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EFFECT OF *TRICHODERMA* ISOLATES, DELIVERY SYSTEMS AND HOST GENOTYPE ON BIOLOGICAL CONTROL OF COTTON SEEDLINGS DISEASE

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Abstract: Six isolates of *Trichoderma* spp. (belonging to species; *Trichoderma harzianum* and *T. longibrachiatum*) were applied as seed or soil treatments to suppress damping-off of seedlings of ten cotton cultivars under greenhouse conditions. In most cases, cultivar x isolate interaction was a highly significant ($p < 0.01$) source of variation in the tested seedling growth parameters: incidence of disease, seedling height, and seedling dry weight. This interaction implies that a single isolate of *Trichoderma* can be highly effective in controlling the disease on a cotton cultivar but may have minimal efficiency in controlling the disease on another cultivar. It was also found that, in most cases, cultivar x isolate x application method was a highly significant source of variation ($p < 0.01$) in the tested growth parameters. Cotton cultivars showed differences in the disease reaction to the biocontrol agents. In the experiments evaluating the *Trichoderma* antagonists and their effect on seedling disease, a highly significant ($p < 0.01$) experimental treatment interaction was found. This interaction suggests that the outcome of cultivar x isolate interaction is markedly affected by the application method. Thus, the application method should be chosen to maximize the outcome of this interaction. The degree of the control of seedling disease in cotton differed according to the isolates of antagonists, the application method and cultivars.

Key words: seed coating, seedling emergence, *Trichoderma*, cotton, growth parameters

INTRODUCTION

Cotton (*Gossypium* spp.) is the most important fiber crop, grown world-wide in over 80 countries. It is the first crop in terms of economic value in Egypt. Cotton seedling disease is one of the most serious diseases in all cotton-producing areas in Egypt. The soilborne fungi most often isolated from diseased cotton seedlings in-

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clude *Fusarium*, *Rhizoctonia*, *Macrophomina*, and *Pythium* and inflict a significant financial loss for cotton producers (Youssef and Mankarios 1974; Moubasher *et al.* 1984; Omar 1999; Howell *et al.* 2002). These pathogens are capable of colonizing seed within hours from planting and can lead to the following effects: (i) seed decay before germination, (ii) pre-emergence damping-off, (iii) post-emergence damping-off, or (iv) generalized blight (Hillocks 1992; Bell 1999). Due to environmental concerns, there is a considerable interest in finding alternatives to chemical pesticides for suppression of soilborne plant pathogens (Larkin *et al.* 1998). Identification and selection of effective antagonistic organisms is the first and foremost step in biological control (Kamalakannan *et al.* 2004). Antagonism by *Trichoderma* species to various fungi has been well documented (Harman *et al.* 1989, 2004; Kubicek and Harman 1998; Elad 2000; McBeath *et al.* 2001; Batta 2004). Biocontrol with beneficial microorganisms seems to be a promising approach to managing cotton seedling damping-off (Howell 1982; Howell *et al.* 1997; Howell and Puckhaber 2005). A number of *Trichoderma* isolates collected from the cotton rhizosphere were effective in suppressing seedling disease on cotton under greenhouse conditions (Asran-Amal *et al.* 2005).

Several factors affect the ability of *Trichoderma* spp. to provide systemic disease control (Hoitink *et al.* 2006). Abiotic and biotic environmental parameters may have a negative influence on the biocontrol efficacy of *Trichoderma* strains, therefore it is very important to collect information about the effects of environmental factors on different activities of *Trichoderma* strains with biocontrol potential (Kredics *et al.* 2003).

Additionally, as different isolates of fungal biocontrol agents are known to vary in biocontrol efficacy, mode of action, and physiology, it is important to determine whether isolates of fungal antagonists all respond similarly to changes in the environment and, consequently, help in the selection of isolates most suitable for mass production (McQuilken *et al.* 1997).

The purpose of this study was to evaluate the effects of delivery methods of antagonistic, *Trichoderma* isolates, and host cultivar on the efficacy of biological control of cotton seedling disease.

MATERIALS AND METHODS

Fungal isolates

Pathogenic and antagonistic isolates used in this study are listed in Table 1. Isolates of pathogenic soil-borne fungi were isolated from roots of cotton seedlings with damping-off disease symptoms and collected from cotton-growing areas. Isolation of *Trichoderma* spp. was made on potato dextrose agar (PDA) from rhizosphere of healthy cotton grown in agro-climatically different locations. Monosporic cultures were made and stored on PDA slants for further use.

Production of pathogen inoculum

Inoculum of the pathogens was prepared by wetting 40 g sorghum seeds with 50 ml water, autoclaving at 15 psi for 30 min, infesting with seed-borne pathogens, and incubating at 25°C for 2 weeks. Inoculum was air-dried and stored in a paper bag at 25 to 27°C in the laboratory. Inoculum level for each of the tested isolates was 50 g of fungus-sorghum mixture/kg of soil.

Table 1. Description, characteristics, and sources of biocontrol and pathogen isolates used in the present study

Isolate code	Organism	Characteristics	Geographic origin	Source
Fo	*Fusarium oxysporum*	cotton root-borne fungi	Beheria	Cotton Disease Depart.
Fs	*F. solani*	cotton root-borne fungi	Dagahliya	Cotton Disease Depart.
Rs	*Rhizoctonia solani*	cotton root-borne fungi	Fayium	Cotton Disease Depart.
Mp	*Macrophomina phaseolina*	cotton root-borne fungi	Gharbiya	Cotton Disease Depart.
Sr	*Sclertium rolfsii*	cotton root-borne fungi	Beheria	Cotton Disease Depart.
Pu	*Pythium ultimum*	cotton root-borne fungi	Daqahliya	Cotton Disease Depart.
T1	*Trichoderma harzianum*	biocontrol agent	Gharbiya	Asran-Amal *et al.* 2005
T2	*T. harzianum*	biocontrol agent	Gharbiya	Asran-Amal *et al.* 2005
T3	*T. harzianum*	biocontrol agent	Daqahliya	Asran-Amal *et al.* 2005
T4	*T. longibrachiatum*	biocontrol agent	Minufiya	Asran-Amal *et al.* 2005
T5	*T. longibrachiatum*	biocontrol agent	Minufiya	Asran-Amal *et al.* 2005
T6	*T. longibrachiatum*	biocontrol agent	Giza	Asran-Amal *et al.* 2005

Production of antagonist inoculum

The six fungal antagonists were grown on molasses yeast medium (Papavizas et al. 1984) by liquid fermentation for 14 days, and formulated by mixing 200 ml of fermentor broth with 500 g of autoclaved talc powder. Five grams of carboxymethyl cellulose (CMC) was added as a sticker to the powder after air-drying and the final dried formulation had a moisture level of 11%.

Application methods of antagonists

Seed treatment

In the first series of tests, 10 g of cotton seeds (10 cultivars) were mixed with 4.0 ml of an aqueous (11%) pelgel (Lipha Tech) solution as a sticker. Ten grams of seeds were mixed with 6 ml of sticker and 1.3 g of each powdered biomass for each fungal isolates. The seeds, sticker, and fungal biomass were mixed thoroughly; the seeds were covered with plastic sheets and stored at 4°C for no more than 7 days before planting. Seeds contained $>10^7$ CFU/1g seed for each fungal antagonist.

Soil amendment

Trichoderma spp. were cultured in sorghum as described by Budge and Whipps (1991). To prepare inoculum for soil treatment, mixtures comprising 2 liters of flaked maize and perlite (15% v/v)and 200 ml tap water in bags were autoclaved twice for 15 min and then inoculated with 100 ml of a suspension of 10^6 spores/ml in distilled water. The bags were incubated at 25°C for 3 weeks. The bags were shaken periodically to distribute the mycelium evenly. The concentration of inoculum of biocontrol fungi was 10^7 colony forming units (CFU) per cm^3 maize colonized with *Trichoderma.* It was mixed with the soil at the rate of 50g/kg soil.

Greenhouse assay for biocontrol activity against cotton seedling disease

The antagonistic capacities of the *Trichoderma* spp. isolates against the pathogen, mixtures of six cotton soil-borne fungi were determined. The autoclaved soil was infested with mixture of the tested fungi *F. solani*, *F. oxysporum*, *R. solani*, *M. phaseolina*, *S. rolfsii* and *P. ultimum* to obtain final concentration of 30, 30, 0.5, 30, 3, and 5 g/kg soil, respectively. Six antagonist isolates were selected, and evaluated for their efficiency in controlling cotton seedling disease on ten commercial cotton cultivars Giza 91 (V1), Giza 89 (V2), Giza 83 (V3), Giza 90 (V4), Giza 85 (V5), Giza 45 (V6), Giza 70 (V7), Giza 80 (V8), Giza 86 (V9), and Giza 88 (V10). These cultivars were selected because they represent important cotton cultivars grown in Egypt. Each experimental unit consisted of pots (15 cm x 20 cm depth) with 10 seeds per pot. Soil treated with fungal pathogens without antagonists was used as control (C1 or positive control). In addition, autoclaved soil treated with CMC was used as control (C2 or negative control).

A completely randomized design with five replications (pots) was used. Irrigation was provided daily. Survival, plant height (cm), and dry weight (mg/plant) were recorded two months after sowing. The temperature regime during cotton-growing period ranged from 23±2 to 38±2.5°C.

Data analysis

All data were analyzed using analysis of variance (ANOVA). Least significant difference (LSD) was used to compare treatment means. Statistical computations were performed using the statistical package STATISTICA 6 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

ANOVA of Table 2 showed that cultivar, isolate, and cultivar x application method interaction were all significant or highly significant sources of variation in disease incidence in 2004 and 2005. The application method was a non-significant source of variation in 2004, while it was a significant source of variation in 2005. Cultivar x isolate interaction was a highly significant source of variation in 2004 and non-significant source of variation in 2005. Isolate x application method was a non-significant source of variation each year. The second order interaction of cultivar x isolate x application method was a highly significant source of variation in 2004 and non-significant source of variation in 2005.

Table 2. Analysis of variance of the effect of cotton cultivar, *Trichoderma* isolate, method of application of *Trichoderma* isolate, and their interactions on incidence of cotton seedling disease under greenhouse conditions

Year and source of variation	D.F.	M.S.	F. value
2004			
Cultivar (V)	9	4551.6113	20.2632*
Method (M)	1	40.4800	0.1802
Isolate (T)	7	36359.7148	161.8685 **
V x M	9	1363.5577	6.0704 **
V x T	63	450.7857	2.0068**
M x T	7	126.7886	0.5644
V x M x T	63	350.7935	1.5617**
2005			
Cultivar (V)	9	3226.1111	17.0412**
Method (M)	1	2414.990	12.7566**
Isolate (T)	7	46686.5352	246.6110**
V x M	9	607.9177	3.2112**
V x T	63	200.9206	
M x T	7	260.2871	
V x M x T	63	191.4560	

D.F. – degrees of freedom
M.S. – mean square
** highly significant value

Relative contribution of cultivar, isolate, application method, and their interactions with the variation in disease incidence (Fig. 1) revealed that *Trichoderma* isolates were the most vital source of variation in disease incidence as they accounted for 70.9 and 83.7% of the explained (model) variation in 2004 and 2005, respectively.

A significant of cultivar x isolate x application method interaction in 2004 implies that the cultivar x isolate interaction was clearly affected by isolate T2 (Table 3) which reduced disease incidence on cultivar V2 by 78.3% relative to pathogen-infested control when it was applied as seed treatment (AM1). However, when this isolates was applied as soil treatment (AM2) its efficiency in controlling the disease was decrease to 38.1% on the same cultivar. Another example was isolate T4, which significantly reduced disease incidence by 81.8% on cultivar V6 when it was applied as seed treatment. Nevertheless its efficiency was reduced to 55% on the same cultivar when it was applied as soil treatment. A very highly significant interaction of cultivar x application method in 2005 indicates that cultivars responded differently to the two application methods. Least significant difference (LSD) was used to compare he effectiveness of the two application methods used in cultivars (Table 4).

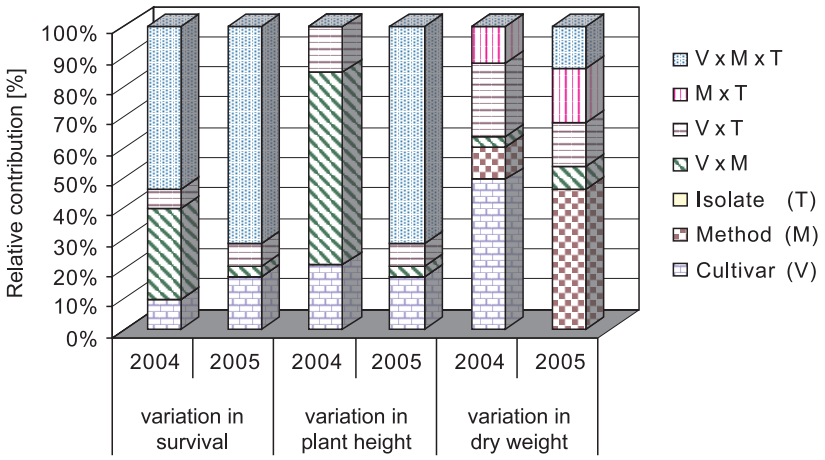

Fig. 1. Relative contribution of the effect of cotton cultivar, *Trichoderma* isolate, method of application of *Trichoderma* isolates, and their interaction to variation in survival, plant height, and dry weight of cotton seedlings under greenhouse conditions

These comparisons showed that the interaction between cultivars and application methods was due to changes in the magnitude of the differences between the application methods within cultivars. For instance, the difference in disease incidence between the two application methods was highly significant on cultivar V1, while it was non-significant in case of cultivar V4. The lack of significance isolate x cultivar and the isolate application method in 2005 (Table 2) indicates that isolate efficiency in controlling the disease was not affected by cultivar nor the application method.

Therefore, LSD was used to compare between the general means of the isolates and this comparison showed that isolate T4 was the most effective isolate in reducing disease incidence since it reduced it by 60.8% (Table 5) relative to the pathogen infested control.

Seedling height was significantly affected by all the sources of variation each year (Table 5). Cultivar was the first in importance as a source of variation in seedling height every year (Fig. 1). Isolates showed almost the same relative contribution to variation in seedling height each year; however, it was the third and the second in importance in 2004 and 2005, respectively. Application method was the second and third in importance in 2004 and 2005, respectively.

The significance of cultivar x isolate x application method interaction implies that the effect of cultivar x isolate interaction on seedling height was markedly affected by the application method. For example, isolate T4 (Table 6) significantly increased seedling height of cultivar V2 by 23.59% when it was applied as seed treatment, while height of seedlings of the same cultivar was increased by 57.05% when the isolate was applied as soil treatment.

An additional example was isolate T1, which was ineffective in increasing seedling height of cultivar V6 when it was applied as seed treatment. However, the isolate increased seedling height of the same cultivar by 32.20% when it was applied as soil treatment.

Table 3. Effect of cotton cultivar (V), *Trichoderma* isolate (T), application method (AM) and their interaction on incidence of cotton seedling disease [%] in 2004

Application method[a]	Isolate[b]	Cultivars[c]										Mean
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	
AM1	T1	36	40	47	30	48	26	44	48	34	46	40.1
	T2	40	20	52	56	50	28	38	48	48	36	41.6
	T3	26	30	54	46	26	32	40	52	38	46	39.0
	T4	52	24	46	32	46	16	50	54	48	32	40.0
	T5	46	22	62	30	32	34	26	58	32	60	41.2
	T6	42	32	36	48	40	22	26	38	56	48	38.8
	C1	84	82	90	88	94	88	90	84	92	82	88.4
	C2	20	20	30	18	24	22	14	32	28	28	23.6
AM2	mean	43.2	33.7	52.1	43.5	45	33.5	41	51.7	47.2	47.2	43.9
	T1	40	68	50	60	54	22	46	42	38	38	42.2
	T2	29	48	74	52	52	24	38	40	34	24	41.9
	T3	45	44	74	58	38	40	52	38	46	18	46.5
	T4	34	68	64	56	46	36	32	36	40	18	39.4
	T5	42	72	64	48	44	30	24	46	46	32	44.4
	T6	36	58	60	42	50	24	24	44	40	18	39.8
	C1	86	16	90	94	90	80	88	96	96	90	89.4
	C2	18	82	30	16	22	16	10	28	26	14	19.8
	mean	41.2	44.2	63.3	53.2	40.6	44	55.2	46.2	45.7	31.5	45.4
Overall mean		42.2	39.6	57.8	38.4	47.2	33.7	40.4	48.9	46.4	39.3	44.7

[a] application methods seed treatment (AM1) and soil treatment (AM2)
[b] *Trichoderma* isolates were *T. harzianum* (T1, T2, and T3) and *T. longibrachiatum* (T4, T5, and T6)
C1 pathogen – infested soil and C2 autoclaved soil
[c] cotton cultivars were Giza 91(V1), and Giza 88(V10)
LSD for cultivar x isolate x application method interaction = 24.42 ($p < 0.01$) or 18.58 ($p < 0.05$)

Table 4. Effect of cotton cultivar (V), *Trichoderma* isolates (T), application method (AM) and their interaction on incidence of cotton seedling disease [%] in 2005

Application method[a]	Isolate[b]	Cultivars[c]										Mean
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	
AM1	T1	40	32	46	46	48	36	42	38	38	26	38.2
	T2	26	16	60	52	46	24	38	40	34	24	36.0
	T3	30	12	42	46	42	40	48	38	42	38	37.8
	T4	34	40	46	30	40	22	32	36	40	18	33.8
	T5	24	24	58	42	44	30	44	46	42	32	38.6
	T6	18	42	60	42	44	24	38	44	36	38	38.6
	C1	86	88	92	94	88	84	88	90	90	90	89.0
	C2	10	8	26	12	16	8	16	16	12	4	13.0
AM2	mean	33.5	67.2	56.3	44.3	46	33.5	43.3	43.5	47.8	33.8	40.9
	T1	38	70	56	54	52	34	50	50	38	38	44.0
	T2	38	38	50	36	34	22	52	56	38	24	62.2
	T3	44	30	62	42	38	48	40	40	32	18	47.8
	T4	24	24	48	50	36	48	46	42	28	32	38.0
	T5	40	42	44	60	48	34	48	42	42	36	44.0
	T6	42	34	44	44	54	30	46	42	52	18	40.6
	C1	92	95	96	94	94	96	92	96	92	94	94.1
	C2	22	22	28	16	18	10	10	24	14	12	17.6
	mean	42.5	39.7	51	49.5	46.8	39	48.5	49	42	34	44.2
Overall mean		38	36.2	53.7	64.9	46.4	36.7	45.9	46.3	41.9	33.9	42.6

[a] application methods seed treatment (AM1) and soil treatment (AM2)
[b] *Trichoderma* isolates were *T. harzianum* (T1, T2, and T3) and *T. longibrachiatum* (T4, T5, and T6)
C1 pathogen – infested soil and C2 autoclaved soil
[c] cotton cultivars were Giza 91(V1), and Giza 88(V10)
LSD for cultivar x isolate = 5.01 ($p < 0.01$) or 3.81 ($p < 0.05$)
LSD for cultivar x application method = 7.9342 ($p < 0.01$) or 6.03 ($p < 0.05$)

Table 5. Analysis of variance of the effect of cotton cultivar, *Trichoderma* isolate, method of application of *Trichoderma* isolate, and their interaction on height of cotton seedlings under greenhouse conditions

Years and source of variation	D.F.	M.S.	F. value
2004			
Cultivar (V)	9	590.6174	218.5318**
Method (M)	1	4028.4475	1490.5488**
Isolate (T)	7	376.9914	139.4890**
V x M	9	103.1651	38.1717**
V x T	63	26.0605	9.6426**
M x T	7	41.7557	15.4499**
V x M x T	63	15.3979	5.6973**
2005			
Cultivar (V)	9	351.1113	112.4282**
Method (M)	1	1255.7887	402.1122**
Isolate (T)	7	289.2909	92.6329**
V x M	9	190.6290	61.0407**
V x T	63	14.3367	4.5907**
M x T	7	84.8411	27.1825**
V x M x T	63	3.1230	6.0330**

D.F. – degrees of freedom
M.S. – mean square
** highly significant value

Table 6. Effect of cotton cultivar (V), *Trichoderma* isolate (T), application method (AM) and their interaction on height of cotton seedlings (cm) in 2004

Application method[a]	Isolate[b]	Cultivars[c]										Mean
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	
AM1	T1	15.64	15.34	20.75	18.58	20.6	18.54	20.6	15.3	15.04	15.62	17.6
	T2	16.26	17.86	20.16	18.06	21.5	20.44	20.86	18.9	15.46	15.82	18.53
	T3	18.54	19.24	20.44	18.8	23.1	19.86	22.5	18.38	15.1	16.5	17.48
	T4	17.68	19.28	18.7	19.62	22.86	21.36	20.94	19.02	15.56	17.5	19.25
	T5	16.94	17.98	17.2	20.3	22.66	21.94	21.34	15.48	15.48	14.16	18.44
	T6	16.28	16.96	17.9	17.9	18.6	21.4	19.42	16.7	17.3	15.14	17.76
	C1	14.52	15.6	17.1	15.32	15.1	18.76	14.3	11.86	14.02	12.26	14.88
	C2	16.2	16.6	17.9	17.02	17.24	18.98	17.48	18.24	14.8	15.86	18.78
AM2	mean	16.63	17.35	18.76	18.2	20.20	20.16	19.68	16.73	15.34	15.40	17.84
	T1	19	21.1	25.3	27.5	25.36	27.1	28.4	19.14	20.1	20.04	23.30
	T2	20.44	26.6	28.3	29.5	25.9	28.5	27.6	17.2	18.9	17.6	24.05
	T3	19.5	28.66	22.9	28.4	26.4	28.9	28.1	15.72	18.9	18.52	23.6
	T4	20.94	28.74	25.2	29.2	29.4	26.2	28.3	17.3	20	19.08	24.43
	T5	21.66	27.2	27.9	22.2	26.88	29.3	27.4	17.6	18.6	18.76	23.75
	T6	20.4	26	24.9	19.2	28.6	33.4	23.9	18.9	21.2	14.5	24.5
	C1	17.4	18.3	16.74	17.4	22.16	20.5	15.6	12.28	18.3	10.2	16.88
	C2	19.3	19.74	19.74	21.4	22.8	20.5	18.28	19.7	19.96	16.2	19.76
	mean	19.83	24.54	23.89	24.35	25.93	26.8	24.69	17.23	19.49	16.92	22.36
Overall mean		18.23	20.94	21.32	21.27	23.06	23.48	22.18	16.98	17.4	16.16	20.1

[a] application methods seed treatment (AM1) and soil treatment (AM2)
[b] *Trichoderma* isolates were *T. harzianum* (T1, T2, and T3) and *T. longibrachiatum* (T4, T5, and T6)
C1 pathogen – infested soil and C2 autoclaved soil
[c] cotton cultivars were Giza 91(V1), and Giza 88(V10)
LSD for cultivar x isolate x application method = 2.68 ($p < 0.01$) or 2.04 ($p < 0.05$)

Table 7. Effect of cotton cultivar (V), *Trichoderma* isolate (T), application method (AM) and their interaction on height of cotton seedlings (cm) in 2005

Application method[a]	Isolate[b]	Cultivars[c]										Mean
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	
AM1	T1	18.74	20.16	22.72	17	22.05	20	20.48	21.06	17.1	17.96	19.72
	T2	17.36	21.66	17.1	18.52	23.5	21.62	20.44	20.76	19.5	17.86	19.83
	T3	20.16	20.63	19.5	18.62	23.36	21.1	17.96	20.86	20.7	16.98	19.98
	T4	21.66	20.3	18.24	18.52	24.4	21.16	18.9	20.46	18.9	18.3	20.08
	T5	18.3	21	18.6	19.62	21.4	19.22	19.2	19.26	18	17	19.16
	T6	21.48	21.5	19.6	17.87	23.5	19.74	19.2	19.5	22	17.64	20.20
	C1	17.52	17.5	15.24	15.35	20.2	17.4	17.46	16.98	16.5	15.7	16.98
	C2	18.44	20.8	18.68	19.35	21.3	21.16	19.8	18.12	19.5	17.5	19.46
AM2	mean	19.20	20.8	18.7	18.17	22.46	20.17	19.18	19.62	19.5	17.36	19.51
	T1	18.5	21.2	24.7	26.6	26.7	26.7	27.6	17.6	20.5	20.6	23.07
	T2	19.9	24.8	26.98	28.3	28.3	27.7	27.4	16.7	20.1	18.2	23.8
	T3	19.1	27	21.6	27.4	28.2	27.3	26.5	16.38	19.4	18.68	23.15
	T4	20.5	27.24	26.58	29.2	28.6	25.6	25.5	17	20.6	19.8	24.06
	T5	21	26.2	25.2	22.8	23.98	28.6	23.9	17.5	19.2	15.82	22.42
	T6	19.9	25.38	24.1	19.6	20.4	30.2	24.28	18.6	21.9	15	21.93
	C1	16	18	18.46	17.4	17	20.2	15.8	13.1	17.1	12	16.49
	C2	19.18	20.12	19.8	22.18	21.18	23.3	20.6	17.1	20.2	16.8	20.04
	mean	19.26	23.74	23.42	24.18	24.37	26.23	23.9	16.73	19.87	17.11	21.88
Overall mean		19.23	22.27	21.06	21.17	23.41	23.2	21.5	18.17	19.68	17.23	20.69

[a] application methods seed treatment (AM1) and soil treatment (AM2)
[b] *Trichoderma* isolates were *T. harzianum* (T1, T2, and T3) and *T. longibrachiatum* (T4, T5, and T6)
C1 pathogen – infested soil and C2 autoclaved soil
[c] cotton cultivars were Giza 91(V1), and Giza 88(V10)
LSD for cultivar x isolate x application method = 2.88 ($p < 0.01$) or 2.19 ($p < 0.05$)

Table 8. Analysis of variance of the effect of cotton cultivar, *Trichoderma* isolate, method of application of *Trichoderma* isolate, and their interaction on dry weight of cotton seedling under greenhouse conditions

Years and source of variation	D.F.	M.S.	F. value
2004			
Cultivar (V)	9	231480.5781	34.4385**
Method (M)	1	185199.0469	27.5529**
Isolate (T)	7	160276.8906	23.8451**
V x M	9	66445.7891	9.8854**
V x T	63	21377.0313	3.1804**
M x T	7	94026.8594	13.9888**
V x M x T	63	14735.1035	2.1922**
2005			
Cultivar (V)	9	105525.8672	12.9621**
Method (M)	1	2273027.7500	279.2043**
Isolate (T)	7	277453.0625	34.0806**
V x M	9	42903.4023	5.2700**
V x T	63	12440.1826	1.5281**
M x T	7	135355.5313	16.6262**
V x M x T	63	11663.3926	1.4327**

D.F. – degrees of freedom
M.S. – mean square
** highly significant value

The formerly mentioned conclusions concerning cultivar and isolate application method interaction hold true for 2005 data shown in Table 7.

Dry weight of seedlings was significantly affected by all sources of variation each year (Table 8). Cultivar was the main important source of variation each year as it accounted for 30.08 and 28.35% of the explained (model) variation in 2004 and 2005, respectively.

Cultivars and application method was the second in importance as a source of variation in 2004 as it accounted for 19.46% of the explained (model) variation. Isolate was the third and second in importance in 2004 and 2005, respectively. Application method was almost as important as application method x isolate interaction as a source of variation each year (Fig. 1).

The interaction of cultivar and isolates was significantly affected by dry weight. For example, isolate T1 (Table 9) significantly increased dry weight of V7 seedlings by 71.2% when it was applied as soil treatment. Almost the same results were obtained when the experiment was repeated in 2005 (Table 10).

Table 9. Effect of cotton cultivar (V), *Trichoderma* isolate (T), application method (AM) and their interaction on dry weight of cotton seedling [mg] in 2004

Application method[a]	Isolate[b]	Cultivars[c]										Mean
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	
AM1	T1	212.8	296.4	347.6	297.4	355.2	254.2	317.6	233	289.4	252.4	285.6
	T2	197.4	269.8	403	332.8	393.8	221	341	238	273.6	290.4	296.08
	T3	240.2	383.2	380.4	306.2	304.4	229.4	317.4	323.4	286.4	313.8	308.4
	T4	321.4	322.2	375.8	271.1	319.2	235.6	289.4	339.4	341	276.8	309.19
	T5	260.8	275.2	400.6	343.4	329.2	303	302.6	232.6	240.8	264	295.22
	T6	218.4	297	325.4	335	300	249.6	239.2	303.2	313.6	285.4	286.68
	C1	198.2	235.4	225.8	193.4	241	237.6	185.6	129.5	128.6	148.8	182.39
	C2	255.8	308.6	325	213	295	246	246.2	336.8	265.6	262.1	294.36
AM2	mean	238.1	298.4	347.9	286.5	317.22	247.05	279.7	2791.4	278.1	274.6	294.33
	T1	306.8	283.8	481.6	483	478.4	332.4	516.6	373.4	248.4	294	379.84
	T2	249.2	474	609.6	513.8	616.8	488.8	453.8	267.8	265.2	210.8	414.9
	T3	213.6	451.8	487.2	449.2	363.4	401	454.4	234.6	244.2	244.4	354.38
	T4	312.4	523.4	480.2	587.6	412.8	363.4	400.6	370.8	289.4	268.4	400.9
	T5	248.8	306.6	526	340.6	464	344.8	400.6	244.8	213	156.6	324.58
	T6	244.4	448.4	555.2	351.2	487.8	477.6	287	370.2	257.8	176	365.56
	C1	198	239.2	279.4	233	260.2	210.4	141	159	211.6	117.8	204.96
	C2	210.2	248.6	301.4	250.4	299.6	249.2	295.8	275.6	242	147.4	252.02
	mean	247.9	371.9	465.6	401.1	422.6	358.4	368.7	287	246.4	201.9	337.15
Overall mean		243	335.1	406.4	343.8	370.03	302.7	324.2	283.2	256	232.0	309.64

[a] application methods seed treatment (AM1) and soil treatment (AM2)
[b] *Trichoderma* isolates were *T. harzianum* (T1, T2, and T3) and *T. longibrachiatum* (T4, T5, and T6)
C1 pathogen – infested soil and C2 autoclaved soil
[c] cotton cultivars were Giza 91(V1), and Giza 88(V10)
LSD for cultivar x isolate x application method = 133.57 ($p < 0.01$) or 101.63 ($p < 0.05$)

Table 10. Effect of cotton cultivar (V), *Trichoderma* isolate (T), application method (AM) and their interaction on dry weight of cotton seedling [mg] in 2005

Application method[a]	Isolate[b]	Cultivars[c]										Mean
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	
AM1	T1	307.4	341.6	416.6	307.4	416.4	241.6	274.4	306.6	280	265.6	315.74
	T2	330.4	337.2	432	330.4	432	237.2	336	347.8	268.4	246.4	329.78
	T3	448.8	336.8	324	448.8	324	336.8	301.2	316.6	241	247.4	332.54
	T4	347.8	349	400.6	347.8	400.6	249	286.8	344.6	284.4	298.6	340.90
	T5	300.6	311.6	335.6	400.6	335.6	211.6	286.2	305.4	325	227.4	293.90
	T6	288.8	315.4	339	288.8	339	215.4	302.8	366.6	298.4	251.4	300.50
	C1	209.2	2008	173.6	209.2	213.6	245.8	167.2	203.4	183.4	177.8	204.90
	C2	294	230.6	312.4	290	212.4	230.6	293.6	319.2	230.6	240.4	284.78
	mean	315	296	341.1	317.8	329.2	246	281.5	308.4	263.9	244.2	290.8
AM2	T1	426	462.2	507.6	451.8	555.8	380.8	387.2	509	372.6	349	440.2
	T2	364	502.6	483.6	562.4	509.8	417.4	372	393.2	445.2	313.2	436.34
	T3	422.4	502.4	479.8	570.4	482.4	430	374.8	399.4	448.4	308.8	441.8
	T4	454.4	516	467.2	453.7	571	465.6	472	418.8	388.6	322.6	447.2
	T5	426.4	433.8	517.6	549.2	520.4	493.6	326.8	488.2	338.6	400.4	449.5
	T6	459.6	491.2	512	428.4	540.6	374.2	347.2	400	324.4	319.4	419.0
	C1	258.4	237.4	264	249.2	291.8	217.8	210.4	252.8	202.2	206.6	239.0
	C2	357.6	251.6	383.6	347	317.8	365.8	204.8	259.4	329	262.4	307.8
	mean	361.1	424.6	451.9	451.5	473.7	393.1	336.9	390.1	356.1	310.3	394.9
Overall mean		338.4	360	396.6	389.6	401.4	319.55	309.2	349.25	310	277.2	345.09

[a] Application methods seed treatment (AM1) and soil treatment (AM2)
[b] *Trichoderma* isolates were *T. harzianum* (T1, T2, and T3) and *T. longibrachiatum* (T4, T5, and T6)
C1 pathogen – infested soil and C2 autoclaved soil
[c] cotton cultivars were Giza 91(V1), and Giza 88(V10)
LSD for cultivar x isolate x application method = 147.00 ($p < 0.01$) or 111.85 ($p < 0.05$)

DISCUSSION

Numerous attempts in biological control have resulted in second-rate disease control under varying environmental conditions and sites. This inconsistency possibly was related, at least partially, to a general lack of understanding of how these biocontrol systems work and under which conditions they may or may not be expected to function. This has resulted in the introduction of biocontrol organisms into environments in which they are ecologically unsuitable (Deacon 1991). Any individual biocontrol microorganism can only be expected to perform within a limited set of physical, biological, and environmental conditions. Hitherto, generally, these conditions are inadequately clear (Larkin and Fravel 2002).

For eco-friendly and sustainable management of the disease, 6 isolates belonging to two species of *Trichoderma* (*T. harzianum*, and *T. longibrachiatum*) were applied as seed and soil treatments to suppress damping-off of cotton seedlings on ten cotton cultivars under greenhouse conditions. On the whole, cultivar and isolate interaction was a highly significant source of variation ($p < 0.01$) in the tested seedling growth parameters (disease incidence, seedlings height, and seedling dry weight).

This interaction implies that a single isolate of antagonist can be vastly effective in controlling the diseases on a cotton cultivar but may have minimal efficiency in controlling the disease on another cultivar. Antagonists also varied in their efficiency as biocontrol agents, and a relative effectiveness of different antagonists varied among growing seasons (Ryan *et al.* 2004). The efficacy of biological control agents can also vary relative to each other and overall when assayed on different host cultivars (Schisler *et al.* 2000). *T. longibrachiatum* conferred varying levels of protection to the cotton seedling disease, depending on isolate, host and pathogen (Sreenivasaprasad and Manibhushanrao 1990).

The interaction also indicates that apparently many genes from both cotton and *Trichoderma* interact to regulate the number of cotton cultivars and *Trichoderma* isolates (Wells and Bell 1983). The methods of applying biocontrol agents to a target area are critical in the development of biocontrol strategies for protection against different diseases (Mao *et al.* 1997).

These findings have an important bearing on antagonism testing methods. Isolates of *Trichoderma* should be tested on as many cotton cultivars as possible, as this will improve the chance of identifying antagonist isolates effective in controlling the disease on more than a few cotton cultivars.

The interaction also suggests that it may be more prudent to evaluate blends of antagonist isolates for wild application on more cotton cultivars. In this investigation, the interaction between cotton cultivars and *Trichoderma* isolates was evaluated under greenhouse conditions favourable for the growth of both cotton cultivars and *Trichoderma* isolates.

Under field conditions, environmental conditions during the different periods of cotton growing season may be more favourable for cotton cultivars or the antagonist isolates. Thus, the findings of this work are not expected to be necessarily related to the degree of biological control that may be observed in the field, but should reflect the capacities and genetic variability of the antagonist isolates and of the various cotton cultivars to response to antagonisms (Bell *et al*. 1982).

It was also found that, in most cases, cultivar x isolate x application method was a highly significant source of variation ($p < 0.01$) in the tested growth parameters.

This interaction suggests that the outcome of cultivars x isolates interaction is markedly affected by the application method. Thus, application method should be chosen to maximize the outcome of the interaction.

CONCLUSIONS

Environmental factors play an important role in restricting the activity of potential biological control agents, detailed information on the environmental requirements of *Trichoderma* are required in order to improve its efficacy and also to assist in optimizing large-scale inoculum production. The results obtained here, demonstrated that significant complex interactions occur between methods of application, cultivars and antagonistic isolates. Further tests also are required to improve our understanding for this complex interaction.

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POLISH SUMMARY

WPŁYW IZOLATÓW TRICHODERMA, SYSTEMÓW ICH APLIKACJI I GENOTYPU ROŚLINY ŻYWICIELSKIEJ NA BIOLOGICZNE ZWALCZANIE CHOROBY SIEWEK BAWEŁNY

W celu ograniczenia zgorzeli siewek 10 odmian bawełny wykorzystano 6 izolatów *Trichoderma* spp., zaliczanych do gatunków *T. haraianum* i *T. longibrachiatum*. Przy ich użyciu stosowano zaprawianie nasion bawełny, lub wprowadzano je do ziemi w doświadczeniu prowadzonym w warunkach szklarniowych. W większości przypadków źródłem występującej, wysoce istotnej ($p < 0,01$) zmienności badanych parametrów wzrostu siewek (występowanie choroby, wysokość roślin i ich sucha masa), było współdziałanie odmiany z izolatem grzyba antagonistycznego. To współdziałanie pokazuje, że izolat *Trichoderma* może być wysoce efektywny w zwalczaniu choroby na jednej odmianie, ale może wykazywać minimalną efektywność zwalczania na innej odmianie. Stwierdzono także, że w większości przypadków współdziałanie: odmiana x izolat x metoda jego aplikacji było wysoce istotnym ($p < 0,01$) źródłem zmienności ocenianych parametrów wzrostu. Wykryto różnice w reakcji chorobowej odmian bawełny na zastosowane izolaty *Trichoderma*. Oceniając wpływ antagonistycznych izolatów oraz sposób ich zastosowania na zgorzel siewek bawełny stwierdzono wysoce istotne ($p < 0,01$) współdziałanie tych czynników. Sugeruje to, że współdziałanie: odmiana x izolat jest w dużym stopniu zależne od zastosowanej metody ich aplikacji.

Należy więc wybierać metodę pozwalającą na maksymalizację korzystnego aspektu tego współdziałania. Stopień zwalczania zgorzeli siewek bawełny różnił się zależnie od izolatu grzyba antagonistycznego, metody jego aplikacji oraz odmiany bawełny.