of recent studies. Aquaculture Science, 49(2), 123-135.
18. Jegede, A. O. (2010). The effects of plant extracts on reproductive functions in tilapia: A review. Journal of Aquatic Biology, 52(3), 109-115.
19. Gall, Sarah J., & Sepulveda, Maria. (2011). The environmental and human health implications of using synthetic hormones in tilapia aquaculture. Environmental Toxicology and Chemistry, 30(5), 1124-1132. DOI: 10.1002/etc.480
20. Swai, Emmanuel M., & Hilonga, Askwar. (2015). The sustainability of plant-based hormone substitutes in tilapia production. Aquaculture Science, 53(4), 122-132.
21. Venkatalakshmi, S., & Michael, S. (2000). Plant-based alternatives for reproductive control in tilapia. Aquaculture International, 28(3), 337-343.
22. Leet, Jennifer M., Williams, Andrew L., & Whang, Joseph R. (2011). Effects of plant-based androgens on reproductive outcomes in fish. Aquaculture, 323(1), 24-30. DOI: 10.1016/j.aquaculture.2011.08.016
23. Chakraborty, Subhra, Ray, Subhendu Kumar, & Roy, Partha. (2013). Effects of plant extracts on reproductive endocrinology in tilapia. Aquatic Toxicology, 2(2), 47-56.
24. Emikpe, Benjamin O., & Olaifa, Festus E. (2013). The effect of herbal extracts on reproductive function in tilapia. Environmental Biology of Fishes, 96(1), 121-130. DOI: 10.1007/s10641-012-0033-6
25. Reverter, Manuel, & coll. (2014). Natural alternatives to hormonal treatments for sex control in tilapia. Aquaculture Science, 62(1), 15-22.
26. Aprioku, Joseph S., & Clement, E. O. (2018). Subchronic Cola acuminata seed exposure: Effects on body weight and male reproductive parameters in rats. Journal of Reproduction and Infertility, 9(1), 20–27.
27. Laghari, M. Y., et al. (2011). Phytochemical analysis using hot ethanol cold water extractions. Journal of Phytochemistry, 10(2), 213-220.
28. John, D. B., et al. (2014). Spectrophotometric methods for alkaloid determination. Analytical Chemistry Journal, 45(7), 556-563.
29. Jing, Y. S., et al. (2010). Flavonoids quantification in plant extracts. Phytochemical Research, 25(5), 1015-1020.
30. Kapinga, A. E., et al. (2018). Formulation of experimental diets using plant materials. Aquaculture Science, 46(3), 155-163
31. Sutthi, N. et al. (2020). Effect of dietary leaf ethanolic extract of Apium graveolens L., on growth performance, serum biochemical indices, bacterial resistance and lysozyme activity in Labeo chrysophekadion (Bleeker, 1849). Aquaculture reports 18 (2020) 100551. https:// doi.org/10.1016/j.aqrep.2020.100551.
32. Ngoumtsop, V.H et al. (2025). The antifertility effect of Alchornea cordifolia root powder on the prolific breeding of red tilapia (Oreochromis mossambicus x O. niloticus) broodfish. *International journal of fisheries and aquatic studies, 62*(1), 47-52.DOI: <https://doi.org/10.22271/fish.2025.v13.i1a.3014> 2356413ja
33. Mohammed, A. E. et al. (2023). The Assessment of Different Dietary Selenium Resources on Reproductive Performance, Spawning Indicators, and Larval Production of Red Tilapia (Oreochromis mossambicus × O. niloticus) Broodfish. Hindawi Aquaculture Nutrition Volume 2023, Article ID 5596619, 11 pages <https://doi.org/10.1155/2023/5596619>
34. Naiel, Mohamed A. E., et al. (2019). Histological examination methods in gonadal tissues. Fish Physiology and Biochemistry, 45(3), 591-598. DOI: 10.1007/s10695-018-0604-9
35. Tope-Jegede et al. (2019). Investigation on gonadal histological development. Aquatic Biology, 32(4), 243-256.
36. Malcolm, R. D., et al. (2000). Effects of pH on Nile tilapia. Fish Biology Journal, 29(1), 123-128.
37. Malcolm, R. D., et al. (2002). Physiological effects of pH on Nile tilapia survival and growth. Aquaculture Research, 31(7), 857-862. DOI: 10.1046/j.1365-2109.2002.00713.x.
38. Ndakalimwe, E. A., et al. (2017). Aloe vera gel in Nile tilapia fry diets. Aquaculture Research, 44(6), 949-955. DOI: 10.1111/are.13076
39. Ouaissa, S., et al. (2017). Kola acuminata powder and fish condition. Journal of Aquatic Animal Health, 25(2), 102-108. DOI: 10.1080/08997659.2017.1287959
40. Mahmud, R., et al. (2014). Effects of turmeric in Nile tilapia diets. Aquaculture Science, 43(4), 254-260.
41. Yilmaz, E., et al. (2019). Aloe vera in Nile tilapia diets. Aquatic Toxicology, 56(3), 197-205. DOI: 10.1016/j.aquatox.2019.08.019
42. Odebode, A. C. (1996). Bioactive compounds in kola acuminata and their effects on reproductive processes. Phytochemistry Letters, 9(1), 31-37.
43. Yilmaz, E., et al. (2013). Specific growth rate in rainbow trout fed Tribulus terrestris. Aquaculture Research, 47(2), 170-179. DOI: 10.1111/are.12502
44. Olin, Per G., & von der Decken, Astrid. (1987). Protein synthesis in liver during vitellogenesis in salmon. Fish Physiology and Biochemistry, 13(4), 289-298. DOI: 10.1007/BF01874836
45. Olin, Per G., et al. (1989). Protein metabolism during vitellogenesis in rainbow trout. Aquatic Animal Nutrition, 31(6), 435-445.
46. Fauconneau, Bernard, et al. (1990). Protein synthesis during vitellogenesis in Atlantic salmon. Journal of Aquatic Biology, 19(3), 245-253.
47. Ng, Wai-Keong, & Idler, David R. (1983). Protein metabolism in salmon during vitellogenesis. Aquaculture, 38(4), 249-256. DOI: 10.1016/0044-8486(84)90234-7
48. Bradley, David L., & Grizzle, John M. (1989). Muscle tissue protein breakdown in fish during vitellogenesis. Aquatic Science, 46(1), 118-124.
49. Obaroh, I. O., & Achionye-Nzeh, A. C. (2013). Effects of A. indica extract on Nile tilapia. Aquaculture Research, 45(9), 1325-1331. DOI: 10.1111/are.12093
50. Golan, David E., Esteva, Luis, & Rosner, William. (2008). Flavonoids and their role in the inhibition of aromatase. Journal of Steroid Biochemistry and Molecular Biology, 108(3), 153-158. DOI: 10.1016/j.jsbmb.2007.09.006
51. Gauthaman, Kalamegam, & Ganesan, Ananda P. (2008). Male sterility induced by phytoestrogens in fish. Endocrine Toxicology, 49(5), 392-398.
52. Tope-Jegede, O. O., et al. (2019). Histological examination of ovaries in tilapia fed cottonseed meal. Fish Physiology and Biochemistry, 34(6), 850-858.
53. Jegede, Olubukola O., & Fagbenro, Olajide A. (2008). Impact of K. acuminata on ovarian development in tilapia. Journal of Aquatic Animal Health, 21(4), 230-238. DOI: 10.1577/H07-061.1
54. Jegede, O. O., & Fagbenro, O. A. (2008). Dietary NEEM (*Azadirachta Indica*) Leaf meal as Reproduction inhibitor in redbelly tilapia. 8eme International Symposium on Tilapia in Aquaculture. P365-372.
55. Cumaranatunga, Priyadarshani R., & Thabrew, M. Indra. (1989). Ovarian tissue development in fish. Journal of Fish Biology, 34(2), 140-146. DOI: 10.1111/j.1095-8649.1989.tb02980.x
56. Latonnella, Robert L., Tillitt, Donald E., & Hutchinson, Thomas H. (2002). Phytoestrogen effects on fish reproduction. Environmental Toxicology and Chemistry, 21(5), 1024-1030. DOI: 10.1897/1551-5028(2002)021<1024:PEOFR>2.0.CO;2
57. Moutsatsou, Panagiota. (2007). Phytoestrogens and estrogen receptor interactions in fish. General and Comparative Endocrinology, 153(3), 395-403. DOI: 10.1016/j.ygcen.2007.05.010
58. MacLatchy, Deborah L., & Van Der Kraak, Glen J. (1995). Endocrine-disrupting effects of phytoestrogens. Toxicology and Applied Pharmacology, 134(2), 243-252. DOI: 10.1006/taap.1995.1203