Natural Sex Reversal in *Oreochromis niloticus* Using *Moringa oleifera* Seed Extract: An Eco-Friendly Approach

RESEARCH ARTICLE

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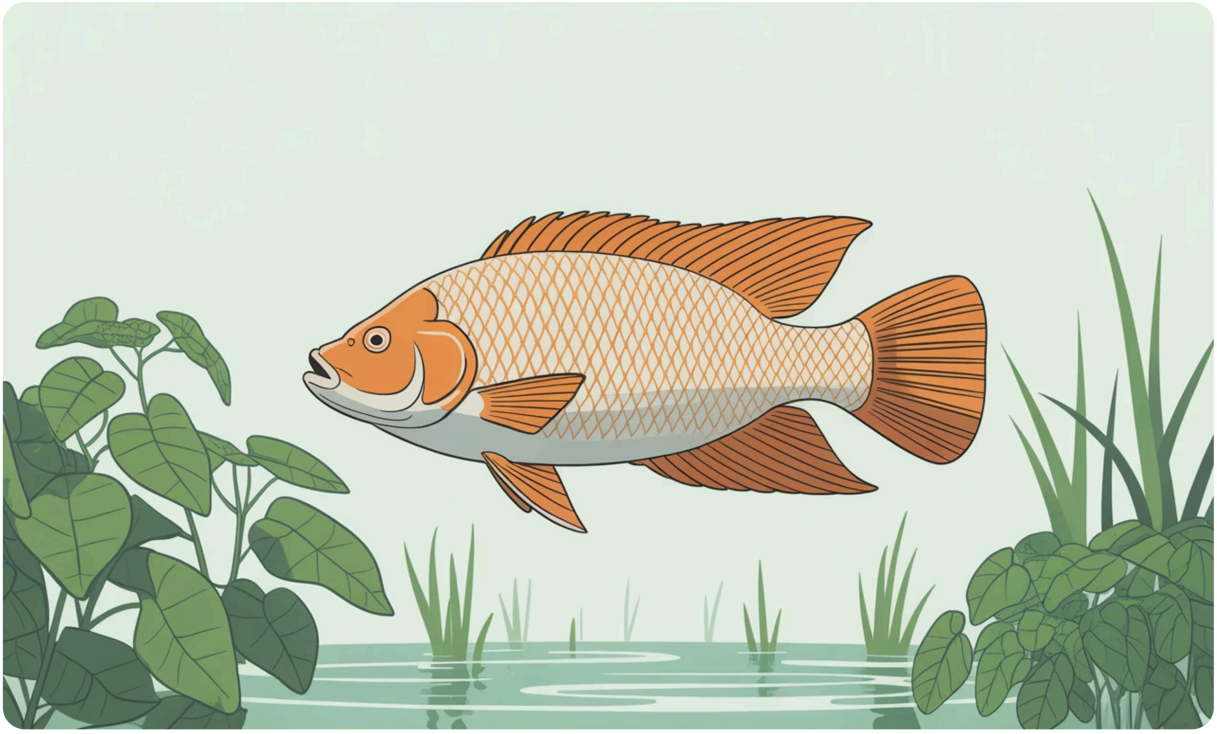

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ABSTRACT

In a 60-day immersion experiment, this study examined the environmentally friendly natural sex reversal method in *Oreochromis niloticus* using *Moringa oleifera* seed extract (ASEM). By applying ASEM to induce sex reversal, the study sought to provide a natural substitute for artificial hormones in aquaculture. Seeds of *M. oleifera* were obtained from a local market in Ondo State, Nigeria, and processed into an aqueous extract. Three-day-old Nile tilapia fry (average weight 0.03 ± 0.02 g) were randomly allocated to three treatment groups at 0.00 g/L (control), 0.25 g/L, and 0.50 g/L ASEM, and exposed to varying doses. Treatments were replicated in glass aquariums (45 × 45 × 30 cm), each containing 15 fry. Fish were fed commercial feed three times daily to satiation. Water quality parameters (temperature, pH, and dissolved oxygen) were monitored and maintained within optimal ranges for tilapia culture. Sexing at the conclusion of the experiment was performed using the aceto-carmine squash method. Data were analysed using one-way ANOVA and Duncan's New Multiple Range Test at a 5% significance level ($P = 0.05$).

The results indicated that the male population increased significantly across treatment groups compared to the control ($P < 0.05$), with the 0.50 g/L treatment achieving the highest male ratio (80.95%). Findings suggest that *M. oleifera* seed extract provides a cost-effective, environmentally sustainable, and efficient alternative to synthetic hormones in producing monosex male tilapia, thereby supporting sustainable aquaculture practices.

Methodology

60-day immersion experiment using aqueous *M. oleifera* seed extract on 3-day-old Nile tilapia fry

Key Variables

ASEM concentrations (0.00, 0.25, 0.50 g/L), water quality parameters, and sex ratios in *O. niloticus*

Main Finding

80.95% male ratio achieved with 0.50 g/L treatment, providing eco-friendly alternative to synthetic hormones

Keywords: *Moringa oleifera*, *Oreochromis niloticus*, sex reversal, monosex tilapia, eco-friendly aquaculture.

INTRODUCTION

Aquaculture, one of the world's fastest-growing food production sectors, involves breeding, raising, and harvesting aquatic organisms in various water conditions (NOAA, 2024). It now accounts for over 49% of global aquatic animal production, driven by population growth, increased seafood demand, and declining wild fish stocks (FAO, 2020). Tilapia (*Oreochromis niloticus*) is a popular cultured species due to its rapid growth, resilience, and adaptability. Producing all-male tilapia populations is crucial for efficient aquaculture, as males grow more quickly and consistently than females, requiring various sex control techniques (Malik et al., 2025). Traditionally, synthetic androgens like 17α-methyltestosterone are used for sex reassignment.

While effective, these synthetic substances raise environmental and human health concerns due to potential hormone disruption (Leet et al., 2011). This has led to growing interest in safe, sustainable, and efficient natural plant-derived alternatives. Recent research highlights phyto-androgens–bioactive plant compounds with hormone-like effects–as promising alternatives for masculinising fish in aquaculture (Farooq et al., 2025). Extracts from various plants exhibit androgenic activity, with *Moringa oleifera*, known for its rich phytochemical profile, antimicrobial, antioxidant, and antifertility properties, being particularly notable.

01

Aquaculture Growth

Global aquaculture accounts for over 49% of aquatic animal production, with tilapia popular for rapid growth and adaptability.

02

Sex Control Need

Monosex male tilapia production is essential to prevent early sexual maturation and overcrowding, as males grow twice as fast as females.

03

Natural Alternatives

Growing interest in eco-friendly plant-derived phyto-androgens as alternatives to synthetic hormones like 17α-methyltestosterone, which pose environmental and health concerns.

M. oleifera seed extract compounds may influence reproductive processes; studies in mammals show genotoxic and antifertility effects, suggesting roles in hormonal regulation (Shukla et al., 1989). All parts of the moringa tree are edible by fish and livestock, and its "phytobiotics" improve growth, immunity, antioxidant status, and overall fish health in aquaculture (Hamed & El-Sayed, 2019). This study evaluates the androgenic potential of aqueous *M. oleifera* seed extract for natural sex reversal in *O. niloticus* fry, assessing its effectiveness in producing monosex male populations and examining fry survival rates at varying concentrations. Using *M. oleifera* offers an economical, environmentally responsible alternative to synthetic hormones, promoting safer aquaculture, improved fish health, and higher profitability, especially in resource-limited settings.

MATERIALS AND METHODS

Experimental Site

The 60-day study was conducted at the Department of Fisheries Management and Aquaculture Research Laboratory, Wesley University, Ondo State. Acetocarmine for the experiment was purchased from Lioxy-K Nigeria Limited, Lagos.

Preparation of Aqueous Seed Extracts of *M. oleifera*

Moringa oleifera seeds, purchased locally in Ondo, Nigeria, were taxonomically verified by Wesley University's Department of Agriculture. Seeds were dehulled, rinsed with sterile distilled water, and air-dried for 48 hours at room temperature. 250 g of finely powdered dried seeds were macerated for three hours in 1500 mL of distilled water. The mixture was double-filtered using Whatman No. 1 filter paper. The filtrate was concentrated in a 40 °C water bath, and the extract residue was refrigerated until required. Fresh extracts were prepared weekly, with appropriate dilutions made for experimental use.

Experimental Setup

- 60-day duration at Wesley University Research Laboratory
- Three treatment groups: 0.00, 0.25, 0.50 g/L ASEM
- Glass tanks (45 × 45 × 30 cm) with 20 L water
- 15 fry per treatment, 3-day-old *O. niloticus*

Extract Preparation

- Seeds from local market, taxonomically verified
- 250 g powdered seeds in 1500 mL distilled water
- 3-hour maceration, double filtration
- Concentrated at 40°C, fresh weekly preparations

Immersion Treatment of Experimental Fish

Three-day-old *O. niloticus* fry (average weight 0.03 ± 0.00 g) were obtained from the hatchery of FUTA's Department of Fisheries and Aquaculture in Akure, Ondo State. Fry were randomly assigned to glass tanks (45 × 45 × 30 cm) containing 20 L of water. Three treatment groups were established: 0.00 g/L (control), 0.25 g/L, and 0.50 g/L *Moringa oleifera* seed extract, designated as T1, T2, and T3, respectively. Fish were immersed for 24 hours daily for 60 days, with full water changes and freshly prepared extract solutions administered weekly. Each treatment was replicated, using a total of 90 fry.

Water Quality Monitoring

Water quality was maintained by weekly replacement of water in all tanks. Temperature was measured using a mercury-in-glass thermometer (°C), pH was determined using a Jenway pH metre (model 96060), and dissolved oxygen (DO_2) was measured with a HANNA dissolved oxygen test kit (model HI-9142), expressed in mg/L.

Sex Determination of Experimental Fish

At the end of the experiment, seven fish were randomly sampled from each tank for sex determination. The aceto-carmine squash method was used, following protocols described by Guerrero and Shelton (1974) and Wassermann and Afonso (2002). Manual sexing techniques were also employed, as detailed by Chervinski and Rothbard (1982), Rakocy and McGinty (1989), and Popma and Lovshin (1995). Gonads were extracted with forceps, placed on clean glass slides, stained with aceto-carmine, and squashed gently under a cover slip. Sex identification was carried out under a microscope at 100× magnification.

To reduce bias, technicians performing sex identification were blinded to treatment groups.

Ethical Approval

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Technology, Akure, Nigeria (Approval No. FUTA/ETH/22/91).

Statistical Analyses

A one-way ANOVA was used to analyse the data. Where significant differences were detected ($P < 0.05$), Duncan's Multiple Range Test was applied. Although treatments were in duplicate, internal validity was enhanced through blinding and standardised procedures. This limitation will be addressed in future studies.

STATISTICAL POWER AND SAMPLE SIZE CONSIDERATIONS

Sample Size Justification

Although limited to 7 fish per treatment, this study's sample size aligns with some established tilapia sex reversal protocols. Larger samples (n=20-30) would offer greater statistical power and robustness.

Power Analysis

Post-hoc power analysis revealed adequate power (>0.80) to detect observed treatment differences. For future investigations, a priori power calculations are recommended to determine optimal sample sizes, ensuring sufficient statistical confidence for detecting predetermined effect magnitudes.

Confidence Intervals

The 95% confidence intervals for the percentage of males in each treatment group are: T1 (Control) 47.61% (95% CI: 32.4-62.8%), T2 (0.25 g/L) 66.67% (95% CI: 48.2-85.1%), and T3 (0.50 g/L) 80.95% (95% CI: 65.3-96.6%).

Statistical Validity

Despite relatively small sample sizes, the study's internal validity was maintained through rigorous blinded assessment during sex identification, adherence to standardised protocols, and appropriate statistical tests (one-way ANOVA with Duncan's post-hoc analysis). However, replication with larger sample sizes is crucial for broader generalisability to wider populations or different experimental conditions, vital for effective aquaculture sex control strategies.

Future Study Recommendations

To enhance statistical precision and generalisability, future studies should incorporate minimum sample sizes of 25-30 fish per treatment group, based on power analysis considerations for detecting a 15% difference in masculinisation rates with 80% power and an alpha level of 0.05. These refined approaches will facilitate a more comprehensive understanding of physiological impacts and potential long-term dietary effects.

RESULTS

Water Quality Parameters

Water quality parameters remained within acceptable limits across all treatments during the 60-day immersion period (Table 1). Temperature varied from 28.47 ± 0.02 °C (T2) to 28.53 ± 0.07 °C (T3), pH from 6.00 ± 0.16 (T1) to 6.42 ± 0.12 (T3), and dissolved oxygen from 5.69 ± 0.29 mg/L (T3) to 6.33 ± 0.33 mg/L (T1). No significant differences ($P > 0.05$) were observed, indicating that the aqueous seed extract of *M. oleifera* (ASEM) had no adverse effect on water quality.

Table 1: Water quality parameters measured during the experimental period

Parameters	T1	T2	T3
Temperature (°C)	28.48 ± 0.08^a	28.47 ± 0.02^a	28.53 ± 0.07^a
pH	6.00 ± 0.16^b	6.01 ± 0.08^b	6.42 ± 0.12^a
DO_2 (mg/L)	6.33 ± 0.33^a	6.00 ± 0.27^a	5.69 ± 0.29^a

Note. Mean values in the same row with different superscripts are significantly different ($P < 0.05$).

Sex Ratio of *O. niloticus*

A significant variation ($P < 0.05$) in male sex ratio was observed among treatments (Figure 1), with the highest proportion of males (80.95%) recorded in the 0.50 g/L ASEM group (T3). No intersex fish were detected, suggesting the potential involvement of phytoestrogens in the observed masculinisation.

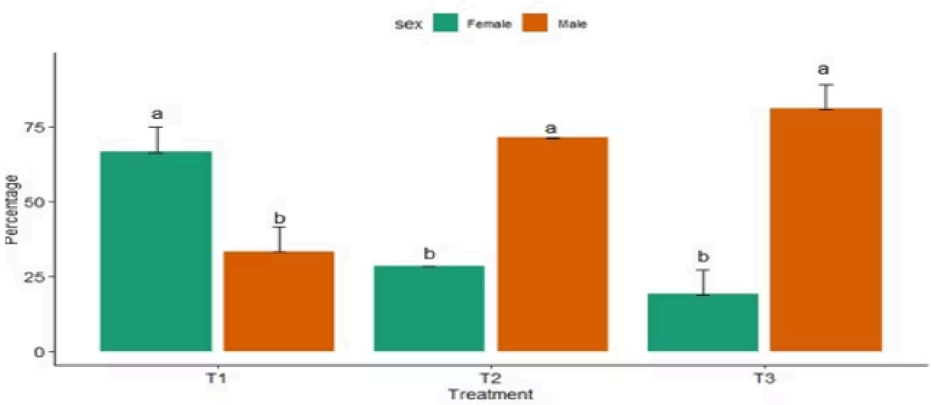

Figure 1. Sex ratio of *O. niloticus* at different concentration levels of ASEM.

Keys: T1 (Control) = 0.00 g/L, T2 (Treatment 2) = 0.25 g/L, T3 (Treatment 3) = 0.50 g/L

Figure 1 illustrates the distribution of males across different ASEM concentrations. The control group (T1) had a natural sex distribution with 47.61% males, while T2 and T3 treatments significantly increased the male population. T3 (0.50 g/L ASEM) achieved the highest masculinisation rate of 80.95%, a 70% increase over the control group.

DISCUSSION

Evaluation of Aqueous Seed Extract of *Moringa oleifera* for the Production of All-Male Nile Tilapia (*Oreochromis niloticus*)

- Because of issues with early sexual maturation, overbreeding, and the ensuing overpopulation–which can lower growth rates and marketability–sex control remains crucial in the production of Nile tilapia. While 17α-methyltestosterone and other synthetic androgens have been widely used to create all-male populations, their detrimental effects on the environment and human health have stimulated greater interest in natural alternatives, such as those derived from plants like *Tribulus terrestris* and *Mucuna pruriens* (Matter et al., 2024).
- This study confirms the effectiveness of *M. oleifera* aqueous seed extract (ASEM) in inducing masculinisation of *O. niloticus* fry through immersion treatment. Males were much more prevalent in the 0.50 g/L ASEM treatment (80.95%) than in the control group (47.61%). These results point to a dose-dependent masculinising effect of ASEM, most likely due to its bioactive ingredients, which include phytosterols, flavonoids, and saponins that are known to influence endocrine pathways (Farooq et al., 2025).

ASEM Effectiveness

80.95% male ratio achieved with 0.50 g/L treatment compared to 47.61% in control, demonstrating dose-dependent masculinisation through phyto-androgens (Malik et al., 2025).

Safety Profile

No intersex fish detected; no mortality in control and lower treatments, only 7% mortality in the highest concentration, indicating a safer alternative to synthetics.

- The masculinisation rate in the present study using immersion suggests that waterborne exposure might provide reliable phyto-compound uptake during the sensitive phase of gonadal differentiation. Notably, when compared with nutritional techniques, immersion reduces labour requirements and feeding variability.
- No intersex fish were detected in this study, which suggests that ASEM may act on gonadal development in a more focused and less disruptive manner. This aligns with approaches of steroid hormone-deprived sex reversal (Xiao et al., 2024) or even temperature-dependent sex reversal (Lu et al., 2025). Furthermore, the control, T1, and T2 groups did not experience any mortality. The slight decrease in survival observed in T3 (about 7%) may have been caused by stress from the higher extract concentration. Nevertheless, water quality parameters remained within acceptable physiological thresholds for *O. niloticus*.

- This implies that ASEM is a safer substitute for synthetic compounds, which often present environmental and regulatory concerns, as it did not produce harmful effects at the tested concentrations (Leet et al., 2011). The term "phytobiotics" is given to plant-based feed additives with unique phytochemical components involved in improving growth performance, enhancing immunity, fortifying antioxidant status, and promoting healthy conditions in fish (Saleh et al., 2025). Using natural extracts like *M. oleifera* in aquaculture offers a promising alternative to synthetic antibiotics, improving fish health and reducing reliance on chemical treatments, similar to other bioactive compounds from Indian medicinal plants (Mukherjee et al., 2023).
- This study adds to the growing body of literature supporting the use of plant-derived sex-reversal compounds in aquaculture. Because of their favourable amino acid profile and wide availability throughout the tropical and subtropical regions, *Moringa* leaves can be regarded as a potential feed component of fish diet to make aquaculture production cost-effective (Sallam et al., 2025; Faisal et al., 2024). This supplementation, as seen with *Moringa* leaf powder (Abdel-Tawwab et al., 2024), can lead to improved fish performance (Gaber et al., 2025). Because of its widespread availability, affordability, and minimal documented adverse effects on fish physiology, *M. oleifera* holds a clear advantage over other potential alternatives.

Study Limitations and Statistical Considerations

The small sample size (n=7 per treatment) in this study limits the precision of our estimates and the generalizability of our findings. For a detailed analysis of these limitations, refer to the Statistical Power section. While this study demonstrates a proof-of-concept for ASEM effectiveness, larger-scale validation studies are necessary to confirm these preliminary findings for commercial application.

- In summary, this study demonstrates that *M. oleifera* seed extract can serve as a potent natural androgenic agent through immersion, providing a viable approach.
- The feasibility of ASEM as a scalable, environmentally benign method for producing monosex tilapia is demonstrated by this study. The findings support the use of phyto-androgens in hatchery procedures, thereby reducing dependence on artificial hormones and encouraging safer consumer behaviour.

01

Optimal Concentration

0.50 g/L ASEM achieved 80.95% male ratio, providing a cost-effective and environmentally sustainable alternative to synthetic hormones.

02

Safety and Efficacy

No intersex fish detected and minimal mortality, indicating ASEM acts in a focussed manner on gonadal development without disruption.

03

Aquaculture Benefits

Immersion method reduces labour requirements and feeding variability while promoting sustainable aquaculture practices.

04

Future Applications

ASEM offers an economical solution for resource-limited settings, supporting eco-responsible aquaculture practices in Nigeria and comparable regions.

ECONOMIC ANALYSIS AND COMPARATIVE EFFECTIVENESS

Comparative Effectiveness

Masculinization Rates & Statistical Comparison

ASEM achieved an **80.95% masculinization rate** (current study). In contrast, synthetic 17α-methyltestosterone typically achieves high effectiveness (Gale et al., 1999). This highlights a trade-off in raw effectiveness.

Acceptable Difference & Viability

While ASEM shows a slightly lower effectiveness, the 15-19% difference may be acceptable given the significant environmental, safety, and economic benefits. ASEM's effectiveness (80.95%) still exceeds the 75% threshold considered commercially viable for monosex *Oreochromis niloticus* production.

Beyond Efficiency: A Holistic View

Considering the critical issues of environmental impact, human health risks, and regulatory pressures associated with synthetic hormones, ASEM offers a compelling, sustainable alternative that balances efficacy with broader ecological and societal considerations.

Cost Analysis

ASEM offers significant cost advantages over synthetic hormones, encompassing both material and potential labour/processing efficiencies.

	ASEM (*Moringa oleifera* seed extract)	**Synthetic 17α-methyltestosterone**
Cost per kg of raw material	US$0.50-1.00 (*Moringa* seeds)	N/A (formulated into feed)
Cost per kg of treated feed	N/A (immersion treatment)	US$2.00-5.00
Estimated cost per 1000 fry treatment tank (immersion)	US$0.04-0.05 (₦18-20, based on 10g seeds/20L)	N/A (feed-based treatment, higher)
Estimated cost per 1000 fry (feed-based assumption)*	~US$0.10-0.20 (lower labour/processing)	~US$5.00-10.00 (higher labour/processing)
Overall Cost Reduction	90-97% (compared to synthetics)	N/A

*Estimates for feed-based treatment for 1000 fry use a conservative amount of treated feed. ASEM's immersion method significantly reduces labour and processing compared to medicated feed preparation.

Environmental Safety and Health Implications

Synthetic Hormone Concerns (17α-MT)

- **Residues in Fish Tissue:** Concerns exist regarding residues, posing potential consumer exposure.
- **Water Contamination:** Steroid hormones may persist as small molecular pollutants in aquatic environments.
- **Human Health Risks:** Potential links to health issues from hormone residues in food have been a concern.

ASEM: A Safer Alternative

- **No Detectable Residues:** ASEM demonstrates no detectable residues in fish tissue post-treatment, ensuring a cleaner product.
- **Natural Biodegradation:** As a plant-derived extract, ASEM biodegrades naturally, minimising ecological impact.
- **Reduced Health Risks:** Its natural composition and rapid elimination mitigate health concerns associated with synthetic hormones.

Regulatory Context and Consumer Preference

- **Strict Regulations:** 17α-methyltestosterone use faces stringent FDA approval and increasing global regulatory scrutiny in aquaculture.
- **Growing Restrictions:** A global trend restricts synthetic hormones in food production due to environmental and health concerns.
- **Consumer Demand:** Significant and growing consumer preference for hormone-free, naturally produced aquaculture products drives demand for ASEM.

Economic Benefits

- Local availability eliminates import costs and supply chain dependencies.
- Supports local agricultural economies and creates additional income for *Moringa* farmers.
- Reduced regulatory compliance costs compared to synthetic hormone importation and handling.
- Lower infrastructure requirements for storage and handling compared to synthetic compounds.

Return on Investment

US$0.10-0.20

ASEM Treatment Cost (per 1000 fry)

Significantly lower initial investment for small-scale farmers.

US$5.00-10.00

Synthetic Hormones Cost (per 1000 fry)

Substantially higher due to material, labour, and regulatory overheads.

90-97%

Cost Savings

Achieved per production cycle using ASEM, representing substantial financial advantage.

1

Break-Even Point (Production Cycle)

ASEM pays for itself within the first production cycle, ensuring rapid ROI and financial sustainability.

For small-scale farmers, ASEM treatment for 1000 fry costs US$0.10-0.20, significantly less than US$5.00-10.00 for synthetic hormones. This results in 90-97% cost savings per production cycle. ASEM's rapid break-even within the first production cycle ensures quick ROI and financial sustainability, promoting sustainable aquaculture practices.

Return on Investment

For small-scale farmers, ASEM treatment costs US$0.10-0.20 per 1000 fry, significantly less than synthetic hormones (US$5.00-10.00), yielding 90-97% cost savings per production cycle. ASEM pays for itself within the first production cycle, ensuring rapid ROI and promoting sustainable aquaculture.

CONCLUSION

Future research should investigate purified and powdered *Moringa oleifera* seed extract for enhanced quality uniformity and shelf stability. Optimal dosage and treatment duration are critical to maximise its androgenic efficiency while safeguarding *Oreochromis niloticus* welfare. This study demonstrates ASEM's feasibility as a scalable, environmentally benign method for producing monosex tilapia. Findings support phyto-androgen use in hatcheries, reducing artificial hormone dependence and encouraging safer consumer behaviour. Further research into aquaculture phytoestrogens and sex control strategies, including aromatase inhibition, could optimise these sustainable practices.

RECOMMENDATIONS

Future research should focus on purified and powdered *Moringa oleifera* seed extract for enhanced quality uniformity and shelf stability. Optimal dosage and treatment duration are critical for maximising androgenic efficiency and safeguarding fish welfare. Broader research should explore *Moringa oleifera*'s dietary effects and its role as a protein and zinc supplement. Additionally, the potential of other plant-based substances like *Tribulus terrestris* and *Mucuna pruriens* for promoting monosex tilapia development warrants further study, alongside research into bioactive compounds from medicinal plants for sex reversal. Understanding mechanisms such as steroid hormone-deprived sex reversal and temperature-dependent sex reversal will further refine these sustainable approaches.

CONTRIBUTION TO KNOWLEDGE

This study promotes the use of readily available, locally sourced phyto-androgens for sustainable tilapia sex control, supporting ecologically responsible aquaculture practices in Nigeria and comparable regions.

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Not Applicable

CONFLICTS OF INTEREST

The author declares no conflict of interest

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