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Assessment Of Efficacies Of 17α Methyl Testosterone Hormone And Some Selected Estrogenic Plants Extracts On The Sex Reversal Of Newly Hatched *Oreochromis niloticus* (tilapia) (Linnaeus, 1758).

***Abdulkarim, B[1,2] & Abdul'azeez, H.R.[3],**

[1]Department of Fishery and Aquaculture, Umaru Musa Yar'adua University Katsina State Nigeria

[2]Department of Biology, Umaru Musa Yar'adua University Katsina State Nigeria

[3]Department of Biology, Federal University Dutsin-ma, Katsina State Nigeria

***Corresponding author: babangida.abdulkarim@umyu.edu.ng, babangidaabdulkarim@gmail.com**
GSM: +2348069132174,+2348029152614

ABSTRACT

The 17α-methyl testosterone is the most common synthetic hormone used in male mono-sex production of Nile tilapia, *Oreochromis niloticus*. The current study was aimed at finding out the efficacies of 17α methyl testosterone hormone, *Carica papaya* (pawpaw) and *Moringa oleifera* (Moringa) seed extracts and *Mangifera indica* (Mango) leaves extracts on the sex reversal of newly hatched *Oreochromis niloticus* (Tilapia) for a period of sixty (60) days.A total of 600 fry were randomly allocated to 24 experimental ponds.The hormonal diets were formulated by adding 17α- methyl-testosterone (Tc), *Carica papaya* seeds extracted with methane (T1), *Carica papaya* seeds extracted with hexane (T2), *Moringa oleifera* seeds extracted with methane (T3) *Moringa oleifera* seeds extracted with hexane (T4) *Mangifera indica* leaves extracted with methane (T5), *Mangifera indica* leaves extracted with hexane (T6) and basal feed with neither hormone nor plant extracts as negative control (T7) at an inclusion of 40ml/ kg diet on the treatments .The fry were fed at 20% body weight during a 30 day feeding trial and a gradual reduction of feed to 10% during the second months rearing period. Results of the growth parameters showed highest weight value of (7.66±0.00 g) in T2 followed by (7.19±0.00 g) in T1, the lowest mean weight value of (5.06±0.01g) was observed in negative control group T7. The maximum length value was recorded as 10.77±0.01 cm in T1, followed by 10.56±0.01cm in T2, followed by 9.96±0.00 cm in TC (positive control). The lowest length value was recorded as 6.99±0.01cm in T7 (negative control). The highest value of SGR (%) occurred at (11.07) from T2 followed by T1 (10.96) and T7 recorded the lowest SGR with (10.37). The maximum survival rate (97.88±2.25) was observed in the negative control group (T7), survival rate of 95.79±3.29 was observed in TC (positive control) and (96.50±2.26) in T1. However, there were no significant differences ($p>0.05$) in the survival rates among the treatment groups. The results of the physicochemical parameters showed no significant differences ($p>0.05$) among all the treatments and the results were within the recommended ranges for the growth and survival of *O. niloticus.* Results from the gonad examination revealed that Nile tilapia fry fed with 17α methyl testosterone hormone diet (Tc) produced the highest phenotypic males of 87%, while T1,T2,T3, T4, T5, T6 gave males of 80%,77%,73%,69%,60%,63% and 27% respectively. Further studies are required to understand the exact mechanism through which the Pawpaw and Moringa seeds and Mango leaves exert their effect of sex differentiation in newly hatched *O. niloticus.*

Keywords*:* Efficacies, growth parameters, Nile tilapia, physicochemical parameters 17α methyl testosterone hormone, Sex reversal

1.1 INTRODUCTION

Tilapia is a global candidate fish of great commercial significance and is currently the second largest farmed fish followed by carps in worldwide production (Prabu et al., 2019). The world's total tilapia production reached approximately 6 million tons in 2020 (Guenard, 2020). The advantageous characteristics of tilapia most importantly faster growth, extreme tolerance capacity to hostile environments, high disease resistivity along with high survival rate make it a predominant culture species (Alam et al., 2014; Dowidar et al., 2018; Hussain, 2021). However, the main obstacle in tilapia culture is its early sexual maturity and frequent spawning in the culture system (Lind et al., 2015). Overpopulation of tilapia results in competition for food and eventually causes decreased production as well as profits. Producing male mono-sex seeds is the best solution to this problem (Beardmore et al., 2001; Omasaki et al., 2017). Besides, male tilapia has a higher growth rate compared to females (Lind et al., 2015). Because metabolic energy in male is used for somatic growth whereas in female large portions of metabolic energy is used for reproductive purposes (Lu et al., 2022; Snake et al., 2020). It has been reported that sex switched tilapia performed three times better growth when treated with ideal hormonal preparation (Kamaruzzaman et al., 2009). Besides, mono-sex stock ensures uniform harvest size and prevents immature courtship activities. Though numerous techniques have been introduced to generate male tilapia, hormonal sex reversal is still treated as the most popular and economically viable practice (Beardmore et al., 2001; Prabu et al., 2019). Therefore, oral administration of feed mixed with androgen hormone has been perceived as the most ideal technique for commercial and mass production across the world (Karaket et al., 2021).

The concerns about the health implications of steroid hormones in fish production, as well as the expensive nature and difficulty in obtaining such hormones, have inspired the need to find suitable, less-expensive, biodegradable alternatives for commercial aquaculture use (Ayotunde and Ofem 2008; Jegede,2010).Various interventions have demonstrated the possibility of utilizing plant extracts as possible options to synthetic steroids in aquaculture in response to the increasing consumer demand for organically produced agricultural products, including fish (Leet*e t al.*, 2011). The acceptance of plants for use in aquaculture is linked to ease of access and being relatively safer for the environment and humans, than synthetic hormones (Chakraborty *e t al.*, 2013).

Carica papaya (Pawpaw) *Moringa oleifera* and *Mangifera indica* (Mango) are among the plants with phytochemicals that have attracted research in this regard (Ampofo-Yeboah 2013). Their seed meal contains many active ingredients, such as Caricain, Carpasemine (a plant growth inhibitor), and oleanolic glycoside (Kobayashi *e t al.,* 2008). Their seed meal has also been reported to have antifertility properties (Udoh and Kekinde 1999). This study was therefore designed to evaluate the efficacy of *Carica papaya, Moringa oleifera* seeds extract and *Mangifera indica* leaves extracts as a natural sex-reversal agent for Nile Tilapia (*Oreochromis niloticus)* and to determine their effect on the growth and survival of the fish during treatment and recovery periods.

The main aim of this study was to induce sex reversal in the fries (newly hatched) *Oreochromis niloticus* (Tilapia) using 17αmethyl testosterone hormone, *Carica papaya* (Pawpaw) seeds extract *Moringa oleifera* seeds extracts and *Mangifera indica* (Mango) leaves extracts.

The specific objectives of the study were: to determine the growth performance of *Oreochromis niloticus* treated with 17αmethyl testosterone hormone, *Carica papaya* seeds extracts, *Moringa oleifera* seeds extract and *Mangifera indica* (Mango) leaves extracts, as well as to determine the survival rate, specific growth rate and length, weight gains and sex ratios of *Oreochromis niloticus* treated with 17αmethyl testesterone hormone, *C. papaya* seeds extracts, *M. oleifera* seeds extract and *M. indica* (Mango) leaves extracts.

2.0 MATERIAL AND METHODS

2.0.1 Study site

The study was conducted at the Fisheries department of the Faculty of Agriculture, Umaru Musa Yar'adua University, Katsina situated at Latitude 12º 53'' 44' N and Longitude 7 º 34'' 51' E. This study involved treating the fries (newly hatched fishes) with dietary 17α methyl testosterone hormone, *C. papaya* seed, *M.oleifera* seed extract and *M. indica* leaves extracts for 60 days (March – April, 2024.)

2.0.3 Source of Experimental Fishes

Six hundred (600) newly hatched (fries) of *Oreochromis niloticus* (Tilapia) were used in this study/ research. They were purchased from fish hatchery of National Biotechnology and Development Agency (NABDA) Yan Katako Katsina State and transported in jerry cans to the department of Fisheries and Aquaculture Umaru Musa Yar'adua University, Katsina State. Collection and transportation of the fishes were done as described by Bolurunduro (2001). Upon reaching UMYUK the fishes were released into storage tanks to acclimatize for 1 week before the beginning of the experiment.

2.0.4 Source of Fish Feeds

The fish feeds comprising of 1 bag of Aler aqua (0.8mm) and 4 bags of Aler aqua (1.5mm) were purchased from Dan Hassan Animal care shop Yahaya Madaki Way, Katsina.

2.0.5 Source of 17α methyl Testosterone hormone and estrogenic plants

The hormone 17α methyl testosterone hormone was purchased at Agro- allied Shop at Ahmadu Bello Way, Kaduna, Kaduna State. Seeds of *Carica papaya,Moringa oleifera* and *Mangifera indica* leaves were collected from local markets and plantations within Katsina metropolis. The seeds and leaves were collected and put in polythene bags and transported to Chemistry Department Umaru Musa Yar'adua University, Katsina for further processing and analysis.

2.0.6 Plant Extracts Preparation

The collected seeds and leaves were washed in sterile distilled water, air-dried under shade for the period of 2 weeks and milled in to powder. The powdered plant materials (250 gm) was extracted with 500 ml of Methanol and Hexane solvents, in a Soxhlet apparatus and the extracts were evaporated to dryness under pressure at 45^0C using a rotary evaporator and stored in glass bottle until ready to use (Hussain *et al*. 2009).

2.0.7 Preparation of Fish Feeds

Three (3) grams of 17α methyl testosterone hormone were dissolved in 2 litres of 95% ethyl alcohol. 200ml of the stock solution was thoroughly mixed with 1kg of the feed (3g / 1kg of feed) based on the procedure of Vinarukwong *et al.,* (2018). The first factor (T0) positive control was mixed with 17α methyl testosterone hormone. It was spread on a clean dry surface allowing the alcohol to evaporate. The second factor was mixed with *C. papaya* seeds extracted with Methanol and hexane (T1 and T2) respectively at concentrations (40ml)/kg diet. The third factor was mixed with *M. oleifera* extracted with Methanol and hexane (T3) and (T4) respectively at concentrations (40ml)/kg diet. The fourth factor was mixed with M. *Indica* leaves extracted with Methanol and hexane (T5) and (T6) respectively at concentrations (40ml)/kg diet. The negative control composed of feed treated with neither extract nor hormone (T7).

2.0.8 Stocking, Rearing and experimental Design.

Six hundred (600) newly hatched fries of *O. niloticus* were randomly distributed evenly in *24*concrete tanks/plastic ponds with sizes of 50 x 100 x 100 cm (H x L x B). Six (6) different treatments were used in this study T1, T2, T3, T4, T5, T6 and compared with 17α methy testosterone hormone (T0) a positive control and negative control (T7) .The fishes were reared for sixty (60) days trial period. The fry were fed at 20% body weight during a 30 day feeding trial and a gradual reduction of feed to 10% during the 2 months rearing period.

Experimental design was composed of eight (8) columns with three rows (3). Seven (7) treatments and one (1) control each with three replications.

2.0.9 Tools for Analysis of Growth Parameters

Major growth parameters were calculated by following the mathematical description of Pechsiri and Yakupitiyage (2005) and Panase and Mengumphan (2015).

$$\%\text{Weight gain} = \frac{\text{Mean final weight - Mean initial weight}}{\text{Mean initial weight}}$$

$$\text{SGR (\% / day)} = \frac{W2 - W1}{T2 - T1} \times 100$$

Where, W1= the initial body weight (g) at time T1 (day), W2 =the final body weight (g) at time T2 (day).
Survival rate was calculated using the formula:

$$\text{S.R.} = \frac{\text{Final number of Fish}}{\text{Initial number of Fish}} \times 100$$

The Fulton's condition factor (K) of each fish was calculated using the formula:

$$K = \frac{100W}{L^3} \times 100$$

Where L= Standard Length (cm)
W= Body weight (g)

2.0.10 Monitoring Water Quality Parameters and Sampling of Fish

The temperature, pH and dissolved oxygen were measured at a 7-day interval by using an automated HORIBA Plus Multi-parameter Instrument (Brannum Lane, USA) Fry were sampled at 7 days intervals during the 60 days hormone treatment period to assess the weight, length, and survival rate. The sampling of fish was done in the morning.

The weight and length of the fry were determined using an electric balance (digital electrical balance, Model EK 3052,) and a measuring plastic ruler respectively.

2.0.11 Confirmatory Squash test for Sex Determination

After 60 days, the sex of Nile tilapia was confirmed by the squash test of the gonad as described byVinarukwong *e t al.,* (2018). Male was identified by their long smooth tubular shape and female was identified by the abundant round shape object in the tube of the gonad.

2.0.11. Statistical Analysis.

Descriptive Statistics were used to analyze% weight gains and SGR, while Analysis of Variance (ANOVA) was used to analyzed growth parameters with treatments as well as the water quality parameters. All the analyses were conducted with Graph pad Prism Version 7.0 at a significant level of $P \leq 0.05$.

3.0. RESULTS

The growth parameters and survival rate were recorded every 14 days interval during 60 days of rearing. The highest weight value (7.66±0.00 g) was observed in T2 followed by (7.19±0.00 g) in T1, 6.71±0.01 in T5, 6.05±0.00 in TC, and the lowest mean weight value of 5.06±0.01g was observed in negative control group T7 (Table 1).The maximum length value was recorded as 10.77±0.01 cm in T1, followed by 10.56±0.01cm in T2, followed by 9.96±0.00 cm in TC (positive control). The lowest length value was recorded as 6.99±0.01cm in T7 (negative control).The highest value of SGR (%) occurred at (11.07) from T2 followed by T1 (10.96) and T5 (10.85) respectively. T7 recorded the lowest SGR with (10.37). The maximum survival rate (97.88±2.25) was observed in the negative control group (T7), followed by (96.50±2.26) in T1 and (96.34±2.23) in T2 respectively. A survival rate of 95.79±3.29 was observed in TC (positive control).However, there were no significant differences ($p>0.05$) in the survival rates among the treatment groups.

Table 1: Growth Parameters and Survival Rates of *O.niloticus* (Nile Tilapia) after 60 days of Treatment

Parameters	Tc	T1	T2	T3	T4	T5	T6	T7
Initial weight(g)	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00a
Final weight (g)	6.05±0.00[a]	7.19±0.00[b]	7.66±0.00[c]	6.02±0.02[d]	5.25±0.01[e]	6.71±0.01[d]	6.03±0.01[e]	5.06±0.01a
Weight gain (g)	6.04±0.00[a]	7.18±0.00[b]	7.65±0.00[c]	6.01±0.02[a]	5.24±0.01[d]	6.70±0.01[e]	6.02±0.00[a]	5.05±0.01f
Specific growth rate %	10.67	10.96	11.07	10.66	10.43	10.85	10.68	10.37
Initial length (cm)	0.94±0.01[a]	0.95±0.01[a]	0.95±0.01[a]	0.95±0.01[a]	0.95±0.01[a]	0.96±0.01[a]	0.95±0.01[a]	0.95±0.01a
Final length (cm)	9.96±0.00[a]	10.77±0.01[b]	10.56±0.01[c]	8.71±0.01[d]	8.67±0.01[e]	7.87±0.01[f]	7.89±0.00f	6.99±0.01g
Length gain (cm)	9.92±0.01[a]	9.82±0.01[b]	9.61±0.01[c]	7.76±0.01[d]	7.72±0.01[e]	6.91±0.01[f]	6.94±0.01[g]	6.04±0.01h
Survival rate %	95.79±3.29[a]	96.50±2.26[a]	96.34±2.23[a]	92.65±2.37[a]	92.45±2.76[a]	91.0±4.50[a]	90.25±2.59[a]	97.88±2.25a
Condition factor (K)	0.61	0.58	0.65	0.91	0.81	1.37	1.23	1.48

Mean and Standard deviation with same superscript along the rows indicate no significant differences at P<0.05

Key: T1-Carica papaya extracted with methane,T2-Carica papaya extracted with hexane T3 -Moringa oleifera extracted with methane T4-Moringa oleifera extracted with hexane, T5- Mangifera indica extracted with methane,T-6 Mangifera indica extracted with hexane TC- Methyl testesterone hormone (Positive control),T7-Negative control

Growth performance of Nile tilapia, including final weight, specific growth rate (SGR), weight gain (g), final length, length gain, a showed significant differences (P<0.05) between the control groups and treated groups, and among the different treatment groups with very few exceptions.

The water quality parameters during the treatment period of 60 days are shown in Table 2. The temperature ranged between 27.43-27.90^{0}C and there were no significant differences (p= 0.4145) among the treatment and the week /months. The pH value ranged between 7.17-7.56 and no significant differences (p>0.9999) were observed during the treatment period. Dissolved oxygen during the study was recorded within the range of 5.26-5.57mg/l and no significant differences (p=0.2915) were observed during the treatment period.

Table 2: Water Quality Parameters during the treatment Period of 60 days

Month / week	Temperature (^{0}C)	pH	Dissolved Oxygen (mg/l)
First week	27.43±0.51[a]	7.17±0.29[a]	5.26±0.19[a]
March	27.53±0.50[a]	7.56±0.17[a]	5.34±0.32[a]
April	27.90±0.17[a]	7.20±0.35[a]	5.57±0.12[a]

Mean and Standard deviation with same superscript along the columns indicate no significant differences at P<0.05

At the end of the 60 days treatment in the negative control group (T7) which was fed with the basal diet only had the lowest sex ratio of 0.37:1 male to female (M:F).(Table 3). There was higher number of males to females among the positive control group (TC) 3.8:1; however, T1 recorded the highest sex ratio of 4.0:1 followed by T2 with 3.3: 1 sex ratio respectively.

Table 3: The Male and Female Sex Ratio in a Mixed population of *Oreochromis niloticus* after 60 days treatment

Treatment	N	Male	Female	% Male	%Female	M:F Ratio
Tc	75	65	10	87	23	3.8:1
T1	75	60	15	80	20	4.0:1
T2	75	58	17	77	23	3.3:1
T3	75	55	20	73	27	2.7:1
T4	75	52	23	69	31	2.3:1
T5	75	45	30	60	40	1.5:1
T6	75	47	28	63	37	1.7:1
T7	75	20	55	27	73	0.37:1

Key: T1-Carica papaya extracted with methane,T2-Carica papaya extracted with hexane T3 -Moringa oleifera extracted with methane T4-Moringa oleifera extracted with hexane, T5- Mangifera indica extracted with methane,T-6 Mangifera indica extracted with hexane TC- Methyl testesterone hormone (Positive control),T7-Negative control

4.0 DISCUSSION

Growth parameters

Final weight and weight gain in this study were higher in T1 and T2 (*C. papaya*) extracted with methane and hexane respectively, followed by Tc (hormone treated i.e. methyl testosterone hormone). This was in agreement with the findings of Abdul'azeez (2023) who reported final and weight gain to be higher in *O. niloticus* fed with 40ml/g of *C. papaya* extracted with methane.The findings was also in line with the findings of Zaki *et al.,* (2021) who reported higher growth performance from two extracts of (1g and 2 g) of *Tribulus terrestris* then followed by 60mg/l of methyl testosterone hormone. Specific growth rate (SGR) was higher in *C. papaya* (40ml/g) extracted with methane and hexane. This was in agreement with the findings of Zaki *e t al.,* (2020) who recorded higher SGR in *O. niloticus* fed with feed extracted with 1 g and 2 g of *Tribulus terrestris.* The findings agreed with the work of Abdul'azeez (2023) who reported higher SGR in 40ml/g of *C. papaya* extracted with methane.

A higher survival rate in this study was recorded in the negative control followed by the hormone treated. This agreed with the findings of Ampo-yeboah (2013) who reported higher survival rates in negative control in *Oreochromis mossambicus* treated with *C. papaya* seed powder and methyl testosterone hormone. However, this was in disagreement with the findings of Abdul'azeez (2023) who reported higher survival rates in feed mixed with 40 ml *C. papaya* extracted with methane. The higher survival rates in both *C.papaya* extracts (treatments) from this study might be due to the therapeutic properties of the plant extract and several compounds, as vitamins A, C, E, fatty acid and essential amino acid contents (Gharaei *e t al.,* 2020).

Water Quality Parameters

The growth performance of juvenile *O. niloticus* were affected by different environmental factors such as water quality parameters including water temperature, pH, dissolved oxygen concentration, transparency e.t.c. (Abdulkarim *et al;* 2019). Temperature recorded in this study was in the range of 27.43- 27.90^{0}C. This finding was in agreement with Bwathondi and Abdulkarim (2017) who reported 27.13^{0}C from Juveniles *Oreochromis niloticus* reared with two different diets in Kunduchi Ponds, Dar es salaam, Tanzania. This finding was also in accordance with the findings of Abdul'azeez (2023) who reported 27.83^{0}Cin ponds reared with *O. niloticus.* This finding was however higher than those reported by Sarker *et al*, (2022) values of 21-25^{0}C. and lower those reported by Zaki *etal.,* (2021) values of 29.67°C. The differences in the temperature between this study and others might be attributed to location different weather and climatic conditions. pH recorded in this study ranged between7.17-7.56 which was almost similar to the findings of Bwathondi. And Abdulkarim (2017)values of7.87-7.99 , also similar to the

findings of Zaki et al.,(2021) values of 7.6 to7.8 and the values were lower than those reported by Abdulkarim et al., (2019). Dissolved Oxygen recorded in this study ranged between 5.26-5.57 was in line with the findings Abdu'lazeez (2023) who reported pH values of 5.0- 5.33, however, the values were lower than those reported by Zaki etal., (2021) 5.76 to 6.05 mg/l and Bwathondi and Abdulkarim (2017) values of 6.82-9.52mg/l. All the water quality parameters recorded during this study were within suitable range for the normal growth performance and survival of *O. niloticus* as reported by Boyd (2003) and Abdulkarim *et al.,* (2019).

Sex Ratio

The success of sex-reversal techniques in tilapias is to produce all-male populations. Findings of this study showed that Tc- methyl testosterone produced higher sex ratio 87%: 23% (M: F) compared with the other treatment groups. However, T1- negative control was found to have low sex ratio 27%:73 % (M: F). This study wassimilar to the findings of Beaven & Muposhi (2012) and Ajiboye *et al*. (2015) who reported a 90 % male percentage at 60 mg 17- α MT / kg feed and it also correspond to the findings of Orose *e t al.,* (2016) who reported a 90 % male percentage at 60 mg 17- α MT / kg feed. This finding was also in agreement with the findings of Zaki et al., (2021) who reported highest male percentage of (90%) at the dose of 60 mg methyl testosterone hormone,/kg. This was in line with the work of Abdul'azeez (2023) who reported (86% males to 14% females) in *O. niloticus* treated with methyltestosterone hormone, *Carica papaya* and *Moringa oleifera* seed powders. This study indicated that 40 mg/l of methyltestosterone hormone reversed sex of Tilapia at higher % than followed by *C. papaya*, *M. oleifera* and *M. indica* respectively. The study has shown that these plants extracts are capable of reversing the sex of newly hatched *O. niloticus* with less mortality and without changing the physicochemical parameters of the pond water.

5.0 CONCLUSION

It can be concluded that the inclusion of the crude form of Pawpaw and Moringa seeds and Mango leaves resulted in the sex reversal or masculinisation of fry of *O. niloticus* up to 80%,77% and 69%. In addition, the appreciable growth performance and less mortality exhibited by the fishes fed with the treated diets indicated that the water environment and the necessary water parameters were within normal level for the successful survival of *O. niloticus*

6.0 RECOMMENDATION(s)

This study recommends the following:

i. Further studies are required to understand the exact mechanism through which the Pawpaw and Moringa seeds and Mango leaves exert their effect of sex differentiation in newly hatched *O. niloticus*
ii. The biochemical composition of Pawpaw and Moringa Seed and Mango Leaves should be investigated further to establish the relative quantities and range of the phytoestrogens required for adequate and safe sex reversal in *O. niloticus*
iii. The results obtained provide an indication of the use of Pawpaw, Moringa seeds and Mango leaves as a possible alternative to induce sex reversal in mixed sex tilapia populations at a small scale and this could be extended to a large scale for mass production of monosex tilapia for commercial purposes.

Conflict of Interest: No conflict of interest whatsoever among the authors.

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