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DIFFERENTIAL ANTAGONISM OF *TRICHODERMA* SP. AGAINST *MACROPHOMINA PHASEOLINA*

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Abstract: In view of the ecological hazards of chemicals, pot experiments were conducted to determine the efficacy of *Trichoderma* sp. against *Macrophomina phaseolina*. Greenhouse evolution of the interaction between *M. phaseolina* isolates and *Trichoderma* sp. isolates revealed a very highly significant (p=0.0000). *M. phaseolina* isolate x antagonist isolate interaction for all the following parameters: 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 *M. phaseolina*, but may have only minimal effects on other isolates of *M. phaseolina.* Therefore, isolates of antagonist should be tested against as many isolates of *M. phaseolina* as possible, as this will improve the chance of identifying antagonist isolates effective against several isolates of *M. phaseolina*.

Key words: cotton, *Macrophomina*, *Trichoderma*, seed coating

INTRODUCTION

Charcoal root rot (CRR) is an economically important disease of many crops especially in cotton and soybean. *Macrophomina phaseolina* (Tassi) Goid. a soilborne fungus causes charcoal root rot. The fungus can infect the root and lower stem of over 500 plant species (Wyllie 1989). The pathogen is a widespread distribution in the Egyptian soil, and it is easily and frequently isolated from cotton roots particularly during the late period of the growing season (Omar 1999). Although initial infections of cotton by *M. phaseolina* occur at seedling stage, they usually remain latent until the cotton plant approaches maturity (Dhingra and Sinclair 1978). Aly et al. (2006) found that resistance to *M. phaseolina* was completely lacking in the commercial Egyptian cottons (*Gossypium barabadense* L.). Thus, the use of seed-dressing fungicides for con-

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trolling the disease has become indispensable under Egyptian conditions. While effective fungicides are available (Abdel-Aziz et al. 1996; Aly et al. 2001), 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 resistance races of the pathogen, and the possible carcinogenicity.The continuous use of chemical treatments has resulted in control failure when the pathogens become resistant to the active ingredient (Williams and Gisi 1992). Other problems include gradual elimination and phasing out of some compounds (Zaki et al. 1998).

Biological control has been considered as a serious alternative to seed-dressing fungicides. Regarding *M. phaseolina*, a number of reports demonstrated that some fungi, in particular *Trichoderma* spp. could be effectively used for the suppression of this pathogen (Chowdhury 1998; Khan and Gupta 1998; Raguchander et al. 1997, 1998; Singh and Sindhan 1998; Rajurkar et al. 1998; Adekunle et al. 2001; Aly et al. 2001). Other workers (Kumar and Khare 1990; Parveen and Ghaffar 1991; Mathur 2006) have also proposed T. *harziahum* as a potential biocontrol agent of *M. phaseolina.*

The objective of this study was to evaluate the interaction between 5 isolates of *Trichoderma* sp. and 14 isolates of *M. phaseolina*, pathogenic on cotton, under greenhouse conditions.

MATERIALS AND METHODS

Fungal isolates

Isolates of *Trichoderma* sp. and *M. phaseolina* used in the current study (Tables 1, 2) were obtained from the fungal collection of Cotton Disease Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. *Trichoderma* sp. were originally isolated from cotton roots, while *M. phaseolina* isolates were recovered from cotton and other hosts.

Table 1. Geographic origin of *Trichoderma* spp. used in studying the interaction between isolates of *M. phaseolina* and isolates of *Trichoderma* spp.

Isolate No.	Geographic orgin
1	Sharqiya
2	Daqahliya
3	El-Minya
4	Giza
5	Assiut

Production of *M. phaseolina* inoculum used in soil infestation

Substrate for growth of 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 three weeks.

Table 2. Geographic origin and sources of *M. phaseolina* isolates

Isolate No.	Geographic origin	Source
1	Giza	soybean
2	Giza	sunflower
3	Beheira	cotton
4	Kafr El-Sheikh	cotton
5	Faiyoum	sesame
6	Giza	sesame
7	Beheira	cotton
8	Giza	cotton
9	Daqahliya	cotton
10	Daqahliya	cotton
11	Kafr El-Sheikh	cotton
12	Giza	soybean
13	Gharbiya	cotton
14	Sharqiya	cotton

Production of *Trichoderma* sp. inoculum used in seed treatment

Inoculum of *Trichoderma* sp. isolates was prepared as previously mentioned; however, 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).

In vivo interaction between *Trichoderma* sp. and *M. phaseolina* isolates

Fourteen batches of autoclaved clay loam soil were placed on greenhouse benches and individually infested with inoculum of each *M. phaseolina* isolates at the rate of 40 g/kg soil. The inoculum consisted of mycelia and scleroia growing on sorghum. After thoroughly mixing, infested soil was dispensed into 15-cm-diameter clay pots. Seeds of cultivar Giza 89 were treated with the powdered inoculum of each isolate of *Trichoderma* sp. at the rate of 6 g/kg seeds.

In the control treatment, seeds were treated with sorghum powder at the same rate. Slightly moist 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 *M. phaseolina*-infested soil. The pots (5 for each treatment) were randomly distributed on a greenhouse bench under a temperature regime ranging from 19.5±1.5 to 34±4°C. Preemergence damping-off was recorded 15 days after planting. Preemergence damping-off, survivals, plant height (cm), and dry weight (mg/plant) were recorded 45 days after planting.

Analysis of statistical data

The experimental design of the present study was a randomized complete block design with 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 between means of *Trichoderma* sp. isolates within *M. phaseolina* isolates. Percentage data were transformed into arc sine angles before carrying out the ANOVA to produce approximately constant variance.

RESULTS

ANOVA (Table 3) showed very highly significant (p=0.0000) effects of *Trichoderma* isolate, *M. phaseolina* isolate, and their interaction on all the tested parameters. *Trichoderma* isolate x *M. phaseolina* isolate interaction was the most important factor in determining variation in all the tested parameters.

Table 3. Analysis of variance of *Trichoderma* isolates, *M. phaseolina* isolate and their interaction on cotton seedling disease variables (cultivar Giza 89) under greenhouse conditions

Parameter and source of variation	D.F.	M.S.	F. value	P > F
Preemergence damping-off				
Replication	4	18,011	0,4479	
Trichoderma isolate (T)	5	1102,647	27,4206	0.0000
M. phaseolina isolate (M)	13	929,316	23,1102	0.0000
T x M	65	336,265	8,3622	0.0000
Error	332	40,212		
Postemergence damping-off				
Replication	4	373,663	5,3389	0.0004
Trichoderma isolate (T)	5	2522,831	36,0461	0.0000
M. phaseolina isolate (M)	13	2077,617	29,6849	0.0000
T x M	65	941,618	13,4538	0.0000
Error	332	69,989		
Survival				
Replication	4	371,363	5,7446	0.0002
Trichoderma isolate (T)	5	5770,213	89,2595	0.0000
M. phaseolina isolate (I)	13	1648,693	25,5036	0.0000
T x M	65	680,381	10,5248	0.0000
Error	332	64,645		
Plant height				
Replication	4	79,403	2,3832	0.0513
Trichoderma isolate (T)	5	744,693	22,3514	0.0000
M. phaseolina isolate (M)	13	139,608	4,1902	0.0000
T x M	65	97,324	2,9211	0.0000
Error	332	33,318		
Dry weight				
Replication	4	61562,640	2,2453	0.0639
Trichoderma isolate (T)	5	712015,734	25,9690	0.0000
M. phaseolina isolate (M)	13	82193,485	2,9978	0.0000
T x M	65	100785,166	3,6759	0.0000
Error	332	27417,890		

[a] replication is random, while each of *Trichoderma* isolate and *M. phaseolina* isolate is fixed
D.F. – Degrees of freedom
M.S. – mean square
F. – F. value used to test the hypothesis of equal population means
P-value is the area to the right of the F statistic under an F distribution
with g-1 and N-g degrees of freedom

Trichoderma isolate was the second in importance as a source of variation in survival, plant height, and dry weight, while *M. phaseolina* isolate was the second in importance as a source of variation in preemergence damping-off and postemergence damping-off (Table 4).

Table 4. Relative contribution of *Trichoderma* isolate, *M. phaseolina* isolates and their interaction to variation in cotton seedling disease variables (cultivar Giza 89) 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 isolate (T)	13.96	12.33	30.05	30.57	31.16
M. phaseolina isolate (M)	30.57	26.40	22.33	14.89	9.35
T x M	55.30	59.82	46.07	51.93	57.34

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

Due to the very highly significant effect of *Trichoderma* isolate x *M. phaseolina* isolate interaction on preemergence damping-off, LSD was calculated to compare means of *Trichoderma* isolates within each isolate of *M. phaseolina* (Table 5). These comparisons showed that the differences in preemergence damping-off between *Trichoderma* isolates and the control were not the same for each *M. phaseolina* isolate that is, *M. phaseolina* isolates responded differently to the application of *Trichoderma* isolates. For example, *Trichoderma* 1 was the only isolate, which significantly reduced preemergence damping-off caused by *M. phaseolina* 1. Preemergance damping-off caused by *M. phaseolina* 5 was significantly suppressed by all the *Trichoderma* isolates; however, *Trichoderma* isolates showed different levels of efficiency in suppressing this isolate of *M. phaseolina*. It is worth noting that some *Trichoderma* isolates proved to be stimulatory for pathogenicity of some *M. phaseolina* isolates like *Trichoderma* 3 and *Trichoderma* 4, which significantly increased pathogenicity of *M. phaseolina* 12 and *M. phaseolina* 10, respectively. It was also found that the magnitude of the differences between *Trichoderma* isolates differed from one *M. phaseolina* isolate to another. For example, the difference between *Trichoderma* 1 and *Trichoderma* 2 was highly significant against *M. phaseolina* 1, while it was nonsignificant against *M. phaseolina* 3. The difference between *Trichoderma* 4 and *Trichoderma* 5 was nonsignificant against *M. phaseolina* 13, while it was highly significant against *M. phaseolina* 14. The previously mentioned conclusions regarding preemergence damping-off hold true for postemergence damping-off data shown in Table 6, survival data shown in Table 7, and data of seedling growth parameters (Table 8).

Table 5. Effect of *Trichoderma* isolate, *M. phaseolina* isolates and their interaction on preemergence damping-off of cotton seedlings (cultivar Giza 89) under greenhouse conditions

M. phaseolina isolate	Isolate of *Trichoderma* sp.													
	Trichoderma 1		*Trichoderma* 2		*Trichoderma* 3		*Trichoderma* 4		*Trichoderma* 5		Control		Mean	
	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed
1	14.00[a]	(21.69)[b]	34.00	(35.02)	24.00	(28.80)	16.00	(23.02)	22.00	(27.60)	26.00	(30.55)	22.67	(27.78)
2	16.00	(20.95)	36.00	(36.82)	20.00	(26.27)	18.00	(22.28)	26.00	(30.55)	42.00	(40.33)	26.33	(29.54)
3	28.00	(31.88)	24.00	(29.09)	10.00	(16.38)	14.00	(19.33)	20.00	(26.27)	18.00	(24.35)	19.00	(24.55)
4	36.00	(36.65)	10.00	(18.44)	34.00	(35.49)	26.00	(30.55)	22.00	(27.60)	36.00	(36.65)	27.33	(30.90)
5	34.00	(35.62)	42.00	(40.33)	74.00	(47.31)	30.00	(32.96)	18.00	(24.35)	68.00	(55.59)	44.33	(39.36)
6	34.00	(35.02)	50.00	(45.00)	56.00	(48.46)	20.00	(25.85)	30.00	(32.78)	54.00	(47.31)	40.67	(39.07)
7	38.00	(37.98)	26.00	(30.55)	68.00	(55.59)	34.00	(35.49)	48.00	(43.85)	54.00	(47.31)	44.67	(41.79)
8	26.00	(30.55)	24.00	(29.22)	28.00	(31.28)	60.00	(50.77)	54.00	(47.26)	44.00	(41.54)	39.33	(38.44)
9	32.00	(34.29)	28.00	(31.75)	50.00	(45.00)	38.00	(39.13)	48.00	(43.85)	62.00	(51.97)	43.00	(40.99)
10	22.00	(27.89)	38.00	(38.03)	30.00	(32.66)	56.00	(48.46)	32.00	(34.24)	18.00	(24.64)	32.67	(34.32)
11	10.00	(16.38)	18.00	(24.64)	28.00	(31.33)	28.00	(31.88)	42.00	(40.33)	24.00	(28.80)	25.00	(28.89)
12	12.00	(20.06)	14.00	(21.69)	60.00	(48.51)	36.00	(36.65)	16.00	(23.31)	34.00	(35.27)	28.67	(30.92)
13	20.00	(26.56)	26.00	(30.42)	40.00	(39.13)	14.00	(21.69)	12.00	(20.06)	32.00	(34.29)	24.00	(28.69)
14	10.00	(16.38)	34.00	(38.03)	40.00	(38.95)	60.00	(50.87)	12.00	(20.06)	46.00	(42.64)	33.67	(34.49)
Mean	23.71	(27.99)	28.86	(32.07)	40.14	(37.51)	32.14	(33.50)	28.71	(31.58)	39.86	(38.66)	32.24	(33.55)

[a] mean of five replicates

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

LSD (transformed data) for isolate of *Trichoderma* sp. x isolates of *M. phaseolina* interaction = 7.89 ($p \leq 0.05$) or 10.39 ($p \leq 0.01$)

Table 6. Effect of *Trichoderma* isolate, *M. phaseolina* isolate, and their interaction on postemergence damping-off of cotton seedlings (cultivar Giza 89) under greenhouse conditions

M. phaseolina isolate	Isolate of *Trichoderma* sp.													
	Trichoderma 1		*Trichoderma* 2		*Trichoderma* 3		*Trichoderma* 4		*Trichoderma* 5		Control		Mean	
	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed
1	50.00[a]	(45.05)[b]	10.00	(14.02)	72.00	(58.37)	10.00	(14.31)	4.00	(7.38)	52.00	(46.15)	33.00	(30.88)
2	8.00	(10.62)	8.00	(12.69)	20.00	(25.85)	12.00	(18.00)	22.00	(24.69)	34.00	(35.62)	17.33	(21.25)
3	14.00	(19.62)	50.00	(45.00)	22.00	(27.60)	26.00	(30.00)	8.00	(10.33)	52.00	(46.20)	28.67	(29.86)
4	2.00	(3.69)	40.00	(39.18)	42.00	(40.33)	42.00	(40.00)	12.00	(18.00)	34.00	(35.32)	28.67	(29.47)
5	8.00	(14.75)	40.00	(39.18)	8.00	(12.69)	40.00	(39.18)	54.00	(47.36)	28.00	(31.75)	29.67	(24.90)
6	10.00	(14.31)	2.00	(3.69)	4.00	(7.38)	20.00	(26.27)	44.00	(41.44)	40.00	(39.18)	20.00	(22.04)
7	12.00	(18.00)	12.00	(15.94)	30.00	(33.21)	10.00	(16.38)	16.00	(23.31)	34.00	(35.49)	19.00	(23.72)
8	12.00	(18.00)	10.00	(14.31)	6.00	(9.00)	4.00	(7.38)	16.00	(23.31)	38.00	(37.80)	14.33	(18.30)
9	54.00	(49.67)	52.00	(53.35)	20.00	(26.27)	14.00	(19.33)	22.00	(27.60)	34.00	(35.62)	32.67	(35.31)
10	40.00	(39.18)	34.00	(35.62)	44.00	(41.54)	26.00	(30.55)	4.00	(7.38)	74.00	(60.29)	48.33	(35.76)
11	44.00	(41.49)	50.00	(45.00)	64.00	(47.36)	60.00	(51.05)	8.00	(12.69)	64.00	(53.18)	48.00	(41.79)
12	54.00	(47.36)	50.00	(45.00)	22.00	(27.89)	46.00	(42.64)	14.00	(19.62)	60.00	(50.99)	41.00	(38.92)
13	72.00	(58.25)	30.00	(32.96)	46.00	(42.64)	66.00	(54.51)	56.00	(48.51)	48.00	(43.80)	53.00	(46.78)
14	30.00	(32.49)	10.00	(16.38)	52.00	(46.20)	4.00	(7.38)	58.00	(49.67)	42.00	(40.33)	32.67	(32.07)
Mean	29.29	(29.46)	28.43	(26.92)	32.29	(31.88)	27.14	(28.41)	23.43	(25.81)	45.29	(42.27)	30.98	(30.78)

[a] mean of five replicates

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

LSD (transformed data) for isolate of *Trichoderma* sp. x *M. phaseolina* isolates interaction = 10.41 ($p \leq 0.05$) or 13.71 ($p \leq 0.01$)

Table 7. Effect of *Trichoderma* isolate, *M. phaseolina* isolate and their interaction on survival of cotton seedlings (Cultivar Giza 89) under greenhouse conditions

M. phaseolina isolate	Isolate of *Trichoderma* sp.													
	Trichoderma 1		*Trichoderma* 2		*Trichoderma* 3		*Trichoderma* 4		*Trichoderma* 5		Control		Mean	
	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed	[%]	Transformed
1	36.00[a]	(36.65)[b]	56.00	(49.16)	4.00	(7.38)	74.00	(59.87)	74.00	(59.87)	24.00	(29.22)	44.67	(40.36)
2	76.00	(60.78)	56.00	(48.51)	60.00	(50.87)	70.00	(57.04)	52.00	(46.15)	24.00	(29.22)	56.33	(48.76)
3	58.00	(49.72)	26.00	(30.42)	68.00	(55.84)	56.00	(50.82)	72.00	(58.25)	30.00	(32.96)	50.00	(46.33)
4	62.00	(52.20)	50.00	(45.00)	24.00	(29.22)	32.00	(34.29)	66.00	(54.38)	30.00	(32.96)	44.00	(41.34)
5	58.00	(49.67)	18.00	(24.64)	38.00	(37.98)	30.00	(32.96)	28.00	(28.80)	40.00	(7.38)	35.33	(30.24)
6	56.00	(48.56)	48.00	(43.85)	40.00	(39.18)	60.00	(50.92)	26.00	(30.42)	6.00	(9.00)	39.33	(36.99)
7	50.00	(45.00)	62.00	(52.02)	2.00	(3.69)	56.00	(48.56)	28.00	(36.82)	12.00	(15.64)	35.00	(33.62)
8	62.00	(52.15)	66.00	(54.51)	66.00	(55.03)	36.00	(36.82)	30.00	(32.73)	18.00	(24.64)	46.33	(42.65)
9	10.00	(16.38)	8.00	(10.62)	30.00	(32.91)	46.00	(42.69)	30.00	(32.96)	4.00	(7.38)	21.33	(23.82)
10	38.00	(37.98)	28.00	(31.75)	28.00	(30.42)	18.00	(24.64)	64.00	(53.40)	8.00	(12.69)	30.67	(31.82)
11	46.00	(42.69)	32.00	(34.29)	18.00	(24.64)	12.00	(15.64)	50.00	(45.00)	12.00	(15.64)	30.00	(29.65)
12	34.00	(35.49)	36.00	(36.60)	22.00	(25.11)	18.00	(24.64)	70.00	(57.04)	6.00	(9.00)	31.00	(31.31)
13	8.00	(12.69)	44.00	(41.54)	14.00	(21.69)	20.00	(26.27)	32.00	(34.29)	20.00	(26.27)	23.00	(27.12)
14	60.00	(51.05)	52.00	(46.15)	8.00	(12.69)	36.00	(36.82)	28.00	(31.75)	16.00	(18.60)	33.33	(32.84)
Mean	46.71	(42.21)	41.57	(39.22)	30.14	(30.47)	40.29	(38.71)	46.43	(42.99)	17.86	(19.33)	44.31	(35.49)

[a] mean of five replicates

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

LSD (transformed data) for isolate of *Trichoderma* x *M. phaseolina* isolates interaction = 10.00 ($p \leq 0.05$) or 13.17 ($p \leq 0.01$)

Table 8. Effect of *Trichoderma* isolates, *M. phaseolina* isolates, and their interaction on plant height and dry weight of cotton seedlings (cultivar Giza 89) under greenhouse conditions

M. phaseolina isolate	Plant height [cm]						
	Trichoderma 1	*Trichoderma* 2	*Trichoderma* 3	*Trichoderma* 4	*Trichoderma* 5	control	mean
1	22.86[a]	24.90	9.85	24.88	22.81	20.10	20.90
2	27.27	26.32	22.29	27.24	20.77	18.60	23.75
3	26.17	29.36	23.07	25.86	24.85	20.85	25.03
4	22.16	22.73	25.89	27.88	23.87	22.28	24.13
5	23.82	20.56	21.09	29.57	19.46	10.50	20.84
6	23.47	29.53	20.84	20.52	20.76	10.72	20.97
7	22.51	19.80	5.70	33.19	21.18	13.01	19.23
8	28.95	23.39	20.31	27.11	22.91	21.65	24.05
9	17.61	10.44	21.90	23.43	21.57	9.75	17.45
10	22.98	21.21	25.20	24.00	23.96	14.33	21.95
11	23.95	25.02	26.07	12.55	24.01	14.95	21.09
12	23.91	22.11	20.52	23.81	20.40	9.24	20.00
13	14.10	19.91	22.09	24.13	25.26	21.46	21.16
14	23.64	25.85	12.51	24.80	17.44	12.35	19.43
Mean	23.10	22.94	19.81	24.93	22.09	15.70	

[a] mean of five replicates
LSD for isolate of *Trichoderma* sp. x isolate of *M. phaseolina* interaction = 7.18 ($p \leq 0.05$), 9.46 ($p \leq 0.01$)

M. phaseolina isolate	Dry weight [mg/plant]						
	Trichoderma 1	*Trichoderma* 2	*Trichoderma* 3	*Trichoderma* 4	*Trichoderma* 5	control	mean
1	598.40	665.40	303.60	766.00	632.20	516.20	580.33
2	604.40	510.00	343.20	673.80	542.60	536.40	535.07
3	339.00	641.80	515.80	679.20	618.80	516.00	551.77
4	573.80	551.00	608.80	796.60	682.80	431.20	607.37
5	671.40	651.80	647.40	704.20	531.80	295.80	583.73
6	704.00	394.80	741.60	636.80	662.60	283.20	570.50
7	333.20	257.80	164.00	812.80	709.80	382.80	443.40
8	720.60	432.40	612.20	757.40	640.80	455.60	603.17
9	676.00	298.60	566.00	742.80	751.60	221.00	542.67
10	575.80	482.40	240.60	598.20	587.80	409.20	482.33
11	697.80	825.40	620.80	499.40	556.20	399.20	599.80
12	618.40	765.20	480.40	581.60	849.80	264.40	593.30
13	327.20	724.60	623.80	825.40	399.20	511.40	568.60
14	500.40	616.00	502.00	587.20	349.60	273.20	471.40
Mean	567.17	558.37	497.87	690.10	608.26	392.56	

LSD for isolate of *Trichoderma* sp. x isolate of *M. phaseolina* interaction = 206.00 ($p \leq 0.05$), 271.30 ($p \leq 0.01$)

DISCUSSION

Five isolates of *Trichoderma* sp. were evaluated in vivo, to assess their antagonistic potential against *Macrophomina phaseolina* causing CRR of cotton. Greenhouse evaluation of the interaction between *M. phaseolina* isolates and *Trichoderma* isolates revealed a very highly significant (p = 0.0000) *M. phaseolina* isolate x antagonist isolate interaction for all the tested parameters. This interaction implies that a single isolate of antagonist can be highly effective against an isolate of *M. phaseolina*, but may have only minimal effects on the other isolates of *M. phaseolina.* The interaction also indicates that apparently many genes from both organisms interact to regulate the amount of antagonism between *M. phaseolina* and *Trichoderma* isolates (Wells and Bell 1983). Aly et al. (2001) reported similar interaction when they studied the in vitro antagonism of *Trichoderma* spp., *Penicillium* spp., and *Aspergillus* spp. against *M. phaseolina* isolates. The results of Cardona and Rodriguez (2006) show that there was no any effect of *T. harzianum* on the incidence of the charcoal rot disease in sesame. Isolates of *T. koningii* and *T. harzianum* were selected from soil dilutions and tested in vitro for their antagonistic behaviour against cowpea pathogen *M. phaseolina* before use in the field (Adekunle et al. 2006).These findings have an important bearing on antagonism testing methods. Isolates of antagonists should be tested against as many isolates of *M. phaseolina* as possible, as this will improve the chance of identifying antagonist isolates effective against several isolates of *M. phaseolina*. The interaction also suggests that it may be more prudent to evaluate blends of antagonist isolates for wider application against more isolates of *M. phaseolina*. In this investigation, the interaction between *M. phaseolina* isolates and the antagonist isolate was evaluated under greenhouse conditions in a soil and at temperatures favourable for the growth of both *M. phaseolina* and the antagonist. Under field conditions, soil nutrients and temperatures during the different periods of cotton growing season may be more favourable for *M. phaseolina* isolates or the antagonist isolates. Thus, the results 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 *M. phaseolina* isolates to resist antagonism (Bell et al. 1982).

CONCLUSIONS

Biological control of cotton charcoal root rot using antagonistic fungi (*Trichoderma* sp.) was evaluated. It is worth noting that some *Trichoderma* isolates proved to be stimulatory for pathogenicity of some *M. phaseolina* isolates. This result is in agreement with that of Khan and Gupta (1998) who demonstrated that *T. polysporum* was stimulatory for radial growth of *M. phaseolina* on PDA. In contrary, *T. viride* and *T. harzianum* were the most effective in reducing the mycelial growth and sclerotial formation of *M. phaseolina*. Culture filtrates of *T. viride* inhibited the growth of the pathogen as well as sclerotial germination to a greater extent (Karthikeyan et al. 2006). Differences in the antagonistic performance of the pathogens were observed depending on the isolates with which they interacted. Further investigations are needed on pathogen-antagonist interactions in the complex soil ecosystem to select *Trichoderma* isolates, which could be utilized in field to manage soilborne plant pathogens.

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POLISH SUMMARY

ZRÓŻNICOWANY ANTAGONIZM *TRICHODERMA* SP. WOBEC *MACROPHOMINA PHASEOLINA*

Doświadczenia szklarniowe nad współdziałaniem pomiędzy izolatami *M. phasedina* i izolatami *Trichoderma* sp. wykazały bardzo wysoką istotność (p = 0,0000) współdziałania izolatu *M. phaseolina* z isolatem grzyba antagonistycznego dla wszystkich nastepujących parametrów: zgorzel przedwschodowa, zgorzel powschodowa, przeżywalność, wysokość roślin i sucha masa. To współdziałanie sugeruje, że pojedynczy izolat grzyba antagonistycznego może być wysoce efktywny wobec izolatu *M. phaseolina,* ale może on mieć tylko minimalny efekt w stosunku do innych izolatów *M. phaseolina*. Właściwości izolatów antagonistycznych powinny więc być przebadane przy wykorzystaniu możliwie dużej liczby izolatów *M. phaseolina,* ponieważ to rozszerzy możliwość zidentyfikowania ich efektywności wobec pewnej liczby izolatów tego patogena.