Trichoderma Species in Egypt and Their Biocontrol Potential against Some Plant Pathogenic Fungi

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ABSTRACT

Eighty-one *Trichoderma* isolates were collected from diverse sources and locations in Egypt, to evaluate their biocontrol potential against certain common plant pathogens. The majority of isolates were obtained from Beni-Suif (36.1%), followed by Kafr El-Sheikh (22.2%) and Behera, Mnoufeya, Sharkia; each yielded (13.9%). *Trichoderma* isolates were characterized and tentatively identified on the basis of morphological characters. Twenty-one representative isolates were verified at the species level by using Online Interactive *Trichoderma* Key, where 36.5% of isolates were identified as *T. harzianum, T. hamatum(*7.4%) and *T. aureoviride, T. viride* and *T. strictipile* were (7.7% each). It was obvious that *T. harzianum* was the predominant species that is widely distributed in Egyptian fields compared with the other 5 species. All *Trichoderma* isolates demonstrated varied degrees of antagonistic action over *Botrytis fabae, Fusarium oxsporum, Rhizoctonia solani* and *Pythium ultimum*. Isolates of *T. harzianum* had the most antagonistic ability. On agar media, isolates of *Trichoderma* overlapped the colony of either *R. solani* or *P. ultimum* within 120 hours, coiling around pathogen hyphae, penetrate producing haustoria and finally leads to hyphae lyses. Under greenhouse conditions, six antagonistic *Trichoderma* isolates suppressed chocolate spot disease of faba bean. The most effective strain was *T. hamatum* (T73). All tested *Trichoderma* isolates significantly reduced damping off disease of chick-pea, faba bean and cabbage caused by *F. oxysporum, R. solani* and *P. ultimum*, respectively. However, the degree of disease reduction varied among these isolates. Treatments with *T. hamatum* (T73) provided the best control followed by *T. strictipile* (T100).

Key words: Biodiversity, *Trichoderma*, Biocontrol, Plant pathogenic fungi, Egypt.

INTRODUCTION

Scientific interest in biological control of soil borne plant pathogens is a response, in part, to growing public concerns over chemical pesticides. However, there is an equally great or greater need for biological control of pathogens that presently uncontrolled or only partially controlled (Cook, 1983). Crop loss due to disease-causing fungi can be reduced using environmentally friendly means known as biological control agents. Such control measures are urgently needed to decrease the use of chemical pesticides in farming.

Although microfungi in the *Hypocrea-Trichoderma* group are effective for biological control of crop pathogens, their use in biological control is hindered by lack of knowledge about their classification and characterization (Harman *et al.* 2004). The genus *Trichoderma* and its sexual state *Hypocrea* is a confused complex of species that are difficult to identify. Moreover, the probability of finding new *Trichoderma* species and biotypes for use as biological control agents is high in unusual habitats and unexplored geographic areas. *Trichoderma* the most of fungi that used in biological control of fungus-induced plant diseases are species of that genus (Harman and Kubicek, 1998). Countless studies have been undertaken to apply *Trichoderma* to control mostly fungal plant diseases. Knowledge concerning the behavior of these fungi as antagonists is essential for their

effective use as biocontrol agents since they can act against target organisms in several ways (Jeffries, and Young, 1994). Much of the known biology and many of the uses of these fungi have been documented (Harman and Kubicek, 1998; and Kubicek and Harman, 1998).

Some of the most fungal species used in biocontrol all over the world belong to the genus *Trichoderma*. In particular, isolates of *T. harazianum, T. viridae* and *T. hamatum* are used against diseases in a wide variety of economically important crops. They have been used with success against soil borne diseases, seed borne diseases and foliar and storage diseases (Papavizas, 1985; Chet, 1987; Khare *et al.*, 2010 and Shabir and Lawrence, 2010). Concluded that *Trichoderma* spp. have many advantages as biocontrol agents. Many *Trichoderma* isolates possess three antagonistic mechanisms: competition, antibiosis, and mycoparasitism (Dennis and Webster (1971).

In Egypt, *Trichoderma* spp. have been reported more frequently as effective biocotrol agents (Asran-Amal *et al,.* 2005; El-Katatny *et al,.* 2006; El-Fiki *et al,.* 2006 and Mohamed and Haggag, 2006). Occurrence of *Trichoderma* spp. in Egyptian soils has been the subject of several earlier studies (Moubasher and Abdel-Hafez, 1987; El-Naghy, *et al,.* 1998 and Gherbawy *et al,.* 2004). However, in the abovementioned studies, investigators used morphological analysis for taxon identification and

the species identity of the detected isolates is thus unclear. Many more species are thus remained to be discovered and described.

Therefore, the purpose of the present article was to assess the biocontrol capacity of *Trichoderma* spp., from diverse locations and sources in Egypt, against certain plant pathogens.

MATERIALS AND METHODS

Collection of soil and plant samples

Soil samples were collected from farms at different districts, with different soil types, representing five governorates in Egypt; *i.e*, Behera, Sharkeya, Kafr El-Sheikh, Beni-Sweef and Mnoufeya. Soil samples (approximately 500 g) were collected from rhizosphere of different plant species, by using a weeding hoe to a depth of 5-20 cm. The samples were put in polyethylene bags and transferred to the laboratory. Soil of each site was mixed thoroughly, and composite samples (200g) were stored in paper bags at 4°C, in storage room until the time of isolation of *Trichoderma* spp. In addition, leaf and seed samples of different plant species (faba bean, lentil, chickpea, flax and citrus) were collected, and the leaves were kept in polyethylene bags in ice box and transferred to laboratory, to isolate *Trichoderma* spp. on the following day.

Isolation of *Trichoderma*

Isolation of *Trichoderma* spp. from soil samples was determined following the soil dilution plate technique (Johnson *et al,*. 1959). Three 10g of soil samples each was suspended in 90 ml water, giving a two successive dilutions (1:100 and 1:1000) and aliquots of 0.1 mL of each suspension were evenly spread on 9cm diam. Petri plates containing rose bengal agar medium (Martin, 1950) or Potato Dextrose Agar (PDA) amended with streptomycin sulphate (100 μg mL-1).

Isolation from leaves and seeds was carried out using the washing technique (Dickinson, 1971). About 10 grams of leaves and/ or seeds of faba bean, lentil, chick pea, flax and citrus were agitated in 90 ml sterilized water for about 1 hour on reciprocating shaker. A dilution series was then prepared from the wash water and aliquots (0.1ml) were plated out on PDA medium containing 50 μg/ml streptomycin sulphate. Plates were incubated under near ultra violet light with a 12h photoperiod at approximately 26±2°C. Three plates were used for each suspension. After 4-7 days, putative *Trichoderma* colonies were purified by two rounds of sub-culturing on potato-dextrose agar (PDA) medium.

Hyphal tips from the growing colonies were transferred to plates containing PDA medium and incubated for 4 days at 25°C. Single spore technique was used for purification of the isolates *Trichoderma*.

Source of known *Trichoderma* species

Four taxonomy confirmed species of *Trichoderma*; *i.e. T. harzianum, T. atroviride, T. hamatum* and *T. viride* from culture collection of Biological Resource Center Standard (BRCS), Plant Pathology Research Institute were included in the present study as reference isolates.

Identification of *Trichoderma* species

All obtained *Trichoderma* isolates were characterized and tentatively identified on the basis of morphological characters according to the key of Kubieck and Harman, (1998), which employs the classification into four sections similar to that proposed by Bissett (1991a, b, c). A representative 21 isolates were verified at the species level by using online Interactive *Trichoderma* Key (Samules *et al.,* 2004)

Assay of biological control potential

The antagonistic capacity of the collected *Trichoderma* isolates was evaluated *in vitro* and *in vivo*, against four different fungal plant pathogens; *i.e. Botrytis fabae (*chocolate spot of faba bean), *Rhizoctonia solani, Pythium ultimum* and *Fusarium oxysporum* the causal of seedling damping-off disease on faba bean, cabbage and chickpea, respectively.

Assay of antagonism *in vitro*

Dual culture plates with PDA medium were used to study the antagonistic effect of 81 *Trichoderma* isolates against *R. solani, P. ultimum, F. oxysporum* and *Botrytis fabae,* as described by Dennis and Webster (1971). Each plate was inoculated at one side with a disk (5 mm in diameter) obtained from the periphery of 4 days-old culture of each pathogen. The opposite side of each plate was inoculated with a disc (5 mm in diameter) of each tested *Trichoderma* isolate, obtained from 3 days old culture. Three plates were used for each *Trichoderma* isolate. Plates inoculated only with the pathogenic fungus were prepared to serve as a control. Inoculated plates were incubated at 25°C for 6-10 days. When mycelial growth covered the medium surface in the control plates, plates were then examined and the linear growth of the pathogens was measured. The growing cultures were observed visually and microscopically for evidence of antibiosis and/ or mycoparasitism. Percentage of reduction in mycelial growth of plant fungal pathogens was calculated using the following formula:

$$X = 100- [G_2 / G_1 x 100]$$

Where: X: % of reduction in growth.

G_1: growth of pathogenic fungus in control plates.

G_2: growth of pathogenic fungus in dual plates with *Trichoderma*.

Assay of biocontrol capacity, *in vivo*

Six selected *Trichoderma* isolates (T73 (00325F), T55 (00327F), T102 (00333F), T58 (332F), T2 (330F) and T100 (00328F)) (Abou-Zeid *et al.*, 2009), representing the species identified in the present study were tested for their biocontrol potential against the four fungal pathogens; *i.e. B. fabae , R. solani, P. ultimum,* and *F. oxysporum* in greenhouse experiments.

Inocula of *Trichoderma* antagonists were prepared, as conidial suspension from 10 days–old culture of each *Trichoderma* isolate grown on PDA plates. Number of conidia were counted using haemocytometer slide and adjusted to 10×10^6 conidia /ml. Inoculum of each tested fungal pathogen was produced using different media according to the pathogen and experiment as follows:
- Faba bean leaves extract agar medium for *B. fabae*: to prepare spore suspensions (10^5 spores ml^{-1}).
- Barley-grain medium (Tuite, 1969) for inoculum of *R. solani, P. ulimum* and *F. oxysporum*. The inoculated medium was incubated at 25 ^{0}C for 15 days and the resultant inoculum was used for soil infestation at rate of 5g/kg soil.

A- Biocontrol of chocolate spot of faba bean (*Botrytis fabae*)
1. Assay using the detached leaf technique

The detached leaves technique described by Mansfield and Deverall (1974) was followed. Bifoliate faba bean leaves, cv Giza 40, were cut from 8-week-old plants, surface-sterilized by soaking in 2% Sodium hypochlorite solution for 3 min, rinsed twice in distilled water and dried between sterilized filter papers before being placed abaxial surface uppermost in Petri dishes (15 cm in diameter) containing moist news-paper sheets. The cut ends of the petioles were wrapped with moist tissue paper to prevent desiccation. Leaves were sprayed with the suspension of each *Trichoderma* isolate containing of 10×10^6 conidiospore. After 24 h, each leaflet was inoculated with 20 µL droplets of spores of *B. fabae* (10^5 spores/ml) (Abou-Zeid *et al.*, 1985, Abou-Zeid and Hassanien, 2000 and Abou–Zeid and Zaid, 2006) and incubated at 20 °C, with a 12 h photoperiod provided by fluorescent Phillips Cool white lamps. Disease development was recorded every 12h at which time the Petri dishes were aerated for 5 min. Disease was measured as percentage of browning and lesion spreading according to the key devised by Mansfield and Deverall (1974).

2. Assay using whole plants under greenhouse conditions

Faba bean plants grown in 25 cm diam. clay pots were sprayed twice to run-off with a handy atomizer,

45 and 55 days after sowing, with sore suspension (108cfu/ml) of each tested *Trichoderma* isolates. Control plants were sprayed with water only. Three days after the second spray, plants were sprayed with spore suspension (105conidia/ml) of *Botrytis fabae*. The inoculated plants were kept in the greenhouse at 22±2C under high relative humidity conditions (Abou-Zeid *et al.*, 1981 and Abou-Zeid, 2000). Incidence of chocolate spot disease was recorded after 21 days. Severity of chocolate spot disease was assessed using an arbitrary rating scale described by (Abou-Zeid and Le-Normand, 1979),

B- Biological control of seedling damping-off

Seeds of faba bean, cabbage and chickpea were obtained from the commercial sector in Egypt. Seeds were surface sterilized in 2% sodium hypochlorite solution for 2 min. before sowing. Pot experiments were carried out during 2006 at greenhouse of Faba bean Project, Plant Pathology Research Institute, Giza. Plastic pots (25cm diam.), filled with autoclaved sterilized sandy-loam soil, were infested with (5 gm /Kg soil) of *R. solani or P ultimum or F. oxysporum*. Inoculum of each pathogen was mixed separately with soil. Infested pots were irrigated and kept for 7 days before seed sowing. Five seeds were sown in each pot, 4 replicates were specified for each treatment. Then, the pots were kept in the greenhouse until the end of the experiment (Abou-Zeid *et al.*, 2001).

Disease assessment for incidence of pre-emergence damping-off was recorded after about 2 weeks from planting seeds.

Percentage of pre emergence damping off =

$$\frac{\text{No. of non emerged}}{\text{No. of sown seeds}} \times 100$$

The percentage of post-emergence damping-off or infected plants with root-rot and/or wilt was recorded as dead plants up to 6 weeks after seed sowing as follows:

Percentage of dead plants =

$$\frac{\text{No. of infected plants}}{\text{Total emerged seedlings}} \times 100$$

Statistical Analysis

Results of all experiments were statistically analyzed using One-Way Analysis of Variance (ANOVA) to test for significance, and the Fisher Test was used for mean separations by SPSS computer package system. Means were compared by least significant difference test (LSD) or Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Characterization and identification of *Trichoderma* isolates

Seventy-two isolates of *Trichoderma* were established in pure cultures, from soil and plant

materials collected from various districts in five governorates in Egypt (Table 1). The majority of isolates were obtained from Beni-Suif (36.1%), followed by Kafr El-Sheikh (22.2%) and Behera, Mnoufeya, Sharkeya; each yielded (13.9%). A total of 46 *Trichoderma* isolates were obtained from soil samples, while 13 isolates were obtained from either phyllosphere or seeds. About ٣٨.٩% of *Trichoderma* isolates from soil in this study were obtained from faba been and chick-pea rhizosphere.

All obtained *Trichoderma* isolates were characterized for colony morphology, colony color, conidiophores branching pattern, phialides, conidia morphology and measurements as well as growth rate on PDA medium at 26°C. The majority of isolates showed that fast growth rate did not vary greatly. It ranged from 59 to 90 mm/ 4days at 26°C. Microscopic observation of hyphae, branching pattern, conidiophores, phyalides conidia and the presence of sterile hyphal elongations and revealed that all obtained isolates are *Trichoderma* spp.

Identification data, using Online Interactive Identification Key (Samules *et al,.* 2004), indicated that these isolates belong to six *Trichoderma* spp.; *i.e. T. viride* (4.8%), *T. strictipile* (4.8%), *T. atroviride* (4.8%), *T. aueroviride* (14.2%), *T. hamatum* (23.8%) and *T. harzianum* (47.6).

Biological control potential *of Trichoderma* spp.

Biocontrol potential of the 72 obtained isolates in addition to 6 known *Trichoderma* isolates were assayed against 4 different plant pathogens; *i.e. B. fabae, R. solani, P. ultimum,* and *F. oxysporum in vitro;* while the promising isolates were further assessed *in vivo.*

Assay of antagonistic activity in vitro

Inhibition of radial growth of *B. fabae, R. solani, F. oxysporum and P. ultimum* was assessed only over four days due to the rapid growth rate of the *Trichoderma* isolates. This assay showed variations in the percentage of inhibition of radial growth of these phytopathogens by the different isolates of *Trichoderma* (Table 2 and Figs.1-4). The highest mean inhibition values more than 80% (Table 1). Figure (6) illustrates the variation among the different *Trichoderma* isolates in suppressing the four pathogens.

The *Trichoderma* isolates could be grouped into four categories (Table 2) based on the reduction percentages of pathogen mycilial growth as follows: A) very high antagonists- isolates caused more than 80% reduction in pathogen growth; B) high antagonists- isolates caused ≥60-80% reduction in pathogen growth; C) moderate antagonists-isolates caused more than 40-60% reduction in pathogen growth; D) low antagonists-isolates caused less than 40% reduction in pathogen growth. For *B. faba* (Fig.

6): about 50.6% of *Trichoderma* isolates fall in group (B), while only 8.6% are in Group (A), in Group (C) 34.6 and 6.2% in group (D). For *F. oxysporum* (Fig. 6): about 44.4% of *Trichoderma* isolates fall in group (C), while only 23.5% were in group (D), in group (B) 22.2% and 9.9% in group (A).

For *R. solani* (Fig. 6), about 60.5% of *Trichoderma* isolates fall in group (C), while only 30.9% are in group (B) and 8.6% in group (D). For *P. ultimum* (Fig. 6), about 45.7% of *Trichoderma* isolates fall in group (C), while only 34.5% were in group (D), 17.3% in group (B) and 2.5% in group (A).

It could be concluded that there is a great diversity among the 81 *Trichoderma* isolates in suppression the mycilial growth of the tested pathogens (Table 2). For example, 30.9% of *Trichoderma* isolates showed high antagonism (≥60-80% reduction) against *R. solani*, while none caused the same degree of antagonism when against *Rhizoctonia*. Generally, *Trichoderma* isolates showed less antagonistic degrees against *Pythium* compared to the other three pathogens (Table 2).

Figure (5) illustrates the interaction between an isolate of *Tricoderma harzianum* (T55) and each of *R. solani* and *P. ultimum* on water agar coated glass-slide. It is clear that *Trichoderma* hyphae started overlapping the colony of *R. solani or P. ultimum* within 3-d days of incubation, coiling around the *R. solani* (Fig. 5 A & B) or *Pythium* hyphae (Fig. 5 C & D) and penetrate producing haustoria within the large hyphae (Fig. 5 E, F, G and H). Two days later, lysis of *Rhizoctonia* or *Pythium* hyphae was evident (Fig. 5 J, K, L and M).

Biocontrol of chocolate spot of faba bean

Data in table (٢) indicate that all *Trichoderma* isolates suppressed chocolate spot disease of faba bean using the detached leaves technique. It was observed that inoculum droplets (20μl) containing 2000 *Botrytis fabae* conidia began to develop lesions after 12h beyond inoculation droplets, however, browning of inoculum sites was increased rapidly and a dark brown to black spreading lesions were formed on the non-treated control plants 48h after inoculation. Data in table (٢) indicate also that the antagonistic *Trichoderma isolates* varied in their ability to reduce *Botrytis* chocolate spot lesions on the detached leaves and caused slightly red and small lesions with *T. hamatum* (T73), to black gray spreading lesions with *T. harzianum* (T55). *T. viride* (T2) was the least effective isolates, while, other isolates showed moderate effect.

Further evaluation, in pot experiments under greenhouse conditions revealed the same trend of

Table (1): Origin and source of isolates of *Trichoderma* spp. obtained from different locations in Egypt

Location	Rhizospheric Soil					Phylosphere					Seeds				
	Faba bean	Flax	Chic k pea	Lenti l	Citru s	Faba bean	Flax	Chic k pea	Lenti l	Citru s	Faba bean	Flax	Chic k pea	Lent il	Citru s
Mnoufeya															
Shebin El-Kom	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Khatatba	-	-	-	-	6	2	-	-	-	-	-	-	-	-	-
Kafr El-Sheikh															
Sakha	3	7	-	-	-	1	-	-	-	-	5	-	-	-	-
Behera															
Nubaria	3	-	-	-	-	2	-	-	-	-	-	-	-	-	-
Abou El-Matameir	2	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Zarzora	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sharkia															
Hehia	3	-	-	-	-	2	-	-	-	-	2	-	-	-	-
Abou -Kebier	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beni-Suif															
Seidec	-	-	4	3	-	-	-	2	1	-	-	-	2	3	-
Nasser center	-	-	8	-	-	-	-	2	-	-	-	-	1	-	-
Total	16	7	12	3	8	8	-	4	1	-	7	-	3	3	-
	46					13					13				

Table (2): Summary of antagonistic efficiency of 81 *Trichoderma* isolates against four plant pathogenic fungi, *in Vitro*

Antagonistic capacity	B. fabae		F. oxysporum		R. solani		P. ultimum	
	No.	%	No.	%	No.	%	No.	%
More than 80% Very high	7	8.6	8	9.9	-	-	2	2.5
<60-80% High	41	50.6	18	22.2	25	30.9	14	17.3
40-60% Moderate	28	34.6	36	44.4	49	60.5	37	45.7
less than 40% Low	5	6.2	19	23.5	7	8.6	28	34.5

Table (3): Effect of six *Trichoderma* spp. on suppressing infection of faba bean leaves with *Botrytis fabae* the causal of chocolate spot disease, using the detached leaf technique [x]

Isolate	Disease severity[y] after			
	24 hours		48 hours	
	Lesion browning	% Disease reduction	Lesion browning	% Disease reduction
T. hamatum (T73)	6.0g[z]	90.9	7.2g	91.4
T. harizianum (T55)	12.0d	81.8	16.8e	80
T. aureoviride (T102)	14.4c	78.2	18.0d	78.6
T. atroviride (T58)	8.4f	87.3	12.0f	85.7
T. viride (T2)	28.8b	56.4	36.0b	57.1
T. strictipile (T100)	10.8e	83.6	20.4c	75.7
Non- traetaed	66.0a	0.0	84.0a	-

[x] Percentage of browning beneath the inoculum droplet.
[y] Assessed based on scale described by Deverall and Mansifield (1974).

Table (4): Evaluation of antagonistic *Trichoderma* isolates for controlling chocolate spot disease, caused by *Botrytis fabae* under greenhouse conditions [y]

Isolate	Disease severity at days after inoculation				% Disease reduction
	2	4	7	21	
T. hamatum (T73)	0.3c[z]	0.4c	0.5c	0.7c	92.2
T. harizianum (T55)	0.8b	0.9c	1.3b	1.6bc	82.2
T. aureoviride (T102)	1.2b	1.4b	1.6b	1.9b	78.8
T. atroviride (T58)	0.6c	0.7c	0.9b	1.2b	86.6
T. viride (T2)	1.3b	1.4b	1.7b	1.9b	78.8
T. strictipile (T100)	1.1b	1.4b	1.5b	1.8b	80.0
Non- traetaed	2.4a	3.0a	8.0a	9.0a	

y) Plants were sprayed, to run-off with bioagents, twice after 45 and 55 days from sowing.
z) Values followed by the same letter in each column for each period are not significantly different at P≤ 0.05 according to Duncan's multiple range test.

238

Table (5): Efficiency of six isolates of representing *Trichoderma* spp. for controlling damping-off disease caused by *Rhizoctonia solani* on faba bean under green-house conditions

Isolate	Damping-off (%)		Plant Survival (%)
	Pre-emergence*	Post-emergence**	
T. hamatum (T73)	0.0e[z)]	10.0c	90.0
T. harzianum (T55)	10.0c	10.0c	80.0
T. aureoviride (T102)	12.0b	20.0b	78.0
T. atroviride (T58)	0.0e	8.0d	92.0
T. viride (T2)	0.0e	24.0a	76.0
T. strictipile (T100)	0.0e	24.0a	76.0
Infested-control	8.0d	4.0e	88.0
Non-Infested control	26.0a	24.0a	50.0

*14 days after sowing   **35 days after sowing

aa) Values followed by the same letter in each column for each treatment are not significantly different at $P \leq 0.05$ according to Duncan's multiple range tests.

Table (6): Efficiency of six isolates representing *Trichoderma* spp. for controlling   damping-off disease caused by *Pythium ultimum* on cabbage under green-house conditions

Tested isolate	Damping-off (%)		Plant Survival (%)
	Pre-emergence*	Post-emergence**	
T. hamatum (T73)	10.0d[z)]	6.66c[z)]	84.0
T. harzianum (T55)	26.0b	10b	664.0
T. aureoviride (T102)	4.0f	10b	86.0
T. atroviride (T58)	20.0c	0.0d	80.0
T. viride (T2)	10.0d	· 6.66c	84.0
T. strictipile (T100)	6.60e	6.66c	88.0
Non-Infested control	40a	20a	40

*14 days after sowing   **30 days after sowing

z) Values followed by the same letter in each column for each treatment are not significantly different at $P \leq 0.05$ according to Duncan's multiple range tests.

Table (7): Efficiency of six isolates representing *Trichoderma* spp. for controlling damping-off disease caused by *Fusarium oxysporum* on chickpea under green-house conditions

Isolate	Damping-off (%)		Plant Survival (%)
	Pre-emergence*	Post-emergence**	
T. hamatum (T73)	2.0f[z)]	4.0f	94
T. harzianum (T55)	10e	8.0d	82.0
T. aureoviride (T102)	12.0d	6.0e	82.0
T. atroviride (T58)	30.0b	14.0b	56.0
T. viride (T2)	26.0c	12.0c	62.0
T. strictipile (T100)	12.0d	0.0g	88.0
Non-Infested control	46.0a	26.0a	28.0

*14 days after sowing   **35 days after sowing

z) Values followed by the same letter in each column for each treatment are not significantly different at $P \leq 0.05$ according to Duncan's multiple range tests.
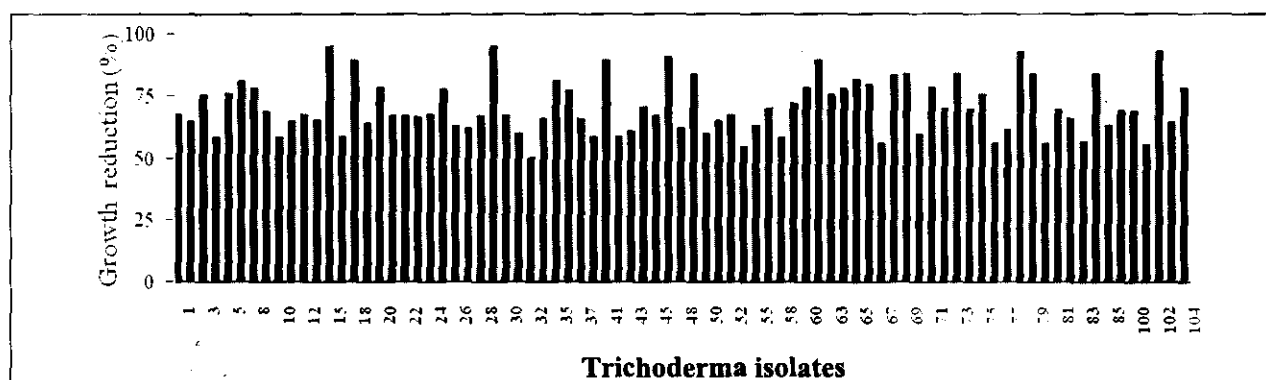
Fig. (1): Growth reduction of *B. fabae* isolate by different *Trichoderma* isolates when grown in dual culture plates on PDA medium, for 7 days at 26°C.
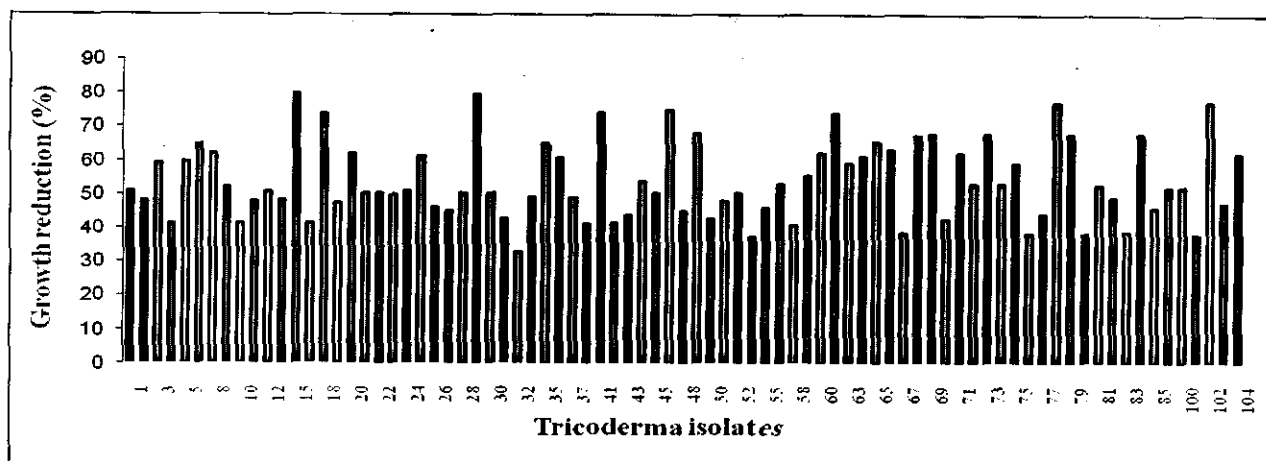
Fig. (2): Growth reduction of *Fusarium oxysporum* isolate by different *Trichoderma* isolates when grown in dual culture plates on PDA medium, for 7 days at 26°C.
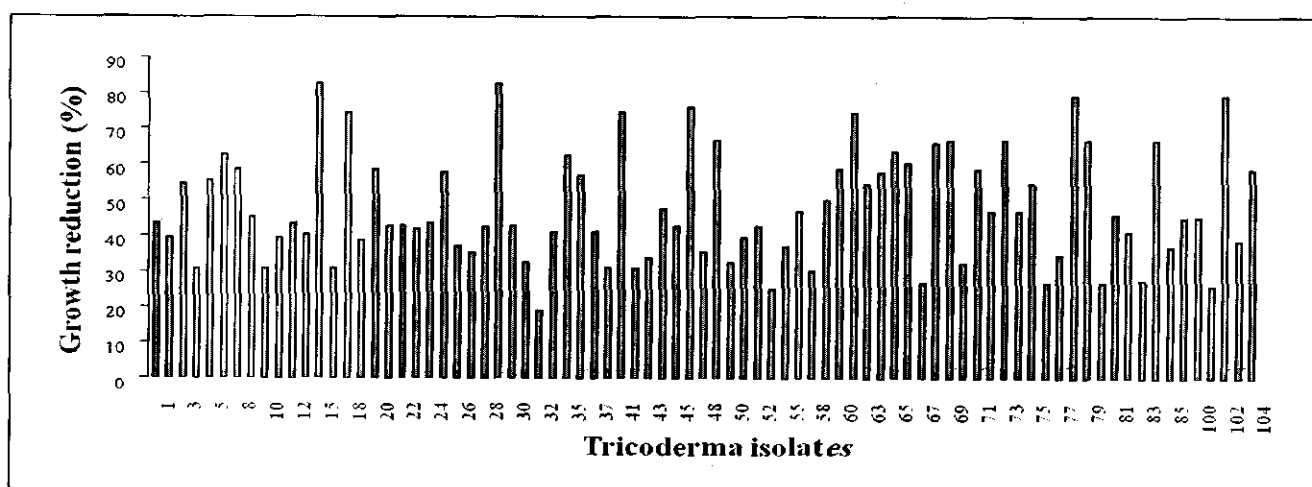

Fig. (3): Growth reduction of *Rhizoctonia solani* isolate by different *Trichoderma* isolates when grown in dual culture plates on PDA medium, for 7 days at 26°C.
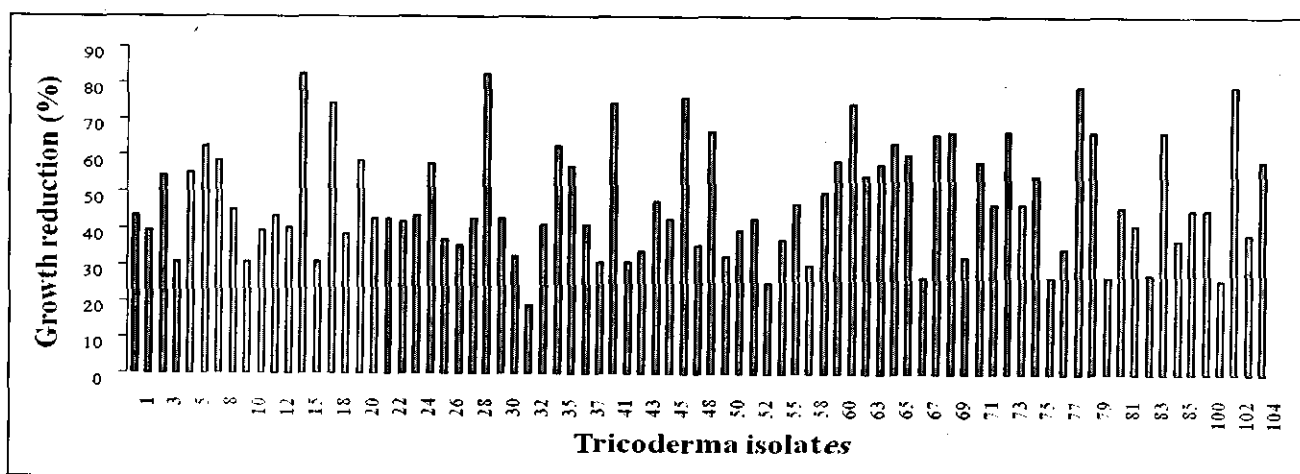

Fig. (4): Growth reduction of *Pythium ultimum* isolate by different *Trichoderma* isolates when grown in dual culture plates on PDA medium, for 7 days at 26°C.
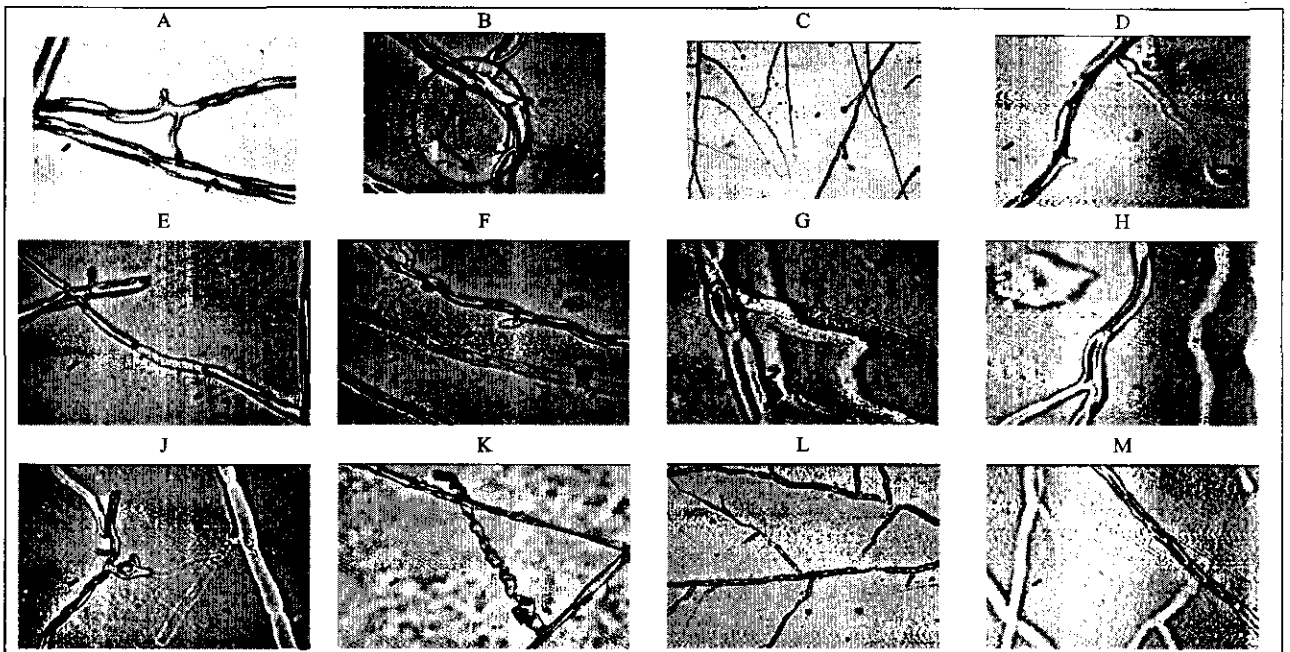
Fig. (5): Photographes of hyphal interaction between different *Trichoderma* isolates and *R. solani* and *Pythium ultimum,* respectively grown on slides of water agar medium, for 3-6 days at 26°C. (A, B, C, D) Coiling of *Trichoderma* around *R. solani* and *Pythium ultimum,* respectively hyphae. (E, F, G, H) Penetration of *R. solani* and *Pythium ultimum* hyphae, respectively. (J, K, L, M) Lysis of *R. solani* hyphae.
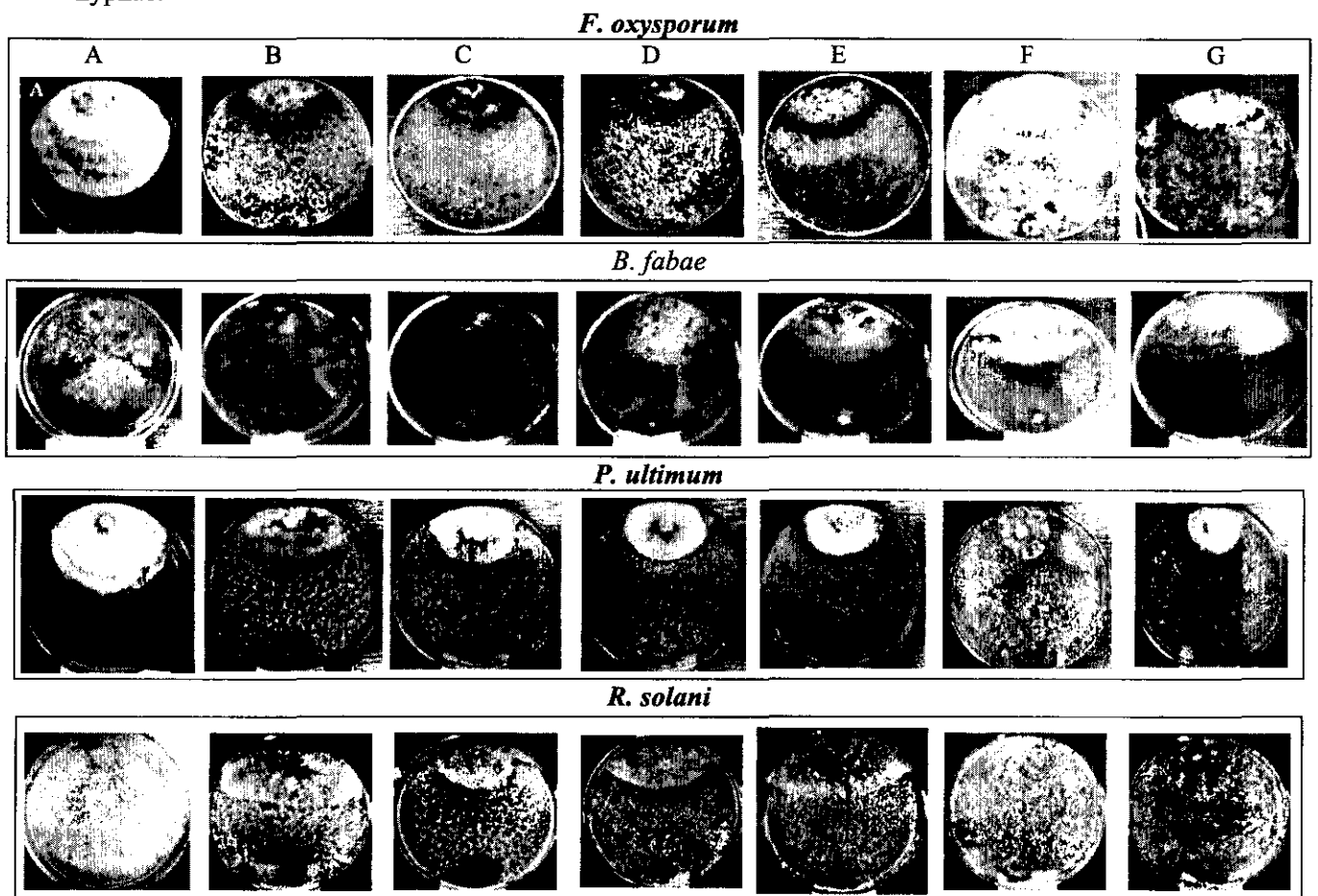

Fig. (6): *In vitro* interaction between *Trichoderma* isolates and *Botrytis fabae isolate, F. oxysporum* isolate, *Rhizoctonia solani* isolate and *Pythium ultimum* isolate, grown on dual culture plates on PDA medium for 7 days at 26°C.

A- *Botrytis fabae* (control)   B- *Trichoderma viride* (T2)   C- *Trichoderma hamatum* (T73)
D- *Trichoderma harzianum* (T55)   E- *Trichoderma aureoviride* (T102)   F- *Trichoderma strictipile* (T100)
G- *Trichoderma atroviride* (T58)

results (Table 4). The most effective isolates were *T. hamatum* (T73) which reduced disease severity by 92%, up to 21 days after pathogen inoculation. Other isolates showed also high efficiency; it ranged from 78.8 to 82.0%.

Biocontrol of seedling damping-off

Six of the most antagonistic *Trichoderma* isolates were tested for their biocontrol capacity against damping-off disease of seedlings caused by three distinct pathogens (Tables 5, 6 and 7).

1- Biocotrol of *Rhizoctonia solani on faba bean*

Results in table (5) indicate that all the tested *Trichoderma* isolates significantly reduced damping-off of faba bean seedlings caused by *R. solani*. The most effective strain was *T. atroviride* (T58) which reduced damping off and increased survival of seedling to 92% compared to non-treated control (50%). Other isolates; *i.e.T. strictipile* (T100), *T. harizianum* (T55), *T. aureoviride* T102n and *T. viride* (T2) were also efficient in controlling the disease and increased seedling survivals (Table 5).

2- Biocotrol of *Pythium ultimum on cabbage*

Results in table (6) indicate that all the tested *Trichoderma* isolates significantly reduced damping-off of cabbage seedlings caused by *Pythium ultimum*. The most effective strain was *T. strictipile* (T100) which reduced damping off and increased seedling survival to 88% compared to non-treated control (40%). Other isolates; *i.e. T. hamatum* (T73), *atroviride* (T58), *T. harzianum* (T55), *T. aureoviride* (T102) and *T. viride* (T2) were also efficient in controlling the disease and increased seedling survivals (Table 6).

3- Biocotrol of *Fusarium oxysporum* on chickpea

Results in table (7) indicate that all the tested *Trichoderma* isolates significantly reduced damping off of chick-pea seedlings caused by *F. oxysporum*. The most effective Strain was *T. hamatum* (T73) which reduced damping off and increased seedling survival to 94% compared to non-treated control (28%). Also, *T. strictipile* (T100), *T. harzianum* (T55), *T. aureoviride* (T102) and *T. viride* (T2) showed high seedling survival (88-82%).

It could be concluded that all tested *Trichoderma* isolates were able to reduce seedling damping-off on 3 different crops with three different pathogens; however, the degree of disease reduction was varied among these isolates. Treatment with *T. hamatum* (T73) provided the best control followed by *T. strictipile* T100.

The results of the present study demonstrate the biodiversity of *Trichoderma* species in selected five

different governorates in Egypt, to obtain an overview on the relative abundance of individual species and eventually to identify biocontrol potentially new taxa. Seventy-two isolates of *Trichoderma* were established in pure cultures, from soil and plant materials collected from various districts in five Governorates in Egypt. Since the isolates were collected in a non-random fashion, emphasizing *Trichoderma* groups within the isolates, the sample may not accurately represent all species found in locations in different Governorates or their relative abundance. The majority of isolates were obtained from Beni-Suif (36.1%), followed by Kafr El-Sheikh (22.2%) and Behera, Mnoufeya, Sharkeya; each yielded (13.9%). The global occurrence of *Trichoderma* was recently investigated in several studies (Wuczkowski *et al.*, 2003; Gherbawy *et al.*, 2004; Druzhinina *et al,.* 2005; Zhang *et al,.* 2005 and Kubicek, *et al,.* 2008). While these studies revealed the worldwide predominance of some species such as *T. harzianum*, they also revealed a putative geographic bias for a number of other taxa.

The occurrence of *Trichoderma* in Egyptian soils has been the subject of several earlier studies (Moubasher and Abd El-Hafez, 1987; El-Naghy *et al,.* 1998 and Shaban and El-Komy, 2000). However, among 20 isolates collected only two taxa were present in soils from the Nile valley (Gherbawy *et al.,* 2004). In the present study, 46 *Trichoderma* isolates were obtained from soil samples, while 13 isolates were obtained from either phyllosphere or seeds. However, 56.5% *of Trichoderma* isolates from soil in this study were obtained from faba been and chick-pea rhizosphere. These findings may be due to the presence of different of organic matter in soil, soil moisture, variation of root exudates of each crop, and so allowing *Trichoderma* to proliferate.

Recent report of Gherbawy *et al.,* (2004) revealed that independent of the crop and the physicochemical properties of the soil, they contained, they only detected two species of *Trichoderma*; viz. *T. harzianum* and the anamorph of *Hypocrea orientalis* from limited survey in the Nile valley. They attributed the reason for this low degree of biodiversity of *Trichoderma* in the Nile valley soil to its alkaline pH value. Danileson and Davey (1973) stressed that the pH of the soil is one of the most critical parameters for *Trichoderma* propagation. Kredics *et al.,* (2003) reported that species of *Trichoderma* grow optimally around pH 4.0–5.0, and exhibit little or no growth below 2.0 and above 6.0. However, in the present study, *Trichoderma* spp. were isolated from various adverse soil habitats, and phyllosphere and seed

surfaces of different plant species. However, we could not detect any correlation between the plant species and the taxa of *Trichoderma* recovered. Correlations between any of these characters and the six identified *Trichoderma* species were essentially random.

Based on morphological and microscopic observations, all obtained isolates were identified as *Trichoderma* spp. Identification data, suggested, although minor morphological characters are existed among isolates, they belonged to six different species; *i.e T. harzianum, T. hamatum, T. viride, T. auroviridae, T atrovidae* and *T. strictiple*. These results were confirmed using Online Interactive Identification Key (Samules *et al.*, 2004). However, based on RAPD analysis data, two new species, *T. auroviridae* and *T. strictipile* were recognized, undescribed previously in Egypt.

The results reported in the present study demonstrate that there is a great variation among the 81 *Trichoderma* isolates in suppression of mycilial growth of 4 fungal plant pathogens; *i.e. B. fabae, R. solani, F. oxysporum* and *P. ultimum, in vitro*; for example, 17.3% of *Trichoderma* isolates showed high antagonism ($\geq$80% reduction) against *R. solani*, while none caused the same degree of antagonism against *P. ultimum*. *Trichoderma* hyphae started overlapping the colony of *R. solani or P. ultimum* within 3-days of incubation, coiling around the hyphea of pathogen and penetrate producing haustoria within the large hyphae and two days later, lysis of *R. solani* or *P. ultimum* hyphae was evident. These results are explained with the complex mechanisms of mycoparasitism, which include directed growth of *Trichoderma* toward target fungi, attachment and coiling of *Trichoderma* on target fungi, and the production of a range of antifungal extracellular enzymes, were elucidated (Chet, 1987; Chet *et al.*, 1998 and Harman, 2006). Some species of *Trichoderma*, e.g. *Trichoderma atroviride* (Dodd *et al.*, 2003), *Trichoderma harzianum, T. virens* and *T. asperellum* are potent mycoparasites against several plant-pathogenic fungi and lysis of the host cell wall has been demonstrated to be an important step in the mycoparasitic attack (Howell, 2003 and Benitez *et al.*, 2004).

Under greenhouse conditions, six antagonistic *Trichoderma* isolates suppressed chocolate spot disease of faba bean. The most effective strain was *T. hamatum* (T73) which reduced diseases severity by 92%, up to 21 days after pathogen inoculation. Other isolates showed also high efficiency; it ranged from 78.8 to 82.0%. The efficiency of certain *Trichoderma* isolates in controlling foliar diseases like leaf spots have also been reported (Mahmoud, *et al.*, 2004; Haggag *et al.*, 2006 and Perello *et al.*, 2009).

In the present study, all tested *Trichoderma* isolates significantly reduced damping-off disease of faba bean, cabbage and chick-pea caused by *R. solani, P. ultimum, and F. oxysporum*, respectively. However, the degree of disease reduction has varied among these isolates. Treatments with *T. hamatum* (T73) provided the best control followed by *T. strictipile* (T100). Clear protective effects of *Trichoderma* spp. under provoking conditions and exposed to a considerable inoculation potential of pathogenic fungi suggest that a similar effect can be obtained in the field. It seems reasonable to conclude that morphological variations among species (Bisset, 1991a, b and c), do not provide sufficient explanation for the variation in biocontrol capacity against the soil borne fungi involved seedling damping-off. There are three main mechanisms by which one microorganism may limit the growth of another microorganism: antibiosis, mycoparasitism and competition for resources (Harman *et al.*, 2004). Species of the *Trichoderma* genus are characteristically saprophytes (Kubicek *et al.*, 1998); however, root colonization by some *Trichoderma* isolates is a common phenomenon in the field (Harman *et al.*, 2004). However, the promotion of plant growth by *Trichoderma* spp., under axenic conditions and in natural soils (Harman *et al.*, 2004) is also demonstrated. Effective biocontrol isolates of *Trichoderma* can also induce the production of defense-related compounds in the roots (Hanson and Howell, 2004). Research in the biocontrol field is making rapid progress in understanding the mechanisms of biocontrol and several biocontrol products are now available for practical, widespread use in plant disease control. Whether the antagonistic *Trichoderma* isolates detected in the present study can be incorporated into a biocontrol regimen will require additional experimentation under field conditions.

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