

## IMPACT OF BIOFERTILIZERS AS A PARTIAL AND SAFE SUBSTITUTE OF NITROGEN FERTILIZER ON SOME GROWTH, PHOTOSYNTHETIC PIGMENTS AND YIELD TRAITS IN COTTON PLANTS

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### ABSTRACT

Attention has been focused on the use of organic and bio-fertilizers due to the pollution factor and high costs of mineral fertilizers. Therefore, two field experiments were conducted at the Experimental Farm of the Faculty of Agriculture at Fayoum in two successive growing seasons 2010 and 2011 to study the effect of bio-fertilizers (Microbin and Cerealin) with different levels of nitrogen. The treated cotton plants with inorganic N combined with microbin or cerealin bio-fertilizers improved all the studied growth traits of cotton, *i.e.* plant height, the number of leaves per plant, the total leaf area per plant and dry weight of leaves per plant as compared to the control treatment. The best results were observed when cotton plants were treated with 100 % inorganic N combined with microbin or cerealin for plant height trait in both seasons. Treatment of 80 % N + microbin or cerealin ranked as the first favorable treatments for enhancing cotton yield and leaf pigments in both seasons. From the obtained results, it could be concluded that the application of 80 % N + microbin or cerealin were favorable treatment for producing significantly higher yield with improved growth traits and seed cotton yield saving 20 % of N fertilizer.

*Key word: biofertilizer, cerealin, cotton, microbin, N inorganic.*

### 1. INTRODUCTION

Cotton is still playing an essential role in the national economy of Egypt. Bio-fertilizers became a positive substitute portion of chemical fertilizers, safe for human, animal and environment. They help in improving crop productivity and quality by increasing biological N fixation, availability and uptake of nutrients, and stimulating the natural hormones (Kannaiyan, 2002).

Singh and Bisoyi (1995) concluded that biofertilizers like *Azotobacter*, *Azospirillum* and *Rizobium* were important for different crops, and addition of bio-fertilizers had an effective role in N fixation and biomass accumulation beside their favorable effect on mineralization and balance of soil N. Further, Neeru *et al.* (2005 a and b) reported that high N fixing, phosphate solubilizing, phytohormones producing isolates of *Azotobacte*, *Azospirillum*, *Acetobacter* and *Pseudomonas* were used as inoculants with varying doses of nitrogen for cotton.

The biofertilizers under optimum conditions could enhance the crop yield by 10-20 % and saved chemical N fertilizer by 15-25 %

(Chowdhury and Mukherjee ,2008).

Saleh *et al.* (2004), El- Sayed and El-Menshawi (2005), Hamed (2006) and Toaima (2007) demonstrated that plant height, boll weight , number of bolls per plant and seed cotton yield per feddan were increased significantly by N application. Generally, N fertilizer is an important and limiting factor for growth and productivity of cotton. It has many functions in plant life, being part of proteins, an important constituent of protoplasm, responsible for the biosynthesis of enzymes, amino acids, plant pigments and encouragement of cell division.

Many authors studied the effect of bio-fertilizers on different crops, *i.e.* Ibrahim *et al.* (2004) and Zeidan *et al.* (2005) for wheat, Wu *et al.* (2005) for maize, Rokhzadi *et al.* (2008) for chickpea and Megawer & Mahfouz (2010) for rape.

Few investigators studied the effect of application of bio-fertilizers to increase the productivity of cotton. (Hamissa *et al.*, 2000; Sobh *et al.*, 2000; Sundaravardarajan *et al.*, 2006; Al-Mohamed *et al.*, 2009).

The main objective of the study was to throw some light on the effect of present bio and N

mineral fertilization on growth, leaf pigment assessments and yield traits of cotton plants.

## 2. MATERIALS AND METHODS

Two field trials were conducted during the two successive seasons of 2010 and 2011 at the Experimental Farm of the Faculty of Agriculture at Fayoum, to study the effect of bio and N fertilization on cotton plants, Giza 90. The soil properties were: coarse sand 5.57 %, fine sand 22.11 %, silt 19.23 %, clay 53.09 %, pH 7.21, organic matter 1.47 %, calcium carbonate 5.43, Ec 1.6, available N, P and K, 72.00, 19.00 and 696.00 ppm, respectively (Page *et al.*, 1982 and Klute, 1986).

N fertilizer levels in the form of urea (46 % N) were applied in two equal doses, the 1<sup>st</sup> dose was after thinning and the 2<sup>nd</sup> one was added at the following irrigation. Phosphorus fertilizer was added at a rate of 22.5 kg P<sub>2</sub>O<sub>5</sub>/ fed. during land preparation. Potassium fertilizer was added at rate of 50 kg in forms of potassium sulphate (48 % K<sub>2</sub>O) in one dose was added by at the time of applying the 1<sup>st</sup> dose of N.

Two K<sub>2</sub>O were used in the present study, *i.e.*, microbin (*Azotobacter chroococcum*) and cerealin (*Bacillus polymyxa*). Arabic gum was melted in an amount of warm water and was added to each of the previous two bio-fertilizers. Cotton seeds were added to the mixture of bio-fertilizer and gum, mixed carefully and spread over plastic sheet far from the direct sun effect for a short time before sowing (Allen, 1971). Sowing irrigation was done immediately to provide a suitable moisture condition for inoculation, while the control treatment was without inoculation.

The experimental design was randomized complete block design with four replications and 9 treatments included:

(T1) 100 % N of (75 kg N/fed.; control treatment).

(T2) 80% N of recommended dose.

(T3) 60 % N of recommended dose.

(T4) 100 % N of recommended dose+ Microbin.

(T5) 80 % N of recommended dose + Microbin.

(T6) 60% N of recommended dose + Microbin.

(T7) 100 % N of recommended dose + Cerealin.

(T8) 80 % N of recommended dose + Cerealin.

(T9) 60 % N of recommended dose + Cerealin.

The plot size was 10.5 m<sup>2</sup> (3.0 × 3.5 m). The ordinary cultural practices for growing cotton were adopted as recommended at the location, except the experimental treatments. Sowing dates were March 15 and 17 for the first and second seasons, respectively. Five individual random

guarded plants were tagged to collect the data but seed cotton yield was weight of the inner rows of each plot in terms of Kg / plot and converted to kentar / feddan.

The studied traits were; I-Growth traits [Plant height (cm), number of leaves per plant, total leaf area per plant (dm<sup>2</sup>) and dry weight of leaves per plant (g)]. II- Photosynthetic pigments assessments [(chlorophyll A and B (mg/g, F.W.) were determined according to Arnon (1949), total carotenoid concentration (mg/g, F.W.) in fresh leaves were estimated by the method described by Welburn and Lichtenthaler (1984) and total sugars (mg/g, D. W.) were determined according to A.O.A.C. (1995)]. III-Yield and its components; [ seed cotton yield (Kentar/fed.), number of bolls /plant, boll weight (g), lint percentage (%), seed index (g) and lint index (g)]. The data were subjected to analysis of variance according to Gomez and Gomez (1984) using MSTAT statistical package. The means were compared using NEW LSD test at the 5 % level.

## 3. RESULTS AND DISCUSSION

### 3.1. Growth parameters

Results presented in Table (1) indicated that growth traits *i.e.*, plant height, number of leaf per plant, total leaves area per plant and dry weight of leaf per plant were significantly positively affected by the treatments of nitrogen fertilizer and/or with inoculation of biofertilizers microbin or cerealin in both seasons. The highest values of plant height were obtained with the biofertilizers cerealin or microbin combined with the chemical fertilizers 80 or 100% N treatments (T4, T5, T7 and T8) in both seasons. The data also indicated that the application of biofertilizers increased plant height significantly compared to that of nitrogen treatments (60 or 80 % N ;(T2 and T3) which in turn scored a significant increments in this parameter as compared to the control (T1). The enhanced plant height obtained occurred by the fact that biofertilizers encouraged a fixing molecular nitrogen and its transfer to the plant as a direct effect on growth hormones auxins.

While, no significant differences were obtained between the two treatments of T6 and T9 for the same trait. The results cleared that the treatment of T8 which is a combination between 80 % N and inoculation of cerealin, followed by T5 and they exerted the highest values for the number of leaves per plant; total leaf area per plant and dry weight of leaves per plant as compared to the control in both seasons. The obtained values were 47.00, 46.00, 66.73, 65.00, 28.33 and 28.66 in the

**Table (1): Mean values of growth traits as affected by nitrogen fertilizer rates and biofertilizers in the two growing seasons.**

Treatments	Plant height at harvest (cm)		Number of leaves		Total leaf Area (dm <sup>2</sup> )		Dry weight of leaves (g)	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
	Season	Season	Season	Season	Season	Season	Season	season
<b>T1: 100% N (control)</b>	114.0	115.3	37.33	35.66	53.00	52.33	21.76	19.33
<b>T2: 80% N</b>	115.3	116.0	36.33	32.66	49.26	44.33	21.00	17.00
<b>T3: 60% N</b>	98.6	95.00	31.66	31.00	48.96	44.33	20.16	17.33
<b>T4: 100% N + Microbin</b>	127.0	128.0	42.00	37.33	62.33	58.33	25.93	23.00
<b>T5: 80% N + Microbin</b>	125.0	126.3	44.33	45.00	63.00	62.33	27.63	27.00
<b>T6: 60% N + Microbin</b>	99.33	96.67	33.33	40.66	49.66	47.00	20.90	17.66
<b>T7: 100% N + Cerealine</b>	128.3	130.3	43.33	42.00	52.66	54.00	27.00	20.00
<b>T8: 80% N + Cerealine</b>	127.7	128.3	47.00	46.00	66.73	65.00	28.33	28.66
<b>T9: 60% N + Cerealine</b>	97.67	98.66	34.66	34.33	50.66	46.33	22.63	19.00
<b>NEW LSD at 5%</b>	4.32	5.27	1.33	2.77	5.06	6.61	1.115	1.117

first and second seasons respectively for T8 treatment. Further, the beneficial effect of the treatments T4, T6, T7 and T9 resulted in improvements of the previously three growth traits. These results are in agreement with those obtained by Kannaiyan (2002) and Hamed (2006).

N fertilizer rate gave a significant effect on the same traits in both seasons. Increasing N rate up to the recommended dose increased significantly all growth traits. This may be due to the fact that the N element stimulates the vegetative growth of cotton plants by increasing amino acids and growth hormones formation which in turn acts positively for cell division and enlargement to produce new tissues. The positive effect of N on cotton has been reported by Saleh *et al.* (2004), El- Sayed and El- Menshawi (2005) and Toaima (2007).

### 3.2. Photosynthetic pigments and total sugar estimations

The data in Table (2) demonstrate that treating cotton plants with mineral-N combined with microbin or cerealine biofertilizers, significantly improved, chlorophyll a, chlorophyll b total carotenoids and total sugars. On the average 80 % N + microbin or cerealine (T5 and T8) showed higher values than the other treatments for the previously traits in the two seasons. T8 treatment

recorded the highest values for chlorophyll b and total carotenoids (*i.e.* 0.86, 0.87, 0.54 and 0.56 for the two aforesaid traits in the first and second seasons, respectively). Moreover, it revealed the highest values for total sugars (34.93) and chlorophyll a (1.17) in the second season. While T5 treatment recorded the highest values of total sugars (32.90) and chlorophyll a (1.13) in the first season compared with the control. These pronounced increments may be due to the fact that organic matter content of the experimental soil (1.47%) increased proliferation and activity of N fixing bacteria of biofertilizer, which led to more efficiency of cotton plant to accumulate N in leaves. Element of nitrogen is one of the essential chlorophyll components. It is as an important part of chlorophyll molecule. Similar trend was obtained by Hamissa *et al.* (2000).

In the meantime, 80 % N, 60 % N and 60 % N+cerealine (T2, T3 and T9) treatments recorded beneficial effect and comparable values for leaf pigments and total soluble sugars traits. Also, the two treatments T4 and T7 recorded comparative values for the previous traits, but more than those values exerted of treatments T2, T3 and T9.

### 3.3. Yield and its components

Average values of seed cotton and its

**Table (2): Mean values of photosynthetic pigments and total sugar estimates as affected by nitrogen fertilizer rates and biofertilizers in the two growing seasons.**

Treatments	Total sugars (mg/g, D. W.)		Chlorophyll a (mg/g, F.W.)		Chlorophyll b (mg/g, F.W.)		Total carotenoids (mg/g, F.W.)	
	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
<b>T1: 100% N (control)</b>	23.93	24.96	0.91	0.93	0.59	0.58	0.44	0.43
<b>T2: 80% N</b>	25.83	26.33	0.96	0.97	0.66	0.65	0.41	0.39
<b>T3: 60% N</b>	20.73	21.23	0.78	0.79	0.51	0.53	0.33	0.33
<b>T4: 100% N + Microbin</b>	27.00	28.00	1.04	0.92	0.61	0.63	0.46	0.48
<b>T5: 80% N + Microbin</b>	32.90	34.30	1.13	1.12	0.83	0.86	0.53	0.52
<b>T6: 60% N + Microbin</b>	21.47	22.20	0.73	0.83	0.55	0.52	0.33	0.35
<b>T7: 100% N + Cerealain</b>	25.00	25.67	1.02	0.95	0.67	0.69	0.48	0.49
<b>T8: 80% N + Cerealain</b>	31.56	34.93	1.02	1.17	0.86	0.87	0.54	0.56
<b>T9: 60% N + Cerealain</b>	24.90	23.50	0.78	0.78	0.54	0.53	0.32	0.33
<b>NEW LSD at 5%</b>	1.427	1.445	0.021	0.029	0.012	0.019	0.006	0.007

**Table (3): Mean values of yield and yield component traits as affected by nitrogen fertilizer rates and biofertilizers in the two growing seasons.**

Treatments	Seed cotton yield (kentar/fed.)*		Number of bolls		Boll weight (g)	
	1 <sup>st</sup> Season	2 <sup>nd</sup> season	1 <sup>st</sup> Season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
<b>T1: 100% N (control)</b>	7.16	6.49	16.36	15.76	2.26	2.22
<b>T2: 80% N</b>	6.10	5.56	13.56	12.63	2.33	2.10
<b>T3: 60% N</b>	5.48	5.10	13.06	13.26	2.17	2.08
<b>T4: 100% N + Microbin</b>	6.90	5.61	16.56	16.40	2.47	1.85
<b>T5: 80% N + Microbin</b>	7.48	7.53	17.93	15.30	2.45	2.42
<b>T6: 60% N + Microbin</b>	5.96	6.08	13.33	14.00	2.31	2.09
<b>T7: 100% N + Cerealain</b>	7.08	6.82	15.33	17.00	2.73	1.85
<b>T8: 80% N + Cerealain</b>	7.81	7.75	17.76	16.66	2.56	2.28
<b>T9: 60% N + Cerealain</b>	6.17	6.15	13.16	13.33	2.42	2.24
<b>NEW LSD at 5%</b>	1.15	1.43	0.66	0.43	0.029	0.023
Treatments	Lint percentage (%)		Seed index (g)		Lint index (g)	
	1 <sup>st</sup> Season	2 <sup>nd</sup> Season	1 <sup>st</sup> Season	2 <sup>nd</sup> Season	1 <sup>st</sup> Season	2 <sup>nd</sup> season
<b>T1: 100% N (control)</b>	35.90	33.96	9.37	9.58	5.25	4.93
<b>T2: 80% N</b>	33.56	32.33	9.50	9.45	4.80	4.51
<b>T3: 60% N</b>	34.03	30.93	6.67	9.20	4.65	4.12
<b>T4: 100% N + Microbin</b>	36.40	35.90	9.30	9.68	5.32	5.42
<b>T5: 80% N + Microbin</b>	38.93	36.53	10.38	10.16	6.62	5.85
<b>T6: 60% N + Microbin</b>	32.13	31.93	9.17	9.10	4.34	4.27
<b>T7: 100% N + Cerealain</b>	36.70	33.33	9.35	9.90	4.42	4.95
<b>T8: 80% N + Cerealain</b>	40.40	37.53	10.45	10.46	7.09	6.32
<b>T9: 60% N + Cerealain</b>	32.73	31.50	9.073	9.30	3.75	4.27
<b>NEW LSD at 5%</b>	0.335	0.740	0.019	0.038	0.017	0.021

\* Kentar = 157.5 kg of seed cotton

components are given in Table (3). It is obvious that the treatments caused highly significant differences regarding, seed cotton yield, the number of bolls, boll weight, lint (%), seed index and lint index. These results showed that microbin

or cerealain combined with mineral 80 % of N fertilizer significantly increased seed yield and its contributors compared with mineral in both seasons except the control treatment. The data showed that the best results were obtained with

the two treatments of T5 and T8 followed by T4, T6, T7 and T9 treatments indicating the beneficial effect of the biofertilizer on these traits. These results also indicated that the importance of the number of bolls and boll weight traits resulted in the positive effect on seed cotton yield. The results showed that the highest values for seed cotton yield (7.53 and 7.75 kantar/fed.) for T5 and T8 treatments occurred in the second seasons, respectively. The corresponding obtained values were 7.48 and 7.81 for the same traits in the first season.

Increasing seed cotton yield and its components after biofertilizers application was also explained by Al- Mohamed *et al.* (2009) and confirmed by Chowdhury and Mukherjee (2008).

These results are in accordance with those obtained by Sundaravardarajan *et al.* (2006) who found that the increased number of bolls, boll weight and seed cotton yield traits may be due to the desirable effect of biofertilizers. In this respect, seed cotton yield may sometimes be limited by photosynthesis. The promoting effect of biofertilization on leaf pigments concentration might be attributed to the enhancing effect of biofertilizer on the nutritional status of cotton plants.

In conclusion, judging from seed cotton yield, the results of the present study clearly indicated that, using of bio-fertilizers reduced the environmental pollution, caused from the chemical ones and to improve the growth and cotton yield traits.

These fertilizers promote the different metabolic processes which increased syntheses of chlorophyll and absorption of essential nutrients, and could be increase seed cotton yield trait. Based on the present results it could be concluded that the application of 80 % N + cerealin or microbin were favorable treatments for producing relatively high yield through improved growth traits, and proved to be a best way for saving 20 % of N fertilizer and avoiding its undesirable effects.

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### تأثير التسميد الحيوي كبديل جزئي وآمن للسماد النيتروجيني على بعض صفات النمو، صبغات البناء الضوئي والمحصول في نباتات القطن

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#### ملخص

تتجه السياسة الزراعية إلى الحد من استخدام التسميد الكيماوي والإتجاه إلى التسميد الحيوي والعضوي بسبب التكاليف العالية للسماد الكيماوي وما يُحدثه من تلوث للبيئة، لذلك يتم الإتجاه الآن إلى التوسع في دراسة المخصبات الحيوية كبديل آمن للإستخدام في التسميد، لهذا الغرض أجريت تجربتان حقليةتان في موسمي 2010، 2011 بمزرعة كلية الزراعة بالفيوم بهدف دراسة تأثير تسعة معاملات مُتمثلة في ثلاث معدلات من النيتروجين 45، 60 و75 كجم نيتروجين/فدان بدون مخصب حيوي مُتضمنه معاملة الكنترول (75 كجم نيتروجين/فدان) وهو المعدل الموصى به في صنف القطن جيزة 9. بالإضافة إلى ستة معاملات عبارة عن تواليف بين نوعين من التسميد الحيوي (ميكروبيين اوسيريالين مع المعدلات السابقة من النيتروجين) على بعض صفات النمو وصبغات البناء الضوئي والمحصول ومكوناته لصنف القطن المصري جيزة 90 في تصميم القطاعات الكاملة العشوائية في أربعة مكررات.

أظهرت النتائج وجود تأثير معنوي على مستوى 5 % على صفات النمو حيث كانت هناك فروقاً معنوية في صفات طول النبات، عدد الأوراق، مساحة سطح الورقة والوزن الجاف للأوراق في كلا الموسمين، حيث أعطت معاملات التسميد الحيوي سيريالين أو ميكروبيين مع السماد النيتروجيني 100 أو 80% أعلى القيم في صفة طول النبات مقارنة بالكنترول وأيضاً كان هناك تأثير ملحوظ لهذه المعاملات على باقي صفات النمو محل الدراسة.

أوضحت الدراسة أن كلوروفيل أ، كلوروفيل ب، الكاروتنويدات الكلية والسكريات الكلية قد تأثرت جميعها معنوياً وإيجابياً بمعاملات التسميد الحيوى (الميكروبيين أو السيريالين) مع التسميد النيتروجينى حيث حققت زيادة معنوية مقارنة بمعاملات التسميد النيتروجينى بدون مخصب حيوى وذلك فى موسمى الدراسة.  
كان لمعاملتى الميكروبيين أو السيريالين مع السماد النيتروجينى 80 % بالمعدل الموصى به تأثيراً معنوياً على صفات المحصول ومكوناته وكذلك على صبغات البناء الضوئى مقارنة بالمعاملات الأخرى، وبناءً على ذلك تعتبر المعاملتين سالفتى الذكر أفضل المعاملات الموصى بهما فى زراعة القطن (صنف جيزة 9) حيث أعطت كل منهما أعلى القيم لمعظم الصفات المدروسة بالإضافة إلى توفير 20% من التسميد النيتروجينى من المعدل الموصى به.

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المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (63) العدد الثالث ( يوليو 2012):236-242.