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EFFECTS OF *Salvia officinalis* EXTRACT ON FISH HEALTH AND REPRODUCTIVE PERFORMANCE OF NILE TILAPIA *(Oreochromis niloticus)*

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Abstract

This study was carried out to evaluate the effect of *Salvia officinalis* extract on reproductive performance and fish health. *S. officinalis* was collected from harplest and extracted using ethanol 95%, purified and identified using GC-mass techniques. *S. officinals* extract was tested as antibacterial *invitro* and added to commercial diet 30% protein in four treatments (0.0, 0.5, 1.0 and 1.5% diet). Total number of 96 apparently healthy Nile tilapia broodstock was collected and selected from Abbassa Fish Farm for reproduction and divided into four groups in three replicates (each 6 females and 2 males). All fish groups were fed diet twice per day for 5 days per week until satiation for 90 days.

At the end of feeding experiment the number and weight of eggs and fries were occurred. Blood and serum were collected for blood and physiological parameters and the fish was challenged with *Aeromonas hydrophila.*

The results indicated that *S. officinals* extract had antibacterial effect against *A. hydrophila in vitro* and the active principal identified as C_{10} H_{18} O Eucalyptol. *S. officinals* extract had good effect on the weight and number of eggs, fries and embryonic development at 0.5 and 1.5%. 0.5 % of *S. officinalis* extract was the best treatment which improved fish health by increasing WBCs count, Hb value, albumin, globulin and total protein. Also it increased fish resistance against *A. hydrophila*.

Key words: *Salvia officinalis*, Nile tilapia, physiological parameters, *Aeromonas hydrophila*, challenge, reproductive performance

INTRODUCTION

Tilapias (Cichlidae) constitute a group of native fishes from Africa that have been introduced into various other countries. Tilapia aquaculture is and will continue to be an important fish, particularly for the lesser-developed countries in the tropics (FAO, 2001). Nile tilapia (*Oreochromis niloticus)* are considered as the most common and popular fish in Egypt. Egypt is a country where, arguably, the farming of tilapia has its roots (Stickney, 2006), where tilapia culture is believed to have originated some 4000 years ago. Tilapia consist 36% of the Egyptian production from fish culture (Sadek, 2000). Hence, Egypt produces 20% of the world tilapia capture and 12% of the world farmed tilapia. The main advantage of tilapia is it's relatively low cost of production, mainly for fry and feed, and the quality of its flesh. The attributes which make Nile's tilapia so suitable for fish farming are its general hardiness, ease of breeding, rapid growth rate, ability to efficiently convert organic and domestic wastes into high quality protein, and good taste (Yi *et al*., 1996).

Broodstock husbandry and spawning techniques are constantly upgraded as Egyptian hatcheries require a high number of good quality eggs to satisfy the needs for aquaculture, so rigorous management of large numbers of broodstock are necessary for mass production of eggs and fry due to the complex reproductive biology and asynchronously spawning with relatively small number of eggs produced per spawning. (Abdelhamid *et al*., 2009).

Fish diseases due to bacterial infection are considered one of the major problems in aquacultures (Robertson *et al*., 2000). The presence of potential danger of many fish pathogens associated with the stress factors may favor the occurrence of outbreaks in cultured fishes causing mass mortalities, reduced production and low quality of aquatic organisms (Ghittino, 1976). *Aeromonas hydrophila* is incorporated in sever outbreaks among fish tilapia's hatcheries *(*Ali and Hossein, 2010*)*. The use of antibiotics in aquaculture as disease prevention and growth promotion may introduce potential hazard to public health and to the environment by the emergence of drug-resistant

microorganisms and antibiotic residues. Furthermore, the normal microbial flora in the digestive tract, which is beneficial to fish, is also killed or inhibited by oral chemotherapy (FAO/WHO/OIE 2006). One of the most recants concepts is replacing the chemical and therapeutic agents with natural components as one of the strategies available and much experimental work is being carried out to assess its commercially applicability (El- Didamony *et al*., 2015). *Salvia officinalis* plant belonging to different species and ecotypes (Biotypes) are widely used in several industries as it has a flavor and cosmetically in pharmaceutical, beverage and food industries. It has also used been as a traditional remedy to treat various ailments such as a spasmodic, antimicrobial, expectorant, carminative and aromatic for whooping and convulsive coughs, digestive disorders and menstrual problems (Aligiannis *et al*., 2001). The chemical compositions of *Salvia officinalis* is Eucalyptol also it contains antimicrobial, antioxidant and other biological activities. (Sivasalnkar *et al*., 2015). So the objective of the present study that evaluate the effect of *salvia officinalis* extract as antibacterial *in vitro* and identified active principle and the possibility for improving reproduction and health of Nile tilapia.

MATERIALS AND METHODS

The present study was conducted at Reproductive Physiology and Hatchery Department, Central Laboratory for Aquaculture Research (CLAR), El Sharkia Governorate, Egypt. The Nile tilapia *(Oreochromus niloticus)* used in present study collected from Central Laboratory Aquaculture Research (CLAR).

Plant:

Salvia officinalis belong to Lamiaceae family, their fragment leaves used in Mediterranean diet as dried and fresh where sage is also drinkable as tea.

Leaves of *Salvia officinalis* were collected from herbalist market and dried at room temperature, gridding and kept in plastic bags until extracted.

Extraction of *Salvia officinalis* leaves:

The powdered of *Salvia officinalis* leaves (150 gm) was extracted using absolute ethanol for 6 hours in a soxhlet, then the extract was filtered using Whitman filter paper No.1. After cooling the excess solvent of crude and partition of aqueous and organic extract removed under vacuum using rotary evaporator. The extract was storage in refrigerator until further biological investigation

Precipitation:

The precipitation process of the antibacterial substance was carried out using petroleum ether. The compound precipitate was centrifuged at 5000 rpm for 15min.The antibacterial effect of the extracted substance of *S. officinalis* (100 l) was tested *in vitro* against *A. hydrophila* which was obtained from Fish Diseases Dept. using disc diffusion method (Anna *et al*., 2004) and the inhibition zone was measured by mm diameter. Another 100 l of absolute ethanol was used as control.

Separation:

Separation of the antibacterial substance into its individual components has been tried by thin layer chromatography using a solvent system composed of chloroform and methanol (24:1 v/v) (kosar *et al*., 2005).

Purification:

The Purification of the antibacterial agent was carried out by using Silica Gel column chromatography. A column of 2.5×50 cm was used for this purpose. Chloroform and methanol 10:1(v/v), was used as an eluting solvent. The column was left for over night until the silica gel (BDH-60-120mesh) was completely settled one –ml crude extract (DIAS *et al*., 2006).

Table 1. Ingredient of fish meal after adding *Salivia officinalis* extract.

Ingredients	• % Control	*Salivia officinalis* extract % in the diets		
		0.5%	1%	1.5%
Fish meal	9.10	9.10	9.10	9.10
Soybean meal	45.50	45.50	45.50	45.50
Ground corn	15.31	15.31	15.31	15.31
Wheat bran	19.21	19.21	19.21	19.21
Starch	4.00	3.50	3.00	2.50
Salivia officinalis	0.00	0.50	1.00	1.50
Cod fish oil	2.23	2.23	2.23	2.23
Corn oil	1.65	1.65	1.65	1.65
Vitamins premix	1.00	1.00	1.00	1.00
Minerals Premix	2.00	2.00	2.00	2.00
Total	100	100	100	100
Chemical analysis %				
Dry matter	91.68	91.46	91.53	91.69
Crude protein	30.11	30.26	30.38	30.41
Crude fat	7.11	7.22	7.39	7.48
Ash	8.13	8.33	8.17	8.06
Fiber	5.45	5.32	5.55	5.32
NFE	49.20	48.87	48.51	48.73
GE(Kcal/100 g)	4390.3	4395.7	4403.8	4423.0
P/E ratio	68.58	68.84	68.99	68.75

Physico-chemical properties of antibacterial agent:

1- *Eletnental analysis*: The element analysis C, H, O, N, and S was carried out by the regional center for Mycology and Biotechnology Al-Azhar University, Egypt

2- Spectroscopic analysis: The GC- mass techniques was determined at the regional center for Mycology and Biotechnology Al-Azhar University, Egypt.

Diet Preparation:

Salvia officinalis extract was added to commercial diet containing 30% crude protein at different levels 0.0, 0.5, 1.0, 1.5 %. The dry ingredients (Table 1) of each diet were thoroughly mixed, 100 ml of water per kg diet was added and all contents were blended using kitchen blender to make a paste. Pelleting

of diets was carried out by passing the blended mixture through laboratory pellet machine with 1 mm diameter matrix. The pellets were dried in a drying oven model (Fisher oven 13 – 261 – 28A) for 24 hours at 85^{o}C, stored in plastic bags and kept in a refrigerator at 4^{o}C during the experimental period to avoid rancidity (NRC, 1994)

Fish for reproduction:

A total number of 96 apparently healthy *Nile tilapia* broodstock males and females with an average body weight of 115: 180 and 85: 150g respectively and average length between 19- 25 cm and 18- 22 cm respectively All fish were acclimatized in tank with freshwater at least for two weeks in wet lab of reproductive physiology. The fish were divided into four groups each group has three replicates.

The sex ratio was six females and two males as showed in figures (3- 8). In middle of May- middle of July, when climatic conditions were suitable for spawning and average daily water temperature was 27± 1^{o}C. They were kept in tank supplied with aeration condition and suitable temperature for living.

Collection of seeds, Swim-up fry first appeared during 30 days of pairing. The seeds (fertile oval, new hatched larvae with yolk sac and Swim-up fry) were collected and counted every 20 days after stocking up. The experiment was completed in three months.

Relative fecundity: This was calculated as described by Kahkesh *et al.* (2010) as follows:

$$\text{The relative fecundity} = \frac{\text{No. of stripped egg and fries}}{\text{Body weight (gm)}}$$

Weight of eggs and fries were occurred and the embryonic development was calculated and time of reproduction.

Blood and serum samples:

At the end of feeding experiment fish did not feed in the 24 our immediately prior to sampling. Fish were anaesthetized with buffered MS222 (30 mg/L) and blood was collected with a hypodermic syringe from the caudal vein. The blood collection lasted less than 3 min in order to avoid cortisol rise induced by the manipulation during sampling. The collected blood was divided in two sets of Eppendorf tubes. One set contained heparin as an anticoagulant, for hematological study (hemoglobin and white blood cell counting and differentiation) according to method described by (Franco, 1984). The second set without anticoagulant and left to colt at 4°C then centrifuged at 5000 rpm for 5 min at room temperature. The collected serum was stored at -20° C for Physiological parameters (total protein content was determined calorimetrically according to Henry (1964), while plasma Albumin was estimated according to Wotton and Freeman (1982).

Challenge Test:

At the end of feeding experiment fish were divided into two subgroups. Each subgroup was challenged I/P with pathogenic *Aeromonas hydrophila* (0.2ml of 4 $\times 10^6$ CFU). The other subgroups were injected I/P with 0.2 ml of sterilized saline as control. Both subgroups kept under observation for 10 days to record the daily mortality rate. Re-isolation and identification of pathogens were carried out according to Schäperclaus *et al*. (1992).

RESULTS AND DISCUSSION

Antibacterial effect of *Salvia officionalis extract in vitro:*

The use of medicinal herbs is an alternative to antibiotics in fish health management (Abdel-Tawwab *et al*., 2008 and Ashraf and Goda, 2008). *Salvia officionalis* extract had antibacterial effect against *Aeromonas hydrophila* with inhibition zone 30 mm in diameter using disc diffusion method at 100µl and no response with absolute ethanol. *S. officinalis* was a herbal had antibacterial effect against *Streptococcus mutans* (ATTC: 35668), Lactobacillus *rhamnosus* (ATCC: 7469) and *Actinomyces viscosu* (Kermanshah *et al*., 2014)

Salvia officionalis is a rich source polyphenols. Rosmarinic acid, carnasol and carnosic acid were the prevalent compounds of *S. officinalis* methanolic extract. Polyphenolic compounds known to be responsible for the main antioxidant activity of *S. officinalis* (Baydar, 2005; Bayram and Sonmez, 2006; Ekren *et al*., 2007; Farhat *et al*., 2009).

Identification of antibacterial activity substance of *Salvia officionalis* extract:

The different filtrates were tested for their antibacterial activity and it the best results obtained with ethyl alcohol extraction. Crude brown powder was tested for their antibacterial activities by using disc diffusion method. The obtained results revealed that two band at R_f 0.86; and one band at R_f 0.86 exhibited obvious inhibitory effects against the growth bacterial strain. The purification of the antibacterial agent was carried out by using silica gel column chromatography. The active fractions were concentrated. The maximum activity was recorded at fraction No. 7 and 8.

Physico – chemical properties of antibacterial agent:

The physical characteristics of the extracted ingredients showed a specific Physico-chemical property such as melting point is 140°C. Regarding the solubility the ingredients are soluble in ethanol, water, chloroform, DMSO and methanol but insoluble in petroleum ether, n-Butanol, hexane and benzene.

A-Elemental analysis:

This analysis indicates suggested empirical formula of the ingredient is

$C_{10}H_{18}O$

B- Spectroscopic characteristics:

GC- mass techniques (Fig. 1). Area = 40.55% which indicates a suggested name Eucalyptol (Fig. 2).

Figure 1. (GC- mass techniques)Area= 40.55%.

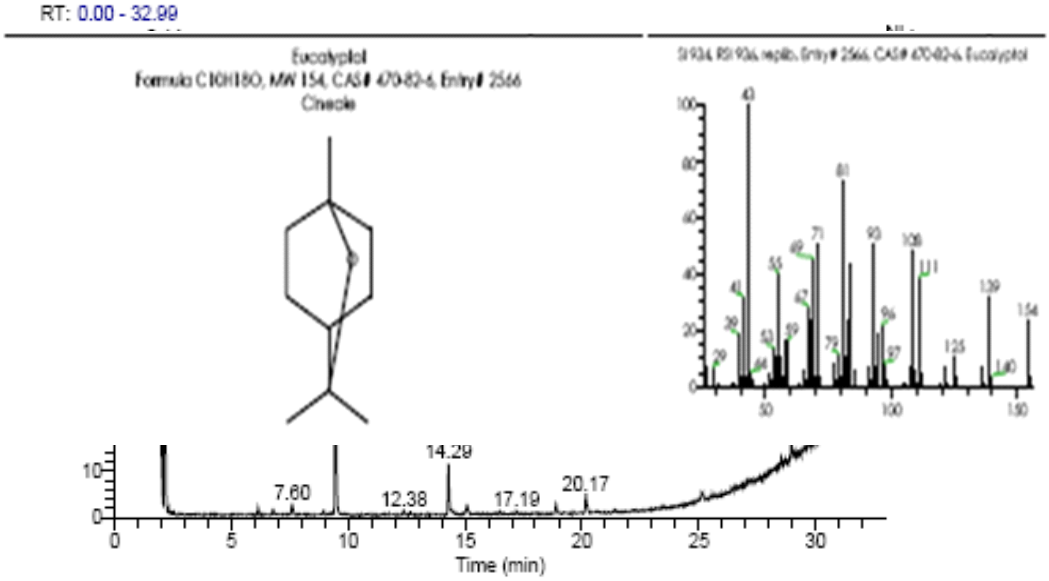

Figure 2. Eucalyptol (C10H18O) treatment and prevention fresh water fishes from bacterial diseases.

Reproduction performance:

The results concerning the good effect of *salvia officinalis* extract on reproductive performance which showed in figures (3- 8).

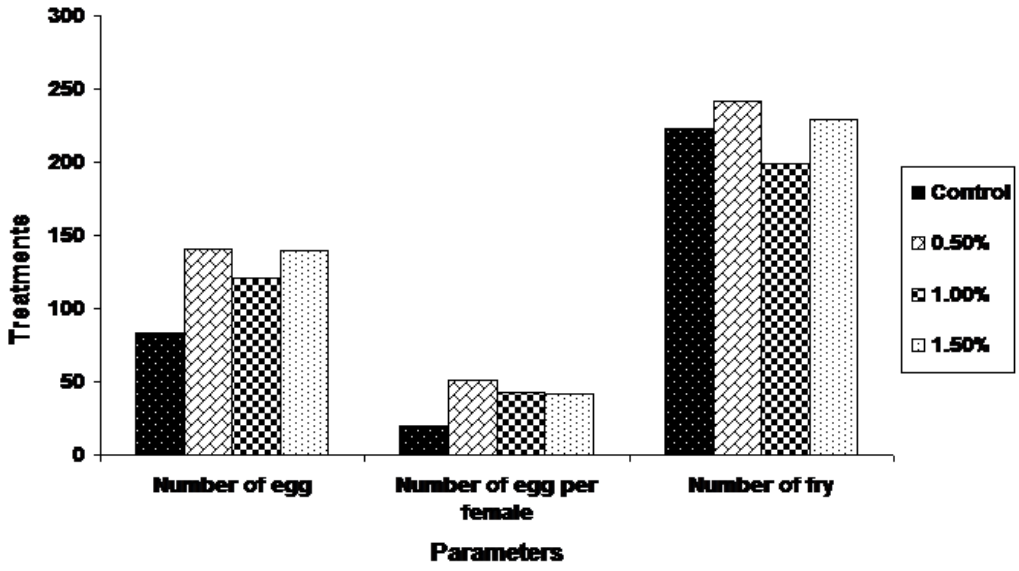

Figure 3. The effect of *salvia officinalis* extract on total number of egg, number of egg per female and number of fry of Nile tilapia *(Oreochromis niloticus)* during 30 day of beginning the feeding experiment.

The results detected in figure (3) showed that increased the total number of egg (141, 120.6 and 139.33 eggs) which produced from broodstock fed supplemented diet with 0.5, 1.0 and 1.5% *S. officinalis* extract for 30 days respectively compare to control group (83.33 eggs). *S. officinalis* extract had good effect on the number of egg per female (51, 43 and 41.6) with 0.5, 1.0 and 1.5% respectively while, control group the number of eggs per female was 19.6. The number of fry slightly increased in broodstock fed diet supplemented with 0.5 and 1.5% *S. officinalis* (241.33 and 229.67) respectively than control group (222.67) where, number of fry of broodstock fed diet contained 1% *S. officinalis* decreased (199.33). In this study, broodstock fed 0.5% *S. officinalis* extract supplemented diet for 30 days had the best positive effect on egg and fry numbers, also the egg number per female.

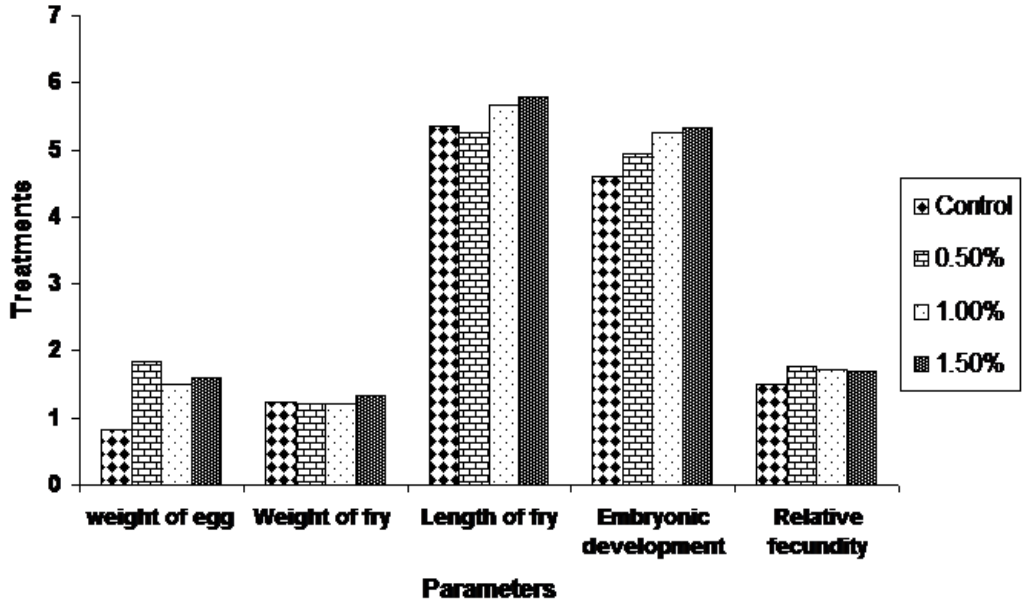

Figure 4. The effect of *salvia officinalis* extract on weight of egg, weight of fry, length of fry, embryonic development and relative fecundity of Nile tilapia *(Oreochromis niloticus)* during 30 day of beginning the feeding experiment.

The observation of egg weight, fry weight, embryonic development and relative fecundity of broodstock fed diet supplemented different treatments of *S. officinalis* for 30 days were described in figure (4). *S. officinalis* extract in different treatments had positive effect on the egg weight by increasing it (1.5 to 1.83 gm) compare to control group (0.83 gm). Feeding diets supplemented with different treatments of *S. officinalis* extract had no effect on fry weight compared control group. *S. officinalis* extract supplemented diets increased embryonic development (4.93, 5.26 and 5.33) at 0.5, 1.0 and 1.5% *S. officinalis* extract supplemented diet respectively than control group (4.6). Also relative fecundity was slightly increased (1.77, 1.72 and 1.70) in the broodstock fed diets supplemented with 0.5, 1.0 and 1.5% *S. officinalis* extract respectively compared control group (1.51). From figure (4) 1.5% *S. officinalis* extract supplemented diet broodstock group gave slightly increasing in fry weight and length and embryonic development.

Reproductive cycles of tilapia broodstock under intensive farming conditions are asynchronous, leading inevitably to variable supplies of fry. Evolution of parental care in tilapia is associated with an increase in egg size and a corresponding reduction in the number of eggs per clutch **Coward and Bromage (1999)**.

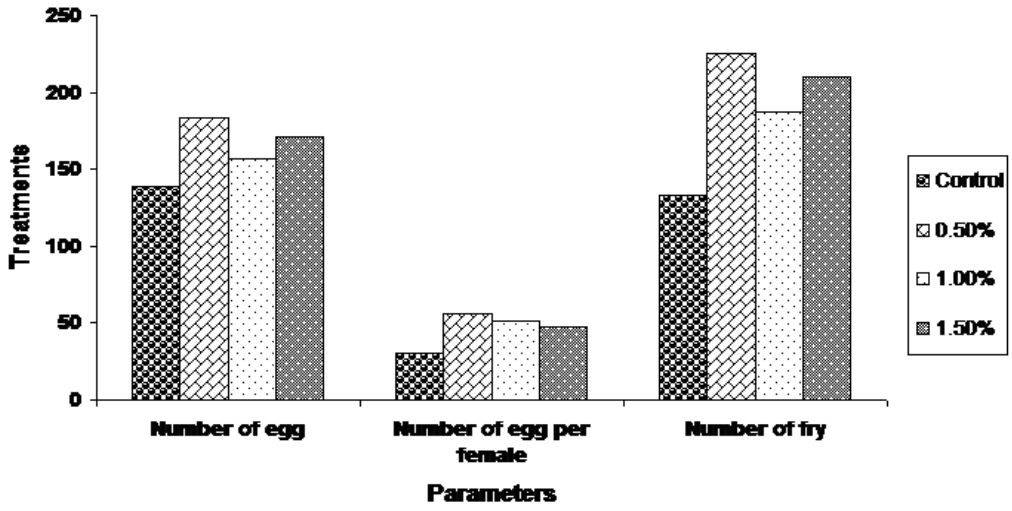

Figure 5. The effect of *salvia officinalis* extract on total number of egg, number of egg per female and number of fry of Nile tilapia *(Oreochromis niloticus)* after 50 day of beginning the feeding experiment.

Figure (5) showed the number of eggs, eggs per female and fry of broodstock fed supplemented diet with different treatments of *S. officinalis* extract for 50 days. *S. officinalis* extract supplemented diet had positive effect on the number of egg, fry and egg per female (183, 157 and 171 eggs), (225.33, 187.33 and 210.33 fries) and (56, 51 and 48 eggs/female) in broodstock groups fed diet supplemented with 0.5, 1.0 and 1.5% *S. officinalis* extract respectively for 50 days compared to control group fed diet free from *S. officinalis* extract (139 eggs, 133.33 fries and 30 eggs/female).

After 50 days of beginning the feeding experiment the egg weight, embryonic development and relative fecundity were described in figure (6). Egg weight of

broodstock groups fed diet supplemented with different treatments (0.5, 1.0 and 1.5%) of *S. officinalis* extract for 50 days were increased (2.26, 1.63 and 1.76 gm) respectively than control group (1.1 gm). From figure (6) 0.5% *S. officinalis* extract supplemented diet broodstock group had the beast effect on the embryonic development (5. 56) than other groups. Broodstock groups fed different treatments (0.5, 1.0 and 1.5%) *S. officinalis* extract supplemented diets for 50 days had highly relative fecundity (1.81, 1.81 and 1.69) respectively than control group (1.09). *S. officinalis* extract had no effect on fry weight or length.

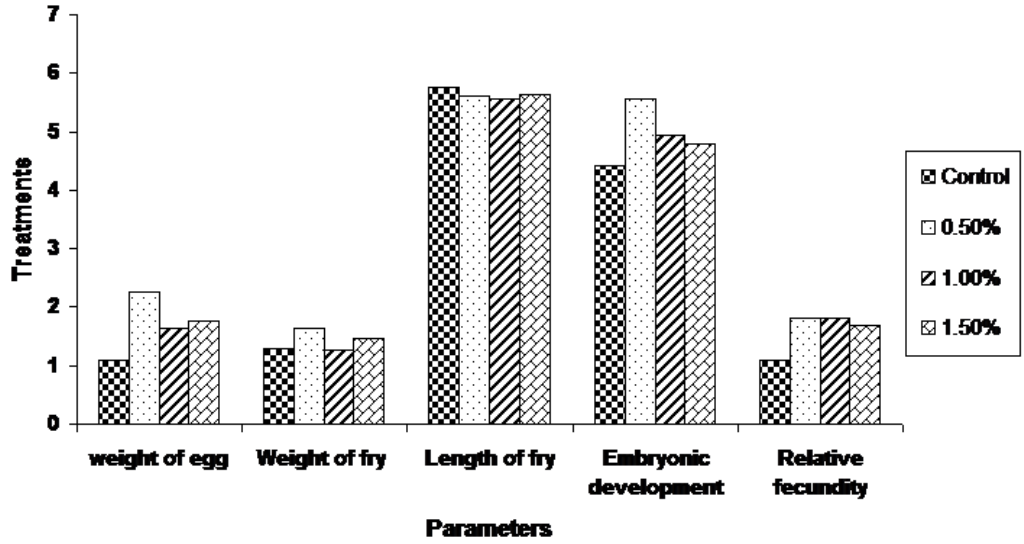

Figure 6. The effect of *salvia officinalis* extract on weight of egg, weight of fry, length of fry, embryonic development and relative fecundity of Nile tilapia *(Oreochromis niloticus)* after 50 day of beginning the feeding experiment.

Tilapia culture is one of the fastest growing farming activities. Tilapia is widely cultured in about 100 countries in the tropical and subtropical regions. As a result, the production of farmed tilapia has increased from 383,654 mt in 1990 to 1,505,804 mt in 2002, representing about 6% of total farmed finfish (FAO, 2004). Nile tilapia is the most important farmed tilapia species in the world. The production of farmed Nile tilapia reached 1,217,055mt representing

about 81% of total production of farmed tilapia in 2002 (FAO, 2004). Broodstock nutrition is one of the most important factors limiting fish fry production and larval quality (Izquierdo *et al.,* 2001). (El-Sayed *et al.,* 2003), it was suggested that the low spawning performance of Nile tilapia fed low protein diets.

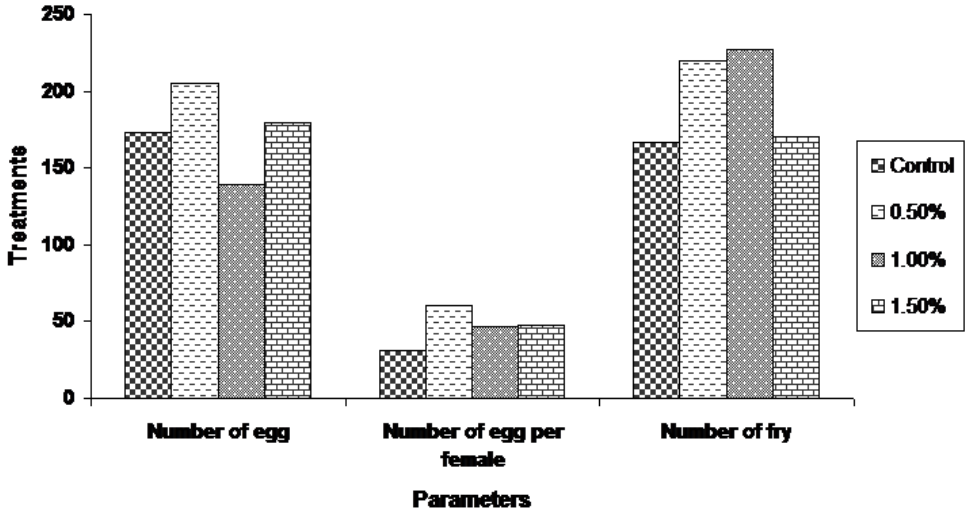

Figure 7. The effect of *salvia officinalis* extract on total number of egg, number of egg per female and number of fry of Nile tilapia *(Oreochromis niloticus)* after 70 day of beginning the feeding experiment.

From figure (7) brood stock group fed diet contained 0.5% *S. officinalis* extract for 70 days had the higher effect on egg and fry number and egg number per female (205 eggs, 220 fries and 60.33 eggs/ female) respectively than other treatments. The number of fry increased in broodstock groups fed diet supplemented with (0.5, 1.0 and 1.5% *) S. officinalis* extract for 70 days (220, 267 and 170 fries) respectively than control group (166.67 fries).

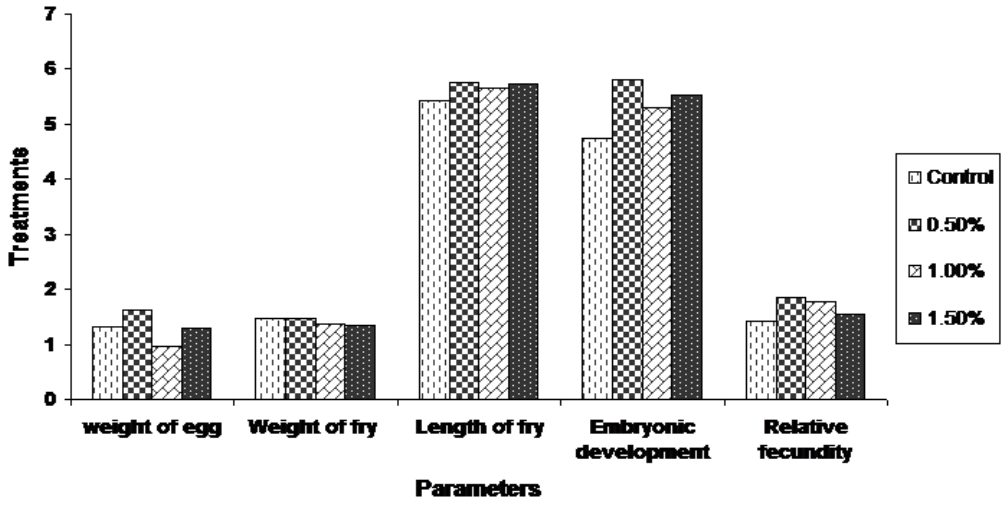

Figure 8. The effect of *salvia officinalis* extract on weight of egg, weight of fry, length of fry, embryonic development and relative fecundity of Nile tilapia *(Oreochromis niloticus)* after 70 day of beginning the feeding experiment.

Figure (8) showed the effect of different treatments of *S. officinalis* extract supplemented diets fed broodstock groups for 70 days on egg and fry weight, fry length, embryonic development and relative fecundity. Broodstock groups fed diets contained 0.5, 1.0 and 1.5% of *S. officinalis* extract for 70 days gave good embryonic development and increased fecundity (5.80, 5.3 and 5.53) and (1.84, 1.78 and 1.53) respectively while, control group gave (4.73 and 1.42) with embryonic development and fecundity respectively. Also 0.5% *S. officinalis* extract supplemented diet was the positive effect on reproductive fish performance at 30^{th}, 50^{th} and 70^{th} day of feeding experiment.

The present results agree with those of Shalaby (2004*)* who found that feeding fenugreek seed meal to Nile tilapia improved growth rate and immunity may be due to the content of essential oil and extracts of *Salvia officionalis species* containing (Eucalyptol) antimicrobial, antioxidant and other biological activities (Milos, *et al*., 2000 and Aligiannis *et al*., 2001).

Health parameters:

Many studies have confirmed that the application of diet herbal additives has a positive impact on the health and resistance of the fish, and also improves their condition rate (Farhat *et al*., 2009).

Results of hemoglobin concentration (fig 9) showed an increasing in the hemoglobin concentration in fish groups fed diet supplemented with *S. offecinalis* extract than control group. Hemoglobin concentration increased by increasing *S. officinalis* extract 8, 10.6, 11 and 11.5 in fish groups fed diet supplemented with 0.0, 0.5, 1 and 1.5% respectively.

Leucocytic count (fig 10) showed that the fish groups fed diets containing 0.5,1 and 1.5 % *S. offecinalis* extract increased (60.5, 80.5 and 95×10^3 cells) respectively comparison with the control group (50.9×10^3 cells). Lymphocyte and Monocyte percent increased in fish groups fed diets containing all treatments 0.5, 1 and 1.5%) of *S. officionalis* extract (11, 20 and 40%) respectively compared with the control group (9 and 8%) respectively. Also *S. officionalis* had positive effect on granulocyte percent where, an increasing it (10, 16 and 35%) in fish groups fed 0.5, 1 and 1.5% *S. officionalis* extract supplemented diets respectively. *S. officionalis* has some constituents that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus and spleen (Jeorg and Lee, 1998) Also; Faisal (2003) reported significantly increased values of haemoglobin concentration in *O. niloticus* and leukocyte count. The employment of hematological techniques, including enaluation of hemoglobin concentration and leukocyte count has provided valuable knowledge for fishery biologists in the assessment of fish health (Blaxhall, 1972). Addition of *S. officionalis* to fish diets increased hemoglobin concentration and leukocyte count (Martins *et al*., 2002). *S. officionalis* contain sallicin, which promotes biogenic performance due to its positive effect on the intestinal flora, thereby improving digestion availability of natural feed, supply of nutrients and utilization of energy which influences the growth of fish (Konning *et al*., 2004).

S. officionalis enhanced immune activates that include promotion of lymphocyte synthesis, cytokine release, phagocytes is and natural killer cell activity (Kyo *et al*., 1998).

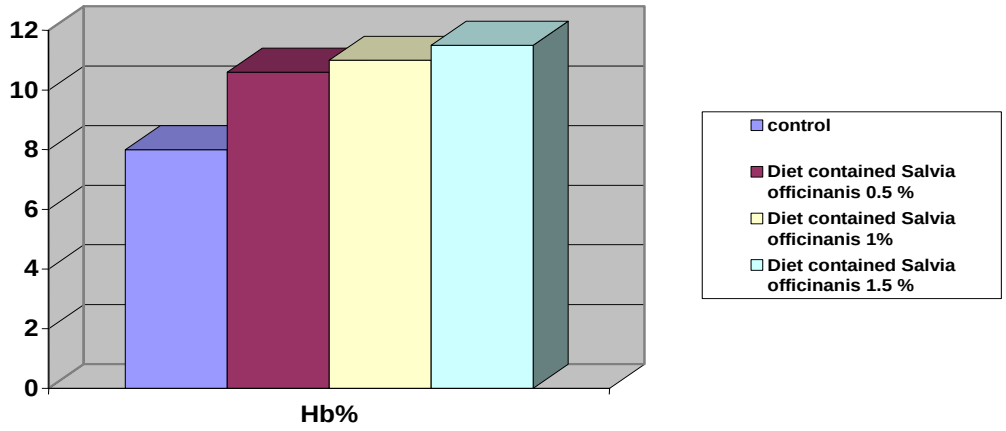

Figure 9. Showing the effect of *S. officinalis* extract supplemented diet on hemoglobin concentration of *O. niloticus*

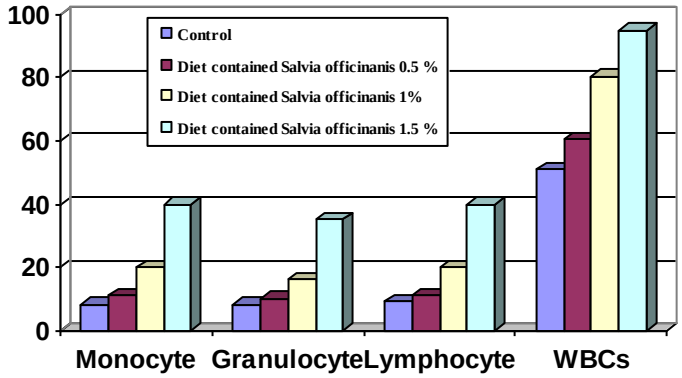

Figure 10. Effect of *S. officinanis* extract supplemented diets on Leucocytic count and differential leucocytic of *O. niloticus*.

Physiological parameters:

Total protein, albumin and globulin values increased with *S. officionalis* extract supplemented diet compared to the control group and the highest value at 0.5 % of the *S. officionalis* extract (fig 11). Albumin value increased from 2.29, 2.30, 2.34 and 2.47 g/dl with 0.0, 0.5, 1 and 1.5% respectively. Globulin was 1.24, 1.29, 1.43 and 1.47 g/dl with 0.0, 0.5, 1 and 1.5% fish groups fed *S. officinalis* supplemented diets respectively. Total protein was 3.30, 3.34, 4.2 and 4.3 g/dl with 0.0, 0.5, 1 and 1.5% fish groups fed *S. officinalis* supplemented diets respectively. The results indicate the improvement of fish health with feeding diet with *S. officionalis* extract. Moreover, the measurement of total protein and albumin in serum or plasma are of considerable diagnostic value in fish, as it affects the general nutritional status as well as the integrity of the vascular system and liver function (**Schäperclaus *et al*., 1992**).

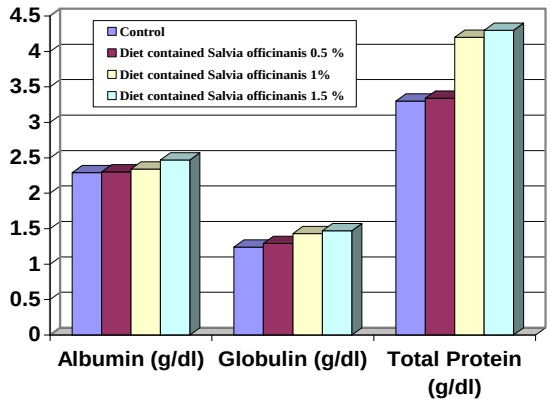

Figure 11. The effect of *S. Officinanis* extract supplemented diet on serum protein, albumin and globulin of *O. niloticus.*

Challenge results:

Diseases of fishes caused by *Aeromonas spp*. are common, have broad host ranges and may cause high mortality. Treatments of captive-reared populations using antimicrobials are limited with concerns for bacterial resistance development and environmental dissemination (Starliper *et al*., 2015).

Results of fish challenged against of *Aeromonas hydrophila* was shown in table (11). No mortalities occurred in fish groups fed *S. officinalis* extract supplemented diets. The highest mortality rates were observed in the control group (90 %). This enhanced immune response may be induced by the essential oils content and extracts of *S. officionalis* species which contains (Eucalyptol) antimicrobial, antioxidant and other biological activities (Aligiannis *et al*., 2001 and Milos *et al*., 2000). Sahin *et al*. (2004) proved the antibacterial effect of oils and extracts of *Eugenia caryophllate* species on many bacterial species like *Escherichia coli, Enterobacter sp., Bacillus sp., Salmonella sp., Staphylococcus aureus, Klebsiella pneumoniae, Listeria monocytogenes and Campylobacter jejuni.* Abdel – Wahab *et al*. (2007) found that 0.5% cinnamon level in the diet is enough to eliminate the harmful microbes in the gut, improve absorption and control blood sugar to a certain extent giving reasonable growth enhancement.

Table 2. Mortality rate of broodstock *O. niloticus* fed diets contained different treatments of ***S. officinanis*** extract for 90 days and challenged by *Aeromonas hydrophila* .

***Salvia Officinanis* extract treatments (%)**		**No, of fish**	**dose**	**injection route**	**mortality**	
					No	**%**
0.0	Subgroup (1)	12	0.2ml of 4x10^6 cells/ml *A. hydrophila*	I/P	11	91.7
	Subgroup (2)	12	0.2 ml of sterile saline	I/P	0	0
0.5	Subgroup (1)	12	0.2ml of 4x10^6 cells/ml *A. hydrophila*	I/P	0	0
	Subgroup (2)	12	0.2 ml of sterile saline	I/P	0	0
1.0	Subgroup (1)	12	0.2ml of 4x10^6 cells/ml *A. hydrophila*	I/P	0	0
	Subgroup (2)	12	0.2 ml of sterile saline	I/P	0	0
1.5	Subgroup (1)	12	0.2ml of 4x10^6 cells/ml *A. hydrophila*	I/P	0	0
	Subgroup (2)	12	0.2 ml of sterile saline	I/P	0	0

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تأثير مستخلص نبات المرمرية على صحة الاسماك والكفاءة التناسلية لأسماك البلطي النيلي

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الملخص العربى

تم تجميع النبات من السوق وعمل مستخلص منه وقياس تأثيره كمضاد للبكتيريا وعمل تنقية وتعريف المادة الفعالة. تم اضافة المستخلص بنسبة 0 ، 0.5 ، 1 ، 1.5 % من العليقة التجارية (30 % بروتين). تم تجميع امهات البلطي من مزرعة العباسة وتم اختيار المناسب منها لعملية التفريخ وقسمت الى اربع مجموعات (3 تكرار) بمعدل كل تكرار (6 اناث + 2 ذكور) وتم التغذية حتي الشبع مرتين يوميا بمعدل خمس ايام للأسبوع لمدة 3 شهور. تم قياس وزن وعدد البيض والزريعة ووزن البيض لكل ام وقياس التطور الجنيني. تم اخذ عينات دم وسيريم لقياس عناصر الدم والانزيمات الفسيولوجيا. كما تم حقن الأسماك صناعيا بالايرو موناس هيدروفيلا لمعرفة مدى مقاومة الاسماك لهذه البكتيريا.

واسفرت النتائج على ان مستخلص المرمرية له تأثير مثبط لبكتيريا الايروموناس هيدروفيلا. وتم تعريف المادة الفعالة ($C_{10}H_{18}O$ EUCALYPTOL) كان لها تأثير اجابي على كفاءة وسرعة التفريخ حيث زاد عدد البيض والزريعة عند الجرعة 0.5 % كما ان المستخلص له تأثير ايجابي على صحة الاسماك حيث حسن من عدد كرات الادم البيضاء– الهيموجلوبين –الجلوبيولين–الالبيومين – والبروتين الكلي كما انه رفع مقاومة الاسماك ضد بكتيريا الايروموناس هيدروفيلا.