

EFFECT OF BLUE GREEN ALGAE AS A BIONITROGEN SOURCE ON YIELD, CHLOROPHYLL, SOME MACRO AND MICRONUTRIENTS CONTENT OF LETTUCE PLANT USING NUTRIENT FILM TECHNIQUE

(Received:11.10.2015)

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ABSTRACT

The present experiment was carried out in a plastic house of Soils and Water Department, Faculty of Agriculture, Al-Azhar University (Nasr City, Cairo, Egypt) to study the effect of two isolates of blue green algae to Hoagland nutrient solution on yield, chlorophyll content of lettuce (*Lactuca sativa* L. cv. Big Bell) using Nutrient Film Technique (NFT). The content of nitrate as well as some macro and micronutrients were taken into consideration. This study aimed to reduce the use of inorganic nitrogen fertilizers for their side effect on human health and high cost, by adding algae as a bionitrogen source to the nutrient solutions. The addition of algal isolates (either *N. pruniformeor* or *A. fertilissima*) to Hoagland nutrient solutions caused a significant increase in the fresh and dry weight of the leaves of lettuce, chlorophyll and nitrogen contents as compared with Hoagland nutrient solution minus N. The most favourable of these results were attained by the addition of algal isolates to 0.75 strength of Hoagland nutrient solution as compared with full strength of Hoagland nutrient solution. Also, the addition of algal isolates to 0.5 strength of Hoagland nutrient solution caused a significant increase in the above parameters as compared with half strength of Hoagland nutrient solution. On the other hand, the poorest results were noted in the treatment of Hoagland nutrient solution minus N whereas; the addition of algal isolates (either *N. pruniformeor* or *A. fertilissima*) to this treatment caused a significant increase in all tested parameters. In conclusion, this study confirmed that the addition of algae to the nutrient solutions under NFT can reduce the use of inorganic nitrogen fertilizers. On the other hand, the relative reduction in the yield of lettuce due to the addition of algae to nutrient solutions minus nitrogen can be acceptable if we take into consideration the safe use of plants due to low content of nitrate and reducing the use of inorganic nitrogen fertilizers.

Key words: *blue green algae, bionitrogen, chlorophyll content, lettuce, NFT.*

1. INTRODUCTION

In general, microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the nutritional need from inorganic fertilizers as much as possible. Plant growth promoting bacteria may be important for plant nutrition by increasing N and P uptake by the plants, and playing a significant role as plant growth promoting in the biofertilization of crops (Cakmakci *et al.*, 2005). In recent decades, it has been observed that the biological N fixation is a valuable tool for agriculture in improving plant growth, yield and soil quality. The usage of inorganic fertilizers may be reduced by exploiting N fixing microorganisms that replaces the usage of inorganic fertilizer and in turn affects the plant

and soil health that causes severe harmful effects to humans (Kumar and Rao, 2012). One of the most important microorganisms is cyanobacterium which represents the largest group of photosynthetic prokaryotes that exist in large diversity and distribution in the world. However, some of them are able to fix nitrogen and also produce a wide range of secondary metabolites (Fish and Codd, 1994). Production of bioactive molecules such as auxins, production of secondary metabolites linked to biocontrol of bacterial and fungal diseases as well as improving soil structure and porosity through secretion of polysaccharides aiding in soil aggregation, are the most important functions of these cyanobacteria (Sergeeva *et al.*, 2002 and Karthikeyan *et al.*, 2007).

Cyanobacteria or blue green algae (BGA) have drawn much attention recently because its application in different aspects (food, feed, fuel, fertilizer, colorant, production of various metabolites including vitamins, enzymes, pharmaceuticals and pharmacological probes) are increasing day by day. Soil is the habitat of some terrestrial cyanobacteria species which are beneficial organisms for maintaining soil fertility by fixing atmospheric nitrogen, binding soil particles, helping to maintain moisture and preventing erosion. Also, blue green algae are able to increase the availability of essential micronutrients in soil which are necessary for plant growth, increase of N content of the surface soil, as well as production of phytohormones substances and other plant growth regulators such as amino acids, sugars and vitamins. These substances are the most important factors for stimulating plant growth which results from the use of blue green algae (Misra and Kaushik 1989 a, b; Irisarri *et al.*, 2001; Karthikeyan 2006; Karthikeyan *et al.*, 2007; Obana *et al.*, 2007).

Beneficial effects of cyanobacterial inoculation on plant growth and soil properties were reported by many investigators. Gupta and Shukla (1967) studied the influence of algae on growth, yield and protein content of rice plants and showed that pre-soaking rice seeds with BGA cultures or extracts enhances germination, promotes the growth of roots and shoots, and increases the weight and protein content of the grain. Svircev *et al.* (1997), also reported that plant growth was enhanced in the presence of cyanobacterium, even without organic N fertilizer application. Beneficial effects of cyanobacterial inoculation were reported, not only for rice, but for other crops such as wheat, soybean, oat, tomato, radish, cotton, sugarcane, maize, chili, bean muskmelon and lettuce (Rodgers *et al.*, 1979; Arif *et al.*, 1995; Thajuddin and Subramanian, 2005; Karthikeyan *et al.*, 2007; Maqubela *et al.*, 2008 and Shariatmadari *et al.*, 2011).

The aim of this investigation was to study the effect of addition of two isolates of blue green algae as a bionitrogen source to nutrient solutions on yield, chlorophyll and some nutrient contents of lettuce using NFT.

2. MATERIALS AND METHODS

The experiment was carried out in a plastic house of Soils and Water Department, Faculty of Agriculture, Al-Azhar University (Nasr City,

Cairo, Egypt) under NFT. The experiment was arranged in a complete randomized block design. Nine treatments were made up as follows: Hoagland nutrient solution minus N, Hoagland nutrient solution minus N plus *A. fertilissima*, Hoagland nutrient solution minus N plus *N. pruniforme*, 0.50 strength of Hoagland nutrient solution, 0.50 strength of Hoagland nutrient solution plus *A. fertilissima*, 0.50 strength of Hoagland nutrient solution plus *N. pruniforme*, 0.75 strength of Hoagland nutrient solution plus *A. fertilissima*, 0.75 strength of Hoagland nutrient solution plus *N. pruniforme* and full strength of Hoagland nutrient solution.

The seedlings of lettuce (*Lactuca sativa* L. cv. Big Bell) were transplanted on the last week of November 2014 in bags (10 cm diameter x 15 cm height) filled with peat moss mixed with perlite (1:1v/v). One plant was planted in each bag. The bags were placed in tubes from plastic polyvinylchloride (PVC), which rise from the ground a distance of 50 cm. The final plant spacing in the tube was 25 cm, while the distance between the tubes was 40 cm. The use of nutrient film technique consists of tubes from plastic (PVC), nutrient solution tank 50 liter (one tank for every tube) and submersible pumps (40 W) to pump the nutrient solution to the upper end of the plastic tube with slop (1.5%).

Four salts were used to prepare three different strengths of Hoagland macronutrients solution (0.5, 0.75 and 1.0) as follows: calcium nitrate, potassium nitrate, potassium mono phosphate and magnesium sulphate. Whereas, Hoagland nutrient solution minus N was prepared from four salts as follows: calcium mono phosphate, calcium sulphate, potassium sulphate and magnesium sulphate. Micronutrients were included according to Hoagland and Arnon (1950). The addition of incubated blue green algae (*N. pruniforme* or *A. fertilissima*) to Hoagland nutrient solutions (minus N, 0.50 and 0.75 strength of Hoagland nutrient solution) was one liter of homogenous algal growth per 50 liter of nutrient solution every two weeks. The pH of all nutrient solutions was kept in the range of 5.5 and 6.5. All nutrient solutions were completely renewed every two weeks.

After two, four, six, eight, and ten weeks from transplanting, chlorophyll content of lettuce was measured as Spad in the second leaf from the outer using chlorophyll meter (Spad-501, Minolta Co., Japan). Also, at the end of the experiment in 7 February 2015, the plants were harvested and fresh weight was recorded. The

plants were washed with distilled water, dried at 70°C and ground, then the samples were wet digested using both HClO₄ and H₂SO₄ acid mixture to determine NPK and micronutrients. Total N was determined by micro-Kjeldahl technique, total P was determined by ascorbic acid method and total K was determined using flame photometer according to Page *et al.* (1982). The micronutrients (Fe, Mn, Zn and Cu) were determined by Inductively Coupled Plasma Spectrometer (ICP) plasma 400. Assessment of NO₃ in the leaves was performed using Brucine method reported by Holty and Potworowski (1972). Statistical analysis was carried out by MSTATC and comparisons of means were made using LSD test according to Snedecor and Cochran (1980).

2.1. Isolation and identification of cyanobacterial isolates

Two algal isolates were isolated from Kafr El-Sheikh soil. The purified isolates were identified to be *A. fertilissima* and *N. pruniforme* according to the methods described by El-Zawawy (2006). The flasks containing fresh liquid Watanabe medium were inoculated with *A. fertilissima* or *N. pruniforme* and incubated under artificial illumination (2500 Lux) up to ten weeks for growth. The mean dry weight and total nitrogen fixation by algal isolates were determined in the laboratory at different times (Table 1) according to Page *et al.* (1982).

dry matter yield of lettuce as compared with non addition of either *N. pruniforme* or *A. fertilissima* to Hoagland nutrient solution minus N. Also, the addition of blue green algae (*N. pruniforme* or *A. fertilissima*) to either 0.5 or 0.75 strength of Hoagland nutrient solution caused a significant increase in the fresh and dry matter yield of lettuce as compared with non addition of them at half and full strength of Hoagland nutrient solution, respectively. It was clear that the difference among treatments was significant except at full and 0.75 strength of Hoagland nutrient solution plus *A. fertilissima*, where the difference between them was not significant. These increases in the fresh and dry matter yield of lettuce as a result of the addition of algal isolates may be attributed to nitrogen fixation and growth regulating substances endogenously produced by these algae (Mahmoud, 2005). Also, these results are in agreement with Sukor (2013), who concluded that nitrogen plays a crucial role in the synthesis of amino acids and proteins, plant growth, chlorophyll formation, leaf photosynthesis and yield of lettuce. The highest values of fresh and dry matter yield of lettuce plants (650 and 54.55 g/plant) were obtained at 0.75 Hoagland nutrient solution plus *N. pruniforme* followed by 0.75 Hoagland nutrient solution plus *A. fertilissima* (581 and 47.72 g/ plant) and full Hoagland nutrient solution (575 and 45.5 g/plant),

Table (1): Mean dry weight and total nitrogen fixation of algal isolates at different times.

Algal isolates	Mean dry weight (mg / 100 ml culture)					Total nitrogen fixation (mg N/ 100 ml culture)				
	Times (weeks)									
	2	4	6	8	10	2	4	6	8	10
<i>A. fertilissima</i>	15	112	167	235	255	5.32	12.43	15.76	19.98	20.18
<i>N. pruniforme</i>	38	150	189	260	290	7.98	13.77	16.83	20,12	21.13

A. fertilissima = *Anabaena fertilissima*

N. pruniforme = *Nostoc pruniforme*

3. RESULTS AND DISCUSSION

3.1. Effect of addition of blue green algae to Hoagland nutrient solution on fresh and dry weight (g/plant) and nitrate content (mg/kg) of lettuce

Data in Table (2) revealed that the addition of blue green algae (*N. pruniforme* or *A. fertilissima*) to Hoagland nutrient solution minus N caused a significant increase in the fresh and

respectively; while the lowest values (52.99 and 5.66 g/plant) were obtained with Hoagland nutrient solution minus N. Also, the obtained values with other treatments were found to be in between. On the other hand, the superiority treatment of full Hoagland nutrient solution on both Hoagland nutrient solution minus N plus *N. pruniforme* or *A. fertilissima* may be due to containing the full Hoagland nutrient solution on

all the required nutrients in balanced formulation including nitrogen.

Concerning nitrate content in the leaves of lettuce plants, the data in Table (2) showed that the highest content of NO₃⁻ (702 mg kg⁻¹ fresh weight) was obtained when lettuce plants were treated with full Hoagland nutrient solution in comparison with other treatments. Whereas, NO₃⁻ not detected at the following treatments; Hoagland nutrient solution minus nitrogen,

lettuce harvested in summer, 4500 mg kg⁻¹ fresh weight for that harvested in winter, and 2500 mg kg⁻¹ fresh weight for outdoor grown lettuce.

3.2. Effect of addition of blue green algae to Hoagland nutrient solution on chlorophyll content (Spad) of lettuce plants

Data of Table (3) showed that the chlorophyll content (Spad) of lettuce at different stages of growth was significantly affected by the addition

Table (2): Effect of different treatments on fresh and dry weight (g/plant) and NO₃⁻ content (mg/kg) of lettuce plants grown under NFT.

Treatments	Fresh weight (g/plant)	Dry weight (g/plant)	Nitrate content (mg/kg fresh weight)
Hoagland nutrient solution minus N	52.99	5.66	ND
Hoagland nutrient solution minus N plus <i>A. fertilissima</i>	344.97	24.35	ND
Hoagland nutrient solution minus N plus <i>N. pruniforme</i>	402.21	30.22	ND
Half Hoagland nutrient solution	463.01	36.66	302.00
0.5 Hoagland nutrient solution plus <i>A. fertilissima</i>	510.39	40.21	312.66
0.5 Hoagland nutrient solution plus <i>N. pruniforme</i>	542.18	43.61	326.00
Full Hoagland nutrient solution	575.00	45.50	702.00
0.75 Hoagland nutrient solution plus <i>A. fertilissima</i>	581.00	47.72	513.33
0.75 Hoagland nutrient solution plus <i>N. pruniforme</i>	650.00	54.55	530.00
L.S.D. at 5%	30.85	2.68	33.42

ND= Not Detected

Hoagland nutrient solution minus nitrogen plus *A. fertilissima* and Hoagland nutrient solution minus nitrogen plus *N. pruniforme*. While, the obtained values with other treatments were found to be in between. It may be worth mentioning that the nitrate contents under the experimental conditions were lower than those established by the European Commission Legislation (Gent, 2003) which has established a maximum acceptable content of NO₃⁻ in lettuce of 3500 mg kg⁻¹ fresh weight for greenhouse

of blue green algae to different treatments of Hoagland nutrient solution. Where the addition of blue green algae (either *N. pruniforme* or *A. fertilissima*) to different treatments of Hoagland nutrient solution (Hoagland minus N, 0.5 and 0.75 strength of Hoagland nutrient solution) caused a significant increase in chlorophyll content at different stages of growth as compared with Hoagland nutrient minus N. Also, addition of algal isolates (*A. fertilissima* or *N. pruniforme*) to either 0.5 or 0.75 strength of

Hoagland nutrient solution caused a significant increase in chlorophyll content (Spad) at different stages of growth as compared with non addition of them at half and full strength of Hoagland nutrient solution, respectively. The highest content of chlorophyll at different stages of growth was recorded with the addition of *N. pruniforme* to 0.75 strength of Hoagland nutrient solution followed by the addition of *A. fertilissima* to 0.75 strength of Hoagland nutrient solution and full strength of Hoagland nutrient solution, respectively. While the lowest content of chlorophyll at different stages of growth was recorded with Hoagland nutrient minus N. These increases in chlorophyll content (Spad) of lettuce plants at different stages of growth as a result of the addition of algal isolates may be attributed to nitrogen fixation where, nitrogen is an integral part of chlorophyll manufacture through photosynthesis. Moreover, nitrogen is a constituent of amino acids, which are required to the synthesise of proteins and other related compounds (Marschner, 1995).

affected by the application of blue green algae as a bionitrogen source combined with different treatments of Hoagland nutrient solution. It was observed that, the content of nitrogen had the same trend previously recorded with their fresh and dry weights of lettuce plants. Whereas, the highest values were obtained at the addition of *N. pruniforme* to 0.75 strength of Hoagland nutrient solution followed by the addition of *A. fertilissima* to 0.75 strength Hoagland nutrient solution and full Hoagland nutrient solution, respectively; while the lowest values were obtained at Hoagland nutrient solution minus N. Also, the obtained values affected by other treatments were found to be in between. These results could be interpreted on the basis that nitrogen is the main building nutrient for fundamental plant material where, it is considered as the characteristic constituent of functional plasma, an integral part of chlorophyll molecules, proteins, amino acids, nucleic acids (RNA and DNA), nucleotides, phosphotides, alkaloids, enzymes, coenzymes, hormones and

Table (3): Effect of different treatments on chlorophyll content (Spad) of lettuce plants at different stages of growth.

Treatments	Chlorophyll content (Spad)				
	Weeks after transplanting				
	2	4	6	8	10
Hoagland nutrient solution minus N	1.42	1.66	2.21	3.38	5.07
Hoagland nutrient solution minus N plus <i>A. fertilissima</i>	3.80	5.67	7.27	10.55	14.76
Hoagland nutrient solution minus N plus <i>N. pruniforme</i>	6.33	9.61	13.18	18.98	24.67
Half Hoagland nutrient solution	8.80	13.37	16.87	25.31	33.16
0.5 Hoagland nutrient solution plus <i>A. fertilissima</i>	11.21	16.34	20.84	31.46	42.48
0.5 Hoagland nutrient solution plus <i>N. pruniforme</i>	13.57	19.13	24.93	37.89	50.92
Full Hoagland nutrient solution	15.83	22.08	29.08	43.91	59.72
0.75Hoagland nutrient solution plus <i>A. fertilissima</i>	15.90	22.35	29.35	44.23	61.88
0.75 Hoagland nutrient solution plus <i>N. pruniforme</i>	18.33	25.40	33.20	50.26	70.33
L.S.D. at 5%	1.82	2.52	3.07	5.01	7.18

3.3. Effect of addition of blue green algae to Hoagland nutrient solution on some macro (%) and micronutrients (mg/kg) content in lettuce plants

Data presented in Table (4) represent the content of some nutrients in lettuce plants as

vitamins (Owen, 2003).

Results of P and K contents in the leaves of lettuce plants were in harmony with their concentrations in Hoagland nutrient solution. Whereas, the addition of *N. pruniforme* or *A. fertilissima* to Hoagland nutrient solution minus

N caused a significant increase in P and K percentages as compared with Hoagland nutrient solution minus N. This effect may be attributed to fixed and released nitrogen in its surrounding environment and therefore positively influenced the growth of the plant and the uptake of other nutrients in the nutrient solution (Ladha and Reddy, 2003). Also, growth regulating substances which produced by algae may be important for growth promotion and plant nutrition by increasing N and P uptake by the plants, and playing a significant role as plant growth promoting the biofertilization of crops (Cakmakci *et al.*, 2005). The highest values of P and K contents were recorded when the lettuce plants were treated with full Hoagland nutrient solution. This effect may be due to contains full Hoagland nutrient solution on all required nutrients in balanced formulation including nitrogen as compared with Hoagland nutrient solution minus N whereas, the lowest values were obtained at the treatment of Hoagland nutrient solution minus N.

different treatments, where, full Hoagland nutrient solution gave the highest content values of these nutrients followed by the treatments of Hoagland nutrient solution minus N supplemented by the addition of algae (*N. pruniforme* or *A. fertilissima*), respectively. The least values were recorded with Hoagland nutrient solution minus N, whereas the obtained values with the other treatments were found to be in between. These data are agreement with Alan (2012), who found that the different combinations of Hoagland's solution and *Azolla filiculoides* positively affected the uptake of macro and micro nutrients in the shoots and roots of *Beta vulgaris* grown in hydroponic cultures.

Based on the above results, it may be concluded that the addition of blue green algae as a bionitrogen source to the growth media can reduce the use of inorganic nitrogen fertilizers and consequently it can reduce the hazard effect resulted from excess consumption of mineral fertilizers.

Table (4): Effect of different treatments on some macro (%) and micronutrients contents (mg/kg) in lettuce plants grown under NFT.

Treatments	N	P	K	Fe	Mn	Zn	Cu
	(%)			(mg/kg)			
Hoagland nutrient solution minus N	0.13	0.12	1.03	89.67	21.27	10.35	8.00
Hoagland nutrient solution minus N plus <i>A. fertilissima</i>	1.11	0.42	2.94	200.00	61.78	33.41	25.50
Hoagland nutrient solution minus N plus <i>N. pruniforme</i>	1.55	0.43	3.00	209.00	64.90	35.31	26.33
Half Hoagland nutrient solution	2.04	0.32	1.80	114.00	30.00	20.00	13.95
0.5 Hoagland nutrient solution plus <i>A. fertilissima</i>	2.33	0.33	2.00	122.33	32.57	21.00	15.00
0.5 Hoagland nutrient solution plus <i>N. pruniforme</i>	2.61	0.34	2.10	126.00	36.90	22.33	16.33
Full Hoagland nutrient solution	2.90	0.45	3.17	242.50	72.83	45.13	36.00
0.75Hoagland nutrient solution plus <i>A. fertilissima</i>	2.92	0.38	2.38	165.00	52.57	27.43	20.50
0.75 Hoagland nutrient solution plus <i>N. pruniforme</i>	3.45	0.38	2.47	169.33	54.40	28.17	21.67
L.S.D. at 5%	0.24	0.03	0.41	19.30	6.14	4.15	3.42

Concerning micronutrient (Fe, Mn, Zn and Cu) contents in the leaves of lettuce plants (Table 4), it maybe concluded that the contents of these nutrients were associated with their concentrations in the growth media according to

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تأثير الطحالب الخضراء المزرقة كمصدر للنيتروجين الحيوى على محصول ومحتوى الكلورفيل
و بعض العناصر الكبرى والصغرى لنبات الخس باستخدام نظام الفيلم المغذى

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قسم الأراضى والمياه - كلية الزراعة - جامعة الأزهر

ملخص

أجريت تجربة في صوبة بلاستيكية بقسم الاراضى والمياه - كلية الزراعة جامعة الأزهر (مدينة نصر- القاهرة - مصر) تحت نظام الفيلم المغذى (NFT) لدراسة تأثير اضافة سلالتين من الطحالب الخضراء المزرقة الى محلول هوجلاند المغذى على محصول نبات الخس صنف (Big Bell) ومحتوى الكلورفيل الكلى فى الأوراق وكذلك محتوى النترات وبعض العناصر المغذية الكبرى والصغرى. تهدف هذه الدراسة الى تقليل استخدام الأسمدة النيتروجينية المعدنية نظرا لخطورتها على صحة الانسان والتكلفة العالية لهذه الأسمدة وذلك عن طريق اضافة الطحالب كمصدر للنيتروجين الحيوى الى المحاليل المغذية تحت نظام الفيلم المغذى. يمكن تلخيص اهم النتائج المتحصل عليها فى النقاط التالية:- ادت اضافة الطحالب الخضراء المزرقة (سواء سلالة *N. pruniformeor* او سلالة *A. fertilissima*) الى محلول هوجلاند غير المحتوى على النيتروجين إلى زيادة محصول نبات الخس والمحتوى الكلى من الكلورفيل والنيتروجين بالمقارنة بعدم اضافة هذه الطحالب الى معاملة محلول هوجلاند غير المحتوى على النيتروجين. كانت افضل النتائج (محصول نبات الخس ومحتوى كل من الكلورفيل الكلى و النيتروجين) عند اضافة الطحالب الخضراء المزرقة مع معاملة 0,75 هوجلاند. اظهرت اضافة الطحالب الخضراء المزرقة (سواء سلالة *N. pruniformeor* او سلالة *A. fertilissima*) مع معاملة 0,5 هوجلاند تفوق معنوى فى محصول نبات الخس ومحتوى الكلورفيل الكلى والنيتروجين بالمقارنة بعدم اضافة هذه الطحالب الى تلك المعاملة. تؤكد الدراسة بان اضافة الطحالب الخضراء المزرقة الى المحاليل المغذية تحت نظام الفيلم المغذى تقلل من استخدام الأسمدة النيتروجينية المعدنية وان الانخفاض النسبى فى المحصول الناتج عن اضافة هذه الطحالب (كمصدر حيوى للنيتروجين) الى المحاليل المغذية (الغير المحتوية على النيتروجين) يمكن قبوله إذا أخذنا فى الاعتبار الاستخدام الآمن لهذه المحاصيل نظراً لانخفاض محتواها من النترات و الحد من الاسراف فى استخدام الأسمدة المعدنية.

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (66) العدد الرابع (أكتوبر 2015) 343-350.