Egyptian Journal for Aquaculture
P-ISSN: 2090-7877
E-ISSN: 2636-3984
www.braesd.org
Gad *et al.*, 2019; 9 (1):77-95

Biochemical changes of Moringa seeds extracts on Nile Tilapia breeding fish

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Received: March. 3, 2019; Accepted: March. 24, 2019 published: Vol.9 (1):77-95; 2019

Abstract

The present study was conducted to evaluate the effect of *Moringa oleifera* seeds extracts in two different solvents ethanol and petroleum ether on the reproductive performance and physiological state of Nile Tilapia. Fish were stocked in 15 concrete ponds, grouped into 5 treatments in triplicates for each treatment. The first treatment T1 (control without any moringa extract), the second T2 and the third T3 (containing respectively 0.5 g and 1 g of moringa seed ethanol extract (MSEE)/kg diet), the fourth T4 and the fifth T5 (containing respectively 0.5 g and 1 g of moringa seed petroleum ether extract (MSPEE)/kg diet). The experiment extended to 70 days. The results showed that the highest numbers of fry production and ovaries weights were obtained with T4 and T5. The best treatment for blood hematological parameters is T4 and the highest level of testosterone and esteradiol hormones were observed in all moringa treatments. Thus it was concluded that 0.5 g of Moringa seed petroleum ether extract/kg was useful for the improvement of the reproductive performance and physiological blood characteristics of brooding stock Nile tilapia fish.

Keywords: *Moringa oleifera*, seeds, extracts, Biochemical, Nile Tilapia, and Breeding.

INTRODUCTION

Aquaculture is the fastest growing sector of food production, about 50 percent of total global food fish production now comes from aquacultures. It is estimated that by2030, the world will require the production of an additional 27 million tons of fishery products to satisfy the growing demand for food fish (**FAO 2016)**. Tilapia is a widespread fresh water

cultured fish that can be reared under wide ranges of environmental conditions and can accept different protein sources in its diet (**Welker and Lim, 2011)**. Reproduction process constitutes the main factor affects on any production yield, thus the financial outcome from aquaculture projects. Reproductive process is regulated by many elements; fish species, nutrition and environment are the master leading elements. Unfortunately, the effects of these neutraceuticals on reproduction and gamete quality, is still poorly studied **(Giorgini *et al.*, 2010).** Feeding chickens with Moringa leaves and seeds will improve egg production. The inclusion of *Moringa oleifera* leaves meal up to 30% in the diet of growing traditional chickens had no negative impact on live body weight **(Worku, 2016).**

Plant oil from *Moringa oleifera* seeds are in high demand for their medicinal value. Apart from the medicinal uses, *M. oleifera* was reported to be a good source of vitamins and amino acids. The seeds of *Moringa* are considered to be antipyretic, acrid, and bitter and reported to show antimicrobial activity **(Olugbemi *et al*, 2010)**.

Moringa seed oil is considered equivalent to olive oil in terms of its chemical properties and contains a large quantity of tecopherol **(Mani *et al*, 2007)**. The unique property is the ability of its dry, crushed seed and seed press cake, which contain polypeptides, to serve as natural coagulants for water treatment. The aqueous solution of *Moringa* seed is a heterogeneous complex mixture having various functional groups, mainly low molecular weight organic acids (amino acids). These amino acids have been found to constitute a physiologically active group of binding agents, working even at a low concentration, which because of the ability to interact with metal ions is likely to increase the sorption of metal ions **(Anwar *et al.*, 2007)**.

Ogunjinmi and Oladipo (2012) showed that hexane and methanolic extracts of *Moringa* seed contained the secondary metabolite such as alkaloid, glycoside, flavonoid, tannins, saponin, steroid and reducing sugar which make the seed of *Moringa oleifera* to posses the biological properties. The Moringa seed oil is high (80.4%) in polyunsaturated fatty acid; The dry matter basis of *Moringa oleifera* seeds contained 34.80% ether extract, 31.65% protein, 7.54% fiber, 8.90% moisture, and 6.53% ash contents **(Ogbunugafor *et al.*, 2011)**.

Jabeen *et al.* (2008) mentioned that the antimicrobial properties of the *M. oleifera* seed extracts may be due to lipophilic compounds. These compounds may attach to the cytoplasmic membrane. *M. oleifera* seed powder is a good water purifier; and contains polyelectrolytes, which

constitute active ingredients in water treatment. Aqueous extract of mature seeds from trees and shrubs of Moringacae family are effective in clarifying turbid and waste water in tropical countries.

The most common phytochemical contents present in moringa seeds include flavonoids (13.09%), alkaloids (12.28%), tannin (5.32%), saponins (1.37%) and cyanide (0.05%), while the proximate analysis were reported as; moisture (4.77%), ash (1.71%), protein (31.04%), crude fiber (1.17%), fat (21.25%) and carbohydrate (40.06%) **(Olorode *et al.*, 2014).** The effect of Moringa seed powder on the gonadal integrity of Mozambique Tilapia, *Oreochromis mossambicus* indicated that *Moringa* seed powder at 5.0 g/kg basal diet and higher inclusion levels have an antifertility effect, significantly affecting gonad integrity and sperm production in sexually mature *O. mossambicus* males **(Ampofo-Yeboah *et al.*, 2013)**. These studies carried out on the use of plant products (MSEE and MSPEE) on fish aquaculture and determination their biological effects on fish such as improvement of reproduction performance and physiological state of Nile Tilapia by improving in brood stock nutrition. The study aimed to evaluate the effect of different levels (0.5gm and 1gm/kg diet) of moringa seeds ethanol extract MSEE and moringa seeds petroleum ether extract MSPEE on the reproduction performance, physiological state of Nile tilapia.

MATERIAL 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 and extended 70 days.

1- Collection and processing of plant materials:

The fully mature dry seeds were round or triangular in shape and the kernel is surrounded by light wooded shell with three wings. The seeds were air-dried, the testa and wings were manually removed and the white kernel was ground to fine powder, using the coffee mill attachment of a Moulinex domestic food blender **(Price, 2000)**.

2- Preparation of *M. oleifera* seeds extracts:

The powder samples of seeds were weighed individually accurately to the 50 g, 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 (*Moringa* seeds ethanol extract MSEE and *Moringa* seeds petroleum ether extract MSPEE) 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, labeled appropriately and stored at -20°C. **(Abdel-Naby, 2014).**

3-Determination of chemical components of two extracts (MSEE and MSPEE) by Gas chromatographic analysis (GC-MS):

The identification of constituents 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:

MSEE and MSPEE were analyzed by gas chromatography using (Agilent Technologies 7890A) 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 mm i. 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 1µl of the sample. The MS operating parameters were as follows: ionization potential 70 electrons volt, interface temperature 250 ºC, and acquisition mass range 50-800 **(Abdel-Naby, 2014).**

4- Diet preparation:

Table (1) showed all dry ingredients which mixed with *Moringa* seeds extracts (MSEs) 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 1 mm diameter matrix. The resulting wet pellets were dried at room temperature for two days. Five treatments were prepared (T2, T3, T4 and T5); T1 (control treatment without any extract), T2 and T3 contain 0.5 and 1 g of *Moringa* seeds ethanol extract/ kg (MSEE) respectively , T4 and T5 contain 0.5 and 1 g of *Maringa* seeds petroleum ether extract/ kg (MSPEE) 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 34.85% crude protein

and 6.2 % crude fat. Experimental diets were formulated according to **NRC, (1993).**

Table (1): Ingredients and chemical analysis of the experimental diet.

Ingredients	**Quantity %**
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
Total	100
Total chemical analysis (%):	
Crude protein	34.85
Crude fat	6.20
Ash	7.70
Crude fiber	6.13
NFE	45.12

5- 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 of 220 g. Fish were distributed in 15 concrete ponds; each pond contained 16 females and 8 males; concrete ponds (9 x 2.5x 1.2 m), where water depth in each pond was 0.7m. Fish were acclimated for 10 days in the same conditions. Fish *Nile Tilapia* was divided to 5 treatments, each treatment was triplicates. The first treatment (T1) left as control group, the second and the third treatment (T2 and T3) fed with 0.5 and 1 g of MSEE/ kg. The fourth and the fifth (T4 and T5) fed with diet contain 0.5 and 1 gm of MSPEE/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.

6- 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.

7- Fish growth and economic evaluation of brood stock.

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

Weight gain = W2 – W1

Where W1 and W2 are the initial and final weights, respectively.

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

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

8- Hematological examination.

6 fish (3 males and 3 females) from each of the treatments and control group were taken for physiological investigations at the end of the experimental period. Fish were not fed for 24 hours prior to blood sampling. Fish blood was collected from each fish caudal vein by a sterile syringe**.** Blood samples were divided into two parts; the first part was transferred on dry and clean tube with EDTA solution for measuring hemoglobin (Hb), red blood cells (RBCs), hematocrite (Hct) in blood after good mixing. 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 analysis (testosterone, estradiol and cortisol).

Hematocrit was measured as packed cells volume by using micro hematocrit tubes method as described by **Dacie and Lewis (1991)**. Haemoglobin (Hb) concentration and blood indices (MCV, MCH and MCHC) were determined immediately according to **(Vankampen, 1961).,** 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, Finland according to **(Abraham, 1977)**. But estradiol in females was determined by the radioimmunoassay method according to **Xing *et al*. (1983)** using Immun. Chem. kit, provided by Diagnostic Products Corporation, Los Angeles. All the brought fish were weighed to the nearest 0.1 g 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) and Hepato or somatic index (HSI) 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 breeding 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 yellow extracts obtained from MSEE and MSPEE 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 tables 2 and 3, respectively). Tables results showed that *Moringa* extracts contain antioxidants compounds like (Quercetin 3´-methyl ether, 7, 4´-Dimethoxy-3-hydroxyflavone, Citronellic acid and Squalane); fatty acids compounds like (Linolenic acid, Tetradecanoic acid and Tetracosanoic acid); vitamins like (Ascorbic acid and Vitamin E).

Table (4) the results in this table showed that significantly increased in the fry number /pond, the fry number /female and female average wt (g) in all *Moringa* treatments when compared with the control group. The best results were showed in fish fed with 0.5 and 1 g of MSPEE /kg diet (T4 and T5) as compared with control group throughout the breeding season.

The hematological parameters 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 T4 ($2.59 \pm 0.13X10^6$/cmm). Hemoglobin (Hb) content was significantly increased in all treatments compared with control group (5.85 ± 0.33 g/dl). Also the hematocrit value was significantly increased in T2 and T4 but no significant in T3 and T5.

Blood indices parameters were recorded in table (6). There were significant decreases in mean values of MCV in all treatments. There were variable changes of MCH and MCHC values.

Table (7) showed significant increases in estradiol hormones in T3, T4and T5 in blood serum of females *Nile Tilapia* after fed with diet containing 0.5 and 1 g of MSEs/kg when compared with 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 in T4 (MSPEE) and no significant decreases in HSI.

Table (2): Chemical composition of ethanol *Moringa* seeds extract analyzed by gas chromatography.

No	Retention time (min)	Name	Area sum %
1	10.574	Quercetin 3´-methyl ether	3.0
2	12.375	Tetradecanoic acid	0.41
3	13.577	(S)-(-)-Citronellic acid	4.24
4	13.715	4´,6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	9.51
5	13.841	7, 4´-Dimethoxy-3-hydroxyflavone	1.67
6	14.603	Serotonin	0.95
7	14.835	Linolenic acid	38.09
8	14.962	Quercetin 3´, 4´, 7 trimethyl ether	9.10
9	15.931	Ascorbic acid, permethyl	0.53
10	16.253	Dodecanedioic acid	5.27
11	16.486	3-Hydroxy-6,3´,4´- trimethoxyflavone	7.78
12	16.657	3-(3, 4 –Dimethoxyphenyl)-6-methyl-4-phenylcoumarin	0.54
13	18.726	Quercetin 3,5,7,3´,4´-pentamethyl ether	13.42
14	18.983	6-methoxyluteolin	1.06
15	19.842	Vitamin E	1.57
16	21.337	Tetracosanoic acid	0.84
17	21.704	β-Citronellol	0.80
18	22.967	Squalane	0.61
19	20.466	Phytanic acid	0.59
Total			99.98

Table (3): Chemical composition of petroleum ether Moringa seeds extract analyzed by gas chromatography.

No	Retention time (min)	Name	Area sum %
1	5.47	2- Decanol	0.58
2	6.635	Undecane	3.5
3	7.706	D-(+)-Mannose	6.15
4	10.623	6-methylchromone	1.22
5	10.700	3,6-dimethylchromone	0.71
6	10.855	Santonox	0.92
7	11.360	Germacrene D	5.87
8	11.466	Nylidrin	3.28
9	11.633	Thuga-2,4 (10)-diene	2.26
10	11.922	Nopol (terpene)	2.06
11	12.085	α-cubebene	2.13
12	12.118	Cumic aldehyde	2.30
13	12.212	P- Mentha-1,3,8- triene	2.57
14	12.374	Cosmene	2.38
15	12.656	2H-1-benzopyran-2-one, 7-hydroxy-4-methyl-3-phenyl	2.24
16	11.774	(-)-perillyl alcohol	3.38
17	12.831	Aristolene	1.9
18	12.920	β- Caryophyllene	2.0
19	13.364	3, 3´- Dihydroxyflavone	1.04
20	13.792	Afromosin 7-O-glucoside	1.06
21	13.865	Zearalenone	2.51
22	14.827	9-Octadecenoic acid, (E)-	13.88
23	14.921	Cis-Vaccenic acid	12.63
24	15.405	Retinol	7.43
25	19.915	Vitamin E	15.99
Total			99.99

Table (4): The effect of different levels of *Moringa* seeds extracts on reproductive performance of Nile tilapia.

Items	T1	T2	T3	T4	T5
Fry no./pond	17407 ±467^{d}	20505 ±719^{c}	21377 ±668^{bc}	22820 ±291^{a}	22357 ±487^{ab}
No of fry/ female	1088 ±29^{d}	1282 ±45^{c}	1336 ±42^{bc}	1426 ±18^{a}	1397 ±30^{ab}
Female final wt (g)	234.33 ±1.53^{d}	243.00 ±1.15^{b}	240.00 ±1.04^{c}	247.50 ±2.52^{a}	243.83 ±1.53^{b}

Means having the same letter in the same row is not significantly different at ($P < 0.05$).

Table (5): The haematological changes (Hb, RBCs, Hct) of the blood of Nile tilapia after fed with diet containing extract of moringa seeds.

Items	T1	T2	T3	T4	T5
RBCs (X10^{6}/cmm)	1.71 ± 0.09^{c}	2.10 ± 0.05^{b}	2.01 ± 0.15^{b}	2.59 ± 0.13^{a}	2.01 ± 0.15^{b}
HB (g/dl)	5.85 ± 0.33^{c}	6.90 ± 0.18^{b}	6.79 ± 0.40^{b}	7.14 ± 0.11^{a}	6.79 ± 0.40^{b}
Hct (%)	16.87 ±0.89^{c}	18.90 ± 0.46^{b}	16.83 ± 0.53^{c}	21.64 ±1.12^{a}	16.83 ±0.53^{c}

Table (6): The haematological changes (MCV, MCH and MCHC) of the blood of Nile tilapia after fed with diet containing extract of *Moringa* seeds.

Items	T1	T2	T3	T4	T5
MCV(μ$^{3)}$	98.76 ±4.39^{a}	82.33± 3.74^{b}	83.31±4.97^{b}	90.91±6.78^{b}	83.93 ±4.97^{b}
MCH (Pg)	34.27 ±1.67^{a}	28.99±0.31^{ab}	28.02±1.52^{a}	31.75± 1^{b}	33.82 ±1.52^{a}
MCHC(%)	34.78 ±3.03^{b}	35.22±1.35^{b}	33.76 ±1.21^{a}	34.94±1.95^{b}	40.33 ±1.21^{a}

Table (7): The effect of different levels of *Moringa* seeds extracts on estradiol, cortisol, ovary weight, GSI and HSI of females *Nile Tilapia*.

Items	T1	T2	T3	T4	T5
Estradiol (pg/ml)	204.00 ± 5.58^{c}	202.87 ± 3.01^{c}	227.33 ± 6.51^{b}	239.40 ± 5.77^{a}	231.53 ± 3.40^{a}
Cortisol (ng/ml)	146.73 ± 6.75^{c}	164.13 ± 4.00^{a}	177.30 ± 6.65^{a}	156.50 ± 4.05^{b}	165.80 ± 3.67^{a}
Ovary wt (gm)	4.00 ± 0.79^{c}	4.40 ± 0.97^{c}	4.84 ± 0.44^{b}	6.03 ± 1.17^{a}	4.41 ± 1.12^{c}
GSI (%)	1.71 ± 0.32^{c}	1.81 ± 0.36^{b}	2.02 ± 0.32^{b}	2.43 ± 0.97^{a}	1.81 ± 0.44^{b}
HSI (%)	1.87 ± 0.13^{b}	1.22 ± 0.22^{c}	1.47 ± 0.43^{bc}	1.56 ± 0.19^{b}	2.10 ± 0.13^{a}

Means having the same letter in the same row is not significantly different at ($P < 0.05$).

Table (8) showed significant increases in T2, T3 and T4 in testosterone hormone level in serum of blood of male *Nile Tilapia* compared with control group after fed with diet containing 0.5 and 1 g/kg of MSEs. There was no significant increase in testosterone level in T5. However there was a significant increase in all treatments in cortisol compared with control group. There were significant increases of testis weight and GSI in T4 and T5 compared with control group. There were significant decreases of HSI in T2, T3 and T5 compared with control group.

Table (8): The effect of different levels of *Moringa* seeds extracts on testosterone, cortisol, testis weight, GSI and HSI of males *Nile tilapia*.

Items	T1	T2	T3	T4	T5
Testosterone (ng/ml)	3.41 ± 0.48^{c}	4.84 ± 0.66^{b}	4.73 ±0.23^{b}	5.62± 0.42^{a}	3.98 ± 0.45^{c}
Cortisol (ng/ml)	150.33±9.0^{c}	162.67±5.86^{b}	178.13±2.^{a}	170.83±1.8^{b}	170.43±10.1^{b}
Testis wt(g)	1.09±0.22b^{b}	1.10± 0.20^{b}	1.02 ±0.18^{b}	1.99 ± 0.19^{a}	1.73 ± 0.21^{a}
GSI (%)	0.49 ± 0.11^{b}	0.5 ± 0.08^{b}	0.47 ±0.06^{b}	0.90 ± 0.09^{a}	0.78 ± 0.08^{a}
HSI (%)	1.44± 0.15^{a}	1.10 ± 0.21^{b}	1.05 ±0.05^{b}	1.27 ± 0.21^{a}	0.99 ± 0.07^{b}

Means having the same letter in the same row is not significantly different at ($P < 0.05$).

Table (9) showed that feed required to 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 T4 diet supplemented with 0.5 g of *Moringa* seeds petroleum ether extract.

Table (9): Economic evaluation and feed utilization efficiency of *Nile Tilapia* brood stock as affected with different levels of *Moringa* seeds extracts.

Items	T1	T2	T3	T4	T5
Fry no./pond	17407±467^{d}	20505±719^{c}	21377±668^{bc}	22820±291^{a}	22357±487^{ab}
Total feed used,gm/pond	2663±6.65^{b}	2707±1.52^{ab}	2720±3.46^{ab}	2747±1.52^{a}	2730±4.58^{ab}
Feed required to produce1000 fry, (gm)	153±7.21^{a}	132±5^{b}	127.3±4.16^{ab}	120.33±1.53^{c}	122.33±0.58^{c}
The cost of feed/1000 fry, LE	1.38±0.06^{a}	1.24±0.04^{b}	1.24±0.04^{b}	1.13±0.02^{c}	1.19±0.01^{bc}

Discussion

Aquaculture is the main source to increase fish supply. Fast development of aquaculture and increasing fish demand lead to intensification of fish culture, Plant extracts on fish physiology as well as a lack of homogenization in the extract preparation and fish administration of the plant extracts. These studies carried out on the use of plant products on fish aquaculture and their biological effects on fish such as improvement of reproductive performance. Improvement in brood stock nutrition and feeding has been shown to greatly improve not only egg and sperm quality but also gonads development and fecundity are affected by certain essential dietary nutrients, especially in continuous spawning with short vitellogenic periods. Thus, more attention has been paid to the level of different nutrients in brood stock diets. However, studies on brood stock nutrition are limited.

From the results of GC analysis; MSEE contains antioxidants such as (quercetin 3´-methyl ether**,** quercetin 3,5,7,3´,4´-pentamethyl ether **,** ascorbic acid, permethy, l7, 4´-Dimethoxy-3-hydroxyflavone, Citronellic acid and Squalane)); highly unsaturated fatty acids (Linolenic acid, Tetradecanoic acid and Tetracosanoic acid)**;** vegetable vitamins (Ascorbic acid and Vitamin E) is soluble in alcohol;. The results of GC analysis; MSPEE contains a high ratio of vitamin E due to miscible with petroleum ether**.**

The results showed significantly increased in the fry number /pond, the fry number /female and female average wt (g) in all *Moringa* treatments

when compared with the control group and this results agree with **Latoya *et al.*, (2013)** who reported that animal studies have shown that the supplementation of *Moringa* on the diet as a source of vitamins and minerals can improve the quality of reproduction and overall growth performance; and concluded that nutritive content of *Moringa* is sufficient to maintain and enhance the quality of reproductive and overall health in zebrafish. These study results were similar with **(Wahbi and Sangak, 2017**) who concluded that the *Spirulina* in fish feeds improve reproduction performance of *Tilapia* fish. The major bioactive compounds of phenolics were flavonoid groups such as quercetin and these agree with our results of GC. On the basis of the results obtained, *Moringa* seeds are found to be a potential source of natural antioxidants due to their marked antioxidant activity **(Perumal and Klaus 2003)**. The results of (RBC) and (Hb) were significantly increased in all treatments compared with control group. This elevation in erythrocytic count, haemoglobin content 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 and Rashid, 2007**).

Moringa seed contains a large quantity of tecopherol **(Mani *et al, 2007)**.* Lipid and fatty acid composition of brood stock diet have been identified as major dietary factors that determine successful reproduction and survival of spring. During spawning season, highly unsaturated fatty acids (HUFA) in brood stock diets increases fecundity, fertilization and egg quality. Ascorbic acid has also been shown to play an important role in salmonid reproduction, Vitamin E deficiency affects reproductive performance, causing immature gonads and lower hatching rate and survival of offspring. Elevation of dietary α-tocopherol levels has been found to reduce the percentage of abnormal eggs and increase fecundity in the gilthead seabream (*Sparus aurata*) **(Izquierdo *et al*., 2001)**.

O´donnel *et al.*, (2001) stated that, testosterone produced by Leydig cells is responsible for spermatogenesis as well as for the development and maintenance of male secondary sex characteristics. This means that the amount of plasma testosterone is related to the capacity of the Leydig cells to secrete testosterone in the animal testis **(Ewing *et al.*, 1979).** Our results showed significant increases in T2, T3 and T4 in testosterone hormone level in serum of male *Nile Tilapia* and significant increases of testis weight and GSI in T4 and T5. This results were agree with the finding of **(Zade *et al.*, 2013)** who concluded that *Moringa* seeds aqueous

extract enhanced sexual behavior of male albino rats. On the other hand, estradiol (E2) is the main sex hormone in female which play an important role in sexual development and responsible for estrogenic activity. It is produced mainly by the ovaries and in smaller amounts by the adrenal glands. Estradiol levels hypophysiotropic hormones and neuromodulators of reproductive behaviors **(Ibrahem, 2013**).The significant increases in ovary weight, gonad somatic index and estradiol hormones in T3, T4and T5 in serum of females Nile tilapia after fed with diet containing 0.5 and 1 mg of MSEs/kg. may be due to MSEs contains antioxidants such as (quercetin**,** ascorbic acid, highly unsaturated fatty acids (Linolenic acid**)** and vitamin E. These results agree with the result of **Mehrad and Sudagar (2010)** who reported that vitamin E caused higher gonadosomatic index, larger ova, and more eggs than a control in a study on the effect of vitamin E and growth hormone on the gonadal maturity of fresh water fish (*Cyprinus carpio*). In addition, **Mehrad and Sudagar (2010)** concluded that complete spawning occurred in fish fed a diet containing vitamin E, but only partial spawning occurred in the fish fed diets without vitamin E. The number of fries of fish was increased with increasing the level of dietary vitamin E.

The HSI is a useful biomarker to detect hazardous effects of environmental stressors. There were significant decreases of HSI among the treatments compared with control group. These results indicate that *MSEs* had no hazardous effect on the fish. These finding was in agreement of that **Gbadamosi *et al.*, (2016)** who concluded that moringa leaf protected the membrane integrity of the liver cells against stressors. These results seed also similar with **Aja *et al., (2014***) who concluded that administration of aqueous, ethanolic and methanolic seed extracts of *Moringa oleifera* significantly reduced the levels of some liver enzymes in albino rats. They also observed that the liver pathology showed that no significant lesions were observed and this may point to the fact that the seed of *Moringa oleifera* is relatively safe for use medicinally**.**

Conclusion

M. oleifera seeds petroleum ether extract gives high yield of vitamin E than ethanol extract than petroleum ether extract. Supplementation of *M. oleifera* seeds extracts in feeds for *O.niloticus* brood stock increase number of fry, sexual hormones and GSI for males and females of brood stock; we conclude that *M. oleifera* seeds petroleum ether extract in lower dosage (0.5g/kg) has the potential to be used as a supplement in fish diets. The use of Moringa seeds extractions as feed additive in fish increase reproduction performance and enhance physiological state.

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التغيرات البيوكيميائية على تكاثر أسماك البلطى النيلى بعد تغذيتها بمستخلصات بذور نبات المورينجا

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الملخص العربى

تم عمل هذه الدراسه لتقييم تاثير مستخلصات بذور نبات المورينجا بمذيبين مختلفين (كحول اثيلي وبترولیم ايثر) علي الاداء التكاثري والعناصر الفسيولوجية لاسماك البلطي النيلي. وقد سكنت الاسماك في ١٥ من الاحواض الاسمنتية وقد قسمت التجربة الي خمس مجموعات كل مجموعة لها ثلاث مكررات في احواض اسمنتية وهذه المجموعات هي المجموعة الاولي (T1) وهي عليقة بدون اي اضافة لمستخلص المورينجا والمجموعة الثانية (T2) تتغذى علي عليقة مضاف إليها ٠,٥ جم / كجم من المستخلص الكحولي لبذور المورينجا والمجموعة الثالثة(T3) مضاف إليها ١جم/ كجم من المستخلص الكحولي لبذور المورينجا. أما المجموعة الرابعة (T4) تتغذى علي عليقة مضاف إليها ٠,٥ جم / كجم من المستخلص لبذور المورينجا بالبترولیم إيثر والمجموعة الخامسة (T5) مضاف إليها ١جم/ كجم من المستخلص لبذور المورينجا بالبترولیم إيثر وقد استمرت التجربه ٧٠ يوم.

وأوضحت النتائج زيادة معنوية في عدد الزريعة ووزن المبيض الناتج عن المجموعة الرابعة والخامسة 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 المجموعة الضابطة. كما أظهرت النتائج زيادة معنوية في الاستراديول وحساب مؤشر الأعضاء التناسلية فى إناث الأسماك فى المجموعة الرابعة, ووجد زيادة معنوية في هرمون التيستوستيرون فى تحليلات الدم لذكور الأسماك. ولذلك نوصي باستخدام المستخلص لبذور المورينجا بالبترولیم ايثر فى علائق الاسماك بنسبة ٠,٥ جم / كجم حيث أنها تحسن الاداء التكاثري والحاله الفسيولوجية للأسماك.