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EFFECT OF *NIGELLA SATIVA* ON GROWTH PERFORMANCE AND SOME BLOOD CONSTITUENTS OF NILE TILAPIA (*OREOCHROMIS NILOTICUS)*

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ABSTRACT

A total number of 135 Nile tilapia (*O. niloticus)* were used to study the effect of dietary supplementation with black cumin seed (*Nigella sativa*) on growth performance and some blood constituents. The fish were randomly divided into three treatment groups each of 45 fish, and each group was divided into 3 subgroups (15 fish / unit). These groups received N. sativa at 1% (T1) and 2% (T2) and control group (C) which fed on basal diet without *N. sativa*. Body weight and length were recorded biweekly all over the experimental period (12 weeks). Blood samples from each fish were collected to determine each of hemoglobin (Hb), packed cell volume (PCV%), serum glucose, total protein, albumin, globulin, and alkaline phosphatase. Ten fish were scarified to determine hepatosmatic index (HSI), spleen somatic index (SSI), and gonadosomatic index (GSI). Other ten fish were transported to wet laboratory and reared without oxygen supplementation to determine the resistance under oxygen deficiency. The results showed that there was insignificant increase in body weight, body weight gain, total length, and length increment in T2. Also, the hemoglobin increased insignificantly ($P > 0.05$) in T2 than the control. Moreover, the serum albumin improved insignificantly ($P > 0.05$) in T1 and T2 than the control. Serum alkaline phosphatase was significantly ($P < 0.05$) increased in treated fish with *N. sativa;* while serum glucose decreased significantly ($P < 0.05$) as compared with control ones. The hepatosoatic index (HSI) insignificantly (*P > 0.05*) decreased in T1 and increased insignificantly (*P > 0.05*) in T2 compared to the control. Also, the dietary 1 and 2 % *N. sativa* did not affect spleenosomatic index (SSI). The fish group which reared under oxygen deficiency exhibited remarkable decreased activity and increased random movement elicited by hypoxia changes in direction of swimming, while the fish groups fed diets with *N. sativa* showed slightly abnormal behavior changes. The mortality rates under oxygen deficiency were 100, 40 and 20% for control, T1 and T2, respectively. Under oxygen deficiency stress, the Hb and glucose did not differ between T1 and T2, while PCV% was increased significantly (*P > 0.05*) in T2 than that of T1; the same occurred ($P < 0.05$) with serum total protein concentration, globulin, and alkaline phosphatase activity, but serum albumin in T1 was higher than that of T2 ($P < 0.05$). There were no significant ($P > 0.05$) in HIS, SSI and GSI between treated groups with *N. sativa* and control ones. To conclude, the dietary supplementation with *N. sativa* at 1% and 2% displayed slight beneficial effects on growth performance and major effects on some blood constituents of *O. niloticus*.

Keywords: Nigella sativa, growth, blood, Nile tilapia, feed supplements.

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INTRODUCTION

In the world, very important investments have been made in cultural fisheries to meet the protein needs for humans. In parallel with the development of this industry, diseases and stress factors have also increased (FAO, 2016). For a healthy and efficient production, the main cause of these problems must be identified and solved. Tilapia is cultured in aquaculture farms all over the world. This fish has been recognized as the potential aquaculture fish species of the twenty-first century (Shelton, 2002). Among a number of species of tilapia (FAO, 2018), Nile tilapia (*Oreochromis niloticus*) is most widely cultured in a diverse group of aquatic environments because of its fast growth, stress tolerance, and reproduction both in freshwater and saline water condition (Chervinski, 1982; Abdel-Fattah and El-Sayed, 2006; Gibtan *et al*. 2008).

Currently, Egypt is one of the countries where aquaculture is growing fastest with Nile tilapia (*Oreochromis niloticus*) as the most widely farmed species. Unfortunately, intensive aqua-farming is accompanied with several problems where infectious diseases and oxygen deficiency come in limiting the production with consequent negative impact on growth, fecundity and productivity.

Due to the European Union has been decided prevent the use of antibiotic as feed additive in 2006, scientists have turned to research on natural medicines. According to many researchers, it has been reported that medicinal plant having many properties such as growth promotion, appetite stimulation, antimicrobial, immune-stimulant, anti-inflammatory, anti-stress, and anticancer (Bulfon *et al*., 2013). Using natural feed additive is becoming useful for fish feeding rather than classic chemical feed additives due to the cumulative effects of the chemical components induced deterrent effects on human health (El-Dakar *et al*., 2008).

From these, *N. sativa,* which belongs to the Ranunculacea family, is a medicinal herb having many therapeutic characteristics (Ziaee *et al*., 2012). *N. sativa* has been used for treatment for more than 2000 years, which is commonly known as black cumin seed. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern, Mediterranean region, South Europe, India, Pakistan, Turkey, and Saudi Arabia (Ahmad *et al.*, 2013). *N. sativa* seeds and oils have been commonly used as treatment for a variety of health conditions pertaining to the respiratory system, digestive tract, kidney and liver functions, cardiovascular system, and immune system support, as well as for general well-being (Ahmad *et al.*, 2013).

Moreover, black cumin (*N. sativa*) have been used as enhancer for performance, growth and immune system of some fish species (Abdel-Ghaffar *et al.,* 2003; Diab *et al.,* 2008) where the nutritional content is as follows: 20.8% raw protein, 3.7% raw cinder, 7.0% moisture, 34.8% fat and 33.7% carbohydrates (Atta, 2003). The main objective of this experiment, therefore, is to evaluate the effect of *N. sativa* on growth performance, some blood variables, in addition to immunity under oxygen deficiency in Nile tilapia (*O. niloticus*).

MATERIALS AND METHODS

One hundred and thirty five fish *(Oreochromis niloticus)* were collected from the experimental fish farm belonging to the Poultry Production Department, Faculty of Agriculture, Assiut University. All the experimental fish appeared to be clinically normal and in a good health at the experiment start. Average body weight and body length were 101 ± 1g and 18.11 ± 0.41cm, respectively. The fish were reared

in floating cage and adapted for two weeks in a water pond.

At the start of the experiment the fish were weighed and the total length was measured. Three experimental groups (45 fish each, 15/ subgroup) where distributed randomly as follow:

1 -The first group was considered as a control which fed on a basal diet (Table 1).

2 - The second group was fed on the basal diet supplemented with 1% *Nigella Sativa*.

3 - The third group was fed on the basal diet supplemented with 2% *Nigella Sativa*.

The dry ingredients of the experimental diets were thoroughly grinded, mixed and pelletized. The experimental fishes were offered the dietary treatments twice daily at 9.00 AM and 3.00 PM at a rate of 3% of their live body weight. The feed quantity was readjusted biweekly on the basis of the actual average biomass of the fish in each treatment.

1- Growth variables

The individual body weight (g) and total body length (cm) for all fishes per treatment were recorded biweekly. The average body weight gain (ABWG) was estimated according to the following equation:

Weight gain = Average final weight (g) – Average initial weight (g)

The body length increment (cm) was estimated according to the following equation:

Length increment (cm) = Average final length (cm) - Average initial length (cm)

Table (1): Composition and Chemical analysis on dry matter basis) of the experimental basal diet

Ingredient	Weight (Kg)
Yellow corn grain	52
Soya bean meal	22
Fish meal (72% protein)	25
Vitamin Mix.[1]	0.5
Mineral Mix.[2]	0.5
Total ingredients	100
Calculated analysis	
Crude protein (%)	30.44
Gross energy (Kcal/Kg)	4038.50

[1]Vitamin Mix contained mg per kg diet: alpha tocopherol, 20; Menadione, 5; thiamine, 5; riboflavin, 5; calcium pantothenate, 10; piridoxine, 100; folic acid, 2; cyanocobalamin, 0.5; biotin, 0.5; ascorbic acid, 200; p-aminobenzoic acid, 50; inositol, 500; choline chloride, 500; (UI/kg diet): retinol 10000; cholecalciferol, 2000.
[2]Vitamin Mix contained mg per kg**:** cobalt sulphate, 0.4; copper sulphate, 5.0; iron sulphate, 40; sodium fluoride, 1.0; potassium iodide, 0.6; magnesium oxide, 100; manganesium oxide, 10; zinc oxide, 12.5.

2- Blood sampling

At the end of the experimental period, 10 fish were randomly taken from each experimental group. Individual blood samples were collected by severing the caudal peduncle. Aqduate amounts of whole blood were collected in two small plastic vials; one containing heparin and one without anticoagulant. The first sample was used for determination of hemoglobin (Hb) by using suitable Kits and hematocrit (PCV) according to Stoskopf (1993).

The other sample was centrifuged at 3000 (rpm) for 15 minutes to obtain serum. The serum was stored at -20 C^o until analysis. Serum total protein (g/dl), albumin (g/dl), alkaline phosphatase (u/l), and glucose

(mg/ml) were determined calorimetrically using Kits purchased from the Egyptian company for biotechnology (S.A.E) Pharmaceutical Chemicals (Egypt). Globulin was calculated by difference between serum total protein and albumin. After blood sample collection, all the fish samples (10/group) were scarified and the abdominal cavity was opened to remove spleen, gonads and liver to be weighed. The gonad, liver and spleen indices were calculated as follow:

$$\text{Spleenosomatic index (SSI)} = \frac{\textit{Spleen weight (g)}}{\textit{Body weight (g)}} X100$$

$$\text{Gonadosomatic index (GSI)} = \frac{\textit{Gonad weight (g)}}{\textit{Body weight (g)}} X100$$

$$\text{Hepatosomatic index (HSI)} = \frac{\textit{Liver weight (g)}}{\textit{Body weight (g)}} X100$$

3- Stress experiment (Oxygen deficiency):

This experiment aimed to study the effect of *Nigella Sativa* on Nile tilapia (*Oreochromis niloticus*) under oxygen deficiency. Ten / treatment fish were transported to the aquaculture laboratory at the same farm; each group was distributed randomly into two sub-groups each was placed in a metallic aquarium (1.5 m X 0.6 m X 0.5 m diameters and 180 L water capacity). The air supplementation was stopped and there was no water change for all the fish groups. The fish were fed with the same diet for each group at a rate 3 % of live body weight daily. The temperature and pH of the water were measured three times daily. This experiment lasted for 4 days (96 hours). During this experimental period the fish behavior was recorded. At the end of the treatment, the fish from each aquarium were weighed and measured for the total length. The gonads, liver and spleen indices were calculated as above mentioned. Blood samples were collected from each fish by the same above mentioned method. Moreover, the hemoglobin and hematocrit levels were estimated according to the same method above mentioned. Serum samples were obtained as above mentioned and stored under -20 Cº. The serum was stored for determination of the total protein, albumin, alkaline phosphatase, and glucose by the same above mentioned methods. Body indices (HSI, SSI and GSI) were calculated as above shown.

4. Statistical analysis

The obtained data were subjected to statistical analysis using one-way ANOVA according to the following model:

$$Y_{ij} = \mu + T_i + E_{ik}$$

Where: Y_{ij} = an observation, μ = overall mean, T_i = effect of treatment, and E_{ik} = random error.

Differences among means of the experimental groups were tested for significance by Duncan's multiple range tests (Duncan, 1955). Differences were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

1- Growth performance

Body weight (BW) and body weight gain (BWG)

The presented data in Table (2) showed that the average body weight at the start of the experiment for all groups [control (C), 1% N. sativa (T1), 2% *N. sativa* (T2), was 101.36, having not significant differences in between.

At the second week of the experiment there was insignificant differences between the treatments and control ones; it increased for T_1 by 0.58 %. Also, the results in Table (3) showed that the averages of body weight gain were 14.80 and 12.91 for T1 and T2 versus 13.53 for the control group at the second week. Also, the results showed that treated groups had insignificant ($P > 0.05$) higher ABWG compared to the control group.

Table (2): Average body weight ± SE of *Oreochromis niloticus* treated with different levels of Nigella sativa.

Treatments	Experiment weeks						
	0	2	**4**	**6**	**8**	**10**	**12**
Control	101.67 ±2.55	115.20 ±3.12	126.07 ±3.52	145.33 ±4.29	152.53 ±4.08	176.61 ±5.04	182.11 ±5.25
T1 (1% NS)	101.07 ±2.64	115.87 ±2.70	126.13 ±2.94	145.96 ±3.44	144.6 ±3.76	172.15 ±4.14	183.9 ±4.64
T2 (2% NS)	102.16 ±2.34	115.07 ±2.68	130.09 ±3.28	145.18 ±3.43	155.79 ±3.27	180.12 ±4.92	196.44 ±5.55

At the fourth week, the average body weight increased insignificantly (P > 0.05) for T1 and T2, as compared with control ones. At the sixth week of the experiment, the average body weight for the same groups (C, T1 and T2) were 145.33and 145.96**,** and 145.18**,** respectively (Table 2).

Also, the fish groups fed 2% *N. sativa* had ABWG insignificant (*P > 0.05*) differences as compared with the control group (Table 3).

Table (3): Average body weight gain of *Oreochromis niloticus* treated with different levels of *Nigella sativa*

Treatments		Experimental weeks						Overall Mean
		2	**4**	**6**	**8**	**10**	**12**	
Control		13.53	10.87	19.26	6.2	24.08	5.5[a]	13.24
Nigella sativa	T1 (1%)	14.8	10.26	19.83	-1.36	27.55	11.75[b]	13.81
	T2 (2%)	12.91	15.02	15.09	10.61	24.33	16.32[b]	15.71

Means with different superscripts in the same row are significantly different (P ≤ 0.05).

On the sixth week, the supplementation of 1% *N. sativa* maximized the average body weight gain. At the eighth week the average body weight increased insignificantly (P > 0.05) for T2 only where it increased by 2.14 % as compared with control ones (Table 2). Moreover, the body weight gain of the treated fish at the eighth week for the fish groups treated with 2% *N. sativa* increased by 71.13% compared with the control group.

At the tenth week and the twelfth week, the average body weight there for the groups (T1 and T2) and analysis of variance for body weight at these periods showed that there were insignificant (*P > 0.05*) differences compared to the controls. At tenth week the body weight gain for the same groups were 24.08, 27.55 and 24.33g (Table 3) for C, T1 and T2, respectively. While, at the twelfth week, the body weight gain for the same groups treated with 1% and 2% *N. sativa* had a significant (P ≤ 0.05) increase compared with control ones (Table 3).

Moreover, at the end of the experimental period the overall mean averages of body weight for the groups were 142.79, 141.38 and 146.41, and AVBWG were 13.24, 13.81 and 15.71 for control, T1 and T2, respectively (Table 2, 3).

However, the obtained results showed that the level of *N. sativa* (1% and 2%) had insignificant (*P > 0.05*) effect on body weight of Nile tilapia (*Oreochromis*

niloticus) all over the experimental period. These results are supported by Dey *et al*. (2020), who concluded that dietary 2% *N. sativa* did not significantly change the body weight and body weight gain in *O. niloticus* when compared to the control. Also, Bektaş *et al*. (2018) showed that there were no significant differences among the fish group fed on 2.5 g/k *N. sativa* and control. In the same trend, Al-Dubake *et al.* (2012) declared that the diet containing 1% black seed improved insignificantly ($P > 0.05$) growth rate compared with control and diet containing 3% *N. sativa* in *cyprinus carpio*. Some researchers attributed the relative increase in BW and BWG of Nile tilapia fed on *Nigella sativa* seeds to its digestive stimulating effect through their aromatic substances or essential oils (Abou-Zeid, 1998)**.**

Bilen *et al.* (2011) didn't acquire any significant increase in growth rate and average weight due to N. sativa supplementation. In another study, John *et al*. (2007) used 3% *N. sativa* on the growth of *Oreochromis niloticus*, and the observed mean final weight in the treatment was higher than the control**.** In Red tilapia, Abd Elmonem *et al*. (2002) found that dietary addition of black seed *(Nigella sativa)* at levels 0, 3, 6 and 9 % displayed positive effects on growth performance.

2- Body length (BL):

The differences in body length among the experimental groups were insignificant (*P > 0.05*) (Table 4), which indicate that experimental groups were homogenous at the beginning of the experiment.

Table (4): Average body total length ± SE (cm) of *Oreochromis niloticus* treated with different levels of *Nigella sativa* for 12 weeks

Treatments		Experiment period (weeks)						
		0	**2**	**4**	**6**	**8**	**10**	**12**
Control		17.97± 0.16	18.90 ±0.21	19.96 ±0.16	20.37 ±0.2	20.78 ±0.20	21.39 ±0.20	21.98 ±0.21
Nigella sativa	**T1 (1%)**	18.13 ±0.16^{a}	18.82 ±0.14^{a}	19.68 ±0.13^{a}	20.44 ±0.15^{a}	20. 49 ±0.17^{b}	21.19 ±0.17^{a}	21.92 ±0.18^{a}
	T2 (2%)	18.28 ±0.15	19.04 ±0.15	20.01± 0.18	20.34 ±0.17	21.9 ±0.14	21.68 ±0.18	22.33 ±0.19

Means with different superscripts in the same row are significantly different ($P \leq 0.05$).

Table (5): Average length increment ± SE (cm) of *Oreochromis niloticus* treated with different levels of *Nigella sativa* for 12 weeks

Treatments		Period (weeks)						
		2	**4**	**6**	**8**	**10**	**12**	**Overall Mean**
Control		1.04	0.95	0.41	0.65	0.63	0.58	0.87
Nigella sativa	**T1 (1%)**	0.69	0.85	0.77	0.01	0.74	0.37	0.57
	T2 (2%)	0.77	1.01	0.28	0.59	1.28	0.65	0.76

After two weeks the analysis of variance for the body length and length increment showed that there were no significant ($P > 0.05$) differences between the treatment groups and control ones. On the fourth and six week of the experiment the average

body length for T2 only increased by 0.25% versus the control ones (Table 4). While, there were insignificant (*P > 0.05*) differences in body length and length increment compared with control ones. On the sixth, eighth**,** tenth and twelfth week no significant (*P > 0.05*) differences in body length and length increment for all groups comparing with the control ones. These results are in coincided with Bektaş *et al*. (2018) who showed that there was insignificant (P > 0.05) differences in length between groups fed dietary *N. sativa*.

3. Hepato, gonado and spleeno – somatic indices:

Data presented in Table (6) showed that the, dietary 1% *N. sativa* decreased hepatosoatic index (HSI) insignificantly (*P > 0.05*), while, 2% N. sativa increased it insignificantly (*P > 0.05*). Also, the dietary 1 and 2 % *N. sativa* has effects on spleenosomatic index (SSI) compared to groups control ones. Also, the gonadosomatic index for male (MGSI) decreased insignificantly (*P > 0.05*) in T1 and T2 as compared with control group.

Table (6): Average hepato, gonado and spleeno – somatic indices ±SE. of *O. niloticus* fed dietary *Nigella sativa* for 12 weeks.

Treatment	Control	Nigella sativa	
	C	T1 (1%)	T2 (2%)
HSI	1.41±0.15	1.33 ±0.09	1.83±0.08
SSI	0.10±0.01	0.11±0.02	0.11±0.01
Male GSI	0.46±0.12	0.44±0.12	0.35±0.08

4- Blood parameters:

A- Hematological parameters:

Hemoglobin level was insignificantly increased (*P > 0.05*) for T1 and T2 as compared with the control group. The hematocrit (PCV %) increased insignificantly (*P > 0.05*) in fish group fed 2% *N. sativa* (T2) as compared with the control group.

These results are in harmony with Bektaş *et al.* (2018) who recorded that higher HCT levels were found at doses of 0.5, 2.5 and 10.0 g/kg *N. sativa*. Also, John *et al.* (2007) recorded significant hematocrit values in *O. niloticus* fed with 3*% N. sativa*.

Means with different superscripts in the same row are significantly different (P ≤ 0.05).

B- Serum constituents:

The achieved results illustrated in Table (7) showed that dietary supplementation with *N. sativa* displayed higher serum total protein (*P > 0.05*) in T2 than T1. However, such increase in total protein was mainly due to the increase in the globulin concentration rather than albumin. This increase in serum total protein supported the higher growth of T2 fishes (Table 7).

These results were supported by Yang and Chen (2003) who mentioned that total serum protein is one of the most important indicators of general health of fish. Also, Ahmed and Ali (2013) reported that the total protein concentration in fish serum differ, depending on a series of factors such as food diet, species, season, degree of sexual maturity and water temperature. Awad *et al.* (2013), reported an enhancement in total protein and lysozyme activity in groups fed with *N. sativa* oil and Quercetin, especially with higher doses that recorded the highest significant value compared to the controls.

Table (7): The average blood components ± S.E (hematological parameters) and serum variables of *O. niloticus* fed dietary Nigella sativa for 12 weeks

	Treatments		
Variables	**Control**	**T1. 1% *N. sativa***	**T1. 2% *N. sativa***
Blood components			
a) Hematology			
1) Hemoglobin (g/dl)	7.63±0.43	8.81±0.37	8.88±0.47
2) Hematocrit (%)	28.75±1.04[a]	24.40±1.23[b]	29.50±1.44[a]
b) Serum constituents			
1- Total protein (g/dl)	6.03±0.51	5.77±0.42	5.68±0.34
2- Albumin (g/dl)	4.02±0.47	4.42±0.27	4.61±0.34
3- Globulin (g/dl)	2.01±0.83	1.35±0.40	1.07±0.32
4- Alkaline phosphatase (U/I)	89.63±8.34[c]	103.90±2.44 [b]	133.50±3.98 [a]
7- Glucose (mg %)	113.40±55.97[b]	162.52±31.27[a]	159.94±26.79[a]

The results in Table (7) showed that the supplementation with 1% and 2% *N. sativa* had significantly (*P < 0.05*) higher alkaline phosphatase activity as compared with control the fish group. It is clear that the alkaline phosphatase increased in the case of high level of *N. sativa* (2%) where it increased by 48.95% as compared with the control ones.

The present results agree with the findings of Wache *et al.* (2006) who found that feeding trout on *S. cerevisiae* led to significant (*P < 0.05*) higher activity of alkaline phosphatase. They attributed this increase to the earlier maturation of digestive system in fish. In addition, Tovar-Ramíreza *et al.* (2004) reported that the probiotics stimulated amylase secretion and the activity of alkaline phosphatase in larval sea bass. The dietary *N. sativa* resulted in a significant (*P < 0.05*) decrease in the serum glucose concentrations by 44.13 and 52.14% for 1% and 2% *N. sativa* as compared with the control ones. These findings are coincided with findings of Al-Dubakel *et al.* (2012) who showed that blood glucose of common carp fed different experimental diets (0, 1, and 3% *N. sativa*) were significantly different (P < 0.05) between control and diet; the 3% *N. sativa* treatment sowed higher blood glucose versus those of the control and 1% treatment (120.88 mm/dl versus 67.66 and 75.33 mm/dl). However, most authors concluded that fish behave like diabetic mammals with respect to blood glucose (Hepher, 1988).

5. The effect of dietary *N. sativa* on Nile tilapia on behavioral changes under oxygen deficiency

At the beginning of the experiment, the control fish exhibited remarkable decreased activity. Also, increased random movement elicited by hypoxia changes in direction of swimming. The fish groups fed diets with *N. sativa* showed slightly abnormal behavior changes due to reduction of dissolved oxygen. These signs were reported by Doudoroff and Shumway (1970). Moreover, the control fish group exhibited remarkable signs of distress, including fast swimming, erratic movement, great surfacing frequency to gulp atmospheric air, loss of body equilibrium and decreased attention to the

feeding. At the end, the fish died with opened mouth.

In contrast, fish groups maintained at the same condition of oxygen deficiency and supplemented with dietary *N. sativa* showed less above-mentioned abnormal behavior than the control group. Such response to oxygen deficiency may be due to increase of respiration rate and heart rate (Dheer, 1988; Mason, 1991). Also, reduction of water dissolved oxygen content which may be attributed to the inhibiting effect of photosystems I and Π and community respiration (Ojala, 1966).

The improvement of dietary *N. sativa* on Nile tilapia (*Oreochromis niloticus*) behavior under oxygen deficiency may supported by Chanock *et al.* (1994) who reported that phagocyte activation is usually associated with abrupt rise in oxygen consumption, leading to production of reactive oxygen species (ROS) which play an important role in the host defense. This metabolic event is called the respiratory burst and the main enzyme involved in this process is an $NADP^+$ oxidase. The respiratory burst activity of fish phagocytes can be modulated *in vivo* and *in vitro* by many substances (Tahir and Secombes, 1996), glucans (Couso *et al.,* 2001), levamisole (Mulero *et al.*, 1998) and animal extracts (Bøgwald *et al.*, 1996). Li and Gatlin (2003) found that feeding juvenile hybrid striped bass a diet supplemented with growth promotion for 16 weeks increased blood neutrophil oxidative radical production and extracellular superoxide anion production of head kidney leucocytes.

6. Effects on mortality rate under oxygen defficiency:

The mortality rate of *Oreochromis niloticus* exposed to oxygen deficiency was 100, 40 and 20 for control, T1 and T2, respectively. It is clear that dietary (1% and 2%) N. sativa had significant effect on survival rate of *O. niloticus* under oxygen deficiency where the mortality rate was 40 and 20, for T1 and T2, as compared with 100% for control group. For these, Khondoker *et al.* (2016) used *Nigella sativa* at 2%, 4% and 6% doses and investigated immunological parameters such as bactericidal activity and phagocytic activity. For the role of *N. sativa* on mortality rate of *O. niloticus* under oxygen deficiency; it was obvious that high level (2%) of *N. sativa* has significant ($P < 0.05$) effect on mortality rate where it reduced the mortality rate, from 100% in the control to 20% in 2% *N. sativa* (T2). While, the treatment received low level (1%) of *N. sativa* (T1) showed high mortality rate (40%).

Moreover, the positive role of *N. sativa* in reduction of mortality rate in the fish is supported by some studies, as that of Dorucu *et al.* (2009) who used 1%, 2.5% and 5% of *N. sativa* on the immune response of *O. mykiss* and they found higher serum protein levels ($P < 0.05$) higher than the controls. Survival rate was improved and all treatments showed no mortality all over the experimental period (Al-Dubakel, 2012). Moreover, black seed (*Nigella sativa*) enhanced T cell immunity and production of cytokines (Haq *et al.*, 1995), and natural killer cell and compliment (Mahdi, 1993). Also, *Nigella sativa* extract has positive effect on leukocytes (Mona *et al*., 2002). In the same trend, Diab *et al*. (2008) argued that it could increase the survival rate and the resistance of fish to some infectious diseases and may enhance the growth performance especially after prolonged application.

Similarly, Bektaş *et al.* (2018) reported that the application of medicinal plants and their derivates as immunostimulants in fish culture is an effective and safe method to enhance the immune responses against pathogens during periods of stress, such as intensive farming culture, grading, vaccination and reproduction.

7- Blood components:

A-Hematological parameters:

Hemoglobin (Table 8) levels did not significantly (*P > 0.05*) alter by oxygen deficiency for all the fish groups. While, the hematocrit (PCV%) levels increased significantly (P < 0.05) in T2 as compared with T1.The improvement in hemoglobin and hematocrit levels due to dietary probiotics may be due to their improvement of the spleen status as SSI (Table 9) which is the main organ for the processing, storing and maturing of the erythrocytes.

Similar results were found by Hussein and Kobeisy (1999) on the same fish (*O. niloticus*) who reported that oxygen deficiency led to increase of hemoglobin and hematocrit. Tun and Houston (1986) attributed these results to the stimulating effect of oxygen deficiency on red blood cells liberation from spleen and subsequently erythropoiesis.., Abdelhamid (2006) reported that hypoxia was responsible for increased PCV % and decreased Hb in *O. niloticus*.

Table (8): Average blood components (hematological parameters and serum constituents) of Nile tilapia (*Oreochromis niloticus*) under stress factors (Oxygen deficiency) ± S.E

	Treatments		
Variables	**Control**	**T1. 1% N. sativa**	**T1. 2% N. sativa**
Blood components			
a) Hematology			
1) Hemoglobin (g/dl)	N.D.	8.80±35	8.86±46
2) Hematocrit %	N.D.	23.40 ± 1.23^{b}	30.51 ± 1.44^{a}
b) Serum constituents			
1- Total protein (g/dl)	N.D.	3.80 ± 36^{b}	6.40 ± 82^{a}
2- Albumin (g/dl)	N.D.	9.93 ± 48^{a}	4.58 ± 33^{b}
3- Globulin (g/dl)	N.D.	1.33 ± 0.40^{b}	3.04 ± 0.35^{a}
4- Alkaline phosphatase (U/I)	N.D.	103.90 ± 2.42^{b}	132.48 ± 3.96^{a}
7- Glucose (mg %)	N.D.	158.91±23.79	160.50±30.26

N.D : not determined according to dying of all fish in control group after one day.
Means with different superscripts in the same row are significantly different (P ≤ 0.05).

B- Serum constituents

Serum total protein concentration tended to be significant (*P < 0.05*) higher in fish groups fed dietary 2% *N. sativa*. Such increase in total protein levels mainly due to the increase of serum globulin rather than albumin concentration (Table 8). In fact, the improvement of globulin level in fish groups fed dietary *N. sativa* may attributed to immune system response under oxygen deficiency. These results are supported by Hussein and Kobeisy (1999) who found similar results in *O. niloticus*. Also, Shulʹman (1974) stated the same results.

Serum alkaline phosphatease activity was significantly increased (*P < 0.05*) for the fish groups T2 as compared with the T1. The increase in alkaline phosphatase of *O. niloticus* fed dietary *N. sativa* may be throughout their effects on immune response and counteracting the adverse effect of oxygen deficiency. However, these results are confirmed by some researchers such as Wilson (1973) and Lovell and Lim (1978), who reported that the increase of serum alkaline phosphatase activity coincided with the elevation of some anti-stress supplementation.

Serum glucose concentration did insignificantly (*P > 0.05*) affected due to oxygen deficiency. This result may be attributed to the physiological role of *N. sativa* on decreasing glucocorticoids synthesis. These results are in harmony with those found by Thaxton and Pardue (1984) and consequently gluconeogenesis. Moreover, Abdelhamid (2006) recorded highly significant increase in plasma glucose and significant decrease in plasma total protein in tilapia suffered from hypoxia for 3 days.

C- Hepato, gonado and spleeno-somatic indices

Data presented in Table (9) showed that there were insignificant (*P > 0.05*) differences in hepatosomatic (HSI) index of *O. niloticus* under oxygen deficiency for T1 and T2. These results are coincided with the findings of Hussein and Kobeisy (1999), who reported that HSI of the same fish (*O. niloticus*) was not affected by oxygen deficiency. Also, the present results are in harmony with the findings of Hussein and Kobeisy (1999), which indicated that *O. niloticus* exposed to oxygen deficiency had higher SSI than the controls. They attributed this increase in SSI to the reduction in the concentration of plasma corticosterone. Moreover, Halver (1985) reported that the high level of corticosterone led to a prominent decrease in spleen size. In addition, this improvement in SSI is attributable to acceleration of iron absorption in the spleen. However, it could refer the improvement in SSI of *O. niloticus* fed 1% *N. sativa* to their content of some enzymes and protein which may improve the immune response.

There were significant differences (*P < 0.05*) in gonadosomatic index among the fish fed groups 1% and 2% *N. sativa*. The significant (*P < 0.05*) increase in GSI may be due to the improvement effect on fish appetite and increase the energy intake which is required for reproduction (Spieler *et al.*, 1977). Moreover, the dietary supplementation counteracted insignificantly the adverse effects of oxygen deficiency on GSI may be through its stimulative effect in biosynthesis of sex steroids (Levine and Morita, 1985).

To conclude the results, the *dietary supplementation with N. sativa* at 1 and 2% displayed slight beneficial effects on growth performance and major beneficial effects on some blood constituents of *O. niolticus*. In point of view, additionally, *N. sativa* has high potent on immunity of *O. niloticus*.

Table (9): Average hepato, spleeno and gonado – somatic indices of Nile tilapia (*Oreochromis niloticus*) under stress factors (Oxygen deficiency) ±S.E. for different treatment at experimental period

Variables	Treatments		
	Control	**(1% *N. sativa*) T1**	**(2% *N. sativa*) T2**
HSI	N.D.	1.39±0.11	0.41±0.06
SSI	N.D.	0.17±0.08	0.08±0.00
GSI	N.D.	0.56 ± 0.12^{b}	1.49 ± 0.21^{a}

N.D.: Not determine

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تأثير بذور حبة البركة على أداء النمو وبعض مكونات الدم للبطى النيلى

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تم استخدام عدد ١٣٥ سمكة من أسماك البلطى النيلى لدراسة تأثير بذور حبة البركة على أداء النمو وبعض مكونات الدم. وقد تم تقسيم الأسماك عشوائيا إلى ثلاث مجموعات بكل معاملة ٤٥ سمكة وتم تقسيم كل مجموعة رئيسية الى ثلاثة مكررات بكل منها ١٥ سمكة. تم تغذية هذه المجاميع على عليقة أساسية مضافا إليها ١% حبة بركة والمجموعة الثانية على على العليقة الاساسية مضافا اليها ٢% حبة بركة، أما مجموعة الكونترول فقد تم تغذيتها على العليقة الاساسية بدون حبة البركة. وقد تم تسجيل الوزن والطول كل أسبوعين طوال فترة التجربة وهى ١٢ اسبوع. وقد تم جمع عينات الدم من كل سمكة لتقدير نسبة الهيموجلوبين والهيماتوكريت ومحتوى سيرم الدم من كل من الجلوكوز والبروتين الكلى والألبيومين والجلوبيولين وإنزيم الفوسفات القاعدى. وبعد ذلك تم تشريح عدد ١٠ سمكات لتقدير معامل الكبد ومعامل الطحال ومعامل الغدة الجنسية. بالإضافة إلى ذلك فقد تم نقل عشرة سمكات أخرى الى المعمل الرطب وتم تربيتها بدون الإمداد بالأكسجين لتقدير مقاومة الأسماك لنقص الأكسجين.

وقد اوضحت النتائج المتحصل عليها بأنه لايوجد زيادة معنوية فى وزن الجسم، معدل زيادة وزن الجسم والطول الكلى والزيادة فى الطول، بينما ازداد الهيموجلوبين معنويا ولكن لم يحدث تغير فى الهيماتوكريت. وفى نفس السياق، فقد حدث تحسن بزيادة غير معنوية فى كل من بروتين السيرم والالبيومين والجلوبيولين بينما انزيم الفوسفات القاعدى فقد زاد زيادة معنوية فى الأسماك التى تغذت على حبة البركة بينما انخفض معنويا جلوكوز السيرم فى الأسماك المعاملة مقارنة بمجموعة الكونترول. إنخفض معامل الكبد انخفاضا غير معنويا، بينما فى المجموعة المعاملة بمستوى ٢% حبة بركة ازداد زيادة غير معنوية وفى نفس الاتجاه فان المستوى ١ & ٢% حبة بركة كانتا لهما تأثير على معامل البنكرياس .

اما مجموعة الأسماك التى تم تربيتها تحت نقص الاكسجين فان مجموعة الكونترول اظهرت علامات السباحة الغير عادية ولكن المجموعات التى تم تغذيتها على حبة البركة اظهرت تغيرات غير طبيعية ضعيفة أما نسبة النفوق فقد كانت ١٠٠ ، ٤٠ ، ٢٠ % لمجوعة الكونترول والمعاملة الاولى والمعاملة الثانية بينما لم يحدث تغير فى مستوى الهيموجلوبين بينما زاد الهيماتوكريت زيادة معنوية وكذلك كانت هناك زيادة معنوية فى تركيز البروتين الكلى وقد ازداد انزيم الفوسفات القاعدى زيادة معنوية بينما لم يتأثر مستوى الجلوكوز وكذلك لم يوجد اية فروق معنوية فى معامل الكبد ومعامل الطحال ومعامل الغدة الجنسية بين الاسماك المعاملة بحبة البركة ومجموعة الكونترول. يتضح من نتائج هذه الدراسة أن إضافة بذور حبة البركه الى علائق (بمعدل ١% ، ٢%) أسماك البلطى النيلى لمدة ١٢ أسبوعا أظهرت تأثيرات مفيده غير معنويه على صفات النمو، وأدت إلى تحسن بعض صفات الدم بصورة معنويه.