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Antagonistic Activities of *Trichoderma* spp. on Phytophthora Root Rot of Sugar Beet

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ABSTRACT- *Trichoderma* species are among important antagonists of plant pathogenic fungi. The main purpose of this study was to evaluate the biocontrol potential of native *Trichoderma* spp. isolates from sugar beet fields against *Phytophthora drechsleri* and *P. cryptogea*, the causal agents of Phytophthora root rot of sugar beet. During 2007–08, 85 isolates of *Trichoderma* spp. from sugar beet fields in eight parts of Fars province, Iran were isolated. Based on morphological and physiological characteristics, eight species could be distinguished: *T. asperellum*, *T. atroviride*, *T. brevicompactum*, *T. harzianum*, *T. longibrachiatum*, *T. spirale*, *T. tomentosum* and *T. virens*. Their antagonistic activities including competition, colonization and antibiosis against *Phytophthora* isolates both *in vitro* and *in planta* were investigated. Different species and even different isolates of one species differed in their antagonistic capacities. High competitive abilities were shown by *T. asperellum*, *T. atroviride*, *T. harzianum* and *T. virens* in pre colonized plates, and by the former three species in dual cultures. *T. harzianum* and *T. atroviride* efficiently colonized both species of *Phytophthora*. *T. asperellum* Ksh_2, *T. virens* DB_{6r}, *T. virens* DB_2 and *T. virens* DB_3 and *T. harzianum* MS_3 gave the highest growth inhibition. In *in planta* experiments, *T. harzianum* MS_3 and FG_4 and *T. virens* DB_{6r} were the most efficient in suppressing root rot of sugar beet seedlings. Our results suggest that *T. harzianum* MS_3 is the best native candidate for the biocontrol of this disease.

Keywords: Biological Control, *Phytophthora cryptogea*, *Phytophthora drechsleri*, Sugar Beet, *Trichoderma* spp.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is an important economic crop in the world (57) and in Iran (17). Phytophthora root rot is a major constraint to sugar beet production in different parts of Iran, especially Fars province (3), and it causes serious economic losses (25). Various species of *Phytophthora* are reported to cause Phytophthora root rot on sugar beet, but the most prevalent and aggressive are *P. cryptogea* and *P. drechsleri* (8, 25, 27, 53, 54 and 55). The principal procedures for controlling Phytophthora root rot on sugar beet include cultural practices and the use of fungicides (8 and 55). Fungicides are expensive and involve risks to the environment; they are not totally effective and often lead to the appearance of resistant strains of the pathogens (16). Application of environment-friendly

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biological agents is more suitable in view of sustainable agriculture and can be incorporated in integrated control programs (34, 36 and 14).

Trichoderma spp. has been widely used in biological control studies against several commercial phytopathogens (2, 7, 16, 26 and 52). However, little attention has been paid to their ability to control *Phytophthora* spp. in general, and *P. cryptogea* and *P. drechsleri* in particular; their inhibitory effect on some zoosporic pathogenic fungi suggests that they may have a role to play (51). Therefore employing *Trichoderma* spp. against *Phytophthora* spp. represents an alternative for the control of the Phytophthora root rot on sugar beet. The success of biocontrol largely depends on the ability of the antagonists to proliferate under conditions of the given environment. Hence, the application of native fungal strains in biocontrol may be advantageous or even desirable. Equally important is the fact that native strains do not represent a foreign element potentially endangering local biodiversity. The main purpose of the present study was to evaluate the biocontrol potential of native *Trichoderma* spp. isolates in sugar beet fields against *P. drechsleri* and *P. cryptogea*, the causal agents of Phytophthora root rot of the sugar beet.

MATERIAL AND METHODS

Trichoderma and *Phytophthora* Isolates

Isolation and Identification of *Trichoderma* spp.

During 2007–08, 85 *Trichoderma* spp. isolates were collected from 25 samples of sugar beet soil and rhizosphere in sugar beet fields from eight parts of Fars province, Iran (Marvdasht, Fasa, Kavar, Zarghan, Beyza, Darab, Eghlid and Abadeh). Isolates were recovered from soil by a serial dilution method. Three media were used for isolation including: Davet (calcium nitrate 1 gL^{-1}, calcium chloride1 gL^{-1}, sucrose 2 gL^{-1}, potassium nitrate 0.25 gL^{-1}, $MgSO_4 \cdot 7H_2O$ 0.25 gL^{-1}, phosphate 0.125 gL^{-1}, citric acid 0.05 gL^{-1}, agar 25 gL^{-1}); potato dextrose agar (PDA; extract of 300 g potato, dextrose 20 gL^{-1}, agar 16 gL-1, amended with 0.1 gL^{-1}NPX "nonyl phenyl polyethylene glycol with 10.5 moles of ethylene oxide" and 0.5 gL^{-1}ampicillin) and peptone rose bengal agar (sucrose 5 gL^{-1}, peptone 0.5 gL^{-1}, magnesium sulfate 0.5 gL^{-1}, potassium phosphate 0.5 gL^{-1}, rose bengal 0.05 gL^{-1}, agar 20 gL^{-1}). After serial sub-culturing, single-spore cultures were isolated and maintained on PDA slants at 4 °C in the dark. Species were identified based on morphological characteristics using available keys (9, 10, 11, 12, 22, 29 and 48).

Phytophthora Rot Agents

Isolates of *Phytophthora drechsleri* (SCRP232) and *P. cryptogea* (St1) were originally isolated from rotted sugar beets obtained from the Department of Plant Protection, College of Agriculture, Shiraz University, Iran and used in all tests. The pathogenesis of these isolates was demonstrated on roots and seedlings of sugar beet *in vitro* and *in planta.*

Trichoderma Strains Used in Most Experiments

T. asperellum (BAB_1, BAB_2, $EghA_4$, Ksh_2), *T. atroviride* (FG_6), *T. harzianum* (FG_1, FG_4, MS_3, MS_4, KK_1, ZG_{6r}, BM_1, DA_1, DA_2, $EghB_1$) and *T. virens* (DB_1, DB_2, DB_3, DB_4, DB_{6r}).

Treatments included: the two *Phytophthora* isolates with each *Trichoderma* isolate, and the *Phytophthora* isolates with sterile filter paper disc.

There were three replicate plates per treatment in all laboratory experiments and each experiment was performed twice. Treatments were arranged in a completely randomized design and compared with Tukey's test ($P \leq 0.05$).

Antagonistic Activity of *Trichoderma* spp. against *Phytophthora* spp. *In Vitro* (Laboratory Experiments)

Precolonized Plates

The precolonized plate method (39) was used to assay mycoparasitic abilities of all *Trichoderma* isolates (Table 1) towards *Phytophthora* isolates. Sterile filter paper discs (5 mm diam, Whatman No. 1) were placed on top of the mycelium of *Trichoderma* isolates that had been grown 5 days on cornmeal dextrose agar (CMD; corn meal 40 gL^{-1}, dextrose 10 gL^{-1}, agar 16 gL^{-1}, sterile distilled water 1 L), and the fungal cultures were incubated for an additional 4 days. Filter paper discs, overgrown with hyphae of individual *Trichoderma* isolates, were placed on top of mycelia of 10-day-old cultures of the pathogen on CMD agar. Growth and progress rates of each *Trichoderma* isolate on mycelia of the pathogen were observed daily following a 7 day incubation period at 25°C. The following system was used to rate mycoparasitism: 3+, the entire culture of the pathogen was colonized within 7 days; 2+, the entire culture of the pathogen was colonized between 7 and 14 days; 1+, the entire culture of pathogen was colonized after 14 days; -, no growth on the pathogen mycelia was observed.

Table 1. Origins and codes of the isolates studied

Species	Isolates	Source*
T. asperellum	(BAB_1**+,2,3,4,5,6,7,8,9r,10r***), ($EghA_{4,5r,6r}$), ($Ksh_{2,3,4r}$, $SG_{3,4r}$)	Beyza, Eghlid, Abadeh
T. atroviride	FG_6, KK_5	Fasa, Kavar
T. brevicompactom	($FG_{2,3,5,8r}$), ($MS_{1,2,6,7}$), ($KK_{4,6r,7r}$), ($ZG_{1,2,3,5,7r}$), ($BDK_{1,2,3}$), ($EghA_{1,2,3}$)	Fasa, Marvdasht, Kavar, Zarghan, Beyza, Eghlid
T. harzianum	($FG_{1,4}$), ($MS_{3,4,5}$), ($KK_{1,2,3}$), ($ZG_{4,6r}$), ($BM_{1,2,3r}$), ($DA_{1,2,3r}$), ($EghB_{1,2,3r}$), ($Ksh_{1,5r}$, $SG_{1,2}$)	Fasa, Marvdasht, Kavar, Zarghan, Beyza, Darab, Eghlid, Abadeh
T. longibrachiatum	$EghC_{1,2,3r,4r}$	Eghlid
T. spirale	FG_{7r}, KK_{8r}	Fasa, Kavar
T. tomentosum	($BAA_{1,2,3r,4r}$), ($BH_{1,2,3r}$)	Beyza, Abadeh
T. virens	$DB_{1,2,3,4,5,6r,7r}$	Darab

* All isolates recovered from Fars province, south-western Iran

** Each number represents one isolate

*** r = isolation from rhizosphere

\+ Origin of isolation for each symbol: Abadeh (Ksh, SG, BH), Beyza (BDK, BM, BAA, BAB), Darab (DA, DB), Eghlid (EghA, EghB, EghC), Fasa (FG), Kavar (KK), Marvdasht (MS), Zarghan (ZG)

Culture on Slide Method

All antagonist–pathogen combinations were examined on CMD agar on slides in 10 cm empty Petri dishes. For dual cultures on slides, mycelial discs (5 mm diam and 3 mm thick), taken from actively growing, 5-day-old cultures of *Phytophthora* and *Trichoderma* isolates, were placed at a distance of 4 cm. discs of both *Phytophthora* isolates were applied one day prior to those of the *Trichoderma* isolates. As controls, discs of agar were added to similar plates inoculated with *Phytophthora* isolates. Any interactions were microscopically observed on a daily basis for six days (18). The following system was used to rate mycoparasitism: 3+, the entire culture of pathogen was colonized within 3 days; 2+, the entire culture of the pathogen was colonized between 3 and 5 days; 1+, the entire culture of the pathogen was colonized after 5 days; -, no colonization of the pathogen. Treatments included: each *Phytophthora* isolate with each *Trichoderma* isolate and each *Phytophthora* isolate alone with CMD agar disc.

Effect of *Trichoderma* spp. on the Development of Phytophthora Rot in Sugar Beets

Promising isolates from the precolonized plate method and culture on slide assay were screened for suppression of *Phytophthora* on sugar beet roots using a modification of the method of Krauss *et al*. (39). *Trichoderma* and *Phytophthora* isolates were grown for 10 days on CMD agar in 10 cm Petri dishes. A 5 × 20 mm strip of a *Phytophthora* colony was removed from the 5-day-old culture, inverted and placed on top of the mycelium of *Trichoderma* isolates on CMD agar. This agar strip was cut, into 5 mm pieces after 7 days. Sugar beet roots were surface-disinfested by soaking in 0.5 % sodium hypochlorite for 5 min and then rinsed three times in sterile distilled water. A core, 8 mm diam and 20 mm deep, was removed aseptically from each root by a cork borer, and a 5 mm piece of the pathogen or the pathogen-antagonist combination was inserted into the hole. The core of the root tissue was replaced and the wound sealed with Parafilm (Pechiney Plastic Packaging, USA). Roots were placed individually in paper bags and incubated at 25 °C in the dark. Symptoms were recorded in dissected roots 2–4 weeks after inoculation and the extent of discoloration of the root was estimated as a percentage of the total area. The following rating system was used: 3+, 15–20 mm development of rot (discoloration); 2+, 20–25 mm development of rot; 1+, 25–30 mm development of rot; and –, 30–35 mm development of rot. Root colonization by *Trichoderma* and *Phytophthora* isolates evaluated by placing fragments of the dissected root on PARP-CMA (extract of 40 g corn mealL^{-1}, agar 16 gL^{-1}, PCNB 0.1 gL^{-1}, ampicillin 0.5 gL^{-1}, rifampciine 0.01 gL^{-1}, delvacide 0.02 gL^{-1}), or PDA amended with 0.1 g NPX and 0.5 g ampicillin.

Dual Culture Method

All antagonist–pathogen combinations were examined on CMD agar in 10 cm Petri dishes. For dual cultures, mycelial discs (5 mm diam), taken from actively growing, 5-day-old cultures of *Phytophthora* and *Trichoderma* isolates, were placed at a distance of 4 cm. Discs of each *Phytophthora* isolates were applied one day prior to those of the *Trichoderma* isolates. As controls, discs of agar were added to similar plates inoculated with *Phytophthora* isolates. Growth rate and sporogenesis of *Trichoderma* isolates on the *Phytophthora* colony were surveyed daily for six days.

Vegetative growth of the pathogens was recorded by measuring each colony's radius after 3 days and recording the inhibition percentage according to the following formula (50):

$$I = \frac{A-B}{A} \times 100$$

Where I: inhibition percentage of vegetative growth of the *Phytophthora* isolates, A: radius of the control colony in mm, and B: the distance in mm traveled by the *Phytophthora* isolates colony over the *Trichoderma* isolates.

Effect of Non-Volatile Metabolites of *Trichoderma* spp. on Mycelial Growth of the Pathogen

Two mycelial discs (5 mm diam), taken from actively growing, 5-day-old cultures of each *Trichoderma* isolate were placed into 100 ml Davet broth and incubated at 25 °C on a shaker for 7 days. The cultures were filtered through sterile filter paper and then through 0.2 µm millipore filters (Gema Medical, Spain). Five ml aliquots of the filtrate were placed in sterile Petri dishes and 20 ml molten CMD agar was added. After the agar had solidified, mycelial discs of *Phytophthora* isolates (5 mm diam), obtained from actively growing colonies were placed in the centre of the agar plates. The Petri dishes were incubated at 25 °C for 3 days. Vegetative growth of the pathogen was recorded by measuring each colony's radius after 3 days and recording the percentage inhibition (19). Treatments included: each *Phytophthora* isolate with each *Trichoderma* isolate and each *Phytophthora* isolate with CMD agar.

Effect of Volatile Metabolites of *Trichoderma* spp. on Mycelial Growth of the Pathogen

Petri dishes with 1/4 strength CMD agar were inoculated in the centre with a 7 mm diameter disc taken from an actively growing, 5-day-old culture of either *Phytophthora* or *Trichoderma*. *Phytophthora* Petri dishes were inverted, placed above the *Trichoderma* dish and sealed with Parafilm. These Petri dishes were incubated at 25 °C for 3 days. Growth of the pathogen was recorded by measuring the radius after 3 days (20).

Antagonistic Activity of *Trichoderma* spp. against *Phytophthora* spp. *In Planta* (Greenhouse Experiments)

Preparation of Inoculum of *Phytophthora* and *Trichoderma* Isolates

To prepare inoculum from the pathogen, 60 ml of hemp seed broth (extract of 60 g hemp seed L-1) + vermiculite (100 ml) was poured in a 250 ml Erlenmeyer flask and autoclaved for 15 min at 121 °C on each of the two consecutive days and then inoculated with 5 mycelial discs (5 mm diam), taken from actively growing, 5-day-old cultures of each *Phytophthora* isolate (4). *Trichoderma* inoculum was prepared by the method proposed by Smith *et al*. (51). Five discs (5 mm diam) were cut from the edge of the actively growing, 5-day-old cultures of each *Trichoderma* isolate, and transferred into a 250 ml Erlenmeyer flask containing a mixture of peat and wheat bran (3:1, v/v) which had been moistened with 15 ml of distilled water and sterilized for 15 min at 121 °C on two consecutive days. The Erlenmeyer flasks were incubated at 25 °C in darkness for 15 days.

Seed Planting and Inoculation of Seedlings

The surface of sugar beet seeds (cultivar 7233) was sterilized in 0.5 % sodium hypochlorite for 2 min and then rinsed three times in sterile distilled water. Five sugar beet seeds were sown in each pot containing a 2:1 mixture (v/v) of soil and sand that had been sterilized twice at 121°C for 1 hour on two consecutive days. The seedlings were grown in a greenhouse with a 16 h photoperiod at 25°C and a relative humidity of 60–70% for 2 months. After 2 months, single seedlings were transferred into pots containing sterilized soil and inoculated with a *Trichoderma* isolate at a rate of 4% (v/v). After seedling stabilization (about one week), the soil in each pot was inoculated with *Phytophthora* isolates at a rate of 4% (v/v). Plants were maintained in the glasshouse with a 16 h photoperiod at 25°C and a relative humidity of 60–70% with plastic bags placed around the pots to retain moisture (50).

Evaluation of the Treatments

Root rot of sugar beet was assessed 4–6 weeks after inoculation by the observation of plant wilting and root rot. Plants were harvested 6 weeks after inoculation and the severity of rot was assessed using a 1–5 scale modified from Nemec *et al*. (45): 1 for healthy roots and 5 for all roots infected. Root and rhizosphere colonization by *Phytophthora* and *Trichoderma* were evaluated by culture of root sections on PARP-CMA and PDA amended with 0.1 g NPX and 0.5 g ampicillin. Fresh and dry weights of roots from each pot were recorded to show the effect of *Trichoderma* isolates on the growth of sugar beet plants (50). Treatments included both *Phytophthora* isolates with all *Trichoderma* isolates, *Phytophthora* and *Trichoderma* isolates alone, and plants without fungi. Utilized *Trichoderma* isolates included: *T. asperellum* (Ksh_2), *T. harzianum* (MS_3), *T. harzianum* (FG_4), *T. virens* (DB_2) and *T. virens* (DB_{6r}). There were four replicate pots per treatment and the experiment was performed twice. Treatments were arranged in a completely randomized design and compared with Tukey's test (P = 0.05).

RESULTS

Isolation and Identification of *Trichoderma* spp.

Recovered isolates are listed in Table 1. Eight different species of *Trichoderma* were isolated from the sugar beet fields of Fars province: *T. asperellum*, *T. atroviride*, *T. brevicompactum*, *T. harzianum*, *T. longibrachiatum*, *T. spirale*, *T. tomentosum* and *T. virens.*

Antagonistic Activity of *Trichoderma* spp. against *Phytophthora* spp. *In Vitro* (Laboratory Experiments)

Precolonized Plates

Growth, progress and colonization rate of each *Trichoderma* isolate on mycelia of the pathogens were recorded. One *T. atroviride* isolate (FG_6), 8 *T. asperellum* isolates (BAB_1, BAB_2, BAB_3, BAB_{9r}, BAB_{10r}, $EghA_{5r}$, $EghA_{6r}$, Ksh_2), 10 *T. harzianum* isolates (FG_4, FG_1, MS_3, ZG_4, ZG_{6r}, BM_{3r}, DA_1, DA_{3r}, $EghB_{3r}$, Ksh_{5r}) and 3 *T. virens* isolates (DB_{7r}, DB_{6r}, DB_5) showed the highest growth rates on both *P. drechsleri* and *P. cryptogea* colonies and colonized mycelia of the *Phytophthora*

isolates within 7 days. Whereas *T. spirale* and *T. tomentosum* isolates had the lowest growth rate on the *Phytophthora* isolates colonies and needed more than 21 days for colonization of the mycelium of the *Phytophthora*.

Culture on Slide Method

After microscopic observation of the initial contact of some *Trichoderma* hyphae with *Phytophthora* hyphae, parallel growth and then hyphae coiling around those of *Phytophthora* isolates were observed (Fig. 1). One *T. atroviride* isolate (FG_6) and 11 *T. harzianum* isolates (FG_4, FG_1, MS_3, MS_4, KK_1, ZG_{6r}, BM_1, DA_1, DA_2, DA_{3r}, $EghB_1$) showed the highest colonization rates of mycelia of the pathogens, completely colonizing them within 3 days. Four *T. asperellum* isolates (BAB_1, BAB_2, $EghA_4$, Ksh_2) and 5 *T. virens* isolates (DB_2, DB_3, DB_4, DB_5, DB_{6r}) had relatively high rates of colonization of pathogen mycelia and completely colonized mycelia of *Phytophthora* isolates within 3-5 days. *T. brevicompactum*, *T. longibrachiatum*, *T. spirale* and *T. tomentosum* isolates did not colonize mycelia of the pathogens.

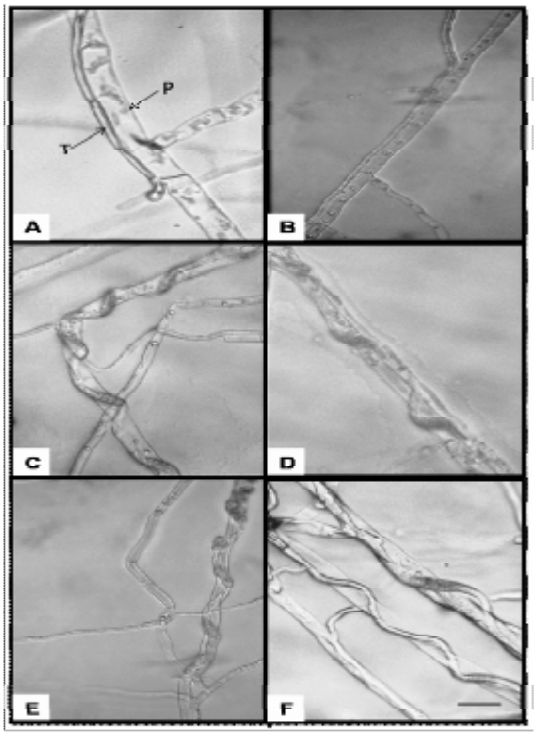

Fig. 1. Colonization of *Phytophthora* spp. hyphae by *Trichoderma* spp. in culture on slide method after 3 days. A: *T. harzianum* MS_3 (T) and *P. drechsleri* SCRP232 (P); B: *T. harzianum* FG_4 and *P. drechsleri* SCRP232; C: *T. harzianum* DA_1 and *P. cryptogea* (St1); D: *T. harzianum* DA_1 and *P. drechsleri* SCRP232; E: *T. asperellum* Ksh_2 and *P. cryptogea* St1; F: *T. virens* DB_{6r} and *P. cryptogea* St1. Bar =10 µm

Effect of *Trichoderma* spp. on the Development of Phytophthora Rot on Sugar Beet Roots

When inoculated with *Phytophthora* isolates alone, roots became extensively discolored, both externally and internally 2-4 weeks after inoculation. Roots inoculated with both *Trichoderma* and *Phytophthora* isolates showed less discoloration than the control (Fig. 2, Table 2), but in no case was the damage completely suppressed.

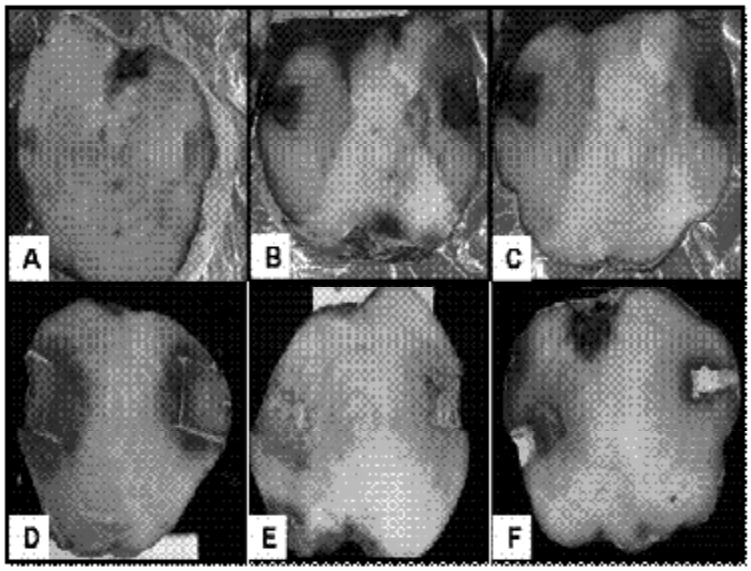

Fig. 2. Symptoms of internal rot in sugar beet roots after inoculation with *Trichoderma* spp. and *Phytophthora* spp. A: control; B: *P. drechsleri* (SCRP232); C: *P. cryptogea* (St1); D: *T. harzianum* MS_3 and *P. drechsleri* (SCRP232); E: *T. virens* DB_3 and *P. cryptogea* (St1); F: *T. harzianum* BM_1 and *P. drechsleri* (SCRP232)

Table 2. Phytophthora rot restriction ability of *Trichoderma* isolates in sugar beet roots (2–3 weeks after inoculation)

Species	Isolates	rot restriction ability of isolates	
		P. cryptogea	*P. drechsleri*
T. sperellum			
	BAB_1	++*	++
	BAB_2	++	++
	$EghA_4$	++	++
	Ksh_2	+++	+++
T. atroviride			
	FG_6	-	-
T.harzianum			
	FG_4	+++	+++
	FG_1	++	+
	MS_3	+++	+++
	MS_4	++	++
	KK_1	++	+
	ZG_{6r}	++	++
	BM_1	+	+
	DA_1	+	+
	DA_2	+	+
	$EghB_1$	++	++
T. virens			
	DB_1	+++	++
	DB_2	+++	+++
	DB_3	+++	++
	DB_4	+++	++
	DB_5	++	++
	DB_{6r}	+++	+++
Control		-	-

*+++ = 15–20 mm development of rot; ++ = 20–25 mm development of rot; + = 25–30 mm development of rot; - = 30–35 mm development of rot

Dual Culture Method

Growth inhibition of the *Phytophthora* isolates (Table 3) by different *Trichoderma* isolates varied over a broad range. *T. harzianum* MS_3, MS_4 and FG_4, *T. asperellum* Ksh_2, and *T. virens* DB_{6r} showed the strongest growth inhibition of both *Phytophthora* isolates. Whereas *T. asperellum* BAB_2 and *T. atroviride* FG_6 had the least effect.

Effect of Non-Volatile Metabolites of *Trichoderma* spp. on Mycelial Growth of Pathogen

Several *Trichoderma* species had significant effects on the mycelial growth of *Phytophthora* isolates (Table 3). *T. asperellum* Ksh_2, *T. virens* DB_{6r}, DB_2 and DB_3 gave the strongest inhibition of both *Phytophthora* isolates, while *T. harzianum* DA_1, and DA_2, *T. atroviride* FG_6, and *T. asperellum* $EghA_4$ did not inhibit their growth.

Effect of Volatile Metabolites of *Trichoderma* spp. on Mycelial Growth of Pathogen

Volatile metabolites generally had lower effects on the mycelial growth of *Phytophthora* (Table 3) than the non-volatile ones. *T. virens* DB_{6r}, DB_3, DB_2, *T. asperellum* Ksh_2 and *T. harzianum* MS_3 had the strongest effect on both *Phytophthora* isolates. *T. asperellum* BAB_1 and BAB_2 showed least inhibition.

Table 3. Growth inhibition ratio of *Phytophthora drechsleri* and *P. cryptogea* by *Trichoderma* isolates in different laboratory experiments. Mean values within a column followed by the same letter are not significantly different at P ≤ 0.05 by Tukey's test

Trichoderma isolates	Growth inhibition (%)					
	in dual culture method		of non volatile metabolites		of volatile metabolites	
	P. drechsleri	*P. cryptogea*	*P. drechsleri*	*P. cryptogea*	*P. drechsleri*	*P. cryptogea*
***T. asperellum* BAB_1**	**20j**	**21j**	**6.6l**	**6.6l**	**8.2g**	**10.3g**
***T. asperellum* BAB_2**	**14.3k**	**14.3k**	**12.4k**	**13.3k**	**8.2g**	**10.4g**
***T. asperellum* $EghA_4$**	**33.3i**	**34.6i**	**27.6j**	**26.6j**	**0h**	**0h**
***T. asperellum* Ksh_2**	**63.1c**	**64.3c**	**75.4c**	**76.6c**	**32a**	**33.3a**
***T. atroviride* FG_6**	**12.3k**	**13k**	**25.3j**	**25.6j**	**0h**	**0h**
***T. harzianum* FG_4**	**63.1c**	**64.3c**	**68.2e**	**70e**	**20.1e**	**20.8e**
***T. harzianum* FG_1**	**40.1g**	**41j**	**58f**	**60f**	**13.8f**	**15.7f**
***T. harzianum* MS_3**	**67a**	**71a**	**75.4c**	**76.6c**	**21e**	**21.5e**
***T. harzianum* MS_4**	**65.5b**	**67.6b**	**70.3d**	**73.3d**	**21e**	**16.6f**
***T. harzianum* KK_1**	**44.3f**	**44.3f**	**56.3f**	**59.3f**	**8.5g**	**10.7g**
***T. harzianum* ZG_{6r}**	**40.1g**	**41j**	**58f**	**60f**	**14.8f**	**16.6f**
***T. harzianum* BM_1**	**49.8e**	**51e**	**38.3h**	**40h**	**8.1g**	**103g**
***T. harzianum* DA_1**	**44.3f**	**44.3f**	**44.3g**	**46.6g**	**0h**	**0h**
***T. harzianum* DA_2**	**44.3f**	**44.3f**	**43.2g**	**45.6g**	**0h**	**0h**
***T. harzianum* $EghB_1$**	**43.4f**	**45.6f**	**34.3i**	**34.3i**	**7g**	**10g**
***T. virens* DB_1**	**33.3i**	**35.3i**	**67.6e**	**69.6e**	**20.3e**	**21.3e**
***T. virens* DB_2**	**52.3d**	**54.3d**	**78b**	**80b**	**23.1c**	**25.3c**
***T. virens* DB_3**	**49.8e**	**51e**	**81.3a**	**83.3a**	**23.3d**	**23.3d**
***T. virens* DB_4**	**35.6h**	**37.6h**	**68.2e**	**70e**	**19e**	**20e**
***T. virens* DB_{6r}**	**62c**	**64c**	**81.3a**	**83.3a**	**28.3b**	**30b**

Antagonistic activity of *Trichoderma* spp. against *Phytophthora* spp. *in planta* (greenhouse experiments)

Antagonistic Activity of *Trichoderma* spp. against *Phytophthora* spp. *In Planta* (Greenhouse Experiments)

Plants inoculated with the pathogen alone showed significantly stronger damage after 6 weeks than those inoculated with antagonists alone or combinations with a pathogen.

Fresh and dry weights of plant roots and shoots differed widely when inoculated with different antagonists. Plants inoculated with *Trichoderma* isolates alone had the highest fresh and dry weights and plants inoculated with pathogens alone had the lowest. Fresh and dry weights of plants inoculated with combinations of *Trichoderma* and *Phytophthora* isolates were higher than those of plants inoculated with pathogens alone and lower than those of plants inoculated with *Trichoderma* isolates (Figs 3, 4).

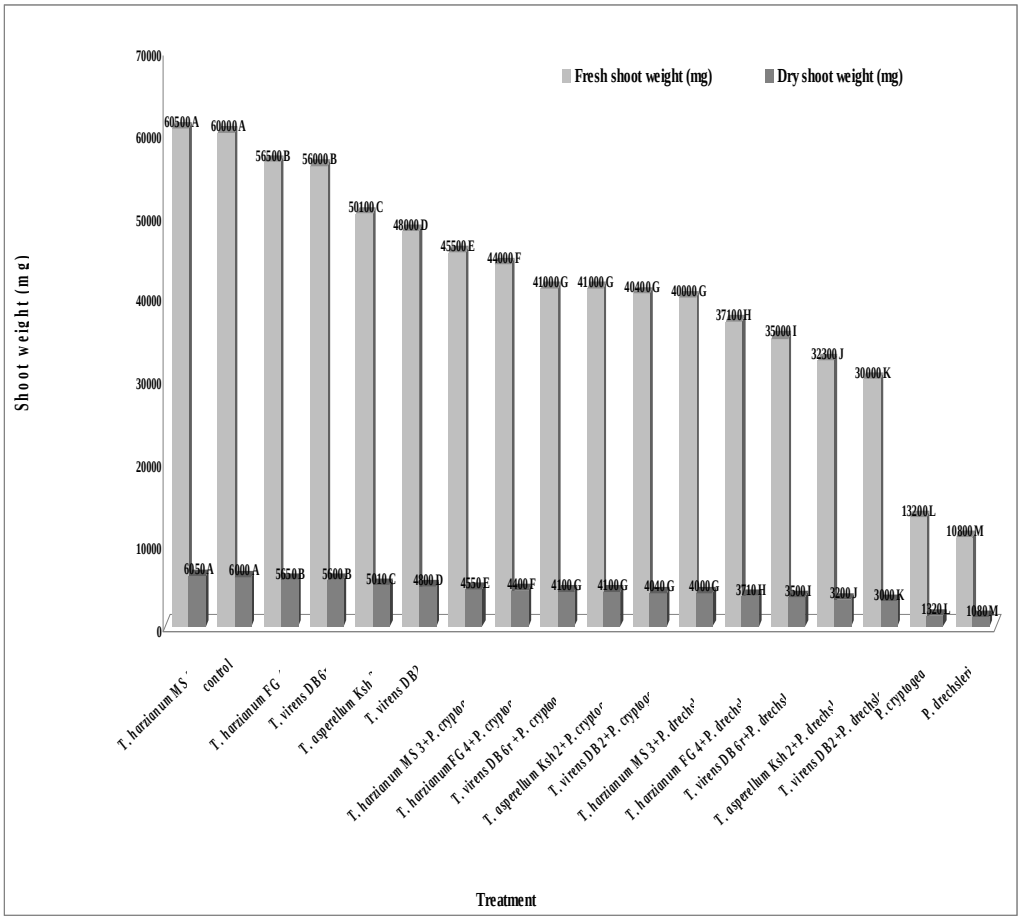

Fig. 3. Shoot weight (fresh and dry weight) of sugar beet in the presence of *Trichoderma* spp. and *Phytophthora drechsleri* and *P. cryptogea* after six weeks. Mean values at the column peaks followed by the same letter are not significantly different at $P \leq 0.05$ by Tukey's test

DISCUSSION

Isolates of *Trichoderma* spp. have demonstrated to be mycoparasitic on a number of fungi (34, 35 and 49). We reported here the isolation of a variety of mycoparasitic *Trichoderma* isolates that have the potential to suppress Phytophthora root rot on

sugar beet caused by *P. drechsleri* and *P. cryptogea*. We screened several *Trichoderma* isolates using both *in vitro* and *in planta* tests. Antagonism involving *Phytophthora* spp. has so far received less attention (50). To our knowledge this is the first report on the isolation and application of biological control agents of the Phytophthora root rot on sugar beet *in planta* or *in vitro*.

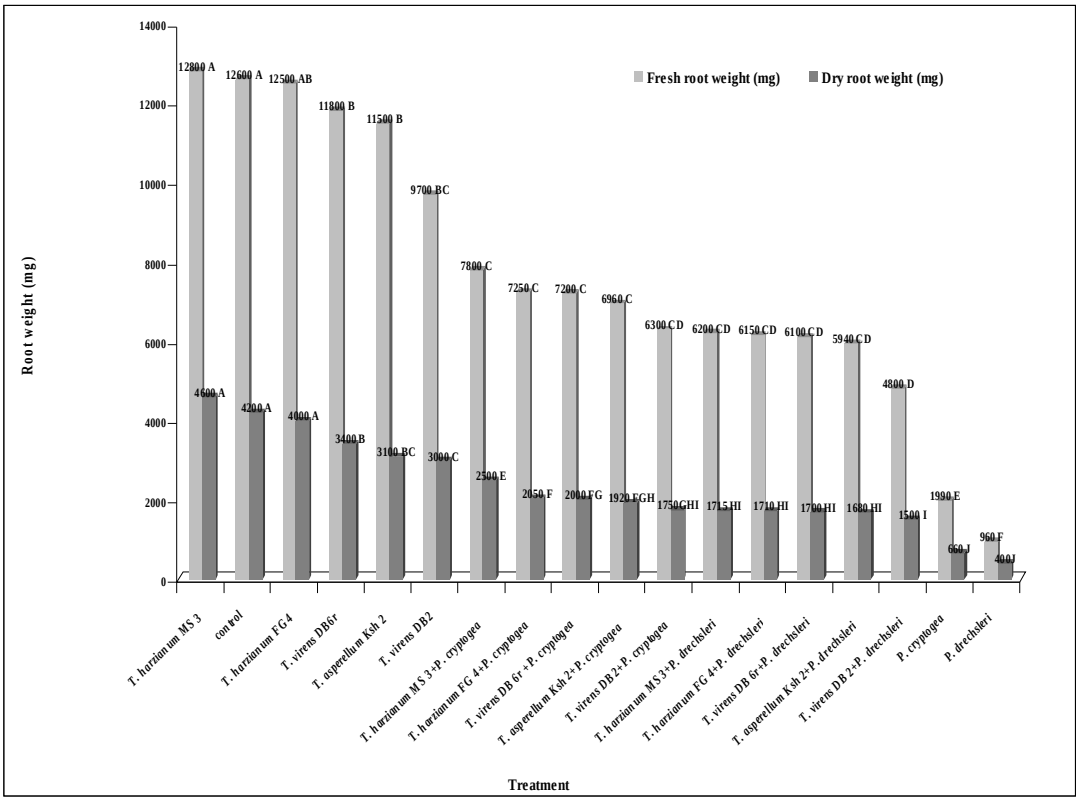

Fig. 4. Root weight (fresh and dry weight) of sugar beet in the presence of *Trichoderma* spp. and *Phytophthora drechsleri* and *P. cryptogea* after six weeks. Mean values at the column peaks followed by the same letter are not significantly different at P ≤ 0.05 by Tukey's test

The *in vitro* precolonized plate method and culture on slide assay narrows the initial collection of candidate isolates to a manageable number for subsequent, more time-consuming assays (38). These *in vitro* assays were used to demonstrate that the selected *Trichoderma* isolates had a potential to be mycoparasitic on a number of causal agents of Phytophthora root rot on sugar beet.

In cultures on slides, growth of *Phytophthora* isolates was generally inhibited; the host cell contents disorganized and the hyphae lysed and were parasitized. Similar reactions were reported previously by Barnett & Binder (5), Elad *et al*. (24) and Sid Ahmed *et al*. (50) who noticed inhibition of growth, lysis and parasitism of some species of *Phytophthora* by *Trichoderma* spp., but not of *P. drechsleri* and *P. cryptogea*. According to Elad *et al*. (24), hyphal lysis is due to enzyme activity of *Trichoderma* isolates at the contact points.

Five *Trichoderma* isolates (*T. asperellum* Ksh_2, *T. harzianum* FG_4, *T. harzianum* MS_3, *T. virens* DB_2, and *T. virens* DB_{6r}) showed the best effects in reducing root rot (Table 2). These isolates were selected for use in greenhouse experiments.

Progress, colonization and sporulation of *Trichoderma* isolates on *Phytophthora* colonies were observed. Similar results with other fungi had been

reported previously by Etebarian *et al*. (26), Behbudi *et al*. (6), and Sid Ahmed *et al*. (50).

Non-volatile metabolites produced by some of our *Trichoderma* isolates inhibited the growth of the plant pathogens tested. Volatile metabolites also had inhibitory effects but the non-volatile metabolites seem to be more effective in growth inhibition.

Mycoparasitism, antibiosis, competition, promotion of plant growth and induction of systemic resistance in plants are mechanisms suggested to be involved in the antifungal activity by *Trichoderma* spp. (12, 32, 34, 36 and 48). Inhibitory effects of *Trichoderma* metabolites were reported in numerous studies (*e. g.* 19, 20 and 31) and this phenomenon and related mechanisms have been analysed by many authors (13 and 37). *T. virens* is well known to produce gliotoxin, viridin and gliovirin (43). It also produces endochitinase, which acts synergistically with gliotoxin to inhibit germination of conidia of some fungi (21). *T. harzianum* is known to produce various toxic and antibiotic metabolites (15, 19, 20 and 41) and enzymes (41) and peptaibiotics compounds which are involved in the inhibition and lysis of pathogenic fungi.

Our laboratory experiments show great variation in the inhibition of radial growth of the phytopathogen by different isolates of *Trichoderma*. *Trichoderma* species in general show antifungal activity against *Phytophthora* isolates by mycoparasitism, antibiosis and competition (26, 6, 50 and 52).

T. harzianum still is a heterogenous complex of genetically different strains (23); its isolates mostly show antifungal activity through mycoparasitism, and competition, whereas *T. virens* isolates act by producing mainly nonvolatile metabolites with high antibiotic effects. In our laboratory experiments *P. cryptogea* (St1) was generally more sensitive to antagonism than *P. drechsleri* (SCRP232).

Laboratory bioassays employing plant parts do not completely imitate natural field conditions, but were helpful in eliminating inefficient isolates from the screening tests. Our greenhouse experiments show that *T. harzianum* MS_3, FG_4, and *T. virens* DB_{6r} significantly reduce sugar beet seedling root rot. The reduction seems to be related to reduced population density of *Phytophthora* isolates in the substrate and perhaps to alterations caused by *Trichoderma* isolates in the *Phytophthora* hyphae, and also to production of volatile and nonvolatile metabolites with fungistatic effects, as observed *in vitro*. These results are supported by reports of other authors (32).

The establishment of an antagonist in a soil or substrate and its subsequent proliferation may be an important factor in biological control of pathogens (40). Malajczuk (44) described *Phytophthora* spp. as weak competitors and *Trichoderma* spp. as active parasites of *Phytophthora* spp., contributing to their breakdown and decay in soil. Also the addition of wheat bran led to increases in the populations of both organisms when they were applied to the substrate together, suggesting that this nutrient source stimulated not only *Trichoderma* isolates but also *Phytophthora* isolates although to a lesser degree. The pathogens can obviously also use the added nutrient.

The reduction of root rot was not related to the stimulation of plant growth. In the pots inoculated with *Trichoderma* isolates alone and mixtures of *Phytophthora* and *Trichoderma* isolates, fresh and dry weights were less than those of the uninoculated plants. This suggests that in our case the *Trichoderma* isolates, while reducing the Phytophthora root rot, did not stimulate plant growth. *Trichoderma* isolates alone reduced apical growth and fresh and dry weight of plants. Results of

other authors support these observations. Ghisalberti *et al*. (30) observed that *T. harzianum* isolates reduced take-all even when they did not seem to promote plant growth. Fang & Tsao (28) showed that *Pythium nunn* reduced the incidence of the disease caused by *Phytophthora* spp. but did not favor plant growth. Similar results are reported by Abada (1), Etebarian *et al*. (26) and Sid Ahmed *et al*. (50). Certain *Trichoderma* metabolites can be harmful to plants (30 and 56).

In laboratory experiments, antibiotic metabolites produced by *T. virens* had higher antagonistic effects on pathogens than those of other species. However, in greenhouse experiments *T. harzianum* isolates were more effective than other species in reducing Phytophthora root rot.

In this study the antagonistic activities of *T. asperellum* and *T. atroviride* isolates were also considerable. Only recently their antagonistic potential is being studied. Our results showed the ability of these species for employing them in biological control research. However, according to our results *T. harzianum* MS_3 appears to be the most effective isolate for future biocontrol studies.

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فعالیت آنتاگونیستی گونه‌های تریکودرما روی پوسیدگی فیتوفتورایی ریشه‌ی چغندر قند

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چکیده - شبه‌گونه‌های تریکودرما از مهم‌ترین آنتاگونیست‌های قارچ‌های بیمارگر گیاهی می‌باشند که شناسایی و تعیین توان آنتاگونیستی آنها کمک زیادی به مطالعات کنترل زیستی می‌کند. هدف اصلی از این مطالعه تعیین توان کنترل‌زیستی جدایه‌های بومی تریکودرما، جدا شده از مزارع چغندرقند، علیه گونه‌های *Phytophthora drechleri* و *P. cryptogea* عوامل پوسیدگی ریشه چغندرقند می‌باشد. طی دو فصل زراعی (1386 و 1387)، 85 جدایه از گونه‌های *Trichoderma* از مزارع چغندر استان فارس جدا سازی شد. بر اساس خصوصیات ریخت‌شناختی و فیزیولوژیکی حداقل هشت گونه شامل *T. asperellum*، *T. atroviride*، *T. brevicompactum*، *T. harzianum*، *T. longibrachiatum*، *T. spirale*، *T. tomentosum* و *T. virens* تشخیص داده شد. فعالیت آنتاگونیستی جدایه‌های به دست آمده مانند توان رقابتی، کلونیزه‌کردن و پادزیستی، در شرایط آزمایشگاهی و گلخانه‌ای مورد بررسی قرار گرفت. شبه‌گونه‌ها و حتی جدایه‌های مختلف یک شبه‌گونه از نظر سازوکارهای آنتاگونیستی متفاوت بودند. در شرایط آزمایشگاهی جدایه هایی از *T. asperellum*، *T. atroviride*، *T. harzianum* و *T. virens* در روش تشتک‌های از قبل کلونیزه شده، هم‌چنین جدایه‌هایی از *T. asperellum*، *T. atroviride* و *T. harzianum* در روش کشت متقابل، از نظر قدرت رقابتی توانایی بالایی نشان دادند. جدایه هایی از *T. harzianum* و *T. atroviride* از نظر قدرت کلونیزه‌کردن ریشه‌ی جدایه‌های بیمارگر *Phytophthora*، توانایی بالایی نشان دادند. جدایه‌های *T. asperellum* Ksh_2، *T. virens* DB_{6r}، *T. virens* DB_2 و *T. virens* DB_3، *T. virens* DB_{6r}، *T. virens* DB_3، *T. asperellum* Ksh_2 و *T. harzianum* MS_3 بیشترین درصد بازدارندگی رشد جدایه‌های بیمارگر *Phytophthora* را داشتند. در شرایط گلخانه‌ای سه جدایه‌ی *T. harzianum* MS_3، *T. harzianum* FG_4 و *T. virens* DB_{6r} بیشترین تأثیر را در کنترل پوسیدگی فیتوفتورایی گیاهچه‌های چغندرقند نشان دادند. با توجه به نتایج این تحقیق به نظر می‌رسد که جدایه‌ی *T. harzianum* MS_3 بهترین جدایه‌ی بومی برای مطالعات بعدی کنترل‌زیستی این بیماری می‌باشد.

واژه‌های کلیدی: ***Phytophthora cryptogea*، *Phytophthora drechsleri*، *Trichoderma* spp.، چغندر قند، کنترل زیستی**

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