



## Field evaluation of beneficial and deleterious effects of rhizobacteria on cotton stand and yield

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### Abstract

Thirteen *Bacillus* strains, one *Lactibacillus* strain, and two *Pseudomonas* strains were isolated from the rhizosphere of cotton seedlings collected at various locations in Egypt. Three field trials were conducted at Sakha (North Delta Region), El-Gemmeiza (Middle Delta Region), and Sirs El-Lian (Middle Delta Region) in 2015 to evaluate the beneficial and deleterious effects of rhizobacteria on cotton stand and yield. Field evaluation revealed inconsistent performance among strains from one site to another. Thus, while certain strains effectively controlled seedling damping-off at some locations, they were ineffective in controlling the disease at other locations or even increased disease. Seedling stand counts were not correlated with seed cotton yield at Sakha and El-Gemmeiza, while they were highly correlated at Sirs El-Lian. Regarding each of seedling stand counts (survival) and yield, the performance of the tested strains at any site was not correlated with their performance at the other two sites. Grouping the strains by cluster analysis based on their effect patterns was neither related to their taxonomic position nor to geographic origins. Of the 16 strains, strain no. 10 of *Bacillus circulans* is a promising strain for commercialization for two reasons: firstly, it significantly increased stand at all sites and secondly, it significantly increased seed cotton yield at Sirs El-Lian.

**Key words** – cotton – rhizobacteria – stand – yield

### Introduction

Cotton seedling damping-off is caused by a complex of seed-borne and soil-inhabiting organisms. These organisms are found in all cotton producing areas of Egypt. Although the populations of inciting organisms differ from area to area, the pathogens most commonly involved in the disease complex are *Rhizoctonia solani* and *Fusarium* spp. (El-Samawaty et al. 1999). The disease occurs as pre-germination decay of the seed, decay of the seedling on the way to the soil surface (pre-emergence damping-off), partial or complete girdling of the emerged seedling at or near the soil surface (“sore shine” or post-emergence damping-off), and seedling root rot (Watkins et al. 1981).

Cotton seedling damping-off is difficult to control due to the broad host range of the pathogens involved in the disease, and long viability of their resting structures. Commercially

acceptable resistant cultivars are unavailable in Egypt. Large-scale application of solarization and fumigation to reduce resting structures in soil is expensive and difficult to achieve. Thus, the widespread use of seed-dressing fungicides for controlling the disease has become indispensable under Egyptian conditions. While effective fungicides are available (Abdel-Aziz et al. 1996, El-Samawaty et al. 1999), it is becoming increasingly evident that their widespread use is associated with some problems, such as the potential harmful effect on non-target organisms, the development of resistant races of the pathogens, and possible carcinogenicity. Other problems include gradual elimination and phasing out of some compounds (Zaki et al. 1998). Therefore, rhizobacteria-mediated biological control has become a promising alternative for controlling the disease.

*Bacillus* spp. and *Pseudomonas* spp. have been widely used for biological control of cotton seedling damping-off because of their rapid growth rate and their ability to colonize rhizospheres and spermospheres (Ardakani 2009, 2010, Mansoori 2013, Khiyami et al. 2014). *Bacillus* is a Gram-positive aerobic endospore forming genus. Endospore forming, universally found in the genus is thought to be a strategy for survival in the soil, wherein these bacteria predominate and endospores make them resistant to unfavorable environmental conditions (Kumar et al. 2012). *Pseudomonas* infests the rhizosphere abundantly and aggressively colonizes plant roots (Elliott & Lynch 1984). The relatively rapid growth rate of *Pseudomonas* spp. and the selectivity of roots, due to the high affinity of Gram-negative bacteria for amino acid exudates, give *Pseudomonas* a greater rhizosphere competence than other rhizosphere microflora (Azad et al. 1985).

In general, rhizobacteria, in particular *Bacillus* and *Pseudomonas*, antagonize phytopathogenic fungi through various mechanisms such as competition for niches (e.g. iron through siderophores synthesis); parasitism, that may involve production of hydrolytic enzymes such as chitinase,  $\beta$ -1,3-glucanase, protease, and cellulase, that lyse fungus cell walls; inhibition of the pathogen by secreting antimicrobial compounds such as antibiotics, toxins, and biosurfactants; and induction of systemic resistance in host plants (Mavrodi 2006, Kumar et al. 2012).

Lactic acid bacteria (LAB) such as *Lactobacillus amylovorus* have previously been shown to play a crucial role in the preservation and microbial safety of food because of their beneficial influence on nutritional and shelf-life characteristics, and are naturally occurring in many food systems (Guo et al. 2014). Few studies have been aimed at antifungal activity of LAB against phytopathogenic fungi, e.g., citrus fungal pathogens *Geotrichum citriauranti* (Gerez et al. 2010) and the potato late blight pathogen *Phytophthora infestans* (Guo et al. 2014).

Contrary to common belief that *Bacillus* and *Pseudomonas* are effective biocontrol agents, among members of these two genera are numerous species that are pathogenic to plants (Peng et al. 2013). In addition to the pathological effects, some *Bacillus* spp. and *Pseudomonas* spp. may be involved in deleterious synergistic interactions with phytopathogenic fungi, particularly root-invading fungi. The objective of the present study was to evaluate the beneficial and deleterious effects of rhizobacteria on cotton stand and yield under field conditions.

## Materials & Methods

### Bacterial strains

Sixteen bacterial strains were randomly selected from the bacterial culture collection of Cotton Diseases Research Section, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt. All strains were originally isolated from the rhizosphere of cotton seedlings collected in various locations in Egypt (Table 1).

### Preparation of bacterial inoculum

Bacterial strains were grown in nutrient glucose broth at 30° C for 72 h on a shaker. The growth was adjusted turbidimetrically to 10<sup>8</sup> cfu/ml using spectro 2000 RSP 220v. 50Hz. Bacterial cultures were formulated in powder form by mixing 400 ml of cell suspension with 1 kg talc as carrier, which previously had been autoclaved for 30 min. over two successive days, 10 g of carboxy methylcellulose (CMC) was added to 1 kg of the carrier and mixed well. The pH of all

materials was adjusted to 7.0 by adding calcium carbonate. The bacterial population was assessed as  $4 \times 10^7$  cfu/g talc.

### Field trials

The strains evaluation trials were conducted in 2015 growing season at the agricultural research stations of Sakha (North Delta), El-Gemmeiza (Middle Delta), and Sirs El-Lian (Middle Delta). Soil type was clay in all the experimental sites. Each experiment was designed as a randomized complete block of three replicates, each replicate consisted of two, 5 m rows. Each row included 25 hills, each contained 10 seeds. Cotton (*Gossypium barbadense* L.) cultivars (Table 3) were chosen according to the recommendations of each experimental station. Slightly moist seeds were treated with the powdered inoculum of each strain at a rate of 10 g/kg seeds, and thoroughly shaken in plastic bags for 5 min. and allowed to dry for 2 hours before being planted. Planting dates were 12 April in Sakha, 31 March in El-Gemmeiza, and 24 March in Sirs El-Lian. Stands (healthy surviving seedlings) were recorded 45 days from sowing. Seed cotton yield (cottonseed and lint before ginning) was picked from 15–30 October at each site.

### Isolation and identification of fungi in soils of the experimental sites

Infected seedlings, which showed typical damping-off symptoms, were removed from the field and washed under tap water to remove any adhering soil. Small pieces of necrotic root and hypocotyle tissue were surface sterilized with 10% Clorox solution for 2 min. and washed several times with sterilized water. The surface sterilized pieces were then dried on sterilized filter papers and plated on potato dextrose agar (PDA) medium amended with streptomycin sulfate and rose bengal to eliminate bacterial contaminations. The plates were incubated at  $26 \pm 3^\circ$  C for 3–7 days. The developing colonies were identified according to Gilman (1966), Barnet & Hunter (1979). Colonies of each fungus were expressed as percentage of the total developing colonies.

### Statistical analysis of the data

The experimental design was a randomized complete block with three replicates. Least significant difference (LSD) was used to compare treatment means. Analysis of variance (ANOVA) was carried out by MSTAT-C statistical package. Data from each field site were subjected to ANOVA independently because of differences in environmental conditions and site management. Correlation and cluster analyses were performed with the software package SPSS 10.0.

### Results

Identification of the selected strains (Table 1) revealed that 13 strains (81.3%) belonged to genus *Bacillus*, two strains (12.5%) belonged to genus *Pseudomonas*, and only one strain (6.3%) belonged to genus *Lactobacillus*. *B. subtilis* was represented by four (25%) strains, *B. circulans*, *B. coagulans*, and *B. cereus* were each represented by two strains (12.5%). *B. globisprus* and *B. pumilus* were each represented by only one strain (6.5%). Species of *Bacillus* strain no. 5 was unidentified.

Nine of the strains (56.3%) were isolated from the Nile Delta governorates (Daqahliya, Sharqiya, Qalyubiya, Beheira and Minufiya), four strains (25%) from Middle Egypt governorates (Giza and Minia), one strain (6.3%) from Upper Egypt (Assiute governorates), and two strains (12.5%) from unknown origins. *Bacillus* strains were isolated from widely separated governorates in Nile Delta, Middle Egypt, and Upper Egypt, while *Pseudomonas* strains were restricted to Nile Delta.

The infected seedlings used in isolation yielded *Fusarium* spp. and unidentified fungi at all the experimental sites, while they yielded any of the other fungi at only two sites. The difference in isolation frequency between *Fusarium* spp. and any of the other fungi was significant only in El-Gemmeiza (Table 2).

Certain strains did effectively control seedling damping-off at some locations (Table 3); however, there was a lack of consistency among locations, that is, they were ineffective in controlling the disease at other locations or even increased the disease. For example, strains no. 2, 4, 13, and 16 significantly increased the disease at Sakha, suppressed it at El-Gemmeiza, and had no effect on it at Sirs El-Lian. Strains no. 9, 11, and 14 had no effect on the disease at Sakha, while they suppressed it in El-Gemmeiza and Sirs El-Lian. Strain no. 10 was the only strain, which effectively controlled the disease in the three experimental sites. None of the tested strains failed in controlling the disease at all the sites. Only one strain (6.3%) effectively controlled the disease at Sakha, 15 strains (93.8%) at El-Gemmeiza, and six strains (37.5%) at Sirs El-Lian.

**Table 1** Identification and geographic origins of bacterial strains isolated from rhizosphere of cotton seedlings.

Strain No.	Classification	Geographic origin	
		Governorate	Region
1	<i>Bacillus coagulans</i>	Unknown	Unknown
2	<i>B. globisporus</i>	Daqahliya	East Delta
3	<i>B. pumilus</i>	Giza	Middle Egypt
4	<i>Lactobacillus amylovorus</i>	Sharqiya	East Delta
5	<i>Bacillus</i> sp.	Qalyubiya	South Delta
6	<i>B. subtilis</i>	Assiute	Upper Egypt
7	<i>B. subtilis</i>	Minya	Middle Egypt
8	<i>B. circulans</i>	Beheira	West Delta
9	<i>B. coagulans</i>	Sharqiya	East Delta
10	<i>B. circulans</i>	Beheira	West Delta
11	<i>B. subtilis</i>	Minya	Middle Egypt
12	<i>B. cereus</i>	Unknown	Unknown
13	<i>B. subtilis</i>	Sharqiya	East Delta
14	<i>B. cereus</i>	Giza	Middle Egypt
15	<i>Pseudomonas putida</i>	Minufiya	Middle Delta
16	<i>P. fluorescens</i>	Daqahliya	East Delta

**Table 2** Frequency of fungi isolated from cotton seedlings infected with post-emergence damping-off in the experimental sites.

Fungus	Isolation frequency (%) <sup>a</sup>		
	Sakha (cv. Giza 86)	El-Gemmeiza (cv. Giza 89)	Sirs El-Lian (cv. Giza 89)
<i>Fusarium</i> spp.	20.82	52.96	27.50
<i>Rhizoctonia solani</i>	12.50	17.06	0.00
<i>Alternaria</i> spp.	0.00	6.68	21.66
<i>Aspergillus</i> spp.	26.26	0.00	10.00
<i>Rhizopus</i> spp.	40.40	0.00	16.68
Unidentified	33.36	13.34	10.07

Colonies of each fungus are expressed as percentage of the total developing colonies. Each value is the mean of 5 replications (plates).

LSD (p=0.05) for fungus × experimental site = 29.79.

**Table 3** Effects of treating cotton seeds with bacterial strains on surviving seedlings and yield under field conditions in 2015.

No.	Treatment	Sakha (Giza 86)		El-Gemmeiza (Giza 89)		Sirs El-Lian (Giza 89)	
		Survival (%)	Yield (Kentar <sup>a</sup> /Feddan <sup>b</sup> )	Survival (%)	Yield (Kentar/Feddan)	Survival (%)	Yield (Kentar/Feddan)
1	<i>Bacillus coagulans</i>	52.60	3.04	46.67	9.75	68.33*(+)	10.84
2	<i>B. globisporus</i>	47.00*(-)	3.49	48.42*(+)	9.75	66.67	9.86
3	<i>B. pumilus</i>	48.50	2.91	49.58*(+)	11.29	63.33	9.10
4	<i>L. amylovorus</i>	44.60*(-)	1.98*(-)	61.17*(+)	11.29	66.67	10.15
5	<i>Bacillus</i> sp.	47.70	1.83*(-)	57.25*(+)	9.75	63.33	10.37
6	<i>B. subtilis</i>	47.50	2.35*(-)	48.58*(+)	11.29	61.67	8.57*(-)
7	<i>B. subtilis</i>	46.80*(-)	2.37*(-)	51.00*(+)	10.60	55.00*(-)	5.70*(-)
8	<i>B. circulans</i>	44.85*(-)	2.72	51.25*(+)	7.95	58.33*(-)	7.35*(-)
9	<i>B. coagulans</i>	51.00	2.22*(-)	59.92*(+)	10.09	71.67*(+)	11.61*(+)
10	<i>B. circulans</i>	55.40*(+)	2.81	48.75*(+)	9.66	68.33*(+)	11.38*(+)
11	<i>B. subtilis</i>	55.20	2.74	51.67*(+)	8.98	70.00*(+)	10.19
12	<i>B. cereus</i>	50.50	3.64	53.25*(+)	10.26	56.67*(-)	6.63*(-)
13	<i>B. subtilis</i>	46.00*(-)	2.85	55.25*(+)	8.89	65.00	10.47
14	<i>B. cereus</i>	48.35	2.62*(-)	52.22*(+)	10.06	90.00*(+)	15.90*(+)
15	<i>Pseudomonas putida</i>	43.25*(-)	3.39	57.00*(+)	9.24	70.00*(+)	9.99
16	<i>P. fluorescens</i>	40.35*(-)	4.11*(+)	50.92*(+)	8.81	66.67	9.99
17	Control 1 <sup>c</sup>	51.25	3.17	45.17	10.60	63.33	10.05
18	Control 2 <sup>d</sup>	37.60	2.24	45.67	7.10	28.33	1.49
	LSD (p≤0.05)	4.05	0.55	3.08	1.06	4.08	1.28

<sup>a</sup> One kentar = 157.50 kg

<sup>b</sup> One feddan = 4200 m<sup>2</sup>

<sup>c</sup> Control 1 was cotton seeds treated with the carrier (talc + carboxymethylcellulose).

<sup>d</sup> Control 2 was untreated cotton seeds

\*Significant difference from control 1. The tested strain caused significant increase (+) or significant decrease (-)

Strains no. 4 and 5 reduced yield at Sakha, while they had no effect on yield at the other two sites. Isolates no. 6 and 7 significantly reduced yield at Sakha and Sirs El-Lian, while they had no effect on yield at El-Gemmeiza. Isolates no. 9, 10 and 14 increased yield in Sirs El-Lian. Isolates no. 9 and 14 showed deleterious effects on yield at Sakha and had no effect on yield at El-Gemmeiza. Isolate no. 10 had no effect on yield at Sakha and yield was not affected by any strain at El-Gemmeiza. Isolate no. 16 was the only isolate which significantly increased yield at Sakha. Regardless of the tested strain, cotton plants produced the lowest seed cotton yield at Sakha due to the high level of late season insect infestation. For all sites combined, 22 biological treatments increased stands; for each individual site the number ranged from one at Sakha to 15 at El-Gemmeiza. On the other hand, only four biological treatments increased yield; for each individual site the number ranged from one at Sakha to three at Sirs El-Lian. As to the deleterious effects, 10 biological treatments reduced stands; for each individual site the number ranged from three at Sirs El-Lian to seven at Sakha. Ten biological treatments reduced yield; for each individual site the number ranged from four at Sirs El-Lian to six at Sakha.

Seedlings stand counts were not correlated with seed cotton yield at Sakha and El-Gemmeiza, while they were highly correlated at Sirs El-Lian ( $r: 0.956, p=0.000$ ) (Table 4). Regarding each of seedling stand counts (survival) and yield, the performance of tested strains at any site was not correlated with their performance at the other two sites (Table 5).

Five groups of similar strains (strains 3, 6, 2; strains 1, 10, 11; strains 15, 16; strains 9, 13, 5, 8, 4 and strains 7, 12) were identified by cluster analysis (Fig.1). The effect patterns of strains 4 and 14 were quite different from the others. Grouping the strains by cluster analysis was neither related to their taxonomic position nor to geographic origin. For instance, strains 6, 7, 11 and 13 belonged to *B. subtilis*; however, they were placed in remotely related sub-clusters. Another example was strains 1 and 9, which were placed in remotely related sub-clusters although they belonged to *B. coagulans*. Strains 7 and 11 of *B. subtilis* came from Minya, however, they were placed in remotely related sub-clusters. Similarly, strains 8 and 10 of *B. circulans* were placed in remotely related sub-clusters although they were isolated from Beheira.

**Table 4** Correlation between surviving cotton seedlings and yield under the effects of 16 bacterial strains used for treating seeds in three locations.

Correlation	Location
-0.223 <sup>a</sup> (0.406) <sup>b</sup>	Sakha
0.066 (0.807)	El-Gemmeiza
0.956 (0.000)	Sirs El-Lian

<sup>a</sup> Linear correlation coefficient, which measures the degree of association between the surviving seedlings and yield.

<sup>b</sup> probability level and  $n=16$

**Table 5** Correlation between effects of locations on the performance of 16 bacterial strains used for treating cotton seeds.

Variable	Location	Location	
		1	2
Survival	1- Sakha		
	2- El-Gemmeiza	-0.273 <sup>a</sup> (0.306) <sup>b</sup>	
	3- Sirs El-Lian	0.147 (0.586)	0.111 (0.681)
Yield	1- Sakha		
	2- El-Gemmeiza	-0.382 (0.145)	
	3- Sirs El-Lian	-0.120(0.658)	-0.062(0.821)

<sup>a</sup> Linear correlation coefficient, which measures the degree of association between effects of two locations on the performance of the bacterial strains.

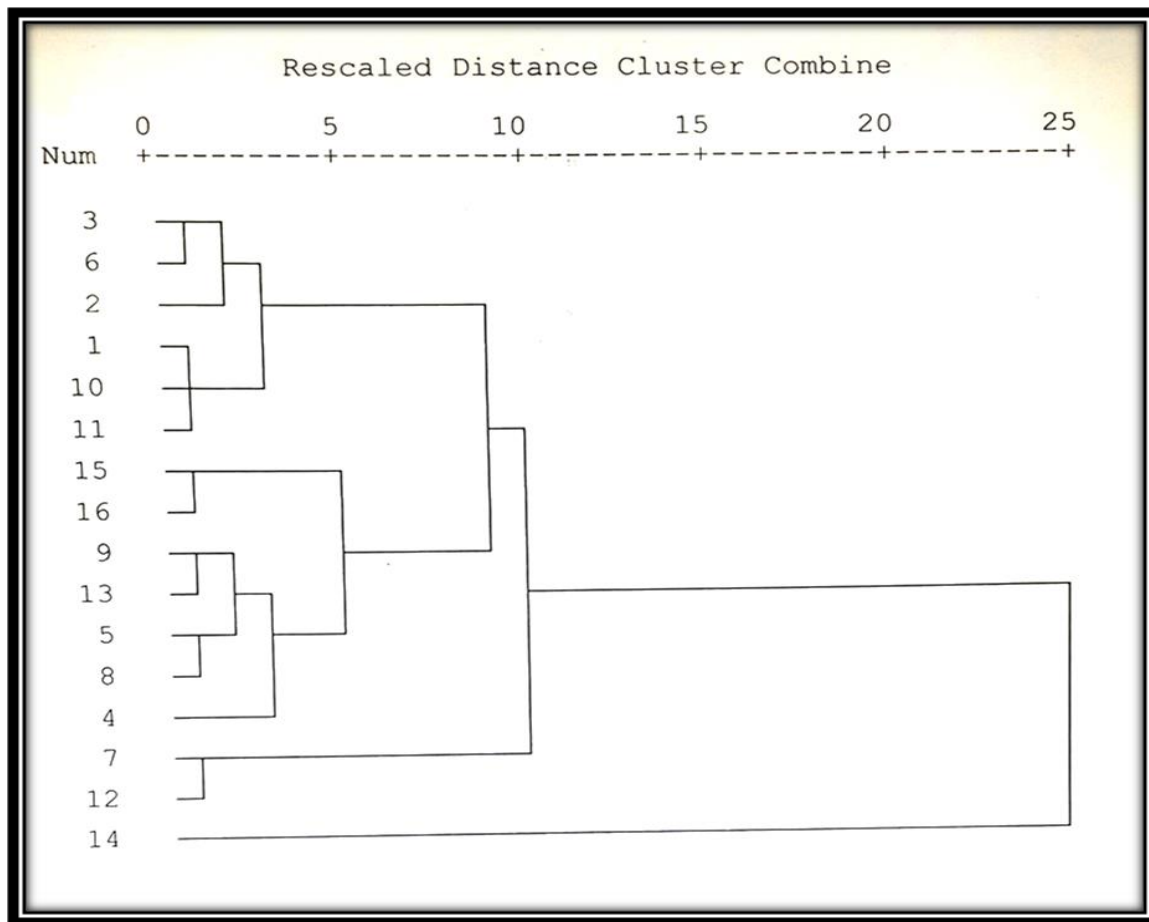
<sup>b</sup> probability level and  $n=16$

## Discussion

Successful application of rhizobacteria for control of cotton seedling damping-off requires development of a delivery system in which the bacteria can survive for a considerable length of time, and development of a suitable method of application to control early and late stages of disease development.

Previous studies demonstrated the successful management of various plant diseases using talc-based bioformulation of rhizobacteria (Manikandan et al. 2010). Talc formulations of spore-forming strains of *Bacillus* have an advantage over the non-spore forming strains such as *Pseudomonas* (formulated as vegetative cells). Spores are more robust and resistant to the elevated temperature and high concentrations of chemicals. Moreover, the shelf-life of biological products based on *Bacillus* spores can survive up to 1–3 years. A disadvantage of the use of spores is that

after application they need time to return to the metabolic active stage of vegetative cell (Kumar et al. 2012).



**Fig 1** – Phenogram based on average linkage cluster analysis of beneficial and deleterious effects of 16 rhizobacterial strains on cotton stand and yield under field conditions at three experimental sites. The tested strains were *Bacillus coagulans* (1), *B. globisporus* (2), *B. pumilus* (3), *Lactobacillus amylovorus* (4), *Bacillus* sp. (5), *B. subtilis* (6), *B. subtilis* (7), *B. circulans* (8), *B. coagulans* (9), *B. circulans* (10), *B. subtilis* (11), *B. cereus* (12), *B. subtilis* (13), *B. cereus* (14), *Pseudomonas putida* (15), and *P. fluorescens* (16).

In the present study, talc and CMC-based powder formulation of bacterial strains was used as a delivery system and seed treatment was used as a delivery system that is, the powder formulation was used for treating seeds. When treated seeds were sown in soil, the bacteria moved from spermosphere to rhizosphere and established there; in other words, bacterial strains colonized the rhizosphere at the time of seed germination. Once a portion of root was occupied by the introduced bacteria, the native bacteria in soil were unable to displace them (Vidhyasekaran & Muthamilan 1995). The establishment of the introduced bacteria in the rhizosphere of plants is due to an improved capacity to compete for root exudates (Gamliel & Katan 1992).

It is worth noting that the talc (magnesium silicate) and CMC-based powder formulation in itself, and regardless of any beneficial effects of the introduced strains, significantly increased stand and yield. It seems reasonable to relate these increases to the positive benefits that magnesium and silicon brought to cotton (Kirkpatrick & Rothrock 2001, Polanco et al. 2014).

The infected seedlings used in isolation yielded *Fusarium* spp. and *Rhizoctonia solani*, which are considered major causes of cotton seedling damping-off, while the other isolated fungi are considered minor disease agents except when the cotton seedlings are weakened (Watkins 1981).

The disease pressures at the experimental sites were due to the pathological effects of the isolated fungi. Plant stands of control 2 (untreated seed) provided the basis for disease pressure estimates, which were high at Sirs El-Lian, moderate at Sakha, and low at El-Gemmeiza.

The results of the present study showed that the performance of the tested strains was site-specific. Thus, for all sites combined, 48 biological treatments were evaluated as to their beneficial or deleterious effects on stand and yield. Twenty-two (45.83%) treatments showed beneficial effects on stand. Most of the beneficial treatments (15 treatments) were observed at El-Gemmeiza probably due to the low disease pressure at this site. On the other hand, the relatively high disease pressure at Sakha and Sirs El-Lian may obscure any potential antagonism by the treatments.

Iron availability in soil is inversely correlated with pH (Misaghi et al. 1998). The pH of the Egyptian soils range from 7.92 to 9.15 (Aly & Kandil 1999). In these alkaline soils, iron is expected to be sparingly soluble and too low in concentration to support microbial growth. Thus, it seems reasonable to speculate that siderophore-mediated iron deprivation may be the operative mechanism of damping-off suppression at least by some of the beneficial (antagonistic) treatments. However, one should keep in mind that the extent of disease suppression as a consequence of bacterial siderophore production is affected by several factors including the specific pathogen, the specific bacteria, the soil type, the crop, and the affinity of the siderophore for iron (Bashan & de-Bashan 2005).

Chitin is a major structural component of most fungal cell walls (Sahai & Manocha 1993). Various species of *Bacillus* have been shown to secrete chitinase, including *B. circulans*, *B. licheniformis*, *B. cereus*, and some others (Pleban et al. 1997). Moreover, there is a wealth of data supporting the important role of chitinolytic enzymes in bacterium-fungus antagonistic interactions (Haran et al. 1996). The chitinase exhibited activity over the pH range of 4.5–7.5 (Pleban et al. 1997). Therefore, it is unlikely that chitinase played an important role in the successful antagonism exhibited by the beneficial strains because the range of pH values in the Egyptian soils is unfavorable for its activity.

It is noteworthy that strain no. 10 was the only strain, which was effective in increasing stand at all sites. It seems that this strain had some type of stable mechanism(s) for repressing the disease. From practical stand point, strain no. 10 seems to be a promising strain for commercialization for two reasons; firstly, it significantly ( $p \leq 0.05$ ) increased stand at all sites and secondly, it significantly ( $p \leq 0.05$ ) increased seed cotton yield at Sirs El-Lian. However, this will require more comprehensive evaluation at as many sites as possible.

Ten (20.83%) of the biological treatments showed deleterious effects on stand (significantly reduced survival). These deleterious treatments could be due to the deleterious rhizobacteria (DRB) that affect plants by their metabolites without parasitizing plant tissues (Schippers et al. 1987). Fluorescent and nonfluorescent pseudomonads as well as *Bacillus* spp. among rhizobacteria conform to this definition (Suslow et al. 1982). DRB induce growth retardation, root discoloration, wilting, necrotic reactions, distortions of leaves and roots, or stunting of plants (Schippers et al. 1987). Increase in the susceptibility of plants to parasitic pathogens is another possible mode of action by which DRB exert their detrimental effects on stand and yield (Schippers et al. 1987). However, one should keep in mind that the deleterious synergistic interactions between rhizobacteria and root-invading fungi are not simple or universal phenomena. Pathogen – bacteria compatibility may be an important factor in determining the outcome of the interaction between rhizobacteria and fungi pathogenic on cotton seedlings (Aly et al. 1996).

The deleterious effects of rhizobacteria on cotton stand were also reported under field conditions in natural soil (Hagedorn et al. 1993) and under greenhouse conditions in autoclaved soil (Khiyami et al. 2014).

The incidence of damping-off of cotton seedlings traditionally has been attributed to fungal infections, and the pathogenicity of several fungi has been demonstrated (Watkins et al. 1981). To our knowledge, no attempts have been made to identify organisms other than fungi, and usually bacteria that are detected are treated as contaminants. We believe that rhizobacteria can play a synergistic role in cotton damping-off disease, and it is likely that with more comprehensive



isolation, a greater involvement of rhizobacteria in cotton damping-off disease will be demonstrated.

Sixteen (33.33%) of the biological treatments had no effect on stand. A key factor involved in this lack of success in biocontrol activity could be a rapid decline of the size of populations of active cells, to levels ineffective to achieve antagonism following introduction into soil. This rapid decline (microbiostasis) occurs when the introduced bacterial strains are unable to cope with the adverse and fluctuating biotic and abiotic soil conditions, to survive, and to remain active. For instance, declines were observed for newly introduced population of typical soil bacteria such as fluorescent pseudomonads and *B. subtilis* (Veen et al. 1997). The lack of correlation between seedling stand counts and seed cotton yield at Sakha and El-Gemmeiza, after the application of rhizobacteria, was also observed by Hagedorn et al. (1993) and Aly et al. (2000).

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