DIFFERENTIAL RESPONSE OF COTTON GROWTH, YIELD AND FIBER QUALITY TO FOLIAR APPLICATION OF CYANOBACTERIA EXTRACT AND UREA FERTILIZER

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Abstract

Cotton is an economically important cash crop in Egypt. Along with the classical mineral fertilization practices, foliar spray by selective biological sources can be contributive enhancers to its growth and performance. Mineral fertilization by common treatments with P and K along with quantities of N fertilizer is conventionally considered the main source of nutrition for best plant growth and performance. This work aimed to introduce dual fertilization practice comprising N fertilizer along with foliar spray of cyanobacteria. Two-seasons field experiment were carried out at the experimental farm of the Sakha Agriculture Research Station, Kafr Elsheikh, Egypt (31°18′30″N & 30°48′14″E) to study the response of plant growth, yield attributes and fiber qualities of the cotton cultivar Giza-94 to foliar application of 6, 12 or 18 g/ha destructed cells of Spirulina platensis and 96, 144 or 192 kg urea (46% N) / ha. Application of the cyanobacteria positively affected area of mean of the leaf area, contents of photosynthetic pigments, gibberellins, plant height at harvest, number of fruiting branches and open bolls/plant, seed yield, and improved fiber length, fineness (Micronair) and strength (Pressely) comparing with the corresponding non-sprayed control plants. Application of 18 g/ha destructed cells of Spirulina along with urea at 192 kg N/ha exhibited the superior enhancement effects comparing to the other treatment combinations. The results strongly suggesting contributive effect of foliar application of Spirulina for better growth and performance of cotton.

Introduction

Egyptian cotton (Gossypium barbadense L.) is one of the most important fiber crops in the world for its best fiber quality. Its vegetative and reproductive growth proceeds simultaneously Nitrogen plays a crucial role in synthesis of amino acids, proteins and chlorophyll contents, photosynthesis, plant growth and crop performance. Excessive vegetative growth due to over doses of mineral fertilization aiming for higher growth, yield and better fiber quality may cause fruit abortion leading to less productivity. Nitrogen plays a crucial role in synthesis of amino acids and proteins, plant growth, chlorophyll formation, leaf photosynthesis, and yield development of cotton.

Microalgae bio-stimulants are products that can be utilized in small quantities for stimulation of growth and development of many crops under both optimal and stressful conditions. They can be used, in conjunction with synthetic fertilizers, for crop protection, growth regulators, yield enhancers and supporters to plant tolerance towards stress conditions (Tamoi et al. 2006 and Ronga et al. 2019). Mechanisms of positive effects of its utilization were reported to be due to increase of antioxidants, leaf chlorophyll, enhanced cellular metabolism (Khan et al. 2009), along with longer shelf life of harvested products (Garcia-Gonzalez and Sommerfeld 2016; Rouphael and Colla 2018). Cyanobacterial preparations were proved to be a potential biofertilizer when used for field soil inoculation or foliar application of their extracts during the vegetative growth stage (Howard et al, 2007). Cyanobacterial extracts contain substances that proved to support plant growth and development of many crops containing, among others, cotton, barley, oats, tomato, sugarcane, maize, chili, lettuce (Ordog 1999 and Grzesik and Romanowska-Duda, 2015). They reported activation mechanisms of plant growth enhancement involving production of vitamins, amino acids, polypeptides, along with antibacterial and antifungal bio-controllers that can, directly and/or indirectly, improve plant growth and crop productivity.

This work targeted assessment of the ability of nitrogen fertilization along with foliar application of cyanobacteria extract for possible improvement of cotton growth and performance as assessed by mean of the leaf area, chlorophyll a, b, carotenoids, gibberellin contents during vegetative growth, plant height, number of fruiting branches, open bolls at harvest , and yield of cotton cultivated under agrochemical circumstances dominant during the cotton cultivation season (April through October) in the Egypt Nile delta. This cotton fertilization management practice, if found

successful, can contribute to more economic production, higher yield and fiber qualities and better environmental soundness.

Materials and Methods

The study was conducted at The Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt (31°18′30″N & 30°48′14″E) during the two consecutive cotton cultivation seasons 2016 and 2017. The fertilization management comprised foliar application of ascending quantities of cyanobacteria extract and N-fertilization by urea (46% N) on growth, yield and fiber quality of the cotton cultivar Gossipium barbadense L, cv. Giza 94. The experimental design comprised 3 main-plot treatments for urea application at 96, 144 or 192 kg N/ha and 4 sub-plot treatments for foliar application of broken cells of Spirulina platensis at 6, 12 and 18 g dried cells/ha, along with a non-sprayed control treatment.

Preparation of mass culture of *Spirulina platensis* and field application of destructed cells

The cyanobacterium was obtained from The Algae Biotechnology Unit of The National Research Center, Cairo, Egypt (www.nrc.sci.eg), grown on 100 ml (**Zarrouk medium,1966**) and incubated for 7 days under 30+2oC under continuous illumination of 5500 – 6500 lux. The produced culture density was found to be around 108 colony-forming units (CFU)/ml. For assessment of mass production possibility, the culture was used as a starter for inoculation of a glass basin containing 288 liter of the same medium and incubated for 25 day. Sterile air was contentiously pumped for culture circulation all over the propagation course. The culture was then filtered through a sterile cotton cloth to remove debris. Cell biomass of the product was 2.5 g dried Spirulina cells/L.

Chemical analysis of the produced ***Spirulina platensis***

Chemical analysis of the cells produced using the previous cultivation method and conditions were performed. Total proteins were measured by the Kjeldahl method . Amino acids were measured using the high-performance amino acid analyzer (Biochrom 30) and contents of P, K, Mn and Fe were measured by ICP (optima 2000 DV – Perkin Elmer). Both were obtained using the methods described in **(AOAC, 2012)**.

Table 1. Protein, amino acids and mineral contents of dried cells of *Spirulina platensis*

Amino acids (ppm)			
Aspartic (ASP)	34000	Tyrosine (TYR)	11400
Threonine (THR)	17600	Phenylalanine (PHE)	17700
Serine (SER)	12200	Hisitidine (HIS)	4500
Glutamic (GLU)	35200	Lysine (LYS)	15500
Glycine (GLY)	18200	Arginine (ARG)	19600
Alanine (ALA)	26200	Proline (PRO)	11700
Valine (VAL)	20900	Cysteine (CYS)	17100
Isoleucine (ILE)	15900	Methionine (MET)	10500
Lucien (LEU)	25500	Total protein	36.4%
Elements (ppm)			
Potassium	189.7	Phosphorus	52.3
Manganese	7.7	Iron	20.5

Preparation of Spirulina for field use

Spirulina platensis culture containing 6 g of dried cells/L was blended until be free of culturable cells. For field application, a volume containing 6 g of destructed cells/L was suspended in 500 L water and refrigerated at 4 ºC until field use.

Field characteristics

The field soils in the two experimentation seasons were clayey originated over thousands of years from annual the Nile flood sediments. Mechanical, chemical and physicochemical analysis **(Jackson 1973)** of field soils used in the seasons 2016 and 2017 were clayey soil textures had 50.7 and 50.7 clay, 38.3 and 38.1 silt, and 11.0 and 11.2% sand, respectively. Electrical conductivities (EC) were 2.6 and 2.7 ds/m-1 and pH of 8.15 and 8.15. Soluble Ca2+ was 7.8 and 8.8, Mg2+ 6.6 and 6.6, K+ 3.5 and 3.7, Na+ 8.1 and 8.3, HCO2 -1 3.0 and 3.1, Cl-1 17.0 and 18.0, and SO42- 6.0 and 5.9 meq/100 g, available N 36 and 33, P 7.7 and 7.2 and K 235 and 220 ppm, respectively. Source of irrigation water was Nile attributes. The fields had efficient field drainage systems. Phosphate fertilizer was applied as common

treatment with calcium super phosphate (15.5% P2O5) during soil tillage before the third perpendicular plough. Potassium sulphate (48% K2O) at the rate of 57.6 kg K2O/ha was applied later at 70 days after sowing.

Experimental layout and treatments distribution

Cotton seeds were sown during the last week of April. In the two seasons, the experiments were designed in split-plot with 3 main-plot treatments for N fertilization by urea (46% N) at 96, 144 or 192 kg N/ha, applied in 2 equal doses, the first was after thinning to 2 seedlings/hole (followed by the first irrigation) and the second was at 45 days after sowing. Four sub-plot treatments were designed for foliar application with destructed cells of *Spirulina platensis* in the rate of 6, 12 or 18/ha, plus a non-sprayed control sub treatment. Cotton leaf worm insecticide(s) was applied as a common treatment for all the field area when necessary.

Foliar application of destructive Spirulina cells

All the cyanobacteria treated sub-plots received the first dose of 6 g cells/ha at 30 days after sowing. A similar quantity of cells were applied to the 12 g treated sub-plots after 25 days later, at the beginning of the squaring growth stage (55 days from sowing). Third Spirulina dose targeted the third set of subplots designed to receive 18 g cell/ha, applied at the beginning of the flowering stage (70 days from sowing). Regarding field application practice, the three levels of Spirulina application were 7.5, 15.0 or 22.5 mg dried Spirulina cells/12.5 m^2 field plot area.

Determination of growth characters

Twenty grams of fresh green fourth upper leaves on the main stem were taken randomly from each experimental plot at 75 days after sowing. They were used for spectrophotometric determination of chlorophyll a, b and carotenoids contents using the wave lengths 440, 644 and 662, respectively (Wettstein 1957). The results are presented here as mg / L using the following formula: chl. a = (9.784 x E662) - (0.99 x E644), chl. b = (21.426 x E644) - (4.65 x E662), and for the carotenoids = (4.695 x E 440) - 0.268 (Chl. a + Chl. b) where E is the optical density at the given wave length. Concentration of gibberellins in leaves was determined at 90 days after sowing using the method described by Shindy and Smith (1975). Plant height, number of fruiting branches, number of open bolls/plant and mean of leaf area / plant at 75, 90 and 105 days after sowing was determined.

Yield components and technological characteristics

Mean of boll weight was calculated. Earliness percentage was determined using seed yield of the first picking/total seed yield of the first and second pickings. Seed cotton yield and seed index (100-seed weight) were determinated using yield of the 3 central rows of each replicated subplot. Technological characteristics of lint cotton comprised lint percent (weight of lint / weight of cotton seed x 100), fineness (Micronair) and strength (Pressely). They were determined in the laboratories of The Cotton Technology Research Division, Cotton Research Institute - ARC, Giza, Egypt (http://www.arc.sci.eg/InstsLabs/Default.aspx?OrgID=2&lang=en) using the high volume instrument model statex fiberotex 900. All according to the methods described in ASTM: D 3818-1986.

Statistical analysis

The collected data were statistically analyzed as split-plot with 3 main plot treatments for N fertilization and 4 subplot treatments for the levels of foliar spray with the destructed cells of Spirulina, and 3 replications. The mean differences were compared using the Duncan's multiple range test (Duncan 1955) using the 95% confidence level (Snedecor and Cochran 1967).

Results

Effects of interaction between N-fertilizer doses and destructed cells of *Spirulina platensis* on leaf area, photosynthes pigments and gibberellin contents.

Data in Table (2) indicating that the mean of leaves area /plant at 75, 90 and 105 days after sowing and also gibberellins content at 90 days, expressed their maximum values by application of 192 kg N/ha along with foliar application of 18 g destructed Spirulina cells/ha. However, the data of statistical analysis indicated no negative first order interaction, but rather a sort of synergism, between application of the N-fertilizer and foliar spray of the destructed cells of Spirulina cell.

Data of Table (3) indicating concomitant increases in leaf chlorophyll a, chlorophyll b, total chlorophyll and carotenoids contents with application of the N fertilizer and destructed cells of Spirulina. Maximum concentrations of the photosynthesis pigments were also obtained by application of 192 kg N/ha along with 18 g of destructed Spirulina

cells. No statistically significant first order interaction was found supporting negative effect of one factor in presence of the other.

Table 2. Effect of N fertilizer doses and foliar spray of ascending quantities of destructed cells of *Spirulina platensis* on gibberellins content and leaf area at different cotton growth stages in the growing seasons 2016 and 2017.

Growing season	N (kg /ha)	Destructed cells of *Spirulina platensis* (g/ha)	Gibberellins (mg / 100g fresh leaves	Mean of leaves area/plant (cm^2)		
				75 DAS	90 DAS	105 DAS
2016	96	Control	10.05 g	18.2 f	24.8 e	27.1 h
		6	10.08 g	18.6 f	25.1 e	27.6 h
		12	10.08 g	19.8 e	25.5 e	28.1 g
		18	10.20 fg	20.1 d	26.4 d	28.6 fg
	144	Control	10.32 ef	20.5 d	26.7 d	30.2 f
		6	10.41 e	20.8 d	27.2 cd	31.7 e
		12	10.48 de	21.4 cd	27.5 cd	31.9 e
		18	10.62 d	22.1 c	28.1 c	33.6 d
	192	Control	10.94 c	22.9 b	29.0 b	35.5 c
		6	11.43 b	23.8 ab	29.8 b	36.3 b
		12	11.56 b	24.4 a	30.2 ab	36.4 b
		18	11.74 a	25.8 a	31.2 a	38.4 a
2017	96	Control	10.03 h	17.8 i	24.3 h	26.7 i
		6	10.09 h	19.2 h	24.9 h	27.1 h
		12	10.22 h	20.3 g	25.6 g	27.9 h
		18	10.41 g	20.3 g	26.6 f	28.7 g
	144	Control	10.65 f	20.7 g	27.0 f	29.1 f
		6	10.93 e	21.3 f	28.3 e	29.8 e
		12	11.03 de	22.1 e	25.9 d	29.9 g
		18	11.19 cd	23.2 d	29.6 c	33.5 d
	192	Control	11.32 c	24.8 c	30.2 b	35.3 c
		6	11.54 b	26.1 b	30.7 b	35.6 c
		12	11.69 ab	26.8 b	31.0 ab	37.2 b
		18	11.82 a	27.5 a	31.6 a	41.1 a

Table 3. Effect of N fertilizer doses and foliar spray of destructed cells of *Spirulina platensis* on chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents in the cotton growing season 2016 and 2017.

Growing season	N- doses (kg N/ha)	Destructed cells of *Spirulina platensis* (g/ha)	Chl. a	Chl. b	Total chl.	Carotenoids
2016	96	Control	2.95 e	1.66 g	4.56 i	1.13 h
		6	3.00 e	1.70 g	4.66 i	1.24 gh
		12	3.08 e	1.83 g	4.91 h	1.29 gh
		18	3.09 e	2.03 f	5.10 g	1.45 fg
	144	Control	3.59 d	2.06 f	5.65 f	1.47 efg
		6	3.60 d	2.14 f	5.75 f	1.63 def
		12	4.04 c	2.35 e	6.39 e	1.64 def
		18	4.05 c	2.56 d	6.61 d	1.70 cde
	192	Control	4.15 bc	2.81 c	9.69 c	1.85 cd
		6	4.30 b	3.04 b	7.46 b	1.91 c
		12	4.31 b	3.20 b	7.52 b	2.09 b
		18	4.62 a	3.43 a	8.05 a	2.33 a
2017	96	Control	3.21 c	2.43 d	4.18 g	1.43 g
		6	3.70 b	2.75 c	4.58 g	1.57 fg
		12	3.63 b	2.80 c	4.61 g	1.68 ef
		18	3.60 b	3.02 b	5.32 f	1.75 def
	144	Control	3.65 b	3.10 b	6.22 e	1.83 cde
		6	4.00 b	3.80 a	6.61 de	1.92 bcd
		12	3.32 c	3.33 ab	6.84 cd	2.00 bc
		18	3.98 b	3.54 ab	7.13 bc	2.05 bc
	192	Control	4.07 b	3.88 a	7.30 bc	2.10 b
		6	4.40 a	3.04 b	7.33 bc	2.13 b
		12	4.43 a	3.18 b	7.68 b	2.31 a
		18	4.89 a	3.98 a	8.30 a	2.47 a

Effects of interaction between N-fertilizer doses and destructed cells of *Spirulina platensis* on yield and its components

Plant height in both seasons increased by adding N fertilizer at 192 kg/ N / ha along with 6 g of destructed spirinulina cells. On the other hand, Number of fruited branches and percentage of earliness in both seasons increased concomitantly and significantly with increasing the amount of fertilizer-N up to 192 kg N/ha along with 18 g of destructed *Spirulina* cells (Table 4). However, statistically significant differences between means of the different ascending quantities of the *Spirulina* were recognized.

Table 4. Effect of different application doses of N fertilizer and amount of destructed cells of *Spirulina platensis* on cotton plant height, number of fruited branches/plant and earliness percentage.

Growing season	N- doses (kg N/ha)	Destructed cells of *Spirulina platensis* (g/ha)	Plant height (cm)	Fruiting branches / plant	Earliness (%)
2016	96	Control	178.72 c	18.32 ab	63.57 ab
		6	181.91 c	18.06 ab	66.23 ab
		12	182.28 c	17.91 b	65.15 ab
		18	183.65 bc	17.81 b	64.96 ab
	144	Control	185.52 bc	18.82 ab	64.54 ab
		6	185.62 bc	18.69 ab	64.47 ab
		12	185.62 bc	18.66 ab	64.30 ab
		18	187.03 bc	18.61 ab	63.97 ab
	192	Control	194.72 ab	19.60 ab	63.33 b
		6	198.27 a	19.92 a	66.88 a
		12	195.64 ab	19.48 ab	63.05 b
		18	189.68 abc	18.94 ab	63.05 b
2017	96	Control	192.56 e	17.34 b	56.00 d
		6	194.00 c	17.03 c	55.88 e
		12	193.23 d	17.40 b	56.00 d
		18	193.87 d	17.00 c	56.03 d
	144	Control	194.00 c	17.54 b	56.87 cd
		6	194.24 c	17.23 b	57.09 c
		12	196.00 b	17.00 c	57.54 c
		18	195.55 c	17.03 c	58.00 bc
	192	Control	197.54 ab	18.00 ab	58.12 b
		6	200.65 a	18.76 a	60.45 a
		12	195.16 b	18.34 a	59.00 ab
		18	196.09 b	18.02 ab	58.09 b

Data of Table (5) show that in season 2016, the maximum values of open bolls/plant were obtained by using 192 kg fertilizer-N/ha along with application of either 6, 12 or 18 g destructed Spirulina cells/ha. However, results of the subsequent season 2017 came different. At least 12 g of destructed cells of Spirulina/ha was needed for scoring the highest number of open bolls/plant. Concerning the mean of boll weight/plant, 192 kg N/ha along with at least 6 g destructed cells of Spirulina were needed to obtain the heights values. Results of seed cotton yield and seed index (weight of 100 g seeds after ginning) telling different stories. The highest figures of both in season 2016 were obtained by application of 192 kg N/ha along with foliar spray of 6 g destructed Spirulina cells/ha, indicating no statistically significant differences that can be attributed to application of the Spirulina. In season 2017, results of seed index did not show significant differences that could be attributed to differences between the N application doses nor the amount of the applied Spirulina cells. The two year results strongly suggest that weight of seed is likely controlled by a genetical factor(s) that proceed independently regardless of the amount of produced ginned seeds from a similar field area. However, factors attributed to seasonal variation may be involved.

Table 5. Effect of different application doses of N fertilizer and amount of destructed cells of *Spirulina platensis* on open bolls/plant, mean of boll weight, seed index and seed cotton yield

Growing season	N- doses (kg N/ha)	Destructed cells of *Spirulina platensis* (g/ha)	No. of open bolls/plant	Mean of bolls weight (g)	Seed index (g)	Seed cotton yield (ton/ha)
2016	96	Control	31.74 d	2.47 d	11.44 d	3.68 c
		6	31.81 d	2.50 cd	11.63 cd	3.73 c
		12	32.69 cd	2.52 bcd	11.67 cd	3.83 c
		18	33.11 bcd	2.52 bcd	11.86 bc	3.84 c
	144	Control	33.90abcd	2.63 bcd	11.88 bc	3.91 c
		6	34.22 abc	2.64 bcd	11.92 bc	4.08 bc
		12	34.67 abc	2.73 abcd	11.92 bc	4.18 b
		18	34.69 abc	2.74 abcd	11.92 bc	4.47 ab
	192	Control	35.00 b	2.91 a	12.00 b	4.28 b
		6	36.38 a	2.96 a	12.53 a	4.78 a
		12	35.21 ab	2.79 ab	11.99 bc	4.59 ab
		18	35.23 ab	2.47 d	12.15 b	4.49 ab
2017	96	Control	30.14 g	2.32 c	11.60 a	3.78 d
		6	31.23 f	2.31 c	11.65 a	3.85 c
		12	31.54 f	2.34 c	11.45 a	3.86 c
		18	32.32 ef	2.30 c	11.99 a	3.89 bc
	144	Control	33.50 e	2.32 c	12.00 a	3.93 bc
		6	33.87 e	2.86 b	11.89 a	3.94 bc
		12	34.00 d	2.80 b	11.78 a	3.96 bc
		18	34.02 d	2.43 d	12.00 a	4.06 b
	192	Control	34.19 c	2.89 b	12.12 a	4.00 b
		6	36.64 a	3.10 a	12.34 a	4.32 a
		12	35.12 b	2.80 b	12.08 a	4.10 b
		18	36.51 a	2.65 c	12.00 a	4.08 b

Effect of different application doses of N fertilizer and amount of *Spirulina cells* on technological characters of cotton fiber

Lint percentage and fineness in both seasons (Table 6) positively responded to N application up to 192 kg N/ha with adding 12 g of *Spirulina* cells/ha. In season 2016, the strength did not show statistically significant differences that could be attributed to either increasing quantities of the N fertilizer or application of the tested different amounts Spirulina. In contrast, highest figures of strength in season 2017 could not be obtained unless N fertilizer at 192 kg N/ha was applied. No significant differences could be obtained due to application or not of the destructed cyanobacterial cells.

Table 6. Effect of different application doses of N fertilizer and amount of destructed cells of *Spirulina platensis* on lint percent, fineness (Micronair) and strength (Pressely)

Growing season	N- doses (kg N/ha)	Destructed cells of *Spirulina platensis* (g/ha)	Lint percent	Fineness (Micronair)	Strength (Pressely)
2016	96	Control	38.65 d	4.0 b	10.0 a
		6	38.67 d	4.0 b	10.1 a
		12	39.09 d	4.0 b	10.1 a
		18	40.35 cd	4.1 b	10.1 a
	144	Control	40.38 cd	4.2 ab	10.2 a
		6	40.40 cd	4.2 ab	10.2 a
		12	41.02 c	4.2 ab	10.2 a
		18	41.71 bc	4.2 ab	10.2 a
	192	Control	43.29 b	4.2 ab	10.3 a
		6	42.18 bc	4.2 ab	10.5 a
		12	44.99 a	4.4 a	10.3 a
		18	42.14 bc	4.2 ab	10.5 a
2017	96	Control	41.00 a	4.0 c	10.0 c
		6	42.25 a	4.2 b	10.1 b
		12	42.17 a	4.0 c	10.1 b
		18	41.34 a	4.1 b	10.0 c
	144	Control	42.00 a	4.1 b	10.0 c
		6	42.34 a	4.0 c	10.1 b
		12	41.98 a	4.0 c	10.2 a
		18	42.00 a	4.2 b	10.1 b
	192	Control	41.56 a	4.1 b	10.2 a
		6	41.76 a	4.1 b	10.2 a
		12	42.56 a	4.3 a	10.3 a
		18	41.67 a	4.2 b	10.2 a

Discussion

Cyanobacterial preparations were proved to be a potential biofertilizer when used for field soil inoculation or foliar application of their extracts during the vegetative growth stage (Howard et al, 2007). Cyanobacterial extracts contain substances that proved to support plant growth and development of many crops containing, among others, cotton, barley, oats, tomato, sugarcane, maize, chili, lettuce (Ordog 1999, Aly et al. 2008 and Grzesik and Romanowska-Duda, 2015). They reported activation mechanisms of plant growth enhancement involving production of vitamins, amino acids, polypeptides, along with antibacterial and antifungal bio-controllers that can, directly and/or indirectly, improve plant growth and crop productivity. Foliar application of *Spirulina* extract, in particular, was found to increase carotenoids, chlorophyll a and b (Yassen et al. 2018), plant height, plant biomass and element contents (Godlewska et al. 2019). Conclusively, members of the genus *Spirulina,* specially *Spirulina platensis,* is rich in amino acids which have biological roles in detoxification of plant toxins and heavy metals (Rizwan et al. 2017 and Bashir et al. 2018), chlorophyll synthesis (Amin et al 2011), optimization of nutrient uptake, translocation and metabolism, vitamin biosynthesis, increased plant dry matter (Khalilzadeh et al 2012), maintenance of protein structures required for cell division and enlargement, production of hormones involved in cell division, differentiation, production of growth-efficient polyamines (Kakkar et al. 2000), amino acids (Mohamed and Mohamed 2012) which are involved in growth bio-stimulation and alleviation of stress conditions (Souri and Hatamian 2019). Fawzy et al (2012) found that foliar spray of amino acids increases protein, N, Cu and Mn in the plant tissues; reduce the flow of ammonium and the transcription of the root tissue. They also increase the concentrations of gibberellic acid and indole acetic acid and uptake of NPK (Talaat et al. 2005). For instance, Methionine which appeared in the chemical analysis of the *Spirulina platensis* used in this study (Table1) was found to involve in synthesis of growth regulation substances comprised cytokines and auxins in plant tissue, led to increases in NPK content and dry weight of plant shoots (Chen et al. 2005; El-Awadi et al. 2011). Other components in this organism, comprised tryptophan and phenylalanine, were found to enhance leaf surface area (Dahab and El-Aziz 2006; Boopathi et al, 2013), while cysteine has an important role in cytosol and mitochondria in plant cells and improve hairy plant roots which found enhancers to nutrients uptake mechanisms (Romero et al. 2014). Recently, glycine and glutamine stimulates of plant growth when used for foliar application (Noroozlo et al. 2019). However, glycine was found by Mohammadipour and Souri (2019 a, b) to increase N, Ca, K, P, Fe, Mg, Zn contents, plant height, shoot and root fresh weights, soluble solids (TSS) and vitamin C antioxidant activity in plant.

Summary

From our results, nitrogen application showed significant enhancement effects on cotton plant growth, performance and fiber quality. The collected results involved statistically significant concomitant increases in the leaf area / plant, chlorophyll a, b, carotenoids, gibberellin contents, plant height (Howard et al. 2007), number of open bolls at harvest, seed yield and seed index, enhanced technological characteristics involving lint percent, fineness (Micronair) and strength (Pressely) with application of ascending quantities of N-fertilizer up to 192 kg N/ha individually, confirming results of Zhenan et al 2001, with further increases due to foliar application of the destructed/homogenized *Spirulina platensis* cells. The results came supportive and in conformity with most of the above-mentioned research results which related additional enhancement effects (to that which related to N application), to application of the destructed cyanobacterial cells, with potential synergism between each other as no significant first order interaction but rather contributive positive effects of each one in presence of the other. In this concern, Howard et al. (2007) and Rodríguez et al (2006) found that taller plants induced by N application and further supported by cyanobacterial extract enhanced reproductive growth due to enough light penetration that facilitates boll opening, leading to more seed cotton yield. Anyway, the positive interactions here found were significant in both the two cultivation seasons. However, in some cases, application of 192 kg N / ha along with only 6 g of destructed *Spirulina* cells/ha surpassed other combinations under study as the treatment induced the tallest plants (198.27 and 200.65 cm) , largest number of fruiting branches (19.92 and 18.76) and the highest number of open bolls / plant. However, for most of the studied growth, yield and fiber quality parameters, they needed the highest *Spirulina* application dose of 18 g cells/ha to express their maximum values.

The results expressed statistically significant increases in mean of leaves area /plant, contents of chlorophyll *a* and *b*, carotenoids, gibberellins at 18 g/ha along with urea at 192 kg N/ha, plant height at harvest, number of fruiting branches and open bolls/plant, seed cotton yield and seed-index; along with improvement of cotton fiber length, fineness (Micronair) and strength (Pressely) with application of *Spirulina* at 6 g/ha along with urea at 192 kg N/ha. Conclusively, both the urea and *Spirulina* cells acted synergic ally for better plant growth and performance. Results

of this translational study, if successfully considered by cotton producers in Egypt and worldwide can contribute to better cotton growth, fiber qualities and quantities and production economy.

References

Aly, M.H.A., A.M.A Azza, and S.M.M Soha S.M. 2008. Enhancement of sugar beet seed germination, plant growth, performance and biochemical compounds as contributed by algal extracellular products. J. Agric. Sci., Mansoura Univ., 33:8429-8448.

Amin, A., F. Gharib, M. El-Awadi and E. Rashad. 2011. Physiological response of onion plants to foliar application of putrescine and glutamine. Scientia Horticulturae 129: 353-360.
ASTM 1986. American stander for testing material (ASTM) D 1440-50, D 1578- 67, D 1425.

A.O.A.C. (1995): Association of Official Agriculture Chemists. Official Methods of Analysis. 16th Ed.

Bashir, A., M. Rizwan, S. Ali, M.Z. Zia-ur Rehman, W. Ishaque , M.A. Riaz and A. Maqbool 2018. Effect of foliar-applied iron complexed with lysine on growth and cadmium (Cd) uptake in rice under Cd stress. Environmental Science and Pollution Research 25:20691–20699.
Boopathi, T., V. Balamurugan, S. Gopinath, and M. Sundararaman. 2013. Characterization of IAA Production by the Mangrove Cyanobacterium Phormidium sp. MI405019 and its Influence on Tobacco Seed germination and organogenesis. Journal of Plant Growth Regulation 32:758-766.

Chen, M., B. Cheng, Q. Zhang, Y. Ding and Z. Yang. 2005. Effects of applying L-methionine, L-phenylalanine and L-tryptophan on Zea mays growth and its nutrient uptake. The Journal of Applied Ecology 16:1033-1037.

Dahab, T. and N. El-Aziz. 2006. Physiological effect of diphenylamine and tryptophan on the growth and chemical constituents of Philodendron erubescens plants. World Journal of Agricultural Sciences 2:75-81.

Duncan, D.B. 1955. Multiple range and multiple F-Test. Biometrics 11:1-5.

El-Awadi, M., A. El-Bassiony, Z. Fawzy and M. El-Nemr. 2011. Response of snap bean (Phaseolus vulgaris Lants) to nitrogen fertilizer and foliar application with methionine and tryptophan. Nature and Science 9:87-94.

Fawzy, Z., Z. El-Sha, L. Yunsheng, O. Zhu and O. Sawan. 2012. Response of garlic (Allium Sativum L) plants to foliar spraying of some bio-stimulants under sandy soil condition. Journal of Applied Sciences Research 8:770-776.

Garcia-Gonzalez, J. and M. Sommerfeld. 2016. Biofertilizer and biostimulants properties of the microalga Acutodesmus dimorphus. J. Appl. Phycol., 28:1051–1061.

Godlewska, K., I. Michalak, P. Pacyga, S. Baśladyńska and K. Chojnacka. 2019. Potential applications of cyanobacteria: Spirulina platensis filtrates and homogenates in agriculture. World Journal of Microbiology and Biotechnology 35:1-18.

Grzesik, M. and Romanowska-Duda Zdzisława. 2015. Ability of cyanobacteria and green algae to improve metabolic activity and development of willow plants. Pol. J. Environ. Stud. 24: 1003-1012.

Howard, D.D., C.O. Gwathmey, M.E. Essington, R.K. Roberts, M.D. Hua-Jing, W. Liang-Huan, W. Min-Yan, Z. Hussain, A., S. Ali, M. Rizwan, M.Z. Zia-ur Rehman, A.A. Hameed, F. Hafeez, S.A. Alamri, M.N. Alyemeni and L. Wijaya. 2007. Role of zinc– lysine on growth and chromium uptake in rice plants under Cr stress. Journal of Plant Growth Regulation 37:1413-1422.

Jackson, M.L. 1973. Soil Chemical Analysis. Prentic-Hall, Inc, Englewood Cliffs, NJ, USA.

Kakkar, R., P. Nagar, P. Ahuja and V. Rai. 2000. Polyamines and plant morphogenesis. Biologia. Plantarum 43:1-11.

Khalilzadeh, R., M. Tajbakhsh and J. Jalilian. 2012. Effect of foliar application of bio-organic fertilizers and urea on yield and yield components characteristics of mung bean. International Journal of Agriculture Research and Review 2:639-645.

Khan, S.A., M.Z. Hussain, S. Prasad and U.C. Banerjee. 2009. Prospects of biodiesel production from microalgae in India. Renew. Sustain. Energy Rev. 13:2361–2372.

Mohamed E. and S. Mohamed. 2012. Improvement the growth and quality of green onion (Allium cepa L.) plants by some bioregulators in the new reclaimed area at Nobaria region, Egypt. New York Science Journal 5:114-120.

Mohammadipour, N. and M.K. Souri. 2019a. Effects of different levels of glycine in the nutrient solution on the growth, nutrient composition and antioxidant activity of coriander (Coriandrum sativum L.). Acta Agrobotanica 72:6-9.

Mohammadipour, N. and M.K. Souri. 2019b. Beneficial effects of glycine on growth and leaf nutrient concentrations of coriander (Coriandrum sativum) plants. Journal of Plant Nutrition 42:1637-1644.
Noroozlo, Y.A., M.K. Souri and M. Delshad. 2019. Stimulation effects of foliar applied glycine and glutamine amino acids on Lettuce Growth. Open Agriculture 4:164–172
.
Ordog, V. 1999. Beneficial effects of microalgae and cyanobacteria in plant/soil systems, with special regards to their auxin- and cytokine- like activity. International workshop and training course on microalgal biology and biotechnology. Hungary, June 1999, p. 13-26.

Rizwan, M., S. Ali, A. Hussain, Q. Ali, M.B. Shakoor, M. Zia-ur-Rehman, M. Farid and M. Asma. 2017. Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (Triticum aestivum L.) and health risk assessment. Chemosphere 187:35-42.

Rodríguez, A.A., A.M. Stella, M.M. Storni, G. Zulpa and M.C. Zaccaro. 2006. Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in Oryza sativa L, Saline Systems 7-10.

Romero, I., J. Téllez, L. Yamanaka, M. Steindel, A. Romanha and E.C. Grisard. 2014. Transsulfuration is an active pathway for cysteine biosynthesis in Trypanosoma rangeli. Parasites & Vectors 7:2-11.

Ronga, D., E. Biazzi, K. Parati, D. Carminati, E. Carminati and A. Tava. 2019. Microalgal biostimulants and biofertilisers in crop productions. Agronomy 9:1-22.

Rouphael, Y. and G. Colla. 2018. Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture. Front. Plant Sci. 9:1-6.

Shindy, W.W. and O. Smith. 1975. Identification of plant hormones from cotton ovules. Plant Physiology 55:550-554. http://dx.doi.org/10.1104/pp.55.3.550.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical methods. 6th Edition, Ames, Iowa, the Iowa State University.

Souri, M.K. and M. Hatamian. 2019. Aminochelates in plant nutrition: a review. Journal of Plant Nutrition 42:67-78.

Tamoi, M., M. Nagaoka, Y. Miyagawa and S. Shigeoka. 2006. Contribution of fructose-1, 6-bisphosphatase and sedoheptulose-1, 7-bisphosphatase to the photosynthetic rate and carbon flow in the Calvin cycle in transgenic plants. Plant Cell Physiology 47: 380-390.

Wettstein, V.D. 1957. Chlorophyll letale and der sub-mikroskopishe formweschselder plastiden. Experimental cell Research 12:427. Doi:10.1016/0014-4827(57)90165-9.

Yassen, A.A., E.A.A. Abou ELNour, M.A. Abou Seeda, M.M.S. Abdallah and S.A.A. El- Sayed. 2018. Effect of potassium fertilization levels and algae extract on growth, bulb yield and quality of onion (Allium cepa L.). Middle East Journal of Agriculture Research 7:625-638.

Zarrouk, C. 1966. Contribution a L'etude D'une Cyanophycee: Influence de Divers Facteurs Physiques et Chimiques sur la Croissance et la Photosynthese de Spirulina maxima (Setch et Gardner) Geitler. Ph.D. Thesis, Faculte des Sciences de l'Universite de Paris, Paris, France.

Zhenan, H., L. Pinfang, L. Baoguo, G. Jiang and W. Yanna. 2001. Effects of fertigation scheme on N uptake and N use efficiency in cotton. Plant Soil 290:115–126.