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EFFECT OF GARDEN CRESS (*Lepidium sativum*) AND WATERCRESS (*Eruca sativa*) SEEDS ON SOME PHYSIOLOGICAL PROFILES AND REPRODUCTIVE PERFORMANCE IN NILE TILAPIA (*Oreochromis niloticus)*

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Abstract

Medicinal herbs have curative powers and are used in making medicines because of their healing properties. The present study provides a probable insight on the beneficial effects of medicinal plant in improving fertility and physiological parameters of Nile tilapia (*Oreochromis niloticus)* through the effect of dietary graded levels (0, 1, 1.5 & 2 %) of garden cress *(Lepidium sativum)* and watercress (*Eruca sativa*) seed meal. A total of 126 male and female of *O. niloticus* at average body weights of 163.49±4.65 g were distributed as sex ratio 1:2 (one male per two female) at a density of 6 fish per a glass aquaria in for 6 weeks. The obtained results revealed improving effect of this feed additive (garden cress and watercress seed meal) on Hematological parameters such as red blood cells (RBCs), hematocrit (Hct), hemoglobin (Hb, RBCs) index (MCV, MCH & MCHC) as well as, glucose, total protein, albumin and globulin levels and also AST, ALT, Creatinine and uric acid. The most enhanced results were obtained from addition of 1% garden cress to fish meal followed by 1% of watercress compared to the controls and other treatments. Also, addition of 1% of garden cress (T2) in diet of *O. niloticus* accelerated hatching and gave highly dynamic seed production after 4th week. At the end of the 5th week, females, *O. niloticus* hatched and produced less efficient seeds in T5 (1% of watercress). On the other hand, the females in control group (T1) and other remaining treatments (T3, T4, T6 & T7) were hatched at the end of the experiment (6th week) but less in number and efficiency.

Keywords: *Oreochromis niloticus*, Garden cress, Watercress, Reproduction, Physiology.

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* is currently considered to be the most important and commonly cultured tilapia species around the world and constitutes over 70% of the cultured tilapia (Fitzsimmons, 2004). Tilapia species constitute a major and important item in the Egyptian fish farming. It displays many favorable attributes as culture species, on the basis of its general hardness, resistance to diseases, high yield potential, and ability to grow on wide range of natural and cheap artificial foods (El-Sayed, 2006). So, tilapias are the second only to carps as the most widely farmed freshwater fish in the world (FAO, 2010).

Plants still remain the basis for development of modern drugs and medical plants have been used for years in daily life to treat diseases all over the world (Ates, 2003). Recently, several efforts have been made to replace chemical drugs by herbal medicine in aquaculture industry in many countries (Obaroh and Achionye-Nzeh, 2011).

Garden cress *(Lepidium sativum)* has been used widely in different parts of the world for its wide therapeutic application, plant and seeds are considered one of the popular medicinal herbs used as a good mediator for bone fracture healing in the human skeleton (Gill and MacLeod, 1980). It is related to Brassicaceae (Cruciferae) family and called as *Hab el Rashaad* or *Thufa* (Bafeel and Ali 2009).

The most effective ingredient present in *Lepidium sativum* is isothiocyanates, which is formed with glucosinolates (Kassie *et al*., 2002). *Lepidium sativum* seeds are recommended for the good health and reproduction. The edible whole seed is known to have health promoting properties. Hence, it was assumed that these seeds can be a functional food (Abdelhamid and Soliman, 2012).

Watercress (*Eruca sativa*) is a perennial plant which growths in clear, cold water and is found in ditches and streams everywhere. Watercress, which

is cultivated for its pungent leaves which are used in cooking especially in soups, garnishes and salads, is one of the most important herbal medicines used in traditional treatment of some diseases such as oxidative stress (Yazdanparast *et al*., 2008) and immune depression (Sonnenbichler *et al*., 1986). Raw watercress leaves are used as salad greens, or can be steamed and consumed as a normal processed vegetable. It also known as arugula, or rocket, it is called "Jarjeer" in Arabic, it is an edible plant and also is considered a medical plant with many reported properties, including its strong aphrodisiac effect known since Roman times (Font *et al*., 2003).

Watercress is a valuable source of vitamins and a good detoxifying herb. This plant contains a relatively large amount of vitamins B1, B2, C and pro-vitamin A, folic acid, glucosinolates, iodine, iron, protein, and especially calcium and sulphur compounds, which influence its characteristic odor, but also adds to its nutritional benefits (Palaniswamy *et al*., 2003). It is the richest source of glucosinolates, which can be hydrolyzed to produce phenethyl isothiocyanate. Isothiocyanates can prevent carcinogen activation (Conaway *et al*., 1996).

The major glucosinolate in seeds is Erucin which is potentially capable of protecting cells against oxidation performance (Khalil *et al*., 2015). They also contain Zn, Cu, Fe, Mg, Mn, and other elements (Abdo, 2003) which increase immune response and the reproductive performance. Kolawole *et al*. (2011) stated that one way to distinguish the appropriate or inappropriate prescription of medical plants is the assessment of their effects on hematological and biochemical parameters in experimental animals.

Some studies have been done in which herbs, as dietary additives, were fed to fish. The focus of these studies includes their effects on hematological and plasma biochemical parameters of the fish. However, few attempts incorporate rocket seeds in diets of red tilapia (Abd Elmonem *et al.*, 2002), Nile tilapia (Mahmoud *et al*., 2009) and African catfish (Fagbenro, 2004) and also cresson seeds in the diets of Nile tilapia (Abdelhamid and Soliman, 2012) and

Common carp (Jayarama *et al*., 2015). More nutritional and physiological evaluations of dietary cresson and rocket seeds by fish are still required.

Therefore, the objectives of the present study were to evaluate the effects of graded levels (0, 1, 1.5 and 2% kg diet) of dried garden cress (*L. sativum)* and watercress (*E. sativa*) seeds on hematological, plasma biochemical parameters and reproduction of Nile tilapia; *O. niloticus*.

MATERIALS AND METHODS

Fish and experimental management:

This study was conducted in Central Laboratory for Aquaculture Research Abbassa, Abu- Hammad, Sharkia governorate, Egypt.

Fish were stocked in rearing tank for two weeks as adaptation period. During this time they were fed the basal diet. A total of 126 male and female; *O. niloticus* at average body weights of 163.49±4.65 g were distributed as one male per two females at a density of 6 fish per aquarium. The experimental treatments were tested at three aquaria (replicates) for each. Each glass aquarium (170×50×40 cm) was supplied with 255L de-chlorinated tap water and an air stone connected with electric compressor. The replacement of the aquaria water was done partially every day to re-new the water and to remove the wastes. All treatments and experimental design are showing in Table A.

Table A. Experimental design and dietary treatments.

Treatments	Details seeds
Control (T1)	Basal diet+0% dried seeds of Garden cress and Watercress kg $^{-1}$diet
T2	Basal diet + 1% dried seeds of Garden cress kg $^{-1}$diet
T3	Basal diet + 1.5% dried seeds of Garden cress kg $^{-1}$ diet
T4	Basal diet + 2% dried seeds of Garden cress kg $^{-1}$ diet
T5	Basal diet + 1% dried seeds of Watercress kg $^{-1}$ diet
T6	Basal diet + 1.5% dried seeds of Watercress kg $^{-1}$ diet
T7	Basal diet + 2% dried seeds of Watercress kg $^{-1}$ diet

Experimental diet:

Dried seed meal of Garden cress (*L. sativum***)** and also Watercress (*E. sativa*) were added at levels of 1, 1.5 and 2 % to Nile tilapia fish diets. The ingredients and additives (seeds of *L. sativum & E. sativa*) were bought from the local market. Seeds of *L. sativum* and *E. sativa* were cleaned and shade-dried in a drying oven at 50°C for 72 hr. Seeds were milled into fine particle size (<250 μm) and kept in a dry, air-tight transparent plastic container. Feed ingredients were ground and the different ingredients mixed manually, then the experimental diets were pressed by manufacturing machine (pellets size 1 mm). The fish diet(30 % prptein) was used, where1, 1.5 and 2 % of the yellow corn was substituted with Garden cress or Watercress seeds powder to form the experimental treatments **(**0 , 1, 1.5, 2 % Garden cress and 1, 1.5 , 2 % Watercress**)**

Experimental procedures:

The experiment continued for 6 weeks. Experimental diets were introduced manually to the fish twice daily, at 8.00 am and 2.00 pm at a rate of 2% of their live body weight daily, six days per week.

Samples of water were taken from each aquarium to determine water quality parameters included temperature in degree centigrade which was recorded every day (via a thermometer), pH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter) were measured weekly. All tested water quality criteria were suitable for rearing and reproduction of Nile tilapia (*O. niloticus*) as cited by Abd El-Hakim *et al*. (2002). Since, water temperature ranged between 27.6 – 28.5°C, pH values 7.7-8.1 and dissolved oxygen 5.50-8.80 mg/L.

Hematological and biochemical analyses:

Blood samples were collected from the caudal veins at the end of the experimental period (6 weeks). The blood parameters (RBCs, Hb and Hct) were determined according to Sarder *et al*. (2001) and the blood indices (MCH, MCHC and MCV) were calculated as previously mentioned by Brown (1988).

The rest of the blood samples were centrifuged to obtain the plasma for biochemical analyses. All samples were kept in the deep freezer. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel (1957). Total protein and albumin was determined according to Henry (1964). Globulin levels were calculated by subtracting albumin values from total protein. Plasma glucose was determined according to Trinder (1969). Creatinine and uric acid were determined according to Henry (1974).

Statistical analysis:

One-way ANOVA and Duncan multiple range test were used to evaluate the difference between the concentrations of different studied variables with respect to sites. The software CoStat version 6.311(CoStat, CoHort software, USA) was used for data analysis. A probability at level of 0.05 or less was considered significant (Bailey, 1981).

RESULTS AND DISCUSSION

The present results (Tables, 1&2) showed an increasing of WBCs, RBCs, Hb, Hct values, mean corpuscular hemoglobin concentration (MCHC) plasma total protein, albumin and globulin in T2 and T5 (1% of garden cress and watercress) compared with the control group (T1) and decreasing of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). These results in hematological and biochemical parameters led to increasing the immune responses and healthy status of fish. These positive findings were related to garden cress seeds which are rich source of proteins, dietary fiber, omega-3 fatty acids, iron, other essential nutrients and phytochemicals (Snehal and Manisha, 2014) and also watercress inclusion of antioxidant constituents; carotenoids, vitamin C, flavonoids such as appiin and luteolin and glucosinolates the precursors of isothiocyanates and sulfaraphene (Hanafi *et al*., 2010), volatileoils like myristicin and apiole β-phellandrene (Leung and Foster, 1996). Glucosinolates were found in both garden cress and watercress to have several biological activities including anticarcinogenic, antifungal and

antibacterial plus their antioxidant action (Kim *et al*., 2004). The major glucosinolate in seeds is potentially capable of protecting cells against oxidative stress. In addition, garden cress contains Zn, Cu, Fe, Mg, Mn and other elements (Abdo, 2003) which increase immune response.

Table 1. Effect of garden cress (*Lepidium sativum*) and watercress (*Eruca sativa*) on some blood parameters of female Nile tilapia; *Oreochromis niloticus.*

	WBCs x 10^3 cell/µl	RBCs x 10^6 cell/ µl	Hb g/dl	Hct %	MCV Fl	MCH Pg	MCHC g/dl
Control (T1)	15.17 ±0.01 ^{b}	2.44 ±0.01 ^{b}	8.63 ±0.07 ^{b}	23.22 ±0.48 ^{b}	95.06 ±2.15 ^{a}	35.35 ±0.37 ^{a}	37.21 ±0.05 ^{b}
T2	17.72 ±0.44 ^{a}	2.84 ±0.02 ^{a}	9.83 ±0.03 ^{a}	24.75 ±0.28 ^{a}	87.03 ±0.96 ^{b}	34.58 ±0.19 ^{a}	39.72 ±0.12 ^{a}
T3	15.40 ±0.05 ^{b}	2.56 ±0.01 ^{b}	8.70 ±0.06 ^{b}	23.55 ±0.12 ^{b}	91.83 ±0.57 ^{a}	34.02 ±0.21 ^{a}	36.72 ±0.02 ^{b}
T4	15.26±0. 56 ^{b}	2.45 ±0.01 ^{b}	8.63 ±0.07 ^{b}	23.38 ±0.20 ^{b}	95.02 ±0.96 ^{a}	35.28 ±0.19 ^{a}	36.91 ±0.22^b
T5	17.28 ±0.19 ^{a}	2.86 ±0.01 ^{a}	9.63 ±0.03 ^{a}	24.81 ±0.01 ^{a}	86.75 ±0.15 ^{b}	34.14 ±0.05 ^{a}	38.80 ±0.15 ^{a}
T6	15.58 ±0.15^b	2.45 ±0.01 ^{b}	8.73 ±0.07 ^{b}	23.23 ±0.01 ^{b}	94.77 ±0.06 ^{a}	35.63 ±0.25 ^{a}	37.20 ±0.21 ^{b}
T7	15.35 ±0.09 ^{b}	2.43 ± 0.01 ^{b}	8.33 ±0.07 ^{b}	23.35 ±0.01 ^{b}	95.02 ±0.23 ^{a}	34.26 ±0.76 ^{a}	35.66 ±0.31^b

Data are represented as means ± S.E.

Means with the same letter in the same column are not significantly different at P≤.05.

In other words, adding of garden cress and watercress to fish diet may concentrate hemoglobin in red blood cells of fish. These results were agreed with Salem (2012) who concluded that watercress meal improved blood parameters (RBCs, WBCs), total plasma protein, albumin and globulin in Nile tilapia due to increase of immunity and reduce the negative effect of aflatoxin B1 on fish. In this trend, many studies on tilapia indicated that using medical plants led to increase the immune status of fish such as Nigella sativa (Elkamel and Mosaad, 2012); *Trigonella foenum-graecum*, *Eucalyptus citriodora* and *Matricaria recutita* (Zaki *et al*., 2012).

Table 2. Effect of garden cress (*Lepidium sativum*) and watercress (*Eruca sativa*) on plasma glucose, total protein, albumin and globulin of female Nile tilapia (*Oreochromis niloticus).*

	Glucose mg/dl	Total Protein g/dl	Albumin g/dl	Globulin g/dl
Control (T1)	68.48±0. 85 [a]	4.63±0.04 [b]	2.21±0.08 [a]	2.41±0.13 [b]
T2	70.20± 0.89 [a]	5.21±0.17 [a]	2.21±0.06 [a]	2.99±0.16 [a]
T3	62.37±0. 52 [b]	4.65±0.05 [b]	2.19±0.03 [a]	2.43±0.07 [b]
T4	59.83±0. 64 [b]	4.64±0.04 [b]	1.89±0.04 [b]	2.42±0.01 [b]
T5	69.36±0. 68 [a]	5.38±0.14 [a]	2.41±0.02 [a]	2.87±0.08 [a]
T6	62.40±0. 32 [b]	4.67±0.02 [b]	1.93±0.03 [b]	2.48±0.04 [b]
T7	59.90±0.32 [b]	4.64±0.02 [b]	1.92±0.02 [b]	2.41±0.02 [b]

Data are represented as means ± S.E.

Means with the same letter in the same column are not significantly different at P≤.05

Our results, also agreed with Khalil *et al*. (2015) who concluded that dietary dried watercress seeds gave the best results compared with dried watercress leaves and the inclusion of 3% dried watercress seeds or 2% dried watercress leaves as feed additives of all male monosex *O. niloticus* increased hematological and biochemical parameters. Increase in hemoglobin content (Hb), hematocrit (Hct), and numbers of leucocytes (WBCs) were reported in Nile tilapia (Shalaby *et al*., 2006) and hybrid tilapia (Ndong and Fall, 2011) fed with diet enriched by garlic. These results were in agreement with previous findings where feeding with other herbal supplementary food led to an increase in hemoglobin levels (Ji *et al*., 2007).

Data presented in Table (2) indicated that the glucose concentration of the control group was 68.48±0. 85 (mg %). feeding of *O. niloticus* with 1.5 and 2 % of garden creass and watercress caused significant decrease in glucose concentration to (62.37±0. 52, 62.37±0. 52, 62.40±0.32 and 59.90±0.32 mg%, respectively). The decrease in blood glucose may be due to the presence of antioxidants such as alkaloids, flavonoids, cysteine and glycine which have blood glucose-reducing properties. These results are in agreement with Nadar *et al.* (2009) and Mehran *et al.* (2017) who demonstrated that adding of watercress

and garden cress seeds extract to diabetic rats decreased blood glucose levels significantly and increase in blood insulin levels and liver glycogen in diabetic rats treated with alloxan.

The increased total protein levels in plasma of fish fed with watercress is accompanied by the increased levels of immune parameters which have a protein structure, such as globulins and total complements. Several authors reported an increase in complement activity following administration of different immunostimulants such as herbal derivatives (Christybapita *et al*., 2007), sodium alginate (Cheng *et al*., 2008) and vitamins C and E (Ortuno *et al*., 2001).

There is a close relationship between the level of protein synthesis in liver tissue and plasma protein pools, total protein levels in plasma may be elevated due to the increased levels of protein synthesis in liver tissue of fish feed with garden cress and watercress (Asadi *et al*., 2012).

Banaee *et al*. (2011) reported that oral administration of some herbal medicine such as silymarin may improve protein synthesis in fish liver tissue. Consequently, significant increase of the total protein levels in plasma in treated fish is probably reflecting the increase of the protein synthesis in liver tissue. Similarly, the highest serum protein level was recorded in Nile tilapia fed yellow leader and Japanese honeysuckle (Ardó *et al*., 2008), ginger, mistletoe and stinging nettle (Dügenci *et al*., 2003). Proteins include albumin and globulin; some globulins are produced in the liver, while others are made by the immune system (Sandnes *et al*., 1988). Globulin is made up of subunit of α1, α2, β, and γ globulins, which are considered as the source of almost all the immunologically active proteins in the blood (Jha *et al*., 2007). Commonly, Increases in the levels of plasma total protein, albumin and globulin in fish are thought to be associated with a stronger innate immune response (Wiegertjes *et al*., 1996).

Data described in Table 3 indicated that, liver functions were not disturbed in all experimental groups as reflected on the estimated values of

activities of plasma AST (22.55±0. 03, 22.78±0.05, 22.44±0. 05, 22.54±0.05, 22.94±0.06 and 22.62±0.05 Iu/L in fish after fed with 1, 1.5 and 2% of garden cress and watercress, respectively when compared the control group (23.05±0. 05 Iu/L). The assessment of plasma ALT activity after treatment with garden cress and watercress revealed non-significant changes to (16.32±0. 05, 17.02±0. 02, 17.01±0.04, 16.38±0. 03, 16.92±0. 05 and 16.81±0.08 Iu/L) after fish fed with 1, 1.5 and 2% of garden cress and watercress, respectively when compared to the control value (17.02±0. 03Iu/L). The present findings could argue that garden cress may have hepatoprotective effect (Al Hamedan, 2010). Also, Kidney functions were not affected in all experimental treatments, as reflected on the unchanged values of creatinine and uric acid. Our results of liver function and kidney function were in agreement with Emtenan *et al*. (2010), where they used watercress seed oil in meal of rabbits. Also, Ahmed *et al.* (2013) recorded that the administration of garden cress meal did not cause any significant change in the activity of uric acid and creatinine functions of the rats. Our results were taken as a guide to evaluate the safety of the garden cress and watercress.

Table 3. Effect of garden cress (*Lepidium sativum*) and watercress (*Eruca sativa*) on liver and kidney functions of female Nile tilapia (*Oreochromis niloticus).*

	AST µ/l	**ALT µ/l**	**Creatinine mg/dl**	**Uric Acid mg/dl**
Control (T1)	23.05±0. 05 [a]	17.02±0. 03 [a]	0.32±0.003 [a]	1.42±0.004 [a]
T2	22.55±0. 03 [a]	16.32±0. 05 [a]	0.30±0.003 [a]	1.40±0.005 [a]
T3	22.78±0. 05 [a]	17.02±0. 02 [a]	0.33±0.006 [a]	1.43±0.006 [a]
T4	22.44±0. 05 [a]	17.01±0. 04 [a]	0.34±0.006 [a]	1.38±0.005 [a]
T5	22.54±0. 05 [a]	16.38±0. 03 [a]	0.31±0.007 [a]	1.33±0.001 [a]
T6	22.94±0.06 [a]	16.92±0. 05 [a]	0.34±0.006 [a]	1.33±0.005 [a]
T7	22.62±0. 05 [a]	16.81±0.08 [a]	0.35±0.005 [a]	1.36±0.004 [a]

Data are represented as means ± S.E.

Means with the same letter in the same column are not significantly different at P≤.05

Data in Table 4 indicated that addition of 1% of garden cress (T2) in diet of *O. niloticus* accelerated hatching and gave highly dynamic seed production after 4th week. At the end of the 5th week, females, *O. niloticus* hatched and produced less efficient seeds in T5 (1% of watercress). On the other hand, the females in control group (T1) and other remaining treatments (T3, T4, T6 & T7) were hatched at the end of the experiment (6th week) but less in number and efficiency.

Table 4. Spawning of female Nile tilapia (*Oreochromis niloticus)* feed on different levels of garden cress and watercress through 6 weeks.

Item	IFBW	FFBW	SN	TSP	SP/F	RF	WG/F	Time (week)
Control (T1)	162.9 ±1.9	164.1 ±0.8	12	0828	69	0.42	1.2	6
T2	166.7 ±1.7	170.2 ±2.2	12	2160	180	1.06	3.5	4
T3	166.8 ±0.9	169.4 ±1.2	12	1392	116	0.68	2.6	6
T4	166.7 ±1.9	168.2 ±2.2	12	1188	99	0.59	1.5	6
T5	164.2 ±2.0	167.3 ±2.0	12	1920	160	0.96	3.1	5
T6	166.8± 0.8	169.1± 1.2	12	1200	100	0.59	2.3	6
T7	167.1 ±1.3	168.4 ±2.2	12	1020	85	0.50	1.3	6

FBW: initial female body weight, FFBW: final female bodyweight, SN: surviving number, TSP: total seed production, SP/F seed production per female, RF: relative fecundity, WG/F: weight gain per female**.**

Garden cress possess tocopherol and some other endogenous metabolites, which are rich in antioxidant activity (Muanda *et al.*, 2011).This tocopherol plays a vital role for the activity of the body (Jaiswal *et al.*, 2004), and its fat-soluble antioxidant and kept the body from various chemical reactions of free radicals, which poison the cells (Braun and Cohen, 2007). The results of the present research agree with the findings of the Nada and Faris (2013) in their study on male rabbits treated with seeds extract of *Lepidium sativum* plant, which led to improved fertility of rabbits and increasing sperm concentration.

Watercress seeds are rich source of vitamin A which is considered the most important vitamin in the protective mucous membranes and reproduction, glucosinolates were found also to have antioxidant action where the major glucosinolate in seeds is potentially capable of protecting cells against oxidative stress (Kim *et al*., 2004). Emtenan *et al*. (2010) confirmed these positive effects of watercress on reproduction, where they studied effect of watercress seeds on testes of male rabbits. The same results were recorded by Zena (2013) where she studied the effect of garden cress on fertility in albino mice.

From this study, it could be concluded that addition 1% grinding seeds of the garden cress or watercress to the diet of the Nile tilapia can improve their healthy status and shorten the period required for their spawning.

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تأثير بذور حب الرشاد والجرجير على بعض النواحى الفيسيولوجية واداء التكاثر لاسماك البلطي النيلي

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الملخص العربي

للكثير من الأعشاب الطبيعية خصائص علاجية حيث تستخدم حديثا في صنع الأدوية ومحسنات غذائية للإنسان والحيوان على السواء.

توضح هذه الدراسة التأثيرات الأيجابية والمفيدة لمسحوق بذور حب الرشاد والجرجير في تحسين الخصوبة وبعض القياسات الفسيولوجية لسمكة البلطي النيلي من خلال إضافة 1 ، 1.5 ، 2٪ من مسحوق بذور حب الرشاد أو الجرجير للعليقة حيث تم توزيع 126 من ذكور وإناث سمكة البلطي النيلي بمتوسط وزن 163.49 ±4.65 جرام ، حيث تم توزيعهم على اساس 2 ذكور لكل4 إناث في حوض واحد وقد قسمت هذه الاسماك الى سبعة مجموعات لكل مجموعه 3 مكررات ، وتغذت المجموعه الاولى على علائق متزنة لاتحتوى على اى اضافات من حب الرشاد او بذور الجرجير بينما المجموعة الثانيه والثالثه والرابعة اضيف الي عليقتها (1 ، 1.5، 2 %) بالترتيب من حب الرشاد ، والمجموعه الخامسه والسادسه والسابعه اضيف إلى عليقتها نفس الكميه (1 ، 1.5 ، 2 %) من الجرجير، وقد لوحظ بعد مرور 4 اسابيع أن المجموعة الثانية (1% من حب الرشاد) قد حدث بها تفريخ في كل المكررات وأن الزريعة كان عددها كبير وذات حيوية عاليه ، وبعد مرور 5 اسابيع لوحظ أن المجموعة الخامسة (1% من الجرير) حدث بها تفريخ ايضا ولكن بعدد اقل وحيويه اقل. وكذلك بعد 6 اسابيع (نهايه التجربه) لوحظ أن المجموعة الضابطه (0% من الجرجير وحب الرشاد) وكذلك باقى المعاملات قد حدث بها تفريخ ايضا ولكن بعدد زريعه اقل بكثير.

وبعد انتهاء التجربة تم اخذ عينات الدم وقد لوحظ تحسن ملحوظ لاضافة حب الرشاد والجرجير علي قياسات الدم المختلفة وكذلك نسبة السكر. ولوحظ ايضا زيادة في مستوي البروتين الكلي والالبيومين و الجلوبيولين اما وظائف الكبد والكلي وحمض اليوريك فلم تبدي اي تغير مقارنة بالكنترول وقد وجد أن افضل النتائج كانت عند تركيز(1% من حب الرشاد) يليه (1% من الجرجير).

من هذه الدراسة يمكن استنتاج أن إضافة 1٪ من مسحوق بذور حب الرشاد أو الجرجير إلى النظام الغذائي للبلطي النيلي يمكن أن يحسن الحالة الصحية للأمهات ويسرع من عملية التفريخ وبكفاءة أعلى وذلك بإضافة 1% من مسحوق بذور أي منهما.