IMPACTS OF *SPIRULINA PLATENSIS* IN FISH DIETS ON GROWTH PERFORMANCE AND IMMUNITY OF *OREOCHROMIS NILOTICUS* AND THE ANTIMICROBIAL ACTIVITY OF SPIRULINA EXTRACTS

Reham A. E. Abd - El hay[1]; Walaa T. El Ekiaby[2] and Samah A.A.[2]

[1]*Limnology Dept., Central Lab. for Aquaculture Research, Agricultural Research Center.*

[2]*Fish health Dept., Central Lab. for Aquaculture Research, Agricultural Research Center.*

Received 17/ 2/ 2016 *Accepted 5/ 3/ 2016*

Abstract

This study was conducted in two experiments. The first experiment aimed to test *Spirulina* extracts against pathogenic bacteria by three types of solvents "petroleum ether ,diethyl ether and acetone" The second experiment aimed to evaluate of the use of *Spirulina* at different rates "10-15 and 20 g/kg feed" on growth performance of *Oreochromis niloticus* and fish immunity (Heamatocrite, Lysozome, Respiratory Brust and Total Antioxidant Capacity).The results clear that: First :the acetone extract had significantly antibacterial against bacteria. Second: The addedtive of *Spirulina* in fish diets were significantly increased growth performance of Nile Tilapia. The 15g *Spirulina* in fish diets were significantly increased of fish immunity. The permissible level of *Spirulina* to fish diets was 15g/kg feed. The study recommended using *Spirulina* in fish diets on a commercial scale.

INTRODUCTION

Spirulina are multicellular and filamentous blue-green microalgae belonging to two separate genera *Spirulina* and *Arthrospira* and consists of about 15 species. *Spirulina* has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquaculture diets. It grows in water, can be harvested and processed easily and has very high macro- and micro-nutrient contents. It has long been used as a dietary supplement by people living close to the alkaline lakes where it is

naturally found for instance those living adjacent to Lake Chad in the Kanem region have very low levels of malnutrition, despite living on a Spartan millet-base diet. This traditional food, was rediscovered in Chad by a European scientific mission, and is now widely cultured throughout the world.

Some researcher has revealed that *S. platensis* or its extract could show physiological benefits as antioxidant, antibacterial, anti-inflammatory, antiviral or antifungal (Careri *et al.* , 2001 and Subhashini *et al.,* 2004). Some volatile components and various extracts of Spirulina also showed antibacterial activities (Ozdemir *et al.,* 2004). Recently (Mendiola *et al.,* 2007) applied supercritical fluid extraction to obtain functional extracts with antioxidant and/or antimicrobial activities from *S. platensis*. Studies on antibacterial and antifungal effect of Spirulina as also studies against various organisms that incite diseases of plants and humans (Sharma *et al.,* 2005; Rania and Hala 2008) have reported the protective role of Spirulina feed in freshwater fish. In vitro antibacterial activity of laboratory cultured S. platensis was tested against one Gram-positive bacterium (*Staphylococcus aureus*) and four Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*).

Immunostimulants enhance the innate immune system, thereby preventing infectious diseases. In fish, several immunostimulants such as chitin (Sakai *et al.,* 1992 and Esteban *et al.,* 2001), lactoferrin (Sakai *et al.,* 1993), dimerized lysozyme (Siwicki *et al.,* 1998), substances play a promising role in aquaculture by enhancing the resistance of cultured fish against diseases.

Fish culture is increasing to compensate the shortage of animal protein all over the world. Fish under intensive culture conditions will be badly affected and often fall prey to different microbial pathogens that have been treated with chemotherapeutic substances of which antibiotics were intensively used. These curative substances produce the problem of bacterial drug fastness on one hand and the public health hazards on the other hand (Odowd and Austin, 2000). These awaited drawbacks enforced the fish pathologists to seek for other

alternatives; the use of natural immunostimulants in fish culture for the prevention of diseases is a promising new development and could solve the problems of massive antibiotic use. Natural immunostimulants are biocompatible, biodegradable and safe for both the environment and human health. Moreover, they possess an added nutritional value (Jessus *et al.*, 2002).

The aim of the present study is directed to:

1- Study the ability of *Spirulina plantensis* extracts to inhibit microbial growth.
2- Natural immunostimulants of Nile tilapia fish after feeding on *Spirulina plantensis*.

MATERIALS AND METHODS

This study was carried out in Central Laboratory for Aquaculture Research at Abbassa, Agriculture Research Center, Egypt.

Cultivation of *Spirulina plantensis*:

In this study the used medium was *Spirulina* Medium (modified) (Aiba and Ogawa, 1977).The first isolation of *Spirulina plantensis* was obtained from botany department, faculty of science, Cairo university. *Spirulina plantensis* was cultivated in Aiba and Ogawa medium. The culture was incubated at 30 ± 2ºC under continuous illumination produced by white fluorescent light (3000-5000 lux). Alga was harvested at the late exponential growth phase.

Harvesting, extraction and processing the algal biomass:

In stationary phase the algal culture reached maximum growth, in this case the circulation provided by the pumping system was stopped and the algal cells harvested by filtrated the carboy using a cheese cloth of cotton or nylon. The algal cells (Cyanophyta) were dried in oven at 60ºC and ground in an electric coffee mill. Resulting powder was submitted to lipid-soluble extraction with (petroleum ether, diethyl ether and acetone) 1: 10 (p: v) using a soxhlet extractor at 55-60ºC all samples were refluxed until saturation (24 hours) and the respective extracts were dried in an oven at 50ºC or rotavapor (José Vitor *et al.*, 2002).

Test microorganisms:

Gram negative bacterial strains *Aeromonas hydrophila, A. jandaei*, *A. sobria* and *Pseudomonas sp.* and gram positive *Staphylococcus aureus* were used for this study. These microorganisms were isolated from Abbassa fish farm.

Tests for antimicrobial activity after extraction of *Spirulina plantensis* by solvents (*In vitro*):

Using Paper disk assay:

The sterilized tryptic soy agar medium poured in sterilized Petri dishes. After solidification the plates were inoculated with 0.1ml of fresh bacterial suspension, immediately sterilized paper discs impregnated with 30 µl of the extracted materials and air dried, were placed over the agar surface, this process was done for each extract on the surfaces of bacteria. The plates incubated for 24 hrs and examined for inhibition zones. Also impregnate sterilized paper discs in the solvents that used in extraction and put in the surface of agar as a control.

Experimental design:

A total of 120 with individual initial weight (16 ± 2 g/fish*) Oreochromis niloticus* the fish were acclimatized for one week divided in twelve glass aquaria (210 L/aquaria) and supplied with continuous aeration using air pumping compressors. Fish were allocated into 4 groups (30 fish / group); each in three replicates (10 fish / aquarium).

Experimental diets preparation:

Commercial basal diet (crude protein 36 %) was crushed, and then divided into four treatments. The first treatment was mixed with (10 g/kg) dried pure powder of *Spirulina plantensis*. The second treatment was mixed with (15 g/kg) dried pure powder of *Spirulina plantensis*. The third treatment was mixed with (20 g/kg) dried pure powder of *Spirulina plantensis* and the fourth

treatment was mixed with *Spirulina* medium free from dried algae (control treatment). The diet was reformed into pellets, spread to air dry and stored at 4˚C. Fish were hand-fed twice daily in 60 days, at a rate of 3.5% of body weight. Quarter volume of aquaria water were siphoned daily.

Table 1. Composition and proximate chemical analysis of experimental diet

Ingredient	%
fish meal	72
Soybean meal	44
yellow corn	7.7
vegetable oil	0.2
Corn gluten	60
Fish oil	0.2
milling wheat	16
Chemical analysis of diet	
Energy kcal/kg	4013
crude protein	36
crude fat	6.45
crude fiber	4.17

The fish were weighed at day 15, 30, 45 and 60 biweekly during the experimental period.

Growth Performance:

Fish of all replicates were weighed individually and their body weight gain was measured. Specific growth rate (SGR) was calculated according to Goodwin *et al.* (2007). SGR = Ln [final mean body weight (g)]-Ln [initial mean body weight (g)] ÷ time interval (days) × 100

Blood and serum sample for immunity assay:

In the second, fourth, sixth and eighth week of the feeding experiment, the fish were anaesthetized by immersing the fish in water containing 0.1 ppm tricaine methane sulphonate (MS-222). Blood-samples were collected as pooling from the caudal vein of fish, by using needles previously rinsed in heparin (15 unit / ml) for the evaluation of haematocrit and respiratory burst activity. For plasma separation, the heparinzed blood was centrifuged at 3000

rpm for 5 minutes. The plasma was stored at -20˚C in screw cap glass vials until used for lysozyme.

Haematocrit level:

Haematocrit level is a method used to determine the volume of packed cells in the blood. The haematocrit value will vary depending on the health and the physiological condition of the individual fish. Haematocrite capillary tubes were used and measured according to (Purves *et al.*, 2004).

Respiratory burst activity by measuring Nitro Blue Titrazolium activity (NBT):

The NBT (yellow) is reduced to formazan (blue) in the reaction with oxygen radicals from neutrophils and monocytes. The production of oxygen radicals analyzed using NBT can be done by spectrophotometer. According to (Siwicki *et al.*, 1985).

Serum Lysozyme activity:

The lysozyme activity was measured using photoelectric colorimeter with attachment for turbidity measurement according to (Schaperclaus *et al*., 1992).

Total antioxidant capacity

The muscle homogenates (10% w/v) prepared in ice-cold 150mM KCl were used for in vitro assays. Homogenate was centrifuged at 1000 x g for 15 min and supernatants (cell free homogenate) were stored at -70°C until used. The most common total antioxidant capacity tests were summarized by Rice-Evans (2000).

Statistical analysis:

Statistical analysis was performed using the one way analysis of variance (ANOVA). It was performed with SPSS statistical software (version 10.0, SPSS). The data were subjected for test of homogeneity of variances and

Duncan test and were considered significantly different when $P \leq 0.05$ as described by Dytham (1999).

RESULTS

Antimicrobial activities of *Spirulina plantensis* extracts:

The antimicrobial activities of *Spirulina platensis* petroleum ether extract against different tested bacteria:

As shown in Table (2) Petroleum ether extract of *Spirulina platensis* had large inhibition zone with *Aeromonas sobria, Staphylococcus aureus and Pseudomonase sp.* where the diameter of inhibition zones were 34.3, 27.7 and 26.3mm respectively.

Table 2. The antimicrobial activities of *Spirulina platensis* petroleum ether extract against different tested bacteria (inhibition zone measured as mm).

Bacterial species	Algal petroleum ether extract (30µl)	
	Spirulina platensis	**Control**
Aeromonas hydrophila	0.0	0.0
Aeromonas sobria	34.3	9
Aeromonas Jandaei	0.0	9
Pseudomonase sp.	26.3	9
Staphylococcus aureus	27.7	0.0

The antimicrobial activities of *Spirulina platensis* diethyl ether extract against different tested bacteria:

Diethyl ether extract of *Spirulina platensis* had inhibition zones only with *Aeromonas sobria and A. Jandaei* with diameter inhibition zones 38.3 and 12.7 mm. while, no response to *Spirulina platensis* diethyl ether extract only (control) except in *Aeromonas sobria* had inhibition zone with diameter 19.3mm. as shown in Table (3).

Table 3. The antimicrobial activities of *Spirulina platensis* diethyl ether extract against different tested bacteria (inhibition zone measured as mm).

Bacterial species	Algal diethyl ether	
	Spirulina platensis	**Control**
Aeromonas hydrophila	0.0	0.0
Aeromonas sobria	38.3	19.3
Aeromonas Jandaei	12.7	0.0
Pseudomonase sp.	0.0	0.0
Staphylococcus aureus	0.0	0.0

The antimicrobial activities of *Spirulina platensis* acetone extract against different tested bacteria:

Acetone extract of *Spirulina platensis* had inhibition zones with all examined bacteria 29.3, 12.7, 24.3, 21.7 and 17.7mm with *Aeromonas hydrophila, Aeromonas Jandaei, Pseudomonase sp., Aeromonas sobria and Staphylococcus aureus* respectively. The control had no effect on the bacteria as shown in Table (4).

Table 4. The antimicrobial activities of *Spirulina platensis* acetone extract against different tested bacteria (inhibition zone measured as mm).

Bacterial species	Algal acetone extract (30µl)	
	Spirulina platensis	**Control**
Aeromonas hydrophila	29.3	0.0
Aeromonas sobria	21.7	0.0
Aeromonas Jandaei	29.3	0.0
Pseudomonase sp.	24.3	0.0
Staphylococcus aureus	17.7	0.0

Growth Performance:

Body weight (BW):

The means of body weights gain for the experimental treatments are presented in Fig.. (1) after four weeks from the begging of feeding experiment .

T2 showed higher significant ($p \leq 0.05$) live body weight than the other experimental treatments.

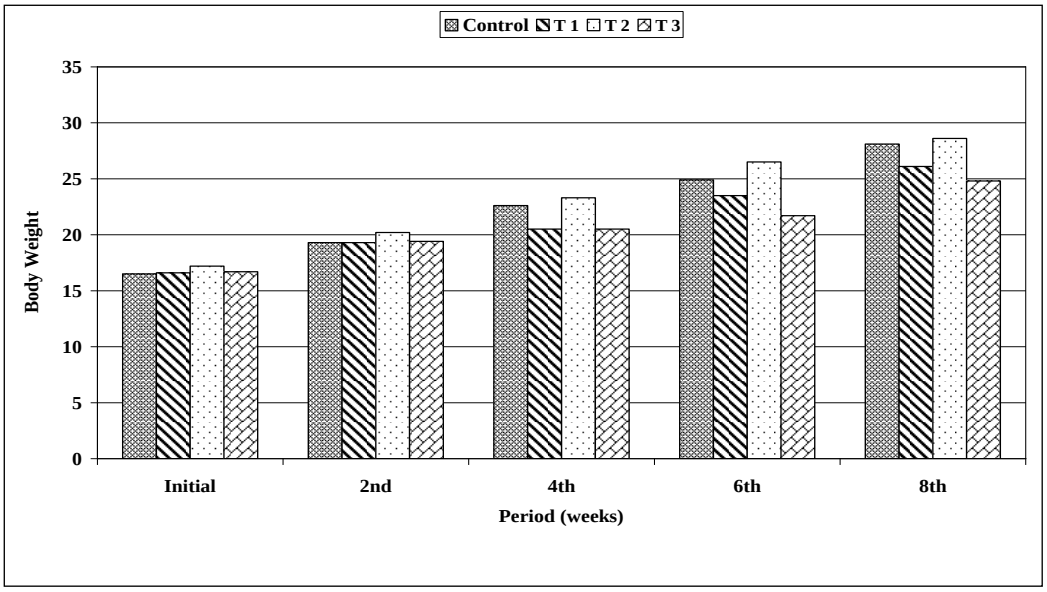

Fig.. 1. The average body weight of Nile Tilapia biweekly under different rate of *Spirulina* in fish diets during the experimental period.

From the presented data in Fig. (1), the average body weight were significantly increased with increasing period **.** Also, show the body weight in treated 3 were significantly increased ($p \leq 0.05$) than other treatment.

Specific Growth Rate (SGR %):

In the Fig.(2) there was increased in the specific growth rate % in T_2 and followed by the control in the second week, but in the fourth week the SGR% decreased in the three treatments T2 > T1 >T3. After that happened decreased in SGR% of the control in the 6^{th} and 8^{th} week, increased in SGR% of T1, T2 in the 6^{th} week and in T1 and T3 in the 8^{th} week.

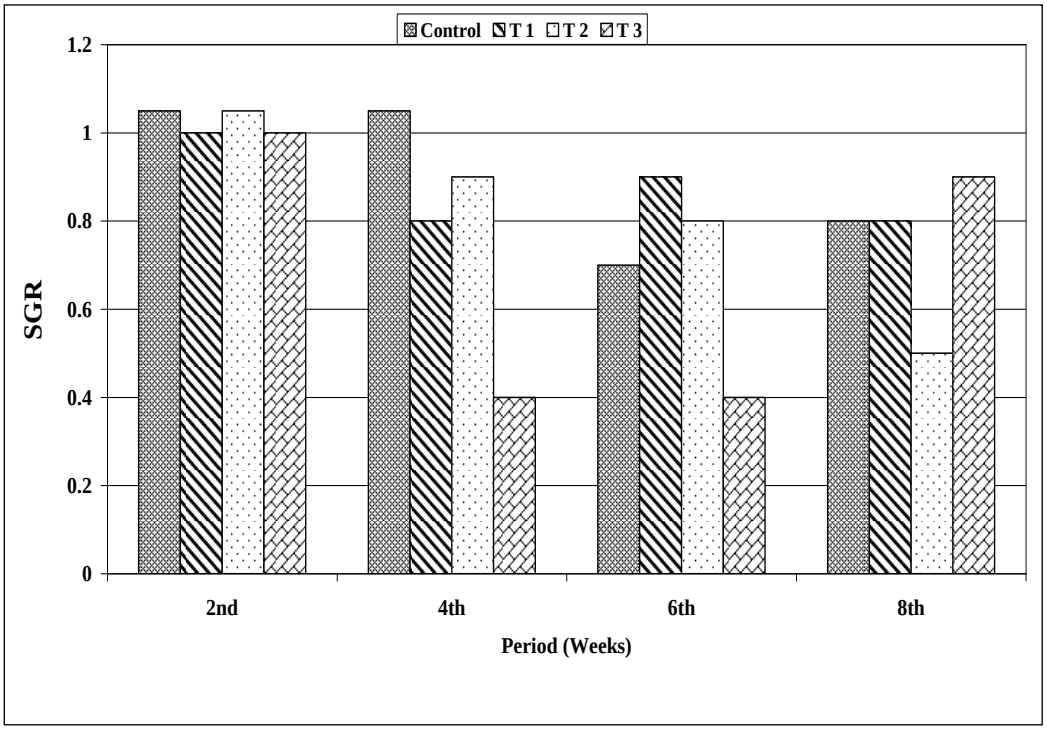

Fig. 2. The average SGR of Nile Tilapia biweekly under different rate of *Spirulina* in fish diets during the experimental period.

Blood and serum sample for immunity assay:

Haematocrit Value

From Table (5), the haematocrit values ranged from 23.3 ± 6 to 63.7a ± 0.88% among the three treatments in the fourth week of feeding experiment. The haematocrit value increased significantly in the fourth week of feeding experiment with T1 and T2 than T3. Haematocrit value was high in T2 than control in 2^{nd}, 3^{rd} and 4^{th} week.

Table 5. Effect of different treatments on Haematocrit values (%).

	2nd week	4th week	6thweeks	8th weeks
Control	26.7 ± 0.88	22.7^{c} ± 1.45	38.7^{b} ± 3.75	24.7^{c} ± 0.33
T 1	30 ± 0	45^{b} ± 7.6	35^{b} ± 2.9	57.7^{a} ± 4.33
T 2	32.7 ± 4.3	61.7^{a} ± 6	62.7^{a} ± 1.45	63.7^{a} ± 0.88
T3	23.3 ± 6	32.7^{bc} ± 1.45	37.7^{b} ± 1.45	32.3^{b} ± 1.45

Means carrying different superscripts are significant at (p≤ 0.05) in the same column.C= basal diet free from spirulina in fish diets. T1= Fish fed basal diet supplemented with 10g/kg spirulina in fish diets. T2= Fish fed basal diet supplemented with 15g/kg spirulina in fish diets. T3= Fish fed basal diet supplemented with 20g/kg spirulina in fish diets.

Respiratory burst activity:

The results from Table (6) showed that no significant difference in the NBT value between the control (0.66 ± 0.02) and the three treatments. T1 (1.21 ± 0.03) and T2 (1.22 ± 0.03) were high significant than the value in control (0.94 ± 0.07) in the 4th week of feeding with *Spirulina plantensis* supplemented diet. In the 6th week of feeding experiment, there was no significant difference in NBT between the control and the three treatments, while the control group decreased significantly in 4th and 8th week (0.94 ± 0.07) and (0.84 ± 0.3) respectively. Generally, T1, T2 had respiratory burst higher than control and T3 from the 4th to 8thweek of feeding experiment.

Table 6. Effect of different treatments on NBT values (%).

	2ndweek	4th week	6th week	8th week
Control	0.66 ± 0.02	0.94^{b} ± 0.07	0.99 ± 0.09	0.84^{b} ± 0.3
T 1	0.62± 0.01	1.21^{a} ± 0.03	1 ± 0.05	1.49^{a} ± 0.3
T 2	0.59 ± 0.03	1.22^{a} ± 0.03	1.2 ± 0.09	1.09^{ab} ± 0.2
T3	0.65 ± 0.02	0.89^{b} ± 0.05	1.2 ± 0.3	0.9^{bc} ± 0.05

Means carrying different superscripts are significant at (p≤ 0.05) in the same column. C= basal diet free from spirulina in fish diets. T1= Fish fed basal diet supplemented with 10g/kg spirulina in fish diets. T2= Fish fed basal diet supplemented with 15g/kg spirulina in fish diets. T3= Fish fed basal diet supplemented with 20g/kg spirulina in fish diets.

Lysozme activity:

The result in Table (7) indicated that the initial value of lysozyme activity increased significantly at the second week of feeding experiment. T1 (0.84 ± 0.04) and T2 (0.95 ± 0.002) were high significant than the value in control (0.68 ± 0.09), while there was no significant difference in lysozyme activity between the control and the three treatments. Generally, T1 and T2 had lysozyme activity higher than control and T3 from the 4th week to 8thweek of feeding experiment.

Table 7. Effect of different treatments on Lysozme values (%).

	2nd week	**4th week**	**6th week**	**8th week**
Control	0.68^{ab} ± 0.09	0.67 ± 0.2	0.53 ± 0.02	0.45 ± 0.2
T 1	0.84^{a} ± 0.04	0.71 ± 0.02	0.54 ± 0.09	0.82 ± 0.05
T 2	0.95^{a} ± 0.002	0.53 ± 0.09	0.42 ± 0.14	0.65 ± 0.01
T3	0.65^{b} ± 0.009	0.47 ± 0.04	0.67 ± 0.11	0.62 ± 0.19

Means carrying different superscripts are significant at ($p \leq 0.05$) in the same column. C= basal diet free from spirulina in fish diets. T1= Fish fed basal diet supplemented with 10g/kg spirulina in fish diets. T2= Fish fed basal diet supplemented with 15g/kg spirulina in fish diets. T3= Fish fed basal diet supplemented with 20g/kg spirulina in fish diets.

Total antioxidant capacity(TAC):

From Fig. (3), revealed that TAC decreased nonsignificantly at first and second month of feeding of all treatment except T3 at second month increased nonsignificant comparing with control.

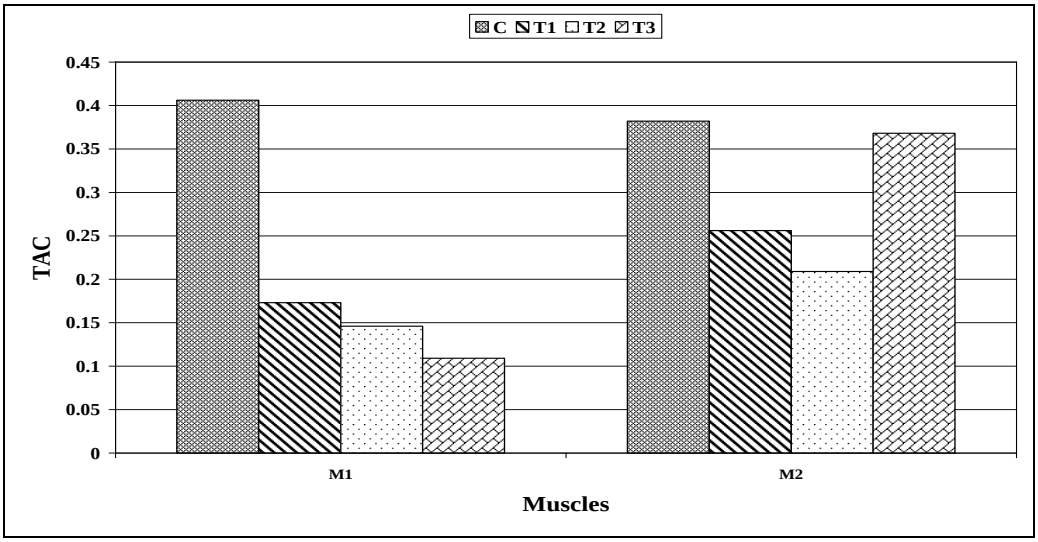

Fig. 3. Showing total antioxidant capacity (TAC) in muscle of tilapia fish during the experimental of feeding the fish during two months

DISCUSSION

Among the microalgae used as foodstuffs for food supplements and animal feed in many parts of the world, *Spirulina* spp is the most popular due to high nutrient values and cost effectiveness at the farm scale (Meng-Umphan, 2009). spirulina in diet of fish induce resistance of fish to environmental stress that it can be related to effective compounds attributed Spirulina powder (Khorshid *et al.,* 2014).

The antibacterial activity of algal compounds extracted from algae depends upon the type of solvent used for extraction. The present study revealed that the use of organic solvents in the preparation of algal extracts provide more consistent antimicrobial activity. Antimicrobials active lipids and active fatty acids are present in a high concentration in this alga. Lampe *et al.* (1998) who reported microorganisms was hypothesized by that lipids and kill it by leading to disruption of the cellular membrane as well as bacteria, fungi and yeasts because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration.

The antibacterial of *Spirulina platensis* extracts was determined against all tested bacteria. petroleum ether extract had antibacterial against (*Aeromonas sobria, Pseudomonase sp.* and *Staphylococcus aureus).* Diethyl ether extract of S. *platensis* had antibacterial effect against *Aeromonas sobria and A. Jandaei***.** and acetone extract of S. *platensis* had antibacterial against all tested bacteria. Our results are in agreement with those by (Kaushik and Chauhan, 2008) who reported that extracts of *Spirulina platensis* inhibited the growth of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumonia.* Parisi *et al.,* 2009 and Vinay Kumar *et al.* (2011) found that the high antimicrobial activity of *Spirulina platensis* extracts was against *Staphylococcus aureus* and *Salmonella yphimurium*. Usharani *et al.,* 2015 indicated that the *Spirulina platensis* extracts had antimicrobial activity against pathogenic bacteria and fungi used in there study.

Present investigations is contradictory with the results of other studies (Kaushik and Chauhan, 2008, El-Baky *et al.,* 2008, Vinay Kumar *et al.,* 2011). The enhanced antibacterial activity expressed in sequential extraction might be due to the fact that both hydrophobic and hydrophilic bioactive compounds were extracted .

In our study the treatment that supplement by 15 g/kg (T2) dry Spirulina to fish diets was the highest treatment in the increased the body weight of fish. the results in our study in agreement with Watanabe *et al.* (1990) and Takeuchi *et al.* (2002) who found that feed supplemented with *S. platensis* powder improved the feed conversion ratio and growth rates in striped jack, *Pseudocaranx dentex*. Lu *et al.* (2002) demonstrated that raw *S. platensis* can be an effective uni-feed for larval tilapia at a feeding rate of 30% (on a dry basis) of body weight. Abdel-Tawwab and Ahmed (2009) recorded that the growth and feed utilization of *O. niloticus* were obtained at 5 g fresh culture of *S. platensis* /kg diet. On the contrary, Ungsethaphand *et al.* (2010) recorded that the final weight gain, specific growth rate, feed conversion ratio of hybrid red tilapia were not affected by *S. platensis* supplementation. Mai *et al.,* (2013)

recorded that the optimum concentration of dried *S. platensis* in the *O. niloticus* practical diet is 10 g/kg for 2 months, to positively improving health conditions, enhanced the non-specific immunity of *Oreochromis niloticus*, as well as its resistance to challenge by *P. fluorescens* infections. It is recommended to supplement Spirulina in the diet of Nile tilapia especially those grow in farms under immunosuppressive/stressful conditions. Additional researches are needed to study additional desired effects of the blue green algae in cultured fish. These variations might be attributed to the difference in the *S. platensis* concentration to exert the intended effects, the form of *S. platensis*, raw or dried *S. platensis* or even its products, fish species and size in addition to the rearing conditions.

In the present study, higher hematocrit were observed in fish fed *spirulina* containing diets indicating better fish health condition in agreement with the results of studies by (Sung *et al.*, 2013) and Aysel *et al.*, 2015) on parrot fish and *O. niloticus* respectively.

The immunomodulatory activity of spirulina has been attributed to its content of C-phycocyanin (Venkataraman, 1997). Recent studies showed that polyphenolic compounds possess protective effects on immune system (Aquilano *et al.*, 2008; Franova *et al.*, 2010). In our study, significantly higher respiratory burst activity was found in all supplementation levels of spirulina in agreement with the results of studies by Watanuki *et al.* (2006), Abd el Tawwab and Ahmad (2009) and Sung *et al*.(2013) on the common carp *Cyprinus carpio* and Nile tilapia. In addition, Aysel *et al.* (2015) who showed a significant increase respiratory burst activity was found in the *O. niloticus* dietary spirulina 10g/kg. Duncan and Klesius (1996) who reported enhancement of the peritoneal phagocytes from channel catfish, *I. punctatus* fed *S. platensis*, Spirulina algae contain carotenoids, which specifically improving fish health and increasing the ability to Fig.ht off infections through the reduction of stress levels. Also, in our study, significantly higher lysozyme activities were detected for all supplementation levels of spirulina, and the highest activity was observed in T1 group. Lysozyme is found in a wide range of vertebrates, including fish and is

one of the defensive factors against invasion by microorganisms (Mesaeli and Phillipson, 2004). Results were in accordance with Tayag *et al.*, (2010) who concluded that the white shrimp *L. vannamei* that received the hot-water extract of *S. platensis* had enhanced innate immunity as lysozyme and increased resistance against *V. alginolyticus* infection. Similar results obtained by Ragap *et al.* (2012) mentioned that oral administration of *Spirulina platensis* at a dose of 10 mg / fish for 4 weeks to tilapia *(O. niloticus)* leads to enhanced lysozyme activities .Similarly, Promya and Chitmanat (2011) showed a significant increase in serum lysozyme activity in African sharptooth catfish *Clarias gariepinus* fed 3% or 5% dietary *spirulina.* The importance of TAC as a novel instrument to estimate the relationship between diet and oxidative stress-induced diseases, is presented in recent studies(Serafini *et al.*, 2002) and (Brighenti *et al.*, 2005). Total antioxidant capacity (TAC) may be an appropriate tool to determine the additive antioxidant properties of plant foods(Pellegrini *et al.*, 2003). In our study, higher TAC was found in T3 at 2nd month may be attributed to antioxidant activity of phenolic compounds such as salicylic, trans-cinnamic, synaptic, chlorogenic, quimic and caffeic acids found in the extract of *spirulina (*Vasudha *et al.,* 2009*)* .

Finally, from the present investigation, it was concluded that the extracts of the *Spirulina platensis* were determined antibacterial activity against all tested bacteria (*Aeromonas sobria, Pseudomonase sp.* and *Staphylococcus aureus).* Optimum concentration of dried *S. platensis* in the *O. niloticus* practical diet is 15g/kg for 2 months, to positively improving health conditions, enhanced the non-specific immunity of *Oreochromis niloticus.*

REFERENCES

Abdel-Tawwab, M. and M.H. Ahmad, 2009. Live spirulina (Arthrospira platensis) as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus* (L.), challenged with pathogenic *Aeromonas hydrophila*. Aquac Res 40, 1037-1046.

Aiba, S. and T. Ogawa, 1977. Assessment of growth yield of a blue-green alga: Spirulina platensis, in axenic and continuous culture. J. Gen. Microbiol. 102: 179-182.

Aquilano, K.; S. Baldelli; G. Rotilio, and M.R. Ciriolo, 2008. Role of nitric oxide syntheses in Parkinson's disease:a review on the antioxidant and antiinflammatory activity of polyphenols. Neurochemical Research, 33: 2416–2426.

Aysel, Ş; T .Oğuz; A. Fatmagün; Ö .Sevkan; E .Celal; D .Selçuk; U. Leyla.and Ö .Filiz, 2015. Determination of some hematological and nonspecific immune parameters in Nile Tilapia (*Oreochromis niloticus L*.) fed with Spirulina (*Spirulina platensis*) added diets. Journal of Aquaculture Engineering and Fisheries Research 1(3): 133-139.

Brighenti, F.; S.Valtueña; N.Pellegrini; D. Ardigo and D.Del Rio, 2005. Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high-sensitivity C-reactive protein in adult Italian subjects. Br J Nutr, (9): 619-625.

Careri, M.; L.Furlattini; A.Mangia; M.Musci; E.Anklam; A. Theobald and C.V. Holst, 2001. Supercritical fluid extraction for liquid chromatographic determination of carotenoids in Spirulina Pacifica algae.

Duncan, P.L. and P.H. Klesius, 1996. Effects of feeding Spirulina on specific and non-specific immune responses of channel catfish. Journal of aquatic animal, 8 (4): 308–313. Robertson P.A.W.,C.

Dytham, C., 1999. Choosing and Using Statistics: A Biologist's Guide. Blackwell Science Ltd., London, UK.

El-Baky, H.; K. Baz and G. Baroty, 2008. Characterization of nutraceutical compounds in blue green alga Spirulina maxima, J. Med. Pl. Res., 2: 292-300.

Esteban, M.; A.Cuesta; J.D. Ortuno and J.Meseguer, 2001. Immuno modulatory effects of dietary intake of Chitin on gilthead seabream (*Sparus aurata*) innate immune system. Fish shell fish immunol., 11: 303-315.

Franova, S.; M.Joskova; M. Sutovska; E. Novakova; K. Adamicova; O. Pechanova and G. Nosalova, 2010. The efficiency of polyphenolic compounds on allergen induced hyperreactivity of the airways. Biomedicine and Preventive Nutrition, 1: 232–235.

Goodwin, J.E.; M.Glaister; G.Howatson; R.A.Lockey and G.McInnes, 2007. .Effect of pre-performance lower-limb massage on thirty-meter sprint running. Journal of Strength and Conditioning Research, 21(4): 1028-1031.

Jessus, O.; A. cuesta; A. Rodriguez; A. Eesteban and J. Meseguer, 2002. Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the celluler innate immune response of gillhead seabream, *Sparus aurata* L. J. Veterinary immunology and immunopathology, 85: 41-50.

José Vitor; M. Lima-Filho; F.F.U. Ana; Carvalho; Sissi M. freitas and M.M. Vania, 2002. Antibacterial activity of some extracts of six macroalgae from The Notheastern Brazilian Coast. Brazilian J. of Mic., 33: 311-313.

Kaushik, P.and A. Chauhan, 2008. In vitro antibacterial activity of laboratory grown culture of Spirulina platensis. Ind. J. Microbial., 4: 348-352.

Khorshid, G.; H. Mohammadiazarm and H.Mabudi, 2014. Increases resistance of convict cichlid (*Amatitlania nigrofasciata*) to environmental stress by spirulina powder, International Journal of Biosciences, 5 (8): 128-134.

Lampe, M.F.; L.M. Ballweber; C.E.Isaacs; D.L Patton and W.E. Stamm, 1998. Killing of Chlamydia trachomatis by novel antimicrobial lipids adapted from compounds in human breast milk. Antimicro. Agents Chemo., 45: 1239-1244.

Lu, H.K.; V. Hsieh; J.J. Hsu; Y.K. Yang and H.N. Chou, 2006. Preventive effects of Spirulina platensis on skeletal muscle damage under exercise-induced oxidative stress. Eur J Appl Physiol, 98 (2): 220-226.

Mai, D.I.; F.M. Mohamed and A.I. Marwa, 2013. The Role of Spirulina platensis (*Arthrospira platensis*) in Growth and Immunity of Nile Tilapia (*Oreochromis niloticus*) and Its Resistance to Bacterial Infection Journal of Agricultural Science, 5 (6).

Mendiola, J.A.; L. Jaime; S.Santoyo; G.Reglero; A. Cifuentes; E. Ibanez and F.J. Senorans, 2007. Screening of functional compounds in supercritical fluid extracts from Spirulina platensis. Journal of food chemistry, 102: 1357 -1367.

Meng-Umphan, K., 2009. Growth performance, sex hormone levels and maturation ability of pla pho (*Pangasius bocourti*) fed with Spirulina supplementary pellet and hormone application. International Journal of Agriculture and Biology, 11: 458-462.

Mesaeli, N. and C.Phillipson, 2004. Impaired p53 expression, function, and nuclear localization in calreticulin-deficient cells. Mol Biol. Cell, 15: 1862-70.

Odowd, P.W. and B. Austin, 2000. Use of Carnobacterium sp. as a probiotic for atlantic salmon (*Salmo salar L.*) and rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture, 185: 235-243.

Ozdemir, G.; N.U. Karabay; M.C. Dalay and B.Pazarbasi, 2004. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytotherapy Research, 18: 754–757.

Parisi, A.S.; C.O. Younes; Reinehr and Colla L.M., 2009. Assessment of the antibacterial activity of microalgae *Spirulina platensis*.Revista de Ciências Farmacêuticas Básicae Aplicada, Araraquara, 30 (3): 97-301.

Pellegrini, N.; M. Serafini; B. Colombi and D. Del Rio; S. Salvatore 2003. Total antioxidant capacity of plant foods, beverages and oils

consumed in Italy assessed by three different in vitro assays. J. Nutr., 133: 2812–2819.

Promya, J. and C. Chitmanat, 2011. The effects of *Spirulina platensis* and Cladophora algae on the growth performance, meat quality and immunity stimulating capacity of the African Sharptooth Catfish (*Clarias gariepinus*). Int. J. Agric. Biol., 13: 77-82.

Purves, W.K.; D.Sadava; G.H. Orians; H.C. Heller, 2004. Life: The Science of Biology (7th Ed.). Sunderland, Mass: Sinauer Associates. p. 954. ISBN 0-7167-9856-5.

Ragap, H.M.; R.H. Khalil and H.H Mutawie, 2012. Immunostimulant effects of dietary *Spirulina platensis* on tilapia *Oreochromis niloticus.* Journal of Applied Pharmaceutical Science, 2 (2): 26-31.

Rania, M.A. and M.T. Hala, 2008. Antibacterial and antifungal activity of Cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman design for antimicrobial activity of *Spirulina platensis*. Global Journal of Biotechnology and Biochemistry, 3: 22– 31.

Rice-Evans, C., 2000. Measurement of Total Antioxidant Activity as a marker of antioxidant status in vivo: procedures and limitations. Free Rad Res., 33: S59-S66.

Sakai, M.; H. kamiya; S. Ishii; S. Htsuta and M. kobayashi, 1992. The immunostimulating effects of chitin in rainbow trout, *Oncorhynchus mykiss.* In: Shariff, M.; Subasighe, R.P. and Hrthur, J.R. (Eds.), Diseases in Hsian Hquaculture. Fish Health section, Hsion fisheries society, Manila, Philippines, 413-417.

Sakai, M.; T. Rtubo; S. Htsuta and M. kobayashi, 1993. Enhoncement of resistance to bacterial infection to rainbow trout, *Oncorhynchus mykiss* (Walbaum) by oral administration of bovine lacto ferrin. J. fish dis., 16: 239-247.

Schaperclaus, W.; H. Kulow and K.Schreckenbach, 1992. Fish Disease. A.A. Balkema, Rotterdam, the Netherlands.

Serafini, M.; R. Bellocco; A. Wolk and A.M. Ekstrom, 2002. Total antioxidant potential of fruit and vegetables and risk of gastric cancer. Gastroenterology, 123: 985-991.

Sharma, S.; S. Sharma and K.P. Sharma, 2005. Protective role of Spirulina feed in a freshwater fish (*Poecilia reticulate Peters*) exposed to an azo dye-methyl red. Indian Journal of Experimental Biology, 43: 1165–1169.

Siwicki, A.K.; P. Klein; M. Morand; W. Wiczka; M. Studnicka, 1998. Immunostimula-tory effects of dimerized lysozyme (KLP-602) on the nonspecific defense mechanisms and protection against furunculosis in salmonids. Vet. Immunol. Immunopathol., 61: 369–378.

Subhashini, J.; S.V.K. Mahipal; M.C. Reddy; M.M. Reddy; A. Rachamallu and P. Reddanna, 2004. Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562. Biochemical Pharmacology, 68: 453–462.

Sung, S.K.; S.Rahimnejad; K.W. Kim. and J. K. Lee., 2013. Partial Replacement of Fish Meal with *Spirulina pacifica* in Diets for Parrot Fish *(Oplegnathus fasciatus)* Turkish Journal of Fisheries and Aquatic Sciences, 13: 197-204.

Takeuchi, T.J.; G.Lu; Y. Yoshizaki and S. Satoh, 2002. Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw *Spirulina platensis.* Fish Sci., 68: 34-40.

Tayag, C.M.; Y. C.Lin; C.C. Li; C.H. Liou and J.C. Chen, 2010. Administration of the hotwater extract of *Spirulina platensis* enhanced the immune response of white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish Shellfish Immunol., 28: 764-73**.**

Ungsethaphand, T.; Y. Peerapornpisal; N. Whangchai and U. Sardsud, 2010. Effect of feeding *Spirulina platensis* on growth and carcass composition

of hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*). *Maejo Int. J. Sci. Technol.,* (4): 331-336.

Usharani, G.; G. Srinivasan; S. Sivasakthi and P. Saranraj, 2015. Antimicrobial Activity of *Spirulina platensis* Solvent Extracts Against Pathogenic Bacteria and Fungi. Advances in Biological Research, 9 (5): 292-298.

Vasudha, S.; V.Manish and N.S. Som, 2009. Evaluation of antioxidant profile and activity of Amalaki (*Emblica officinalis*), *Spirulina* and wheat grass Indian Journal of Clinical Biochemistry, 24 (1): 70-75

Venkataraman, L.V., 1997. *Spirulina platensis* (Arthospira): physiology, cell biology and biotechnologym, edited by Avigad Vonshak. J. Appl. Phycol., 9: 295-296.

Vinay, K.; A.K. Bhatnagar and J.N. Srivastava, 2011. Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compound. Journal of Medicinal Plants Research, 5 (32): 7043-7048.

Watanabe, T.; W. Liao; Y.T. Takeuchi and H. Yamamoto, 1990. Effect of dietary *Spirulina platensis* supplement on growth performance and flesh lipids of cultured striped jack. J. Tokyo Univ. Fish., 77: 231-239.

Watanuki, H.; K. Ota; A. Tassakka; T. Kato and M. Sakai, 2006. Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*. Aquaculture, 258: 157-163.

أثر إضافة الأسبيرولينا بلاتينيس فى علائق الأسماك على أداء و مناعة أسماك البلطى النيلى والنشاط المضاد لمستخلصات الأسبيرولينا للميكروبات

ريهام عبدالوهاب عبدالحى[١]، ولاء طلعت الإكيابى[٢]، سماح عطية على[٢]

[١] قسم بحوث الليمنولوجى، المعمل المركزى لبحوث الثروة السمكية، مركز البحوث الزراعيه.

[٢] قسم بحوث صحه الاسماك ورعايتها، المعمل المركزى لبحوث الثروة السمكية ، مركز البحوث الزراعيه.

الملخـــص العربـــى

قد أجريت هذه الدراسة بالمعمل المركزى لبحوث الثروة السمكية بالعباسة، مركز البحوث الزراعية. وقد اجريت فى تجربتين .التجربه الاولى وكانت بغرض اختبار مستخلصات الاسبيرولينا بلاتينيس ضد البكتريا الممرضه وكانت بواسطه المذيبات (أثير البترولييم ، ثنائى أثيل الأيثر والأسيتون) والتجربه الثانيه كانت بغرض اختبار التاثير المحفزللمناعه (الهيماتوكريت، الاكسجين النشط، الليزوزيم ومضادات الاكسدة) باستخدام طحلب الاسبيرولينا الجاف وفيها استخدام ثلاث تركيزات من الطحلب فى العليقه المستخدم وكانت ١٠-١٥-٢٠جم طحلب جاف /كيلوجرام عليقه مقارنه بالعليقه المفارنه بدون اضافه طحلب وكانت اهم النتائج:فى التجربه الاولى:اظهرت النتائج ان مستخلص الاسيتون كان له قدرة كبيرة فى تنشيط البكتريا المختبرة وفى التجربه الثانيه لوحظ اضافه الطحلب الى العليقه ادى الى زيادة فى اداء الاسماك مقارنه بالكنترول كما لوحظ ان معدل الاضافه الثانى "١٥جم/كجم علف:كانت افضل من حيث انها اعطت اعلى معدلات نمو الاسماك البلطى واشارت النتائج ايضا ان استخدام طحلب الاسبيرولينا فى تغذيه الاسماك مع العليقه ادى الى تحسيين المناعه بشكل عام (الهيماتوكريت، الاكسجين النشط، الليزوزيم ومضادات الاكسدة) واظهرت ايضا ان الاضافه الثانيه ١٥جم طحلب /كجم كانت الافضل.ومن النتائج الدراسه السابقه يمكن التوصيه باستخدام طحلب الاسبيرولنا الجاف بمعدل ١٥جم طحلب جاف /كجم مما يساعد على زيادة معدلات النمو بالاضافه الى تحسين مناعه الاسماك واجراء الدراسه على نطاق واسع لبحث التقييم الاقتصادى لها وان البحث من الناحيه التطبيقيه يمكن استخدامه على الاقل فى الزراعات الشبه مكثفه للاسماك حيث انها تزيد معدلات النمو وتحسن مناعه الاسماك.