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Efficacy of *Basella alba* L. Extract on the Masculinization of Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758) Using Immersion and Oral Administration Techniques

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Abstract

The study evaluated the efficacy of *Basella alba* L. extract in inducing masculinization in Nile tilapia (*Oreochromis niloticus*) fry and eggs using oral administration and immersion techniques. Tilapia seedstocks were randomly assigned to negative control (no hormonal treatment), positive control (17α – methyltestosterone or 17α-MT), and *B. alba* extracts. Each treatment was administered via egg immersion, fry immersion, and oral administration, with the setup replicated three times. Yellow eggs were immersed for 96 hours in aqueous *B. alba* extract and 17α-MT hormone, while fish fry was immersed two hours weekly over 28 days. Oral administration involved feeding fish fry with feeds added with *B. alba* ethanol extract and 17α-MT. The study employed the standard acetocarmine squash technique of gonads to analyze the sex reversal percentage. The findings demonstrated that immersion of eggs and fry in *Basella alba* extract resulted in higher sex reversal percentages compared to orally treated fish fry, suggesting the effectiveness of both egg and fry immersion as masculinization techniques. Moreover, there were no significant differences in the percentage of males between groups treated with 17α-MT and those treated with *B. alba* extract, regardless of whether the treatment was administered orally or through immersion. These findings highlight the potential of *Basella alba* extract as a viable alternative to synthetic steroid hormones for achieving masculinization in Nile tilapia. Furthermore, egg and fry immersion can be considered an alternative technique to the traditional sex reversal method of feeding the fry with hormone-treated feeds.

Keywords: Nile tilapia, *Basella alba*, Sex reversal, Immersion, 17α-MT

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INTRODUCTION

Although tilapia (*Oreochromis niloticus*) is an introduced species in the Philippines, it has become the second largest freshwater cultured species by volume, following milkfish (Guerrero, 2017). Globally, tilapia ranks as the third most widely farmed finfish, surpassed only by grass carp (*Ctenopharyngodon idellus*) and silver carp (*Hypophthalmichthys molitrix*) as reported by Radkhah and Eagderi (2021a) and the Food and Agriculture Organization (FAO) in 2022. This highlights the importance of Nile tilapia as a significant source of protein for human consumption worldwide (Arumugam et al., 2023).

The significant increase in tilapia production can be attributed to its rapid growth rate and short production cycle. In commercial aquaculture, monosex "all-male" tilapia populations are preferred due to their higher energy efficiency and faster growth than females (Megbowon and Mojekwu, 2014). Techniques to produce all-male populations include manual sex separation (Radkhah and Eagderi, 2021b) genetic manipulation or YY-male technology (Mair et al., 1997; Jordaan, 2004), and hormonal sex reversal (Desprez et al., 2003). Among these methods, synthetic hormones, particularly 17α-methyl testosterone (17α-MT), are the most common, achieving high masculinization success rates (Wassermann and Afonso, 2002). The efficacy of 17α-MT is linked to its ability to inhibit aromatase activity, thus preventing estrogen production and promoting androgenesis in developing gonads (Golan and Levavi-Sivan, 2014). However, synthetic hormones have raised health concerns for hatchery workers and environmental issues (Megbowon and Mojekwu, 2014; Abaho et al., 2021), prompting research into phytochemical alternatives.

Plant parts and extracts are increasingly utilized in aquaculture, although research has focused on their effects on fish growth and immunity. Reverter et al., (2014) and Van Hai (2015) indicated that plant extracts containing phytochemicals such as isoflavonoids, flavonoids, and saponins, which possess estrogenic and androgenic properties, could potentially replace synthetic steroid hormones for sex reversal in tilapia. Notable plants with phytochemicals capable of altering the sex ratio in favor of males include pine pollen (Abaho et al., 2022), aloe vera (Gabriel et al., 2016), *Glycine max* (Kefi, 2014), *Tribulus terrestris (*Amer et al., 2021; Ghosal and Chakraborty, 2014), ginseng (Mansour et al., 2018), *Basella alba* (Ghosal et al., 2016), *Quillaja saponaria*, and *Trigonella foenum-graecum* (Stadtlander et al., 2012).

Basella alba L. commonly known as Malabar spinach, is a succulent, branched, smooth, twining herbaceous vine. The leaves of *B. alba* contain phytochemicals such as flavonoids, tannins, steroids, and saponins (Ghosal et al., 2016). Previous studies have demonstrated the androgenic activities of *B. alba* in the masculinization of Nile tilapia (Asad et al. 2024; Radkhah and Eagderi, 2021b; Yusuf et al., 2019). Therefore, this study aimed to validate the effect of *B. alba* on the masculinization of *O. niloticus* through different techniques and determine the most effective method of administration for achieving the highest percentage of male tilapias.

MATERIALS and METHODS

Experimental Design and Treatment

The experiment was conducted in a Completely Randomized Design (CRD). Experimental fish seeds were randomly distributed to different treatments and replicated three (3) times with 60 fry/egg in each replicate. The experimental design encompassed three main treatments: negative control (no hormonal treatment), positive controls (17α -MT), and *B. alba* extracts, all administered in three different techniques, namely, egg immersion, fry immersion, and oral administration.

Conditioning and Pairing of Broodstock

Tilapias are capable of mating at an early age of at least 2-3 months from the start of culture. Female and male broodstock were conditioned separately in hapa nets for 10 days and were fed a commercial diet at 3% of their body weight.

The broodstock was paired by stocking three females for every single male in a hapa measuring 2m × 1m × 1m (L×W×H).

Egg Collection

Egg collection commenced 10 days after the first day of pairing. The female tilapia normally holds the fertilized eggs in their buccal cavity for incubation. Yellow and/or eyed eggs were collected and transferred to artificial incubation units for incubation and treatment.

Egg Incubation and Hatching

A simple tilapia egg incubation system was developed using inverted 1.5 L capacity empty plastic soda bottles. The bottoms of the bottles were cut off to make ideal downwelling incubators. A fixed pipe above the container provides water entry. Water flow was adjusted to gently circulate the eggs, simulating the movement of eggs in the buccal cavity of the female to enable hatching. The larvae were moved to several trays immediately after hatching and kept separately until they reached the yolk sac fry stage.

Leaf Extract Preparation

Matured leaves from *Basella alba* plant of at least 100 days old were procured from a Malabar spinach farmer. The leaves were washed with distilled water, air-dried, and pulverized. Aqueous leaf extract was prepared by heating 18 g of powdered leaves in 1500 ml distilled water at 100 ºC for 30 minutes then filtered with Whatman filter paper of 11µm pore size, twice (Ghosal and Chakraborty, 2014). On the other hand, ethanolic extract was prepared according to the method described by Mukherjee et al., *(2018).* Briefly, 250 g of powdered leaves was soaked in 500 ml ethanol for 5 hours. The ethanolic extract was evaporated using a rotary evaporator and stored at -20^{o}C.

Egg Immersion Technique

Sixty yellow eggs per replicate were immersed for 96 hours in one liter of *B. alba* extract (0.12g/L) and 17α-MT (800µg/L). Aeration was provided into plastic containers that measured 30cm × 20cm × 20cm (L×W×H) to facilitate the continuous movement of eggs and provide sufficient oxygen for the developing embryos. The solutions (*B. alba* extract and 17α-MT) were replaced regularly with freshly prepared solutions using a siphon hose (0.5cm Ɵ). After immersion, hatched eggs were transferred to hapa nets measuring 1m × 2m × 1m (L×W×H). The Nile tilapia fry was reared and fed a commercial diet for 60 days for further development.

$$SGR = \frac{lnW_f - lnW_i}{t} \times 100$$

$$Hatching\ rate = \frac{no.of\ hatched\ larvae}{initial\ no.ofeggs} \times 100$$

$$Survival\ Rate = \frac{final\ stock}{initial\ stock} \times 100$$

Fry Immersion Technique

Batches of sixty Nile tilapia yolk-sac fry (5 days old) were immersed in one liter of *B. alba* extract (0.12g/L) and 17α-MT (800µg/L) for two hours every seven days for 28 days. The solution/s was aerated and replaced regularly by siphoning or using a siphon hose (0.5cm Ɵ). The experimental fish were then reared in hapa nets and were fed with a commercial diet for further development.

Table 1. Composition of the commercial diet used in the experiment

Composition	Percentage
Crude Protein	42
Crude Fat	5
Crude Fiber	5
Ash or Mineral	16
Moisture	12

Oral Administration Technique

Ethanolic leaf extract of *B. alba* was sprayed evenly on one kilogram of fry booster feeds. Meanwhile, 0.06 g of 17α-MT was dissolved in 500 ml ethanol and evenly sprayed on one kilogram of fry booster feeds. The treated feeds were spread on a platform for air drying at room temperature. Fry with fully absorbed yolks were fed the treated air-dried feeds (10% BW) three times daily for 28 days. For continued development, the fish were nursed in hapa net cages.

Sex Identification

Ten (10) fish from each replicate were randomly selected and prepared for sex identification following the gonad squash technique by Guerrero and Shelton (1974). The experimental fish were dissected, and the gonads were placed on a glass slide. A few drops of acetocarmine were added, and the gonads were gently squashed with a cover slip. Subsequently, the mounts were observed under a microscope for sex identification.

$$\%\, male = \frac{no.\ of\ identified\ males}{total\ no.of\ fish\ samples} \times 100$$

Statistical analysis

The data collected were: the percentage of males, hatching rate, and survival rate. Statistical analyses were carried out using SPSS version 20. One-way analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to determine the significant difference among treatments. The least significant difference (LSD) test was used to compare treatment means at a 5% level.

RESULTS and DISCUSSION

Table 2 presents the growth rates of Nile tilapia subjected to various sex reversal techniques. Findings showed that the gain in weight of fry hatched from eggs immersed in 17α-MT was significantly higher (9.32±0.32) compared to both the negative control (7.74±0.03) and those immersed in aqueous *B. alba* extract (8.32±0.41).

On the other hand, no significant differences were observed among treatment means in the oral administration and fry immersion techniques.

Table 2. Effect of *B. alba* extracts on the specific growth rate (mean±SD) of Nile Tilapia subjected to sex reversal using the egg/fry immersion and oral administration techniques

Treatment	Specific Growth Rate (SGR)		
	Egg Immersion Technique	**Fry Immersion Technique**	**Oral Administration Technique**
Negative Control	7.74 ± 0.03^{b}	7.74 ± 0.03^{b}	7.74 ± 0.03^{b}
Basella alba	8.32 ± 0.41^{b}	8.30 ± 0.49^{b}	7.76 ± 0.19^{b}
17 α-MT	9.32 ± 0.32^{a}	7.77 ± 0.04^{b}	8.29 ± 0.03^{b}

**Values with different superscripts are significant at the 5% level.*

Medicinal plants containing diverse groups of phytochemicals such as phenolics, flavonoids, alkaloids, polysaccharides, and volatile oils have been reported to act as antimicrobial agents and to stimulate both specific and non-specific immunity in fish by modulating the functions of the immune cells increasing antibody production (Ghosal et al., 2020b; Chakraborty and Hancz, 2011) and eliminating undesirable constituents in the intestine. These phytochemicals enhance the absorption and/or stability of essential nutrients (Holst and Williamson, 2008) and have been linked to increased fish growth (Makkar et al., 2007; Chakraborty et al., 2013).

However, the results of this study showed that eggs immersed in 17α-MT had better growth performance compared to those immersed in the aqueous leaf extract of *Basella alba* and those that received no hormone solution. It was initially suspected that the higher growth rate observed could be attributed to the lesser number of fry survivors in this treatment, which means that there is a lower degree of food, dissolved oxygen, and space competition between fishes. It was mentioned by Ahmadoon et al., (2023) that growth depends on the number of factors divided into exogenous and endogenous factors. Exogenous factors include environmental parameters such as dissolved oxygen, degree of competition, amount of the nutrient, salinity, and temperature. In addition, the difference in growth rate established by young fish is not indeterminate and may not persist throughout life. Initially, slow-growing fishes may surpass fast-growing fish and finally reach a greater length and size (Kefi, 2014). However, the results revealed that survival has no effect ($p>0.05$) on the differences in SGR.

Table 3 demonstrates comparable sex inversion rates for Nile Tilapia using egg and fry immersion techniques in both *B. alba* and 17α-MT treated groups. However, 17α-MT resulted in a notably higher ($p<0.05$) sex inversion rate when using the oral administration technique. It is also evident from the results that, among the three techniques used, the egg immersion technique produced the highest percentage of males.

Table 3. Effect of *B. alba* extracts on the sex inversion (mean±SD) of Nile Tilapia using egg immersion, fry immersion, and oral administration techniques

Treatment	Percent Male (%)		
	Egg Immersion Technique	**Fry Immersion Technique**	**Oral Administration Technique**
Negative Control	46.71±8.72^{bc}	46.71±8.72^{bc}	46.71±8.72^{c}
Basella alba	80.20±7.86^{a}	75.00±6.90^{a}	64.96±13.14^{b}
17 α-MT	82.63±8.24^{a}	70.00±6.50^{ab}	74.76±4.63^{ab}

**Values with different superscripts are significantly different at the 5% level.*

The efficacy of *B. alba* extract on sex inversion was investigated in this study using three modes of introduction: egg immersion, fry immersion, and in-feed administration. The findings of the study revealed that the use of *B. alba* extract for sex inversion is as effective as the use of the synthetic hormone 17α-methyltestosterone. The study also demonstrated, through egg immersion, the efficacy of masculinization treatment during embryonic development, supporting the report of Rougeot et al., (2008) that the development of primordial germ cells and/or future somatic cells of the presumptive gonads is influenced by hormones. The egg immersion technique is rarely used in the sex manipulation of Nile tilapia. The most used method is oral administration, which takes 21 to 28 days to complete. However, unlike fry immersion and oral administration, the egg immersion technique provides a short-term procedure for masculinization in about 96 hours. The studies of Ghosal and Chakraborty (2014) and Ghosal et al., (2016) on *B. alba* did not include the egg immersion technique. To the knowledge of the authors, this is the first report on the efficacy of *B. alba* extract using the egg immersion method.

In this study, egg immersion using *B. alba* extract attained sex inversion rates of 80.20% which is comparable to 17α-methyltestosterone (82.63%). Additionally, the inversion rate of 75.00% for *B. alba* using the fry immersion technique surpassed the 70.30% rate reported by Ghosal and Chakraborty (2014). This study used a higher concentration of 0.12 g/L, whereas Ghosal and Chakraborty (2014) used a lower concentration of 0.10 g/L.

Previous studies indicated that the extract of *B. alba* leaves was found to stimulate the production of testosterone in testicular fractions and Leydig cell cultures in healthy adult albino male rats (Moundipa et al., 2006; Nantia et al., 2011). The result shows that phytochemical extracts, such as *B. alba* extracts, are more effective when used during the early stages of embryonic development. As the stage of development progresses, the efficiency of *B. alba* extract in sex manipulation decreases. Results indicate that the highest percentage of males was obtained when eggs were used, and the sex inversion rate decreased when yolk-sac fry and fry of advanced age were used.

In the early stage of development, Nile tilapia is sexually undifferentiated. Sexually undifferentiated gonads are bipotent and can either develop into testes or ovaries depending on genetic or environmental factors (Nishimura and Tanaka, 2016). External conditions and exogenous endocrine-active substances play a determining role in the development of sex characteristics. In the natural environment, the endocrine system, especially during the embryonic development of aquatic animals, can be affected by chemical effluents from different industries (Hoy and Benson, 1998). Early exposure to endocrine-disrupting chemicals (EDCs) can have immediate effects on the development of the reproductive tract or the establishment of the various gonad cell types; subsequent effects may be seen on hormonal homeostasis, somatic cell differentiation, gamete production, and gamete quality (Marlatt et al., 2022).

Phytochemicals modulate the endocrine system of fishes; hence these are referred to as endocrine-disrupting compounds (EDCs). The phyto-compounds can disrupt the biosynthesis, distribution, and functions of steroid hormones subsequently interfering with the reproductive physiology of fish (Abajo et al., 2021; Zhou et al., 2019;). It could be that the primordial cells in fish are present in the endodermal layer of the yolk sac (as observed in humans), where the phytochemicals present in *B. alba* extract can easily penetrate. As embryonic development proceeds, the primordial cells migrate into the gonads where they are harder to reach by the phytochemicals. Thus, the time of introduction of an environmental or exogenous endocrine-active chemical stimulus to manipulate the sex of an organism is highly important to attain success. In the case of masculinization, missing the perfect timing could result in a lower number of males being produced (Capel, 2017; Marlatt et al., 2022).

The result of the oral administration in this study (64.96%) is slightly higher than the findings of Omar et al., (2014) on the sex reversal of Nile tilapia using *T. terrestris* (64.48%) and Date palm pollen (56.67%) but is lower than the results obtained by Ghosal *et al*. in 2015 for *B. alba* (83.2±0.7) and *Tribulus terrestris* (88.9±1.1). The immersion technique resulted to significantly higher masculinization percentage than oral treatment. This result could be attributed to the statement of Abaho et al., (2021), that variations in the amount of sex steroids available to the fish, due to the non-uniform distribution of the extracts in the diets during mixing, limit the efficiency of the in-feed technique.

The hatching and survival rates of Nile Tilapia are presented in Table 4. Findings indicate a significantly higher (p<0.05) hatching rate of tilapia eggs immersed in aqueous *B. alba* extract (88.33%±2.08) compared to the negative control (56.67%±8.02), and 17α-MT (53.33%±4.51). No significant variations (p>0.05) in survival rates were observed among treatment means in both fry immersion and oral administration techniques.

Table 4. Hatching and survival rate (mean±SD) of experimental eggs/fish after immersion and oral administration

Treatment	Hatching Rate (%)	Survival Rate (%)	
	Egg Immersion Technique	Fry Immersion Technique	Oral Administration Technique
Negative Control	56.67±8.02^{b}	56.67±8.02^{b}	56.67±8.02^{b}
Basella alba	88.33±2.08^{a}	61.67±4.25^{b}	58.33±3.79^{b}
17 α-MT	53.33±4.51^{b}	58.33±6.51^{b}	53.33±7.02^{b}

**Values with different superscripts are significantly different at the 5% level.*

The highest hatching rate was observed in eggs immersed in aqueous *B. alba* extract. Major biological activities exhibited by *Basella alba* show androgenic, anti-inflammatory, antimicrobial, antioxidant, antiviral, central nervous system depressant, hepato-protective, and wound-healing properties (Chakraborty and Hancz, 2011). These properties are believed to have contributed to the higher hatching rate and comparable survival rate of eggs immersed in *B. alba* extracts.

CONCLUSION

Basella alba extracts can be used with promising results as an alternative to steroid hormones for the masculinization of Nile Tilapia. Both immersion techniques and oral administration of *B. alba* extracts resulted in masculinization. Immersion technique particularly egg immersion resulted in higher sex inversion rates.

Thus, egg immersion can be regarded as an alternative technique to the traditional sex reversal method of feeding the fry with hormone-treated feeds. Further studies, however, are recommended to identify the concentration of the extract that will result in optimal masculinization in Nile Tilapia using the egg immersion technique.

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REFERENCES

Abaho I., Masembe C., Akoll P. & Jones C. L. W. 2021. The use of plant extracts to control tilapia reproduction: Current status and future perspectives, *Journal of the World Aquaculture Society*, 53(3), 593–619.

Abaho I., Akoll P., Jones C. L. W. & Masembe C. 2022. Dietary inclusion of pine pollen alters sex ratio and promotes the growth of Nile tilapia (*Oreochromis niloticus*, L. 1758), *Aquaculture Reports*, 27, 1-10.

Ahmadoon H. G. a. B., Elawad A. N., Alamin A. M. & Babiker A. A. 2023. Chemical Composition of <i>Sphyraena barracuda</i> with Different Area, Size and Season, in Sudanese Red Sea Coast, *Food and Nutrition Sciences*, 14(05), 415–420.

Amer M. A., Zaki F. G., Tahoun A. & Said M. M. 2021. Effects of 17 α-Methyltestosterone and *Tribulus Terrestris* Extract on Sex Ratio and Gonads Histology of Red Tilapia Hybrid, *Egyptian Journal of Nutrition and Feeds*, 24(2), 189–198.

Arumugam M., Jayaraman S., Sridhar A., Venkatasamy V., Brown P. B., Abdul Kari Z., Tellez-Isaias G. & Ramasamy T. 2023. Recent Advances in Tilapia Production for Sustainable Developments in Indian Aquaculture and Its Economic Benefits, *Fishes*, 8(4), 176.

Asad F., Naz S., Ali T., Gul Y., Jamal R., Shaheen Z., Tasadaq M., Nadeem A., Anwar N., Batool N. & Bano S. 2024. Effect of natural and synthetic androgen hormone on sex reversal of Nile Tilapia (*Oreochromis niloticus*), *Brazilian Journal of Biology*, 84, 1-7.

Capel B. 2017. Vertebrate sex determination: evolutionary plasticity of a fundamental switch, *Nature Reviews Genetics*, 18(11), 675–689.

Chakraborty S. B., Horn P. A. & Hancz C. 2013. Application of phytochemicals as growth-promoters and endocrine modulators in fish culture, *Reviews in Aquaculture*, 6(1), 1–19.

Chakraborty S. B. & Hancz C. 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture, *Reviews in Aquaculture*, 3(3), 103–119.

Desprez D., Géraz E., Hoareau M. C., Mélard C., Bosc P. & Baroiller J. 2003. Production of a high percentage of male offspring with a natural androgen, 11β-hydroxyandrostenedione (11βOHA4), in Florida red tilapia, *Aquaculture*, 216(1–4), 55–65.

FAO. 2022. *The State of World Fisheries and Aquaculture*. Towards Blue Transformation. Fisheries and Aquaculture Department, FAO, Rome, Italy

Gabriel N. N., Qiang J., Yu X., He J., Xu P. & Omoregie E. 2016. Sex-Reversal Effect of Dietary Aloe vera (Liliaceae) on Genetically Improved Farmed Nile Tilapia Fry, *North American Journal of Aquaculture,* 79(1), 100–105.

Ghosal I., Mukherjee D. & Chakraborty S. B. 2020b. The effects of four plant extracts on growth, sex reversal, immunological and haemato-biochemical parameters in Nile tilapia, *Oreochmomis niloticus* (Linnaeus, 1758), *Aquaculture Research*, 52(2), 559–576.

Ghosal I. & Chakraborty S. B. 2017. Production of Monosex All-Male Nile Tilapia Using Ethanol Extract of *Tribulus terrestris* Seeds, *Proceedings of the Zoological Society,* 73(2), 188–191.

Ghosal I., Mukherjee D., Hancz C. & Chakraborty S. B. 2016. Production of monosex Nile tilapia, *Oreochromis niloticus* by dietary and immersion treatment with *Basella alba* leaves and *Tribulus terrestris* seeds, *International Journal of Fisheries and Aquatic Studies,* 4(1), 358–363.

Ghosal I., Mukherjee D., Hancz C. & Chakraborty S. B. 2015. Efficacy of *Basella alba* and *Tribulus terrestris* extracts for production of monosex Nile tilapia, *Oreochromis niloticus*. *Journal of Applied Pharmaceutical Science,* 5(8), 152–158.

Ghosal I. & Chakraborty S. B. 2014. Effects of the aqueous leaf extract of *Basella alba* on sex reversal of Nile tilapia, *Oreochromis niloticus, IOSR Journal of Pharmacy and Biological Sciences,* 9(2), 162–164.

Golan M. & Levavi-Sivan B. 2014. Artificial masculinization in tilapia involves androgen receptor activation, *General and Comparative Endocrinology*, 207, 50–55.

Guerrero A. 2017. Farmed Tilapia Production in the Philippines Is Declining: What Has Happened and What Can Be Done, *Philippine Journal of Science,* 148 (2), 11-15.

Guerrero R. & Shelton W. L. 1974. An Aceto-Carmine squash method for sexing juvenile fishes, *The Progressive Fish Culturist*, 36(1), 56.

Holst B. & Williamson G. 2008. Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants, *Current Opinion in Biotechnology,* 19(2), 73–82.

Hoy L. D. & Benson W. H. 1998. Fish reproduction: An ecologically relevant indicator of endocrine disruption, *Environmental Toxicology and Chemistry*, 17(1), 49–57.

Jordaan M. S. 2004. Variation in sex determination and the application of the YY male technology for the production of all-male populations of the tilapia *Oreochromis mossambicus*. PhD thesis, Stellenbosch University, Stellenbosch, South Africa.

Kefi A. S. 2014. Influence of dietary soya beans (glycine max (l.) merr.) protein and lipid combination, and androgen (17 α–methyl testosterone) levels on the growth and reproduction of *Oreochromis andersonii* (Castelnau, 1861). PhD thesis, Bunda College of Agriculture, University of Malawi, Lilongwe, Malawi.

Mair G., Abucay J., Abella T. A., Beardmore J. A. & Skibinski D. O. F. 1997. Genetic manipulation of sex ratio for the large-scale production of all-male tilapia *Oreochromis niloticus, Canadian Journal of Fisheries and Aquatic Sciences,* 54(2), 396–404.

Makkar H. P. S., Francis G., Becker K. 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems, *Animal,* 1, 1371–1391.

Marlatt V. L., Bayen S., Castañeda-Cortés D. C., Delbès G., Grigorova P., Langlois V. S., Martyniuk C. J., Metcalfe C. D., Parent L., Rwigemera A., Thomson P. & Van Der Kraak G. 2022. Impacts of endocrine disrupting chemicals on reproduction in wildlife and humans, *Environmental Research*, 208, 1-20.

Megbowon I. & Mojekwu T. 2014. Tilapia Sex Reversal Using Methyl Testosterone (MT) and its Effect on Fish, Man and Environment, *Biotechnology (Faisalabad)*, 13(5), 213–216.

Moundipa P. F., Ngouela S., Kamtchouing P., Tsamo E., Tchouanguep F. M. & Carreau S. 2006. Effects of extracts from *Hibiscus macranthus* and *Basella alba* mixture on testosterone production in vitro in adult rat testes slices, *Asian Journal of Andrology*, 8(1), 111–114.

Mukherjee D., Ghosal I., Hancz C. & Chakraborty S. B. 2018. Dietary administration of plant extracts for production of monosex tilapia: Searching a suitable alternative to synthetic steroids in tilapia culture, *Turkish Journal of Fisheries and Aquatic Sciences*, 18(2), 267–275.

Nantia E. A., Travert C., Manfo F. T., Carreau S., Monsees T. K. & Moundipa P. F. 2011. Effects of the Methanol Extract of *Basella alba* L (Basellaceae) on Steroid Production in Leydig Cells, *International Journal of Molecular Sciences*, 12(1), 376-384.

Nishimura T. & Tanaka M. 2016. The mechanism of germline sex determination in vertebrates, *Biology of Reproduction*, 95(1), 1-6.

Omar E. A., Yousef M. I., Srour T. M. & Mansour A. T. 2014. Effect of Dietary Natural Phytochemicals on Sex-reversal, Growth Performance, Feed Utilization and Body Composition of Nile Tilapia (*Oreochromis niloticus*) Fry, *Journal of the Advances in Agricultural Researches*, 19(3), 428–441.

Radkhah A. R. & Eagderi, S. 2021a. A brief review of the geographic ranges and ecological effects of three major invasive cyprinid species in Iran. *Journal of Fisheries*, 9(3), 93301.

Radkhah A. R. & Eagderi S. 2021b. Ecological consequences of tilapia species on fish biodiversity of Iran and challenges arising from their introduction. *Iranian Journal of Ichthyology*, 8(4), 342-350.

Reverter M., Bontemps N., Lecchini D., Banaigs B. & Sasal P. 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives, *Aquaculture*, 433, 50–61.

Rougeot C., Prignon C., Kengne C. V. N. & Mélard C. 2008. Effect of high temperature during embryogenesis on the sex differentiation process in the Nile tilapia, *Oreochromis niloticus*, *Aquaculture*, 276(1–4), 205–208.

Stadtlander T., Levavi-Sivan B., Kerem Z., Dweik H., Qutob M., Abu-Lafi S., Francis G., Focken U. & Becker K. 2012. Effects of a saponin fraction extracted from *Trigonella foenum-graecum* L. and two commercially available saponins on sex ratio and gonad histology of Nile tilapa fry, *Oreochromis niloticus* (L.), *Journal of Applied Ichthyology*, 29(1), 265–267.

Van Hai N. 2015. The use of medicinal plants as immunostimulants in aquaculture: A review, *Aquaculture*, 446, 88–96.

Wassermann G. J. & Afonso L. O. 2002. Validation of the Aceto-carmine Technique for Evaluating Phenotypic Sex in Nile Tilapia (*Oreochromis niloticus*) Fry, *Ciencia Rural*, 32(1), 133–139.

Yusuf N. S., Andayani S., Risjani Y. & Rahem Faqih A. 2019. Feed enriched with methanol extract of tongkat ali Eurycoma longifolia Jack root for masculinization of Nile tilapia *Oreochromis niloticus*, *AACL Bioflux*, 12(5), 1481-1490.

Zhou X., Yang Z., Luo Z., Li H. & Chen G. 2019. Endocrine disrupting chemicals in wild freshwater fishes: Species, tissues, sizes and human health risks, *Environmental Pollution*, 244, 462–468.