Egypt. J. Phytopathol., Vol. 35, No. 2, pp. 111-124 (2007)

Suppression of *Pythium ultimum* Involved in Cotton Seedling Damping-off by *Trichoderma* spp.

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Six isolates of *Trichoderma* spp., 4 isolates belonging to *T. harzianum* and 2 isolates belonging to *T. viride* were isolated from cotton seedling, infected with damping-off or from rotted roots of adult plants. The isolates were evaluated for biocontrol capacity against 15 isolates of *Pythium ultimum* causing seedling damping-off of cotton plants under greenhouse conditions. Analysis of variance showed very highly significant effects of *Trichoderma* spp. isolates, *P. ultimum* isolates and their interaction on post-emergence damping-off, survival, plant height and dry weight. Responses of *P. ultimum* isolates to antagonistic effects of *Trichoderma* spp. isolates were different. Some isolates of *P. ultimum* exhibited response to all *Trichoderma* spp. isolates which significantly increased the percentage of survived seedlings, plant height and dry weight. Cluster analysis of *Trichoderma* spp. isolates based on antagonistic patterns showed that isolates was identified to two groups. The first group includes isolates of *T. harzianum*, while the second group includes isolates of *T. viride*. The results of cluster analysis of *P. ultimum* isolates, based on their response patterns to *Trichoderma* spp. isolates, classified the isolates into 4 distinct groups. The first group included the isolates collected from East Delta. The second group included the isolates collected from middle delta, except isolate No. 14 which was collected from south Delta. The third group included the isolates collected from west delta, except isolate No. 9 which was collected from south Delta. Isolate No. 13 which was collected from Giza was the only isolate in the fourth group.

Keywords: Biological control, cotton, damping-off, *Gossypium barbadense* L., *Pythium ultimum* and *Trichoderma* spp.

Pythium ultimum (Trow) is widespread in soil and has a wide host range (Martin and Loper, 1999). This pathogen is involved in seedlings disease complex of cotton and can attack seeds and /or seedlings under favourable conditions of low temperature and high soil moisture at planting (Hillocks, 1992).This disease occurs as seed decay before germination and as pre-emergence damping-off (Davis,1982 and Hillocks, 1992). Many studies demonstrated that some of the isolates of *Trichoderma* spp. showed biocontrol potentiality against several micro-organisms involved in cotton damping-off, root-rot and wilt disease (Mathivanan *et al.*, 1997 and 2000; Silva-Hanlin and Menezes, 1997; Aly *et al.*, 2000; Hanson, 2000; Haq and Khan, 2000; Xueling *et al.*, 2003; Asran *et al.*, 2005; Howell and Pukhaber, 2005 and Aly *et al.*, 2007). *Pythium ultimum* has been effectively controlled through seed and soil treatment with *Trichoderma virens* preparations (Howell, 1982 & 1991 and Nelson, 1994). The objectives of this study were to: (1) isolate and to identify *Pythium* spp. and *Trichoderma* spp. from cotton seedlings. (2) to evaluate the biocontrol capacity of isolated *Trichoderma* spp. against *Pythium* spp. pathogenic to cotton seedlings.

Materials and Methods

Isolation, purification and identification of Pythium ultimum and Trichoderma spp. from cotton (Gossypium barbadense L.*) roots:*

Isolation was made from samples collected from several localities in cotton producing areas in eight governorates, *i.e.* Beheira, Dakahliya, Damietta, Gharbiya, Giza, Kafr El-Sheikh, Qualubiya and Sharkiya (Table 1&2). Each sample consisted of 5 to 15 seedlings showing a variety of damping-off symptoms or rotted roots of 5 adult plants. Seedlings or roots of adult plants were removed from soil and washed thoroughly under running tap water to remove any adhering soil. Small pieces (approximately 0.5cm long) of necrotic root tissues were surface sterilized with 10% Clorox solution for 2 minutes, and washed several times with sterilized water. The surface sterilized pieces were then blotted dry between sterilized filter papers and plated on potato dextrose agar (PDA) medium amended with streptomycin sulphate or penicillin G and rose Bengal to eliminate any bacterial contamination. The plates were incubated at 25±2°C for 3-7 days. *Pythium ultimum* isolates were identified to species level according to Moubasher (1993) while, *Trichoderma* spp. isolates were identified to species level according to Rifai (1969). Identification of isolates to species level was kindly verified by Mycological Centre, Assiut University.

Production of Pythium ultimum inoculum used for soil infestation:

Substrate for growth of isolates was prepared in 500-ml glass bottles, each contained 50g of sorghum grains and 40ml tap water. Contents of each bottle were autoclaved for 30 minutes. Inocula were taken from one-week-old PDA cultures and aseptically introduced into the bottles and allowed to colonize sorghum grains for three weeks.

Production of Trichoderma spp. inoculum used for seed treatment (seed coating):

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

Interaction between Trichoderma spp. and P. ultimum isolates under greenhouse conditions:

Autoclaved clay loam soil was placed on a greenhouse bench and infested with inoculum of each *P. ultimum* isolate at the rate of 2.5g/kg soil. After thoroughly mixing, infested soil was dispensed into 15-cm-diameter clay pots. Seeds of Giza 86 were treated with the powdered inoculums of each isolate of *Trichoderma* spp. at the rate of 6g/kg seeds. In the control treatment, seeds were treated with sorghum powder at the same rate. Slightly moistened seeds were treated with inocula of each isolate, and thoroughly shaken in plastic bags before being planted at the rate of

Table 1. Geographic origins of *Trichoderma* spp. used in the study

Isolate No.	Geographic origin	*Trichoderma* spp.
1	Gharbiya	*T. harzianum*
2	Dakahliya	*T. harzianum*
3	Sharkiya	*T. viride*
4	Sharkiya	*T. harzianum*
5	Beheira	*T. harzianum*
6	Beheira	*T. viride*

Table 2. Geographic origins of *Pythium ultimum* isolates used in this study

Isolate No.	Governorate	Location
1	Sharkiya	Diarb Negm
2	Dakahliya	Mansourah
3	Dakahliya	Aga
4	Sharkiya	Kenayat
5	Gharbiya	Zeftta
6	Kafr El-Sheikh	Sidi Salem
7	Kafr El-Sheikh	EL-Ryad
8	Gharbiya	El-Mahalla
9	Qualubiya	Shebin EL-Kanater
10	Beheira	Damanhour
11	Beheira	El-Delengat
12	Beheira	Etay EL-Baroud
13	Giza	Giza
14	Qualubiya	Toukh
15	Damietta	Kafr Saad

10 seeds/pot of *P. ultimum* infested soil. The pots (5 for each treatment) were randomly distributed on a greenhouse bench under a temperature regime ranging from 20± 2C° to 26±2.5°C. Pre-emergence damping-off was recorded 15 days after planting, post-emergence damping-off, survivals, plant height (cm) and dry weight (mg/plant) were recorded 45 days after planting.

Statistical analysis of data:

The experimental design of the present study was a randomized complete block design with a five replicates. 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. Percentage data were transformed into arcsine angles before carrying out the ANOVA to produce approximately constant variance. Cluster analysis was performed with the software package SPSS 6.0.

Results

In vivo evaluation of Trichoderma spp. antagonism against Pythium ultimum isolates:

ANOVA (Table 3) show very highly significant effects of *Trichoderma* spp. isolates and *Pythium ultimum* isolates on all the tested parameters. The interaction between isolates of *Trichoderma* spp. and isolates of *P. ultimum* was a very highly significant source of variation in post-emergence damping-off, survival, plant height and dry weight. *Trichoderma* spp. isolates were the most important factor in determining variation in survival, plant height and dry weight, while *Trichoderma* spp. isolates x *P. ultimum* isolates interaction was the most important factor in determining variation in pre-emergence damping-off and post-emergence damping-off (Table 4). Due to the lack of significance of Trichoderma x Pythium interaction as a source of variation in pre-emergence damping-off, a least significant difference (LSD) was calculated to compare between general means of Trichoderma isolates and control (Table 5). These comparisons showed that all isolates of *T. harzianum* and one isolate of *T. viride* (T3) have significantly reduced pre-emergence damping-off. On the other hand, no significant differences were observed among Trichoderma isolates, which reduced pre-emergence damping-off .The efficacy of Trichoderma isolates in reducing pre-emergence damping-off ranged from 28.8% to 34.4% compared to the control .In post-emergence damping-off stage (Table 6) the interaction between Trichoderma isolates and *P. ultimum* isolates was very highly significant source of variation, hence a LSD was calculated to compare means of Trichoderma isolates within each isolate of *P. ultimum*. These comparisons showed that the differences in post-emergence damping-off between Trichoderma isolates and the control were not the same for each *P. ultimum* isolates. For example, *T. harzianum* (T1) was the only isolate, which significantly reduced post-emergence damping-off caused by *P. ultimum* isolate No. 1. Post-emergence damping-off caused by *P. ultimum* isolate No. 15 was significantly suppressed by all the Trichoderma isolates which showed different levels of efficiency in suppressing this isolate of *P. ultimum*. It is worthy to mention that some *T. harzianum* isolates proved to be stimulatory for post-emergence damping–off caused by some *P. ultimum* isolates like (T4), which significantly increased post-emergence damping-off of *P. ultimum* isolate No. 9. The difference between *Trichoderma* spp. isolates differed from one *P. ultimum* isolates to another. For example the difference between *T. harzianum* (T1) and *T. harzianum* (T4) was highly significant against *P. ultimum* isolate No. 13, while it was insignificant against *P. ultimum* isolate No. 3. The difference between *T. viride* (T3) and *T. viride* (T6) was insignificant against *P. ultimum* isolate No. 2, while it was highly significant against *P. ultimum* isolate No. 8. The interaction between Trichoderma isolates and *P. ultimum* isolates was a highly significant source of variation in the percentage of surviving seedlings (Table 3). The significance of this interaction implies that a single isolate of the antagonist can be highly effective against an isolate of *P. ultimum*, but may have only minimal effects on the other isolates of *P. ultimum*, (Table 7). For example (T2) isolate was ineffective against *P. ultimum* No. 5, while it was highly effective against *P. ultimum* No. 15. There was variation in efficiency of *Trichoderma* spp. isolates in

Table 3. Analysis of variance of the effect of *Trichoderma* spp. isolates, *Pythium ultimum* isolate and their interaction on cotton seedling disease variables (cv. Giza 86) under greenhouse conditions

Parameter and source of variation	D.F.	M.S	F. value	P>F
Pre-emergence damping off				
Replication[a]	4	504.181	1.0616	-
Trichoderma (T)	6	4867.839	10.2496	0.0000
Pythium (P)	14	961.374	2.0242	0.0151
TXP	84	576.940	1.2148	0.1130
Error	416	474.929		
Post-emergence damping off				
Replication	4	138.448	1.0793	-
Trichoderma (T)	6	3440.199	26.8197	0.0000
Pythium (P)	14	1251.750	9.7586	0.0000
TXP	84	267.404	4.4235	0.0000
Error	416	128.271		
Survival				
Replication	4	106.077	1.1336	-
Trichoderma (T)	6	11096.342	118.5787	0.0000
Pythium (P)	14	611.620	6.5359	0.0000
TXP	84	312.465	3.3391	0.0000
Error	416	93.578		
Plant height				
Replication	4	4.253	0.2301	-
Trichoderma (T)	6	1203.581	65.1128	0.0000
Pythium (P)	14	93.095	5.0364	0.0000
TXP	84	73.522	3.9775	0.0000
Error	416	18.485		
Dry weight				
Replication	4	541.079	0.2570	-
Trichoderma (T)	6	147817.937	70.2089	0.0000
Pythium (P)	14	10631.066	5.0494	0.0000
TXP	84	6961.962	3.3067	0.0000
Error	416	2105.401		

[a] Replication is random, while each of *Trichoderma* spp. isolates and *Pythium ultimum* isolates are fixed.

Table 4. Relative contribution of *Trichoderma* spp. isolates, *Pythium ultimum* isolates and their interaction to variation in cotton seedling disease variables (cv. Giza 86) under greenhouse conditions

Source of variation	Relative contribution [a] to variation in				
	Pre-emergence damping-off	Post-emergence damping-off	Survival	Plant height	Dry weight
Trichoderma (T)	13.95	23.90	65.39	49.07	54.66
Pythium (P)	6.43	20.29	8.41	8.86	9.17
TXP	23.15	55.18	25.78	41.96	36.04

[a] Calculated as percentage of sum squares of the explained (model) variation.

Table 5. Effect of *Trichoderma* spp. isolates, *Pythium ultimum* isolates and their interaction on pre-emergence damping-off of cotton seedlings (cv. Giza 86) under greenhouse conditions

Pythium isolates	Isolate of *Trichoderma* spp.													
	T. harzianum (T1)		*T. harzianum* (T2)		*T. viride* (T 3)		*T. harzianum* (T4)		*T. harzianum* (T5)		*T. viride* (T 6)		Control	
	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed
1	10[a]	(16.38)[b]	22	(27.47)	24	(28.51)	36	(36.77)	30	(33.08)	24	(28.80)	50	(45.00)
2	30	(32.66)	16	(20.95)	22	(27.60)	30	(32.54)	34	(35.26	38	(37.80)	54	(47.34)
3	16	(21.25)	0.0	(0.00)	20	(23.49)	18	(24.64)	30	(24.13)	34	(31.44)	56	(40.56)
4	36	(36.65)	26	(30.13)	16	(23.31)	28	(31.28)	30	(32.96)	28	(31.75)	72	(84.38)
5	44	(41.31)	18	(22.58)	26	(30.13)	16	(23.02)	28	(31.16)	48	(43.62)	52	(46.15
6	36	(34.29)	34	(35.44)	34	(34.85)	18	(19.33)	38	(37.80)	34	(35.02)	50	(45.00)
7	46	(42.64)	24	(20.95)	18	(22.58)	10	(12.69)	38	(38.03)	24	(29.22)	54	(47.36)
8	36	(36.82)	26	(30.55)	18	(24.94)	18	(24.94)	34	(35.61)	48	(43.85)	70	(56.92)
9	36	(36.82)	32	(34.16)	26	(30.55)	28	(31.75)	32	(34.24)	50	(45.0)	74	(59.58)
10	34	(35.62)	26	(30.26)	16	(23.31)	48	(43.85)	26	(30.55)	42	(40.33)	58	(49.67)
11	28	(31.88)	28	(31.88)	20	(26.56)	22	(27.89)	22	(27.60)	24	(28.80)	44	(41.54)
12	18	(24.94)	8	(14.75)	32	(34.24)	26	(30.13)	36	(36.82)	40	(39.65)	56	(48.46)
13	28	(31.75)	24	(28.51)	30	(33.08)	28	(31.88)	30	(33.08)	42	(40.33)	58	(49.62)
14	28	(31.75)	36	(36.95)	44	(41.49)	24	(29.22)	28	(31.58)	28	(31.33)	50	(45.00)
15	10	(18.44)	12	(20.06)	20	(26.27)	20	(25.97)	20	(24.94)	22	(27.89)	44	(41.54)
M	29.0	(31.55)	22.1	(25.63)	24.4	(28.73)	24.6	(28.39)	30.4	(32.46)	35.7	(41.63)	56.1	(47.87)

M= Mean

[a] Mean of five replicates.

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

LSD (transformed data) for isolate of:

Trichoderma isolate (T)........ .... = 6.99 ($P \leq 0.05$)

Pythium isolate (P)................=10.24 ($P \leq 0.05$)

Interaction T x P......... ... .= N.S.

Table 6. Effect of Trichoderma isolates, *Pythium ultimum* isolates and their interaction on post-emergence damping-off of cotton seedlings (cv. Giza 86) under greenhouse conditions

Pythium isolates	Isolates of *Trichoderma* spp.													
	T. harzianum (T1)		*T. harzianum* (T2)		*T. viride* (T 3)		*T. harzianum* (T4)		*T. harzianum* (T5)		*T. viride* (T 6)		Control	
	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed
1	16[a]	(18.60[b])	16	(23.02)	24	28.93	18	(24.22)	20	(20.95)	30	(32.96)	40	(39.00)
2	48	(44.02)	52	(46.20)	22	(24.69)	26	(29.53)	26	(28.69)	14	(17.27)	34	(35.44)
3	26	(30.42)	40	(38.78)	20	(25.38)	32	(33.17)	12	(13.16)	28	(31.33)	38	(37.93)
4	32	(34.24)	42	(40.28)	32	(33.64)	4	(7.69)	18	(22.16)	22	(24.82)	26	(30.42)
5	36	(36.60)	64	(53.53)	14	(16.85)	46	(42.69)	10	(11.53)	8	(10.33)	46	(40.33)
6	34	(32.50)	28	(31.75)	26	(29.06)	58	(49.67)	18	(24.35)	2	(3.69)	40	(39.23)
7	20	(24.22)	16	(23.31)	40	(39.01)	32	(49.54)	8	(10.62)	18	(22.58)	30	(32.96)
8	12	(20.06)	16	(20.95)	34	(35.44)	44	(41.54)	26	(29.53)	8	(10.62)	24	(28.93)
9	20	(23.70)	6	(11.06)	18	(22.16)	50	(45.20)	20	(22.64)	12	(15.64)	12	(20.06)
10	22	(27.00)	8	(14.75)	2	(3.69)	2	(3.69)	4	(7.38)	4	(5.31)	36	(36.65)
11	22	(16.85)	8	(7.85)	2	(3.69)	20	(23.48)	20	(23.49)	0.0	(0.00)	48	(34.11)
12	52	(46.20)	16	(18.57)	16	(20.53)	22	(24.22)	8	(10.33)	8	(10.62)	30	(33.08)
13	4	(7.38)	12	(13.28)	10	(14.02)	26	(29.95)	22	(24.64)	0.0	(0.00)	24	(29.22)
14	4	(7.38)	4	(7.38)	6	(9.69)	32	(33.95)	44	(41.48)	6	(10.52)	38	(37.85)
15	22	(27.89)	12	(18.57)	18	(24.64)	10	(18.44)	22	(24.64)	12	(18.00)	56	(48.46)
M	24	(26.47)	22.6	(24.71)	19	(21.97)	28.13	(30.47)	18.57	(21.17)	11.63	(14.25)	34.53	(34.91)

M= Mean

[a] Mean of five replicates.

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

LSD (transformed data) for isolate of Trichoderma x Pythium isolates interaction = 14.08 ($P \leq 0.05$) or 18.54 ($P \leq 0.01$).

Table 7. **Effect of Trichoderma isolates, *Pythium ultimum* isolates and their interaction on survival of cotton seedlings (cv. Giza 86) under greenhouse conditions**

Pythium isolates	Isolates of *Trichoderma* spp.													
	T. harzianum (T1)		*T. harzianum* (T2)		*T. viride* (T 3)		*T. harzianum* (T4)		*T. harzianum* (T5)		*T. viride* (T 6)		Control	
	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed	%	Trans-formed
1	74[a]	(59.87[b])	62	(52.07)	50	(44.95)	46	(42.59)	50	(45.00)	46	(42.69)	10	(16.38)
2	22	(27.60)	32	(34.41)	56	(48.69)	44	(41.54)	40	(39.13)	48	(43.89)	12	(18.00)
3	58	(49.67)	60	(51.22)	60	(52.67)	50	(45.05)	58	(49.72)	38	(37.28)	6	(10.00)
4	32	(33.69)	32	(34.29)	52	(46.15)	68	(55.76)	52	(46.38)	50	(44.95)	8	(14.75)
5	20	(26.27)	18	(22.58)	60	(51.52)	38	(37.98)	62	(52.38)	44	(41.54)	6	(9.00)
6	30	(32.49)	38	(37.80)	40	(41.36)	24	(31.46)	44	(41.36)	64	(53.82)	10	(18.44)
7	34	(35.42)	60	(51.64)	42	(40.33)	58	(49.84)	54	(47.31)	58	(49.67)	16	(23.31)
8	52	(46.20)	58	(49.84)	48	(43.80)	38	(37.80)	40	(39.00)	44	(41.31)	6	(9.00)
9	44	(41.54)	62	(52.02)	56	(48.51)	22	(24.22)	48	(43.85)	38	(37.80)	14	(16.85)
10	44	(40.89)	66	(54.73)	50	(45.00)	52	(46.20)	70	(57.51)	54	(47.31)	6	(6.64)
11	50	(44.82)	64	(53.82)	78	(62.70)	58	(50.49)	58	(49.89)	76	(61.20)	8	(10.62)
12	30	(32.62)	76	(66.56)	52	(46.33)	52	(46.15)	56	(48.51)	52	(46.38)	14	(21.69)
13	68	(58.25)	64	(53.35)	60	(50.87)	46	(42.47)	48	(43.85)	58	(52.85)	18	(24.94)
14	68	(55.59)	60	(50.87)	50	(45.00)	44	(41.49)	28	(31.33)	64	(53.35)	12	(20.06)
15	68	(55.59)	76	(60.78)	62	(52.02)	70	(57.04)	58	(49.72)	66	(54.51)	0.0	(0.00)
M	46.23	(42.67)	55.17	(48.40)	54.4	(47.99)	47.33	(43.34)	51.03	(45.68)	53.3	(47.23)	9.73	(14.65)

M= Mean

[a] Mean of five replicates.

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

LSD (transformed data) for isolate of Trichoderma *x* Pythium isolates interaction = 12.03 ($P \leq 0.05$) or 15.83 ($P \leq 0.01$).

increasing the number of surviving seedlings ranged from 14% of (T1) to 36% to 76% of (T3). The comparisons between Trichoderma isolates and the control within isolates of *P. ultimum* (Table 8) revealed that the efficiency of the tested Trichoderma isolates in increasing the plant height of surviving seedlings was varied from one isolate to another. Thus, (T2) was effective against *P. ultimum* isolate No. 2 and ineffective against No. 13 Trichoderma (T1) was effective against *P. ultimum* isolate No. 13 and ineffective against *P. ultimum* isolate No. 2. *P. ultimum* isolate responded differently to the application of Trichoderma isolates for example *P. ultimum* Nos. 3, 5 & 15 were highly responsive to Trichoderma isolates, while isolates Nos. 1, 7 & 12 had no response to any Trichoderma isolate. The majority of Trichoderma isolates showed significant effects on dry weight of surviving seedlings Table (8). *T. viride* isolates significantly increased dry weight of seedlings regardless of *P. ultimum* isolate, while, *T. harzianum* showed various significant effects. On the other hand, Trichoderma isolates did not show significant effects in improving dry weight of seedlings in the case of *P. ultimum* isolates Nos. 9, 13 and 14.

Table 8. Effect of *Trichoderma* spp. isolates, *Pythium ultimum* isolates and their interaction on plant height and dry weight of cotton seedlings (cv. Giza 86) under greenhouse conditions

Pythium isolates	Plant height (cm)							Dry weight (mg/plant)						
	(T1)	(T2)	(T 3)	(T4)	(T5)	(T 6)	Control	(T1)	(T2)	(T 3)	(T4)	(T5)	(T 6)	Control
1	8.95	24.64	26.07	21.48	28.05	19.14	21.23	201.0	303.8	316.2	295.6	319.6	214.4	137.0
2	20.76	25.14	31.35	23.47	30.70	27.05	16.31	200.0	292.6	295.2	295.4	339.0	252.6	147.6
3	18.76	24.45	23.55	20.82	30.20	28.51	9.84	296.8	313.8	283.8	245.0	320.4	256.2	140.0
4	20.31	20.90	24.14	25.27	32.98	27.05	15.00	323.8	284.0	281.4	288.8	322.0	279.4	239.6
5	21.57	18.51	28.38	24.19	31.23	29.15	12.55	280.0	218.2	297.4	282.2	289.2	284.2	168.2
6	27.48	21.10	27.30	23.97	32.91	30.90	21.38	267.6	269.2	317.4	260.4	321.8	284.6	196.8
7	20.41	20.91	22.30	21.29	21.76	21.70	20.42	313.0	292.6	267.0	260.0	298.4	292.6	225.0
8	25.96	25.97	25.93	25.74	26.73	29.52	12.52	222.8	319.2	252.6	230.6	306.4	292.8	127.2
9	23.87	27.11	29.13	25.91	27.95	26.51	21.91	313.6	305.4	292.2	298.2	295.6	297.6	256.2
10	23.23	25.59	30.36	27.54	26.68	26.05	6.90	255.6	314.0	297.0	281.4	306.6	300.6	68.4
11	26.98	29.42	29.45	29.60	27.47	27.31	11.52	285.4	309.2	294.8	309.8	301.4	308.2	266.8
12	23.41	23.90	29.44	26.20	25.79	22.54	23.45	281.2	305.2	315.6	311.4	304.6	297.8	130.2
13	29.61	26.50	24.44	26.91	24.80	26.62	22.21	308.8	305.6	314.8	290.0	303.6	301.4	269.2
14	32.73	26.33	27.84	25.84	24.86	25.32	22.53	309.8	305.6	299.6	297.2	309.2	294.2	277.8
15	27.70	26.13	28.50	25.85	24.93	26.59	0.00	300.0	297.0	304.0	295.0	301.4	302.0	0.00
M	24.11	24.44	27.18	24.94	27.80	26.27	15.85	277.3	295.7	295.3	282.7	309.3	283.9	176.67

M= Mean

LSD for isolate of *Trichoderma* spp. x isolate of *Pythium ultimum* interaction= 5.35 ($P \leq 0.05$). 57.04 ($P \leq 0.05$). 7.04($P \leq 0.01$). 75.10($P \leq 0.01$).

Two groups of Trichoderma isolates were classified by cluster analysis (Fig.1). The first group included isolates Nos. 1, 2, 4 and 5 which were belonging to *T. harzianum*. The second group included isolate of *T. viride*. The results of cluster analysis of *P. ultimum* isolates based on their response parameters to Trichoderma isolates are shown in (Fig. 2). There were four main clusters .The first one included isolates Nos. 2, 3, 15, 1 and 4 from east delta. The second cluster analysis included the isolates, which were collected from middle delta except isolate No. 14 collected from south delta. The third cluster included isolates Nos. 10, 11, 12 and 9 which were collected from south delta, while isolate No. 13 which was collected from Giza was placed in the fourth cluster.

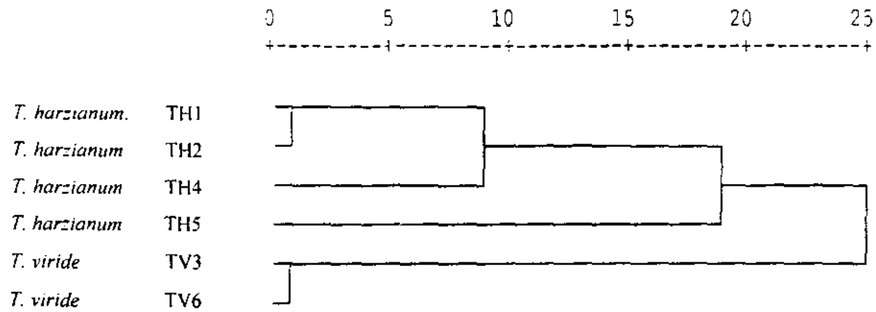

Fig. 1. Phenogram based on average linkage cluster analysis of antagonistic patterns for 6 isolates of *Trichoderma* spp. against 15 isolates of *Pythium ultimum*.

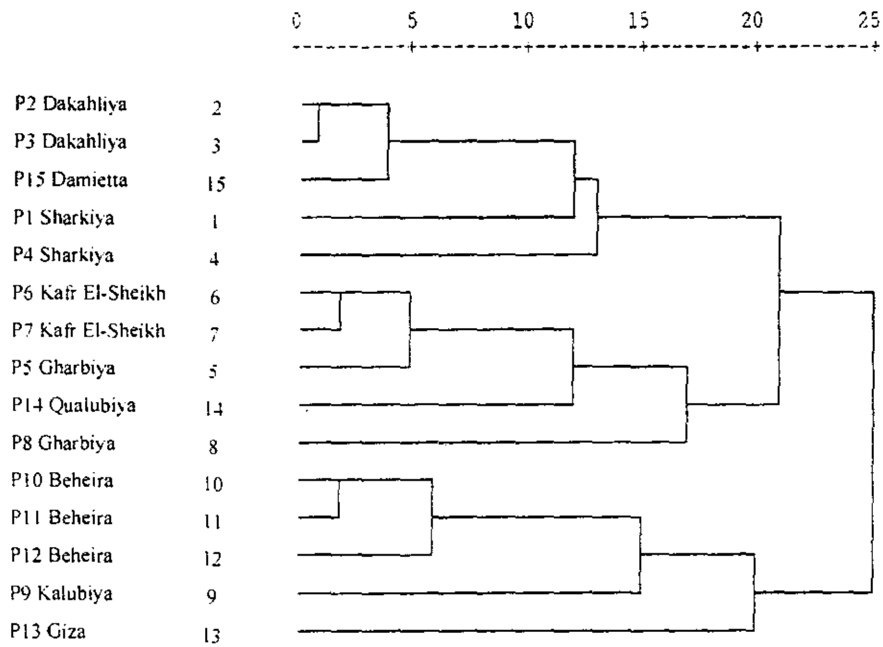

Fig. 2. Phenogram based on average linkage cluster analysis of response patterns of 15 isolates for *Pythium ultimum* to 6 isolates of *Trichoderma* spp.

Discussion

Four isolates of *T. harzianum* and two isolates of *T. viride* were evaluated in *vivo.* to assess their antagonistic potential against *Pythium ultimum* implicated in seedling damping-off of cotton. All *T. harzianum* isolates and *T. viride* (T3) only were significantly reduced pre-emergence damping-off. This result is in agreement with that of Howell (2002) who demonstrated that both strains of G 6-4of *T. virens* and Tk-7of *T. koningii,* gave effective biological control of pre-emergence damping-off of cotton cultivar sure-Grow 77 in soil infested with *P. ultimum*, *P. aphanidermatum*, *Pythium* sp. and *Rhizopus oryzae.* Moreover, the interaction between Trichoderma isolates and *P. ultimum* isolates was very highly significant for the other tested parameters. This interaction implies that a single isolate of antagonist can be highly effective against an isolate of *P. ultimum*, but may have only minimal effects on other isolates of *P. ultimum.* Bell *et al.* (1982) reported similar results when they studied the *in vitro* antagonism of *Trichoderma* spp. against six fungal plant pathogens. The findings of the present study have an important bearing on antagonism testing methods. Isolates of *Trichoderma* spp. should be tested against as many isolates of *P. ultimum* as possible, as this will improve the chance of identifying *Trichoderma* spp. isolates effective against several isolates of *P. ultimum.* The interaction also suggests that it may be more prudent to evaluate blends of Trichoderma isolates for wider application against more isolates of *P. ultimum.* In this investigation, the interaction between *P. ultimum* and the *Trichoderma* spp. isolates was evaluated under greenhouse conditions in a soil and at temperatures favourable for the growth of both *P. ultimum* and *Trichoderma* spp. Under field conditions, soil nutrients and temperatures during the different periods of cotton growing season may be more favourable for *P. ultimum* isolates or for Trichoderma isolates. Thus, the results of present 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 Trichoderma isolates and of the various *P. ultimum* isolates to resist antagonism (Bell *et al.*, 1982). On the other hand, some *T. harzianum* isolates (T4) exhibited deleterious effect as they increased post-emergence damping-off of *P. ultimum,* isolate No. 9. Similar results were reported by other workers. (Harman. 2000 and Habeb, 2007). 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). The method was used to express exactly the genetic similarity among 48 physiological races of *Bremia lactucae* Regel (Lebeda and Jendrulek, 1987) and 20 isolates of *Macrophomina phaseolina* (Tassi) Goide (Omar, 2005). In this study, cluster analysis divided tested isolates of *P. ultimum* into groups based on their response parameters to Trichoderma isolates. Grouping the isolates was somewhat related to their geographic origin. This result may indicate that *P. ultimum* is found as geographically isolated populations. On the other hand, the cluster analysis of *Trichoderma* spp. isolates based on their biocontrol capacity showed that grouping the isolates was related to their morphological taxonomy (Rifai, 1969). Thus, it seems reasonable to conclude that morphological variations among *T. harzianum* and *T. viride*, the basis of the genus taxonomy, may provide sufficient explanation for the variation in their biocontrol capacity against *P. ultimum* isolates.

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(Received 17/10/2007; in revised form 19/12/2007)

تثبيط فطر بيثيم التيمم المسبب لمرض موت بادرات القطن بواسطة أنواع من التريكودرما

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عزلت ست عزلات من فطر التريكودرما تنتمي الـــى النـــوعين هيرزيـــانم وفيريدى من بادرات قطن مصابة بمرض موت البادرات ومن جـــذور نباتـــات بالغة مصابة بمرض عفن الجذور وقيمت قدرة العزلات على المقاومة الحيويـــة لعدد ١٥ عزلة من فطر بيثيم التيمم والمسببة لمرض موت بادرات القطن وذلـــك تحت ظروف الصوبة.أظهرت نتائج تحليل التبـــاين أن عـــزلات التريكودرمـــا وعزلات البيثيم التيمم والتفاعل بينهما كانت جميعا مصادر عالية المعنوية للتباين فى الصفات موضع الدراسة الآتية : النسبة المئوية للبادرات الميتة بعد ظهورهـــا فوق سطح التربة والنسبة المئوية للبادرات السليمة الباقية على قيد الحياة وطـــول البادرات والوزن الجاف للبادرات.

تنوعت استجابة عزلات البيثيم التيمم للتاثير التضادى لعزلات التريكودرمـــا باختلاف عزلات كلا منهما حيث أظهرت بعض عـــزلات البيثـــيم التـــيمم مثـــل العزلات ١٥، ١٠، ٣ أستجابة معنوية لجميع عزلات التريكودرما فيما يخص زيادة نسبة البادرات المتبقية على قيد الحياة وطولها ووزنها الجـــاف.أظهـــر التحليـــل العنقودى لتقسيم عزلات التريكودرما بناءا على قدرتها التضادية انقـــسامها الـــى مجموعتين رئيسيتين أحدهما تضم عزلات تريكودرما هيرزيانم والأخرى شملت عزلتى تريكودرما فيريدى. كذلك أستخدم التحليل العنقودى لتقسيم عزلات البيثيم التيمم الى مجاميع بناء على استجابتها لعزلات التريكودرما فوجـــد أن العـــزلات انقسمت ألى أربعة مجاميع رئيسية: المجموعة الأولى شملت عزلات شرق الدلتا أما المجموعة الثانية فشملت عزلات منطقة وسط الدلتا عدا العزلـــة ١٤ فكانـــت تنتمى ألى منطقة جنوب الدلتا أما المجموعة الثالثة فشملت عزلات منطقة غـــرب الدلتا عدا العزلة رقم ٩ فكانت تنتمى ألى عزلات منطقة جنوب الدلتا أما العزلـــة رقم ١٣ والتى كانت مصدرها الجيزة فانفصلت بمفردها فى المجموعة الرابعة.