Androgenic Potentials of Indian Spinach (Basella alba) Aqueous Leaf Extract for Production of All-Male Nile Tilapia (*Oreochromis niloticus*)

RESEARCH ARTICLE

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ABSTRACT

The precocious maturity and unregulated reproduction of tilapia result in stunted growth and low commercial value, making it difficult for fish farmers to raise them to table size. This study was conducted to assess the androgenic potentials of Indian spinach (Basella alba) aqueous leaf extract (ALEB) for the production of all-male Nile tilapia (Oreochromis niloticus). An experiment was set up for 60 days to evaluate the efficacy of ALEB in the masculinisation of tilapia to address this challenge.

A total of 225 O. niloticus fry were stocked in 15 rectangular tanks (45 cm × 45 cm × 30 cm). Five treatments (T1, T2, T3, T4, and T5: 0.00, 0.25, 0.50, 0.75, and 1.00 g/l) were applied, with each treatment triplicated. ALEB was prepared by boiling 18 g of powder in 1500 ml of distilled water for 30 minutes and filtering with Whatman No. 1 filter paper. Fresh solutions were prepared weekly.

Methodology

60-day experiment using 225 O. niloticus fry in 15 rectangular tanks with five ALEB concentrations

Key Findings

Weight gain increased with ALEB concentration, with 93.33% survival at 0.75 g/l treatment

Main Result

Highest male population (76.19%) achieved at 1.00 g/l concentration with normal gonad formation

Results showed significantly better growth performance ($p < .05$) in fish immersed in ALEB compared to fish without ALEB. Weight gain increased with ALEB concentration. Fish treated with 0.75 g/l ALEB had the best survival (93.33%). Treatment 5 recorded the highest weight gain (2.36 g), while Treatment 2 had the best feed conversion ratio (1.12). Treatment 5 also produced the highest male population (76.19%) at 1.00 g/l concentration. Histological examination of the fish gonads showed normal ovary and testis formation.

The findings indicate that ALEB could be used as an androgenic agent for the production of all-male Nile tilapia (Oreochromis niloticus).

Keywords: all-male tilapia, androgens, Basella alba leaf, sex reversal, phytochemicals

INTRODUCTION

An important part of the world's food production is aquaculture, which is the farming of aquatic species such as fish, molluscs, crustaceans, and aquatic plants. Due to population growth-driven increases in protein demand and overfishing-induced declines in capture fisheries, its importance is growing (FAO, 2024). With 130.9 million tonnes produced in 2022, aquaculture surpassed capture fisheries as the main source of aquatic animal production, accounting for 51% of the overall supply (FAO, 2024).

Aquaculture plays a crucial role in developing nations where food security remains a major concern. By providing a wholesome and reasonably priced source of protein, it enhances the global food supply. Additionally, it raises household income and supports national economic growth, both of which promote food accessibility and economic resilience. To meet the nutritional needs of a growing population, sustainable aquaculture must be prioritised, especially in areas where conventional fisheries are unable to supply demand (FAO, 2024).

01

Tilapia Production Challenge

As one of the most extensively farmed fish worldwide, tilapia species rank third in production among cultivated finfish, after carps and catfishes (FAO, 2024).

02

Early Maturation Problems

Early maturation and frequent spawning present major issues in tilapia aquaculture, leading to overcrowding and impaired growth.

03

All-Male Solution

All-male populations are favoured because of their uniform size and faster growth rates. Sex reversal via hormone therapy during gonadal development is the most common technique.

As one of the most extensively farmed fish worldwide, tilapia species (Cichlidae) rank third in production among cultivated finfish, after carps and catfishes (FAO, 2024). In 2020, tilapia production reached about 7 million tonnes globally and has continued to grow due to their adaptability and rapid growth rates. Early maturation and frequent spawning present major issues in tilapia aquaculture, as they can lead to overcrowding and impaired growth. All-male populations are favoured to address this because of their uniform size and faster growth rates. Sex reversal via hormone therapy during the gonadal development period is the most common technique for creating monosex populations.

Hormonal intervention at the critical stage, usually within the first 10 to 15 days post-hatch, can significantly influence sexual differentiation in tilapia fry that have not yet completed sex differentiation. Androgens such as 17α-methyltestosterone (17α-MT) are frequently employed to induce male traits, resulting in populations that are predominantly male, whereas oestrogens encourage the development of female characteristics.

Hormones are widely used in aquaculture to enhance artificial reproduction and sex reversal, improving seed production and growth potential (Taranger et al., 2010). However, their environmental and health risks are significant concerns (Liu et al., 2011; Guedes-Alonso et al., 2017). Steroid hormone residues, for instance, have been found in wild fish populations, including crucian carp and common carp in Dianchi Lake, indicating widespread contamination. Therefore, best management practices are essential, and research into non-hormonal alternatives like temperature-induced sex reversal and selective breeding is crucial.

One promising non-hormonal strategy is using plant-based substances with inherent androgenic properties. Basella alba, commonly known as Malabar spinach, is a perennial climber rich in nutrients like calcium, iron, vitamin A, and vitamin C (Palada & Chang, 2003).

Phytochemical Properties

- Presence of tannins, saponins, steroids, and alkaloids
- Androgenic properties demonstrated in studies
- Natural alternative to synthetic hormones

Research Evidence

- Male populations increased to 70.3% in 0.1 g/l treatment
- Aromatase inhibition mechanism proposed
- Safe and effective for aquaculture use

Recent studies have shown B. alba extracts can induce masculinisation in Nile tilapia, with immersion treatments yielding up to 70.3% male populations at 0.1 g/l. The extract's androgenic effects are attributed to phytochemicals like tannins, saponins, steroids, and alkaloids. The proposed mechanism involves the inhibition of aromatase, an enzyme that converts androgens to oestrogens, thereby promoting male differentiation by blocking oestrogen biosynthesis (Rempel & Schlenk, 2008).

Although these findings are encouraging, there is considerable variation in the effectiveness of phytochemicals in creating all-male fish populations. Furthermore, more research is needed to ensure the safety and efficacy of these plant extracts in aquaculture, given their possible anabolic and virilising effects. To properly understand the long-term implications of using plant-based compounds such as B. alba in sex reversal procedures, as well as to optimise dosages and treatment periods, further research is required. Because of the potential hazards of chemical hormones, the use of natural sources of methyltestosterone is also being explored. Through tilapia immersion in aqueous leaf extract of B. alba (ALEB), this study aimed to produce all-male populations of O. niloticus and identify the optimal concentration that would yield a high proportion of males.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the Teaching and Research Laboratory of the Department of Fisheries and Aquaculture Technology, The Federal University of Technology, Akure, Ondo State, Nigeria, for 60 days.

Collection of Fish Seed

Newly hatched fry of mixed-sex Nile tilapia were collected from the Fish Hatchery of The Federal University of Technology, Akure, Ondo State, Nigeria.

Experimental Set-Up

A total of 225 O. niloticus mixed-sex fry were randomly collected from the Fish Hatchery of The Federal University of Technology, Akure. The fry were transferred into glass aquaria (45 cm × 45 cm × 30 cm) and acclimatised for 24 hours.

Experimental Design

- 225 mixed-sex Nile tilapia fry
- 15 glass aquaria (45 cm × 45 cm × 30 cm)
- 5 treatments with 3 replications each
- 15 fish per aquarium

Treatment Concentrations

- T1: 0.00 g/l (control)
- T2: 0.25 g/l
- T3: 0.50 g/l
- T4: 0.75 g/l
- T5: 1.00 g/l

Duration and Feeding

- 60-day experimental period
- 168 hours weekly ALEB exposure
- 35% crude protein diet
- Fed to apparent satiation 3 times daily

Table 1: Gross Composition of Experimental Diets (g/100 g) for *O. niloticus*

Ingredients	% Crude Protein	% inclusion (calculated)
Fish meal	64	16.0
Soybean meal	44	33.0
Groundnut cake	42	19.0
Yellow maize	9	22.0
Methionine		0.5
Lysine		0.5
Fish oil		5.0
Mineral-Vitamin premix		2.0
Starch		2.0
Total		100

Proximate Composition (%)	
Moisture	7.35
Ash	10.11
Lipid	9.29
Crude protein	32.22
Crude fibre	2.13
NFE	38.90

Note. NFE = Nitrogen-free extract.

Immersion Treatment of Fish with Plant Extracts

Three-day-old mixed-sex fry of Nile tilapia (mean weight 0.04 ± 0 g) were randomly assigned to 15 glass aquaria (45 cm × 45 cm × 30 cm) across five treatment groups (0.00 g/l as control, 0.25 g/l, 0.50 g/l, 0.75 g/l, and 1.00 g/l). The experiment lasted 60 days. Fish were exposed to the ALEB solution for 168 hours weekly (8 exposures in total). The aquaria were continuously aerated during this period, and the water was completely (100%) replaced manually. Each aquarium was stocked with 15 fish.

The fish were fed finely ground (< 500-1000 µm) formulated diet (Table 1) containing 35% crude protein, to apparent satiation, three times daily. The experiment was conducted simultaneously in triplicate.

Water Quality Monitoring

Water quality parameters (temperature, dissolved oxygen, and pH) were measured fortnightly throughout the experimental period. Temperature was measured using a mercury-in-glass thermometer (◦C). pH was measured with a pH meter (Jenway model 9060). Dissolved oxygen (DO) was measured using a dissolved oxygen test kit (Hanna model HI-9142).

Proximate Composition of Feed

The experimental feed was analysed for moisture, ash, lipid, crude fibre, and crude protein according to AOAC (2010).

Growth Response and Nutrient Utilization

At the end of the experimental period (60 days), performance data were calculated. Fish were counted and batch-weighed. Growth parameters and feed utilisation indices were calculated as follows (Takeuchi, 1988; Tacon, 1990):

Weight gain (g) = Final weight - Initial weight

Specific growth rate

This is calculated from data on changes of body weight over given time interval;

$$SGR(\%\ per\ day) = \frac{(Ln\ final\ weight - Ln\ initial\ weight)}{Time(days)} x100$$

$$Where\ Ln = Natural\ loge$$

Total feed intake (g)

This will be obtained by adding daily mean feed intake (DFI) of fish under each treatment for the experimental period.

$$Feed\ intake\ (g) = \frac{Total\ feed\ intake}{Number\ of\ fish\ survived}$$

$$Feed\ conversion\ ratio\ (FCR)(g) = \frac{feed\ intake(g)}{weight\ gain(g)}$$

$$Survival\ (\%) = \frac{Number\ of\ fish\ harvested}{Number\ of\ fish\ stocked} x100$$

Sexing of Fish

To determine sex, seven fish specimens were sacrificed. Following Guerrero and Shelton (1974) and Wassermann and Afonso (2002), the juvenile fish were sexed using the conventional acetocarmine squash procedure of gonads.

Histological Examination of the Experimental Fish

Histology of the gonads (ovary and testis) was carried out following the method described by Rowley et al. (1990).

Statistical Analysis

All data obtained were subjected to one-way analysis of variance (ANOVA; Steel & Torrie, 1980), followed by Duncan's New Multiple Range Test (Duncan, 1955) to identify differences among mean values at p = .05. Statistical analyses were performed using R programming language (version 4.2.1) and Microsoft Office Excel 2010.

Histological Analysis

Gonad examination using acetocarmine squash procedure for sex determination and tissue analysis

Water Quality Monitoring

Fortnightly measurement of temperature, dissolved oxygen, and pH using calibrated instruments

Statistical Analysis

ANOVA and Duncan's test using R programming language for data analysis and comparison

RESULTS AND DISCUSSION

Water Quality Parameters Measured During the Immersion Period

Water parameter readings recorded during the experimental period are presented in Table 2. The temperature readings (28.03 ± 0.16 °C in T1 and 28.06 ± 0.15 °C in T3 - T5), dissolved oxygen (5.69 ± 0.29 mg/l in T3 and 6.33 ± 0.33 mg/l in T1), and hydrogen ion concentration (6.84 ± 0.12 in T1 and 6.95 ± 0.10 in T5) all fall within favourable limits for initiating sex reversal in tilapia. These values align with findings from previous studies (Popma & Masser, 1999; Phelps & Popma, 2000; Xu et al., 2005; Azaza et al., 2008).

Study Limitations and Statistical Considerations

This study, while providing valuable insights into the efficacy of ALEB concentrations for sex reversal in Nile tilapia, acknowledges certain limitations, particularly regarding statistical power and sample size. The sample size of 225 fry, distributed across 5 treatments with 3 replications, represents a moderate-scale study. While it was adequate for detecting significant differences between the treatment groups observed in this research, it is important to consider the broader implications for statistical power in sex reversal studies.

Sample Size Adequacy

The sample size of 225 fry, while adequate for detecting significant differences, represents a moderate-scale study. Despite this, the significant differences ($p < 0.05$) observed between treatment groups provide confidence in the results.

Comparison to Larger Studies

Statistical power considerations for sex reversal studies often benefit from larger populations. For instance, larger-scale studies like Cagauan et al. (2000) achieved 91% male ratios with larger sample sizes using hormone immersion techniques, providing a benchmark for efficacy.

Future Research Recommendations

Recommendation for larger-scale validation studies to confirm the 76.19% success rate observed at 1.00 g/l ALEB concentration is crucial. Future studies should employ larger sample sizes (>500 fry per treatment) to increase statistical power and provide more robust estimates of treatment efficacy.

Growth Response and Survival Percentage of *O. niloticus* Immersed in ALEB

The results of growth response are presented in Table 3. Weight gain increased with ALEB concentration. Fish treated with 0.75 g/l ALEB had the best survival rate (93.33%), while the control group had the lowest (71.11%). Significant differences ($p < .05$) were observed in survival between the control and treatment groups.

Fish growth is influenced by numerous factors, including species, diet, feed additives, and rearing environment. In this study, ALEB improved growth performance. Treated groups grew faster than controls, possibly due to the enhanced health of fish immersed in ALEB. Notably, *B. alba* methanol extract has been reported to stimulate testosterone synthesis in isolated Leydig cells of Sprague-Dawley rats (Nantia et al., 2011). Similar dietary extracts may enhance androgen production and growth in tilapia.

Other studies support these findings. For example, *Tribulus terrestris* extract increased body weight in young lambs (Georgiev et al., 1988) and in rats (Gauthaman et al., 2002). In this study, treated groups also survived longer than controls. The high survival rate (93.33%) suggests no observable adverse effects, consistent with Chakraborty et al. (2014), who found that *P. reticulata* treated with *B. alba* methanol extract showed no harm. Conversely, other studies reported no significant differences in survival between *Poecilia reticulata* or *P. latipinna* treated with *T. terrestris* extracts and untreated controls (Çek et al., 2007b; Kavitha & Subramanian, 2011).

Janalizadeh et al. (2018) reported lower survival in *Betta splendens* fed *T. terrestris*-enriched *Artemia*. However, in the current study, survival was comparable to that observed by Chakraborty (2017), Ghosal et al. (2016), and Kavitha and Subramanian (2011), who reported high survival when fish were fed *T. terrestris*. For instance, Chakraborty (2017) found 97.5% survival in fish fed 5 g/kg *T. terrestris*. Similarly, Bamba et al. (2008) reported 75-94% survival in *O. niloticus* fed a cocoa bean shell-coconut oil cake diet. Other studies observed 100% survival in *Cichlasoma nigrofasciatum* fed *T. terrestris* (Yeganeh et al., 2017) and 94.96% in *O. niloticus* fed *B. alba* and *T. terrestris* (Ghosal et al., 2015; 2016). These results support claims that *T. terrestris* is not harmful to fish. The present findings are also consistent with Alok et al. (2018), who noted a 76.5% survival rate in *O. niloticus* fed a commercial diet. Overall, the aqueous extract of *B. alba* did not adversely affect *O. niloticus*.

Sex Ratio of *O. niloticus* at Varying Concentrations of ALEB for 60 Days

The sex ratios of males differed significantly ($p < .05$) across concentrations (Figure 1). All ALEB-treated groups had higher proportions of males compared to the control. The highest proportion of males (76.19%) was recorded at 1.00 g/l (T5).

Hormone therapy must begin before gonadal development to induce phenotypic sex reversal (Yamamoto, 1969). All treatment groups recorded significantly more males than the control. The high male ratio in T5 may be attributed to the young age of fry, as younger fry respond better (Popma & Green, 1990). Since the fry were still yolk-dependent, *B. alba* was likely absorbed via the yolk sac. This finding supports Ana et al. (2011), who reported that yolk-sac fry exposed to hormones for longer periods produced higher proportions of males in *O. niloticus*. The present results also compare favourably with Indranath et al. (2015), who treated *O. niloticus* with methanolic *B. alba* extracts at 1.0 g/l.

The current study demonstrates that *B. alba* promotes masculinisation, though serum testosterone levels were not measured at the end of the trial. Thus, it is unclear whether this potency was due to androgen or testosterone. Importantly, even at the highest concentration, *B. alba* extract did not yield 100% males. Similar findings were reported by Francis et al. (2002), who observed increased male ratios in *O. niloticus* fry fed Quillaja saponin but not complete masculinisation.

Variations in masculinisation across studies may result from methodological differences, including the type of extract (aqueous vs. methanol), route of administration (immersion vs. dietary inclusion), treatment duration, and fry developmental stage at exposure. Whereas earlier studies employed methanolic extracts or feed-based delivery systems (Francis et al., 2002; Indranath et al., 2015), the present study used aqueous immersion of yolk-sac fry, which may have improved absorption via the yolk. These methodological differences likely explain the observed variation in masculinisation effectiveness.

Comparative Effectiveness of Plant-Based Sex Reversal Methods

The 76.19% male ratio achieved with ALEB aqueous extract represents a significant improvement over control groups but warrants comparison with other plant-based methods for sex reversal in fish.

ALEB Aqueous Extract (Current Study)

Achieved 76.19% males at 1.00 g/l concentration via immersion in *O. niloticus*. This study focuses on the aqueous extract and immersion method, highlighting its potential.

Ghosal et al. (2015) - *Basella alba* Ethanol Extract

Achieved 83.2% males using *Basella alba* ethanol extract at 1.0 g/kg feed via dietary administration. This suggests that the extraction method and administration route significantly impact efficacy.

Ghosal & Chakraborty (2014) - *Basella alba* Aqueous Extract

Found that *Basella alba* aqueous extract contained phytochemicals like tannins, saponins, steroids, and alkaloids with androgenic properties, achieving 70.3% males at 0.1 g/l via immersion.

Other Plant Extracts

Tribulus terrestris achieved similar results in various studies, and *Asparagus racemosus* showed promise in tilapia masculinization (Mukherjee et al., 2018). These alternatives further broaden the scope of plant-based sex reversal.

Discussion indicates that extraction solvents (aqueous vs. ethanol vs. methanol) and administration methods (immersion vs. dietary) appear to be critical factors affecting the concentration and bioavailability of active compounds. These variations likely explain the observed differences in masculinization effectiveness across studies.

In conclusion, while ALEB shows promise, optimization of extraction methods and administration protocols could potentially improve efficacy beyond the current 76.19% success rate, bringing it closer to or even surpassing other successful plant-based methods.

Histology of Ovaries in *O. niloticus* During Immersion Treatment with ALEB for 60 Days

Sections of ovaries in the control treatment (0.0 g/l) showed normal ovarian tissues and oocytes at the perinucleus stage (Plate 1). Fish treated with ALEB followed a similar trend, showing oocytes at various early and late perinucleus stages. This indicates that the ovaries were still in an early stage of development and had not reached sexual maturity (Plates 2 - 5).

The histological analysis of ovaries from females in the control group revealed the presence of normal ovarian tissues and perinucleus phases. This finding is consistent with Obaroh et al. (2018), who observed normal histological structures of ovarian tissues and perinucleus stages in *O. niloticus* treated with crude guava (*Psidium guajava*) leaf extract. Similarly, sections of ovaries subjected to ALEB treatment exhibited oocytes at various perinucleus and vitellogenic phases, indicating that they remained in a nascent state of growth and had not attained sexual maturity. This finding aligns with Madan (2013), who reported similar results in *O. niloticus* treated with *Aloe vera* leaf extract.

Histology of Testes in *O. niloticus* During Immersion Treatment with ALEB for 60 Days

Testicular tissues in the control treatment (0.0 g/l) showed normal spermatozoa distribution in the lumen (Plate 6). Fish treated with 0.25 g/l exhibited greatly reduced luminal gaps, with mature Sertoli cyst germinal phases observed (Plate 7). Fish treated with 0.50 g/l had prominent maturing germinal stages in Sertoli cysts and reduced luminal gaps (Plates 8 - 9). At 1.0 g/l, testes showed well-developed Sertoli cysts but few luminal spermatozoa (Plate 10).

In the control treatment, testes of *O. niloticus* showed normal testicular tissues and sperm-producing cells within the seminiferous tubule lumen, consistent with Morrison et al. (2006). These findings are also supported by Obaroh et al. (2018), who reported normal testicular tissues and spermatozoa in *O. niloticus* fed crude guava leaf extract. No testicular injury was observed in plant-treated groups across concentrations. Spermatocysts remained encased within Sertoli cysts, indicating normal maturation of spermatozoa.

This agrees with Akin-Obasola and Jegede (2013), who studied the effects of *Momordica charantia* leaf meal on *Coptodon zillii* reproduction, and Sehriban et al. (2007), who reported normal tissues and well-distributed spermatozoa in *P. reticulata* fed powdered *T. terrestris* seed.

Table 2: Water Quality Parameters Measured During the Immersion Period

Parameters	T1	T2	T3	T4	T5
Temperature (°C)	28.03±0.16^{a}	28.05±0.16^{a}	28.06±0.15^{a}	28.06±0.15^{a}	28.06±0.15^{a}
pH	6.84±0.12^{a}	6.86±0.11^{a}	6.88±0.09^{a}	6.92±0.1^{a}	6.95±0.1^{a}
DO_2 (mg/l)	6.33±0.33^{a}	6.00±0.27^{a}	5.69±0.29^{a}	5.73±0.31^{a}	5.77±0.26^{a}

Note. Mean values in the same row with different superscripts are significantly different (p < .05).

Growth Response and Survival Percentage of O. Niloticus Immersed in ALEB

The results of growth response are presented in Table 3. Weight gain increased with ALEB concentration. Fish treated with 0.75 g/l ALEB had the best survival rate (93.33%), while the control group had the lowest (71.11%). Significant differences (p < .05) were observed in survival between the control and treatment groups.

Parameters	T1	T2	T3	T4	T5
IW	0.04±0^{a}	0.04±0^{a}	0.03±0^{a}	0.04±0^{a}	0.03±0.01^{a}
FW	1.81±0.21^{b}	1.72±0.04^{b}	1.73±0.07^{b}	1.82±0.13^{b}	2.39±0.08^{a}
FI	3.91±0^{a}	1.92±0^{d}	2.24±0^{c}	2.75±0^{b}	2.75±0^{b}
WG	1.78±0.21^{b}	1.68±0.04^{b}	1.7±0.07^{b}	1.78±0.13^{b}	2.36±0.08^{a}
% WG	97.89±0.38^{a}	97.86±0.23^{a}	98.07±0.19^{a}	97.78±0.17^{a}	98.73±0.26^{a}
DWG	0.03±0^{ab}	0.03±0^{b}	0.03±0^{b}	0.03±0^{b}	0.04±0^{a}
FER	0.46±0.05^{c}	0.9±0.02^{a}	0.77±0.03^{ab}	0.67±0.05^{b}	0.87±0.03^{a}
FCR	2.21±0.23^{a}	1.12±0.03^{b}	1.3±0.05^{b}	1.52±0.11^{b}	1.15±0.04^{b}
SGR	0.06±0^{a}	0.06±0^{a}	0.07±0^{a}	0.06±0^{a}	0.07±0^{a}
Survival	71.11±5.88^{b}	80±0^{ab}	82.22±2.22^{ab}	93.33±3.85^{a}	86.67±3.85^{ab}

Note. IW = Initial Weight; FW = Final Weight; FI = Feed Intake; WG = Weight Gain; %WG = Percentage Weight Gain; DWG = Daily Weight Gain; FER = Feed Efficiency Ratio; FCR = Feed Conversion Ratio; SGR = Specific Growth Rate. Mean values in the same row with different superscripts are significantly different (p < .05).

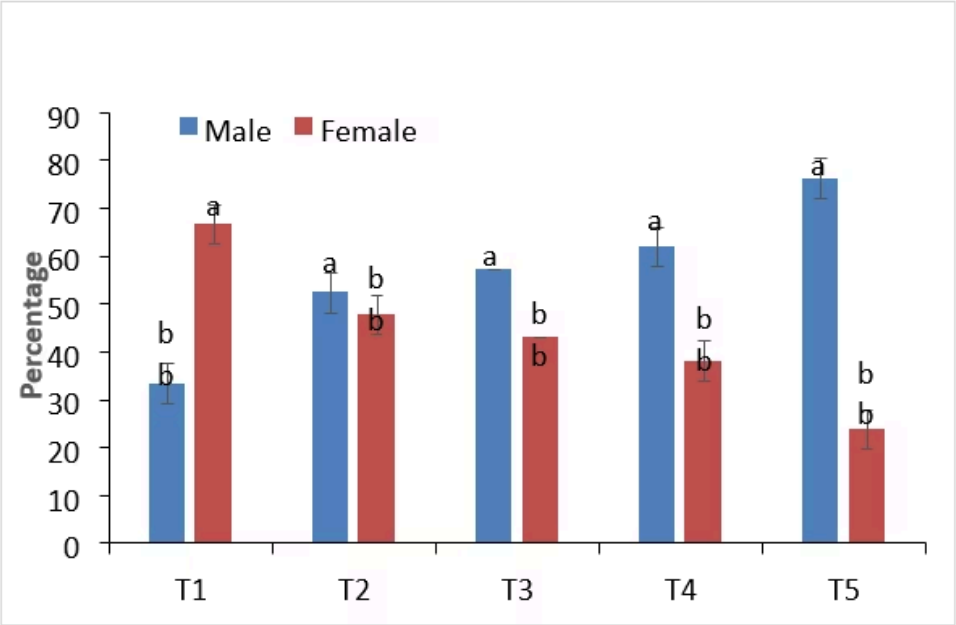
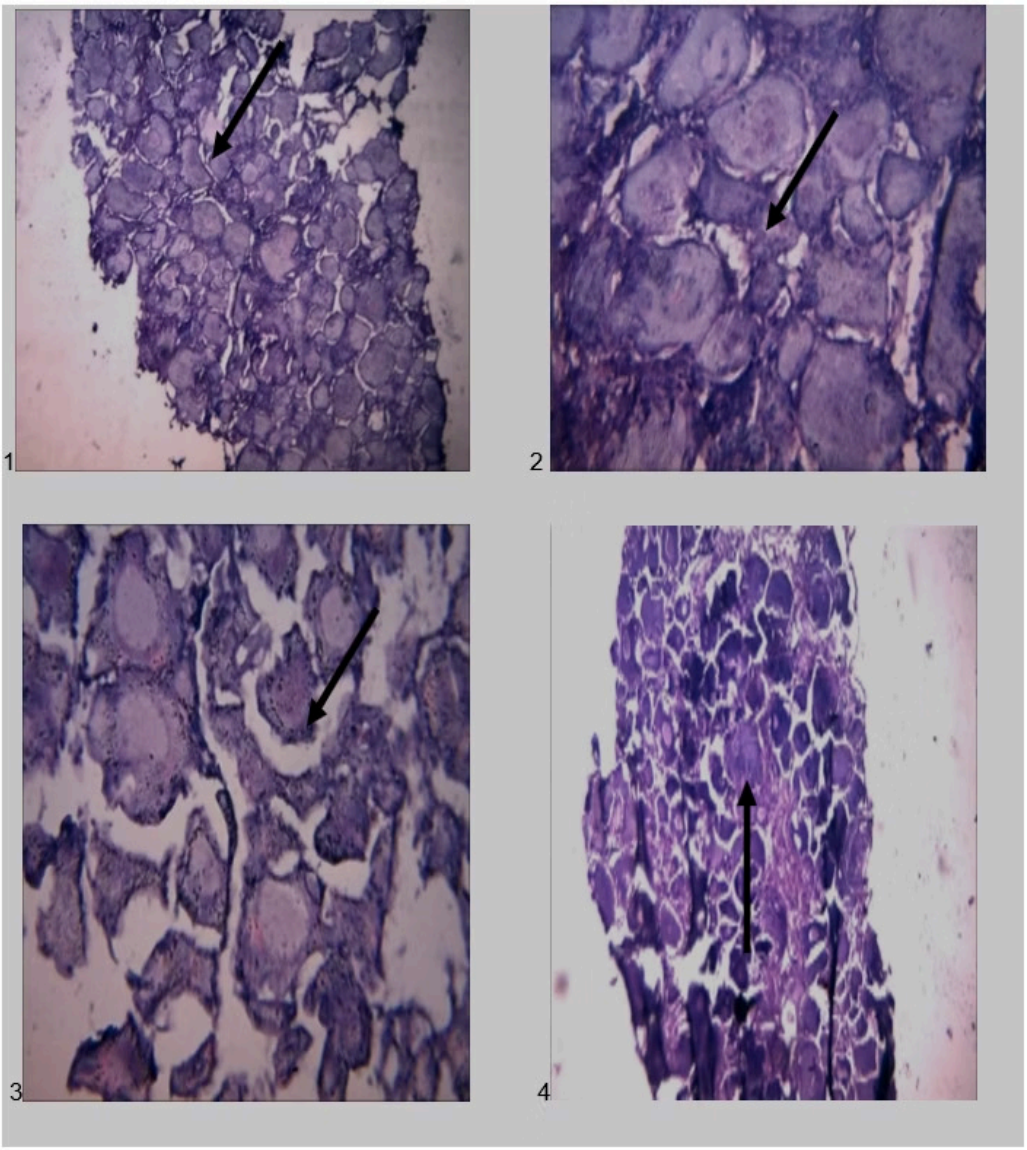
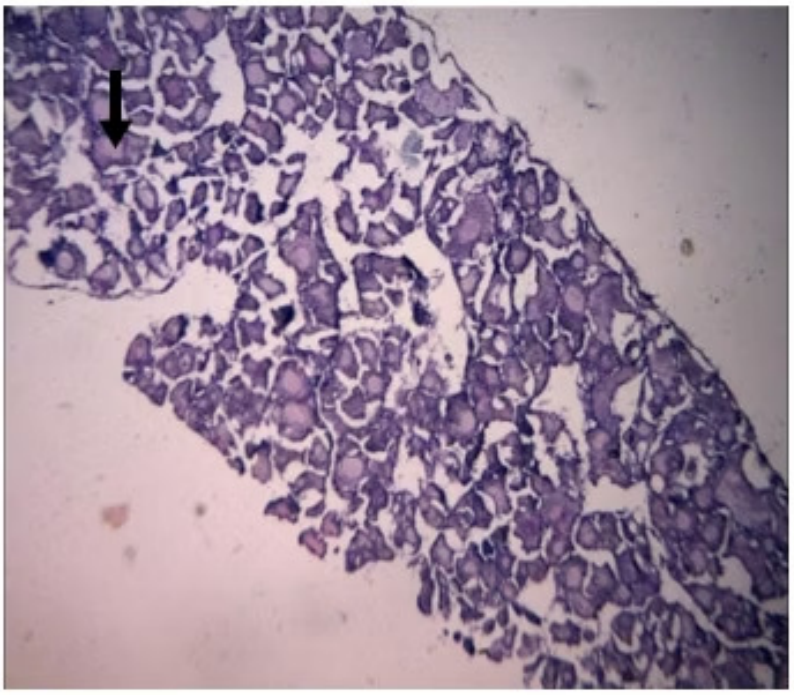


Figure 1: Sex Ratio of *O. niloticus* at Varying Concentration Levels of ALEB





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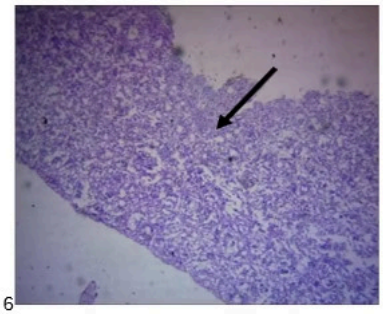

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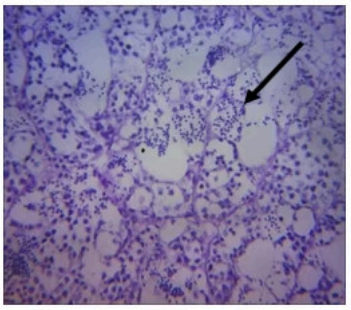

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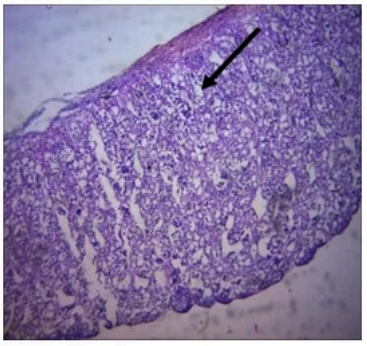

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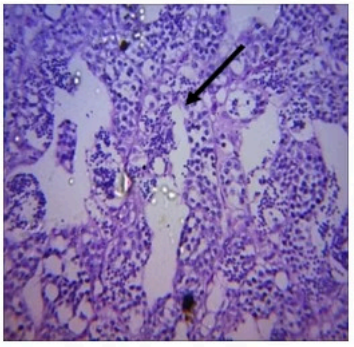

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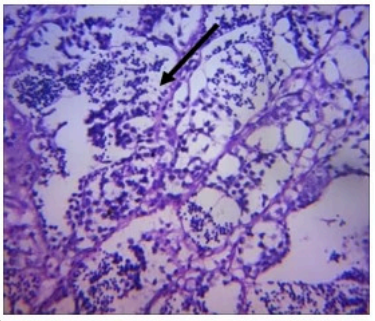

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Plates

- ***Plate 1.*** Section of ovary in O. niloticus immersed in 0.0 g/l, showing oocyte at perinucleus stages (arrow). Mag. ×100. Scale bar = 50 µm.
- ***Plate 2.*** Section of ovary in O. niloticus immersed in 0.25 g/l, showing numerous late perinucleus stages (arrow). Mag. ×100. Scale bar = 50 µm.
- ***Plate 3.*** Section of ovary in O. niloticus immersed in 0.50 g/l, showing numerous late perinucleus stages (arrow). Mag. ×100. Scale bar = 50 µm.
- ***Plate 4.*** Section of ovary in O. niloticus immersed in 0.75 g/l, showing numerous early perinucleus stages (arrow). Mag. ×100. Scale bar = 50 µm.
- ***Plate 5.*** Section of ovary in O. niloticus immersed in 1.0 g/l, showing numerous perinucleus stages (arrow). Mag. ×100. Scale bar = 50 µm.
- ***Plate 6.*** Section of testis in O. niloticus immersed in 0.0 g/l, showing very scanty spermatozoa in the lumen (arrow). Mag. ×100. Scale bar = 50 µm.
- ***Plate 7.*** Section of testis in O. niloticus immersed in 0.25 g/l, showing luminal spaces greatly reduced. Prominent maturing germinal stages in Sertoli cysts (arrow). Mag. ×100. Scale bar = 50 µm.
- ***Plate 8.*** Section of testis in O. niloticus immersed in 0.50 g/l, showing prominent maturing germinal stages in Sertoli cysts (arrow). Mag. ×100. Scale bar = 50 µm.
- ***Plate 9.*** Section of testis in O. niloticus immersed in 0.75 g/l, showing prominent maturing germinal stages in Sertoli cysts and reduced luminal spaces (arrow). Mag. ×100. Scale bar = 50 µm.
- *Plate 10.* Section of testis in O. niloticus immersed in 1.0 g/l, showing well-developed Sertoli cysts and scanty spermatozoa in the lumen (arrow). Mag. ×100. Scale bar = 50 µm.

CONCLUSION

These findings indicate that Basella alba holds promise as a sustainable and potentially environmentally friendly alternative to synthetic hormones for producing male-skewed populations in Nile tilapia. This approach may contribute to improved aquaculture productivity. However, in the absence of toxicological or ecotoxicity data, its environmental safety cannot be conclusively established. Further research is recommended to include hormonal profiling, toxicological assessments, and long-term reproductive evaluations to clarify the underlying mechanisms and confirm the ecological safety and effectiveness of this natural treatment.

01

Demonstrated Efficacy

ALEB successfully induced masculinisation with 76.19% male population at 1.00 g/l concentration without adverse effects

02

Growth Enhancement

Significant improvement in growth performance and survival rates compared to control groups

03

Safe Alternative

Natural plant extract provides environmentally friendly alternative to synthetic hormones in tilapia aquaculture

04

Future Research

Further studies needed for hormonal profiling, toxicological assessment, and long-term reproductive evaluation

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Not Applicable

CONFLICTS OF INTEREST

The author declares no conflict of interest

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