

USE OF *TRICHODERMA* SPP., *Aspergillus* spp., AND *Penicillium* SPP. TO SUPPRESS DAMPING-OFF OF COTTON SEEDLINGS

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ABSTRACT

Fifteen isolates of *Trichoderma* spp., two isolates of *Aspergillus* spp., and four isolates of *Penicillium* spp. were evaluated under field conditions in Assiut and Mallawy as to their efficiency in suppressing cotton seedling damping-off. Seeds of cotton cultivar Giza 83 in both locations were treated with a dry powder of antagonist-sorghum mixture at a rate of 10 g/kg seeds. Of the twenty-one isolates, isolates nos. 3, 9, 10 and 11 of *Trichoderma* spp. and isolate no. 18 of *Penicillium* sp. were effective in increasing stand and yield in both locations. Many of the other isolates were effective in increasing both stand and yield; however, their efficiencies were restricted to one location- that is, their performance lacked stability. It is noteworthy that some isolates of the biocontrol agents were as effective as or even more effective than Rizolex T in suppressing the disease and increasing yield in Assiut or Mallawy.

INTRODUCTION

Cotton seedling damping-off caused by a complex of seed-borne and soil-inhabiting organisms. These organisms are found in all cotton-producing areas of Egypt. Although the populations of inciting organisms differ from area to area, the pathogens most commonly involved in the disease complex are *Rhizoctonia solani* (Rizk, 1980 and Mohamed, 1990), *Fusarium* spp. (Jakob, 1969 and Aly *et al.*, 1996), *Macrophomina phaseolina* (Omar, 1999), *Pythium* spp. (Eisa, 1983), and *Sclerotium rolfsii* (Khashaba, 1972).

The disease occurs as pregermination decay of the seed, decay of the seedling on the way to the soil surface (Preemergence damping-off), partial or complete girdling of the emerged seedling at or near the soil surface ("Sore shin" or postemergence damping-off), and seedling root rot (Watkins, 1981).

The occurrence of major losses from cotton seedling damping-off is not uncommon in all cotton-producing 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 seed cotton 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 (Eisa *et al.*, 1987; Aly *et al.*, 1992; Eisa *et al.*, 1992; and Abdel Azizi *et al.*, 1996), 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 has been considered as a serious alternative to seed-dressing fungicides. Regarding cotton seedling damping-off, a number of reports demonstrated that some fungi, in particular *Trichoderma* spp., could be effectively used for controlling this disease. For example, Elad *et al.* (1982) controlled *R. solani* in cotton by seed-coating with *Trichoderma* spp. spores. The coating reduced disease incidence by up to 83% in the greenhouse. *T. hamatum* was effective at 20°C, *T. harzianum* at 27°C. In 2 field experiments reduction in disease severity was 47-60% equalling that obtained with PCNB (quintozene) fungicide. Lewis and Papavizas (1985) demonstrated that mycelial preparations of 8 of 14 isolates of *Trichoderma* spp. and *Gliocladium virens* reduced survival of *R. solani* at least 50% in pathogen-infested beet seed in soil and in soil infested with sand/cornmeal inoculum of the pathogen. Mycelia preparations, but not conidia, of most isolates of *Trichoderma* spp. and *G. virens* prevented damping-off of cotton seedlings in the greenhouse. Alagarsamy *et al.* (1987) reported that seed pelleting with 3 isolates of *T. viride* and 1 of *T. harzianum* increased the germination rate and reduced postemergence mortality of LRA5166 cotton due to *R. solani*, compared with the untreated controls. *T. harzianum* was effective as quintozene (at 5 g/kg seed) and superior to 2 of the *T. viride* isolates. Lewis and Papavizas (1987) prepared alginate pellets from wet fermentor biomass of 11 isolates of *Trichoderma* spp. and *G. virens*, with wheat bran as a food base carrier. Pellets prepared with some of the 11 isolates prevented damping-off of cotton in the greenhouse. DeVay *et al.* (1988) found that *T. viride* was effective for cotton seedling disease control when applied to cotton seed in an alginate-pyrax gel mixture hardened with calcium gluconate. Lewis and Papavizas (1991) evaluated preparations of isolates of the biological control fungi *Trichoderma* spp. and *G. virens* during 4 growing seasons for their efficacy in preventing damping-off of cotton caused by *R. solani* in field plots artificially infested with the pathogen. Pathogen saprophytic activity and populations of the biological control fungi were monitored periodically in the soil. The preparations included bran/germlings (activity growing hyphae on bran), a powder (pyrax/biomass) and alginate pellets containing milled fermentor biomass of the fungi. Of the 3 preparations, bran/germlings consistently prevented diseases, reduced pathogen saprophytic activity and stimulated proliferation of populations of its biological control fungi. In all 4 years, bran/germlings preparations of an isolate of *T. hamatum* (TRI-4) and an isolate of *G. virens* (G1-21) significantly prevented diseases, and in 3 of the years gave a plant stand comparable to that of the non-infested plots. Pyrax/biomass preparations of G1-21 prevented damping-off in 2 of the 4 years, but stands similar to those of the non-infested plots were never achieved. Pyrax/biomass preparations of most other isolates used were ineffective in preventing disease. Alginate pellets of the isolates studied did not prevent disease in the 2 years in which they were applied to soil. Generally, Pyrax/biomass and alginate pellet preparations did

not reduce pathogen saprophytic activity or stimulate an increase in numbers of antagonistic fungi. Howell *et al.* (1997) found that viridiol (-) mutants of *T. virens* could not synthesize the phytotoxin viridiol but retained the capacity to produce antifungal antibiotics, acted as mycoparasites, and controlled cotton seedlings disease incited by *R. solani*. The use of biocontrol preparations containing viridiol (-) mutants virtually eliminated the phytotoxicity associated with treatment of cotton seed with high concentrations of parent strain preparations.

The objective of this study was to evaluate 15 isolates of *Trichoderma* spp., 4 isolates of *Penicillium* spp., and 2 isolates of *Aspergillus* spp. for their effectiveness as biocontrol agents against soilborne fungi involved in cotton seedling damping-off under field conditions in Assiut and Mallawy.

MATERIALS AND METHODS

Isolates of biocontrol agents

Isolates of the biocontrol agents used in this study (Table 1) were obtained from the fungal collection of Cotton Disease Research Section, Plant Pathology Research Institute, Agric. Res. Center. All isolates were originally isolated from cotton roots.

Table 1: Geographic origins of antagonists used in study.

Isolate No.	Antagonist	Geographic origin
2	<i>Trichoderma</i> sp.	Sharqiya
3	<i>Trichoderma</i> sp.	Sharqiya
4	<i>Trichoderma</i> sp.	Daqahliya
5	<i>Trichoderma</i> sp.	Minya
6	<i>Trichoderma</i> sp.	Giza
7	<i>Trichoderma</i> sp.	Giza
8	<i>Trichoderma</i> sp.	Kafr El-Sheikh
9	<i>Trichoderma</i> sp.	Giza
10	<i>Trichoderma</i> sp.	Sharqiya
11	<i>Trichoderma</i> sp.	Sharqiya
12	<i>Trichoderma</i> sp.	Sharqiya
13	<i>Trichoderma</i> sp.	Domietta
14	<i>Aspergillus</i> sp.	Daqahliya
15	<i>Trichoderma</i> sp.	Domietta
16	<i>Trichoderma</i> sp.	Minufiya
17	<i>Trichoderma</i> sp.	Assiut
18	<i>Penicillium</i> sp.	Giza
19	<i>Penicillium</i> sp.	Kafr El-Sheikh
20	<i>Aspergillus</i> sp.	Gharbiya
21	<i>Penicillium</i> sp.	Qualybiya
22	<i>Penicillium</i> sp.	Giza

Preparation of inocula of biocontrol agents

Substrate for growth of the tested isolates was prepared in 500 ml glass bottles, each bottle contained 100 g of sorghum grains and 80 ml of tap water. Contents of each bottle were autoclaved for 30 minutes. Isolate inoculum, taken from one-week-old culture on PDA, was aseptically introduced into the bottle and allowed to colonize sorghum for 3 weeks. Antagonist-sorghum mixture was air-dried in the greenhouse. The dry mixture was triturated to a fine powder in a blender (Papavizas and Lewis, 1981).

Field evaluation of the effectiveness of biocontrol agents in suppressing cotton seedling damping-off

Experiments were conducted at Mallawy Agricultural Research Station and in the Farm of Faculty of Agriculture in Assiut in 1998. Each experiment was designed as a randomized complete block of five replicates each replicate consisted of four 4-meter rows in Mallawy or two 4-meter rows in Assiut. Each row included 20 hills, each containing 10 seeds. Seeds of cotton (*Gossypium barbadense* L.) cultivar Giza 83 in both locations were treated with the dry mixtures of the tested isolates at a rate of 10 g/kg seeds. The mixtures were added to slightly moist seeds. The seeds were shaken thoroughly in plastic bags for 5 min and allowed to dry before being planted. In the control treatment, no inoculum was added to seeds. Planting dates were 24 and 31 March 1998 in Assiut and Mallawy, respectively. Percentage of preemergence damping-off was recorded 20 days from sowing, while each of postemergence damping-off and survival was recorded 45 days from sowing. Seedcotton yield (cottonseed and lint before ginning) was picked on 15-30 October at each site and determined as g/plant and kg/plot.

Statistical analysis of the data

Analysis of variance (ANOVA) of the data was performed with the MSTAT-C 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 treatment means. Some percentage data were transformed into arc sine angles or square roots before carrying out ANOVA to produce approximately constant variance. Correlation analysis was performed with a computerized program

RESULTS AND DISCUSSION

In Assiut, all isolates, except isolate no. 10, were effective in reducing preemergence damping-off (Table 2). Only 6 isolates significantly reduced postemergence damping-off. All isolates significantly increased survival. Seedcotton yield per plot was not affected by any isolate; however, 9 isolates significantly increased seedcotton yield per plant. These isolates were superior to the fungicide Rizolex, which did not cause significant increase in seedcotton yield per plant.

In Mallawy, only 6 isolates of *Trichoderma* spp., 3 isolates of *Penicillium* spp., and one isolate of *Aspergillus* spp. significantly reduced preemergence damping-off (Table 3). Only 2 isolates significantly reduced postemergence damping-off. The majority of the isolates significantly increased survival. Almost all isolates significantly increased seedcotton yield per plant and seedcotton yield per plot. Almost all isolates were as effective as Rizolex in increasing seedcotton yield.

Table 2: Biological control of cotton seedling damping-off under field conditions in Assiut in 1998.

No.	Treatment	Pre-emergence damping-off (%)	Post-emergence damping-off (%) ^a	Survival (%) ^b	Yield/plant (g)	Yield/plot (kg)
1	Control	59.36a	14.40a	26.24c	73.00f	2.152ab
2	<i>Trichoderma</i> sp.	49.36b-d*	8.36a-c	42.28b*	85.20b-f	2.232ab
3	<i>Trichoderma</i> sp.	45.68cd*	11.16a-c	43.16ab*	98.80a-d*	2.064ab
4	<i>Trichoderma</i> sp.	48.08b-d*	8.16a-c	43.76ab*	101.00a-c*	2.462a
5	<i>Trichoderma</i> sp.	47.44b-d*	10.08a-c	42.48ab*	81.40e-f	2.176ab
6	<i>Trichoderma</i> sp.	50.16b-d*	4.80c*	45.04ab*	94.00a-e*	2.180ab
7	<i>Trichoderma</i> sp.	46.68b-d*	9.64a-c	43.68ab*	85.40b-f	1.975ab
8	<i>Trichoderma</i> sp.	47.44b-d*	8.64a-c	43.92ab*	85.60b-f	1.964ab
9	<i>Trichoderma</i> sp.	47.68b-d*	7.60a-c	44.72ab*	95.40a-e*	2.194ab
10	<i>Trichoderma</i> sp.	52.80a-c	6.88bc*	40.32b*	96.00a-e*	2.294ab
11	<i>Trichoderma</i> sp.	44.18cd*	11.00a-c	44.82ab*	103.20ab*	2.358ab
12	<i>Trichoderma</i> sp.	47.84b-d*	7.44a-c	44.72ab*	92.00a-f	2.376ab
13	<i>Trichoderma</i> sp.	46.88b-d*	13.12a-c	40.00b*	83.00b-f	2.280ab
14	<i>Aspergillus</i> sp.	48.32b-d*	8.00a-c	43.68ab*	83.60b-f	2.236ab
15	<i>Trichoderma</i> sp.	49.12bd*	9.24a-c	41.64b*	94.20a-e*	2.240ab
16	<i>Trichoderma</i> sp.	49.40b-d*	5.30c*	45.30ab*	90.00a-f	2.450a
17	<i>Trichoderma</i> sp.	47.56b-d*	7.16bc*	45.28ab*	92.80a-f	2.326ab
18	<i>Penicillium</i> sp.	49.68b-d*	6.24bc*	44.08ab*	96.20a-e*	2.220ab
19	<i>Penicillium</i> sp.	50.00b-d*	6.00bc*	44.00ab*	85.60b-f	2.025ab
20	<i>Aspergillus</i> sp.	47.16b-d*	8.40a-c	44.44ab*	78.20d-f	2.162ab
21	<i>Penicillium</i> sp.	46.42b-d*	6.70a-c	46.88ab*	83.40b-f	2.160ab
22	<i>Penicillium</i> sp.	42.64d*	7.20a-c	50.16a*	109.20a*	2.386ab
23	Rizolex T	46.20b-d*	10.12ab	43.68ab*	85.20b-f	2.250ab

^a Percentage data were transformed into \sqrt{X} before carrying out the analysis of variance to produce approximately constant variance.

^b Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.

Values in a column 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.

An effective biocontrol agent should meet two requirements. First, it should significantly reduce disease development. Second, it should have a stable performance when it is evaluated under different environmental conditions. Of the 21 isolates evaluated in the present study, isolates nos. 3, 9, 10, and 11 of *Trichoderma* spp. and isolate no. 18 of *Penicillium* sp. were the only isolates which met the two requirements because they were effective in increasing stand and yield in both locations. Many of the other isolates were effective in increasing both stand and yield; however, their efficiencies were restricted to one location- that is, their performance lacked stability. The successful application of biological control for controlling cotton seedling damping-off under field conditions, as we have demonstrated herein, is in agreement with the results of other workers (Elad *et al.*, 1982; Lewis and Papavizas, 1991). It is noteworthy that some isolates of the biocontrol agents were as effective as or even more effective than Rizolex T in controlling the disease and increasing yield in Assiut or Mallawy.

In Assiut, a significant negative correlation was observed between preemergence damping-off and plant yield (Table 4), while in Mallawy, preemergence damping-off was negatively correlated with each of plant yield and plot yield. Taken together, these negative correlations imply that the higher the disease pressure during the pre- and postemergence stages, the

less productive the surviving plants would be. That is, even the plants which survived pre-and postemergence damping-off suffered from a subtle weakness which reduced both plant yield and plot yield. Evidently, such less productive plants were developed from unthrifty seedlings (Watkins, 1981; Minton and Garber, 1983). This interpretation holds true for the significant positive correlation observed in Assiut between survival and plant yield.

Table 3: Biological control of cotton seedling damping-off under field conditions in Mallawy in 1998.

No.	Treatment	Pre-emergence damping-off (%)	Post-emergence damping-off (%) ^a	Survival (%) ^b	Yield/ plant (g)	Yield/ plot (kg)
1	Control	69.25a	4.25ab	26.50c	45.00f	1.628c
2	<i>Trichoderma</i> sp.	68.45ab	5.90ab	25.65a-c	72.80a-c*	2.280a*
3	<i>Trichoderma</i> sp.	66.75ab	3.55b*	27.70ab*	61.60b-e*	2.070a-c
4	<i>Trichoderma</i> sp.	68.30bc*	3.80ab	29.90a*	85.00a*	2.350a*
5	<i>Trichoderma</i> sp.	64.00bc*	6.00ab	30.00a*	64.40b-e*	2.100ab*
6	<i>Trichoderma</i> sp.	68.60ab	5.50ab	25.90a-c	52.80d-f	2.452a*
7	<i>Trichoderma</i> sp.	67.20bc*	4.60ab	28.20ab*	66.00b-e*	2.478a*
8	<i>Trichoderma</i> sp.	69.20ab	3.00b*	27.80ab*	59.40c-f	2.206ab*
9	<i>Trichoderma</i> sp.	60.69c*	8.45ab	30.86a*	63.00b-e	2.430a*
10	<i>Trichoderma</i> sp.	66.10ab	3.65ab	30.25ab*	69.20b-d*	2.160ab*
11	<i>Trichoderma</i> sp.	64.15bc*	7.80ab	28.00a*	77.80ab*	2.386a*
12	<i>Trichoderma</i> sp.	68.95ab	7.44ab	26.80a-c	69.40b-d*	2.070a-c
13	<i>Trichoderma</i> sp.	67.45ab	5.65ab	32.60a-c	62.00b-e*	2.136ab*
14	<i>Aspergillus</i> sp.	68.25ab	3.85ab	27.80ab*	55.40d-f	1.982a-c
15	<i>Trichoderma</i> sp.	69.50ab	3.95ab	26.60a-c	63.80b-e*	2.120ab*
16	<i>Trichoderma</i> sp.	68.85ab	4.50ab	26.65a-c	59.60c-f	2.280a*
17	<i>Trichoderma</i> sp.	66.45bc*	4.60ab	29.00a*	65.80b-e*	2.440a*
18	<i>Penicillium</i> sp.	66.75bc*	5.90ab	29.15ab*	75.00a-c*	2.340a*
19	<i>Penicillium</i> sp.	65.20bc*	5.65ab	30.45a*	67.40b-d*	2.326a*
20	<i>Aspergillus</i> sp.	66.30bc*	3.80ab	31.00a*	69.20b-d*	2.156ab*
21	<i>Penicillium</i> sp.	66.50bc*	4.25ab	29.30a*	66.20b-e*	2.406a*
22	<i>Penicillium</i> sp.	67.45ab	5.90ab	26.65a-c	67.60b-d*	2.260ab*
23	Rizolex T	67.90ab	3.60ab	28.50ab*	63.20b-e	2.250ab*

^a Percentage data were transformed into \sqrt{X} before carrying out the analysis of variance to produce approximately constant variance.

^b Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance.

Values in a column 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 4: Correlation coefficients among variables used for evaluating performance of biocontrol agent isolates in suppressing cotton seedling damping-off under field conditions in Assiut and Mallawy.

Location	Variable	Variable			
		5	4	3	2
Assiut	1 Preemergence damping-off (%)	-0.125a	-0.468*	-0.836**	0.138
	2 Postemergence damping-off (%)	-0.174	-0.335	-0.657**	
	3 Survival (%)	0.201	0.555**		
	4 Plant yield (g)	0.469*			
	5 Plot yield (kg)				
Mallawy	1 Preemergence damping-off (%)	-0.420*	-0.366x	-0.631**	-0.565**
	2 Postemergence damping-off (%)	0.273	0.236	0.053	
	3 Survival (%)	0.163	0.286		
	4 Plant yield (g)	0.503*			
	5 Plot yield (kg)				

^a Linear correlation coefficient (r) is significant at $P \leq 0.10$ (x), $P \leq 0.05$ (*), or $P \leq 0.01$ (**).

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استعمال فطريات التريكوثيرما والأسبرجلس والبنيسليوم لمقاومة مرض موت بادرات القطن

على عبد الهادي على^١، عزت محمد حسين^١، على دياب على علام^٢، عبد المنعم محمود أمين^٢، عبد الرحيم محمد أحمد السمواتي^١.

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اختبرت ١٥ عزلة لفطر التريكوثيرما و ٢ عزلة لفطر الأسبرجلس و ٤ عزلات لفطر البنيسليوم - تحت ظروف الحقل - فى كل من أسيوط وملوى، وذلك لتقييم فعاليتها فى مقاومة مرض موت بادرات القطن. عوملت بذرة صنف جيزة ٨٣ فى كلا الموقعين بمسحوق جاف يتكون من خليط الفطر المضاد والسورجم وذلك بمعدل ١٠ جم/كجم بذرة. اظهرت النتائج أن العزلات ٣ و ٩ و ١٠ و ١١ لفطر التريكوثيرما والعزلة ١٨ لفطر البنيسليوم ذات فعالية فى زيادة الإنبات والمحصول فى كلا الموقعين. على الرغم من أن العديد من العزلات الأخرى أظهرت مثل هذه الفعالية، إلا أنها كانت مقصورة على موقع واحد دون الآخر، مما يدل على ان أداء هذه العزلات كان يفتقر إلى الثبات. الجدير بالذكر أن بعض العزلات كانت تعادل المبيد الفطرى ريزولكس تى أو حتى تتفوق عليه وذلك من حيث القدرة على مقاومة المرض وزيادة المحصول فى أسيوط أو ملوى.