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THE INDIVIDUAL AND COMBINED EFFECT OF BIOINOCULANTS ON COTTON GROWTH AND YIELD UNDER DIFFERENT LEVELS OF N-FERTILIZER

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ABSTRACT: ***Two field experiments were conducted during two successive seasons of 2004-2005&2005-2006 to investigate the influence of two N_2-fixing bacteria,i.e. Azotobacter chroococcum, and Azospirillum brasilense 245 and/or yeast "Sacchromyces cerevisiae"in presence of three levels of mineral nitrogen fertilizer (15, 30 and 60 kg N/fed) on some growth parameters, yield, yield components and fiber quality of cotton crop.***

The results indicated that increasing the level of N fertilizer caused increases of all cotton growth traits, such as plant height, No. of open bolls/plant and bolls weight, seed yield and seed index as well as chemical compositions of leaves and seeds in both seasons.

Application of the bioinoculants, Azotobacter, Azospirillum and/or yeast in presence of the different levels on N-fertilizer augmented all of the obovementioned cotton growth parameters and cotton seed yield as compared with plants amended with N-fertilizer only. The highest value of cotton seed yield was recorded for plants inoculated with Azotobacter chroococcum combined with 60 kg N/fed in both seasons followed by those treated with Sacchromyces cerevisiae and 60 kg N/fed. Application of different levels of N- fertilizer or bioinoculants failed to score any significant increase for fiber quality.

These results suggest that the beneficial effect of Azotobacter or yeast on cotton growth and yield was attributed to the biologically active substances produced by these microbial strains besides the contribution of the diazotrophs Azotobacter chroococcum to nitrogen gains in soil under lower levels of N-fertilizers.

Key words: ***Cotton, Diazotrophs, yeast, Mineral fertilizer***

INTRODUCTION

Cotton crop occupies prominent position in policy of Egyptian agriculture. Cotton is not only the most important fiber crop in the world, but also the second best potential source for plant proteins after soybean and the fifth oil-producing plants (Sawan *et al.,* 2006)

Use of mineral fertilizer is essential for supporting the growth of high yield crops. Despite the importance of mineral fertilizers in providing mankind with

abundant agriculture products, their hazards to the environment have been recognized in the recent years.

Nitrogen plays an important role in balancing vegetative and reproductive plant growth, yield and lint quality. Improving the N nutrition of cotton could substantially increase plant growth and yield. However, increasing the rate of N-fertilization to overcome N limitations may not be successful..Nitrogenous nutrition play a critical role in crop production, and it is very difficult to manage relative to other essential nutrients. Excessive doses of N promotes vegetative development often at the expense of reproductive development, especially at bloom or at early boll fill (Mullins and Burmester, 1990; Tewolde and Fernandez 1997 and Howard et al., 2001).

In search for a solution to the problem, biological fertilizers have been proposed as partially alternative to mineral fertilizers. Naturally occurring N_2-fixing microorganisms including bacteria, such as *Rhizobia, Azotobacter* and *Azospirillum* and fungi such as yeast (*Saccharomyces cereviciae*) have been utilized for suc concern.

The beneficial effects of *Azotobacter spp.* on cereals millets, vegetables, cotton, and sugar cane under both irrigated and rainfed field conditions have been well documented (Hussain et al, 1987; Radwan 1998; Tilak 1993; Mrkovacki and Milic 2001).

Besides dinitrogen fixation, *Azotobacter* is also capable of producing gibberiline, oxines and cytokinens as reported by Mrkovacki and milic (2001); Relix et al., 1987; Martinez Toledo et al (1989).

Azospirillum is another N_2-fixing diazotroph, especially the species *A. lipoferum* and *A. brasilense* which have been shown to associate with a number of cereal plant roots including wheat,maize and sorghum (Döbereiner and Boddy 1981; Fallik and Okon 1996; Malik et al., 1997 and Dobbelaere et al., 2001).

Plant studies have shown that the beneficial effects of *Azospirillum* on plants can be enhanced by co-inoculation with other microorganisms. co-inoculation frequently increased growth and yield compared to single inoculation provided the plants with more balanced nutrition and improved absorption of nitrogen, phosphorus and mineral nutrients(Bashan and Holguin1997 a and b; Bashan 1998).

Associative nitrogen fixation, capability to produce plant growth promoting, antifungal, antibacterial substances and their effects on root morphology are the principal mechanisms responsible for the promotion of crop yields (Tilak and Annapurna, 1993. Inoculation with *Azospirillum* results in enhancing assimilation of mineral nutrients (N, P, K, Rb and Fe^{+2}) and water and offers resistance to pathogens (Waning 1990).

Yeast (*Saccharomyces cerevicia*) plays an important role in soil biofertility because of their capability for producing hormones, amino acids, cytokinines and vitamins (Monib et al., 1982).

The main goal of the current trial was to find out the better biological treatments could be applied to cotton plant to get a high yield with a good quality.

MATERIALS AND METHODS

A field trial was achieved at Sids agricultural research station, Beniswif Governorate, Egypt during two successive seasons of 2004-2005 and 2005-2006 on cotton crop (Gossypium barbadense L.)cultivar Giza 80 to find out the most effective bio-inoculants with 3 rates of mineral nitrogen fertilizer to obtain a high yield of cotton and lowering the unwise doses of N-fertilizer. Chemical and physical properties of soil are shown in Table (1).

Table (1): Physical and chemical properties of experimental soil

Properties	Value
Sand %	19.8
Silt %	38.9
Clay %	40.1
$CaCO_3$	1.3
Textural class	Clay loam
pH (1:2.5)	7.58
E.C. (dS m^{-1})	0.96
O.C. %	0.56
O.M. %	0.96
Total soluble – N (ppm)	42.58
Soluble cations (meql^{-1})	
Ca++	3.72
Mg^{++}	2.23
Na+	2.50
K+	1.19
Soluble anions (meql^{-1})	
CO^-	0.00
HCO_3^-	3.28
CL^-	2.83
SO_4	3.52

The plots area was 7.5m^2 (5 rows, 4m long, 60cm apart and hill spacing was 25cm). The two outer rows were left as a boarder; the remaining three rows were used to determine yield and its components.

The experimental design was split plots with four replicates for each treatment. All plots were fertilized by recommended doses of super phosphate at a rate of 22.5 kg P_2O_5/fed as calcium super phosphate (15.5% P_2O_5) during land preparation while potassium sulphate (48% K_2O) was added at a rate of 24 kg k_2O/fed in one dose with the first dose of nitrogen fertilizer. Three levels of inorganic nitrogen fertilizer 15, 30 and 60 kg N/fed were applied as ammonium nitrate (33.5 %N). The recommended rate (60 kg N/fed) was split into two equal portions. The 1st portion was added after thinning and the 2nd one was added after 15 days. Sowing date was 26th march in both seasons.

The whole scheme of the work is as follow:

1- 15 kg N/fed of N-fertilizer.

2-15 kg N/fed + *Azotobacter.*

3-15 kg N/fed + *Azospirillum.*

4-15 kg N/fed + *Saccharomyces.*

5-15 kg N/fed + *Azotobacter* + *Azospirillum*.

6-15 kg N/fed + *Azotobacter* + *Azospirillum* + *Saccharomyces*.

7-30 kg N/fed of N-fertilizer.

8-30 kg N/fed + *Azotobacter*.

9-30 kg N/fed + *Azospirillum*.

10-30 kg N/fed + *Saccharomyces.*

11-30 kg N/fed + *Azotobacter* + *Azospirillum*.

12-30 kg N/fed + *Azotobacter* + *Azospirillum* + *Saccharomyces*

13- 60 kg. N/fed of N-fertilizer

14-60 kg N/fed *Azotobacter.*

15-60 kg N/fed + *Azospirillum.*

16-60 kg N/fed + *Saccharomyces.*

17-60 kg N/fed + *Azotobacter* + *Azospirillum*.

18-60 kg N/fed + *Azotobacter* + *Azospirillum* + *Saccharomyces.*

Microorganisms used

Two N_2-fixing bacteria (*Azospirillum brasilense*(strain 245)and *Azotobacter chroococcum)* were applied in inoculating cotton seeds and

Saccharomyces cerviciae were obtained from microbiological Dept. Soil, Water and Environ. Res. Inst., ARC., as commercial preparation (Activator Yeast) .*Azospirillum* and *Azotobacter* used in this study were cultured routinely in their proper selective medium as following , *Azospirillum brasilense* (strain 245) was grown in Döbereiner medium (Döbereiner et al., 1976) and *Azotobacter chroococcum* (local isolate) was grown on N-deficient agar medium according to (Abdel-Malek and Ishac , 1968)-

Preparation of inocula:-

Four day old cultures of the assigned bacteria were grown on their proper selective medium at 28ºC to obtain population density of 3 X 10^8 CFU / ml culture. Vermiculite supplemented with 10% Irish peat was packed in polyethylene bags (180 g carrier / bag), then sealed and sterilized by gamma irradiation (5.0 X 10^6 rad.). Bacteria cultures were injected into the carrier to satisfy 60% of water holding capacity.

Cotton grains inoculated with N_2-fixing bacteria at a rate of 600g inoculants/ fed. Arabic gum solution (16%) was used as sticking agent. *Saccharomyces cereviciae*) was added at the surface of the soil at the rate of 5 liter / fed. According to the recommendation of the procedure.

Leaves samples were taken representatively from the upper fourth node of the apex, at 100 days after sowing and prepared for chemical analysis.

Studied characters:

(1) Cotton yield and its components in both seasons:

Ten representative cotton plants were taken randomly from each plot to estimate the following traits: final plant height at harvest (cm), number of fruiting branches/plant, number of open bolls/plant, boll weight (g), earliness%, lint% and seed index (g) and the yield of seed cotton/fed were calculated from the three inner rows of each plot.

Micronaire value and Pressley index were measured at the laboratories of cotton Research Institute, ARC, under standard conditions of test (65± 2% relative humidity and 67°C) according to A.S.T.M. (1975).

II) Chemical analysis of cotton leaves and seeds

Chemical leaves constituents such as chlorophylls, carotenoids, total soluble sugars and N,P,K were determined according to the methods of (Arnon, 1949), (Rolbelen, 1957), (A.O.A.C. 1965), (Chapman and Pratt, 1961). Respectively. While, Seeds oil content was determined according to A.O.A.C. (1975) and Seed protein content was determined using the method outlined by A.O.A.C. (1965).

The statistical analysis of the data in the two successive seasons were done according to Snedecor and Cochran (1981).

RESULTS AND DISCUSSION

Response of cotton plants to bioinoculation under different levels of N-fertilization on :

Cotton Growth Parameters:

Data in Table (2) show that applying 15, 30, 60 kg N/fed caused significant effect on plant height in the first season and non-significant increase in the second season. Combination of the tested inoculants, as compared with uninoculated ones. The Interaction of the bio-inoculants with the different levels of N-fertilizer non significantly increased plant height in both seasons. The highest values of plant height was obtained for the plants fertilized with 60 kg N/fed combined with *Saccharomyces cereviciae* in both seasons followed by the cotton seeds inoculated with *Azotobacter.*

Concerning the number of fruiting branches/plant, data in Table (2) revealed a significant increase in such measure due to application of N-fertilizer. However, non significant increase was determined with cotton seeds inoculated with bacterical strains or *Saccharomyces cereviciae* in both seasons. The interaction was also non significant.

The highest No. of fruiting branches/plant was recorded for the plants fertilized with 60 kg N / fed and inoculated with *Saccharomyces cereviciae* as a biofertilizer. It was clear that application of the biofertilizer enhanced cotton growth parameters in terms of plant height, No. of fruiting branches/plant compared to the uninoculated plants especially when biofertilizer combined with 60 kg N/fed. These results are in agreement with those obtained by Anjum et al., (2007). They recorded that maximum cotton plant height was recorded for NPK applied along with diazotrophs bacterial inoculua e. g. *Azospirillum lipoferum* and *Azotobacter chroococcum* as compared with uninoculated plants. They found also that mineral N significantly increased plant height.

Number of open bolls / plant and bolls weight

Data in Table (2) revealed that N fertilizer levels had a significant effect on No. of open bolls / plant in the first season and non-significant increase in the second season. The highest No. of open bolls / plant was recorded in plants fertilized with 60 kg N /fed combined with inoculation with yeast compared with uninoculated ones followed by seeds inoculated with *Azotobacte*r in both seasons.

Regarding to bolls weight, there was a significant increase in bolls weight as a result of application of different doses of N fertilizer. Inoculation of cotton with biofertilizer had non-significant effect on bolls weight and the interaction of N-fertilizer and bioinoculation was non-significant. The highest bolls weight was observed in treatments amended with yeast and 60 kg N / fed in both seasons. Anjum et al., (2007) indicated that inoculation of cotton seeds with *Azotobacter chroococcum* and *Azospirillum lipoferum* non-significantly increased bolls weight as compared with uninoculated plants. Maximum bolls weight was noticed in NPK treatments of 112-56-60 kg N / ha in combination with bacterial inoculation over its respective control. However, interaction effect of mineral fertilizer and bacterial inoculation was insignificant on bolls weight. Similar results have been observed by Rashid et al., 1999, 2000). Prasad and Prasad (2004) studied the direct and residual effect of N fertilizer 30, 60 and 90 kg N / ha with and without *Azotobacter* on cotton and mustard crop. They found that N significantly increased plant height, No. of bolls / plant and bolls weight up to 90 kg N / ha. *Azotobacter* had no significant effect on cotton growth parameter but *Azotobacter* had significant effect on mustard yield.

Seed cotton yield

Data in Table (3) demonstrate that cotton seed yield was significantly increased with increasing the level of N-fertilizer. The lowest yield (6.5 Kentar / fed) was obtained from plants fertilized with 15 Kg N / fed while the highest one (8.75 Kentar / fed) was obtained from plants fertilized with 60 Kg N / fed in the first season, the same trend was recorded in the second season. These findings were in agreement with those obtained by Sawan et al., 2006 who reported that cotton seed yield significantly increased due to raising the rate of N fertilizer from 95.2 Kg/ha to 142.8 Kg / ha. This could be attributed to the fact that N is an essential nutrient increasing plant dry matter, as well as many rich compounds that regulate photosynthesis and plant production, Feibo et al., 1998).Also, Anjum (2007) stated that N-fertilization significantly influenced seed cotton yield.

Regarding to application of N_2-fixing bacteria and or yeast on cotton plants, data in Table (3) show that application of either *Azotobacter* and /or *Azospirillum* or yeast increased cotton seeds yield in both seasons compared with uninoculated ones, but the increase was non-significant. Plants fertilized with *Saccharomyces cereviciae* scored 8.02 Kentar / fed in the first season , while the uninoculated plants gave 7. 11 Kentar / fed in the second season, the same trend was observed plants treated with yeast scored 8.47 Kentar/ fed compared with 8.10 Kentar / fed in uninoculated ones followed by seed inoculated with Azotobacter in both seasons. Among all treatments the interaction between

Azotobacter **combined with 60 kg N / fed gave the highest cotton seeds yield followed by plants received 60 kg N / fed and fertilized with yeast.**

In this respect, Wankhade et al., (2001) revealed that there were in significant differences in the yield of cotton seeds inoculated with biofertilizers.Inoculation with *Azotobacter* and *Azospirillum* cultures recorded 7.9 and 2.5% increase in cotton seed yield respectively. The present results are in line with those of Anjum et al., (2007), whom reported that inoculation of cotton plants with *Azotobacter* and *Azospirillum* significantly increased the cotton seed yield (21%) compared to treatments without inoculation. The interaction of inoculation and N-fertilization was, however, was insignificant.

In the current work, the application of *Saccharomyces cereviciae*, *Azotobacter* and *Azospirillum* induced promotion effect on cotton seeds yield, which could be attributed to the biologically active substances produced by these microorganisms. In this concern, Malik et al., (2004) used 4-indole 3-acetic acid producing strains of *Bradyrhizobium* and *Azotobacter* as plant growth promoting strains to inoculate 4 cotton cultivars. They found that inoculation with these strains increased the rate of seedling emergence, shoot dry weight, bio mass and N-contents. Also, Sharma and Vasudeva (2005) used azide resistant mutant of *Aacetobacter* diazotroph and *Azospirillum* to inoculate cotton seeds. They found that azide resistant mutants when used as a biofertilizer showed increase in plant height, more yield and high biomass.

Saccharomyces cereviciae **(yeast) produces hormones such as auxins, indole acetic acid, besides it is considered as a source of nutrients and vitamins especially vitamin B12, Monib et al.,(1982). Therefore, the application of yeast as a biologically active microorganism augmented cotton seed yield compared with plants fertilized with N-fertilizer alone.**

Yield earliness percentage.

Data in Table (3) show that earliness % was increased in both seasons among all treatments, as compared with plants receiving mineral N-fertilizer only. The highest percentage of earliness was recorded in plants treated with yeast combined with 60 kg N/fed ,followed by plants inoculated by *Azotobacter* plus 60 kg N/fed .On the other hand , El-shazly and Darwish (2001) reported that application of Microbin as a commercial preparation of biofertilizer for cotton crop with N fertilizer had insignificant effect on earliness % .

Lint percentage.

Data in Table (3) indicate that lint % was affected by application of the bioinoculants with or without nitrogen fertilizer. Plants receiving N-fertilizer

only had lower lint % in comparison compared with plants treated with the biofertilizers. Plants inoculated with *Azotobacter* + 60 kg N/fed gave the highest lint % in the first season while plants treated with yeast + 60 kg N/fed scored the highest value of lint % in the second season. In this respect, El-shazly and Darwish (2001) found that application of Microbin significantly increased lint percentage in cotton plants while the interaction between N-fertilizer levels and Microbin as a biofertilizer gave insignificant effect on lint%.

Seed index.

It is obvious from Table (3) that seed index increased by application of the bioinoculants with or without N-fertilizer in both seasons. In the first season, plants inoculated with *Azotobacte*r combined with 60 kg N/fed scored the highest seed index, while plants treated with yeast +60 kg N/fed gave the highest seed index in the second season.

Fiber quality: **From data in Table (3), non-significant increase was observed due to application of N-fertilizer and/or bioinoculants in micronair reading and Pressley index in both seasons.**

Leaves and seed constituents: **Data presented in Table (4) demonstrate the effect of various tested treatments on chemical analysis of cotton leaves and seeds.It can be noted that Chlorophyll a ,b and total chlorophyll, as well as carotenoids were significantly increased with increasing the level of N-fertilizer. Application of the bioinoculants gave an increase in Chlorophyll A, B and total chlorophyll and carotenoids compared to the uninoculated treatments which received N-fertilizer only. The highest values of Chlorophyll a, b and total chlorophyll as well as carotenoids were recorded in plants inoculated with yeast combined with 60 kg N / fed followed by plants inoculated with *Azotobacter* + 60 kg N / fed. It is also observed from data in Table (4) that total soluble sugars, reducing sugars and non-reducing sugars were significantly increased due to application of N-fertilizer and/or bioinoculants. Inoculation of cotton seeds with *Azotobacter* gave the highest values of total soluble sugars. The increase in Chlorophyll a, b and total chlorophyll and carotenoids due to application of inorganic N-fertilizer and/or bioinoculants could be attributed to increasing of N.**

It is well known that N is an essential nutrient in creating plant dry matter, and many energy rich compounds which regulate photosynthesis. There is an optimal relationship between nitrogen contents in the plant and CO_2 assimilation as reported by Greef (1994), where decreases in CO_2 fixation are well documented for N-deficient plant. Al-Kahal et al., (2007) found that Chlorophyll a, b and total chlorophyll and carotenoids were increases with increase the uptake of N due to application of organic nitrogen.

It is obvious from data in Table (4) that there was a significant difference in N-content of cotton leaves. Application of N-fertilizer alone at different doses gave a significant increase in N-uptake. The highest value was obtained in plants fertilized with 60 kg N / fed without any addition of biofertilizers. These results are on line with those obtained by Al-Kahal et al., (2007). They found that N-content in cotton leafs was increased by increasing the level of inorganic N-fertilizer. Application of the biofertilizers gave an increase in N-uptake. The highest value was recorded in the plants inoculated with *Azotobacter chroococcum* comparied with plants amended N-fertilizer only. The combined application of *Azotobacter chroococcum* together with 60 kg N/fed produced the highest N-content among all treatments. Malik *et al.,* (2004) found that inoculation of 4-cotton cultivars with 4-indole 3-acetic acid producing selected *Bradyrhizibium* strain and *Azotobacter* as plant growth promoting rhizobacteria increased nitrogen uptake in cotton cultivars. Potassium and phosphorus of cotton leaves did not show any significant differences among all treatments.

Concerning crude protein in cotton seeds, The highest crude protein content was recorded in plants amended with *Saccharomyces cereviciae* followed by plants inoculated with *Azotobacter* and/or *Azospirillum* comparied with uninoculated treatments. These results suggest that increasing the uptake of N due to application of inorganic N-fertilizer and / or biofertilizer increases the amino acid synthesis in the leaves and this stimulate the accumulation of protein in the seed. The present results confirmed the findings of Patil et al., (1997).

Regarding to seed oil percentage, increasing the level of N-fertilizer led to a significant increase in the seed oil %. Among all treatments, application of yeast and 60 Kg N / fed gave the highest seed oil% , followed by plants inoculated with *Azotobacter* combined with 60 Kg N / fed.

The fatty acid composition of seed oil crops is mainly under genetic control, but can affected to some extant by N nutrition as reported by Holmes and Bennett (1979) .Synthesis of fats requires both N and carbon skeletons during the course of development as reported by Patil et al., (1996).

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تأثير المعاملة بالمخصبات الحيوية مع مستويات مختلفة من التسميد النيتروجينى على النمو والمحصول لنبات القطن

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الملخص العربى

أجريت تجربتين حقليتين خلال الموسمين الزراعيين المتتاليين ٢٠٠٤ – ٢٠٠٥ ، ٢٠٠٥- ٢٠٠٦ وذلك لدراسة تأثيرسلالتين من البكتيريا المثبتة للآزوت الجوى أزوتوباكتر كروكوكم، أزوسبيريللم برازيلنس ٢٤٥ والخميرة سكاروميسيس سيرفيسيا وذلك فى وجود ٣ مستويات من التسميد النتروجينى ١٥، ٣٠ و ٦٠ كجم نتتروجين/فدان. وذلك على بعض الصفات الخضرية لنبات القطن وعلى المحصول ومكوناتة وصفات الجودة للألياف.

وقد أوضحت النتائج أنه كلما زاد مستوى التسميد النتروجينى كلما زادت قيم المحصول الخضرى للقطن لكل من الموسمين وذلك مثل طول النبات وعدد اللوز المتفتح /نبات ووزن اللوز ومحصول البذرة وكذلك زاد المحتوى الكيماوى للأوراق والبذور لنبات القطن.

ووجد أن استخدام المخضبات الحيوية سواء أزوتوباكتر كروكوكم أو أزوسبيريللم برازيلنس ٢٤٥ أو الخميرة فى وجود المستويات المختلفة من التسميد النتروجينى أدى إلى زيادة صفات المحصول السابق ذكرها مقارنة بالنباتات التى سمدت بالتسميد النتروجينى فقط.

اعطت النباتات التى لقحت با لأزوتوباكتر كروكوكم فى وجود ٦٠ كجم ن / فدان أعلى إنتاج لمحصول القطن تليها النباتا التى عوملت بالخميرة فى وجود ٦٠ كجم ن / فدان وذلك فى كلا الموسمين. لم يكن هناك تأثير معنوى لصفات الجودة للألياف نتيجة استخدام التسميد النتروجينى أو المخصبات الحيوية.

هذه النتائج توضح أن التأثير الناتج من الأزوتوباكتر أو الخميرة على نمو وإنتاجية محصول القطن يرجع إلى المركبات الحيوية التى تنتج بواسطة هذه الميكروبات المستخدمة بجانب تثبيت النتروجين الجوى بواسطة بكتيريا الأزوتوباكتر كروكوكم تحت المستويات المنخفضة من التسميد النتروجينى.

Table (2):Response of cotton growth to biofertilizer treatments under N-fertilizer levels and their interaction

N-Fertilizer (kg/fed) (A)	Treatment (B)	Plant height (cm)		plant dry wt (g)		No. of fruiting branches/plant		No. of open bolls/plant		Average of boll weight (g)/ plant	
		2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
15	Control	103.50	121.80	75.00	78.20	10.25	10.20	12.30	14.20	2.90	2.10
	Azotobacter (1)	115.80	123.70	77.90	80.60	11.00	12.30	14.80	15.00	2.17	2.16
	Azospirillum (2)	108.20	122.80	77.80	79.00	10.80	11.80	13.90	14.90	2.16	2.13
	Sacharomyces (3)	117.70	123.50	78.80	80.70	11.87	13.20	15.60	15.50	2.23	2.18
	(1) + (2)	110.00	122.70	77.90	78.60	10.50	10.76	13.80	14.70	2.13	2.12
	(1) + (2)+ (3)	109.10	122.00	76.00	78.70	10.70	10.90	13.80	14.60	2.10	2.11
	Mean	110.71	122.75	77.18	79.30	10.85	11.52	14.00	14.90	2.15	2.13
30	Control	116.30	127.50	78.60	80.80	12.81	13.80	15.10	16.00	2.26	2.34
	Azotobacter (1)	125.30	129.30	78.80	81.60	14.30	15.30	15.90	16.90	2.30	2.43
	Azospirillum (2)	121.50	121.00	78.73	81.20	13.50	15.00	15.80	16.60	2.29	2.4
	Sacharomyces (3)	126.90	124.10	78.98	81.90	15.30	15.50	15.90	17.10	2.36	2.39
	(1) + (2)	117.80	128.00	78.62	80.90	14.00	14.60	15.60	16.60	2.28	2.39
	(1) + (2)+ (3)	120.00	128.10	78.68	81.00	12.90	14.70	15.50	16.50	2.27	2.35
	Mean	121.28	128.50	78.73	81.23	13.80	14.81	15.60	16.60	2.29	2.40
60	Control	121.80	131.80	78.90	82.60	14.00	15.10	16.00	17.50	2.36	2.40
	Azotobacter (1)	129.10	134.00	81.90	83.90	15.70	16.60	17.10	18.80	2.65	2.98
	Azospirillum (2)	124.60	133.10	80.98	83.20	14.50	15.90	16.90	17.90	2.50	2.69
	Sacharomyces (3)	130.20	133.80	82.00	83.61	15.80	16.90	17.80	18.00	2.59	2.90
	(1) + (2)	128.20	132.01	81.60	83.00	14.60	15.80	16.80	17.80	2.49	2.60
	(1) + (2)+(3)	124.30	132.10	80.89	83.09	14.30	15.70	16.60	17.60	2.43	2.59
	Mean	126.36	132.80	81.04	83.23	14.83	16.00	16.70	17.80	2.50	2.69
Average	Control	113.86	127.03	77.50	80.53	12.35	13.03	14.40	15.70	2.24	2.28
	Azotobacter (1)	123.40	129.00	79.53	82.03	13.66	14.73	15.90	17.00	2.55	2.52
	Azospirillum (2)	118.10	128.53	79.17	81.13	12.96	14.23	15.50	16.40	2.31	2.40
	Sacharomyces (3)	124.93	128.80	79.92	82.07	14.32	15.20	16.10	16.80	2.41	2.52
	(1) + (2)	118.67	127.97	79.27	80.83	13.03	13.72	15.40	16.30	2.30	2.37
	(1) + (2)+ (3)	117.80	127.40	78.52	80.93	12.63	13.76	15.30	16.20	2.26	2.35
L.S.D. at 5%	A	9.28.	N.S.	N.S.	2.78	1.74	1.54	0.94	N.S.	0.09	0.11
	B	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.05
	AB	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.09

Table (3): Seed cotton yield and its attributes as affected by N-fertilizer levels, biofertilizer treatments n,and their interaction

N-	Treatment	Earliness %	Lint %	Seed index	Seed cotton	Micronair	Pressely index

The individual and co

Fertilizer (kg/fed) (A)	(B)							yield		relation			
		2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
15	Control	59.30	61.45	39.56	39.50	7.16	7.73	5.73	6.30	4.2	4.3	10.10	9.90
	Azotobacter (1)	64.90	62.43	40.19	40.40	8.17	8.63	6.74	6.83	4.2	4.3	10.00	10.10
	Azospirillum (2)	62.70	63.99	40.14	39.60	8.13	8.55	6.61	6.82	4.3	4.3	9.90	9.90
	Sacharomyces(3)	66.30	64.33	40.86	40.60	8.95	8.71	7.19	6.87	4.0	4.2	9.90	9.60
	(1) + (2)	60.10	60.64	39.57	39.70	8.02	8.41	6.46	6.66	4.4	4.4	10.10	10.10
	(1) + (2) + (3)	60.30	61.60	39.63	39.60	8.10	8.33	6.49	6.58	4.3	4.2	9.60	9.60
	Mean	62.26	62.40	39.99	39.90	8.08	8.37	6.50	6.64	4.30	4.20	9.90	9.87
30	Control	61.10	59.97	39.90	39.38	9.00	9.07	7.28	8.22	4.2	4.4	10.1	9.8
	Azotobacter (1)	65.70	64.33	40.30	40.72	9.16	9.20	7.69	8.54	4.3	4.2	9.7	9.9
	Azospirillum (2)	63.80	63.07	40.10	40.48	9.13	9.18	7.67	8.67	4.4	4.3	9.5	9.6
	Sacharomyces(3)	66.30	64.70	10.40	40.23	9.18	9.29	7.85	8.58	4.3	4.4	9.5	9.5
	(1) + (2)	61.70	62.60	40.00	39.63	9.10	9.10	7.50	8.27	4.3	4.4	9.9	9.7
	(1) + (2)+ (3)	62.00	59.99	40.20	40.52	9.08	9.09	7.30	8.22	4.2	4.2	9.6	9.5
	Mean	63.43	62.44	40.20	40.16	9.10	9.15	7.54	8.40	4.20	4.30	9.70	9.67
60	Control	62.90	60.60	39.60	39.63	9.23	9.61	8.34	9.79	4.00	4.20	9.70	9.7
	Azotobacter (1)	66.40	69.90	40.30	40.14	9.89	10.00	9.05	10.09	1.10	4.20	9.70	9.9
	Azospirillum (2)	64.90	69.10	40.20	39.73	9.38	9.90	8.98	9.89	4.30	4.30	9.50	9.6
	Sacharomyces(3)	66.90	69.60	40.40	40.45	9.83	9.97	9.02	9.97	4.20	4.20	9.90	9.7
	(1) + (2)	63.20	64.60	39.70	39.72	9.25	9.78	8.78	9.86	4.20	4.10	9.70	9.9
	(1) + (2)+(3)	63.10	66.70	39.75	42.45	9.23	9.86	8.36	9.8	4.10	4.20	9.30	9.9
	Mean	64.56	66.78	40.01	40.02	9.48	9.86	8.75	9.8	4.20	4.20	9.60	9.78
Average	Control	61.10	60.74	39.68	39.50	8.48	8.80	7.11	8.10	4.1	4.3	10.0	9.8
	Azotobacter (1)	65.66	65.55	40.26	40.42	9.05	9.27	7.82	8.47	4.2	4.2	9.8	9.9
	Azospirillum (2)	63.80	65.39	40.14	39.93	8.88	9.21	7.75	8.46	4.3	4.3	9.6	9.7
	Sacharomyces(3)	66.50	66.21	40.55	40.42	9.34	6.32	8.02	8.47	4.2	4.3	9.8	9.6
	(1) + (2)	61.66	62.61	39.82	39.68	8.79	9.12	7.56	8.26	4.3	4.3	9.9	9.9
	(1) + (2)+ (3)	61.80	62.76	39.84	40.19	8.80	9.09	7.38	8.20	4.2	4.2	9.5	9.6
L.S.D At 5%	A	1.64	1.663	N.S.	0.128	0.148	0.034	0.844	0.009	N.S.	N.S.	N.S.	N.S.
	B	1618	3.131	0.216	0.265	0.156	0.075	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	AB	N.S.	N.S.	0.375	0.460	0.271	0.130	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table (4): Effect of individual and mixed bio-inoculation under different levels of N-fertilizer on chemical compositions of cotton leave and seeds

N-Fertilizer (kg/fed) (A)	Treatment (B)	Chlorophyll (mg/g plant)			Caroten-Oid mg/g	Carbohydrates (%)			N %	P %	K %	Protein %	Oil %
		A	B	Total		Total S.S.	R. S.	NR.S.					
15	Control	2.93	2.69	5.62	0.90	14.50	6.07	8.43	2.87	0.31	2.80	15.63	18.05
	Azotobacter (1)	3.60	2.77	6.37	1.20	15.00	6.10	8.90	3.01	0.33	2.80	18.75	18.56
	Azospirillum (2)	3.58	2.47	6.05	0.92	14.85	6.12	8.73	2.90	0.33	2.90	18.75	18.51
	Sacharomyces(3)	3.88	2.76	6.65	1.09	15.10	6.00	9.10	2.88	0.32	2.30	18.75	19.62
	(1) + (2)	3.60	2.77	6.27	1.02	14.90	6.06	8.84	2.99	0.33	2.90	18.75	18.80
	(1) + (2)+(3)	3.38	2.67	6.05	0.87	14.86	6.11	8.75	2.93	0.32	2.90	15.63	18.39
	Mean	3.48	2.68	6.17	1.00	14.87	6.18	8.79	2.93	0.32	2.80	17.71	18.65
30	Control	3.96	2.98	6.14	0.79	16.90	6.70	10.20	3.30	0.35	2.8	18.75	19.02
	Azotobacter (1)	4.70	2.69	7.39	1.04	16.85	6.75	10.10	3.43	0.39	2.9	18.75	20.44
	Azospirillum(2)	4.20	2.19	6.39	0.96	16.90	6.90	10.00	3.41	0.38	2.9	18.75	20.30
	Sacharomyces(3)	4.77	3.00	7.77	0.95	17.01	6.80	10.21	3.30	0.35	2.9	19.79	20.64
	(1) + (2)	3.99	2.55	6.47	0.88	16.50	6.82	9.68	3.40	0.40	2.8	18.75	19.62
	(1) + (2)+(3)	3.94	2.22	6.16	0.87	16.82	6.91	9.91	3.44	0.37	3.0	18.75	19.45
	Mean	4.24	2.60	6.72	0.92	16.83	6.81	10.01	3.38	3.37	2.80	18.91	19.91
60	Control	4.16	2.98	7.14	0.74	17.20	7.01	10.19	3.48	0.34	2.80	18.75	19.24
	Azotobacter(1)	5.12	3.51	8.63	0.98	18.93	7.93	11.00	3.90	0.39	2.90	21.88	21.30
	Azospirillum(2)	4.70	2.41	7.31	0.97	18.70	7.80	10.90	3.70	0.40	3.00	21.88	20.45
	Sacharomyces(3)	5.14	3.81	8.95	0.98	18.20	7.18	11.72	3.50	0.36	2.80	21.88	21.51
	(1) + (2)	4.39	2.67	7.06	1.01	18.40	7.90	10.50	3.80	0.39	2.90	21.88	19.98
	(1) + (2)+(3)	4.99	2.26	7.25	0.93	18.60	7.91	10.44	3.80	0.41	3.10	18.75	20.51
	Mean	4.63	2.94	7.72	0.94	18.45	6.85	10.80	3.69	0.38	2.90	20.24	20.94
Average	Control	3.68	2.88	6.30	0.81	16.53	6.59	9.60	3.21	0.33	2.80	17.71	18.77
	Azotobacter(1)	4.47	2.49	6.47	1.07	16.72	6.92	10.00	3.45	0.37	2.90	19.79	20.10
	Azospirillum(2)	4.16	2.36	6.58	0.95	16.78	6.94	9.84	3.33	0.37	2.90	19.79	19.75
	Sacharomyces(3)	4.37	3.19	7.79	1.01	17.00	6.66	10.34	3.22	0.35	2.80	20.14	20.59
	(1) + (2)	3.94	2.66	6.60	0.97	16.47	6.92	9.67	3.39	0.37	2.90	19.79	17.47
	(1) + (2)+(3)	4.03	2.38	6.48	0.89	16.79	6.97	9.82	3.39	0.36	2.90	17.71	19.45
L.S.D. at 5%	A	0.42	0.07	0.48	0.14	1.02	0.90	0.93	0.09	0.33	0.28	1.70	1.20
	B	1.37	1.03	1.10	1.27	1.03	1.86	1.10	0.08	0.25	0.20	2.10	1.90
	AB	1.75	1.58	1.60	0.14	1.50	0.91	1.20	0.17	0.66	2.47	2.20	2.00