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IMPROVEMENT OF THE REPRODUCTIVE EFFICIENCY OF NILE TILAPIA *(OREOCHROMIS NILOTICUS)* AFTER FED WITH *(MORINGA OLIFERA*) LEAVES EXTRACTS

Adel M. Shalaby[1]; Ahmed A. AbdEl-Rahman[1];

Emad M. Gad[2] and Shimaa M. Metwally[1]

[1]*Fish physiology and hatchery Department, central Laboratory for Aquaculture Research, Agriculture Research Center, Ministry of Agriculture, Egypt.*

[2]*Chemistry Department, faculty of science, Suez Canal University.*

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Abstract

After 70 days of feeding Nile Tilapia *(O. niloticus)* with moringa leaves extracts (MLEs); the reproductive performance and physiological parameters were studied. The experiment was divided into 5 treatments, these treatments were T_1 (control without any moringa extract), T_2 and T_3 were fed on 0.5 and 1 gm of moringa leaves ethanol extract (MLEE)/kg respectively and T_4, T_5 were fed on 0.5 and 1 gm of moringa leaves petroleum ether extract (MLPEE)/kg respectively. The results showed that the highest value for fry production and ovaries weights were obtained with T2 and T3. The hematological parameters red blood cell (RBC), hemoglobin (Hb) and hematocrite (Hct) in the blood of Nile Tilapia were increased significantly in all treatments compared with control group. There no significant changes in AST and ALT activities in T2 and T3 (diet contain 0.5 and 1gm of MLEE/kg) and there are significant increase in total protein all treatments as compared with control group. The results of this study showed significant increase in estradiol hormone, ovary weight and gonad somatic index in females Nile tilapia in all treatments and showed significant increases in testosterone hormone level in serum of blood of male Nile tilapia.

INTRODUCTION

Egypt is considered as one of the developing countries which need to excess the fish production to cover the requirements of growing human population. To produce high quality production from fish, the reproductive processes in fish must be studied especially those mostly been cultivated

(Mohamed, 2008). Nile Tilapia is prolific breeder, easily breeds and does not need special hatchery technology, grows and reproduces in captivity where its sexual maturity is reached at an age of 5-6 months.

Brood stock nutrition is one of the most important factors limiting fish fry production and larval quality (Izquierdo *et al.*, 2001).*Moringa olifera*is one of promising plant protein sources for aquaculture, which grows in many countries under stressful environmental conditions and showing uncountable folk uses due to its various nutritional and pharmacological applications (Ferreira *et al.*, 2008).

Thawatchai *et al.* (2015) concluded that the use of *M.oleifera* leaf extract may provide a cheap and simple approach for improving sexual function under stressful conditions. Therefore, the extract appears to be a potential aphrodisiac, particularly for people with stressful daily life styles. *M. oleifera* contains antioxidants which can inactivate damaging free radicals produced through normal cellular activity and from various stresses (Rapatsa and Moyo, 2014). *M. oleifera* extract was reported to have antibacterial properties and conclusion was made to investigate it as aphytotherapeutic agent to combat infectious agents (Patel, 2011).The leaves and green fresh pods were used as vegetables by man and are rich in carotene and ascorbic acid (vitamin C) with a good profile of amino acids (Makkar and Becker, 1996). The leaves, in particular, have been found to contain phenolics and flavonoids compounds which have various biological activities, including antioxidant, anticarcinogenic, immune-modulatory, antidiabetic and hepatoprotective functions and the regulation of thyroid status in human and animals (Anwar *et al*, 2007).The study aimed to evaluate the effect of different levels (0.5gm and 1gm/kg diet) of ethanol MLE and petroleum ether MLE on the reproduction performance, physiological state of Nile tilapia.

MATERIALS AND METHODS

The present study was carried out on the Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou-Hammad, Sharkia, Egypt. The

feeding experiment was carried out at the hatching and physiology Department for 70 days.

The fresh leaves of *M. oleifera* were collected from the faculty of agriculture farm, Ain Shams University, Cairo, Egypt. Moringa leaves were washed then spread out under the shade to dry for 7-10 days. The leaves were removed manually and grounded into powder by a Moulinex domestic food blender and the powder was kept for later use (Bbole *et al*., 2016). The powder of leaves were weighed individually accurately to the 50 gm and placed on a clean and dry filter paper. The filter paper and its contents were placed in the central syphon portion of the Soxhlet apparatus. The analytical ethanol 95% was placed (250 ml) in the flask and connected to the Soxhelt syphon and condenser. The samples were refluxed for 20 hours. This process was repeated and replaced ethanol as a solvent with petroleum ether (60-80°C). The two extracts (MLEE and MLPEE) were then obtained after the removal of the extraction solvents by using a rotary evaporator at 50 °C. This process was repeated until enough amounts of two extracts were obtained. The obtained dried extracts were weighed, kept in a capped container and stored at -2 °C. (Abdel-Naby, 2014).

Determination of chemical components of two extracts (MLEE and MLPEE) by Gas chromatographic analysis (GC):

The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILLY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

Gas Chromatographic (GC) techniques:

Two extracts MLEE and MLPEE were analyzed by gas chromatography using (Agilent Technologies7890A) interfaced with a mass-selective detector(MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-

phenyl methyl poly siloxane) capillary column (30 m x 0.25 mmi. d. and 0.25µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min. The injector and detector temperatures were maintained at 200 º C and 270 º C, respectively. Volume injected is 1µl of the sample. The MS operating parameters were as follows: ionization potential 70 electron volt, interface temperature 250 º C, and acquisition mass range 50-800 (Abdel-Naby, 2014).

Diet preparation:

All dry ingredients shown in Table (1) were mixed with Moringa leaves extracts (MLEs) of each diet then the mixture of each treatment was blended using kitchen blender to make a paste of each diet. Pelleting of each diet was carried out by passing the blended mixture through a laboratory pellet machine with a 1 mm diameter matrix. The resulting wet pellets were dried at room temperature for two days. Five treatments were prepared; T_1 (control treatment without any extract), T_2 and T_3 contain 0.5 and 1 gm of moringa leaves ethanol extract/ kg (MLEE)respectively, T_4 and T_5 contain 0.5 and 1 gm of moringa leaves petroleum ether extract/ kg (MLPEE) respectively. The diets were stored in plastic bags in a refrigerator (+2°C) until use. The proximate analysis of experimental diets showed that the diet contained 35% crude protein and 6.20 % crude fat. Experimental diets were formulated according to (National Research Council, 1993).

Table 1. Ingredients and chemical analysis of the experimental diet.

Ingredients	**Quantity (gm)** %
Fish meal	30
Soybean meal	15
Rice bran	21
Wheat bran	16
Yellow corn	14
Molasses	2.75
Di calcium phosphate	1
Vit& minerals Premix	0.25
Proximate composition	%
Crude protein	34.85
Crude fat	6.20
Ash	7.70
Crude fiber	6.13
NFE	45.12

Experimental fish:

Healthy fish of 360 males and females *O. niloticus* were collected from Abbassa fish farm, Abu Hammad, Sharkia, Egypt. Fish samples contained 240 females and 120 males (2:1)with an average weight (220 gm).Fish were distributed in 15 concrete ponds; each pond contained 16 females and 8 males; concrete ponds (9 × 2.5 × 1.2 m), where water depth in each pond was 0.7m. Fish were acclimated for ten days in the same conditions. Fish was divided to 5 treatments, each treatment was triplicates. The first treatment (T_1) left as control group, the second and the third treatment (T_2 and T_3) fed with 0.5 and 1 gm of (MLEE)/ kg. The fourth and the fifth (T_4 and T_5) fed with diet contain 0.5 and 1 gm of (MLPEE)/ kg. The feeding ration amounted 0.7% of total body weight daily by hand were divided into two equal parts and fed at 9.00 am and 1.00 pm.

Production of fry:

Fries were collected from the ponds early every morning every fifteen days. All fries samples were taken weighted and counted for each pond. Fries were transferred to nursing ponds.

Measurement of fish growth brood stocks:

Growth performance was determined and feed utilization was calculated as follows:

Weight gain = W2 – W1

Specific growth rate was calculated according to Jauncey and Rose (1982).

Specific growth rate (SGR) = 100 (ln W2 – ln W1) / T

Relative body weight gain (RBWG) was calculated according to the following equation: $RBWG= (wt_2-wt_1) x100/wt_1$

Where W1 and W2 are the initial and final weights, respectively, and T is the number of days of the experimental period.

Feed required to produce 1000 fry, (kg) = total feed used, (kg/pond)/ (fry no. / pond)

The cost of feed/1000 fry, LE=Feed required to produce 1000 fry, (kg)* the cost of one kg feed

Hematological examination:

At the end of the experimental period 6 fish (3 males and 3 females) from each of the treatments and control group were taken for physiological investigations. Fish were not fed for 2 4 hours prior to blood sampling. Fish blood was collected from each fish caudal vein by a sterile syringe (Feldman *et al*., 2000). Blood samples were divided into two parts; the first part of the blood was transferred on dry and clean tube with 0.5 ml of EDTA as an anticoagulant was used for erythrocyte count (Dacie and lewis, 1984), haemoglobin content (Vankampen, 1961) and haematocrit value (Britton, 1963), and blood indices (MCV, MCH and MCHC) were calculated.

The second part was transferred in clean and dry tubes then centrifuged for 15 minutes at 3000 r.p.m for separation of serum (collected serum was stored at -20°C) for hormones measurements and biochemical analysis e.g Serum AST and ALT activity was determined calorimetrically according to the

method described by Reitman and Frankel (1957). Total protein concentration in fish serum was determined by the Henry technique (Henry, 1964). Glucose was determined according to the method of Trinder (1969) ,Teststerone levels in serum of blood were measured by hormonal assays, which were done by Radio Immune Assay (RIA) using the kits supplied by (Coat-A-count) provided by Orion Diagnostic Spectra, Finl and according to (Abraham, 1977). But estradiol in females was determined by the radioimmunoassay method according to Xing *et al*. (1983).

All the brought fish were weighed to the nearest 0.1 gm and plucked out gonads (testes and ovaries) and liver. The gonads and liver were weighed for each fish to calculate the gonado somatic index and hepato somatic index Gonado somatic index (GSI) Hepato or somatic index (HIS) were calculated from the following formula (Munkittrich and Dixon, 1988):

GSI= (Gonads weight / body weight) × 100

HSI= (liver weight / body weight) × 100

Statistical analysis:

One way analysis of variance (ANOVA) was conducted to test the effect of moringa extracts on male/female efficiency on the spawning performance of broad fish. This analysis was done using the computer program SPSS and least Significant difference (LSD) post hoc were done to determine significant differences (Tamhane and Dunlop, 2000).

RESULTS

The green extracts obtained from moringa leaves ethanol extract (MLEE) and moringa leaves petroleum ether extract (MLPEE) were analyzed by gas chromatography and identified by comparing their retention times with that of authentic standards injected under identical conditions. Results of chemical composition of two extracts (ethanol and petroleum ether extracts are shown in Table 2 and 3, respectively).

Table 2. Chemical composition of ethanol Moringa leaves extract analyzed by gas chromatography.

No	RT(min)	Name	Area sum %
1	7.018	3,7,3´,4´,5´-Pentamethoxyflavone	0.65
2	10.574	Quercetin 3´-methyl ether	0.53
3	11.075	1H-Indol-5-ol	6.04
4	12.375	Tetradecanoic acid	1.01
5	12.497	Kampferol-3,4´ dimethyl ether	0.36
6	12.888	Phytol	1.89
7	13.063	2´-Hydroxy-2,3,4´,6´-tetramethoxychalcone	0.61
8	13.189	Astilbin	0.81
9	13.576	Geranylsovalerate	1.12
10	13.715	4´,6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	12.47
11	13.841	7, 4´-Dimethoxy-3-hydroxyflavone	1.43
12	14.334	7, 3´-Dimethoxy-3-hydroxyflavone	0.67
13	14.603	Serotonin	1.85
14	14.835	Linolenic acid	30.14
15	14.962	Quercetin 3´, 4´, 7 trimethyl ether	2.87
16	15.088	5,3´-Dihydroxy-6,7,4´-trimethoxyflavone	0.38
17	15.218	9-Octadecenamide,(Z)-	0.48
18	15.785	3,5,3´,5´-Tetra-tert-butyldiphenoquinone	0.87
19	15.931	Ascorbic acid, permethyl	1.1
20	16.253	Dodecanedioic acid	0.57
21	16.486	3-Hydroxy-6,3´,4´-trimethoxyflavone	1.54
22	16.803	Methyl β-D-galactopyranoside	1.2
23	17.89	Salicylic acid β-D-O-glucuronide	5.05
24	18.726	Quercetin 3,5,7,3´,4´-pentamethyl ether	2.86
25	18.983	6-methoxyluteolin	0.35
26	19.247	Methyl β-D-galactopyranoside	0.96
27	19.842	Vitamin E	2.31
28	20.466	Phytanic acid	0.87
29	20.694	Octacosane	7.44
30	21.337	Tetracosanoic acid	1.82
31	21.704	β-Citronellol	0.55
32	22.026	β-Sitosterol	1.55
33	22.792	Stigmasterol	3.16
34	22.967	Squalane	4.49
		Total	100

Table 3. Chemical composition of petroleum ether Moringa leaves extract analyzed by gas chromatography.

No	RT(min)	Name	Area sum %
1	5.47	2- Decanol	0.57
2	6.619	Phytol	1.32
3	7.706	D-(+)-Mannose	1.63
4	10.615	7- Methoxycoumarin-4-acetic acid	0.9
5	10.692	3,4-Dimethoxycinnamic acid	0.87
6	10.855	β- Patchoulene	1.32
7	11.372	Cannabidiol dimethyl ether	2.09
8	11.47	2´- Hydroxy-3-,4,5´-trimethoxychalcone	1.4
9	11.633	Longipinene	1.8
10	11.91	β- Cuvebene	1.57
11	12.106	ɣ-Cadinene	1.59
12	12.134	6, 3´,4´-Trimethoxyflavanone	1.15
13	12.224	β- Himachalene	1.27
14	12.38	β- Curcumene	1.77
15	12.65	3- Methyl-4-methylenebicyclo[3.2.1oct-2-ene	1.25
16	12.786	7- Hydroxycoumarin-4-ylacetic acid	1.59
17	12.843	1,3-Diisopropylbenzene	0.6
18	12.92	β- Caryophyllene	1.75
19	13.095	A- Copaene	2.02
20	13.336	Kaur-16 –ene	1.61
21	13.792	Afromosin 7- O – glucoside	11.29
22	14.644	(S)-(-)-Citronellic acid	1.95
23	14.8569	5β, 7βH, 10α-Eudesm-11-en-1α-ol	9.4
24	15.006	7,8 – Dihydro- α – ionone	1.17
25	15.088	Genkwanin	3.08
26	15.943	Geranylisovalerate	1
27	16.257	Farnesol	1.4
28	16.509	Cis-Sesquisabinene hydrate	3.17
29	16.864	Gardenin	0.82
30	17.919	Squalane	5.61
31	19.276	Ascorbic acid , permethyl	1.45
32	20.799	Phytan	11.19
33	21.728	Pyrimido[1,2-a]azepine, 2,3,4,6,7,8,9,10-octahydro	4.14
34	22.042	Pregabalin	1.39
35	23.081	Farnesan	14.89
		Total	100.02

Water quality parameters in the concrete ponds used for Nile tilapia spawning throughout the experimental period:

The water quality parameters were the same in all concrete ponds during the experimental period. Temperature was 29.2±0.45°C, the dissolved oxygen was 5.5±0.48 mg/L, pH was 7.2± 0.27 and total ammonia was 0.88±0.27 mg/L. All parameters were within the acceptable levels for spawning and development of Nile tilapia.

Reproductive performance:

The effect of moringa leaves extracts on reproductive performance of Nile tilapia in Table (4) showed that the fry number /pond were significantly increased to (24040, 23140, 21980 and 21113) in fish fed with 0.5 and 1 gm of ethanol and petroleum ether/ kg diet respectively when compared to the control group(18403). Also the fry numbers /female were significantly higher in T_2 and T_3 (1503 and 1446) respectively when compared to the control group (1150); this value was significantly increased in fish fed with 0.5 and 1 gm of petroleum ether /kg diet (T_4 and T_5) as compared with control group.

Table 4. The effect of different levels of moringa leaves extracts on reproductive performance of Nile tilapia.

Items	T1 (Control)	T2	T3	T4	T5
Fry no./pond	18403±155^{e}	24040±247^{a}	23140±202^{b}	21980±95^{c}	21113±120^{d}
No of fry/ female	1150±9^{e}	1503±15^{a}	1446±13^{b}	1373±6^{c}	1319±8^{d}
Female average wt (gm)	234 ±1.53^{e}	247±2.31^{a}	244±3.01^{b}	241±1.14^{c}	238±0.58^{d}

$^{a-d}$ Means having the same letter in the same row is not significantly different at ($P< 0.05$).

Hematological parameters:

They were recorded in Table (5). The results of erythrocytes count (RBC) were significantly increased in all treatments compared with control group (1.71± 0.09 X10^6/cmm). Erythrocytes count (RBC) was significantly high in T2 (2.76 ± 0.15X10^6/cmm) and T3 (2.61 ± 0.02X10^6/cmm) than that in fish of T4 (2.24 ± 0.02X10^6/cmm) and T5 (2.10± 0.05X10^6/cmm). Hemoglobin (Hb) content was significantly increased in all treatments compared with control

group (5.85 ± 0.33g/dl), but fish of T2 (8.00 ± 0.12g/dl) and T3 (7.30± 0.16g/dl) were higher than T4 (7.11 ± 0.06g/dl) and T5 (7.01 ± 0.08g/dl). The results of hematocrit percentage were significantly increased to T2 (22.72 ± 0.69 %, T3 (21.71 ± 1.72 %), T4 (20.37 ± 0.63 %) and T5 (19.50 ± 0.47 %) in all treatments compared with control group (16.87 ± 0.89 %). In contrast there was a significant decrease in mean values of MCV and MCH in T2, T3and T4 compared with control group, however MCHC indicate that there was no significant difference in all treatments.

Table 5. The effect of different levels of moringa leaves extracts on RBCs, Hb, Hct, MCV, MCH and MCHC of male Nile tilapia.

Items	Control (T1)	T_2	T_3	T_4	T_5
RBCs ($X10^6$/Cmm)	1.71 ± 0.09^d	2.76 ±0.15^a	2.61 ± 0.02^b	2.24 ± 0.02^c	2.10 ± 0.05^c
HB (g/dl)	5.85 ± 0.33^c	8.00± 0.12^a	7.30± 0.16^b	7.11± 0.06^b	7.01 ± 0.08^b
Hct (%)	16.87± 0.89^d	22.72 ± 0.69^a	21.71± 1.72^{ab}	20.37± 0.63^{bc}	19.50± 0.47^c
MCV($\mu^{3)}$	98.76± 4.39^a	82.33± 3.22^c	83.31± 6.46^c	90.91 ± 2.07^b	92.95± 1.31^{ab}
MCH (Pg)	34.27 ± 1.67^a	28.99 ±1.09^c	28.02 ± 0.41^c	31.75± 0.09^b	33.41± 0.37^{ab}
MCHC(%)	34.78 ± 3.03^a	35.22 ± 0.79^a	33.76 ± 2.53^a	34.94 ± 0.89^a	35.95 ± 0.60^a

$^{a-c}$ Means having the same letter in the same row is not significantly different at ($P< 0.05$).

Biochemical parameters:

Hepatic enzymes of males Nile tilapia in serum were shown in Table (6). There were no significant increases in aspartate amino transferase (AST) level and glucose concentration in serum of Nile Tilapia fish in all treatments compared with control group. ALT activity significantly increased with increasing levels of leaves extracts in T_3, T4 and T_5, but there no significant differences in T_2 compared with control group. Total protein showed significant increases in T_2 and T_3, but there were significant increases in albumin in all treatments.

Liver functions of females Nile tilapia in serum were shown in Table (7). There were significant increases in AST, ALT levels and glucose concentration in T_3, T_4 and T_5; but no significant increase in T_2 and T_3compared with control group. There were significant increases in total protein and albumin in T_2 and

T_3; but there were no significant increase in T_4 and T_5 compared with control group. Table (9) showed no significant changes in HSI% in all treatments compared with control group.

Table 6. The effect of different levels of moringa leaves extracts on AST, ALT, protein, albumin, glucose and HSI of male Nile tilapia.

Items	Control (T_1)	T_2	T_3	T_4	T_5
AST (U/L)	20.67 ± 1.04^a	21.17 ± 0.58^a	20.17 ± 1.04^a	21.83 ± 1.04^a	22.07 ± 0.9^a
ALT (U/L)	13.4 ± 0.53^{bc}	13.93 ± 0.70^b	17.07 ± 1.01^a	15.17 ± 1.26^{ab}	16.93 ± 0.4^a
Protein (g/dl)	2.74 ± 0.32^c	3.65 ± 0.21^a	3.16 ± 0.07^b	3.07 ± 0.13^{bc}	2.91 ± 0.20^{bc}
Albumin (g/dl)	1.79± 0.04^c	1.96± 0.16^a	1.81 ± 0.02^{ab}	1.74 ± 0.02^b	1.96 ± 0.10^a
Glucose (mg/ dl)	96.33 ± 5.03^a	101.67 ± 6.51^a	107.67 ± 4.93^a	109.00 ± 9.85^a	110.33 ± 10.60^a
HSI (%)	1.86 ± 0.12^a	1.87 ± 0.09^a	1.93 ± 0.19^a	1.99 ± 0.22^a	1.99 ± 0.31^a

$^{a-c}$ Means having the same letter in the same row is not significantly different at ($P < 0.05$).

Table 7. The effect of different levels of moringa leaves extracts on AST, ALT, protein, albumin, glucose and HSI of female Nile tilapia.

Items	Control (T_1)	T_2	T_3	T_4	T_5
AST U/L	18.83 ± 0.65^c	19.50 ± 0.50^c	21.17 ± 0.76^b	23.40 ± 0.69^a	23.33 ± 0.58^a
ALT U/L	19.83 ± 0.76^b	15.60 ± 0.87^b	16.10 ± 0.17^b	18.33 ± 0.76^a	17.67 ± 0.58^a
Protein(g/dl)	3.78 ± 0.10^b	4.13 ± 0.12^a	4.11 ± 0.19^a	3.87 ± 0.06^b	3.72± 0.16^b
Albumin (g/dl)	2.47 ± 0.06^c	2.79 ± 0.09^{ab}	2.99 ± 0.10^a	2.20 ± 0.17^d	2.64 ± 0.23^{bc}
Glucose (mg/dl)	75.20 ± 2.43^c	84.00 ± 5.29^{bc}	88.00 ± 4.58^b	93.50 ± 6.5^{ab}	99.00 ± 5.29^a
HSI (%)	1.84 ± 0.12^a	1.82 ± 0.09^a	1.89 ± 0.03^a	1.95 ± 0.19^a	2.00 ± 0.28^a

$^{a-c}$ Means having the same letter in the same row is not significantly different at ($P < 0.05$).

Hormonal measutments:

Table (8) showed significant increases in estradiol hormone in blood serum of females Nile tilapia after fed with diet containing 0.5 and 1 mg of moringa ethanol extract/kg when compared with the other treatments and control group. There were significant increases in cortisol hormone in all treatments compared with control group. The ovary weight and gonad somatic index in females Nile tilapia were significantly increased inT_2 and T_3 (MLEE) and no significant increases in T_4 and T_5 when compared with control group (5.90 ± 0.18 gm) and (2.69 ± 0.07 %), respectively.

Table 8. The effect of different levels of moringa leaves extracts on estradiol, cortisol, ovary weight and GSI of females Nile tilapia.

Items	Control (T1)	T_2	T_3	T_4	T_5
Estradiol (pg/ml)	204.00 ± 2.58^{d}	279.67 ± 2.60^{a}	252.63 ± 5.63^{b}	202.87 ± 3.01^{d}	231.50 ± 3.40^{c}
Cortisol (ng/ml)	82.97± 3.23^{b}	91.57 ± 6.06^{a}	96.67 ± 1.17^{a}	98.17 ± 2.75^{a}	94.57 ± 6.95^{a}
Ovary wt(g)	5.9 ± 0.18^{c}	8.23 ± 0.19^{a}	7.18 ± 0.34^{b}	6.34 ± 0.39^{c}	6.22 ± 0.37^{c}
GSI (%)	2.69 ± 0.07^{c}	3.73 ± 0.06^{a}	3.26 ± 0.14^{b}	2.88 ± 0.18^{bc}	2.81 ± 0.16b^{c}

$^{a-c}$ Means having the same letter in the same row is not significantly different at (P<0.05).

Table (9) showed significant increases in T2 and T3in testosterone hormone level in serum of blood of male Nile tilapia to (7.39 ± 0.43 and 6.92 ± 0.70ng/ml) compared with all treatments and control group (4.81 ± 0.29 ng/ml) after fed with diet containing 0.5 and 1 mg/kg of moringa ethanol extract. There was no significant increase in testosterone level in T4 and T5 after fed with diet containing 0.5 and 1mg/kg of moringa petroleum ether extract. However there was no significant increase in T_2, T_3 and control group in cortisol but there was a significant increase in T_4 and T_5 cortisol compared with control group. There were significant increases of testis weight and GSI in all treatments compared with control group.

Table 9. The effect of moringa leaves extracts on testosterone, cortisol, testis weight and GSI of males Nile tilapia.

Items	Control (T_1)	T_2	T_3	T_4	T_5
Testosterone (ng/ml)	4.81 ± 0.29^{b}	7.39 ± 0.43^{a}	6.92 ± 0.70^{a}	4.84 ± 0.66^{b}	5.19 ± 0.10^{b}
Cortisol (ng/ml)	101.33 ± 9.02^{c}	108.67± 3.51^{bc}	111.33± 4.04^{bc}	116.00± 3.46^{ab}	125.67 ± 6.11^{a}
Testis wt(gm)	1.39 ± 0.22^{c}	2.28 ± 0.13^{a}	2.20 ± 0.14^{ab}	1.88 ± 0.18^{b}	1.88 ± 0.19^{b}
GSI (%)	0.63 ± 0.11^{c}	1.04 ± 0.07^{a}	0.99 ± 0.06^{ab}	0.85 ± 0.06^{b}	0.85 ± 0.07^{b}

$^{a-c}$ Means having the same letter in the same row is not significantly different at (P<0.05).

Growth performance:

All treatments supplemented with moringa extracts and varying concentrations of MLE induce increase significantly in body weight gain, RBWG **%,** daily wt. gain and improved specific growth rate in treated fish; the

best results are T_2 and T_3moringa ethanolic groups compared with control group.

Table 10. Growth performance of Nile tilapia brood stock as affected with different levels of moringa leaves extracts.

Items	T_1	T_2	T_3	T_4	T_5
Initial weight(gm)	219.33±1.53^{a}	220.33± 1.53^{a}	220± 1.73^{a}	220± 1.53^{a}	221.33 ± 0.58^{a}
Final weight (gm)	254.33±1.53^{c}	269.33 ±1.53^{a}	265.50 ± 1.73^{b}	263.73 ± 1.53^{b}	263.33 ± 0.58^{b}
Weight gain (g)	35± 1.21^{d}	49.00± 1.00^{a}	45.5± 1.53^{ab}	43.40± 0.40^{bc}	42.00± 1.63^{c}
RBWG %	15.96± 0.11^{e}	22.24± 0.15^{a}	20.68± 0.16^{b}	19.70± 0.14^{c}	18.98± 0.05^{d}
Daily wt gain	0.5± 0.05^{d}	0.7± 0.01^{a}	0.65± 0.02^{ab}	0.62± 0.01^{bc}	0.60± 0.05^{c}
SGR (% / day)	0.21^{d}	0.29^{a}	0.27^{b}	0.26^{c}	0.25^{c}

$^{a-d}$Means having the same letter in the same row is not significantly different at (P<0.05).

Economic analysis show that feed required producing 1000 fry by gram and its cost significant decreased in all treatments compared with control group. Although there are significant increases in total, feed used gm/pond in all treatments compared with control group the cost of feed/1000 fry required to produce 1000 fry significant decreased and the best result T_2 diet supplemented with 0.5 gm of moringa leaves ethanol extract.

Table 11. Economic evaluation and feed utilization efficiency of Nile tilapia brood stock as affected with different levels of moringa leaves extracts.

Items	T_1	T_2	T_3	T_4	T_5
Fry no./pond	18403±155^{e}	24040±247^{a}	23140±202^{b}	21980±95^{c}	21113±120^{d}
Total feed used ,gm/pond	2682±15.95^{c}	2750±10^{a}	2730±10^{ab}	2720±10^{b}	2713±15^{b}
Feed required to produce 1000 fry, (gm)	146±2^{a}	114±1.53^{e}	118±1.73^{d}	124±1^{c}	128±0.57^{b}
The cost of feed/1000 fry ,LE	1.31±0.028^{a}	1.05±0.012^{e}	1.12±0.012^{d}	1.14±0.011^{c}	1.22±0.006^{b}

$^{a-d}$Means having the same letter in the same row is not significantly different at (P<0.05).

DISCUSSION

Herbs or medicinal plants have reported to promote various functions like growth, hematological and biochemical status, immune functions**,** egg hatching rates (Yilmaz and Ergün 2012a) and also increase disease resistance in fish culture due to different active component present in herbs plants.(Moyo *et al*., 2011) reported that the profiling of chemical composition, fatty acids, amino acids, minerals and vitamins are essential in animal feed. Such nutrients are used for osmotic adjustment; activate enzymes, hormones and other organic molecules that enhance growth, function and maintenance of life process**.** From the above results at the present previous based on the studies concerned with medicinal plants, the present investigation aimed to study the effect of MLEs as feed additives in Nile tilapia diets on physiological state and reproductive performance.

All water quality parameters were kept within the acceptable limits for spawning according to Pradhan *et al.* (2014) who proved that hematological indices are the most critical ones serving as indicators of fish health.

The improvement in reproduction efficiency of moringa leaves ethanol extract additive feed fed fish reported in this work may be also due to MLE content of micronutrients, this result in agreement with Izquierdo *et al.* (2001) who reported that breeding aquaculture species have high nutritional requirements, thus reproductive capacity depends on appropriate concentrations of lipids, proteins, fatty acids, vitamins C and E, and carotenoids. Furthermore, the relationship of these components influences reproduction in various processes such as fertility, fertilization, birth and development of larvae. The increasing in numbers of fries in fish fed the moringa leaves extracts diet may be due to the presence of B group vitamins; this agreement with these of (Wooster *et al.,* 2000) who demonstrated that the reduction in the mortality of Atlantic salmon fries is due to the beneficial effect of thiamin (vitamin B1). All treatments supplemented with moringa extracts induce the increase in the body weight gain, daily wt. gain and improved specific growth rate in treated fish

especially moringa ethanolic groups compared with control, these results are in agreement with (Hossam *and* Ashraf, 2014).These elevation in the weight gain of fish and improved in feed conversion rate may be attributed to presence of high amounts of vitamins, minerals and amino acids in Moringa leaf (Nkukwana *et al.,* 2014).

Detecting the changes in the blood parameters provides ability to predict the subsequent health of fish (Rehulka, 1996). In the current work, all treatments supplemented diets with MLEs (ethanolic and petroleum ether) induce significant increase in total erythrocytic count, haemoglobin content Hb, packed cell volume, total leukocytic count and phagocytosis % at end of the experiment. These enhancements of these parameters may be due to presence of saponin, alkaloids and flavonoids in chemical composition of MLEs. Same result was reported by (Hossam *et al.,* 2016) in broiler chickens and *O. Niloticus* received MLEs. Elevation in erythrocytic count, haemoglobin content and packed cell volume % post using Moringa may be due to presence of saponin in Moringa (Khalil and Eladawy, 1994)or alkaloids and flavonoids in Moringa induce increase in total erythrocytic count, haemoglobin content and packed cell volume % (Anwar *et al.,* 2007). The calculated blood indices MCV, MCH and MCHC have a particular importance in anemia diagnosis in most animals (Shalaby *et al.,* 2013). The perturbations in these blood indices (decrease MCV, MCH and no significant difference MCHC) these may be related to the increase in Hb and Hct levels.

The enzymes ALT and AST in blood indicate organ dysfunction in aquatic organisms during stress condition (Gabriel and George, 2005). The higher activity of AST and ALT indicates the mobilization of aspartate and alanine via gluconeogenesis for glucose production to cope with stress. (Vutukuru *et al.,* 2007) mentioned that enzymes play an important role in carbohydrate–protein metabolism in fish and determination of transaminases (AST and ALT) can also be used for aquatic biomonitoring. Tekle and Sahu, 2015 reported reduction in cellular enzymes AST and ALT activities when fish diets were supplemented with plant extracts. In the present study, there no

significant changes in AST and ALT activities in T2 and T3 (diet contain 1gm of MLEE/kg) compared with control group, these finding was in agreement of that Gbadamosi *et al.,* (2016) who concluded that moringa leaf supplementation significantly reduced the activities of AST and ALT suggesting that Moringa leaf protected the membrane integrity of the liver cells against stressors. Many studies have shown that an important mechanism of the hepato protective effects may be related to an antioxidant capacity to scavenge reactive oxygen species. Hence, as there was less cellular activity in the Moringa ethanol extracts supplemented groups, it can be inferred that addition of Moringa plant extracts reduced stress and improve growth and health of fish in the present study.

Total protein and albumin activity significantly increase in T1 and T2 compared with all treatments. The obtained results revealed significant increase in serum total proteins, albumin at the end of supplementation of 0.5 gm of MLEs /kg. Our results were coinciding with those obtained by (Hossam Allam *et al.,* 2016) who stated that moringa induce significant increase in total protein and albumin. Elevations in total protein our study may be due to moringa are rich in both essential and sulfur-containing amino acids (Moyo*et al.,* 2011) and due to *M. oleifera* contain large amount of protein.

Glucose and cortisol are important endogenous stress indicators in cells, and their activities are keys indicators of the immunity of the fish (Oliva-Teles, 2012). Khalil, 2018) mentioned that increasing of cortisol level produce inhibitory effects on reproduction of fish as it consider an indicator of stress in fish. Supplementation diets of Moringa leaf extract decreased the level of glucose in fish (Gbadamosi*et al.,* 2016). In this study there was no significant increase between MLEE treatments in glucose and cortisol of females Nile tilapia but there was a significant increase in MLPEE treatments in glucose and cortisol of females; there was a significant increase in T3 and T4 glucose of males compared with control group. This results may be due to MLEE contain large amount of antioxidants like vitamins A, B and C, riboflavin, nicotinic

acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron, and alpha-tocopherol according to chemical analysis of(El Sohaimy *et al*., 2015). This result was in agreement with many studies that reported the role of plant extracts in stimulating the immune system by modulating the activity of metabolic and antioxidative stress enzymes. Tekle and Sahu (2015) reported the ameliorative effects of Moringa flower on *O.niloticus* subjected to *Aeromonas hydrophila* induced stress. *M. oleifera*plant has been widely reported to contain constituents such as nitrile, glycosides and quercetin (Ojiako, 2014) which are believed to be responsible for enhancing immunity against oxidative stress and microbial diseases. The role of the bioactive compounds in the extract is to boost the non-specific defense system including the lysozyme activity. Therefore the presence of potent antioxidants in Moringa supplemented diets was helpful in reducing the negative effects of stressors in *O. niloticus* fish. This result is in agreement with many studies that reported the role of plant extracts in stimulating the immune system and reducing the percentage mortality of fish infected with pathogenic strain of bacteria and by modulating the activity of metabolic and antioxidant stress enzymes Tekle and Sahu (2015).

Testosterone is the most important make sexual hormone in males; it is mainly responsible for development of sex organs and sperm production. The most effective doses of phytochemicals on testosterone were significantly increased in T2 and T3 compared with control group. Testis weight and gonad-somatic index (GSI) of males Nile tilapia significantly increased in all groups fed moringa-supplemented diets compared with the control. The present results indicate that MLEE have positive effect on sexual parameters of fish. These results may be due to presence of flavonoids which are well known antioxidants that can ameliorate oxidative stress- related testicular impairments in animal tissues. flavonoids stimulates testicular and rogenesis and is essential for testicular differentiation and steroidgenic functions. Our results were also corroborate with the finding of (Zade *et al.,* 2013) who concluded that Moringa seeds aqueous extract enhanced sexual behavior of male albino rats. These results are in agreement with Mansour *et al.* (2017) who reported that ginseng

extract increased testosterone levels and gonads for Nile tilapia fingerlings Moreover, the effect of ginseng on testosterone levels could be attributed to its gene regulation effects on testis or due to dietary ginseng up regulated genes that are responsible for steroid hormone metabolism and the top molecular and cellular functions of testes. The results were agree with Gabriel (2017) who indicate that *Aloe. vera* extracts have the potential to be used as sex-reversal agents in Nile Tilapia culture.

Also, the present study revealed that estradiol hormone was increased significantly at 0.5 gm of MLEE/kg diet compared by other treatments and this in agreement with (Izquierdo *et al.*, 2001) who reported that brood stock nutrition is one of the most important factors limiting fish fry production and larval quality. In addition, lipid composition of fish plays a crucial rule in spawning performance and made improvement of all reproductive performance parameters of fish. Gonad somatic index represents the percentage of gonad weight to body weight; GSI seemed to be the best indicator of the fluctuations of the breeding activity of a species throughout the year (El-Agamy*et al*., 1997). In this study GSI showed that significantly increased in females of T2 and T3; there no significantly increased in T3 and T4 compared with control group. But there were significantly increased in GSI of all males 'treatments. These results are in agreement with (Hussein, 2016)who reported that moringa leaves powder can be used as feed additive in the formulated diets for Nile tilapia, *O. niloticus* with improving the performance without cause any adverse effect and no histopathological effect of moringa leaves meal supplemented diets on fish body tissues (liver, gonads and intestine). Otherwise, hepatsomatic index (HSI) which represents a good indication of the liver damage and consequently welfare of fish. In this study HSI showed that no significantly increased in all treatments.

The improved hematological parameters, increased of number of fry and fish growth, may be due to the improved feed intake, which may be possibly has happened due to the presence of *Moringa* leaves ethanol extracts used as a

rich source of essential fatty acids including linolenic acid in the tested feed additive which are required to prevent fatty acid deficiency diseases such as low growth and essential for growth, linolenic acid (C18:3) are the apparent compounds of the omega-3 fatty acid series (Abdel-Naby, 2014), which was found in high amount in moringa leaves ethanol extract which are essential in the diet to increasing immune system. Moreover, the presence of polyphenols, ascorbic acid and flavoniods in moringa extracts which show antioxidant activity. These components could participate on fish metabolism helping to improve health and growth.

The present results suggests that MLEE is better than MLPEE due to MLEE contain more natural compounds and vitamins; this indicate that ethanol have great affinity and high solubility properties for different compositions of *M. oleifera* leaves and this may be due to ethanol is polar solvent and most of bioactive components of moringa leaves are highly polarizable (Dasola *et al*., 2014).

CONCLUSION

Their high polarizability gives high yield of extract compositions in ethanol extract than petroleum ether extract. Supplementation of *M. oleifera* leaves ethanol extract in feeds for *O.niloticus* improves growth performance and increase number of fry; we conclude that *M. oleifera* leaves ethanol extract in lower dosage (0.5gm/kg) has the potential to be used as a supplement in fish diets. The use of medicinal plants in fish will be an efficient tool to achieve sustainable, economical increase, and safe fish production.

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تحسين الكفاءة التناسلية لأسماك البلطي النيلي بعد تغذيتها بمستخلصات اوراق المورينجا

عادل محمد عيسى شلبى[1]، أحمد عبدالله عبدالحمن علي[1]،
عماد محمد أحمد جاد[2]، شيماء محمد متولي [1]

[1] قسم التفريخ والفسيولوجي بالمعمل المركزي لبحوث الثروة السمكية، مركز البحوث الزراعية، وزارة الزراعة، مصر.

[2]قسم الكيمياء بكلية العلوم جامعة قناة السويس، فرع الإسماعلية.

الملخـص العربــى

تم تغذية اسماك البلطي النيلي لمدة 70 يوم علي عليقة تحتوي علي مستخلص اوراق نبات المورينجا فقد تم دراسة الاداء التكاثري والعناصر الفسيولوجية لهذه الاسماك. وقد قسمت التجربة الي خمس مجموعات وكل مجموعة ثلاث مكررات في احواض اسمنتية وكانت هذه المجموعات هي المجموعة الاولي (T1) وغذيت الاسماك بها على عليقة بدون اي اضافة لمستخلص المورينجا كمجموعة ضابطة والمجموعة الثانية (T2) غذيت علي عليقة مضاف إليها 0.5 جم / كجم من المستخلص الكحولي لأوراق المورينجا والمجموعة الثالثة(T3) غذيت علي عليقة مضاف إليها 1جم/ كجم من المستخلص الكحولي لأوراق المورينجا. أما المجموعة الرابعة (T4) غذيت علي عليقة تتغذى علي عليقة مضاف إليها 0.5 جم / كجم من مستخلص أوراق المورينجا بالبتروليم إيثر والمجموعة الخامسة (T5) غذيت علي عليقة مضاف إليها 1جم/ كجم من مستخلص أوراق المورينجا بالبتروليم إيثر.

وأوضحت النتائج زيادة معنوية في عدد الزريعة ووزن المبيض الناتج عن المجموعة الثانية والثالثة وأيضا زيادة معنوية في كل المعاملات في قياسات الدم لأسماك البلطي النيلى مثل عدد كرات الدم الحمراء (RBCs) ومحتوي الهيموجلوبين(Hb) ونسبة الهيماتوكريت (Hct) في الدم وهذا بالمقارنة مع الجموعة الاولى الضابطة. كما أظهرت النتائج زيادة معنوية في هرمون الاستراديول وحساب مؤشر الأعضاء التناسلية فى إناث الأسماك فى المجموعة الثانية والثالثة والخامسة، ووجد زيادة معنوية في هرمون التيستوستيرون فى تحليلات الدم لذكور الأسماك. ولذلك نوصي باستخدام المستخلص الكحولي لأوراق المورينجا فى علائق الاسماك بنسبة 0.5 جم / كجم حيث أنها تحسن الاداء التكاثري والحاله الفسيولوجية للأسماك.