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## Effect of Dietary Natural Phytochemicals on Sex-reversal, Growth Performance, Feed Utilization and Body Composition of Nile Tilapia (*Oreochromis niloticus*) Fry

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**ABSTRACT:** a feeding study was conducted to evaluate the effect of different natural phytochemicals as alternative sex-reversal agents instead of 17 $\alpha$ -methyl testosterone (MT). Newly hatched Nile tilapia (*Oreochromis niloticus*) fry were treated with MT (60 mg/kg), ginseng extract (GE; 0.2 or 0.4 g/kg), *Tribulus terrestris* extract (TTE; 0.6 or 1.2 g/kg) and date palm pollen (DPP; 3 or 6 g/kg) in diet. The treatment period lasted for 28 days for MT and 84 days for other phytochemicals. The results showed that the maximum male percentage recorded with MT (92.30%), followed by TTE (1.2 g/kg; 64.48%), TTE (0.6 g/kg; 57.76%) and DPP (6 g/kg; 56.67%), while the male percentage of the control was 48.36%. All studied treatments showed normal survival rates along the experimental period, which proves that the used phytochemicals are nontoxic. Fish treated with phytochemicals exhibited successful growth acceleration compared to the control group especially with TTE treatments. The same manner was observed with feed conversion ratio. Carcass composition showed that MT treatment has significantly increased moisture and decreased ether extract than other treatments. The effects of phytochemicals showed a slight improvement of protein and ether extract content compared to the control diet. Consequently, it can be concluded that the use of phytochemicals in fish diets as growth promoters is more effective than sex-reversal agents. It is recommended to use TTE in Nile tilapia fry diets.

**Key words:** *Oreochromis niloticus*; Phytochemicals; Sex-reversal; Growth performance

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## INTRODUCTION

The demand for animal protein has gone far beyond supply as a result of the rapid growth of human population in the many countries of the world, especially in the developing countries. An urgent need is therefore necessary to increase the production of protein sources. Nowadays, aquaculture is one of the fastest growing food production sectors and an increasingly important option in animal protein sources in the world. Farmed tilapias have been an important global commodity since the 1990s. The global production of farmed tilapias reached approximately 3.5 million tons in 2012, second only to farmed carps (FAO, 2012). Their ability to grow on a wide range of diets, fast growth, high conversion ratio and reproduce readily in captivity makes them attractive for culture (El-Sayed, 2006).

Despite having many good characteristics, one of the major obstacles in commercial tilapia production is its precocious maturity and the following uncontrolled reproduction, resulting in an increasing competition for feed followed by stunted growth and low commercial value (Wassermann and Afonso, 2003).

Moreover, the sex of fish can be significant in aquaculture because of differences between males and females in growth rate, size, behavior patterns, and breeding time (Turan and Cek, 2007). In populations of tilapia, males grow faster and are more uniform in size than females (Tariq-Ezaz *et al.*, 2004). All male tilapia achieved using methyl testosterone treatment, it was the most popular and successful practice all over the world (Abdelhamid *et al.*, 2009).

Because of consumer concerns and strict regulations in many countries, the use of synthetic chemicals, hormones and antibiotics is becoming unviable and natural compounds are more acceptable to the public (Chakraborty *et al.*, 2013). Furthermore, the synthetic hormones have been reported to have the potential to accumulate in the sediment water and aquatic biota (Contreras-Sanchez *et al.*, 2001; Çek *et al.*, 2004). Farthermost, methyl testosterone (MT) is considered to be carcinogenic (Velazquez and Alter, 2004).

Phytochemicals contained in herbs may enhance the innate immune system, possess antimicrobial capabilities, and are redox active molecules with antioxidant characteristics that may help to improve the general physiological condition of fish (Chakraborty *et al.*, 2013).

Moreover, many of the phytochemicals reported to stimulate testosterone secretion such as ginseng extract (Tsai *et al.*, 2003). Also, *Tribulus terrestris* extract (TTE) increased testosterone level and improve sexual status (Gauthaman and Adaikan, 2008). Furthermore, the effect of DPP on testosterone augmentation were emphasized via numerous authors and different species model e.g. Marbeen *et al.* (2005) on human and Abedi *et al.* (2012) on rat.

Therefore these findings lead to the expected effect of phytochemicals as sex-reversal agent on fish, where Nakari and Erkomaa (2003) reported that phytochemicals modulate endocrine function and changed the sex ratio of the exposed Zebra fish fry. Considering these aspects, the present study was focused to evaluate the efficacy of three phytochemicals (ginseng extract, *Tribulus terrestris* extract and date palm pollen) as natural alternative sex-reversal agents and growth promoters of Nile tilapia (*O. niloticus*) fry instead of methyl testosterone.

## **MATERIALS AND METHODS**

The present work was conducted to investigate the effect of ginseng extract, *Tribulus terrestris* extract and date palm pollen against 17  $\alpha$ -methyl testosterone (MT) on sex reversal, survival rate, growth performance, feed utilization and whole body chemical composition of Nile tilapia (*O. niloticus*) fry. This experiment was carried out at the Laboratory of Fish Nutrition, Faculty of Agriculture (Saba-Basha), Alexandria University, Egypt.

### **Experimental fish and design**

Nile tilapia fry one day old (weighing 0.02 g/fry and measuring 1.13 cm total length/fry) were obtained from a private commercial hatchery sited in Motobas, Kafr–El Sheikh Governorate, Egypt. The fry kept in glass aquaria for one day after transportation without feeding. A total of 800 tilapia fry were randomly distributed on 16 glass aquaria (100×30×40 cm) each 100 L of water, which were continuously aerated with air stone. The aquarium was static bath with changing water

manually, and the used siphon tube was closed by hapa net to prevent fry escaping. The photoperiod was maintained on a (12:12) light:dark schedule and temperature ( $25\pm 2^\circ\text{C}$ ).

The starting fish density was 50 larvae per aquarium with two replicates per treatment. The following eight treatments were performed, control fed basal diet without supplementation, or basal diet supplemented with MT (60 mg/kg), GE (0.2 or 0.4 g/kg), TTE (0.6 or 1.2 g/kg) and DPP (3 or 6 g/kg) in diet. The treatments received experimental diets for 84 days except MT treatment lasted for 28 days and completed with the basal diet, until the end of experiment.

### Experimental diets and sex-reversal agents

The sex-reversal agents were MT (ARGENT laboratories Inc. Philippines), GE (Pharco Pharmaceuticals Co., Alexandria, Egypt), TTE (Nerhadou International Co. for pharmaceuticals and nutraceuticals, Egypt) and DPP: Fresh pollen of date palm was collected in March 2013 from Edku city, Egypt. The pollens were separated from the kernels with a fine gauze sieve and dried at  $50^\circ\text{C}$  for 24 hrs.

The experimental diets were formulated in powder form and its chemical composition is shown in Table (1). The sex-reversal (MT, GE, TTE and DPP) treatments were prepared according to the alcohol evaporation method (Guerrero, 1975; Navarro-Martín *et al.*, 2009). The control diet was treated with ethanol only and then the diet was left to dry at room temperature in front of an electric fan for 12 hours. Fish were fed three times a day in a decreasing rate from 20 to 10% of live body weight for 4 weeks, and then the fish were fed at 8% to the end of the experiment, the feed ratio adjusted every week.

**Table (1): Ingredient and chemical composition (%) of the experimental diets.**

Ingredients	Basal diet
Fish meal (herring; 72%)	56.4
Wheat flour	40
Corn oil	2
Vitamins and Minerals primex <sup>1</sup>	0.5
Vitamin C	0.5
Garlic Extract	0.1
Oxytetracyclin	0.5
<b>Chemical composition (% on dry matter basis)</b>	
Dry matter (DM)	93.70
Crud protein (CP)	44.16
Ether extract	7.23
Nitrogen free extract (NFE)	40.14
Crude fiber	4.17
Ash	6.03
Gross energy (GE; kcal/100g DM) <sup>2</sup>	486.67
P/E ratio (mg protein/kcal) <sup>3</sup>	90.74

<sup>1</sup>Composition of vitamin mineral mixture of 1 kg: Vitamin A - 50,00,000 IU; Vitamin D<sub>3</sub> - 10,00,000 IU; Vitamin B<sub>2</sub> - 2.0 g; Vitamin E - 750 units; Vitamin K - 1.0 g; Calcium pantothenate 2.5 g; Nicotinamide - 10.0 g; Vitamin B<sub>12</sub> - 6.0 g; Choline Chloride - 150.0 g; Calcium - 750.0 g; Manganese - 27.5 g; Iodine - 1.0 g; Ion - 7.5 g; Zinc - 15.0 g; Copper - 2.0 g; Cobalt - 0.45 g.

<sup>2</sup>Gross energy, calculated on the basis of 5.64, 9.44 and 4.11 kcal GE/g protein, ether extract and NFE respectively (NRC, 1993).

<sup>3</sup>Protein/ energy ratio (mg protein/kcal gross energy) = CP/GE x 1000

## Measured parameters

### Sex ratio analysis

At the end of the experiment, gonads were then sampled and examined directly by studying wet-squash preparations according to Guerrero and Shelton (1974). Briefly, the gonads were removed using fine forceps, placed on a slide, covered with few drops of aceto-carmin stain and squashed with a cover slip. The slide was then examined under a compound microscope using magnifications of 10\*40 X.

### Growth performance and feed utilization parameters

At trial termination, all fish were weighed and measured (Total length), and their condition factor (CF) calculated according to the following equation:  $CF = (\text{weight}/\text{length}^3) * 100$ ; Specific growth rate (SGR; %/day) was calculated using the equation:  $SGR = ((\text{natural log of final weight \{g\}} - \text{natural log of initial weight \{g\}}) * 100) / t$  (days), also survival rate was noted throughout the study. Feed conversion ratio (FCR) was calculated according to the following equation:  $FCR = (\text{feed intake}/ \text{weight gained})$ ; protein efficiency ratio (PER) calculated as weight gain/ protein intake.

### body chemical composition

Proximate chemical analyses (%) of the diet and body after the experiment were performed according to the official methods of analysis of the AOAC (1995). Nitrogen free extract (NFE) in the experimental diets were calculated using the following equation:  $NFE = 100 - (\text{Crude Protein} + \text{Ether Extract} + \text{Crude fiber} + \text{Ash})$ .

### Statistical analysis

Data were statistically analyzed with one-way ANOVA and Duncan's multiple range tests and expressed as mean values  $\pm$  SE. In addition, effects with a probability of  $P < 0.05$  were considered significant. Statistical analyses were performed using SPSS for Windows (Standard Version 17 SPSS Inc. Chicago, Illinois).

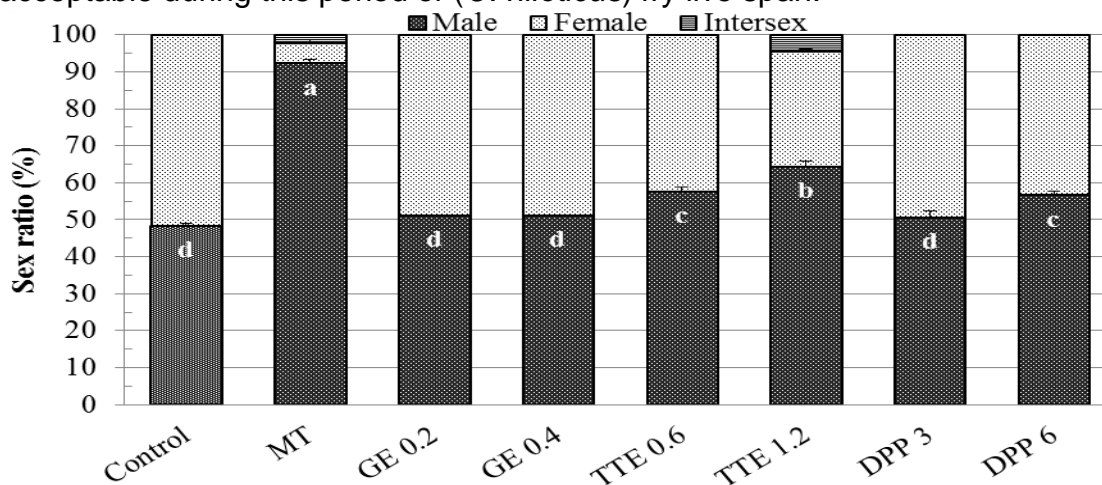
## RESULTS

The highest significant male percent (92.30%) was observed in MT treatment compared to other treatments; and the control group recorded the lowest male ratio (48.36 %). The male percent skewed via dietary TTE (0.6 g/kg diet), TTE (1.2 g/kg diet) and DPP (6 g/kg diet) to be 57.76, 64.48 and 56.67%, respectively. The best sex reversal ratio in TTE in high dose (1.2 g/kg diet) treatment, indicating that TTE affect male percent in dose-dependently.

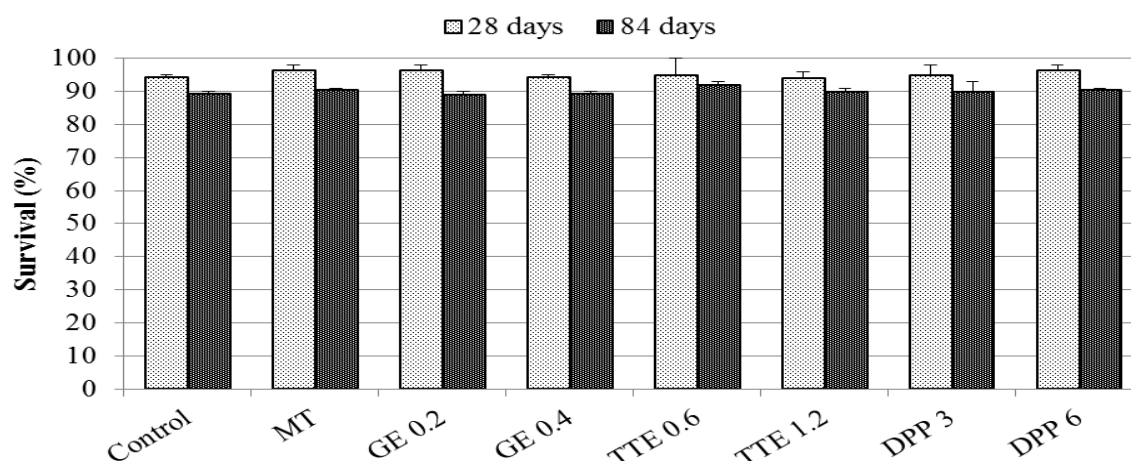
The dietary Ginseng extract treatments (0.2 or 0.4 g/kg diet) and Date palm pollen (3 g/kg diet) didn't affect statistically the male percent compared to control, and were nearly expected ratio of 1:1 (male: female). The inter sex has shown only

with MT and TTE (1.2 g/kg diet) treatments, concomitantly to rise of sex reversal process (Fig. 1).

The overall survival rate (%) ranged from 94% to 96.5% after the first 28 days of treatment, meanwhile after 84 days the survival rate varying from 89% to 92%, without any significant drastic effect of all studied treatments (Fig. 2). The noticed mortality couldn't be attributed to any obvious cases and considered acceptable during this period of (*O. niloticus*) fry live span.



**Figure (1):** Effect of different phytochemicals (g/kg diet) compared with 17  $\alpha$ -methyl testosterone (MT) on sex ratio of Nile tilapia (*O. niloticus*) fry. GE, ginseng extract; TTE, *Tribulus terrestris* extract; DPP, date palm pollen.



**Figure (2):** Effect of different phytochemicals (g/kg diet) compared with 17  $\alpha$ -methyl testosterone (MT) on survival rate (%) of Nile tilapia (*O. niloticus*) fry. GE, ginseng extract; TTE, *Tribulus terrestris* extract; DPP, date palm pollen.

The effect of different sex-reversal agents on growth performance of *O. niloticus* after 28 and 84 days of treatments showed in Table 2. At 28 and 84 days of treatment the final body weight (g/fish) and SGR (%/day) of all studied treatments increased compared with control diet and the highest FBW and SGR after 28 days observed with TTE 1.2 g/kg diet followed by GE 0.4 g/kg diet meanwhile after 84 days the MT treatment recorded the best FBW and SGR followed by TTE 1.2 g/kg diet and GE 0.4 g/kg diet with insignificant differences among them. The total length didn't differ significantly after 28 days of treatment, meanwhile after 84 days the length of MT treated fish significantly surpassed the control total length and the other treatments insignificantly. Moreover, the condition factor didn't differ significantly after 28 or 84 days of treatment.

**Table (2): Effect of different phytochemicals (g/kg diet) compared with 17  $\alpha$ -methyl testosterone on final body weight (g/fish), SGR (%/day), length (cm/fish) and condition factor of Nile tilapia (*O. niloticus*) fry.**

Items	Final body weight (g/fish)		SGR (%/day)		Length (cm)		Condition factor	
	28 days	84 days	28 days	84 days	28 days	84 days	28 days	84 days
<b>Control</b>	0.70±0.01 <sup>d</sup>	5.66±0.12 <sup>c</sup>	13.20±0.05 <sup>d</sup>	6.03±0.01 <sup>c</sup>	3.54±0.05	6.78±0.21 <sup>b</sup>	1.5±0.07	1.82±0.13
<b>MT</b>	0.83±0.01 <sup>bcd</sup>	7.23±0.29 <sup>a</sup>	13.81±0.06 <sup>bc</sup>	6.29±0.06 <sup>a</sup>	3.70±0.04	7.19±0.00 <sup>a</sup>	1.63±0.05	1.95±0.08
<b>GE 0.2</b>	0.89±0.02 <sup>abc</sup>	6.01±0.13 <sup>bc</sup>	14.08±0.16 <sup>abc</sup>	6.10±0.05 <sup>abc</sup>	3.58±0.06	6.99±0.07 <sup>ab</sup>	1.94±0.06	1.76±0.02
<b>GE 0.4</b>	0.97±0.08 <sup>ab</sup>	6.31±0.47 <sup>abc</sup>	14.36±0.36 <sup>ab</sup>	6.15±0.05 <sup>abc</sup>	3.69±0.07	6.97±0.11 <sup>ab</sup>	1.93±0.26	1.86±0.05
<b>TTE 0.6</b>	0.80±0.02 <sup>cd</sup>	6.22±0.39 <sup>abc</sup>	13.70±0.17 <sup>cd</sup>	6.13±0.04 <sup>abc</sup>	3.58±0.02	7.06±0.16 <sup>ab</sup>	1.74±0.01	1.77±0.01
<b>TTE 1.2</b>	1.01±0.07 <sup>a</sup>	6.92±0.31 <sup>ab</sup>	14.53±0.17 <sup>a</sup>	6.24±0.02 <sup>ab</sup>	3.71±0.05	7.18±0.05 <sup>ab</sup>	1.97±0.06	1.87±0.04
<b>DPP 3</b>	0.90±0.01 <sup>abc</sup>	5.87±0.35 <sup>bc</sup>	14.12±0.04 <sup>abc</sup>	6.07±0.09 <sup>bc</sup>	3.64±0.04	6.87±0.08 <sup>ab</sup>	1.88±0.09	1.82±0.17
<b>DPP 6</b>	0.84±0.04 <sup>bc</sup>	5.91±0.34 <sup>bc</sup>	13.88±0.09 <sup>bc</sup>	6.08±0.08 <sup>bc</sup>	3.63±0.04	6.80±0.11 <sup>ab</sup>	1.76±0.15	1.88±0.20

\* Values superscripted by different alphabets within the same column are significantly different ( $P < 0.05$ ). GE, ginseng extract; TTE, *Tribulus terrestris* extract; DPP, date palm pollen.

All diets were acceptable for all treatments, which appeared in insignificant feed intake after 28 and 84 days of feeding treatment, the same pattern reflexed in protein intake. The feed conversion ratio was improved in all supplementation treatments than the control group. The best FCR was observed with TTE 1.2 g/kg diet followed by GE 0.4 g/kg diet after 28 days. Meanwhile, the MT treatment showed the best FCR followed by TTE 1.2 g/kg diet after 84 days of treatment. The same manner was observed with PER (Table 3).

Moisture content (%) of whole fish body ranged from 72.6 to 75.7% without significant differences among all treatments except the significant increase of moisture content of MT treated fish than other treatment. The highest protein content was recorded with TTE 0.6 g/kg diet followed by GE 0.4 g/kg diet with significant increase compared to control. The protein content of other treatment didn't significantly differ from the control. The ether extract significantly dropped with MT treatment by 28.67% compared to control. Meanwhile, other treatment didn't differ significantly than control, except TTE 1.2 g/kg diet and DPP 6 g/kg diet. The ash content of fish body showed significant differences among treatments with the highest values with DDP 6 g/kg diet and control, meanwhile, the lowest ash mean recorded with GE 0.2 g/kg diet (Table 4).

**Table (3): Effect of different phytochemicals (g/kg diet) compared with 17  $\alpha$ -methyl testosterone on feed intake, protein intake, feed conversion ratio (FCR) and protein efficiency ratio (PER) of Nile tilapia (*O. niloticus*) fry.**

Items	Feed intake (g)		FCR (g)		Protein intake (g)		PER (g)	
	28 days	84 days	28 days	84 days	28 days	84 days	28 days	84 days
Control	0.94±0.03	8.10±0.28	1.38±0.03 <sup>a</sup>	1.44±0.02 <sup>a</sup>	0.41±0.01	3.58±0.13	1.64±0.04 <sup>d</sup>	1.58±0.02 <sup>c</sup>
MT	0.95±0.05	8.22±0.01	1.18±0.05 <sup>bcd</sup>	1.14±0.05 <sup>c</sup>	0.42±0.02	3.63±0.00	1.93±0.09 <sup>abcd</sup>	1.99±0.08 <sup>a</sup>
GE 0.2	1.01±0.01	8.11±0.09	1.16±0.04 <sup>bcd</sup>	1.35±0.01 <sup>ab</sup>	0.45±0.00	3.58±0.04	1.96±0.06 <sup>abc</sup>	1.67±0.02 <sup>bc</sup>
GE 0.4	1.03±0.03	8.19±0.05	1.09±0.05 <sup>cd</sup>	1.31±0.10 <sup>abc</sup>	0.46±0.01	3.62±0.02	2.08±0.10 <sup>ab</sup>	1.74±0.14 <sup>abc</sup>
TTE 0.6	1.00±0.00	8.03±0.12	1.28±0.03 <sup>ab</sup>	1.30±0.06 <sup>abc</sup>	0.44±0.00	3.54±0.05	1.77±0.05 <sup>cd</sup>	1.75±0.08 <sup>abc</sup>
TTE 1.2	1.04±0.00	8.06±0.16	1.05±0.07 <sup>d</sup>	1.17±0.03 <sup>bc</sup>	0.46±0.00	3.56±0.07	2.16±0.15 <sup>a</sup>	1.94±0.05 <sup>ab</sup>
DPP 3	1.05±0.03	7.95±0.75	1.19±0.02 <sup>bcd</sup>	1.35±0.05 <sup>ab</sup>	0.47±0.01	3.51±0.33	1.90±0.04 <sup>abcd</sup>	1.67±0.06 <sup>bc</sup>
DPP 6	1.01±0.01	7.99±0.00	1.23±0.05 <sup>abc</sup>	1.36±0.08 <sup>ab</sup>	0.45±0.00	3.53±0.00	1.84±0.07 <sup>bcd</sup>	1.67±0.10 <sup>bc</sup>

\* Values superscripted by different alphabets within the same column are significantly different ( $P<0.05$ ). GE, ginseng extract; TTE, *Tribulus terrestris* extract; DPP, date palm pollen.

**Table (4): Effect of different phytochemicals (g/kg diet) compared with 17  $\alpha$ -methyl testosterone on whole body composition (% of wet weight) of Nile tilapia (*O. niloticus*) fry.**

Items	% on wet weight basis			
	Moisture (%)	Protein (%)	Ether extract (%)	Ash (%)
Control	73.30±0.20 <sup>b</sup>	14.59±0.13 <sup>c</sup>	8.37±0.03 <sup>c</sup>	3.70±0.13 <sup>ab</sup>
MT	75.70±0.60 <sup>a</sup>	14.86±0.11 <sup>bc</sup>	5.97±0.05 <sup>d</sup>	3.45±0.13 <sup>abc</sup>
GE 0.2	73.15±0.35 <sup>b</sup>	14.96±0.13 <sup>bc</sup>	8.64±0.06 <sup>bc</sup>	3.23±0.11 <sup>c</sup>
GE 0.4	73.10±0.10 <sup>b</sup>	15.06±0.15 <sup>b</sup>	8.56±0.14 <sup>bc</sup>	3.28±0.06 <sup>c</sup>
TTE 0.6	73.00±1.00 <sup>b</sup>	15.52±0.19 <sup>a</sup>	8.28±0.25 <sup>c</sup>	3.20±0.12 <sup>c</sup>
TTE 1.2	72.80±0.10 <sup>b</sup>	15.00±0.20 <sup>bc</sup>	8.90±0.16 <sup>ab</sup>	3.30±0.14 <sup>c</sup>
DPP 3	73.35±0.25 <sup>b</sup>	14.67±0.13 <sup>bc</sup>	8.65±0.05 <sup>bc</sup>	3.35±0.15 <sup>bc</sup>
DPP 6	72.60±0.50 <sup>b</sup>	14.61±0.06 <sup>bc</sup>	9.05±0.13 <sup>a</sup>	3.74±0.10 <sup>a</sup>

\* Values superscripted by different alphabets within the same column are significantly different ( $P<0.05$ ). GE, ginseng extract; TTE, *Tribulus terrestris* extract; DPP, date palm pollen.

## DISCUSSION:

The target of the present study was to find an alternative sex-reversal method to induce all male tilapia fry and improve growth performance, for the method currently used to be more effective, easy and environmental friendly. Toward this goal, some phytochemicals were incorporated in diet of *O. niloticus* fry in a comparison with MT treatment. The use of phytochemicals as sex-reversal agents in fish is a recent research area. Mirza and Shelton (1988) reported that the manipulation of biological synthesis of steroids have a similar effect to the administration of exogenous steroid hormones. During the specific critical periods of early gonad development, changes in sex hormone levels can affect the final sex independently of the genetic sex (Andersen *et al.*, 2003). Consequently,

embryonic differentiation of the fetus into a male and its subsequent growth along this line is essentially due to the presence of physiological amounts of androgens (especially testosterone and its metabolite dihydrotestosterone) in the body (Baskin *et al.*, 1997).

From the current findings, the MT treatment was the most effective treatment on sex-reversal of *O. niloticus*. However, synthetic hormones and hormone metabolites persistence and their fate in fish, water and sediment will provide information on the potential risks of using hormonal sex control technology (Contreras-Sanchez *et al.*, 2001). Fish offered to the consumer will not be treated with synthetic hormones and producers may have an alternative method for producing of monosex populations based on natural products (Yilmaz *et al.*, 2009).

Among studied phytochemicals, TTE treatments were the most effective treatment on sex differentiation of *O. niloticus* fry and increasing male ratio in the offspring with the maximum ratio 57.76 and 64.48%, respectively. Meanwhile the other phytochemical treatments didn't have obvious effect on sex ratio skewness. Turan and Cek (2007) reported increase in male sex ratio of *Clarias gariepinus*, as a dose dependent manner with TTE treatment and the highest male ratio (80.42%) recorded with 9g TTE as immersion in 30L water. Moreover, the male percent reached to 87.23% via immersion of newly hatched Convict Cichlid, *Cichlasoma nigrofasciatum*, fry once a week for two months on TTE extract (Çek *et al.*, 2007a). The increase in the sex ratio than the present study may be attributed to the alteration of the TTE application methods, doses and species. In accordance, Omitoyin *et al.* (2013) found that percentage of males in treated fish increased as concentration of TTE increased up to reach 85.7% with 2.5 g/kg diet. This findings mount our results by 20% and this could be the used of double dose of TTE than used in our study. The effect of TTE to increase male percent may be due to the increase of androgens production, where TTE contains a number of different substances including steroidal saponins. Protodioscin, the most dominant saponin in TTE, is thought to be the main substance responsible for increasing testosterone production (Ganzera *et al.*, 2001), dehydroepiandrosterone (Adimoelja and Adaikan, 1997), dihydrotestosterone, and dehydroepiandrosterone sulfate (Gauthaman *et al.*, 2000).

Although, the other studied phytochemicals GE and DPP showed improvement effects on testosterone secretion (Tsai *et al.*, 2003; Abedi *et al.*, 2012), its effect on sex reversal is weak. This can be interpreted to its mechanism to promote testosterone secretion is different than TTE, where each GE and DPP act on the pituitary gland level and promote luteinizing hormone secretion, hence increase testosterone production (El-Desoky, 1995; Salvati *et al.*, 1996). Meanwhile, TTE reported to increase luteinizing hormone secretion (pituitary level) and act as testosterone precursor (testes level) (Bucci, 2000; Gauthaman *et al.*, 2002).

Intersex fingerlings were recorded with MT and TTE (1.2 g/kg diet) treatments. As sexing of fish was performed 84days after the treatment period, it might have been speculated that a 100% sex reversal was induced by MT treatment and the fish reversed back to their original sex when the effects of MT diminished (Piferrer and Lim, 1997). Meanwhile, in the case of TTE, the treatment



continue for last of the experiment, so the intersex occurrence didn't due to the back reverse of sex, it may be caused by incomplete differentiation process.

The survival rate after 28 and 84 days of experiment wasn't reduced by any studied treatments compared to control. These findings revealed that the used treatments didn't have potent toxic effect on studied fish. This result in accordance with the studied of Adimoelja (2000) and Adaikan *et al.* (2000), they have presented evidence that TTE is not toxic to humans and rabbits, respectively. Also, from the previous literature, ginseng (Goda, 2008) and date palm pollen (Iftikhar *et al.*, 2011) didn't report any toxicity cases.

In agreement with MT effect on improving growth performance, considerable information is available on the growth promoting efficiency of anabolic steroid hormones in fishes (Felix, 1989; Tveiten *et al.*, 1998; Asad *et al.*, 2010; Norbeck and Sheridan, 2011). The acceleration of growth due to MT treatment may due to the increase of growth hormone mRNA production in pituitary gland above basal levels when the fish treated with MT (Huggard *et al.*, 1996).

The use of phytochemicals treatments increased growth performance (FBW, SGR and length) rather than control. Meanwhile, the effects on condition factor didn't clearly appear. Furthermore, the phytochemicals inclusion to fish diets increase feed and nutrients utilization compared to control in the case of FCR and PER, without significant effects on feed intake and consequently protein intake. In accordance, ginseng helps regulate body functions, specifically improve adaptability and is believed to help build muscle and endurance (Francis *et al.*, 2002). In accordance, Goda (2008) found a significant increase in growth performance and feed utilization of Nile tilapia (*O. niloticus*) fed diets supplemented with ginseng extract 50, 100, 150, 200, and 250 mg/kg diet. Also, Yan *et al.* (2011) found that the addition of 0.1% wild-ginseng adventitious root to the diet increased growth performance of broilers chicken.

Parallel results to our findings reported by Gauthaman *et al.* (2002) in rats who found an increase in body weight due to TTE treatment. New-born guppies (*Poecilia reticulata*) treated with TTE extract exhibit successful growth acceleration comparing to control group especially with the concentration 0.1 and 0.15 g/L which reached to 4.5 time more than control for the two concentration (Cek *et al.*, 2007b). Moreover, Cek *et al.* (2007a) investigate the effect of higher doses of TTE extract (0.0, 0.10, 0.20 and 0.30 g/l) on growth and survival of convicted cichlid, the 0.3 g/l TTE extract improved growth rate 1.6 times more than control, the survival rate was uniformly high in control and treated group ranging from 88.57 to 90%.

In the case of DPP, the current findings in accordance with Iftikhar *et al.* (2011). The authors found that rat treated with 120 mg/kg daily had normal food intake and didn't have any negative effect on the survival of experimental animals. Attia *et al.* (2011) reported that rabbit offspring received a water solution containing 0, 100, 200 and 300 mg bee pollen/kg body weight, twice per week for 12 week, showed improvement in growth performance in bee pollen groups than control. The Bee pollen at 200 mg/kg BW reveal increase in weight gain and survival rate and reduced feed intake and feed conversion ratio of offspring. Furthermore, Abbass *et al.* (2012) investigate the supplementation of Nile tilapia (*O. niloticus*) diet with 2.5% of honeybee pollen for 21 days. The results showed that dietary honey bee

pollen significantly improved length, specific growth rate, average daily gain and feed efficiency ratio. On the other hand, Hassan *et al.* (2012) study the effect of 240 mg DPP/kg body weight of adult male albino rats daily for 30 days, the weight of treated adult male rat didn't differ compared to the control.

From a chemical composition point of view, the MT treatment increased moisture and decreased ether extract content than control and other treatments, meanwhile other treatments didn't affect the moisture level. The application of phytochemicals slightly increased protein content than control. Moreover, ether extract content decreased with the MT treatment. The effect of phytochemicals didn't clearly appear on the whole body composition in our study. The same trend was observed by Goda (2008), where whole body proximate analysis didn't present any statistical differences for dietary Ginsana<sup>®</sup> levels. In contrast, Yan *et al.* (2011) found that ginseng induced a linear decrease in abdominal fat in broilers chicken.

## CONCLUSION:

The findings of this study indicated that, the use of phytochemicals in fish diets is more growth promoter than sex-reversal agents in Nile tilapia (*O. niloticus*). Meanwhile, the probability of using TTE as an alternative agent for production of monosex Nile tilapia population is likely to occur. However, further investigation will be required to determine the optimum treatment regime for induction of 100% sex reversal with this phytochemical.

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## الملخص العربي

### تأثير التغذية بالفيتوكيميكال الطبيعية علي التحول الجنسي الذكري ومعدلات النمو والاستفادة من الغذاء ومكونات الجسم ليرقات البلطي النيلي

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تهدف الدراسة إلي تقييم استخدام الفيتوكيميكال الطبيعية كمصدر بديل لإحداث التحول الجنسي الذكري بالمقارنة بهرمون التستوستيرون  $17\alpha$ -methyl testosterone. تمت معاملة زريعة البلطي حديثه الفقس بهرمون التستوستيرون مقارنة بمستخلص الجنسج بتركيز 0.2 و 0.4 جم/كجم علف ومستخلص نبات القطب (الحسك) بتركيز 0.6 و 1.2 جم/كجم علف وبطلع النخيل بتركيز 3 و 6 جم/كجم علف. استمرت التغذية بالعلف المعامل بهرمون التستوستيرون لمدة 28 يوم وباقي المعاملات لمدة 84 يوم. أظهرت النتائج أن أعلى نسبة ذكور سجلت مع الأسماك المعاملة بهرمون التستوستيرون (92.3%) تبعها الزريعة المعاملة بمستخلص نبات القطب بتركيز 1.2 جم/كجم علف (64.48%) ثم تركيز 0.6 جم/كجم علف (57.76%) ثم طلع النخيل بتركيز 6 جم/كجم علف (56.67%)، بينما كانت نسبة الذكور في الأسماك غير المعامله (48.36%). جميع المعاملات أظهرت معدلات إعاشة طبيعیه علي مدار فترة التجربة والذي يثبت أن هذه الإضافات غير سامه علي الأسماك. كما أن الأسماك المعاملة بالفيتوكيميكال أظهرت ارتفاع معدلات النمو ومعدل التحول الغذائي بالمقارنة بالغير معاملة وخصوصاً مع الأسماك المعاملة بمستخلص نبات القطب. أظهر التحليل الكيماوي أن المعاملة بهرمون التستوستيرون أدت الي ارتفاع محتوي جسم الأسماك من الرطوبة وانخفاض محتواها من الدهون معنوياً بالمقارنة بالمعاملات الأخرى، في حين أن الأسماك المعاملة بالفيتوكيميكال أظهرت تحسن طفيف في محتوى جسم الأسماك من البروتين والدهون بالمقارنة بالأسماك غير المعاملة. وبالتالي يمكن استنتاج أن استخدام الفيتوكيميكال في علائق الأسماك يعتبر منشط نمو أكثر منه كمحول للجنس. ومن ثم فإنه يوصي باستخدام مستخلص نبات القطب في علائق زريعة البلطي لتأثيره الإيجابي علي النمو.