Effect of Black Cumin Seeds (*Nigella Sativa*) and / or Turmeric *(Curcumin*) On Hematological, Biochemical and Immunological Parameters of *Sea Bass* Vaccinated with Pseudomonas Fluorescence Bacterin

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Abstract: The influence of dietary supplementation of Black cumin seed (***Nigella sativa***) and/or Turmeric (***curcumin***) for 6 weeks on hematological, biochemical and immunological parameters of ***Sea-bass*** vaccinated with ***Pseudomonas florescence*** bacterin was investigated. The present findings indicated that, ration containing black cumin seed and/or turmeric showed a significant increase in the level of all tested hematological and immunological parameters during the most of experiment when compared with the control. Spectrophotometric analysis revealed a significant increase in serum total protein, albumin and globulins in fish fed ration containing black cumin seed and/or turmeric. However, this increase was more pronounced in fish supplemented with a combined mixture of both plants. Electrophoresis of tested fish serum revealed a significant increase in ß-globulin protein fraction especially in fish kept on combined mixture of the plants. In addition, the hypocholestermic and hypolipidemic effects were observed only in fish kept on combined mixture of the plants. The present study concluded that, the combined mixture of both plants (0.5% of diet for each plant) was beneficial than individual administration.
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1. Introduction

Continuous increasing of human population in Egypt is the main cause for increasing the wide scale aquaculture to provide an additional fish source beside the natural one (Nile and seas). Since fish are cold blooded, they do not have to consume energy for maintaining body temperature and they are more efficient at converting food to meat (Lagler et al 1962). Bacterial diseases are the main causes of mortality in both wild and cultured fish. *Pseudomonas florescence* caused serious economic losses among many fish species (Evelyn 1984; Roger & Graham 1986; Soliman 1988). The profuse administration of either antibiotics or vaccines are the most existing solutions to overcome such problem. However, the known adverse effects and the resulted bacterial resistance of the former and the time consuming and high costs of the later are the limiting factors for their uses. In addition, there are increasing demands for using plants in therapy "back to nature" instead of using synthetic drugs of serious side effects (Fluk et al 1976).

Black cumin seed *(Nigella sativa L.)* is a herbaceous plant which is a member of the Ranunculacea family. These seeds or their oil are used by many people for different medicinal purposes. Nowadays, many reports have been written indicating the significant role of black cumin seed in increasing immunity and maintaining good health (El-Kadi et al 1987; Basil & Erwa 1993; Hedaya 1995; Mandour et al 1995; Abd El-Fattah 1996; Abdel-Ghaffar et al 2003; Abeer 2005; Taha et al 2007). However, low dose of Black cumin seed was beneficial than the higher toxic one (Mandour et al 1998; Abeer 2005; El-Bahr 2007).

Turmeric (Curcuma longa) is a perennial herb that grows to a height of three to five feet and is cultivated extensively in Asia (India and China) and other countries with a tropical climate. *Curcumin*, the active ingredient from the spice turmeric is a potent antioxidant and anti-inflammatory agent with hepatoprotective, anticarcinogenic and antimicrobial properties (Pal et al 2001). Literature concerning the effect of Black cumin seeds or turmeric in fish is deficient. Therefore, the present study was aimed to examine the effect of Black seed and Turmeric either alone or in combination on the immune response of *Sea bass*fish which is one of the most consumed and cultured native species. This could be useful to discovering an effective new approach faced the bacterial infection. Therefore, the effect of Black cumin seed and/or Turmeric on haematological, some biochemical and immunological parameters of *Sea*

*bass*fish vaccinated with *Pseudomonas florescence* bacterin were evaluated.

2. Materials and Methods

Plants :

Black cumin seeds *(Nigella sativa)* and Turmeric roots (Curcumin) were bought on a local market in Egypt and identified by botanists in the faculty of Agriculture, Alexandria university, Egypt. The whole seeds and roots were crushed in a blender and mixed well with the basal diet before their administration. Vaccines and Virulent strains

Pseudomonas florescence bacterin was prepared and evaluated according to Badran (1990). Stained *Pseudomonas florescence* bacterin was prepared according to Collins et al (1976) to increase the visibility of the serological reaction.

Fish, Aquaria and experimental design:

A total number of 150 apparently healthy fish with an average body weight of 60 ± 5 gm were obtained from fish farm at Egypt and used in this study. Fish were fed on commercial fish food containing 25% crude protein. The diet was daily provided at 3% of body weight as described by Eurell et al (1979). No drugs or vaccines were given to the fish along the course of the experiment except those under investigation. Glass aquaria measuring 90 × 60 × 50 cm were used for grouping and holding the fish during the experiment. The aquarium supplied with chlorine free tape water with continuous aeration. Throughout the present study the experimental design have included 3 separate subgroups of 10 fish per each treated main group. Each subgroup of fish only represents one observation. Fish were divided into 5 groups (30 fish for each). The first and second groups were kept on basal diet and served as first and second control. Fish of the third group were fed basal diet mixed with 10 grams of powdered Black cumin seed per kilogram of diet (1%). Fish of the fourth group were fed basal diet mixed with 10 grams of powdered Turmeric per kilogram of diet (1%). A total of 5 grams of each powdered Black cumin seed and Turmeric were well mixed together and the whole 10 grams of the mixture was mixed with every kilograms of basal diet (constitute 0.5% of each plant) and provided to the fish of the fifth group. After 2 weeks, Fish of the first group were injected intraperotenially (I/P) with sterile saline 0.2ml/fish and named as non vaccinated control. However, fish of the second group (second control), third, fourth and fifth groups were similarly inoculated with 0.2 ml/fish of formalin inactivated bacterin from *Pseudomonas florescence* . The second group (second control) is now named as vaccinated control. The booster dose of bacterin was provided 2 weeks post injection (4 weeks from the start of the experiment).

After 6 weeks from the start of the experiment, a challenge test was done to determine the protective effect of the tested plants over the vaccine against virulent strain of *Pseudomonas florescence* . This was done through determination of relative level of protection (RLP) of vaccinated control and both tested plants. In this test, fish of all five groups were challenged by inoculation of 0.2ml of virulent strain of *Pseudomonas florescence* of the same strain used for bacterin preparation (2×10^7 bacterial cell/ml^3/100g fish) and fish mortalities were recorded during one week observation period.

Sampling and the analytical methods:

Blood samples were collected weekly from the caudal artery using disposable tuberculin syringe (Hawk et al 1965) for estimation of total erythrocytic count (TEC), total leucocytic count (TLC) and packed cell volume (PCV) according to Stoskopf (1993). Haemoglobin percentage (Hb %) was assessed according to Drubkin (1947), and differential leucocytic count was determined according to Lucky (1977) and Schalm (1986). Phagocytic activity and index were also determined according to Hawk et al (1965). Similarly, blood was collected without anticoagulant for serum separation as described by Leid et al (1975). The obtained sera were used for spectrophotometric determination of the activities of Aspartate transaminase (AST) and Alanine transaminase (ALT) as directed by Reitman & Frankle (1957). In addition, serum total protein, albumin and globulin values were determined spectrophotometrically as implied by the methods of Doumas et al (1981), Reinhold (1953) and Coles (1974), respectively. Furthermore, the obtained sera also were used for spectrophotometric analysis of serum triacylglycerol (TAG), total cholesterol and high density lipoprotein cholesterol (HDL-c) by using of enzymatic method of spin react kits according to the methods of Sidney & Bernard (1973), Zak et al (1954) and Lopesvirella et al (1977), respectively. Very low density lipoprotein cholesterol (VLDL-c) was calculated by division of TAG by 5 (mg/dl) while the low density lipoprotein cholesterol (LDL-c) was calculated (mg/dl) by subtracting the sum of HDL-c and VLDL-c from total cholesterol (Bauer 1982).

In addition, serum samples of each subgroup were pooled and subjected to sodium dodecylsulphate polyacrylamide gel electrophoresis 12% (SDS-PAGE 12%) for determination of different protein fractions. SDSPAGE was carried out according to the procedures of Laemmli (1970) using vertical electrophoresis apparatus (Bio-Rad, Protean® II xi cell). The serum samples (50µl) of different pooled

subgroup per treatment were run parallel with a prepared mixture of molecular weight marker proteins (PageRulerTM Protein Ladder, Fermantas). The proteins of the marker resolved into clearly identifiable bands ranged from 10 to 200 KDa. Individual lanes of the SDS-PAGE containing the protein bands were visualized by staining with coomassie brilliant blue R-250 (Sigma). Electrophoretic gels were analyzed using a laser Densitometer at 600nm. The laser tracings were divided into the following fractions: Albumin, alpha globulin, beta globulin and gamma globulin. The relative percentage of each protein fraction was calculated by the densitometer the area under the curve created by the protein band. The densitometer automatically calculated the absolute value for each fraction by multiplying the total protein of the sample by the corresponding fractional percentage (Gicking et al. 2004).

Moreover, the obtained sera were used also for detection of immune response to *Pseudomonas florescence* at last four weeks of the experiment (Badran 1990). Briefly, in a standard microtiter plate (U-shaped wells), serial two fold dilutions of serum were made in sterile saline solution using a 0.025 ml pipette dropper and 0.025 ml micro diluter. *Pseudomonas florescence* stained antigen (0.025ml) was added to the diluted serum. The suspensions were mixed and incubated overnight in the refrigerator. A positive serological reaction was indicated by bacterial agglutination. Agglutination titers were expressed as logs of the highest serum dilution still giving a clear agglutination. The negative controls consisted of: a) one drops of sterile physiological saline and one drop of tested serum. b) one drop of sterile physiological saline and one drop of stained antigen. The positive controls were carried out using collected positive antisera.

In the challenge test, fish mortalities were recorded and specificity of death was determined by re-isolation of injected bacteria from freshly dead fish during the period of observation (one week following the main experimental period). The potency of bacterin and tested plants were evaluated by calculating the RLP by subtracting the value resulted from the division of percent of mortality of vaccinated fish group on percent of mortality of non vaccinated control {RLP = 1 – (percent of mortality of vaccinated fish group/percent of mortality of non vaccinated control group); Newman & Majnarich 1982}.

Statistical analysis:

The obtained data of biochemical, hematological and immunological parameters were compared between groups within different periods by using student– *t*– test. All data are presented as mean ± standard error of mean (SEM) by using repeated ANOVA between subgroups and related group and between the main groups. The analysis have been done on samples from each subgroup of fish per treatment. Each subgroup of fish only represents one observation. All tests were performed using computer package of the statistical analysis system (SAS 1987).

3. Results

The obtained results demonstrated that, TLC, TEC, Hb and PCV were significantly increased ($P < 0.05$) in all treated fish all over the period of the experiment when compared with the control group (Table 1). This increment was more pronounced in fish supplemented with a combined mixture of both plants followed by that supplemented with black cumin seed alone and finally that kept on diet containing Turmeric alone.

The present study revealed also that, the percentage of lymphocytes and Eosinophils were significantly increased ($P < 0.05$) in fish fed Black cumin seed and/or Turmeric throughout the whole experimental period when all compared with the control (Table 2). The percentage of monocytes was significantly increased ($P < 0.05$) than the control group when fish fed Black seed and/or Turmeric only after three and six weeks from the start of the experiment (Table 2). The percentage of basophils was significantly increased ($P < 0.05$) than the control group in fish fed Black seed and/or Turmeric only at last four weeks from the start of the experiment (Table 2). The percentage of Neutrophils was significantly decreased ($P < 0.05$) than the control group in fish fed Black seed and/or Turmeric only at last four weeks from the start of the experiment. Either increase or decrease in different leucocytes were pronounced in fish kept on a combined mixture of both plants. All leucocytes were calculated as a percentage of the whole leucocytic count which constitute 100 %. The significant decrease of the percentage of neutrophils in Black seed and/or Turmeric received groups was simply attributed to the significant increase of other leucocytes. As they induced significant elevation of other leucocytes in the expense of neutrophils.

Table (1): Effect of daily supplementation of Black cumin seed and/or Turmeric for 6 weeks on TLC (103/mm3), TEC (106/mm3), Hb (g/dl) and PCV(%) in *Sea bass* fish . Values are expressed as mean ± SEM, n = 30 fish

Parameters	Period	Treatment			
		Control	Nigella	Curcumin	Nigella sativa and curcumin
TLC	1st week	20.55 ± 0.33Da	24.67 ± 0.33Ba	22.00 ± 0.58Ca	28.00 ± 0.58Aa
	2nd week	20.67 ± 0.88Da	25.33 ± 0.33Ba	23.67 ± 0.33Ca	27.00 ± 0.58Aa
	3rd week	20.67 ± 0.33Da	26.00 ± 0.58Ba	23.00 ± 0.58Ca	28.33 ± 0.33Aa
	4th week	20.43 ± 0.33Da	25.67 ± 0.88Ba	24.00 ± 0.58Ca	28.67 ± 0.33Aa
	5th week	20.60 ± 0.33Da	25.33 ± 0.33Ba	22.67 ± 0.67Ca	28.33 ± 0.33Aa
	6th week	20.33 ± 0.67Da	23.67 ± 0.33Ba	22.00 ± 0.58Ca	26.33 ± 0.33Aa
TEC	1st week	1.77 ± 0.03Ca	2.17 ± 0.07Ba	2.00 ± 0.10Ba	2.53 ± 0.07Aa
	2nd week	1.78 ± 0.09Ca	2.13 ± 0.03Ba	1.97 ± 0.09Ca	2.80 ± 0.06Aa
	3rd week	1.80 ± 0.06Da	2.20 ± 0.25Ba	1.70 ± 0.06Ca	2.93 ± 0.09Aa
	4th week	1.70 ± 0.12Da	2.53 ± 0.09Ba	2.00 ± 0.06Ca	2.77 ± 0.09Aa
	5th week	1.90 ± 0.21Da	2.57 ± 0.15Ba	1.97 ± 0.09Ca	3.30 ± 0.10Aa
	6th week	1.83 ± 0.03Da	2.60 ± 0.06Ba	2.53 ± 0.12Ca	2.83 ± 0.03Aa
Hb	1st week	8.00 ± 0.58Da	11.33 ± 0.33Ba	10.33 ± 0.33Ca	12.00 ± 0.58Aa
	2nd week	10.00 ± 0.58Da	12.00 ± 0.03Ba	11.33 ± 0.03Ca	12.33 ± 0.03Aa
	3rd week	8.67 ± 0.03Da	11.00 ± 0.03Ba	10.00 ± 0.03Ca	11.67 ± 0.88Aa
	4th week	7.67 ± 0.33Da	10.00 ± 0.03Ba	9.33 ± 0.03Ca	10.33 ± 0.88Aa
	5th week	7.33 ± 0.03Da	10.00 ± 0.58Ba	8.00 ± 0.08Ca	10.33 ± 0.03Aa
	6th week	8.33 ± 0.08Da	11.00 ± 0.08Ba	10.33 ± 0.03Ca	12.00 ± 0.05Aa
PCV	1st week	27.67 ± 0.33Da	33.67 ± 1.45Ba	31.33 ± 0.33Ca	36.00 ± 0.58Aa
	2nd week	30.00 ± 0.58Da	35.33 ± 0.88Ba	33.00 ± 0.58Ca	37.33 ± 0.20Aa
	3rd week	26.33 ± 0.88Da	32.33 ± 0.88Ba	31.00 ± 0.03Ca	34.67 ± 0.03Aa
	4th week	23.33 ± 0.33Da	32.33 ± 1.76Ba	27.67 ± 0.33Ca	33.33 ± 0.19Aa
	5th week	24.33 ± 0.88Da	29.67 ± 0.88Ba	26.33 ± 1.45Ca	32.67 ± 0.33Aa
	6th week	26.00 ± 2.65Da	34.00 ± 1.73Ba	29.00 ± 0.58Ca	36.33 ± 1.45Aa

Capital letter: Indicated that means within the same row carrying different letter are significantly differed at (P< 0.01). Small letter: indicated that means within the same column carrying different letter are significantly differed at (P< 0.05).

Table (2): Effect of daily supplementation of Black cumin seed and/or Turmeric for 6 weeks on the differential leucocytic count (%) in *Sea bass* fish. Values are expressed as mean ± SEM, n = 30 fish.

Parameters	Period	Groups			
		Control	Nigella sativa	Curcumin	Nigella sativa and curcumin
Lymphocytes	1st week	51.33 ± 0.88Ca	56.00 ± 0.58Ba	55.67 ± 0.33Ba	57.00 ± 0.58Aa
	2nd week	53.00 ± 0.58Ca	55.33 ± 0.33Ba	56.33 ± 0.33Ba	60.33 ± 0.33Aa
	3rd week	51.00 ± 0.58Ca	55.00 ± 1.15Ba	56.67 ± 0.33Ba	61.33 ± 0.88Aa
	4th week	51.33 ± 0.88Ca	55.00 ± 0.58Ba	56.00 ± 0.58Ba	61.33 ± 0.33Aa
	5th week	50.67 ± 0.33Ca	54.67 ± 1.20Ba	54.00 ± 0.58Ba	59.00 ± 0.58Aa
	6th week	50.00 ± 0.58Ca	54.33 ± 0.88Ba	53.67 ± 1.20Ba	60.00 ± 0.58Aa
Monocytes	1st week	1.33 ± 0.33Ba	1.33 ± 0.33Ba	1.33 ± 0.33Ba	2.00 ± 0.58Aa
	2nd week	1.33 ± 0.03Ba	1.50 ± 0.58Ba	1.33 ± 0.33Ba	2.67 ± 0.33Aa
	3rd week	1.33 ± 0.33Ca	1.67 ± 0.33Ba	1.67 ± 0.33Ba	2.33 ± 0.88Aa
	4th week	1.67 ± 0.33Ba	1.67 ± 0.88Ba	1.67 ± 0.58Ba	3.00 ± 0.58Aa
	5th week	2.00 ± 0.58Ba	2.00 ± 0.58Ba	2.00 ± 0.33Ba	3.33 ± 0.33Aa
	6th week	1.33 ± 0.33Ca	2.00 ± 0.58Ba	2.00 ± 0.58Ba	3.00 ± 0.58Aa
Basophils	1st week	7.00 ± 0.78Aa	6.67 ± 1.00Aa	7.33 ± 0.33Aa	7.67 ± 0.01Aa
	2nd week	7.67 ± 0.33Aa	7.60 ± 0.58Aa	7.67 ± 0.33Aa	7.67 ± 0.88Aa
	3rd week	6.67 ± 0.33Ca	8.00 ± 0.58Ba	8.00 ± 0.58Ba	9.67 ± 0.67Aa
	4th week	6.00 ± 0.58Ca	8.00 ± 0.58Ba	7.67 ± 0.33Ba	10.67 ± 0.33Aa
	5th week	6.00 ± 0.58Ca	8.00 ± 1.15Ba	8.00 ± 1.45Ba	11.00 ± 0.58Aa
	6th week	7.33 ± 0.33Ca	8.33 ± 0.33Ba	8.67 ± 0.88Ba	10.67 ± 0.33Aa
Eosinophils	1st week	6.33 ± 0.03Ca	7.67 ± 0.88Ba	7.33 ± 0.33Ba	8.67 ± 0.33Aa
	2nd week	7.33 ± 0.03Ca	8.33 ± 0.03Ba	8.67 ± 0.03Ba	10.00 ± 0.58Aa
	3rd week	7.33 ± 0.03Ca	8.00 ± 0.58Ba	8.33 ± 0.03Ba	10.00 ± 0.58Aa
	4th week	5.33 ± 0.33Ca	7.67 ± 0.33Ba	7.33 ± 0.33Ba	9.67 ± 0.33Aa
	5th week	5.33 ± 0.33Ca	7.00 ± 0.58Ba	7.33 ± 0.33Ba	10.33 ± 0.88Aa
	6th week	5.67 ± 0.33Ca	8.67 ± 0.33Ba	8.33 ± 0.67Ba	9.67 ± 0.33Aa
Neutrophils	1st week	34.00 ± 0.00Aa	30.00 ± 1.00Ba	29.33 ± 0.67Ba	24.67 ± 2.03Ca
	2nd week	30.67 ± 0.67Aa	24.57 ± 1.88Ba	26.00 ± 0.58Ba	19.33 ± 0.33Ca
	3rd week	33.67 ± 0.33Aa	28.00 ± 0.00Ba	25.33 ± 0.88Ca	16.67 ± 0.67Da
	4th week	35.67 ± 1.45Aa	28.67 ± 0.67Ba	29.33 ± 0.58Ba	15.33 ± 0.88Ca
	5th week	36.00 ± 0.58Aa	28.00 ± 0.58Ba	28.67 ± 0.88Ba	16.33 ± 1.76Ca
	6th week	35.67 ± 0.67Aa	26.33 ± 1.20Ba	27.33 ± 0.20Ba	16.67 ± 1.76Ca

Capital letter: Indicated that means within the same row carrying different letter are significantly differed at (P< 0.01). Small letter: indicated that means within the same column carrying different letter are significantly differed at (P< 0.05).

Hematological parameters of fish blood, are useful tools that aids in diagnosis of the diseases. It can also be used to study immnuopotentiators. Such tests are general but not conclusive and must be correlated with biochemical tests of the subject. The present findings indicated that, during the first three weeks of the experiment, fish fed Black seed and/or Turmeric showed an elevation of total protein significantly ($P < 0.05$) than the control. This increment was more marked in fish received a combined mixture of both plants (Table 3). Afterwards, total protein was significantly increased in fish fed a combined mixture of both plants while that of fish fed Black seed or Turmeric alone was remained comparable to the control group. The present study revealed also that, albumin was significantly increased ($P< 0.05$) in fish fed a combined mixture of the plants during the first two weeks of the experiment while that of fish supplemented with Black seed or Turmeric alone was remained comparable to the control group. However, fish fed Black seed and/or Turmeric showed a significant increase ($P < 0.05$) of albumin after the third, fifth and sixth weeks of the experiment. Fish kept on Black seed either alone or in a combination with Turmeric showed an increase of albumin when compared with the control after the fourth week of the experiment. Furthermore, during the first two weeks of the experiment, serum globulin was significantly increased ($P < 0.05$) in fish fed Black seed and/or Turmeric. Afterwards, serum globulin was significantly increased ($P < 0.05$) in fish fed Black seed and/or Turmeric when compared with the control. However, the combined mixture of Black seed and Turmeric was superior for increasing serum albumin and globulin followed by Black seed and finally Turmeric. Such effect of course is confirming the synergistic effect of both plants when administered together at the tested dose as mentioned before.

Due to rapid changes that can occur in an animal's plasma, electrophoresis can be used for evaluating the immune response through estimation of gamma globulin fraction. Electrophoresis revealed the following protein fraction: albumin, alpha globulin, beta globulin and gamma globulin in serum of the examined fish . The value for mean ± SEM plasma protein fraction with distinct globulin fractions are presented in Table 3. Significant increase ($P< 0.05$) in alpha and beta globulin were observed in fish fed Black seed and/or Turmeric when compared with the control group throughout the experimental period. However, there were slight significant difference ($P < 0.05$) in alpha and beta globulins of fish fed either Black seed or Turmeric alone (Table 3). Significant increase ($P < 0.05$) in gamma globulin fraction was observed in fish fed Black seed and/or turmeric when compared with the control group throughout the period of the experiment (Table3). Afterwards, this increase was more pronounced in fish fed a combined mixture of both plants followed by that fed Black seed and finally that fed Turmeric when compared with the control. This introduce another evidence of the superiority of the mixture of both plants as immunostimulator than the individual administration.

The results concerning the effect of Black seed and/or Turmeric on the activities of ALT and AST indicated that, there were non significant changes ($P < 0.05$) in the activities of ALT and AST in fish fed ration containing black seed and/or turmeric when compared with the control (Table 4). Such effect indicated that, both plants were safe to the liver which confirmed by the significant increase of total protein, albumin and globulin. However, the administration of the combined mixture of both plants was more preferable in performing the liver function than each separate administration.

The present findings revealed that, TAG and VLDL -c values were significantly decreased ($P < 0.05$) in fish supplemented with Black seed and/or Turmeric either alone or in a combined form throughout the experimental period when compared with the control (Table 5). However, the total cholesterol and LDL-c values were significantly decreased ($P < 0.05$) in fish kept only on a combined mixture of the plants while the individual administration of each plants did not cause such effect (Table 5). Furthermore, HDL-c value was significantly increased ($P < 0.05$) only in fish kept on a combined mixture of both plants as individual administration of each plants also did not cause such effect (Table 5). There were no inter group difference in all values of lipid profiles throughout the experimental period (Table 5).

Table (3): Effect of daily supplementation of Black cumin seed and/or Turmeric for 6 weeks on total protein (g/dl), albumin (g/dl), total globulin (g/dl) and α, B and γ globulins (g/dl) in Sea-bass fish. Values are expressed as mean ± SEM, n = 30 fish

Parameters	Period	Groups			
		Control	Nigella sativa	Curcumin	Nigella sativa and curcumin
Total proteins	1st week	4.94 ± 0.03Ca	5.77 ± 0.06Ba	5.83 ± 0.06Ba	6.20 ± 0.06Aa
	2nd week	4.80 ± 0.06Ca	5.60 ± 0.06Ba	5.55 ± 0.03Ba	6.34 ± 0.03Aa
	3rd week	4.93 ± 0.03Ca	5.60 ± 0.06Ba	5.94 ± 0.03Ba	6.47 ± 0.03Aa
	4th week	4.57 ± 0.03Ca	5.87 ± 0.03Ba	5.07 ± 0.03Ba	6.37 ± 0.03Aa
	5th week	4.67 ± 0.03Ca	5.83 ± 0.03Ba	6.07 ± 0.03Ba	6.27 ± 0.03Aa
	6th week	4.37 ± 0.03Ca	5.87 ± 0.03Ba	5.97 ± 0.03Ba	6.67 ± 0.03Aa
Albumin	1st week	3.77 ± 0.03Ba	3.87 ± 0.03Ba	4.13 ± 0.03Aa	4.13 ± 0.03Aa
	2nd week	3.57 ± 0.03Ba	3.87 ± 0.03Ba	3.87 ± 0.03Ba	4.57 ± 0.03Aa
	3rd week	3.43 ± 0.03Ba	3.63 ± 0.03Ba	4.17 ± 0.03Aa	4.13 ± 0.03Aa
	4th week	3.57 ± 0.03Ba	4.17 ± 0.03Aa	3.87 ± 0.03Ba	4.37 ± 0.03Aa
	5th week	3.47 ± 0.03Ba	3.83 ± 0.03Ba	4.47 ± 0.03Aa	4.17 ± 0.03Aa
	6th week	3.37 ± 0.03Ba	4.17 ± 0.03Ba	4.47 ± 0.03Aa	4.57 ± 0.03Aa
Globulin	1st week	1.17 ± 0.07Ba	1.90 ± 0.03Aa	1.93 ± 0.03Aa	2.07 ± 0.03Aa
	2nd week	1.23 ± 0.09Ba	1.73 ± 0.09Aa	1.68 ± 0.27Aa	1.77 ± 0.03Aa
	3rd week	1.50 ± 0.07Da	1.97 ± 0.03Ba	1.77 ± 0.03Ca	2.33 ± 0.07Aa
	4th week	1.00 ± 0.00Da	1.70 ± 0.06Ba	1.50 ± 0.00Ca	2.00 ± 0.00Aa
	5th week	1.20 ± 0.00Da	2.00 ± 0.06Ba	1.74 ± 0.03Ca	2.10 ± 0.00Aa
	6th week	1.00 ± 0.00Da	1.70 ± 0.03Ba	1.50 ± 0.00Ca	2.10 ± 0.00Aa
α-Globulin	1st week	0.39 ± 0.03Ba	0.50 ± 0.03Aa	0.50 ± 0.03Aa	0.50 ± 0.03Aa
	2nd week	0.42 ± 0.03Ba	0.50 ± 0.03Aa	0.51 ± 0.03Aa	0.53 ± 0.03Aa
	3rd week	0.53 ± 0.03Ba	0.53 ± 0.03Aa	0.53 ± 0.03Aa	0.55 ± 0.03Aa
	4th week	0.35 ± 0.03Ba	0.50 ± 0.03Aa	0.47 ± 0.03Aa	0.50 ± 0.03Aa
	5th week	0.38 ± 0.03Ba	0.50 ± 0.03Aa	0.50 ± 0.03Aa	0.52 ± 0.03Aa
	6th week	0.36 ± 0.03Ba	0.51 ± 0.03Aa	0.50 ± 0.03Aa	0.50 ± 0.03Aa
β-Globulin	1st week	0.31 ± 0.03Ba	0.60 ± 0.03Aa	0.60 ± 0.03Aa	0.60 ± 0.03Aa
	2nd week	0.38 ± 0.03Ba	0.60 ± 0.03Aa	0.58 ± 0.03Aa	0.60 ± 0.03Aa
	3rd week	0.45 ± 0.03Ba	0.64 ± 0.03Aa	0.60 ± 0.03Aa	0.65 ± 0.03Aa
	4th week	0.32 ± 0.03Ba	0.57 ± 0.03Aa	0.60 ± 0.03Aa	0.60 ± 0.03Aa
	5th week	0.38 ± 0.03Ba	0.60 ± 0.03Aa	0.60 ± 0.03Aa	0.62 ± 0.03Aa
	6th week	0.31 ± 0.03Ba	0.56 ± 0.03Aa	0.55 ± 0.03Aa	0.60 ± 0.03Aa
γ- Globulin	1st week	0.43 ± 0.03Ba	0.61 ± 0.03Aa	0.55 ± 0.03Aa	0.97 ± 0.03Aa
	2nd week	0.45 ± 0.03Ba	0.65 ± 0.03Aa	0.59 ± 0.03Aa	0.64 ± 0.03Aa
	3rd week	0.49 ± 0.03Da	0.67 ± 0.03Ba	0.64 ± 0.03Ca	1.13 ± 0.03Aa
	4th week	0.5 ± 0.03Da	0.71 ± 0.03Ba	0.64 ± 0.03Ca	0.90 ± 0.03Aa
	5th week	0.55 ± 0.03Da	0.71 ± 0.03Ba	0.67 ± 0.03Ca	0.96 ± 0.03Aa
	6th week	0.59 ± 0.03Da	0.76 ± 0.03Ba	0.75 ± 0.03Ca	1.00 ± 0.03Aa

Capital letter : Indicated that means within the same row carrying different letter are significantly differed at (P< 0.01). Small letter: indicated that means within the same column carrying different letter are significantly differed at (P< 0.05).

Table (4): Effect of daily supplementation of Black cumin seed and/or Turmeric for 6 weeks on ALT (U/l), AST (U/l) and glucose (mg/dl) in Sea bass fish. Values are expressed as mean ± SEM, n = 30 fish.

Parameters	Period	Groups			
		Control	Nigella sativa	Curcumin	Nigella sativa and curcumin
ALT	1st week	65.00 ± 0.58Aa	66.00 ± 0.58Aa	65.00 ± 0.58Aa	65.00 ± 0.58Aa
	2nd week	65.00 ± 0.58Aa	65.00 ± 0.58Aa	66.00 ± 0.58Aa	65.00 ± 0.58Aa
	3rd week	66.00 ± 0.58Aa	67.00 ± 0.58Aa	67.17 ± 3.84Aa	67.00 ± 0.58Aa
	4th week	65.00 ± 0.58Aa	66.00 ± 0.58Aa	65.00 ± 0.58Aa	66.00 ± 0.58Aa
	5th week	66.00 ± 0.58Aa	66.00 ± 0.58Aa	66.00 ± 0.58Aa	66.00 ± 0.58Aa
	6th week	65.00 ± 0.58Aa	65.00 ± 0.58Aa	66.00 ± 0.58Aa	65.00 ± 0.58Aa
AST	1st week	66.00 ± 0.58Aa	66.00 ± 0.58Aa	66.00 ± 0.58Aa	65.00 ± 0.58Aa
	2nd week	66.00 ± 0.58Aa	65.00 ± 0.58Aa	66.00 ± 0.58Aa	65.00 ± 0.58Aa
	3rd week	65.00 ± 0.58Aa	65.00 ± 0.58Aa	66.00 ± 0.58Aa	65.00 ± 0.58Aa
	4th week	66.00 ± 0.58Aa	66.00 ± 0.58Aa	65.00 ± 0.58Aa	66.00 ± 0.58Aa
	5th week	65.00 ± 0.58Aa	66.00 ± 0.58Aa	65.00 ± 0.58Aa	65.00 ± 0.58Aa
	6th week	66.00 ± 0.58Aa	65.00 ± 0.58Aa	66.00 ± 0.58Aa	66.00 ± 0.58Aa

Capital letter : Indicated that means within the same row carrying different letter are significantly differed at (P< 0.01). Small letter: indicated that means within the same column carrying different letter are significantly differed at (P< 0.05).

The values of phagocytic activity and index were significantly increased *(P <0.05)* in all fish supplemented with Black seed and/or Turmeric when compared with the control. This increment was more marked in fish received a combined mixture of both plants throughout the experimental period followed by those fed Black seed alone and finally with those fed Turmeric (Table 6). However, there was no significant difference ($P < 0.05$) in the values of phagocytic activity and index within the same group throughout the experimental period (Table 6). The increment of phagocytic index and activity in fish fed a combined mixture of both plants than that of the control and that of each alone plant administration introduced another evidence of their synergistic effect.

The values of antibody titer in fish vaccinated against ***Pseudomonas florescence*** bacterin was significantly increased (*p<0.05*) in all fish supplemented with Black seed and/or Turmeric when compared with the vaccinated control (Table 7). This increase was more marked when fish fed a combined mixture of the plants followed by that fed Black cumin seed alone and finally that fed Turmeric alone when compared with the both control groups during the last four weeks of the experiment (Table 7). In addition, the values of antibody titer were time dependant reaching its maximum at fifth and sixth weeks of the experiment in all tested fish (Table 7). The percentage of RLP of fish challenged with *Pseudomonas florescence* virulent strain was significantly increased ($p < 0.05$) in all fish supplemented with Black seed and/or Turmeric when compared with the control (Table 8). This increase was higher in fish fed combined mixture of the plant (80%) than fish administered Black seed alone (60%) or fish fed Turmeric alone (50%) throughout the experimental period (one week, Table 8).

Table (5): Effect of daily supplementation of Black cumin seed and/or Turmeric for 6 weeks on serum TAG (mg/dl), total cholesterol (mg/dl), HDL-c (mg/dl), LDL-c (mg/dl) and VLDL-c (mg/dl) in Sea bass fish. Values are expressed as mean ± SEM, n = 30 fish.

Parameters	Period	Groups			
		Control	Nigella sativa	Curcumin	Nigella sativa and curcumin
TAG	1st week	120 ± 0.58Aa	100 ± 2.03Ba	104 ± 2.58Ba	101 ± 1.03Ba
	2nd week	121 ± 0.58Aa	101 ± 1.58Ba	102 ± 0.58Ba	100 ± 0.58Ba
	3rd week	119 ± 0.58Aa	99 ± 2.03Ba	100 ± 1.58Ba	103 ± 1.58Ba
	4th week	120 ± 0.58Aa	98 ± 2.58Ba	100 ± 1.58Ba	102 ± 1.58Ba
	5th week	122 ± 0.58Aa	100 ± 1.58Ba	103 ± 2.58Ba	100 ± 1.58Ba
	6th week	120 ± 0.58Aa	102 ± 1.58Ba	105 ± 2.58Ba	101 ± 2.58Ba
Total cholesterol	1st week	168 ± 2.58Aa	165 ± 1.58Aa	166 ± 1.58Aa	133 ± 0.58Ba
	2nd week	170 ± 2.58Aa	166 ± 2.58Aa	165 ± 2.58Aa	131 ± 0.58Ba
	3rd week	166 ± 0.58Aa	167 ± 0.58Aa	164 ± 0.58Aa	133 ± 0.58Ba
	4th week	167 ± 1.58Aa	164 ± 2.58Aa	166 ± 1.58Aa	134 ± 0.58Ba
	5th week	169 ± 2.58Aa	165 ± 2.58Aa	167 ± 1.58Aa	136 ± 0.58Ba
	6th week	171 ± 2.58Aa	166 ± 3.58Aa	168 ± 1.58Aa	132 ± 0.58Ba
HDL-c	1st week	168 ± 2.58Aa	37 ± 1.58Ba	39 ± 1.58Ba	46 ± 0.58Aa
	2nd week	170 ± 2.58Aa	37 ± 2.58Ba	40 ± 1.58Ba	44 ± 0.58Aa
	3rd week	166 ± 0.58Aa	36 ± 1.58Ba	38 ± 1.58Ba	45 ± 0.58Aa
	4th week	167 ± 1.58Aa	38 ± 1.58Ba	37 ± 0.58Ba	48 ± 0.58Aa
	5th week	169 ± 2.58Aa	37 ± 2.58Ba	40 ± 2.58Ba	47 ± 0.58Aa
	6th week	171 ± 2.58Aa	36 ± 2.58Ba	39 ± 1.58Ba	46 ± 0.58Aa
LDL-c	1st week	106 ± 0.58Aa	108 ± 1.58Aa	106 ± 1.58Aa	67 ± 0.58Ba
	2nd week	106 ± 1.58Aa	109 ± 2.58Aa	105 ± 2.58Aa	67 ± 0.58Ba
	3rd week	106 ± 2.58Aa	111 ± 2.58Aa	106 ± 2.58Aa	67 ± 0.58Ba
	4th week	106 ± 1.58Aa	106 ± 1.58Aa	109 ± 2.58Aa	66 ± 0.58Ba
	5th week	109 ± 1.58Aa	108 ± 1.58Aa	106 ± 1.58Aa	69 ± 0.58Ba
	6th week	109 ± 1.58Aa	110 ± 1.58Aa	108 ± 1.58Aa	66 ± 0.58Ba
VLDL-c	1st week	24 ± 0.58Aa	20 ± 1.58Ba	21 ± 0.58Ba	20 ± 1.58Ba
	2nd week	24 ± 0.58Aa	20 ± 0.58Ba	20 ± 0.58Ba	20 ± 0.58Ba
	3rd week	24 ± 0.58Aa	20 ± 0.58Ba	20 ± 0.58Ba	21 ± 1.58Ba
	4th week	24 ± 0.58Aa	20 ± 0.58Ba	20 ± 0.58Ba	20 ± 0.58Ba
	5th week	24 ± 0.58Aa	20 ± 0.58Ba	21 ± 1.58Ba	20 ± 0.58Ba
	6th week	24 ± 0.58Aa	20 ± 0.58Ba	21 ± 1.58Ba	20 ± 0.58Ba

Capital letter : Indicated that means within the same row carrying different letter are significantly differed at (P< 0.01). Small letter: indicated that means within the same column carrying different letter are significantly differed at (P< 0.05).

Table (6): Effect of daily supplementation of Black cumin seed and/or Turmeric for 6 weeks on phagocytic activity (%) and index in Sea bassfish. Values are expressed as mean ± SEM, n = 30 fish

Parameters	Period	Groups			
		Control	Nigella sativa	Curcumin	Nigella sativa and curcumin
Phagocytic index	1st week	19.00 ± 0.58Da	24.00 ± 0.58Ba	21.33 ± 0.33Ca	27.00 ± 0.58Aa
	2nd week	19.17 ± 0.33Da	23.33 ± 0.88Ba	21.00 ± 0.58Ca	26.67 ± 0.33Aa
	3rd week	20.00 ± 0.58Da	27.00 ± 0.58Ba	23.00 ± 0.58Ca	30.00 ± 0.58Aa
	4th week	20.13 ± 0.88Da	25.67 ± 0.88Ba	22.00 ± 0.58Ca	31.00 ± 0.58Aa
	5th week	20.00 ± 1.20Da	26.67 ± 0.88Ba	22.00 ± 0.58Ca	30.00 ± 0.58Aa
	6th week	19.50 ± 0.58Da	25.33 ± 0.33Ba	22.00 ± 0.58Ca	25.67 ± 0.33Aa
Phagocytic activity	1st week	1.73 ± 0.12Da	2.60 ± 0.06Ba	2.00 ± 0.06Ca	3.10 ± 0.09Aa
	2nd week	1.87 ± 0.09Da	2.50 ± 0.06Ba	2.00 ± 0.06Ca	2.93 ± 0.12Aa
	3rd week	1.83 ± 0.03Da	2.57 ± 0.03Ba	2.13 ± 0.09Ca	2.95 ± 0.03Aa
	4th week	1.87 ± 0.09Da	2.67 ± 0.09Ba	2.10 ± 0.06Ca	3.00 ± 0.06Aa
	5th week	1.83 ± 0.09Da	2.57 ± 0.09Ba	2.20 ± 0.15Ca	3.17 ± 0.09Aa
	6th week	1.77 ± 0.07Da	2.50 ± 0.06Ba	2.10 ± 0.32Ca	2.97 ± 0.15Aa

Capital letter : Indicated that means within the same row carrying different letter are significantly differed at (P< 0.01). Small letter: indicated that means within the same column carrying different letter are significantly differed at (P< 0.05).

Table (7): Effect of daily supplementation of Black cumin seed and/or Turmeric for 6 weeks on antibody titer in Sea-bass fish vaccinated with Pseudomonas florescence bacterin . Values were measured only at last four weeks of the experiment and expressed as mean ± SEM, n = 30 fish

Period	Control (not vaccinated)	Control (vaccinated)	Black cumin seed (vaccinated)	Turmeric (vaccinated)	Combined mixture of both plants (vaccinated)
3rd week	0.00	3.00 ± 0.05Dc	5.00 ± 0.45Bc	4.00 ± 0.44Cc	6.00 ± 0.55Ac
4th week	0.00	4.00 ± 0.22Db	6.00 ± 0.22Bb	5.00 ± 0.21Cb	7.20 ± 0.23Ab
5th week	0.00	5.00 ± 0.22Da	7.00 ± 0.05Ba	6.00 ± 0.03Ca	8.00 ± 0.05Aa
6th week	0.00	5.00 ± 0.16Da	7.00 ± 0.26Ba	6.00 ± 0.05Ca	8.00 ± 0.27Aa

Capital letter : Indicated that means within the same row carrying different letter are significantly differed at (P<0.01). Small letter: indicated that means within the same column carrying different letter are significantly differed at (P<0.05).

Table (8): Effect of daily supplementation of Black cumin seed and/or Turmeric for 6 weeks on protection of Sea-bass fishagainst virulent strain of Pseudomonas florescence after vaccination with Pseudomonas florescence bacterin.

Groups	Total number of tested fish	Number of dead fish	Mortality %	RLP %
Control (not vaccinated)	30	30	100	1- 100/100 = 0
Control (vaccinated)	30	18	60	1- 60/100 = 40
Black cumin seed (vaccinated)	30	12	40	1- 40/100 = 60
Turmeric (vaccinated)	30	15	50	1- 50/100 = 50
Combined mixture of both plants (vaccinated)	30	6	20	1- 20/100 = 80

4. Discussion

The increment of TLC and TEC may be attributed to the activation of lymphoid and haemopiotic tissues either by Black cumin seed (Satish et al 1991) or Turmeric (Antony et al 1999). The increment of Hb percentage may be attributed either to increasing the synthesis of enzymes needed for biosynthesis of hem (El-Tahir et al 1993) or increasing the size of red blood cells (El-Feki et al 1993). Concerning the effect of Black seed, similar results were obtained in *Orechromus niloticus* fed 1% Black seed (Hussein et al 2000), mice and rats (Zaoui et al 2002), Swiss albino mice infected with schistosomiasis (Soliman & El-Shenawy 2003), rabbits (Meral et al 2004) and Catfish (Abeer 2005). In the contrary, a significant decrease in all

previously tested parameters was observed when black seed extract was used to inhibit snake venom *in vitro* (Sallal & Alkofahi 1996) and when goat administered black seed orally (El-Sarha et al. 1997). However, the conflict observed between the present results and the other mentioned findings (Sallal & Alkofahi 1996; El-Sarha et al 1997) may be attributed to different age or species and/or differences in dose and duration of the administered black cumin seeds. Concerning the effect of Turmeric, similar results (increased TEC and TLC) were obtained either in mice administered curcumin intraperitonealy (Antony et al 1999) or in broiler chicken received turmeric (0.5 and 1%; Al-Sultan 2003). In the contrary, administration of curcumin to male rats, guinea pigs and monkeys at a dose of 300 mg/kg b.wt. caused no effect on the level of TEC, TLC and Hb (Sambaiah et al 1982). Furthermore, treatment of rats with aqueous and alcoholic extract of turmeric for 2 months produced insignificant effect in TEC, TLC, Hb and haematocrite values (Purohit & Bhagat 2004). However, this confliction may attributed to dose, duration and species differences.

The increment of lymphocytes, monocytes, basophils and esionphils perhaps indicated the direct stimulation of Black seed and/or Turmeric to lymphoid tissue (Satish et al 1991 and Antony et al 1999). The superiority of the effect of combined mixture of both plants over their individual administration on all previously discussed hematological parameters perhaps introduce a discovery of the synergistic effect of both plants. The values concerning all hematological parameters were not changed significantly within the same group throughout the experimental period (Table 1, 2).

All values of protein patterns were slight significantly changed within the same group throughout the experimental period (Table 3). Similar results were obtained in catfish injected intraperitoneally with Black seed oil (Abeer 2005). The increment of total protein, albumin and globulin perhaps explained either by the fact that, Black seed contains high percentage of crude protein (20.5%) and free amino acids (Kudryashova et al 1953; Babayan et al 1978; Atta 2003). Such increase perhaps attributed also to the role of Black seed (Hedaya 1995) or Turmeric (Osawa et al 1995) in protein biosynthesis as it is vitally concerned in the growth process. Moreover, the significant increase of serum globulin indicated the immunostimulant effect of Black seed (Aqel et al 1993; Taha et al 2007) or Turmeric (Antony 1999) particularly for the combined mixture used .

The activities of both enzymes were not changed significantly ($P < 0.05$) within the same group (Table 4). In the contrary, liver damage was observed after oral administration of aqueous extract of *Nigella sativa* (10ml/kg of body weight for 14 consecutive days) in rats (Tennekoon et.al 1991) or in mice after oral administration of an aqueous extract of the black cumin seeds in 4 different doses, 6, 9, 14 and 21 g/kg (Vahdati-Mashhadian et al 2005). In addition the same liver damage was observed in rats kept on powdered turmeric (10gm/kg ration, El-Bahr et al 2007). This liver damage was not observed when both plants administered together with the present dose which introduce an evidence that one of the plant perhaps prevent or inhibits the drawbacks of the other when administered together at the examined dose.

The present finding are disagree with those obtained by El-Bahr (2007) who demonstrated that, serum TAG, total cholesterol, very low density lipoprotein cholesterol (VLDL-c) and low density lipoprotein cholesterol (LDL-c) levels were higher while high density lipoprotein cholesterol (HDL-c) were lower than in control ducklings at the end of two weeks of black cumin seeds' administration (2% of diet). However, this confliction may attributed to dose, duration and species differences. The hyperlipidemia caused by Black cumin seed administration in duckling (El-Bahr 2007) is not only prevented in fish when Black cumin seed administered together with Turmeric but also hypolipidemia was observed. This make an evidence that turmeric perhaps prevent or inhibits the drawbacks of Black seed when administered together more stronger.

Administration of turmeric caused an elevation of T and B cells numbers (Churchill et al 2000). Phagocytic index and activity were increased in mice injected intraperitonealy with curcumin (Antony 1999).

The present result concerning the RLP of control vaccinated fish challenged with *Pseudomonas florescence* virulent strain (40%) disagree with those obtained by Khalil et al (2006, 25%). This confliction perhaps attributed to the species difference. The increment of antibody titer against *Pseudomonas florescence* bacterin of fish administered a combined mixture of the plant along with the significant increase of the percentage of RLP against *Pseudomonas florescence* virulent strain confirmed the above mentioned immunostimulant effect of both plants with the superiority of the combined mixture of the both plants which perhaps is essential for biosynthesis and production of immunoglobulins.

The superiority of administration of a combined mixture of the plants over the individual administration perhaps has two explanations. The first is postulated that both plants perhaps are of synergistic effect at the examined dose. The second is

based on that one of the plants perhaps remove or inhibits the drawbacks of the other.

5. Conclusion

The present study concludes that Black seed and/or Turmeric are beneficial to be added in fish diet as both plants have a powerful role in improving the overall performance in fish. However, the combined mixture of both plants at the examined dose was preferable than their individual administration.

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