Bacillus-based biological control of cotton seedling disease complex

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Abstract: To formulate an efficient and eco-friendly strategy for the management of cotton seedling disease complex, pot experiments were conducted and the efficiency of eight *Bacillus* strains against seven fungi involved in the disease were determined. A greenhouse evaluation of the interaction between fungal isolates and *Bacillus* strains was carried out. The evaluation revealed a very highly significant *Bacillus* strains x fungal isolates interaction for all the following parameters: preemergence damping-off, postemergence damping-off, survival, plant height, and dry weight. This interaction implies that a single strain of the *Bacillus* sp. can be highly effective against a fungal isolate, but may have only minimal effects on other fungal isolates. The results of the present study demonstrated that *Bacillus circulans* and *B. coagulans* were the most effective strains in controlling cotton seedling disease. Therefore, strains of *Bacillus* spp. should be tested against as many fungal isolates as possible. The testing will improve the chance of identifying *Bacillus* strains effective against several fungal isolates.

Key words: anatagonastic *Bacillus* spp., cotton damping-off complex

Introduction

Cotton seedling disease complex causes serious annual economic losses in many cotton-producing countries. *Rhizoctonia solani, Pythium* spp., and *Fusarium* spp. are considered the major fungi involved in the disease (Watkins 1981). *Sclerotium rolfsii* and *M. phaseolina* may also be involved in the disease although they are less important. The use of antagonistic bacteria showed promising results in controlling soil-borne fungi (Ramarathnam *et al*. 2011; Samavat *et al.* 2011; Zaim *et al.* 2013). *Pseudomonas* spp., and *Bacillus* spp. are the most common antagonistic bacteria that have been used to control cotton diseases seedling damping-off (Ramarathnam *et al*. 2011; El-Hassan *et al*. 2013; Mansoori *et al.* 2013; Sallam *et al.* 2013; Samavat *et al*. 2014). *Bacillus* spp. are aerobic or sometimes facultative bacteria and catalase positive. Endospore formation, universally found in this group, is thought to be a strategy for survival in soil, wherein these bacteria predominate and the endospores make them resistant to unfavorable environmental conditions (Landa *et al.* 1997). As attractive biocontrol agents and good plant-growth-promoting bacteria, *Bacillus* spp. offer several advantages over fluorescent *Pseudomonas* and other Gram-negative bacteria. These advantages include longer shelf life, their ability to form endospores, and the broad spectrum activity of their antibiotics (Chen *et al.* 2009; Samavat *et al*. 2014).

Because of their disease control properties, *Bacillus* spp., particularly *B. subtilis* and its closely related species, have been widely studied (Huang *et al*. 2012). *B. subtilis* A13 is the best known example of a biocontrol bacterial strain belonging to the genus *Bacillus.* This strain was isolated more than 25 years ago in Australia. The strain was selected based on its *in vitro* inhibitory activity against nine pathogens and was subsequently shown to promote growth of cereals, carrots, and bedding plants when it was applied as seed treatment (Broadbent *et al.* 1971; Merriman *et al.* 1975). Studies done by Hagedorn *et al*. (1989) found that cotton seedlings were initialy colonised by bacteria of many different genera. Populations quickly reached 10^8 CFU/g of root tissue. As the season progressed, the bacteria populations declined while root mass increased and the roots became more woodlike in consistency. There was no correlation between fungal repressive activity of rhizobacteria and the plant growth stage from which the isolates were obtained.

B. subtilis GB03, sold today in the United States as Kodiak for control of damping-off of cotton, is a cotton adapted variant of *B. subtilis* A13 (Mahaffee and Bockman 1993). Pleban *et al*. (1995) reported that endophytic *Bacillius* spp. can survive inside cotton plants and are efficient biocontrol agents against *R. solani* and *S. rolfsii* under greenhouse conditions. These endophytes reduced the disease index by more than 50%. Some *B. subtilis*

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strains that show antifungal activity may act by inducing tolerance in the host plant when tested against *Pythium aphanidermatum* and *Phytophthora nicotiana* in soilless tomato and cucumber culture systems (Grosch *et al.* 1999). The activity of *B. coagulans* as a biological control agent against fungal plant pathogens *Trichothecium roseum, R. solani, Sclerotinia sclerotiorum, Fusarium oxysporum, F. solani*, and *F. culmorum* was studied by Czaczyk *et al*. (2002). The effect of 20 isolates of *Bacillus* spp., obtained from livestock manure composts and cotton-waste composts for *in vitro* antagonism against *F. oxysporum, Phytophtora capsici, R. solani* AG-4, and *S. sclerotiorum* was studied by Kim *et al*. (2008).

Inoculation with both *Bacillus pumilus* and *Pseudomonas alcaligenes*, caused a greater increase in the plant growth of lentil, the number of pods, nodulation, and root colonisation by rhizobacteria, and also caused reduction of *Fusarium* wilting to a greater degree than did individual inoculation (Mohd *et al.* 2010). When using *Bacillus* spp. and other bacteria, for their efficacy against *M. phaseolina* on soybean plants, all tested strains significantly decreased damping-off, and decreased rotted and wilted plants while increasing healthy plants (El-Barougy *et al.* 2009). A wide range of plant pathogens including *Erwinia carotovora, Fusarium* sp., *F. oxysporum, Macrophomina phaseolina, Phytophthora, Pythium* sp., and *R. solani* were controlled by the *Bacillus* spp. (Jiang et *al.* 2006). The broad-spectrum antagonistic activities of *Bacillus* are executed by secretion of a number of metabolites including antibiotics, the volatile compound HCN, siderophores, enzymes chitinase and 1,3-glucanase (Ongena *et al.* 2007; Chen *et al.* 2009; Arrebola *et al.* 2010). The main objective of the present study was to evaluate eight *Bacillus* strains, isolated from rhizosphere of cotton seedlings, as to their efficiency in controlling soil-borne fungi commonly involved in cotton seedling disease complex under Egyptian conditions.

Materials and Methods

Bacterial strains

Soil samples from rhizosphere of cotton (*Gossypium barbadense* L.) seedlings were collected from different governorates (Gharbiya, Dakahliya, Giza, Assuit, Beheira, Minia and Sharkiya). Twenty grams of soil adhering to roots of each sample were suspended in 80 ml of sterile distilled water, shaken for 30 min on a shaker, and then serially diluted in sterile, distilled water. One ml from 10^{-4}, 10^{-5}, and 10^{-6} dilutions were taken form each sample and spread on nutrient glucose agar. The plates were incubated at 25°C for 3 days when the developing colonies were isolated in pure cultures. Selected cultures were identified (Table 1) according to Bergey's Manual of Systematic Bacteriology (Krieg and Holt 1984).

Fungal isolates

The seven pathogenic isolates of *R. solani, M. phaseolina, S. rolfsii, Pythium* sp., *F. oxysporum, F. solani*, and *F. moniliforme* used in this study, were originally isolated from the roots of cotton seedlings infected with damping-off disease. Isolation, purification, and identification of these fungi were carried out at the Cotton Pathology section of the Plant Pathology Research Institute, the Agricultural Research Centre, Giza, Egypt.

Preparation of fungal inoculum

Substrates for the growth of fungi were prepared in 500-ml glass bottles, each bottle contained 50 g of sorghum grains and 50 ml of water. Contents of bottles were autoclaved for 30 min. Fungal inoculum, taken from a one-week-old culture on Potato Dextrose Agar (PDA), was introduced into bottles and allowed to colonise sorghum for two weeks. The fungus-sorghum mixture (inoculum) was stored in plastic bags at 5°C until use. This study was carried out by using autoclaved clay loam soil. Batches of soil were infested separately with inoculum of each fungus at rates of 1g/kg soil for *R. solani*, and 5 g/kg soil for *S. rolfsii,* and *Pythium* sp. The rates of *F. oxysporum, F. solani, F. moniliforme*, and *M. phaseolina* were 50 g/kg soil. Infested soils were dispensed in 15-cm-diameter clay pots. The soil was also infested with a mixture of fungi at the same previously mentioned rates.

Preparation of bioformulations

Strains were grown in nutrient glucose broth at 30°C for 72 h on a shaker. The growth was adjusted turbidimetrically to 10^8 CFU/ml using spectrophotometer 2000 RSP 220 v. 50 Hz. Bacterial cultures were formulated in powder form by mixing 400 ml of cell suspension with 1 kg talc as a carrier, which was previously autoclaved for 30 min

Table 1. Identification of bacterial isolates used in the present study, and their geographic origins

Isolate no.	Identification	Geographic origin
1	*Bacillus coagulans*	Gharbiya
2	*Bacillus globisporus*	Dakahliya
3	*Bacillus pumilus*	Giza
4	*Bacillus subtilis*	Assiut
5	*Bacillus circulans*	Beheira
6	*Bacillus cereus*	Giza
7	*Bacillus coagulans*	Minia
8	*Bacillus cereus*	Sharkiya

for 2 successive days. Ten grams of carboxy methyl cellulose (CMC) were added to 1 kg of the carrier and mixed thoroughly. The pH of all materials was adjusted to 7.0 by adding calcium carbonate. The bacterial population was assessed as 2×10^8 CFU/g talc (Gordon-lennox *et al.* 1987).

Greenhouse pot experiment

Slightly moist seeds of cultivar Giza 89 were treated with the powdered inoculum of each *Bacillus* strain at a rate of 10 g/kg seeds. The treated seeds were then thoroughly shaken in plastic bags before being planted in soil previously infested with the tested fungi. The seeds were planted at a rate of 10 seeds/pot. The pots were randomly distributed on a greenhouse bench. The temperature regime ranged from 19±4 to 30±4°C. The experiment inculded three control treatments: Monceren-treated seeds in infested soil, untreated seeds in infested soil, and untreated seeds in autoclaved soil. The percentages of preemergence and postemergence damping-off were recorded 15 days from sowing while the percentage of surviving seedling, dry weight (mg/plant), and plant height (cm/ /plant) were recorded 45 days from sowing. All tests were repeated once with almost the same results.

Statistical analysis of the data

The experimental design in the present study was a Randomized Complete Block with five replications. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C Statistical Package. Least Significant Difference (LSD) was used to compare treatment means within each fungus ($\alpha = 0.05$ or $\alpha = 0.01$). Percentage data were transformed into arc sine angles or $\sqrt{x}$ before carrying out ANOVA to produce the approximate constant variance.

Results

Fungi, treatments, and fungi × treatment interactions were significant sources of variation in most of the tested parameters (Table 2). Relative contributions of fungi, treatments, and their interactions to variation in the tested parameters are shown in figure 1.

Due to the significant treatment × fungus interaction (Table 2), a LSD was used to compare between treatments within each fungus (Table 3). These comparisons showed that all the tested fungi and their mixtures were pathogenic in the preemergence stage treatments 1, 3, 5, and 8 were highly effective in controlling preemergence damping-off caused by *R. solani* as they decreased infection by 88.9, 83.3, 94.4, and 94.4%, respectively. Strains 5 and 8 were more efficient than the fungicide Monceren in controlling *R. solani* at the preemergence stage. Strain 4 completely suppressed *M. phasolina*, while strain 8 showed complete failure in controlling *M. phasolina*. The other strains showed variable effects. Strains 4 and 7 were effective in controlling *S. rolfsii* while strain 6 was completely ineffective. Strain 6 was effective against *F. oxysporum*, but strain 6 increased infection by *F. solani*. Strain 2 increased infection by *F. moniliforme*, while strains 7 and 2 decreased infection by the mixture. Strain 2 was the most effective strain against the mixture followed by strain no. 7 (efficiency was 82.35% and 76.47%, respectively). Strains 3 and 7 were effective in controlling *Pythium* sp., while strains 1, 6, and 8 were ineffective. Strains 3 and 7 were as effective as the fungicide.

Data in table 4 indicated that all the tested fungi and their mixtures were pathogenic in the postemmergence stage. Treatments 1, 6, 7 and 8 were more efficient than the fungicide (treatment 9) in controlling *M. phasolina* in the postemergence stage as they decreased postemergence by 77.7, 88.9, 55.9, and 55.9%, respectively, while the efficiency of the fungicide (no. 9) was 40.0%.

All the eight strains were highly effective in controlling postemergence damping-off caused by *S. rolfsii* and the efficiency of the treatments was higher than the efficiency of the fungicide. Strain no. 3 completely suppressed *S. rolfsii.* Data shown in table 4 indicate that all treatments were effective in decreasing the postemergence damping-off caused by *Pythium* sp. and *F. oxysporum.* Strain no. 1 was as effective as the fungicide in controlling *Pythium* sp. At the same time, strain no. 5 was more efficient than the fungicide in controlling both fungi. Strains 5, 6, 7, and 8 were highly effective in controlling postemergence damping-off caused by *F. solani* since

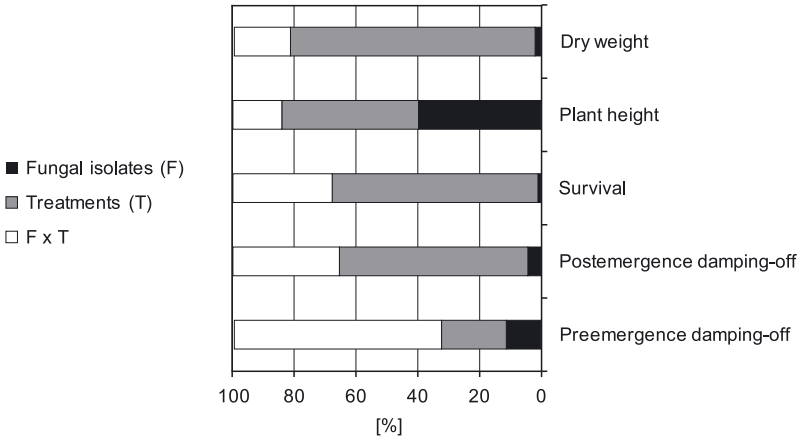

Fig. 1. Relative contribution of treatments, fungi, and their interaction to variation in cotton seedling disease variables (cv. Giza 89) under greenhouse conditions. The relative contribution was calculated as the percentage of the sum squares of the explained (model) variation

Table 2. Analysis of variance of the effect of treatments, fungi, and their interaction on cotton seedling disease variables (cv. Giza 89) under greenhouse conditions

Source	df	MS	F value	P value
Preemergence damping-off				
Replication[a]	3	1.266	1.2330	0.2981
Fungi (F)	7	13.995	13.6360	0.0000
Treatment (T)	10	18.139	17.6737	0.0000
F × T	70	8.295	8.0817	0.0000
Error	261	1.026	–	–
Postemergence damping-off				
Replication	3	1.637	0.0276	
Fungi (F)	7	345.799	5.8225	0.0000
Treatment (T)	10	3,684.831	62.0443	0.0000
F × T	70	298.800	5.0311	0.0000
Error	261	59.390	–	–
Survival				
Replication	3	18.22	0.4775	
Fungi (F)	7	88.126	2.3095	0.0267
Treatment (T)	10	4,980.373	130.5194	0.0000
F × T	70	343.642	9.0057	0.0000
Error	261	38.158	–	–
Plant height				
Replication	3	2.499	0.2824	
Fungi (F)	7	672.393	75.9810	0.0000
Treatment (T)	10	533.089	60.2396	0.0000
F × T	70	26.861	3.0353	0.0000
Error	261	8.849	–	–
Dry weight				
Replication	3	494.082	0.9198	
Fungi (F)	7	684.694	1.3258	0.2382
Treatment (T)	10	19,668.387	37.8088	0.0000
F × T	70	650.269	1.2500	0.0109
Error	261	520.206	–	–

[a] replication is random, while each of the treatments and fungi are fixed
df – degrees of freedom; MS – mean square

these strains decreased infection by 92.6, 77.8, 96.3, and 88.9 while efficiency of the fungicide was 77.8.

On the other hand, strains 1, 2, 3, 4, and 8 were highly effective in controlling postemergence damping-off caused by *F. moniliforme*, and these strains were more effective than the fungicide. Strains 2 and 7 were effective against the mixture as they decreased infection by 79.51% and 79.51%. Strains 3 and 4 followed in effectiveness. At the same time, strains 2 and 7 were as effective as the fungicide (no. 9) in controlling postemergence damping-off caused by the mixture.

Data shown in table 5 indicates that all bacterial strains increased the survival number when compared with treatment no. 10 (infested soil). Strains 1, 3, 5, and 8 were highly effective in increasing survival in the case of *R. solani* where the survival numbers were 67.5, 67.5, 80.0, and 85.0, respectively. Strain no. 8 was equivalent (as high as 85.0) to the fungicide in its effect on the percentage of survival. On the contrary, the bacterial strains 1, 4, 6, and 7 were more effective in increasing survival than the fungicide when used for controlling *M. phaseolina*. Thus, the percentages of survival of strains 1, 4, 6, and 7 were 75.0, 77.5, 850, and 75.0, respectively while that of treatment no. 9 (the fungicide) was 67.5.

All the eight strains were more effective than the fungicide in increasing the survival when used to control *S. rolfsii, F. oxysporum*, and *F. moniliforme*. Regarding *F. solani,* treatments 1, 2, 3, 5, 6, 7, and 8 were less effective than the fungicide in increasing survival. On the other hand, strain no. 4 was more effective than Monceren in increasing survival (to 80.0) while Monceren increased it to 77.5. As to the fungal mixture, treatments 2 and 7 were highly effective in increasing survival. Their percentages were 87.5 and 85.0 while that of the fungicide was 75.0.

Data in tables 6 and 7 show that all strains caused highly significant increases in plant height and dry weight with all the fungi and the mixture, when compared with the control (infested soil). For example, when strain no. 1 was used for controlling *R. solani*, plant height increased to 35.90 cm, whereas plant height increased to 12.72 cm when infested soil (no. 10) was used. Strain no. 1 increased dry weight to 209.0 mg while with the use of infested soil, the dry weight was 69.8 mg. Strain no. 4 was effective in improving plant height and dry weight when used for controlling *M. phasolina* and *S. rolfsii*. With the use of strain 4, plant height was 36.08 cm and 28.82 cm, respectively, while dry weight was 210.83 mg and 204.20 mg. On the other hand, strain no. 4 was effective in increasing plant

Table 3. Effect of treatments, fungi, and their interaction on preemergence damping-off of cotton seedlings (cv. Giza 89) under greenhouse conditions

Treatment[a]	Fungi															
	R. solani		*M. phaseolina*		*S. rolfsii*		*Pythium* sp.		*F. oxysporum*		*F. solani*		*F. moniliforme*		mixed	
	%[b]	transformed	%	transformed	%	transformed	%	transformed	%	transformed	%	transformed	%	transformed	%	transformed
1	5.0	1.58	15.0	3.82	27.5	5.23	35.0	5.91	15.0	3.82	17.5	4.14	27.5	5.23	20.0	4.47
2	25.0	4.98	25.0	4.89	40.0	6.33	15.0	3.82	32.5	5.69	7.5	1.91	35.0	5.91	7.5	2.37
3	7.5	2.37	5.0	1.58	20.0	4.47	5.0	1.58	37.5	6.12	35.0	5.23	20.0	4.47	35.0	5.91
4	37.5	6.12	0.0	0.00	12.5	3.49	20.0	4.47	22.5	4.65	15.0	3.82	25.0	4.98	15.0	3.82
5	2.5	0.79	22.5	4.72	25.0	4.98	10.0	3.16	10.0	3.16	10.0	3.16	10.0	3.16	25.0	4.98
6	10.0	3.16	10.0	3.16	35.0	5.91	30.0	5.48	7.5	1.91	30.0	5.44	15.0	3.82	37.5	6.12
7	32.5	5.68	5.0	1.58	12.5	3.49	7.5	1.91	20.0	4.47	22.5	4.65	7.5	2.37	10.0	3.16
8	2.5	0.79	32.5	5.65	32.5	5.69	35.0	5.91	15.0	3.74	25.0	4.94	15.0	3.82	25.0	4.98
9	7.5	2.37	5.0	1.58	35.0	5.91	7.5	2.37	20.0	4.32	7.5	2.37	17.5	4.14	20.0	4.39
10	45.0	6.70	32.5	5.65	35.0	5.91	37.5	6.12	25.0	4.98	10.0	3.16	22.5	4.72	42.5	5.77
11	7.5	2.37	10.0	3.16	10.0	3.16	5.0	1.58	10.0	3.16	5.0	1.58	7.5	2.37	10.0	2.70

[a] treatments from 1 to 8 are bacterial strains shown in table 1, treatment 9 is Monceren, treatment 10 is infested soil, and treatment 11 is autoclaved soil

[b] percentage data were transformed into $\sqrt{x}$ before carrying out ANOVA to approximately produce constant variance

LSD (transformed data) for treatment × fungi interaction = 1.141 ($\alpha = 0.05$) or 1.81 ($\alpha = 0.01$)

Table 4. Effect of treatments, fungi, and their interaction on postemergence damping-off of cotton seedlings (cv. Giza 89) under greenhouse conditions

Treatment[a]	Fungi															
	R. solani		*M. phaseolina*		*S. rolfsii*		*Pythium* sp.		*F. oxyspoum*		*F. solani*		*F. moniliforme*		mixed	
	%[b]	transformed	%	transformed	%	transformed	%	transformed	%	transformed	%	transformed	%	transformed	%	transformed
1	27.5	31.02	10.03	15.86	7.5	13.83	7.5	11.25	10.0	18.44	22.5	28.22	2.5	4.60	15.0	22.50
2	30.0	33.05	32.5	34.56	5.0	9.22	15.0	19.92	15.0	22.50	20.0	26.56	2.5	4.61	5.0	9.22
3	25.0	29.36	32.5	34.72	0.0	0.00	17.5	24.53	17.5	24.53	27.5	31.55	10.0	18.44	12.5	17.89
4	20.0	26.56	22.5	27.86	5.0	9.22	15.0	22.50	12.5	20.47	5.0	9.22	5.0	9.27	12.5	17.89
5	17.5	24.16	30.0	33.21	20.0	26.19	5.0	9.22	5.0	9.22	15.0	22.50	20.0	76.19	20.0	26.56
6	30.0	33.05	5.0	2.22	17.5	24.53	22.5	28.22	12.0	20.47	2.5	4.61	30.0	33.05	20.0	26.56
7	25.0	29.89	20.0	26.56	15.0	19.92	15.0	16.45	10.0	18.44	7.5	13.83	42.5	40.61	5.0	9.22
8	12.5	20.47	20.0	22.72	10.0	15.86	20.0	26.56	15.0	22.13	17.5	21.58	10.0	15.86	15.0	22.13
9	7.5	13.83	27.5	31.02	27.5	31.55	7.5	13.83	45.0	41.99	15.0	22.50	27.5	31.39	5.0	9.22
10	50.0	45.00	45.0	42.12	60.0	50.83	57.5	49.39	47.5	43.55	67.5	55.29	50.0	45.00	50.0	45.00
11	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00

[a] treatments from 1 to 8 shown in table 1, treatment 9 is Monceren, treatment 10 is infested soil, and treatment 11 is autoclaved soil

[b] percentage data were transformed into arc sine angles before carrying out ANOVA to approximately produce constant variance

LSD (transformed data) for treatment × fungi interaction = 10.73 ($\alpha = 0.05$) or 14.14 ($\alpha = 0.01$)

Table 5. Effect of treatments, fungi, and their interaction on survival of cotton seedlings (cv. Giza 89) under greenhouse conditions

Treatment[a]	Fungi															
	R. solani		*M. phaseolina*		*S. rolfsii*		*Pythium* sp.		*F. oxysporum*		*F. solani*		*F. moniliforme*		mixed	
	%[b]	transformed	%	transformed	%	transformed	%	transformed	%	transformed	%	transformed	%	transformed	%	transformed
1	67.5	55.44	75.0	60.12	65.0	53.78	57.5	49.39	75.0	60.12	60.0	50.93	70.0	56.95	65.0	53.78
2	45.0	42.12	42.5	40.67	55.0	47.89	70.0	56.95	52.5	46.44	72.5	58.61	62.5	52.28	87.5	69.53
3	67.5	55.44	62.5	52.28	80.0	63.44	77.5	61.78	45.0	42.12	45.0	42.12	70.0	56.79	52.5	46.44
4	42.5	40.67	77.5	62.15	82.5	65.47	65.0	53.78	65.0	53.78	80.0	63.81	70.0	57.11	72.5	58.45
5	80.0	63.81	47.5	43.56	55.0	47.89	85.0	67.50	85.0	67.50	75.0	60.12	70.0	56.95	55.0	47.89
6	60.0	50.83	85.0	67.50	45.0	42.12	47.5	43.55	80.0	63.81	67.5	55.29	55.0	47.89	42.5	40.67
7	42.5	40.67	75.0	60.12	72.5	58.45	77.5	62.30	70.0	56.79	70.0	56.95	50.0	45.00	85.0	67.50
8	85.0	63.81	47.5	43.56	57.5	49.33	45.0	42.12	70.0	57.11	57.5	49.33	77.5	60.12	60.0	50.83
9	85.0	67.5	67.5	55.44	37.5	37.73	85.0	67.50	35.0	36.22	77.5	61.78	45.0	42.12	75.0	60.12
10	5.0	9.22	22.5	27.86	5.0	9.22	5.0	9.22	27.5	31.55	22.5	28.22	27.5	31.55	7.5	11.25
11	92.5	76.17	90.0	71.56	90.0	71.56	95.0	80.78	90.0	71.56	95.0	62.89	92.5	76.17	90.0	74.14

[a] treatments from 1 to 8 are the bacterial strains shown in table 1, treatment 9 is Monceren, treatment 10 is infested soil, and treatment 11 is autoclaved soil

[b] percentage data were transformed to into arc sine angles before carrying out ANOVA to approximately produce constant variance

LSD for treatment × fungi interaction = 8.60 ($\alpha = 0.05$) or 11.33 ($\alpha = 0.01$)

Table 6. Effect of treatments, fungi, and their interaction on plant height (cm/plant) of cotton seedlings (cv. Giza 89) under greenhouse conditions

Treatment[a]	Fungi							
	R. solani	*M. phaseolina*	*S. rolfsii*	*Pythium* sp.	*F. oxysporum*	*F. solani*	*F. moniliforme*	mixed
1	35.90	30.59	27.71	21.85	31.00	23.23	23.85	26.15
2	32.54	35.03	24.78	23.90	24.03	25.15	20.73	27.63
3	29.30	32.80	22.83	21.20	25.85	24.58	23.18	30.75
4	32.67	36.08	28.82	24.95	21.90	24.88	21.83	27.90
5	34.89	30.69	26.34	21.70	30.90	22.34	20.00	28.93
6	35.01	36.53	23.69	23.68	28.48	20.25	21.98	23.85
7	30.74	32.79	26.46	21.48	25.95	21.73	25.85	28.90
8	32.93	35.31	24.84	21.66	25.25	21.33	20.95	31.20
9	29.72	29.81	25.57	22.08	29.25	22.00	20.70	23.60
10	12.72	21.10	14.17	9.90	18.23	15.78	13.13	9.35
11	22.86	23.13	20.98	18.73	21.01	18.05	18.05	20.38

[a] treatments from 1 to 8 are the bacterial strains shown in table 1, treatment 9 is Monceren (fungicides), treatment 10 is infested soil, and treatment 11 is autoclaved soil

LSD for treatment × fungi interaction = 4.14 ($\alpha = 0.05$) or 5.46 ($\alpha = 0.01$)

Table 7. Effect of treatments, fungi, and their interaction on dry weight (mg/plant) of cotton seedlings (cv. Giza 89) under greenhouse conditions

Treatment[a]	Fungi							
	R. solani	*M. phaseolina*	*S. rolfsii*	*Pythium* sp.	*F. oxysporum*	*F. solani*	*F. moniliforme*	mixed
1	209.00	186.20	193.93	202.96	185.03	192.73	222.58	201.80
2	192.28	196.43	196.35	211.13	193.80	187.13	197.32	187.80
3	195.25	204.38	202.88	204.30	211.95	203.28	216.38	210.85
4	217.38	210.83	204.20	198.23	205.45	210.68	203.80	196.25
5	182.58	178.63	198.00	196.85	193.00	197.40	195.38	189.00
6	206.30	203.68	210.88	209.75	213.65	207.33	212.50	207.38
7	211.30	197.10	208.68	198.33	204.70	212.83	203.05	193.98
8	196.88	187.13	213.25	201.83	197.18	196.53	193.23	185.68
9	184.80	196.88	197.85	192.48	193.68	183.90	186.23	198.35
10	69.80	143.93	100.63	100.25	160.55	159.03	153.28	92.98
11	167.38	167.10	175.43	175.63	169.33	169.90	169.88	169.88

[a] treatments from 1 to 8 are the bacterial strains shown in table 1, treatment 9 is Monceren, treatment 10 is infested soil, and treatment 11 is autoclaved soil

LSD for treatment × fungi interaction = 31.76 (α = 0.05) or 41.85 Ongena (α = 0.01)

height when used to control *Pythium* sp. while it was not effective in improving dry weight. Strains 2 and 6 were effective in increasing dry weight and plant height in the case of *Pythium* sp. The results of the mixture showed that strains 3, 5, 7, and 8 were effective in increasing plant height to 30.75, 28.93, 28.90, and 31.20 cm, respectively, while the infested soil (no. 10) was 9.35 cm. Strains 1, 3, and 6 were also useful in increasing dry weight. In general, all treatments were more effective than the fungicide in increasing both plant height and dry weight.

Discussion

The genus *Bacillus* is one of the the most important biocontrol agents used against insect plant pathogens. *Bacillus* has been shown to be effective and was commercially applied against plant pathogenic fungi (Jiang *et al.* 2006; Jian 2008). For instance, *B. pumilus* decreased disease incidence caused by *M. phaseolina* in chickpea plants (Akhtar and Siddiqui 2008).

In the current investigation, eight different strains of *Bacillus* spp. were evaluted as biocontrol against seven fungi involved in seedling disease complex of cotton and their mixture. These results are in agreement with the early reports that this group of bacteria are responsible for the biocontrol activity in rhizosphere of cotton seedlings (Aly *et al.* 1996; Wan *et al.* 2012). The results showed that strains 1, 3, 5, and 8 were effective in controlling *R. solani* in the preemergence stage, while strains 4, 5, and 8 were effective in the postemergence stage. These finding indicated that strains 5 and 8 were able to maintain their efficiency in the preemergence and postemergence stages. This phenomena was also observed by Asaka and Shoda (1996) and could be due to the presence of certain fungistatic metabolites secreted by these bacterial strains in preemergence and postemergence stages. For example, *B. subtilis* strain NCD-2 produced fengycin lipopeptide, and the fengycin played a primary role in inhibiting the growth of *R. solani in vitro*, in addition to suppressing cotton seedling damping-off disease *in vivo* (Guo *et al*. 2013).

Results also showed that the bacterial strains may change their efficiency when used for controlling different fungi. For example, strain 8 showed complete failure in controlling *M. phasolina* and *S. rolfsii* while the same strain was effective in controlling *R. solani*. This result implies that the bacterial strain may succeed in controlling some fungi, while it may fail to suppress other fungi (Harris *et al*. 1994). The high significance (p = 0.0000) of the interaction of *Bacillus* isolates × *M. phaseolina* isolates suggests that a single isolate of the antagonist can be highly effective against an isolate of *M. phaseolina*, but may have minimal effects on the other isolates of *M. phaseolina* (Omar *et al*. 2013). There were significant increases in survival and reduced preemergence damping-off showed by *B. coagulans* and *B. cereus* (Amal-Asran 2001).

The results of the present study showed that some bacteria strains may induced or increase the emergence of damping-off in cotton seedling. For example, strain 2 and strain 3 increase the premergence damping-off disease against *F. moniliforme* and *F. solani*, respectively. The afomentioned results indicated that these bacterial strains may be deleterious to plants, by producing toxic metabolites without parasitising plant tissues (Schippers *et al*. 1987).

The results of our studies also showed that *Bacillus* strains sometimes promote plant height and dry weight of cotton seedlings. These effects may be due to the fact that *Bacillus* spp. such as *B. subtilis* is known to produce growth-promoting factors (Swain and Ray 2009; López-Valdez *et al.* 2011).

Bacillus strains protect the roots from pathogen invasion and promote plant growth by solubilising soil phosphorus and producing hydrolytic enzymes, antibiotics, and plant growth hormones (Raza *et al*. 2009; Ling *et al*. 2010; Abdel-Fattah *et al*. 2011).

The introduction of *P. auerofaciens* 30–84 as a biocontrol agent against *R. solani* can result in an increased cotton yield, decreased chemical fungicide application, and protection of the agricultural environment and biological resources (Samavat *et al*. 2014). The results of the present study demonstrated that *B. circulans* and *B. coagulans* were the most effective strains in controlling cotton seedling disease.

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