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BIOLOGICAL CONTROL OF SIX SOIL-BORNE FUNGI OF COTTON USING ANTAGONISTIC *STREPTOMYCES* ISOLATES

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ABSTRACT:

In this study, antagonistic activity of fifteen isolates of *Streptomyces*, *in vitro* or *in vivo*, was determined against six fungi causing cotton seedling diseases. Data showed that all *Streptomyces* isolates were highly antagonistic in-vitro studies, isolate Sc-2 was the most effective against *Fusarium solani* (antagonism distance (AD) was 1.72 mm), on the other hand, isolates Qa-53, Ps-12 showed the lowest antagonism against *F. solani* (AD were 0.80, 0.83 mm, respectively). *Rhizoctonia solani* was the most sensitive fungus *in vitro* to the antagonism of most *Streptomyces* isolates (Qa-84, Qa-51, Is-10, Ps-12, Si-1, Si-4, Si-6, Si-8 and Si-9) with their AD ranged from 2.27 to 2.70 mm. However, Sc-2 was the least effective with AD as low as 0.93 mm. *F. moniliforme* was the least sensitive fungus to the antagonism of *Streptomyces* isolates. Antagonisms of *Streptomyces* isolates against *F. solani*; *Sclerotium rolfsii* and *R. solani* were positively correlated with their antagonisms against *F. oxysporum* ($P \leq 0.01$), *R. solani* ($P \leq 0.05$) and *Macrophomina phasulina* ($P \leq .0.01$), respectively, but negatively correlated against *F. moniforme.* Treated fuzzy seeds with *Streptomyces* significantly reduced seedling disease in Sc-11, Ma-13, Ps-12, Si-1 and Si-6. All *Streptomyces* isolates were ineffective in controlling the disease when the seeds were acid delinted. Some *Streptomyces* isolates showed no efficiency on reducing the seedling disease wheather the seeds were fuzzy or acid delinted such as Sc-2, Qa-44, Qa-51, Qa-53, Qa-84, Da-3, Is-10, Si-4, Si-8 and Si-9.

INTRODUCTION:

Biological control using micro-organisms to suppress plant disease, offer a powerful and environmental safe alternative to the use of pesticides (Emmert and Handel-sman, 1999). Soil-borne plant pathogens in the rhizosphere were biologically controlled with antagonistic bacteria (Tahvonen and Lahdenpera, 1988; Weller, 1988; Crawford *et al*., 1993; El-Tarabily *et al*., 1997; Youssef *et al*., 2001) or yeasts (El-Tarabily, 2004; El-Tarabily and Sivasithamparam, 2006). Bacterial antagonism, responsible for biological control, may operate by antiobiosis, competition or parasitism. Parasitism relies on lytic enzymes for the degradation of cell walls of pathogenic fungi (Chet *et al.*, 1990; El-Tarabily *et al*., 2000). Attempts have been made to develop *Streptomyces* species for controlling root disease agents, since *Streptomyces* spp. are capable of producing a remarkably wide spectrum of antibiotics as secondary metabolites (Franklin *et al*. 1989; Lechevalier and Waksman, 1962; Lechevalier, 1988).

El-Abyad *et al*. (1993) reported in *in-vitro studies that* 80% concentration of the culture filtrate of either *S. pulcher* or *S. canescens* significantly inhibited spore germination, mycelial growth and sporulation of *F. oxysporum* f.sp. *lycopersici*, *V. alboatrum* and *Alternaria solani*, while *in vivo* studies showed that the seed-coating treatment was the most effective in controlling all the pathogens at 42 and 63 days after sowing.

Berg *et al.* (2001) reported that when seeds of sugar beet were coated with the antagonistic *Streptomyces* strain, a statistically significantly enhancement ($p \leq 0.05$) of 17.8% on average (40.0% in maximum) of the emergence rate was found and also significantly reduced up to 47.4% damping-off caused by *Pythium altimum.* Getha and Vikineswary (2002) reported that *Strepto-*

myces violaceusniger strain G10 acted as an antifungal agent antagonistic towards many different phytopathogenic fungi, including different pathogenic races of the *Fusarium* wilt pathogen.

This study aimed to focus on the *in vitro* and *in vivo* antagonistic activities of some *Streptomyces* isolates as biocontrol agents against some soil-borne fungi of cotton to provide significant protection to cotton roots against the fungi under investigation as serious soil-borne root pathogens.

MATERIALS AND METHODS:

***Streptomyces* isolates:** A set of *Streptomyces* isolates obtained from soils of different Governorates, i.e., Alexandria (Sc-2, Sc-11 and Ma-13); El-Fayoum (Qa-44, Qa-51, Qa-53 and Qa-84); Damiatta (Da-3); Ismalia (Is-10);); Port Said (Ps-12) and Sinai (Si-1, Si-4, Si-6, Si-8 and Si-9) were identified by Dr. Sonya H. Mohamed

Fungi species: A total number of six soil-borne fungi species *F. moniliforme, F. oxysporum, F. solani, Macrophomina phasulina, Rhizoctonia solani* and *Sclerotium ralfsii* that cause diseases to cotton seedlings were used. These fungi were obtained from the Collection of Cotton and Fiber Crops Disease Research Department, Plant Pathology Research Institute, ARC, Giza, 12619, Egypt.

Cotton seeds: The seeds of cotton (*Gossypium barbadense* L.) cultivar Giza 89 were obtained from Cotton Research Institute, ARC, Giza, 12619, Egypt, and were used as fuzzy and acid delimited .

Preparation of *Streptomyces* for antagonistic activity: The starch nitrate broth medium was prepared as described by W*aksman* and *Lechevalier* (+1961) with 3.5% NaCl and was taken in 15 flasks while nutrient broth medium was prepared by *Sambrook* et. al., (1989) with 3.5% NaCl and was taken 15 in flasks (250ml). The streptomyces isolates were inoculated on media separately and incubated at 28±2^{o}Cfor 6 days on a rotary shaker (160-rpm). The mixture of mycelium and spores was used for *in vitro* and *in vivo* antagonistic studies.

***In vitro* antagonism:** Antagonistic activity of the *Streptomyces* isolates under study against the *F. moniliforme, F. oxysporum, F. solani, M. phaseolina, R. solani* and *Sc. rolfsii* was carried out based on the method of Zaki *et al.* (1991). The fungi were seeded on potato dextrose agar (PDA) medium using a sterile cork borer, holes of about 0.6 mm diameter were made filed with 0.1 ml of supernatants of *Streptomyces* isolates. After incubation at 4^{o}C for one hour, the plates were incubated at 30±2^{o}C for 24-48 hours. Antagonistic activity was determined by measuring the inhibition zones (mm) using the diffusion methods as described by British Pharmacopeia (1968). For control, 0.1 ml of un-inoculated broth media was poured in other holes.

Substrate preparation: The substrate was prepared in 500ml glass bottles contained 50 g of sorghum grains and 40 ml of tap water in each bottle and were autoclaved for 30min at 121°C.

Fungal inoculum: Inoculum of one-week old fungal isolate culture grown on PDA medium was especially introduced into the substrate and allowed to colonize sorghum for 3 weeks.

Fungi preparation: Six fungal species (*F. moniliforme, F. oxysporum, F. solani, M. phaseolina, R. solani* and *Sc. rolfsii*, respectively) were inoculated separately in sterilized clay loom soil at rate of 50, 50, 50, 30, 1 and 10g/kg of soil respectively. Infested soil was dispensed in 10-cm-diameter clay pots.

Antagonisms activity: Cotton seeds (Fuzzy and acid delinted) were separately coated with the suspension of the 15 *Strepomyces* isolates at a rate of 10 ml per kg cotton seeds. Three treatments named 16, 17 and 18 were applied as controls: seed treated with fungicide monceren at a rate of 3 g/kg seeds; untreated seeds grown in infested soil and untreated seeds grown in autoclaved soil respectively.

Pots were planted with cotton seeds (10 cotton seeds per pot) in 5 replicates. Pots were distributed in a randomized complete block design on a greenhouse bench. Percentage of fungus-infected seedlings was recorded 45 days post planting.

Statistical analysis of the data: Analysis of variance (ANOVA) of the data was performed with the MSTAT-C statistical package (A Micro Computer Program for the Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA). Least significant difference (LSD) was used to compare isolate means. Correlation and regression analysis were performed with a computerized program.

RESULTS:

Fifteen isolates of *Streptomyces* were isolated from different Governorates were used to determine their *in vitro* and *in vivo* effect on six soil-borne fungi, which cause cotton seedling diseases. ANOVA presented in Table-1 showed that *Streptomyces* (S), fungi (F) and *Streptomyces* x fungi (F x S) interaction were significant sources of variation *in vitro* antagonism of 15 *Streptomyces* spp. Due to the interaction a least significant difference (LSD) was used to compare between the individual means of *Streptomyces* isolates within fungi. Data in Table-2 showed that all *Streptomyces* isolates were highly antagonistic. Isolate Sc-2 was the most effective one against *F. solani* (antagonism distance (AD) was 1.72 mm), on the other hand, isolates Qa-53, Ps-12 and Si-9 showed the lowest antagonism against *F. solani* (AD were 0.80, 0.80 and 0.83 mm, respectively). *R. solani* was the most sensitive fungus *in vitro* to the antagonism of most *Streptomyces* isolates, whereas Qa-84, Qa-51, Is-10, Ps-12, Si-1, Si-4, Si-6, Si-8 and Si-9 isolates were the most effective ones against *R. solani* as the AD was ranged from 2.27 to 2.70 mm. However, Sc-2 isolate was the least effective one with AD 0.93 mm against *R. solani F. moniliforme* was the least sensitive fungus to the antagonism of *Streptomyces* isolates**.** Results in Table-3 showed that antagonism of *Streptomyces* isolates against *F. solani*, *S. rolfsii* and *R. solani* were positively correlated with their antagonism against *F. oxysporum* ($P \leq 0.01$), *R. solani* ($P \leq 0.01$) and *M. phasulina* ($P \leq .0.01$), respectively. On the other hand, a negative correlation was found between the antagonism of the *Streptomyces* isolates against *R. solani* and their antagonism against *F. moniforme*.

Data in Table-4 show that seed treatments (T) and media (M), (T x M) *Streptomyces* (S) and seed treatments x *Streptomyces* (T x S) were the only significant sources of variation. Due to the significance of seed treatments x *Streptomyces* isolate interaction, LSD was calculated to compare between seed treatments within isolates. The efficiency of *Streptomyces* isolates varied according to type of seeds. Fuzzy seeds were treated with *Streptomyces* isolates (Sc-11, Ma-13, Ps-12, Si-1 and Si-6), significantly reduced seedling disease compared to acid delinted seeds as shown in Table-5. The other ten *Streptomyces* isolates (Sc-2, Qa-44, Qa-51, Qa-53, Qa-84, Da-3, Is-10, Si-4, Si-8 and Si-9) showed no efficiency on reducing the seedling disease whatever the seeds were fuzzy or acid delinted. All *Streptomyces* isolates were ineffective in controlling the disease when the seeds were acid delinted as illustrated in Figures-1, 2 and 3.

Table-1: Analysis of variance of the *in vitro* antagonism of 16 *Streptomyces* spp. isolates against 6 soil-borne fungi involved in cotton seedling disease complex.

Source of variation	D.F.	Mean square	F value	P ≤ F
Streptomyces (S)	016	06.876	236.4715	0.0000
Fungi (F)	005	13.859	476.6159	0.0000
F × S	080	00.919	031.6185	0.0000
Error	510	00.029		

Table-2: Effect of the *in vitro* antagonism of 16 *Streptomyces* spp. against 6 soil borne fungi involved in cotton seedling disease complex

Streptomyces isolates	Fungi						
	F. moniliforme	*F. oxysporum*	*F. solani*	*M. phasulina*	*R. solani*	*S. rolfsii*	Mean
Sc-2	0.90	1.63	1.72[a]	1.17	0.93	1.20	1.26
Sc-11	1.07	1.40	1.40	1.13	1.20	1.00	1.20
Ma-13	1.13	1.23	1.35	1.27	1.30	0.87	1.19
Qa-44	0.87	1.67	1.37	1.83	1.37	0.87	1.22
Qa-51	0.80	1.20	0.87	1.07	2.30	1.27	1.25
Qa-53	1.20	1.03	0.80	1.60	1.63	0.83	1.18
Qa-84	0.83	1.27	1.63	1.10	2.37	1.10	1.38
Da-3	1.00	1.23	1.42	1.13	1.37	0.87	1.17
Is-10	0.93	1.30	1.43	2.53	2.70	1.47	1.72
Ps-12	0.80	1.40	0.83	2.10	2.47	1.43	1.51
Si-1	1.00	1.53	1.67	2.77	2.47	2.30	1.96
Si-4	0.87	1.73	1.60	2.73	2.52	2.33	1.96
Si-6	0.87	1.23	1.27	2.60	2.27	1.58	1.64
Si-8	0.87	1.30	1.17	2.70	2.37	1.57	1.66
Si-9	0.80	1.07	0.80	2.13	2.40	0.90	1.35
17[b]	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	0.88	1.26	1.19	1.73	1.86	1.21	

LSD for:		p≤0.05	p≤0.01
	Streptomyces (S)	0.08	0.10
	Fungus (F)	0.05	0.06
	SXF	0.19	0.25

[a]: Antagonism distance is the distance between *Streptomyces* growth and fungal growth.
[b]: The distance between fungal growth and the desk of growth medium without *Streptomyces* (control treatment).

Table -3: *In vitro* correlation among antagonisms of 15 *Streptomyces* spp. against six soil-borne fungi [a]

Fungi	*F. moniliforme*	*F. oxysporum*	*F. solani*	*M. phasulina*	*R. solani*	*S. rolfsii*
F. moniliforme	---					
F. oxysporum	-0.223	---				
F. solani	0.083	0.650**	---			
M. phasulina	-0.242	0.2220	0.0140	---		
R. solani	-0.529*	-0.131	-0.208	0.675**	---	
S. rolfsii	-0.260	0.506	0.362	0.735**	0.609*	---

[a] : Linear correlation coefficient (r) is significant at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).

Table -4: Analysis of variance of the effect of seed treatment (fuzzy-acid delinted), growth media (Nutrient broth-Starch nitrate), and their interaction on the antagonism of *Streptomyces* spp. isolates used to control cotton seedling disease fungi.

Source of variation	D.F.	Mean square	F value	P > F
Replication	4	412.239	0.9308	
Seed treatments (T)	1	10676.230	24.1067	0.0000
Media (M)	1	3248.586	7.3352	0.0072
T × M	1	3957.381	8.9357	0.0030
Streptomyces isolate (S)	17	1881.051	4.2474	0.0000
T × S	17	986.851	2.2283	0.0040
M × S	17	472.247	1.0663	0.3868
T × M × S	17	704.771	1.5914	0.0656
Error	284	442.875		

Table-5: Effect of seed treatment (Fuzzy and delinted), growth medium (NBM, SNM), and their interaction on the antagonism of *Streptomyces* isolates used to control cotton seedling disease fungi.

Streptomyces isolates	% and Transformed (T)	Seed treatments					
		Fuzzy			Acid delinted		
		Media		Mean	Media		Mean
		NBM	SNBM		NBM	SNBM	
Sc-2	%	48[a]	62	55	70	70	70
	T	46.33	57.69	52.01	57.98	60.05	59.01
Sc-11	%	34	70	52	96	66	81
	T	34.55	62.95	48.75	84.69	58.16	71.42
Ma-13	%	52	46	49	88	94	91
	T	46.80	42.59	44.70	79.85	78.94	79.39
Qa-44	%	72	92	82	68	66	67
	T	64.80	82.15	73.48	58.89	57.51	58.20
Qa-51	%	50	84	67	88	90	89
	T	47.83	74.36	61.09	76.84	78.47	77.65
Qa-53	%	74	80	77	84	92	88
	T	65.36	69.05	67.20	69.05	77.31	73.18
Qa-84	%	74	84	79	68	60	64
	T	65.95	72.00	69.00	59.31	54.00	56.66
Da-3	%	84	62	73	78	84	81
	T	74.53	55.85	65.19	70.67	74.31	72.49
Is-10	%	30	100	65	54	70	62
	T	32.49	90.00	61.24	47.36	61.67	54.51
Ps-12	%	34	62	48	96	74	85
	T	35.49	58.33	46.91	84.69	66.00	75.35
Si-1	%	64	66	65	100	88	94
	T	56.95	60.47	58.71	90.00	74.78	82.39
Si-4	%	54	90	72	96	80	88
	T	50.31	81.00	65.66	84.69	72.00	78.34
Si-6	%	46	56	51	82	80	81
	T	40.11	51.52	45.81	73.15	69.47	71.31
Si-8	%	54	72	63	78	70	74
	T	48.13	61.80	54.96	67.89	65.95	66.92
Si-9	%	62	62	62	58	90	74
	T	55.33	58.28	56.81	50.36	80.00	65.68
Cont.16	%	32	48	40	42	28	35
	T	36.00	46.62	41.31	39.69	31.11	35.40
Cont.17	%	52	52	52	62	88	75
	T	49.16	46.80	47.98	55.38	76.84	66.11
Cont.18	%	22	32	27	46	50	48
	T	27.60	33.77	30.68	42.64	44.35	43.50
Mean	%	52.11	67.78	59.94	75.22	74.44	74.83
	T	48.76	61.40	55.08	66.29	65.66	65.97

NBM: Nutrient broth medium. SNBM: Starch nitrate broth medium

LSD for:		p≤0.05	p≤0.01
	Seed treatment (T)	4.366	5.753
	Media (M)	4.366	5.753
	TXM	6.175	8.135
	Streptomyces (S)	13.10	17.26
	TXS	18.53	24.41
	MXS	NS	NS
	TXMXS	NS	NS

[a]: Cotton seedling damping off (Percentage of infected seedlings) were transformed into arcsine angles before carrying out analysis of variance. Transformed means are shown in parentheses. Each value is a mean of 5 replicates.

Figure -1: Effect of seed treatment (fuzzy), growth medium (NBM or SNBM) and their interaction on the antagonism of *Streptomyces* isolates used to control cotton seedling disease caused by *R. solani*. Pots were planted with 10 cottonseeds per pot, in 5 replicates.

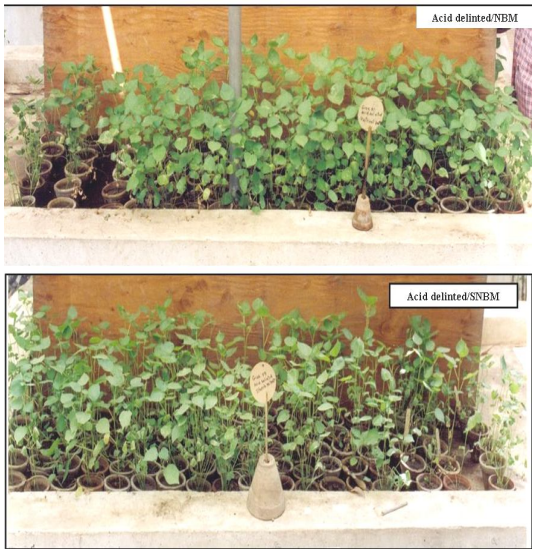

Figure -2: Effect of seed treatment (acid delinted), growth medium (NBM or SNBM) and their interaction on the antagonism of *Streptomyces* isolates used to control cotton seedling disease caused by *R. solani*. Pots were planted with 10 cottonseeds per pot, in 5 replicates.

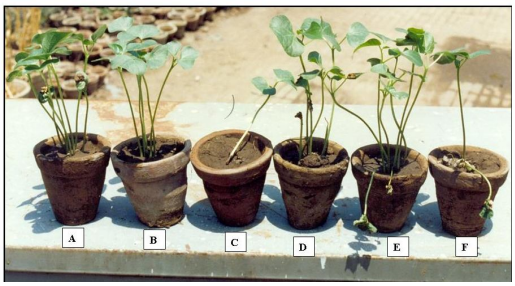

Figure -3: Control treatments: seed treated with fungicide monceren (A & E); untreated seeds grown in infested soil (C & F) and untreated seeds grown in autoclaved soil (B &D). Note, A, B and C represent fuzzy seeds, while, D, E and F represent acid delinted seeds. Pots were planted with 10 cottonseeds per pot.

Results in Figure (4) showed that *Streptomyces* isolates were distributed into 4 groups according to their antagonistic patterns against fungi involved in cotton seedling disease, group A included four isolates, Qa-51, Qa53 (from El-Fayoum), Si-1 and Si-4 (from Sinai), group B included 6 isolates, Sc-2, Sc-11, Ma-13 (from Alexandria), Ps-12 (from Port Said) and Si-6, Si-8 (from Sinai) group C included Qa-44 and Qa-84 (from El-Fayoum), Da-3 (from Damiatta) and Si-9 (from Sinai). However, group D included only one isolate Is-10, which was isolated from Ismailia, according to this isolates distribution, it seems reasonable to conclude that there was no relationship between the *Streptomyces* geographic origin and their antagonistic pattern against soil-borne fungi involved in cotton seedling disease.

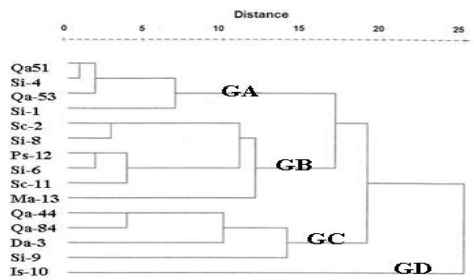

Figure-4: Phenogram based on cluster analysis of antagonistic pattern for 15 isolates of *Streptomyces* spp. obtained from different Governorates

DISCUSSION:

Cotton is an important crop in Egypt and has reputation all over the world. Seedling disease in cotton is a worldwide problem, causing serious stand loss when it is not controlled (Halloin, 1983; Blasingame, 1990). Gram-positive bacteria, such as, species belonging to *Bacillus* or *Streptomyces* that produce spores, which can be formulated readily into stable products (Emmert and Handelsman, 1999). Reddi and Rao (1971) reported that isolates of *Streptomyces ambofaciens* was able to control *Pythium* damping-off in tomato plants and *Fusarium* wilt in cotton plants in an artificially infested soil.

In the present study the antagonistic activities either *in vitro* or *in vivo* of the 15 *Streptomyces* isolates of this study, obtained from different Egyptian soils, were determined against six soil-borne fungi affected cotton. Results revealed that all fungi were adversely affected by all *Streptomyces* isolates. However, *R. solani* was the most sensitive fungus while *F. moniliforme* was the least sensitive one and the other five fungi showed intermediate levels *in vitro*. These antagonistic activities could be due to production of manifold antibiotics or fungal cell wall degrading enzymes such as chitinase (Mohamed 1982; Fravel, 1988; Hamed *et al*., 1998). *In vivo*, types of media whatever NBM or SNBM were significantly effective in the antagonism of the *Streptomyces* isolates against cotton seedling disease fungi. Fuzzy seeds treated with *Streptomyces* significantly reduced disease when Sc-11, Ma-13, Ps-12, Si-1 and Si-6 were used. These results are in agreement with those of De and Gupta (1991) who mentioned that of 27 actinomycete isolates from cultivated fields in Jabalpur, India, 3 *Streptomyces* spp. (A1, A2 and A3) showed strong antifungal activity against *F. solani*, *Helminthosporium oryzae* and *R. solani*. All the *Streptomyces* isolates were ineffective in controlling the disease when the seeds were acid delinted, and this could be due to the decrease in pH range (1.9-3.5) based on cotton cultivar (Mahaffee and Beckman, 1993), while the optimum pH for growing and activation of *Streptomyces* is 7.0 (Yuan and Crawford, 1995; Berg *et al*., 2001). In addition, no relationship was found between the *Streptomyces* geographical origin and their antagonisms against the six applied fungi that infect cotton. Finally this study suggests the potential of *Streptomyces* isolates as antagonists against soil-borne fungi and

paid more attention towards the use of such microorganism as a biocontrol agent and isolation and identification the active substances and their responsible genes.

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