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USE OF *Streptomyces* ISOLATES TO SUPPRESS DAMPING-OFF OF COTTON SEEDLINGS.

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ABSTRACT

Sixteen isolates of *Streptomyces* were evaluated under field conditions in El-Gemmeiza and Sirs El-Lian in 2006 and 2007 as to their efficiency in suppressing cotton seedling damping-off. Seeds of cotton cultivar Giza 89 in both locations were treated with starch nitrate broth-based suspension of the *Streptomyces* isolates at a rate of 10 ml/Kg seeds. The performance of the isolates in reducing infection or increasing seedcotton yield was inconsistent from one site to another. Moreover, in the same site, the performance was variable from year to year. In general, the performance of the isolates was better in Sirs El-Lian compared with El-Gemmeiza. Of the 16 isolates, isolates Nos. 6 and 8 seems to be promising for commercialization for the following reasons: First, they significantly reduced infection in Sirs El-Lian each year and in El-Gemmeiza in 2006. Second, they increased seedcotton yield in Sirs El-Lian each year. Third, in El-Gemmeiza, isolates 8 and 6 increased seedcotton yield in 2006 and 2007, respectively. Cluster analysis was used to differentiate among the isolates based on variation in their antagonistic patterns. No obvious relationship was observed between grouping the isolates by cluster analysis and their geographic origins.

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 another, the pathogens most commonly involved in the disease complex are *Sclerotium rolfsii* (Khashaba, 1972), *Pythium* Spp. (Eisa, 1983), *Fusarium* Spp. (Aly *et al*., 1996), *Rhizoctonia solani* (El-Samawaty, 1999) and *Macrophomina phaseolina* (Omar, 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 Shin or Post emergence damping-off), and seedling root rot (Watkins, 1981).

The occurrence of major losses from cotton seedling damping-off is not uncommon in all cotton-production areas in Egypt. These losses vary over years and locations but characteristically result in poor stands. Stands may be replanted if severely damaged and even if damage is not severe enough for replanting, it may make weed control and other cultural practices difficult for the remainder of the season. Replanting, poor stands and seedling development, and weed competition ultimately affect plant maturity, fiber quality, and seedcotton yield (Kappelman, 1977). Thus, the widespread use of seed-dressing fungicides for controlling the disease has become indispensable under Egyptian conditions. While effective fungicides are available (Aly *et al*., 1992, Eisa *et al*., 1992; Abdel Aziz *et al*., 1996 and El-Samawaty, 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 the possible carcinogenicity. Other problems include gradual elimination and phasing out of some compounds (Zaki *et al*., 1998).

Recently, Biological control using microorganisms to suppress plant disease, offer an effective and environmental safe alternative to the use of pesticides (Emmert and Handelsman, 1999). *Streptomyces* are gram-positive mycelial bacteria. They have been widely used as biocontrol agents for controlling fungi particularly soilborne fungi (El-Tarabily *et al*., 1997). The effectiveness of *Streptomyces* as biocontrol agents has come from several complex factors. They are capable of producing a remarkably wide spectrum of antibiotics as secondary metabolites (Lechevalier, 1988 and Franklin *et al*., 1989). They may also compete with pathogens for space or nutrients in the rhizosphere (El-Tarabily *et al*., 1997).

Some attempts have been made to develop *Streptomyces* Spp. for controlling root disease agents. For example, *in vivo* studies showed that seed-coating with *Streptomyces* was the most effective treatment for controlling *Fusarium oxysporum* f.sp. *Lycopersici, Verticillium alboatrum* and *Alternaria solani* on tomato (El-Abyad *et al*., 1993). Jones and Samac (1996) found that *Streptomyces* strain 93 inhibited the growth of soilborne fungi involved in alfalfa damping-off. Trejo-Estrada *et al*. (1998) found that under greenhouse conditions, a turfgrass seedling disease caused by *Rhizoctonia solani* and a foliar disease caused by *Sclerotinia homococarpa* were partially controlled with the commercial spore formulation of YCED 9 (*S. violacecusniger*). Berg *et al*. (2001) reported a statistically significant enhancement ($P \leq 0.05$) of 17.8% in emergence rate of sugarbeet when seeds were coated with *Streptomyces* and a significant reduction to 47.4% in damping-off caused by *Pythium altimum*. (Getha and Vikineswary, 2002) found that *S. violaceusniger* Strain G 10 was effective in the biocontrol of *F. oxysporum* f.sp. *Cubens* race 4.

The objectives of the present study were to evaluate the effect of 16 isolates of *Streptomyces*, applied as seed treatment on incidence of damping-off and on seedcotton yield under field conditions.

MATERIALS AND METHODS

Streptomyces isolates:

A set of 16 *Streptomyces* isolates from soils of different Governorates, were used in this study (Table 1). These isolates were kindly supplied by Department of Agricultural Microbiology, Soil, Water and Environment Research Institute, ARC, Giza, Egypt. Preparation of *Streptomyces* formulation for antagonistic activity:

The starch nitrate broth medium was prepared as described by (Waksman and Lechevalier 1961) with 3.5% NaCl. The growth medium was inoculated with each of the *Streptomyces* isolates and incubated at 28 $\pm$ 2^{o}C for 6 days on a rotary shaker (160 – rpm). The mixture of mycelium and spores were used as seed treatment for antagonistic study under field conditions.

Table 1: Geographic origins of *Streptomyces* isolates, which used in the present study.

Isolate No.	Geographic origin
1	Alexandria
2	Alexandria
3	Alexandria
4	El-Fayoum
5	El-Fayoum
6	El-Fayoum
7	El-Fayoum
8	Damiatta
9	Ismalia
10	Port Said
11	Sinai
12	Sinai
13	Sinai
14	Sinai
15	Sinai
16	Sinai

Field evaluation of *Streptomyces* isolates to suppress Damping-off of cotton seedlings:

Field trials were conducted in 2006 and 2007 growing seasons at the Agricultural Research Stations of El-Gemmeiza and Sirs El-Lian. The experiment consisted of a randomized complete block design of six and four replicates in the first and in the second growing seasons respectively, in El-Gemmeiza. In Sirs El-Lian the experiment consisted of a randomized complete block design of three replicates each growing seasons. Each replicate consisted of six ridges 5-meter long and 70-cm width. Each ridge included 25 hills, and each hill contained 10 seeds. The seeds of cotton (*Gossypium barbadense* L.) cultivar Giza 89 were separately coated with the suspension of the *Streptomyces* isolates at a rate of 10 ml per Kg seeds. Planting dates were 20 March, 29 March in the two growing seasons in El-Gemmeiza, while planting dates were 30 March, 15 April in the two growing seasons in Sirs El-Lian, percentage of fungus-infected seedlings was recorded 45 days post planting. Seedcotton yield (cotton seed and lint before ginning) was picked on 15 – 30 October at each site.

Statistical analysis of the data:

Analysis of variance (ANOVA) of the data was performed with the MSTATC statistical package (A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA). Duncan's multiple range test was used to compare the individual *Streptomyces* isolates means. Correlation and regression analyses were performed with a computerized program.

RESULTS AND DISCUSSION

Four field trials were conducted in El-Gemmeiza and Sirs El-Lian in 2006 and 2007 to evaluate the effectiveness of 16 isolates of *Streptomyces* in suppressing cotton seedling damping off. Typical symptoms of seedling damping-off were observed on infected seedlings in each site. Isolation from infected seedlings yielded 9 fungi (Table 2). Of the isolated fungi, *Rhizoctonia solani* and *Fusarium* Spp. are considered major causes of cotton damping-off, while the other fungi are unimportant disease agents except when the cotton seedlings are Weakened (Watkins, 1981).

Table 2: Isolation frequency (%)[a] of fungi from cotton seedlings (Giza 89) infected with post emergence damping-off in the experimental sites.

Fungus	El-Gemmeiza		Sirs El-Lian	
	2006	2007	2006	2007
Rhizoctonia solani	20.40	0.00	0.00	55.28
Fusarium Spp.	72.96	26.49	37.50	30.00
Aspergillus Spp.	3.34	0.00	0.00	0.00
Alternaria Spp.	3.34	23.05	21.65	0.00
Rhizopus Spp.	0.00	21.66	26.68	14.72
Cladosporium Spp.	0.00	2.78	0.00	0.00
Macrophomina phaseolina	0.00	13.86	0.00	0.00
Chaetomium Spp.	0.00	12.17	0.00	0.00
Unidentified (Sporulating)	0.00	0.00	14.18	0.00

[a] **Colonies of each fungus were explossed as percentage of the total developing colonies. Each value was the mean of 5 replicates (plates).**

Successful application of *Streptomyces* for controlling cotton seedling damping-off requires development of delivery systems in which the bacteria can survive for a considerable length of time, development a suitable method for application to control early and late stages of disease development, and assessment of field efficacy of these delivery systems in the control of the disease as well as in increasing seedcotton yield. In the present study, starch nitrate broth-based suspension of the *Streptomyces* isolates was used as delivery system and seed treatment was used as delivery method. That is, the cell suspension was used for treating seeds. Seed treatment with cell suspension of *Streptomyces* has been found effective in controlling sugar beet seedling damping-off (Berg *et al*., 2001), and several other diseases of tomato (El-Abyad *et al*., 1993).

Field evaluation of the 16 *Streptomyces* isolates in widely Separated Sites in El-Gemmeiza and Sirs El-Lian revealed inconsistent performance among isolates from one site to another. Moreover, in the El-Gemmeiza site, the performance was variable from year to year (Tables 3 and 4). For example; in 2006, isolates Nos. 1 and 2 were ineffective in reducing infection in El-Gemmeiza, while they were effective in Sirs El-Lian. In El-Gemmeiza, isolates Nos. 1, 2 and 3 were ineffective in reducing infection in 2006, while they were effective in 2007. Data in Tables 3 and 4 suggest that the

performance of the isolates in reducing infection or increasing seedcotton yield was highly sensitive to environmental conditions, which may vary from year to year and from site to site. In general, the performance of the isolates was better in Sirs El-Lian compared with El-Gemmeiza where the seedlings could be subjected to high disease pressure, which obscured any potential antagonism by the isolates. Another possibility is that the populations of the pathogenic fungi in El-Gemmeiza were less sensitive to the antagonistic effects of *Streptomyces* isolates. However, these interpretions dose not ruled out the possibility that other factors may also be responsible for such a difference in performance like edaphic factors in the experimental sites.

Table 3: Effect of *Streptomyces* isolates on incidence of cotton seedling damping-off and on seedcotton yield under field conditions in El-Gemmeiza in 2006 and 2007 growing season.

Isolated	2006		2007	
	Infection (%)	Seedcotton yield (Kenter/Feddan)	Infection (%)	Seedcotton yield (Kenter/Feddan)
1	56.33[a] A	9.75 BC	10.00[b] F*	14.24 A*
2	47.50 A-E	7.78 BC	22.75 DEF*	9.75 CD
3	49.50 A-D	8.98 BC	24.00 C-F*	10.13 BCD
4	42.00 B-E	9.32 BC	39.25 ABC	10.65 A-D
5	45.67 A-E	10.09 BC	28.75 B-C	8.34 CD
6	41.33 CDE*	9.23 BC	37.50 A-D	11.41 ABC*
7	39.00 DE*	11.03 AB*	27.00 CDE*	9.75 CD
8	37.00 E*	13.68 A*	45.00 A	10.26 BCD
9	40.33 CDE*	10.95 AB*	25.50 CDE*	9.62 CD
10	48.00 A-E	10.26 BC	24.00 C-F*	11.93 ABC*
11	46.67 A-E	9.58 BC	24.00 C-F*	11.67 ABC*
12	48.83 A-E	9.66 BC	26.00 CDE*	11.28 A-D
13	42.17 B-E	10.43 ABC	21.25 EF*	13.85 AB*
14	47.33 A-E	10.77 AB*	25.50 CDE*	11.28 A-D
15	46.33 A-E	10.35 ABC	25.75 CDE*	11.28 A-D
16	51.67 ABC	8.98 BC	31.50 A-E	13.85 AB*
Control	53.50 AB	7.10 C	42.50 AB	7.44 D

[a] Mean of 6 replicates, Means followed by the same letter(s) are not significantly different (P < 0.05) according to Duncan's multiple range test. An asterisk denotes a significant difference from the control.

[b] Mean of 4 replicates.

In El-Gemmeiza and Sirs El-Lian, a significant negative correlation was observed each year between infection and seedcotton yield (Table 5 and Figs. 1 and 2). Infection accounted for 24 to 90% of the total variation in seedcotton yield. This relationship implied that the higher the disease pressure during the seedling stage, the less productive the surviving plants would be in their seedcotton yield. That is, even the plants, which survived damping-off suffered from a subtle weakness, which reduced seedcotton yield (Watkins, 1981). Of the 16 isolates, isolates Nos. 6 and 8 seems to be promising for commercialization for the following reasons: First, they significantly reduced infection in Sirs El-Lian each year and El-Gemmeiza in 2006. Second, they increased seedcotton yield in Sirs El-Lian each year. Third, in El-Gemmeiza, isolates 8 and 6 increased seedcotton yield in 2006 and 2007, respectively.

The phenogram in Fig. 3 was constructed based on rescaled distances generated from cluster analysis of correlation similarity coefficients shown in Table 6. In this phenogram, the smaller the distance, the more closely the isolates were related in their antagonistic patterns. Three groups of isolates were identified at the distances of 5, 2 and 7. Isolates Nos. 1 and 7 were unrelated to the other groups. Within each group, the isolates were associated strongly, whereas between groups, the isolates were associated weakly. No obvious association was observed between grouping the isolates based on their antagonistic patterns and their geographic origins.

Table 4: Effect of *Streptomyces* isolates on incidence of cotton seedling damping-off and on seedcotton yield under field conditions in Sirs El-Lian in 2006 and 2007 growing season.

Isolated	2006		2007	
	Infection (%)	Seedcotton yield (Kenter/Feddan)	Infection (%)	Seedcotton yield (Kenter/Feddan)
1	33.33[a] DE*	11.67 A*	36.67 A-D	6.66 B*
2	35.00 CDE*	11.29 A*	46.67 AB	5.44 BC*
3	41.67 ABC	9.02 A-D	36.67 A-D	6.43 BC*
4	33.33 DE*	10.83 AB*	30.00 CD*	5.75 BC*
5	31.67 E*	11.02 AB*	36.67 A-D	5.51 BC*
6	33.33 DE*	10.24 AB*	33.33 BCD*	5.66 BC*
7	46.67 A	7.07 CD	13.33 E*	11.08 A*
8	33.33 DE*	10.98 AB*	23.33 DE*	11.32 A*
9	45.00 A	6.43 D	30.00 CD*	6.41 BC*
10	45.00 A	6.55 D	26.67 CDE*	6.14 BC*
11	36.67 B-E	9.33 ABC*	33.33 BCD *	5.88 BC*
12	40.00 A-D	8.50 BCD	36.67 A-D	5.55 BC*
13	35.00 CDE*	10.39 AB*	40.00 ABC	5.19 CD
14	45.00 A	6.55 D	50.00 A	4.02 D
15	36.67 B-E	9.0 A-D	36.67 A-D	5.35 BC*
16	43.33 AB	7.03 CD	33.33 BCD*	5.97 BC*
Control	43.33 AB	7.01 CD	50.00 A	4.04 D

[a] **Mean of 3 replicates, Means followed by the same letter(s) are not significantly different (P < 0.05) according to Duncan's multiple range test. An asterisk denotes a significant difference from the control.**

Table 5: Regression equations that describe the effect of infection with cotton seedling damping-off (X) on seedcotton yield (Y) under field conditions.

Year	Location	Linear regression equation	R[a]	R^2 [b]	F. value	P > F
2006	El-Gemmeiza	Y = 17.99 - 0.176X	0.643	0.413	10.56	0.005
	Sirs El-Lian	Y = 22.29 - 0.343X	0.949	0.901	137.03	0.000
2007	El-Gemmeiza	Y = 13.94 - 0.105X	0.491	0.241	4.76	0.045
	Sirs El-Lian	Y = 12.496 - 0.179X	0.826	0.683	32.306	0.000

[a] **Linear correlation coefficient.**
[b] **Coefficient of determination.**

(A)

(B)

Fig. 1: Regression equations that describe the effect of infection with cotton seedling damping-off (X) on seedcotton yield (Y) under field conditions in El-Gemmeiza (A) and Sirs El-Lian (B) in 2006.

(A)

(B)

Fig. 2: Regression equations that describe the effect of infection with cotton seedling damping-off (X) on seedcotton yield (Y) under field conditions in El-Gemmeiza (A) and Sirs El-Lian (B) in 2007.

Fig. 3: Phenogram based on average linkage cluster analysis of variation in antagonism against soilborne fungi involved in cotton seedling damping-off for 16 isolates of *Streptomyces* Spp.

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استعمال عزلات الإستربتوميسس لمقاومة مرض موت بادرات القطن٠

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اختبرت ١٦ عزلة إستربتوميسس – تحت ظروف الحقل فى كل من الجميزة وسرس الليان فى موسمى ٢٠٠٦ و ٢٠٠٧، وذلك لتقييم فعاليتها فى مقاومة مرض موت بادرات القطن٠ عوملت بذرة القطن صنف جيزة ٨٩ فى كلا الموقعين بمعلق العزلات فى بيئة نترات النشا السائلة وذلك بمعدل ١٠ مل/كجم بذرة٠ تباينت كفاءة العزلات فى تقليل الإصابة أو زيادة المحصول من موقع لموقع ومن عام لآخر٠ عموماً كان آداء العزلات أفضل فى سرس الليان٠ أظهرت النتائج أن السلالتين ٦، ٨ هما الوحيدتان اللتان يمكن استعمالهما على نطاق تجارى وذلك للأسباب التالية:

١- أحدثتا انخفاض معنوى فى مستوى الإصابة فى سرس الليان فى كل عام، وفى الجميزة عام ٢٠٠٦.
٢- أحدثتا زيادة معنوية فى محصول القطن الزهر فى سرس الليان فى كل عام٠
٣-أحدثت العزلة ٨ والعزلة ٦ زيادة معنوية فى محصول القطن الزهر فى الجميزة خلال عامى ٢٠٠٦ و٢٠٠٧ على الترتيب٠

باستعمال التحليل العنقودى لتصنيف هذه العزلات إلى مجموعات، بناءً على ما بينهما من تباين فى أنماط التضاد لم يلاحظ ارتباط واضح بين المجموعات التى إنقسمت إليها العزلات وأصولها الجغرافية٠

Table 6: Correlation similarity coefficient matrix among antagonistic patterns of 16 isolates of *Streptomyces* Spp.

Isolate No.	Isolate No.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		0.924	0.924	0.715	0.879	0.745	0.654	0.500	0.822	0.874	0.926	0.913	0.924	0.878	0.885	0.869
2			0.963	0.854	0.970	0.893	0.649	0.668	0.889	0.873	0.961	0.965	0.990	0.979	0.994	0.916
3				0.898	0.970	0.918	0.826	0.755	0.969	0.971	0.997	0.998	0.976	0.969	0.951	0.981
4					0.945	0.995	0.801	0.951	0.895	0.882	0.914	0.918	0.858	0.859	0.859	0.945
5						0.963	0.738	0.814	0.915	0.909	0.977	0.979	0.962	0.951	0.964	0.960
6							0.776	0.924	0.907	0.886	0.931	0.938	0.896	0.899	0.901	0.953
7								0.805	0.910	0.927	0.818	0.819	0.701	0.717	0.636	0.875
8									0.804	0.782	0.771	0.780	0.674	0.697	0.680	0.841
9										0.982	0.959	0.967	0.922	0.940	0.891	0.972
10											0.969	0.968	0.910	0.906	0.861	0.981
11												0.999	0.975	0.961	0.950	0.987
12													0.978	0.971	0.958	0.987
13														0.991	0.990	0.941
14															0.988	0.934
15																0.911
16																