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ANTAGONISTIC SPECIFICITY OF ISOLATES OF *Trichoderma* SPP. AGAINST ISOLATES OF *Rhizoctonia Solani* FROM COTTON ROOTS

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ABSTRACT

Biocontrol efficiency of four isolates of *T. harzianum* and *T. viride* were evaluated against twelve isolates of *Rhizoctonia solani* under greenhouse conditions. *R. solani* and *Trichoderma* isolates were isolated from cotton seedlings. Six of *R. solani* isolates belonged to AG4 and six belonged to AG2-2. Analysis of variance showed very highly significant effects of *Trichoderma* spp. isolates, *R. solani* isolates, and their interaction on preemergence damping-off, postemergence damping-off, survival, plant height, and dry weight. This interaction implies that a single isolate of antagonist can be highly effective against an isolate of *R. solani*, but may have only minimal effects on other isolates of *Rhizoctonia solani*. The correlation among variables used for evaluating pathogenicity of *R. solani* isolates under effect of *Trichoderma* isolates was studied. It was found that the application of *Trichoderma* as biocontrol agent changed the relationship between these variables. Cluster analysis of *Trichoderma* isolates based on their antagonistic patterns showed that isolates were divided to two groups. The first group included isolates of *T. harzianum* and isolate 2 of *T. viride*, while the second group included the other isolate of *T. viride*. It seems that grouping of *Trichoderma* spp. isolates was not related to either geographic origin or0morphological taxonomy. Cluster analysis of *R. solani* isolates based on their response patterns to *Trichoderma* isolates suggests that AG4 isolates were more homogeneous in their response patterns than those of AG2-2.

INTRODUGTION

Rhizogtonia solani Kühnis a widespread, soilborne pathogen responsible for serious damage in many crops including cïtton. The widm host range of this pathogen as well as its ability"to survive under adverse environmental conditions as sclerotia have markedly reduced the potential of crop rotation as a management strategy (Benhamou and Chet, 1993). Some Fungicides have been successfully used to control *R. solani*. Although in many cases, these fungicides appear to be the most economical and efficient means of controlling this pathogen. Toxicological, environmental, and sociological concerns have led to drastic reduction in the availability of efficient commercial fungicides, and the use of fungicides may also lead to the appearance of new resistant strains of the pathogen (Hajieghrari *et al.*, 2008).

Biological control of plant pathogens, especially soilborne plant pathogens, by microorganisms has been considered a more natural and environmentally acceptable alternative to existing chemical treatment methods (Barker and Panlitz, 1996; and Eziashi *et al.*, 2007). *Trichoderma* spp., that are common saprophytic fungi found in almost any soil and rhizosphere microflora, have been investigated as potential biocontrol agents

because of their ability to reduce the incidence of disease caused by plant pathogenic fungi, particularly many common soilborne pathogens (Papavizas, 1985; Calvet *et at.*, 1990; Spiegel and Chet, 1998; Elad, 2000; Freeman *et al.*, 2004; Ashrafizadeh *et at.*, 2005; and Dubey *et al.*, 2007). Although some have been occasionally recorded as plant pathogen (Menzies, 1993), *Trichoderma harzianum*, *T. viride*, *T. vimes* and *T. hamatum* are the species that most often used in biological control of pathogens (Hajieghrani *et al.*, 2008).

Hadar *et al.* (1979) found that *T. harzianum* directly attacked *R. solani* mycelium. Wheat-bran-grown cultures of this antagonist added to soil in greenhouse plantings reduced damping-off caused by *R. solani* in beans, tomato, and eggplants.

Elad *et al.* (1982) found that coating cotton seeds with *Trichoderma* spp. reduced incidence of disease caused by *R. solani* by up to 83% in the greenhouse.

The objective of this study was to evaluate the biocontrol specificity of isolates of *Trichoderma* spp. against *R. solani* isolates the causal agent of cotton seedling disease under greenhouse conditions.

MATERIALS AND METHODS

Fungal isolates

Isolates of *R. solani* and *Trichoderma* spp. used in the present study (Tables 1 and 2) were obtained from the fungal collection of Cotton Disease Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. *R. solani* and *Trichoderma* spp. were originally isolated from cotton roots.

Table 1. Isolates of *Rhizoctonia Solani* used in this study

Isolate No.	Geographic Origin	Anastomosis Group (AG)
1	Gharbiya	4
2	Assiut	2-2
3	Sharkiya	4
4	Assiut	2-2
5	Sohag	2-2
6	Sharkiya	4
7	Dakahliya	2-2
8	Minya	2-2
9	Minufiya	4
10	Beheira	4
11	Dakahliya	4
12	Gharbiya	2-2

Table 2. Geographic origins of *Trichoderma* spp. used in the study

Isolate No.	Geographic Origin	*Trichoderma* spp.
1	Assuit	*T. harzianum*
2	Sharkiya	*T. viride*
3	Dakahliya	*T. viride*
4	Beheira	*T. harzianum*

Production of *Rhizoctonia solani* inoculum:

Inoculum of *R. solani* isolates was prepared in 500-ml glass bottles, each contained 40g of sorghum grains and 50ml tap water. The bottles then autoclaved at 15psi for 30min. Inocula, taken from one-week-old PDA cultures, were aseptically introduced into the bottles and allowed to grow and colonize sorghum grains for 2 weeks at 25°C.

Production of *Trichoderma* spp. inoculum:

Inoculum of *Trichoderma* spp. isolates was prepared as previously mentioned; however Trichoderma-sorghum mixtures were air-dried in the greenhouse and then triturated to a fine powder in a blender (Papavizas and Lewis, 1981).

Greenhouse assay for biocontrol activity of *Trichoderma* spp. against *Rhizoctonia solani*:

Autoclaved clay loam soil was placed on greenhouse benches and individually infested with inoculum of each *R. solani* isolates at rate of 1g/kg soil. After thoroughly mixing, infested soil was dispensed into 15-cm-diameter clay pots. Seeds of cultivar Giza 86 were treated with the powdered inoculum of each isolate of *Trichoderma* spp. at the rate of 10g/kg seeds. In the control treatment, seeds were treated with sorghum powder at the same rate. Slightly moist cotton seeds were treated with inoculum of each isolate, and thoroughly shaken in plastic bags before being planted at the rate of 10 seeds/pot of *R. solani* infested soil. Temperature regime in the greenhouse ranged from 20 ± 2°C to 34 ± 3°C. Preemergence damping-off was recorded 15 days after planting. Postemergence damping-off, survival, plant height (cm), and dry weight (mg/plant) were recorded 45 day after planting.

Statistical analysis of data:

The experimental design of the present study was a randomized complete block designed with four replicates. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C statistical package. Least significant difference (LSD) was used to compare between means of *Trichoderma* spp. isolates within *R. solani* isolates. Percentage data were transformed into arc sine angles before carrying out the ANOVA to produce approximately constant variance.

RESULTS AND DISCUSSION

Two isolates of *T. viride* (T2 and T3) and two isolates of *T. harzianum* (T1 and T4) were evaluated *in vivo* to study their antagonistic potential against six isolates of *R. solani* AG4 and six isolates of AG2-2 implicated in seedling damping-off of cotton cultivar Giza 86. ANOVA (Table 3) showed very highly significant (P=0.0000) effects of *Trichoderma* isolates, *R. solani* isolate, and their interaction on all the tested parameters. *R. solani* and *Trichoderma* spp. isolates were almost equally important factors in determining variation in pre-emergence damping-off, while *Trichoderma* spp. isolates were the most important factor in determining variation in survival, plant height, and dry weight (Table 4). The interaction between isolate of *Trichoderma* spp. and isolate of *R. solani* was the most important factor in determining variation in

postemergence damping-off (Table 4). Due to the very highly significant effect of the interaction of *Trichoderma* spp. isolate x *R. solani* isolate on all the tested parameters, LSD was calculated to compare means of *Trichoderma* isolates within each isolate of *R. solani* (Tables 5, 6, and 7).

Table 3. Analysis of variance of the effect of *Trichoderma* isolate, *Rhizoctonia solani* isolate, and their interaction on cotton seedling disease variables (cultivar Giza 86) under greenhouse conditions

Parameter and source of variation	D.F.	M.S.	F. value	P >F
1. Preemergence damping-off				
Replication	3	1.619	0.0780	0.0000
Trichoderma isolate (T)	4	3650.155	175.7276	0.0000
Rhizoctonia isolate (R)	11	1375.000	66.1959	0.0000
T x R	44	299.469	14.4172	0.0000
Error	177	20.772		
2. Postemergence damping-off				
Replication	3	35.215	1.2428	0.2957
Trichoderma isolate (T)	4	5685.239	200.6394	0.0000
Rhizoctonia isolate (R)	11	518.677	18.3048	0.0000
T x R	44	828.632	29.2435	0.0000
Error	177	28.336		
3. Survival				
Replication	3	28.375	0.5482	0.0000
Trichoderma isolate (T)	4	14357.774	277.3975	0.0000
Rhizoctonia isolate (R)	11	718.428	13.8803	0.0000
T x R	44	418.498	8.0855	0.0000
Error	177	51.759		
4. Plant height				
Replication	3	50.456	2.5281	0.0414
Trichoderma isolate (T)	4	1985.558	99.4869	0.0000
Rhizoctonia isolate (R)	11	90.183	4.5186	0.0000
T x R	44	100.63	5.0421	0.0000
Error	177	19.958		
5. Dry weight				
Replication	3	2631.355	1.5256	0.1954
Trichoderma isolate (T)	4	169178.272	98.0862	0.0000
Rhizoctonia isolate (R)	11	4570.883	2.6501	0.0032
T x R	44	7279.701	4.2206	0.0000
Error	177	1724.792		

These comparisons showed that the differences in preemergence damping-off (Table 5) between *Trichoderma* isolates and the control were not the same for each *R. solani* isolate that is, *R. solani* isolates responded differently to the application of *Trichoderma* isolates. For example, all *Trichoderma* isolates caused highly significant reduction in preemergence damping-off caused by *R. solani* isolate no. 1, while *Trichoderma* T1 and T2 were the only isolates, which significantly reduced preemergence damping-off caused by *R. solani* isolate no. 5.

Table 4. Relative contribution of *Trichoderma* isolate, *Rhizoctonia solani* isolate,and their interaction to variation in cotton seedling disease variables (cultivar Giza 86) under greenhouse conditions

Source of variation	Relative contribution [a] to variation in				
	Preemergence damping-off	Postemergence damping-off	Survival	Plant height	Dry weight
Trichoderma isolate (T)	34.03	34.98	68.51	63.97	58.56
Rhizoctonia solani isolat (R)	35.25	8.78	9.43	4.75	7.31
T X R	30.71	56.08	21.97	30.28	32.64

[a] Relative contribution was calculated as percentage of sum of squares of the explained (model) variation

Table 5. Effect of *Trichoderma* isolate, *Rhizoctonia solani* isolate, and their interaction on preemergence damping-off of cotton seedlings (cultivar Giza 86) under greenhouse conditions

R. solani isolate	Isolate of *Trichoderma* spp.											
	T. harzianum (T1)		*T. viride* (T2)		*T. viride* (T3)		*T. harzianum* (T4)		Control		Mean	
	(%)	Transformed	(%)	Transformed	(%)	Transformed	(%)	Transformed	(%)	Transformed	(%)	Transformed
1	7.50[a]	(13.83)[b]	17.50	(24.43)	17.50	(24.53)	12.50	(20.47)	42.50	(40.67)	19.50	(24.79)
2	25.00	(29.89)	12.50	(20.47)	25.00	(29.89)	25.00	(29.89)	47.50	(43.56)	27.00	(30.74)
3	15.00	(22.50)	20.00	(26.56)	22.50	(27.86)	32.50	(34.56)	57.50	(49.33)	29.50	(32.16)
4	32.50	(34.72)	25.00	(29.89)	37.50	(37.73)	45.00	(42.12)	55.00	(47.89)	39.00	(38.47)
5	42.50	(40.67)	32.50	(34.72)	50.00	(45.00)	55.00	(47.89)	55.00	(47.89)	47.00	(43.23)
6	12.50	(20.47)	20.00	(26.56)	30.00	(33.21)	12.50	(20.47)	42.50	(40.67)	23.50	(28.28)
7	15.00	(22.50)	25.00	(29.89)	7.50	(13.83)	5.00	(9.22)	45.00	(42.12)	19.50	(23.51)
8	2.50	(4.61)	15.00	(22.50)	2.50	(4.61)	35.00	(36.22)	52.50	(46.44)	21.50	(22.88)
9	0.00	(00.00)	5.00	(9.22)	17.50	(24.52)	2.50	(4.61)	22.50	(28.22)	9.50	(13.31)
10	12.50	(20.47)	0.00	(00.00)	20.00	(26.56)	10.00	(18.44)	32.50	(34.72)	15.00	(20.04)
11	0.00	(00.00)	32.50	(34.72)	22.50	(28.22)	10.00	(18.44)	47.50	(43.56)	22.50	(24.99)
12	10.00	(18.44)	22.50	(28.22)	10.00	(18.44)	0.00	(00.00)	35.00	(36.22)	15.50	(20.26)
Mean	14.58	(19.01)	18.96	(23.93)	21.87	(26.20)	20.42	(23.53)	44.58	(41.77)		

[a] Mean of four replicates.

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

LSD (tranformed data) for isolate of *Trichoderma* spp. x isolates of *Rhizoctonia solani* interaction = 6.36 (P<0.05) or 8.39 (P<0.01)

It was also found that the magnitude of the differences between *Trichoderma* isolates differed from one *R. solani* isolate to another. For example, the difference between T3 and T4 was nonsignificant against *R. solani* isolate no. 2, while it was highly significant against isolate no. 8. The difference between T1 and T2 against *R. solani* no.1 was highly significant, while the difference between the same bioagent isolates against isolate no. 3 was nonsignificant (Table 5).

Table 6 showed that isolate T4 caused highly significant reduction in postemergence damping-off caused by pathogen isolate no. 3, whereas postemergence damping-off was 0.0, while T2 and T3 failed to suppress this isolate in the postemergence stage. It is worth noting that some *Trichoderma* isolates stimulate pathogenicity of some *R. solani* isolates like T4 which significantly increased postemergence damping-off caused by isolate no. 7, while the other *Trichoderma* isolates completely suppressed the effect of isolate no. 7 on postemergence damping-off (Table 6). This result is in agreement with other workers (Harman, 2000; Habeb, 2007; and Aly *et al.*, 2007). Aly *et al.* (2007) demonstrated that *Trichoderma* spp. was stimulatory for pathogenicity of some *Macrophomina phaseolina* isolates.

It is noticeable that all isolates of *Trichoderma* caused significant or highly significant increases in percentage of survival (Table 7). All the antiagonistic isolates failed to improve plant height or dry weight of surviving seedlings, in the case of the pathogen isolates nos. 1 and 6 (Table 8).

The very highly significant interaction (p=0.0000) between *Trichoderma* isolates and *R. solani* isolates for all tested parameters implies that a single isolate of antagonist can be highly effective against an isolate of *R. solani* but may have only minimal effects on other isolate of the same fungus. Bell *et al.* (1982) reported similar results when they studied the *in vitro* antagonism of *Trichoderma* spp. against six fungal plant pathogens. The interaction also indicated that apparently many genes from both organisms interact to regulate the amount of antagonism between *R. solani* and *Trichoderma* isolates (Wells and Bell, 1983). Therefore, isolates of antagonists should be tested against as many isolates of *R. solani* as possible, as this will improve the chance of identifying antagonist isolates effective against several isolates of *R. solani* (Aly *et al.*, 2007). The interaction also suggests that it may be more prudent to evaluate blends of antagonist isolates for wider application against more isolates of *R. solani* (Asran *et al.*, 2005). In this investigation, the interaction between *R. solani* isolates and *Trichoderma* isolates was evaluated under greenhouse conditions in a soil and at temperature favourable for the growth of both *R. solani* and *Trichoderma*. Under field conditions, soil nutrients and temperature during the different periods of cotton-growing season may be more favourable for *R. solani* isolates or the antagonist isolates. Thus, the results of this work are not expected to be necessarily related to degree of biological control that may be observed in the field, but should reflect the capacities and genetic variability of the antagonist isolates and the various *R. solani* isolates to resist antagonism (Bell *et al.*, 1982). Soil conditions may strongly affect pathogenicity of *R. solani* (Aly and Kandil, 1999). If these sorts of soil effects commonly occur, conceivably such effects could also include the antagonist isolates.

Table 6. Effect of *Trichoderma* isolate, *Rhizoctonia solani* isolate, and their interaction on postemergence damping-off of cotton seedlings (cultivar Giza 86) under greenhouse conditions

R. solani isolate	Isolate of *Trichoderma* spp.											
	T. harzianum (T1)		*T. viride* (T2)		*T. viride* (T3)		*T. harzianum* (T4)		Control		Mean	
	(%)	Transformed	(%)	Transformed	(%)	Transformed	(%)	Transformed	(%)	Transformed	(%)	Transformed
1	12.50[a]	(20.47)[b]	20.00	(26.91)	37.50	(37.72)	22.50	(27.85)	32.50	(34.71)	25.00	(29.39)
2	32.50	(34.56)	10.00	(18.43)	0.00	(00.00)	17.50	(24.53)	45.00	(42.05)	21.00	(23.91)
3	12.50	(20.47)	37.50	(37.72)	45.00	(42.11)	0.00	(00.00)	40.00	(39.17)	27.00	(27.89)
4	40.00	(39.17)	12.50	(20.47)	0.00	(00.00)	27.50	(31.55)	37.50	(37.72)	23.50	(25.78)
5	0.00	(00.00)	17.50	(24.53)	0.00	(00.00)	17.50	(24.53)	35.00	(36.06)	14.00	(17.02)
6	7.50	(11.25)	17.50	(24.16)	15.00	(22.50)	0.00	(00.00)	45.00	(42.11)	17.00	(20.00)
7	0.00	(00.00)	0.00	(00.00)	0.00	(00.00)	40.00	(39.17)	27.50	(31.39)	13.50	(14.11)
8	37.50	(37.72)	40.00	(39.23)	0.00	(00.00)	20.00	(26.56)	47.50	(43.56)	29.00	(29.41)
9	0.00	(00.00)	30.00	(33.05)	5.00	(9.22)	20.00	(26.56)	75.00	(60.27)	26.00	(25.82)
10	12.50	(20.47)	7.50	(11.25)	37.50	(37.72)	10.00	(15.86)	60.00	(50.83)	25.50	(27.23)
11	10.00	(13.28)	0.00	(00.00)	32.50	(34.56)	2.50	(4.61)	47.50	(43.56)	18.50	(19.20)
12	15.00	(22.50)	42.50	(40.67)	10.00	(18.44)	0.00	(00.00)	65.00	(53.78)	26.50	(27.08)
Mean	15.00	(18.32)	19.58	(22.98)	15.21	(16.86)	14.75	(18.43)	46.46	(42.94)		

[a] Mean of four replicates.

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

LSD (tranformed data) for isolate of *Trichoderma* spp. x isolates of *Rhizoctonia solani* interaction = 7.43 ($P<0.05$) or 9.80 ($P<0.01$)

Table 7. Effect of *Trichoderma* isolate, *Rhizoctonia solani* isolate, and their interaction on survival of cotton seedlings (cultivar Giza 86) under greenhouse conditions

R. solani isolate	Isolate of *Trichoderma* spp.											
	T. harzianum (T1)		*T. viride* (T2)		*T. viride* (T3)		*T. harzianum* (T4)		Control		Mean	
	(%)	Transformed	(%)	Transformed	(%)	Transformed	(%)	Transformed	(%)	Transformed	(%)	Transformed
1	80.00[a]	(63.44)[b]	62.50	(52.49)	45.00	(42.11)	65.00	(53.78)	25.00	(29.88)	55.50	(48.34)
2	42.50	(40.67)	77.50	(61.78)	75.00	(60.11)	57.50	(49.33)	7.50	(11.25)	52.00	(44.63)
3	72.50	(58.45)	42.50	(40.67)	32.50	(27.21)	67.50	(55.44)	2.50	(4.61)	43.50	(37.28)
4	27.50	(31.55)	62.50	(52.27)	62.50	(52.27)	27.50	(31.39)	7.50	(11.25)	37.50	(35.75)
5	57.50	(49.33)	50.00	(45.00)	50.00	(45.00)	27 50	(31.39)	10.00	(15.86)	39.00	(37.32)
6	80.00	(63.81)	62.50	(52.34)	55.00	(47.88)	87.50	(69.39)	12.50	(17.89)	59.50	(50.26)
7	85.00	(67.50)	75.00	(60.11)	92.50	(63.48)	55.00	(47.95)	27.50	(31.39)	67.00	(54.09)
8	60.00	(50.83)	45.00	(42.11)	97.50	(47.31)	45.00	(42.11)	0.00	(00.00)	49.50	(36.47)
9	100.00	(39.23)	65.00	(53.78)	77.50	(61.78)	77.50	(61.78)	2.50	(4.61)	64.50	(44.24)
10	75.00	(60.11)	92.50	(73.36)	42.50	(40.67)	80.00	(63.80)	7.50	(13.83)	59.50	(50.35)
11	90.00	(51.33)	67.50	(55.28)	45.00	(42.05)	87.50	(69.53)	5.00	(9.22)	59.00	(45.48)
12	75.00	(60.11)	35.00	(36.06)	80.00	(63.44)	100.00	(39.23)	0.00	(00.00)	58.00	(39.77)
Mean	70.42	(53.03)	61.46	(52.10)	62.92	(49.44)	64.79	(51.26)	8.96	(12.48)		

[a] Mean of four replicates.

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

LSD (tranformed data) for isolate of *Trichoderma* spp. x isolates of *Rhizoctonia solani* interaction = 10.04 ($P<0.05$) or 13.25 ($P<0.01$)

Table 8. Effect of *Trichoderma* isolate, *Rhizoctonia solani* isolate, and their interaction on plant height and dry weight of cotton seedlings (cultivar Giza 86) under greenhouse conditions

R. solani isolate	Plant height (cm)						Dry weight (mg/plant)					
	Isolate of *Trichoderma* spp.						Isolate of *Trichoderma* spp.					
	T. harzianum (T1)	*T. viride* (T2)	*T. viride* (T3)	*T. harzianum* (T4)	Control	Mean	*T. harzianum* (T1)	*T. viride* (T2)	*T. viride* (T3)	*T. harzianum* (T4)	Control	Mean
1	26.73	26.72	24.89	25.06	23.02	25.28	211.20	229.20	202.40	215.00	182.60	208.08
2	25.78	27.70	27.98	27.97	15.43	24.97	214.00	233.80	234.80	218.40	184.00	217.00
3	27.44	27.57	27.69	26.84	21.14	26.14	236.00	238.00	236.40	242.40	163.60	223.28
4	26.35	28.05	28.00	27.66	11.42	24.30	232.20	248.60	241.20	237.80	99.00	211.76
5	26.05	26.19	26.78	28.10	17.22	24.87	237.00	233.60	214.20	211.00	139.60	207.08
6	24.81	25.89	26.87	28.13	24.62	26.06	236.60	232.40	232.40	202.60	196.00	220.00
7	26.96	27.56	27.80	26.63	25.45	26.88	249.60	236.40	257.60	240.00	204.20	237.56
8	27.70	27.86	28.22	26.82	0.00	22.12	246.40	243.20	263.80	242.60	0.00	199.20
9	28.30	26.20	27.42	27.94	15.45	25.06	242.80	239.00	232.60	234.00	198.60	229.40
10	27.64	28.24	27.41	27.98	21.20	26.49	252.20	243.00	235.60	234.60	170.80	227.24
11	27.00	25.80	27.12	25.98	10.76	23.33	246.20	241.40	240.80	233.60	101.60	212.72
12	26.50	25.90	26.90	27.00	0.00	21.26	239.60	248.20	242.00	254.80	0.00	196.92
Mean	26.77	26.97	27.26	27.18	14.19		237.00	238.90	236.10	230.60	117.10	

LSD for isolate of *Trichoderma* spp. x isolate of *Rhizoctonia solani* interaction

(P < 0.05) = 5.57 **(P < 0.05) = 51.75**

(P < 0.01) = 7.34 **(P < 0.01) = 68.21**

Table 9. Correlation [a] among variables used for evaluating pathogenicity of *Rhizoctonia solani* isolates under the effect of *Trichoderma* isolates on cotton seedlings (cultivar Giza 86) under greenhouse conditions

Trichoderma isolate	Disease variable	Disease variable			
		2	3	4	5
T. harzianum (T1)	1. Preemergence damping-off %	0.129	-0.726**	-0.491	-0.358
	2. Postemergence damping-off %		-0.776**	-0.127	-0.317
	3. Survival %			0.401	0.448
	4. Plant height (cm)				-0.486
	5. Dry weight (mg/plant)				
T. viride (T2)	1. Preemergence damping-off %	-0.181	-0.439	-0.427	-0.012
	2. Postemergence damping-off %		-0.804**	-0.070	0.144
	3. Survival %			0.323	-0.124
	4. Plant height (cm)				0.227
	5. Dry weight (mg/plant)				
T. viride (T3)	1. Preemergence damping-off %	-0.080	-0.549	-0.141	-0.544
	2. Postemergence damping-off %		-0.789**	-0.397	-0.329
	3. Survival %			0.419	0.611*
	4. Plant height (cm)				0.823**
	5. Dry weight (mg/plant)				
T. harzianum (T4)	1. Preemergence damping-off %	0.149	-0.845**	0.120	-0.187
	2. Postemergence damping-off %		-0.655*	-0.036	-0.710**
	3. Survival %			-0.072	0.527
	4. Plant height (cm)				0.213
	5. Dry weight (mg/plant)				
Control	1. Preemergence damping-off %	-0.769**	0.038	0.238	0.244
	2. Postemergence damping-off %		-0.667*	-0.650*	-0.672*
	3. Survival %			0.738**	0.767**
	4. Plant height (cm)				0.994**
	5. Dry weight (mg/plant)				

[a] **Linear correlation coefficient (r) is significant at P< 0.05 (*) or P< 0.01 (**)**

The correlation among variables used for evaluating pathogenicity of *R. solani* isolates under the effect of *Trichoderma* isolates are shown in table 9. It clears that the correlation between preemergence damping-off and postemergence damping-off was negative and highly significant (r = -0.77, P<0.01) in the control, but this correlation became nonsignificant under the effect of all antagonist isolates. On the other hand, correlation between preemergence damping-off and survival was nonsignificant in the control, while it was negative and highly significant (r = -0.73, P<0.01) and (r = 0.85, P< 0.01) respectively under the effect of application of the two isolates of *T. harzianum* T1 and T4. The correlation between postemergence damping-off and plant height was negative and significant (r = -0.65, P<0.05) in control, while it changed to nonsignificant correlation under effect of application all isolates of the antagonist. While correlation between survival and each of plant height and dry weight were highly significant (r = 0.74, P<0.01) and (r = 0.77, P<0.01) respectively, these correlations became nonsignificant as a result of applications of T1, T2, and T4. The highly significant correlation (r = 0.99, P<0.01) between plant height and dry weight in control disappeared under effect of all *Trichoderma* isolates. Results of correlation indicated that

the application of *Trichoderma* as biocontrol agent changed the relationship between seedlings disease variables.

Cluster analysis of *Trichoderma* isolates based on their antagonistic patterns are shown in table 10 and fig. 1. *Trichoderma* isolates divided into two groups. The first group included both *T. harzianum* isolates (T1 and T4) and *T. viride* isolate (T2), while the second group included only one isolate (T3) which belonging to *T. viride*. The application of cluster analysis has been suggested previously for assessing similarity and/or dissimilarity in gene-for-gene host-parasite relationships (Lebeda and Jendrulek, 1987 and Priestley *et al.*, 1984). It seems that grouping isolates of *Trichoderma* spp. based on their antagonistic patterns was not related to their geographical origin. Although isolates of *T. harzianum* were different in their geographic origin, they were in the same group. On the other hand, isolates of *T. viride* divided into two groups although the two isolates were from east Delta region. This result is in agreement with results of Asran *et al.* (2005). It suggests that the variation in antagonistic patterns of *T. harzianum* is limited. Antagonistic pattern of *T. viride* T2 was related to antagonistic patterns of isolates of *T. harzianum* than *T. viride T3*. This result suggests that *T. viride* is more variable in its antagonistic pattern than *T. harzianum*. However, the confirmation of this conclusion requires the use of larger sample of isolates from both species. Grouping isolates of *Trichoderma* spp. was also not related to morphological taxonomy. This result is not in agreement with results of Omar *et al.* (2007) who studied biological control of *Pythium ultimum* by using *Trichoderma* spp. Thus, the different results could be due to the effect of the different pathogens.

Table 10. Similarity matrix of *Trichoderma* isolates based on their antagonistic patterns against twelve isolates of *Rhizoctonia solani*

Trichoderma isolate	*Trichoderma* isolate			
	T1	T2	T3	T4
***T. harzianum* (T1)**				
***T. viride* (T2)**	0.789			
***T. viride* (T3)**	0.397	0.532		
***T. harzianum* (T4)**	1.000	0.431	0.000	

The results of cluster analysis of *R. solani* isolates based on their response to *Trichoderma* isolates are shown in table 11 and fig. 2. The isolates divided into three main cluster groups. The first group (Distance = 10) included all isolates of *R. solani* AG4 in addition to isolate no.12 which belonging to AG2-2 from Gharbiya. The second group (Distance = 20) included only isolate no. 7 which belonged to AG2-2 from Dakahliya. The third group included the remaining isolates of AG2-2 (isolates nos. 2, 4, 8, and 5). It clears that isolates of AG4 were more homogeneous in their response patterns to *Trichoderma* application than isolates of AG2-2. All isolates of AG4 were in one cluster group, although their geographic origin was different; while isolates of AG2-2 divided into three groups.

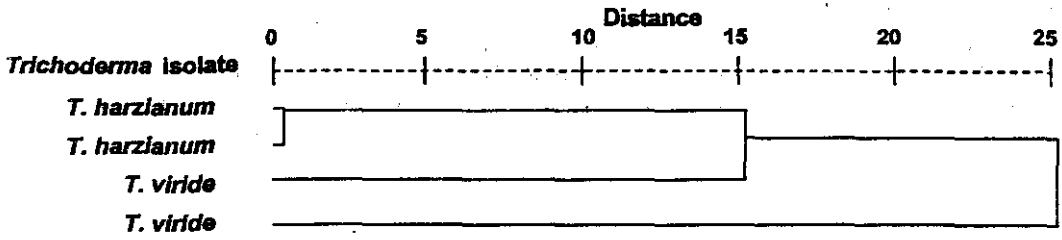

Figure 1. Phenogram of *Trichoderma* spp. isolates based on their antagonistic patterns against twelve isolates of *Rhizoctonia solani*

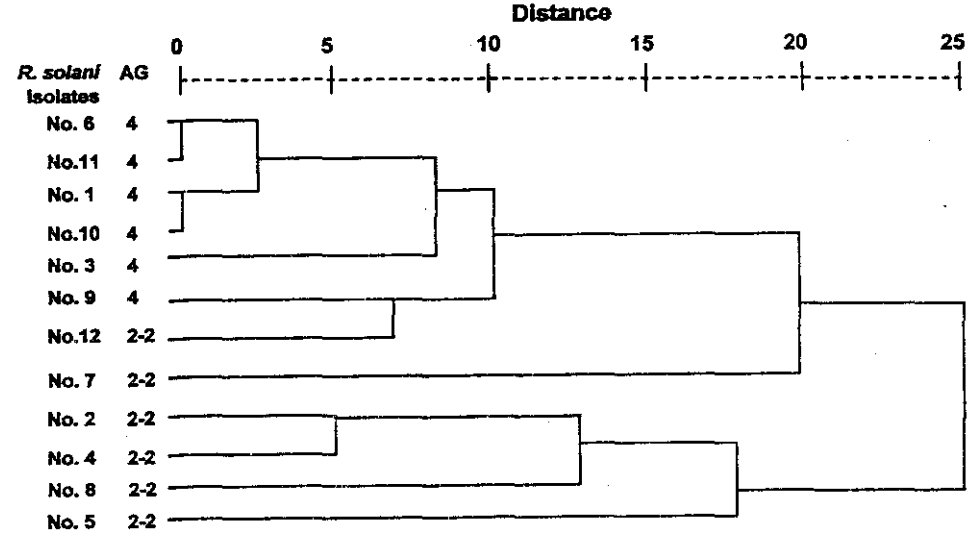

Figure 2. Phenogram of *Rhizoctonia solani* isolates based on their response patterns to four isolates of *Trichoderma* spp.

Table 11. Similarity matrix among *Rhizoctonia solani* isolates based on their response patterns to four isolates of *Trichoderma* spp.

R. solani isolates	*Rhizoctonia solani* isolates										
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
No. 1											
No. 2	0.576										
No. 3	0.868	0.416									
No. 4	0.248	0.896	0.298								
No. 5	0.304	0.514	0.418	0.783							
No. 6	0.937	0.692	0.873	0.275	0.370						
No. 7	0.572	0.676	0.124	0.248	0.274	0.507					
No. 8	0.422	0.779	0.465	0.645	0.337	0.474	0.600				
No. 9	0.888	0.585	0.572	0.030	0.167	0.920	0.815	0.610			
No. 10	0.984	0.657	0.727	0.200	0.192	0.897	0.530	0.242	0.770		
No. 11	0.986	0.508	0.811	0.070	0.208	1.000	0.569	0.276	0.814	0.959	
No. 12	0.714	0.523	0.704	0.043	0.000	0.919	0.399	0.647	0.864	0.506	0.702

It seems that grouping of AG2-2 isolates was somewhat related to their geographic origin; where all middle and upper Egypt isolates (Isolates nos. 2, 4, 8, and 5) were found in one group, while isolates of Delta divided into two groups: one included the east Delta isolate (no. 7) and the other one included the middle Delta isolate (no. 12). These results may indicate that *R. solani* is found as geographically isolated populations.

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تخصص عزلات فطر التريكوديرما فى التضاد تجاه عـزلات فطـر الريزوكتونيـا سولانى المعزوله من جذور القطن

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تم عزل أربعة عزلات من فطر التريكوديرما اثنتان منها تنتمى الى النوع هرزيانم واثنتان منها تنتمى الى النوع فيريدى من جذور بادرات القطن. وتم تقييم لقدرتها التضاديه تجاه اثنى عشر عزلة ريزوكتونيا سولانى من بادرات القطن تحت ظروف الصوبه. وكانت ستة عزلات من الريزوكتونيا سولانى تنتمى الى المجموعه الارتباطيه رقم ٤ وستة عزلات تنتمى الى المجموعه الارتباطيه رقم ٢-٢. أظهرت نتائج تحليل التباين أن عزلات التريكوديرما وعزلات الريزوكتونيا والتفاعل بينهما كانت جميعها مصادر عاليه المعنويه للتباين فى كل من النسب المئويه للبادرات الميته قبل ظهورها فوق سطح التربه والنسبه المئويه للبادرات الميته بعد ظهورها فوق سطح التربه والنسبه المئويه للبادرات السليمه الباقيه على قيد الحياه وطول البادرات والوزن الجاف للبادرات. يدل هذا التفاعل على أن العزله الواحده من التريكوديرما يمكن أن تكون عالية التأثير ضد عزله معينه من الريزوكتونيا سولانى فى حين قد تكون ذات تأثير ضعيف ضد عزله أخرى من نفس المسبب المرضى. عند دراسة الارتباط مابين المتغيرات المستعمله فى تقييم القدره المرضيه لعزلات الريزوكتونيا سولانى على بادرات القطن تحت تأثير عزلات التريكوديرما المختلفه، دلت النتائج على ان اضافة التريكوديرما أدى الى تغيير العلاقه مابين هذه المتغيرات. أظهر التحليل العنقودى لتقسيم التريكوديرما على أساس النمط التضادى أن العزلات انقسمت الى مجموعتين الأولى ضمت عزلتى النوع هرزيانم بالاضافه الى عزله من فيريدى وضمت المجموعه الثانيه العزله الأخرى من النوع فيريدى. ويبدو أن تقسيم التريكوديرما على أساس النمط التضادى لم يكن مرتبطا بالموقع الجغرافى للعزلات، كما انه لم يكن مرتبطا بالتقسيم المورفولوجى لها. عند عمل تحليل عنقودى لتقسيم عزلات الريزوكتونيا سولانى على أساس استجابتها لعزلات التريكوديرما وجد أن العزلات انقسمت الى ثلاثة مجاميع: المجموعه الأولى ضمت جميع عزلات المجموعه الارتباطيه رقم ٤ بالاضافه لعزله واحده من المجموعه الارتباطيه رقم ٢-٢ المعزوله من الدقهليه، وضمت المجموعه الثانيه عزله واحده تابعه للمجموعه الارتباطيه رقم ٢- ٢المعزوله من الدقهليه، أما المجموعه الثالثه فضمت باقى عزلات المجموعه الارتباطيه رقم ٢-٢. ويبدو أن درجة التجانس بين عزلات المجموعه الارتباطيه رقم ٤ من حيث استجابتها للتريكوديرما أكبر من درجة التجانس بين عزلات المجموعه الارتباطيه رقم ٢-٢. ويبدو أن عدم التجانس فى عزلات المجموعه الارتباطيه رقم ٢-٢ مرتبطا الى حد ما بالموقع الجغرافى للعزلات.