Advanced immunological studies on the effect of Spirulina in cultured tilapia

Abdalla O.A., Eissa I. A.*, Omnia E. Kilany and Shimaa M. Elbahar**


English Abstract

This study was undertaken to evaluate the use of spirulina (Arthrospira platensis) as immunity promoter for Nile tilapia, Oreochromis niloticus (L.). A total of 270 fish (50±5 g) were randomly distributed into six groups each at a rate of 15 fish per aquarium and fed on a diet containing 0.0, 5.0 or 10.0 g spirulina/kg diet for 6 weeks. Each subdivided into three equal replicates. After the feeding trial, fish of each treatment were challenged by pathogenic Pseudomonas fluorescens which was given by intraperitoneal (IP) injection. The blood samples were taken after 4 and 6 weeks for immunological examinations. The results showed that the highest white blood cells (WBCs), neutrophils, monocytes and basophils were obtained at 5.0 - 10.0 g spirulina/kg diet before and after the infection. There were non significant changes in lymphocytes after 4 weeks, while after infection with Pseudomonas fluorescens lymphocytes increased in groups supplemented with spirulina. Moreover, spirulina enhanced serum lysozyme activity, bactericidal activity, and antioxidant enzymes (GPx and SOD) of treated groups before and after the infection. Total fish mortality 10-days were decreased after IP injection with Pseudomonas fluorescens with the increase of spirulina level in fish diets. The lowest fish mortality was obtained when fish fed 10.0 g spirulina/kg. These results indicate that spirulina supplementation is promising for disease prevention in tilapia culture, and the optimum level of spirulina in fish diet is 10.0 g per kg diet.

Introduction

Proper nutrition has long been recognized as a critical factor in promoting normal growth and sustaining fish health. Prepared diet not only provide the essential nutrients that are required for normal physiological functioning, but also may serve as the medium by which fish receive other components that may affect their health (Gatlin, 2002). Oreochromis niloticus is one of the most important species within the tilapia species. Abdel-Tawwab and El-Marakby (2004) noted that Nile tilapia, O. niloticus is omnivorous and can utilize a wide range of food.
items including blue green algae. Spirulina (*Spirulina platensis*) is a freshwater blue-green filamentous alga, and it is receiving increasing attention for its bioactive components such as vitamins, protein (60–70%), minerals, polyunsaturated fatty acids, carotenes and other pigments that have antioxidants activity (Madhava et al, 2000; Lin et al, 2007). Researchers have reported the therapeutic effects of spirulina as a growth promoter, probiotic, and booster of the immune system in animals including fishes (James et al, 2006). In fish, several immunostimulants such as Chitin (Esteban et al., 2001), Lactoferrin, dimerized lysozyme (Siwicki et al, 1998), CPG oligodeoxy nucleotides (Tassakka and Sakai, 2003) and nisin have been reported and these substances play a promising role in aquaculture by enhancing the resistance of cultured fish against diseases. Recently, spirulina has been speculated to be associated with modulation of the host immune system (Hironobu et al, 2006). Abdel-Tawwab et al, (2008) reported that the highest red blood cells (RBC), white blood cells (WBC), were obtained when *O. niloticus* fed on diets containing 5.0 - 10.0 g spirulina/kg diet. In the same line James et al, (2009) revealed that the hematological parameters (RBC count and Hb content) were improved in copper exposed *Cirrhinus mirigala* fed spirulina supplemented diets as against copper exposed fish fed spirulina free diet. Also, Ragab et al, (2012) said that The RBCs, WBCs and PCV values had the highest values during addition of spirulina to diet of *O niloticus* at different levels of *S. platensis*, also there was increasing in lymphocytes, monocytes, basophils, eosinophils and neutrophils in groups fed on spirulina. Also, Promya and Chitmanat (2011) reported that by increasing spirulina supplementation fish had higher red and white blood cell counts. Andrews et al, (2011) who reported that the erythrocyte count, haemoglobin concentration and the leucocyte count was significantly higher in *Labeo rohita* fingerlings fed on diets containing spirulina supplementation compare to control group. Also, Kaoud et al, (2012) recorded that the addition of dried *Spirulina platensis* improves the haematological parameters (RBCs, Hb and Hct) as these parameters were of normal values and increased significantly in *O. niloticus* exposed to Hg with *Spirulina platensis*. This study was conducted to study the effects of graded levels of Spirulina (*A. plantensis*) on RBCs and White blood cells (WBC), some oxidative parameters and non-specific immune responses. Besides resistance of Nile tilapia to *Psuedomonas fluorscence* infection.

**Materials and Methods**
Fish: A total number of 270 *O. niloticus* with average body weight of 50±5 g were obtained from the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. They were transported in sterile plastic bags containing water enriched by oxygen (2/3) to the lab of the Dept. of Fish Diseases, faculty of veterinary medicine, Suez Canal University. They kept for two weeks under observation for acclimation in glass aquaria (100×40×50cm). Fish were fed on the basal diet for 2 weeks. The water was changed daily.

Aquaria: These aquaria were used for holding the experimental fish throughout the period of the present study, (triplicate each treatment). Each aquarium was supplied with chlorine free tap water (Innes, 1966). The water temperature was kept at 22±1 °C. The continuous aeration was maintained in each aquarium using an electric air pumping compressors. Settled fish wastes were cleaned daily by siphoned with three quarters of the aquarium’s water, which was replaced by aerated water from the water storage tank.

Diet preparation: A basal diet was formulated to contain 30.6% crude protein diet. The diet was daily provided at a fixed feeding ratio of 3% of body weight of fish according to Eurell et al, (1979). The daily amount of food was offered as two equal meals /day on two occasions over the day (9Am and12 PM).

*S. platensis* used in the present study was obtained from agent chemical laboratories. Redmond, WA, USA.

Pathogen: *Pseudomonas fluorescens* strain was kindly supplied by Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. It was used for serum bactericidal activity and challenge test. Lyophilized *Micrococcus lysodekticus*, which was used for serum lysozomal activity. (Sigma M3770).

Experimental design: The pre-acclimatized fish were divided into six groups each group subdivided into 3 subgroups each group distributed into three aquaria. (Replicates, each 15 fish/ aquarium) Group (I) and (IV) were fed on a basal diet (control), Group (II) and (V) were fed with spirulina supplement at 5.0 g/kg diet and Group (III) and (VI) fed with fed with spirulina supplement at 10 g/kg diet. Groups (VI, V and VI) post feeding trails (at the end of the month) were experimentally infected I/p with *Pseudomonas fluorescens*, the mortalities were estimated post challenge infections till the end of the experiment.

Blood sampling: At the 4 weeks and after 6 weeks, fish were fasted for 24 hours immediately prior to blood sampling and five fish per aquaria were randomly chosen. The blood was extracted from the caudal
blood vessels and divided in two sets of eppendorf tubes. One set contained di potassium salt of EDTA, used as anticoagulant, for the counting of red blood cells (RBC) and white blood cell (WBC), which were done following the methods of Brown (1988). The second set was left with no anticoagulant and centrifuged at 5000 rpm for 5 min at room temperature, the supernatant serum collected and stored at -20 °C in screw caped glass vials until used for serum immunological tests.

**Lysozyme activity:** Serum lysozyme activity was determined through the turbidimetry described by Engstad et al (1992) by using lyophilized Micrococcus lysodekticus (OD=0.3) as the substrate in phosphate buffer(0.1 M, PH 6.4)

**Serum bactericidal activity:** Bacteriocidal activity in fish samples was analyzed according to the Miles–Misra technique Rainger and Rowley (1993).

**Measurement of antioxidant enzymes**

**The activity of Glutathione peroxidase(GPx):** Glutathione peroxidase (E.C.1.1.1.9), activity was determined by measurement of the reduced glutathione substrate (GSH) remaining after the action of the enzyme using the combined methods of Chiu et al (1976) with Ellmans reagent in presence of cumene hydroperoxide as a secondary substrate. The unit of enzyme activity is the amount of Gpx which consumes1μ mol reduced glutathione/ min in presence of cumene hydroperoxide.

**The activity of superoxide dismutase (SOD):** The activity of SOD (E.C.1.1.5.1.1) was determined spectrophotometrically at 480 nm by the epinephrine method by Misra and Fridovich (1972) and it was expressed in (U/g wet wt)/ ml of blood serum (U/ml blood serum).

**Challenge test:** After month, post feeding trials fish from each treatment groups (VI, V and VI) (10fish/ aquarium) were challenged with pathogenic P. fluorescens. The fish were injected intraperitoneally with 0.2 ml sterile saline containing (1.5 x 10^8 / ml) pathogenic strain of Pseudomonas florescens, according to El-Attar and Moustafa (1996).

**Statistical analysis:** The obtained data were subjected to one-way ANOVA to evaluate the effect of spirulina supplement. Differences between means were tested at the 5% probability level using Duncan Multiple Range test. All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, VA, USA) as described by Dytham (1999).
Table 1: Design of the experiment:

<table>
<thead>
<tr>
<th>Treatments (Groups)</th>
<th>Diet</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Basal diet</td>
<td>Not infected</td>
</tr>
<tr>
<td>II</td>
<td>Basal diet containing 5g Spirulina/kg diet</td>
<td>Not infected</td>
</tr>
<tr>
<td>III</td>
<td>Basal diet containing 10g Spirulina/kg diet</td>
<td>Not infected</td>
</tr>
<tr>
<td>IV</td>
<td>Basal diet</td>
<td>Infected</td>
</tr>
<tr>
<td>V</td>
<td>Basal diet containing 5g Spirulina/kg diet</td>
<td>Infected</td>
</tr>
<tr>
<td>VI</td>
<td>Basal diet containing 10g Spirulina/kg diet</td>
<td>Infected</td>
</tr>
</tbody>
</table>

Results

Hematological and leukogram results:
In the present study, the results after 4 weeks showed that fish fed on diets containing spirulina exhibited higher RBCs counts compared with the control (Table2). While, the results after 8 weeks showed that RBCs counts were decreased in all infected groups. While the RBCs counts were stable in infected fish fed on spirulina (Table3). The WBCs counts after 4 weeks had the highest values during addition of spirulina. Also, there was increasing in the neutrophils counts followed by monocytes during the addition of spirulina in groups (II, III). Also after 6 weeks WBCs, neutrophils, monocytes and lymphocytes counts were increased in all infected groups, in which the highest count were obtained in 1% spirulina infected groups (VI), and the lowest count were obtained in the control infected group (IV).

Immunological results:
Lysozyme activity: the results showed that After 4 weeks serum lysozyme activity showed the highest values at spirulina 1% group (III). (Table4). However, after 6 weeks the serum lysozyme activity in non infected groups was increased by the increase of spirulina supplementation in diet. While the challenged groups showed the highest values than non challenged groups, in which the highest values of lysozyme activity at 1% spirulina infected group (VI) (Table5).

Bacterial activity: after 4 and 6 weeks increased significantly with the increase of spirulina supplementation. Moreover, the bactericidal activity in challenged groups showed highest values than non challenged groups, in which the highest values of bactericidal activity were obtained at 1% spirulina infected group (VI) (Table4 and 5).

Antioxidant enzymes: The results showed that After 4 weeks, serum GPx and SOD showed highest values at spirulina 1% group (III), while the lowest values were obtained at control group (I) (Table4). While, After 6 weeks the serum GPx and SOD in non
infected groups were increased by the increase of spirulina supplementation in diet, however challenged groups showed highest values than non challenged groups, in which the highest values of serum GPx and SOD at 1% spirulina infected group(VI) (table 5).

The Mortality Rate and the Relative Level of Protection (RLP): The mortality rate was significantly lower in all S. platensis supplemented groups than the control. Also, the results evoked significant protection in all S. platensis supplemented groups than the control (table 6).

Table (2): Effect of Spirulina platensis on hematological parameters of O.niloticus fed on practical diets containing different levels of spirulina after 4 weeks:

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (I)</th>
<th>Spirulina 0.5% (II)</th>
<th>Spirulina 1% (III)</th>
<th>Control infected (IV)</th>
<th>Spirulina 0.5% infected (V)</th>
<th>Spirulina 1% infected (VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs x 10^9/µl</td>
<td>1.06±0.06^b</td>
<td>1.87±0.50^b</td>
<td>2.3±0.15^a</td>
<td>1.05±0.02^b</td>
<td>1.9±0.51^b</td>
<td>2.4±0.05^a</td>
</tr>
<tr>
<td>WBCs x 10^9/µl</td>
<td>25.9±0.7^a</td>
<td>34.8±0.24^a</td>
<td>63.96±0.26^a</td>
<td>26±0.57^a</td>
<td>35±0.57^b</td>
<td>63.6±0.92^a</td>
</tr>
<tr>
<td>Neutrophils x 10^3/µl</td>
<td>8.6±0.6^a</td>
<td>14±0.57^a</td>
<td>32.75±0.43^a</td>
<td>8.8±0.46^b</td>
<td>13.9±0.51^b</td>
<td>32.6±0.92^a</td>
</tr>
<tr>
<td>Lymphocytes x 10^9/µl</td>
<td>11.5±0.5^a</td>
<td>11.56±0.31^a</td>
<td>11.6±0.35^a</td>
<td>11.3±0.75^a</td>
<td>11.6±0.92^a</td>
<td>11.7±0.98^a</td>
</tr>
<tr>
<td>Monocytes x 10^9/µl</td>
<td>2.7±0.7^c</td>
<td>5.8±0.18^b</td>
<td>14.3±0.17^a</td>
<td>2.7±0.40^b</td>
<td>5.9±0.51^b</td>
<td>14.2±0.69^a</td>
</tr>
<tr>
<td>Eosinophils x 10^9/µl</td>
<td>2.1±0.1^c</td>
<td>2.4±0.23^a</td>
<td>2.64±0.36^a</td>
<td>2.2±0.07^a</td>
<td>2.5±0.28^a</td>
<td>2.6±0.34^a</td>
</tr>
<tr>
<td>Basophils x 10^9/µl</td>
<td>1.0±0.5^c</td>
<td>1.0±0.5^b</td>
<td>2.64±0.36^a</td>
<td>1±0.57^a</td>
<td>1.1±0.57^b</td>
<td>2.5±0.88^a</td>
</tr>
</tbody>
</table>

Data in the same raw with different superscript are significantly different (P < 0.05)

Table (3): Effect of Spirulina platensis on hematological parameters of O.niloticus fed practical diets containing different levels of spirulina after 6 weeks:

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (I)</th>
<th>Spirulina 0.5 % ( II)</th>
<th>Spirulina 1% (III)</th>
<th>Control infected (IV)</th>
<th>Spirulina 0.5% infected (V)</th>
<th>Spirulina 1% infected (VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs x 10^9/µl</td>
<td>1.07±0.04^cd</td>
<td>1.92±0.04^ab</td>
<td>2.53±0.92^a</td>
<td>0.9±0.2^d</td>
<td>1.7±0.40^bc</td>
<td>2.08±0.80^ab</td>
</tr>
<tr>
<td>WBCs x 10^9 /µl</td>
<td>26.2±0.40^f</td>
<td>35.8±0.08^e</td>
<td>64.6±0.20^e</td>
<td>26±0.28^d</td>
<td>84.8±0.28^d</td>
<td>120±0.17^a</td>
</tr>
<tr>
<td>Neutrophils x 10^9/µl</td>
<td>8.7±0.40^f</td>
<td>14.23±0.13^d</td>
<td>32.69±0.39^c</td>
<td>33.6±0.34^c</td>
<td>40.84±0.48^b</td>
<td>52.4±023^a</td>
</tr>
<tr>
<td>Lymphocytes x 10^9/µl</td>
<td>11.5±0.28^d</td>
<td>11.51±0.29^d</td>
<td>11.6±0.35^d</td>
<td>16.9±0.51^c</td>
<td>20.88±0.50^b</td>
<td>30.6±0.34^a</td>
</tr>
<tr>
<td>Monocytes x 10^9/µl</td>
<td>2.7±0.40^f</td>
<td>5.86±0.49^e</td>
<td>14.31±0.17^c</td>
<td>8.2±0.11^d</td>
<td>18.24±0.14^b</td>
<td>33.6±0.35^b</td>
</tr>
<tr>
<td>Eosinophils x 10^9/µl</td>
<td>2.1±0.06^a</td>
<td>2.05±0.02^a</td>
<td>2.64±0.37^a</td>
<td>2.2±0.11^a</td>
<td>2.1±0.16^a</td>
<td>2.2±0.12^b</td>
</tr>
<tr>
<td>Basophils x 10^9/µl</td>
<td>1±0.28^b</td>
<td>1.45±0.03^b</td>
<td>2.64±0.36^a</td>
<td>1.11±0.05^b</td>
<td>1.52±0.30^b</td>
<td>1.2±0.12^b</td>
</tr>
</tbody>
</table>

Data in the same raw with different superscript are significantly different (P < 0.05)
Table (4): Effect of Spirulina platensis on immunity and oxidative stress in serum of O.niloticus fed practical diets containing different levels of spirulina after 4 weeks

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (I)</th>
<th>Spirulina 0.5% (II)</th>
<th>Spirulina 1% (III)</th>
<th>Control infected (VI)</th>
<th>Spirulina 0.5% infected (V)</th>
<th>Spirulina 1% infected (VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysosome activity</td>
<td>7.95±0.15°</td>
<td>9.3±0.288°</td>
<td>11.35±0.23°</td>
<td>8.16±0.20°</td>
<td>9.4±0.80°</td>
<td>11.24±0.69°</td>
</tr>
<tr>
<td>Bactericidal activity</td>
<td>46.35±0.85°</td>
<td>61.1±0.55°</td>
<td>68.00±0.52°</td>
<td>46.33±0.44°</td>
<td>63.4±1.72°</td>
<td>68.16±0.4°</td>
</tr>
<tr>
<td>GPx</td>
<td>0.12±0.05°</td>
<td>0.14±0.01ab</td>
<td>0.18±0.01°</td>
<td>0.11±0.01°</td>
<td>0.13±0.02abc</td>
<td>0.17±0.02abc</td>
</tr>
<tr>
<td>SOD</td>
<td>0.47±0.05°</td>
<td>0.7±0.02</td>
<td>0.80±0.02</td>
<td>0.48±0.03</td>
<td>0.69±0.05ab</td>
<td>0.82±0.06a</td>
</tr>
</tbody>
</table>

Data in the same raw with different superscript are significantly different (P < 0.05)

Table: (5) Effect of Spirulina platensis on immunity and oxidative stress in serum of O.niloticus fed practical diets containing different levels of spirulina after 6 weeks

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (I)</th>
<th>Spirulina 0.5% (II)</th>
<th>Spirulina 1% (III)</th>
<th>Control infected (VI)</th>
<th>Spirulina 0.5% infected (V)</th>
<th>Spirulina 1% infected (VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysosome activity</td>
<td>8.69±0.29°</td>
<td>10.05±0.10°</td>
<td>11.83±0.20°</td>
<td>14.9±0.23°</td>
<td>17.73±0.24°</td>
<td>20.80±0.34°</td>
</tr>
<tr>
<td>Bactericidal activity</td>
<td>46.86±0.54°</td>
<td>62.63±0.40</td>
<td>70.66±0.33</td>
<td>64.86±0.38</td>
<td>70.8±0.26°</td>
<td>83.8±0.45°</td>
</tr>
<tr>
<td>GPx</td>
<td>0.11±0.01°</td>
<td>0.15±0.01</td>
<td>0.18±0.01</td>
<td>0.19±0.01</td>
<td>0.27±0.01</td>
<td>0.31±0.01</td>
</tr>
<tr>
<td>SOD</td>
<td>0.48±0.03°</td>
<td>0.73±0.02</td>
<td>0.85±0.03</td>
<td>0.81±0.04</td>
<td>0.92±0.03</td>
<td>1.09±0.01</td>
</tr>
</tbody>
</table>

Data in the same raw with different superscript are significantly different (P < 0.05)

Table: (6) Mortality and relative level of protection of experimental O. niloticus at end of the study of feeding supplemented diet with Spirulina after challenged with Pseudomonas fluorescens

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (I)</th>
<th>Spirulina 0.5% (II)</th>
<th>Spirulina 1% (III)</th>
<th>Control infected (IV)</th>
<th>Spirulina 0.5% infected (V)</th>
<th>Spirulina 1% infected (VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dead fish</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>15</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Mortality %</td>
<td></td>
<td></td>
<td></td>
<td>75%</td>
<td>40%</td>
<td>20%</td>
</tr>
<tr>
<td>RLP %</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>25.66%</td>
<td>73.34%</td>
</tr>
</tbody>
</table>

Discussion
The blood parameters as leucocytic counts and differential leucocytic counts have diagnostic importance and usually readily respond to identical factors such as physical, chemical and biological stressors (Hicky, 1976 and Soliman, 1996). Erythrocytes are a major and reliable indicator of various sources of stress (Rehulka 1989). In the present study, fish fed on diets containing spirulina exhibited higher RBCs counts. These results in agreement with Abdel-Tawwab et al (2008) who reported that the highest RBCs counts were obtained
when *Oreochromis niloticus* fed on diets containing 5.0 - 10.0 g spirulina/kg diet. Also, Ragab (2009), Andrews et al (2011) and Promya and Chitmanat (2011) who reported that the erythrocyte count was significantly higher in fish fed on diets containing spirulina supplementation compare to control group. Our results may be due to spirulina has 14% phycocyanin and it stimulates the erythropoietin hormone production for hematopoesis (Henrikson, 1994). And/or the increase of RBCs counts is a response to tolerate stress or on the other hand is a measure to maintain general health (Sivagurunathan et al, 2012). The results after 8 weeks showed that RBCs counts were decreased in all infected groups, the lowest count were obtained in the control infected (IV). The decrease in RBCs counts may be attributed to observe hemorrhages and red blood cell lysis (Wafaa, 2007) which results in severe anemia (James et al, 1992) And/or Erythrocytes are a major and reliable indicator of various sources of stress (Rehulka, 2000)

The WBCs counts after 4 weeks had the highest values during addition of spirulina. Also, there was increasing in the neutrophils counts followed by monocytes during the addition of spirulina. Also, after 6 weeks WBCs, neutrophils, monocytes and lymphocytes counts were increased in all infected groups. The results are in agreement to data obtained by (Gupta et al, 1979 and Edvington et al, 1994) who cleared that fish not received any immunostimulants or live under stress conditions showed decreased leucocytes count and increase susceptibility to infection. In the same line with Andrews et al, (2011) who stated that the leucocytic count was significantly higher in *Labeo rohita* fingerlings fed on diets containing spirulina supplementation compare to control group. The high level of WBCs, monocytes, and lymphocytes in infected groups in the agreement with Wafaa (2007) who cleared that the infected fish with *Pseudomonas fluorescens* showed that significant increase in total leucocytic count as well as increased lymphocytic count. Moreover Abdel-Tawwab et al (2010) observed an increase in WBC and Lymphocyte counts in Nile Tilapia fed with feed incorporated with Green Tea and infected with *Aeromonas hydrophila*. Similar increase in WBC, neutrophils, lymphocytes and monocytes were observed in *Cirrhinus mrigala* fed with feed supplemented with Ginger and Turmeric and infected with *P. aeruginosa* by Sivagurunathan et al (2012). The obtained results may be due to there is evidence that c-phycocyanin and polysaccharides of spirulina enhance white blood cell production (Qureshi and Ali, 1996). Studies have shown that phycocyanin affects the stem cells
which found in the bone marrow. Stem cells are the “grandmother” of both white blood cells that make up the cellular immune system and the red blood cells that oxygenate the body (Kithja, 2005). Also, Hayashi et al (2006) cleared that the spirulina extract, hot-water extract, Phycocyanin, and cell-wall component extract, enhanced proliferation of bone marrow cells in mice. The high level of WBCs, monocytes, and lymphocytes in infected groups may be due to leucocytes are centrally involved in phagocytic and as immune responses to parasitic, bacterial, viral and similar challenges (Houston, 1990). Thus increase in the TLC, neutrophils, monocytes and lymphocytes in spirulina incorporated diet fed fishes can be attributed to the non-specific immune response and increase in lymphocytes may be a specific pathogen induced immune response.

Lysozyme is an enzyme found in a wide range of vertebrates including fish and is one of the defensive factors against invasion by microorganisms as it act as non-specific component of innate immunity (Hansen, 1974).

In the present study, the results showed that after 4 weeks serum lysozyme activity was significant in all fish groups given basal diet supplemented with S. platensis. Results were in accordance with (Khalil et al, 2007; Ragab et al; 2012 and Promya and Chitmanat, 2011) who reported that spirulina enhanced responses of lysozyme activity of fish. The increase in the immunity stimulating capacity (measure by a lysozyme activity assay) could be due to the presence of C-phycocyanin in the spirulina alga, which can help build the immunity capacity (Vonshak, 1997). After 6 weeks the serum lysozyme activity in non-infected groups were increased by increase of spirulina supplementation in diet. While the challenged groups showed highest values than non-challenged groups. Results were in accordance with (Manal et al, 2011) that cleared that lysozyme activity in serum samples of P. florescence challenged groups of Nile tilapia was elevated. The elevation of lysozyme in infected groups may be due to high lysozyme activity may be desirable in cultured fish because it may aid against infection when fish are kept at high densities and consequently are exposed to high bacterial loads (Grinde et al, 1988). And/or in fish, lysozyme is an enzyme with antibiotic properties that is released by leucocytes, has a broader spectrum activity than mammalian lysozyme (Demers and Bayne, 1997).

In this study bactericidal activity after 4 and 6 weeks increased significantly with the increase of spirulina supplementation. Moreover, the bactericidal activity in challenged groups showed highest values than non-challenged
groups, in which the highest values of bactericidal activity at 1% spirulina group (VI). This result matches with (Khalil et al, 2007, Abdel-Tawwab et al, 2008 and Ragab et al, 2012) who reported that spirulina enhanced responses of bactericidal activity of Oreochromis niloticus. These results may be attributed to the increase in phagocytosis in blood, which have an important role for prevention of infectious disease (Mohan et al, 2006).

In the present study, the results showed that After 4 weeks serum GPx and SOD showed highest values at spirulina 1%group (III), while the lowest values were obtained at control group (I) This result agreed with (Lin et al, 2010 and Tayag et al, 2010) who stated that White shrimp L. vannamei that had received S. platensis had enhanced innate immunity by significantly increased SOD activity, a GPx activity. These results may be due to antioxidant property of Spirulina, earlier studies reported that Spirulina possess significant antioxidant properties as it is rich in carotenoids, flavonoids which scavenge free radicals (Bhat and Madyatha, 2000). Selenium present in spirulina induced selenium containing enzyme Glutathione peroxidase (GPx), an enzyme catalyzes the reduction of H$_2$O$_2$ and hydroperoxides to non-toxic products (Henrikson, 1989).

After 6weeks the serum GPx and SOD in non infected groups were increased by increase of spirulina supplementation in diet, however challenged groups showed highest values than non challenged groups, in which the highest values of serum GPx and SOD were obtained at 1% spirulina group(VI). These results agreed with (Manal et al, 2011) who cleared that Nile tilapia post bacterial challenge showed that the antioxidant enzyme levels in sera of Pseudomonas florescence challenged group were elevated than control. These results may be due to that pathogenic bacteria could trigger an oxidative stress through-which a group of multifunctional antioxidant enzymes are involved in the detoxification and effective removal of both excessive reactive intermediates and oxygen radicals (Olsvik et al, 2006). These intermediates are responsible for oxidation of biological membranes leading to altered physiological condition, metabolic dysfunction and could ultimately predispose to death (Olsvik et al, 2006).

The challenge infection revealed a significantly lower mortality percentage in the group received 10 g/kg S. platensis in diet (group VI) and significantly high relative level of protection (RLP) after challenge infection using P. fluorescens. These results may be due to spirulina contains phytopigments such as phycobilins, phycocyanin and allophycocyanin, and
xanthophylls, which seem to be related to its antioxidant activity (Miranda et al, 1998 and Bhat and Madyastha, 2000).

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**الملخص العربي**

دراسات مناعية متقدمة على تأثير السبيرولينا في سمك البلطي النيلي

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مازال استخدام الطحالب الدقيقة في مجال تغذية الأسماك يتم على نطاق ضيق. وقد أجريت هذه الدراسة بهدف دراسة التأثيرات الناتجة عن إضافة طحلب السبيرولينا (السببورولا بلانتسيس) لأعلاف أسماك البلطي النيلي بمستويات مختلفة من مسحوق الطحالب. وقد استخدم في هذه الدراسة 270 سمكة بلطي نيلي تم تجميعها من مزرعة مركز بحوث الأسماك بالعباسة بأبوحماد شرقية وكان متوسط وزن السمكة الواحدة 1.5 جرام تقريباً وقد أعطيت الأسمك إلى المعمل بقسم أمراض ورعاية الأسماك بكلية الطب البيطري جامعة قناة السويس في أكياس بلاستيكية مزودة بنسبة أكسجين 0.3%.

تم استخدام 3 علاقات تجريبية بإضافة مسحوق الطحالب في علاقات الأسماك المتزنة بنسبة مختلفة. وقد تم تقسيم الأسماك إلى ست مجموعات بحيث كل مجموعة أسماك مقسمة إلى ثلاث مكررات لكل معاملة لكل مكرر يحتوي كل مكرر على 50 سمكة في أحواض زجاجية وذلك لمدة ستة أسابيع وترتكب هذه الأسماك للأزمة الأولي ببداية التجربة.

- المجموعة الأولى: تم تغذيتها على العليقة الأولى والتي تتكون من عيلقة أسماك متزنة مضاف إليها نسبة 0.5% من مسحوق نفس الطحلب.
- المجموعة الثانية: تم تغذيتها على العليقة الثانية والتي تتكون من عيلقة أسماك متزنة مضاف إليها نسبة 1% من مسحوق نفس الطحلب.
- المجموعة الثالثة: تم تغذيتها على العليقة الثالثة والتي تتكون من عيلقة أسماك متزنة مضاف إليها نسبة 2% من مسحوق نفس الطحلب.
- المجموعة الرابعة: تم تغذيتها على العليقة الأولى والتي تتكون من عيلقة أسماك متزنة بدون إضافة مسحوق نفس الطحلب لها وعُرضت للعدوى بميكروب السواد موناس فلوسرس في الغشاء البوليمر بجرعة 2.0 مل في نهاية التجربة.
- المجموعة الخامسة: تم تغذيتها على العليقة الثانية والتي تتكون من عيلقة أسماك متزنة مضاف إليها نسبة 0.5% من مسحوق نفس الطحلب وعُرضت للعدوى بنفس الميكروب في نهاية التجربة.
المجموعة السادسة: تم تغذيتها على العليقة الثالثة والتي تتكون من علائق أسماك مزنة مضافة إلى النسبة 1% من مسحوق نفس الطحلب وعرضت للعدوى ببنفس الميكروب في نهاية التجربة، وآسفرت هذه الدراسة عن النتائج التالية:

لوحظ أن الإرتفاع واضح في خلايا النتروفيل والالتهابية ولتلوثا في الخلايا مع تقدم التجربة خاصة في المجموعات المعالجة مع زيادة نسبة إضافة مسحوق الطحالب لل العليقة الأسماك بالمقارنة بالمجموعة الأولى وذلك قبل العدوى أما بعد العدوى فلموحظ زيادة زيادة الخيالا النتروفيل والالتهابية والمفاوية. كما أسفرت النتائج المناعية عن زيادة شبات إنزيم سيرم الدم الليزوزيم تدريجيا مع زيادة نسبة مسحوق طلحب السبليرونينا إلى علاق الأسماك بالمقارنة بالمجموعة الضابطة. و بعد العدوى كان هناك ارتفاعا واضحا في نسبة إنزيم الليزوزيم في المجموعة السادسة عن المجموعة الرابعة. أوضحت النتائج أن النشاط القاتل للبكتيريا في سيرم الدم زيادة تدريجية مع زيادة نسبة مسحوق طلحب السبليرونينا إلى علاق الأسماك. و بعد العدوى كان هناك ارتفاعا واضحا في النشاط القاتل للبكتيريا في سيرم الدم في المجموعة السادسة عن المجموعة الرابعة. و كان هناك أيضا ارتفاعا في الانزيمات المضادة للأكسدة وهي جلوتاثيون بروكسيداز و سوبراوكسيد ديميتوتاز في المجموعة الثالثة والثانية عن الضابطة، و بعد العدوى كان هناك ارتفاعا واضحا في المجموعة الرابعة.

تم رصد نسب النفوذ ومعدل الحماية النسبى ولاحظ انخفاض في نسب النفوق مع ارتفاع معدل الحماية بالأسماك المغذة علائق السبليرونينا مقارنة بالمجموعة الضابطة.