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IN VITRO ANTAGONISTIC EFFECTS OF *TRICHODERMA* SPP. ON SEVERAL SOIL-BORNE PLANT PATHOGENIC FUNGI

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Abstract

In vitro studies with *Trichoderma* spp., soil-borne fungal antagonists, demonstrated that a number of isolates produced volatile and non-volatile metabolites capable of inhibiting the growth and sporulation of several soil-borne plant pathogenic fungi. Microscopic observations showed that *T. harzianum* and *T. viride*, isolated from soil samples from Ahwaz and Karaj, adversely affected the mycelial growth of *Rhizoctonia solani*, the causal agent of seed, root rot and damping-off of bean, by hyphal contact, coiling, penetration, necrosis, lysis and in some cases fragmentation of the pathogen hyphae. *T. harzianum* hyphae grew parallel to those of *Phytophthora drechsleri*, the causal agent of root rot of cucumber, and produced appendages that attached themselves to *P. drechsleri* hyphae. Isolates of *T. harzianum* from Ahwaz and *T. viride* from Shahriar, Karaj, significantly reduced the germination of pseudosclerotia of *Colletotrichum coccodes*, the causal agent of brown stem, root rot and black dot of potato. The Ahwaz isolate of *T. harzianum* inhibited mycelial growth and germination of *Phytophthora erythroseptica*, the causal agent of pink rot of potato tubers, without penetrating the pathogen hyphae. *Trichoderma* spp. also reduced the mycelial growth and spore germination of *Fusarium solani*, the causal agent of black root rot of chickpea.

Introduction

Species of *Trichoderma* Pers.: Fr. are considered potent antagonists of many soil-borne plant pathogenic fungi [12]. These antagonists are capable of suppressing the mycelial growth and spore germination of many fungal pathogens and are thus potential biocontrol agents. Trichodermal antagonism may involve mycoparasitism, antibiosis (antimicrobial metabolites and volatile compounds) and competition for food or space.

Keywords: Antagonistic effects; *Colletotrichum coccodes; Fusarium solani; Rhizoctonia solani; Trichoderma* spp.

Dennis and Webster [6] showed that *Trichoderma viride* Pers.: Fr. was able to hydrolyze and also penetrate the hyphae of *Phytophthora erythroseptica* Pethybr., the causal agent of pink rot of potato tubers. Wells *et al.* [15] also demonstrated the colonization of *Sclerotium rolfsii* Sacc., the causal agent of root rot in plants, by *T. harzianum* Rifai. Wright *et al.* [16] showed the antagonistic effects of two isolates of *T. koningii* Qudem and one isolate of *T. viride* on *Sclerotinia sclerotiorum* (Lib.) de Bary and *S. minor* Jagger. Luo *et al.* [9] studied the antagonistic effect of parasitic fungi on sclerotia of *S. sclerotiorum*, a wide-

host range fungal pathogen. Smith *et al.* [14] found that *Trichoderma* spp. were able to prevent root and crown rot diseases on apple trees caused by *Phytophthora* spp. and *P. cactorum* (Leb. & Cohn) Schroet. in particular.

Metabolites from mutants of *Trichoderma* species decreased the effectiveness of soil-borne fungal pathogens in the seed rhizosphere by protecting the roots from fungal infection [1]. Furthermore, a few *Trichoderma* spp. also produced plant growth regulators. Bazgir and Okhovvat [3] were able to identify isolates of *Trichoderma* spp. that were capable of controlling seed rot and damping-off of bean caused by *Rhizoctonia solani* Kühn. The level of control by *Trichoderma* was equal to or more effective than that of fungicides under greenhouse conditions. It has already been shown that an isolate of *T. harzianum* resistant to chlorothalonil, iprodione and benomyl could be used in combination with these chemicals to control soil-borne plant diseases [13].

In this study, isolates of *Trichoderma* spp. were examined against selected species of soil-borne plant pathogenic fungi in order to investigate their antagonistic effects and mechanisms as biocontrol agents.

Materials and Methods

Trichoderma spp. were isolated from field soil and humus using Davet selective medium [5] or were obtained from various agricultural research institutes in Iran [2] (Table 1). Plant pathogenic fungi were also isolated from root tissues of infected plants. Four approaches were adopted to investigate the antagonistic effects of *Trichoderma* spp. on growth and spore germination of different soil-borne pathogenic fungi.

I) In a double-culture test, three methods were used to study mycoparasitism on potato dextrose agar

Table 1. Iranian isolates of *Trichoderma* species and some pathogenic fungi

Fungal name	Isolate and source
Trichoderma Pers.: Fr. species *T. harzianum* Rifai	Soil from a bean field at the College of Agriculture, Karaj
T. harzianum	Soil from a bean field at Golestan, Ahwaz
T. harzianum	Soil from a chickpea field at the College of Agriculture, Karaj
T. koningii Qudem.	The fungal collection of the Arts & Science Research Organization, Tehran
T. viride Pers.: Fr.	Edible fungus from Iran Fungal Culture Company, Karaj
T. viride	Soil from a bean field at Shahriar, Karaj
T. viride	The fungal collection of the Plant Pests and Diseases Research Institute (PPDRI), Tehran
T. viride	University of Tehran, College of Agriculture, Karaj
Rhizoctonia solani Kühn	Bean, College of Agriculture, Karaj
Phytophthora erythroseptica, Pethybr.	Potato, (PPDRI), Tehran
P. drechsleri Tucker	Cucumber, PPDRI
Colletotrichum coccodes (Wallr.) Hughes	Potato, PPDRI
Fusarium solani (Mart.) Apple & Wr.	Chickpea, from a field at the College of Agriculture, Karaj

(PDA): (a) a mycelial disc of *Trichoderma* sp. was placed on one side of a 9 cm-diameter Petri dish and a mycelial disc of a pathogenic fungus on the opposite side. There were four replicate dishes for each *Trichoderma*-pathogen combination. Petri plates were incubated at 25°C for five days and then slides were prepared for microscopic observations; (b) a blank space (1 cm^2) created between the antagonist and the pathogen on PDA was used to study the parasitism of the pathogen's hyphae by *Trichoderma* spp. after five days under a compound microscope; and (c) sterile microscope slides were placed between the antagonist and the pathogenic fungus on PDA in a Petri dish. After five days of incubation at 25°C, the slides were stained with a drop of cotton blue-lactophenol and observed under a compound microscope.

II) Two *Trichoderma* species were used to examine their inhibitory effects upon the viability of pseudosclerotia of *Colletotrichum coccodes* (Wallr). Hüghes. *C. coccodes* was cultured on one side of a PDA plate already colonized by Ahwaz and Institute isolates of *T. harzianum* and Shahriar and Institute isolates of *T. viride* on the opposite side. These plates were incubated at 25°C for 21 days. *C. coccodes* was separately cultured on PDA as a control. In order to determine the effect of *Trichoderma* spp. on the viability of pseudosclerotia of *C. coccodes*, pseudosclerotia were washed into a sieve (pore size, 86 µm), rinsed three times with distilled water and dried on filter paper. Half of the pseudosclerotia from each treatment (five Petri dishes for every isolate with 20 pseudosclerotia per dish) were incubated on PDA at 25°C. After five days, the total number of germinated pseudosclerotia was counted for each treatment. The percent mortality of pseudosclerotia was calculated using the following formula:

Total # of dead pseudosclerotia in a treatment - Total # of dead pseudosclerotia in control / Total # of dead pseudosclerotia in control × 100.

The remaining pseudosclerotia from each treatment were surface-sterilized in 95% ethanol for 30 seconds, rinsed in distilled sterile water and plated on PDA. After five days, the total number of germinated pseudosclerotia was counted and used to determine the percent mortality by the above formula.

III a) The inhibitory effect of non-volatile metabolites secreted by *Trichoderma* spp. on selected soil-borne pathogenic fungi was studied by incubating two agar disks (5 mm diameter) from three-day-old cultures of *Trichoderma* spp. in 100 ml Davet broth medium devoid of toxic compounds at 25°C and 50 rpm on a shaker. After ten days, the mycelial content of each flask was filtered through a Millipore filter (0.22 µm) and the filtrate was diluted 20 and 33 percent with PDA. Isolates of the pathogenic fungi were grown on the PDA containing fungal extract at 25°C. Mycelial growth of each individual pathogenic fungi was measured for seven days. There were four replicates for each treatment. Controls were PDA containing Davet medium only. Percent reduction in mycelial growth of each fungal isolate was computed as follows:

Colony diameter (mm) in control-Colony diameter (mm) in treatment / Colony diameter in control × 100

III b) The inhibitory effect of non-volatile metabolites secreted by *Trichoderma* spp. on the spore germination of *Fusarium solani* was tested on PDA containing *Trichoderma* metabolites in Davet medium. Non-volatile metabolites from *T. harzianum* isolate Ahwaz and *T. viride* isolate Shahriar were prepared at 30% concentration by adding 2 ml of spore suspension of *Trichoderma* species (2.5×10^6 spores/ml) from a six-day-old culture in Davet medium grown under a fluorescent light, to 250 ml of Davet broth medium devoid of toxic compounds. Following this, 6 ml of non-volatile secretions was obtained from a 10-day-old shake culture (60 rpm), filtered through a 0.45 µm filter paper and then mixed with 14 ml of melted PDA. Controls contained 6 ml Davet broth and 14 ml PDA. After the PDA solidified, a drop of *F. solani* isol. 1 spore suspension was evenly spread onto the surface of each Petri plate and incubated at 25°C. After 30 hrs of incubation, germinated and non-germinated spores were counted.

IV) The inhibitory effect of volatile metabolites from different *Trichoderma* spp. on selected pathogenic fungi was investigated by growing the antagonist on PDA for 36 hrs and inverting it over the fungal pathogen in a Petri plate. The two Petri dishes were sealed off with a tape and incubated at 25°C. Mycelial growth rate of each pathogen was recorded after four days and then daily for another eight days. There were four replicates for each fungus-antagonist combination. Controls were pathogens growing individually on PDA in the absence of *Trichoderma* species.

Results

Isolates of *Trichoderma* spp. reacted differently to *Rhizoctonia solani* with respect to mycoparasitism and volatile and non-volatile compounds. In a double culture test, all *Trichoderma* spp. inhibited and overgrew the mycelium of *R. solani* differently (Fig. 1). In most cases, *Trichoderma* spp. sporulated abundantly and seemed to produce metabolites that caused the suppression of *R. solani*. Microscopic observations showed hyphae of *Trichoderma* isolates coiling

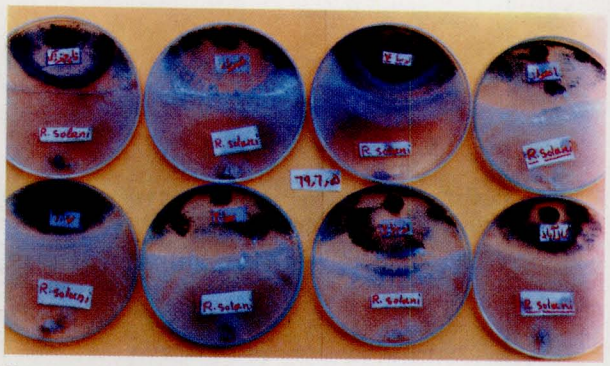

Figure 1. Inhibitory effect of different isolates of *Trichoderma* and *Gliocladium* on mycelial growth of *Rhizoctonia solani* in a double-culture test on PDA medium at 25°C after three days. Top, from left to right: *T. viride* isol. edible fungus; *T. viride* isol. Shahriar, Karaj; *T. viride* isol. 4, College of Agriculture, Karaj; and *T. harzianum* isol. Ahwaz.
Bottom, from left to right: *T. viride* isol. Institute; *T. harzianum* isol. Institute, Tehran (PPDRI); *T. viride* isol. 2 College of Agriculture, Karaj; and *G. virens* isol. bean field in Kamalabad, Karaj.

around and penetrating the *R. solani* hyphae and thus restricting its growth. Ahwaz and Institute isolates of *T. harzianum* caused lytic breakdown and hyphal fragmentation of *R. solani* (Fig. 2). Non-volatile metabolites produced by *T. harzianum* and *T. viride* and diluted 20 and 33 percent in PDA inhibited the mycelial growth of *R. solani* (Fig. 3). Shahriar isolate of *T.*

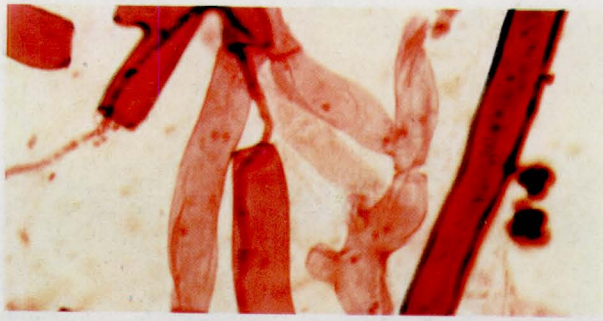

Figure 2. Lytic breakdown of *Rhizoctonia solani* mycelia (thicker hypha) by *T. harzianum* (thinner hypha).

viride was the most potent inhibitor as compared to Ahwaz and Institute isolates of *T. harzianum*. This inhibition is also shown in Table 2 where *T. viride* isolate Shahriar reduced the mycelial growth of *R. solani* by 70% compared to only 5% inhibition by *T. harzianum* isolates.

Volatile metabolites produced by *T. viride* isolate Shahriar inhibited mycelial growth of *R. solani* by 84%, whereas the inhibition by *T. harzianum* isolates was around 63% (Table 3). However, volatile metabolites from *T. harzianum* isolate Ahwaz caused 87% growth inhibition of *Phytophthora drechsleri* Tucker when the former was seeded on PDA 48 hrs in advance of the latter (Table 4). This is compared to only 32% growth inhibition when both were seeded simultaneously. Microscope slide observation revealed that

Table 2. Inhibitory effect of non-volatile metabolites from three isolates of *Trichoderma* species on mycelial growth of *Rhizoctonia solani* on potato dextrose agar after 72 hours at 25°C

Trichoderma species	Isolate	%Dilution of metabolites in PDA (1)	Mean colony diameter of *R. solani* (mm)	%Reduction in mycelial growth of *R. solani* (2)
T. harzianum	Ahwaz	20	84.0	5.6 b
		33	85.0	4.5 b
T. harzianum	Institute	20	86.7	2.4 b
		33	83.3	6.4 b
T. viride	Shahriar	20	26.7	70 a
		33	27.6	69 a
Control	Extract from Davet Medium	20	89.0	0 b
		33	89.0	0 b

(1) Metabolites extracted from shake culture of the *Trichoderma* spp. after 10 days. Each treatment had four replicates.
(2) Numbers in column followed by the same letter are not significantly different at α= 0.01.

Figure 3. Inhibitory effect of non-volatile compounds produced by *Trichoderma* isolates on mycelial growth of *Rhizoctonia solani*. Top: *T. harzianum* isol. Ahwaz (left); *T. harzianum* isol. Institute (right). Middle: control.
Bottom: *T. viride* isol. edible fungus (left); *T. viride* isol. Shahriar, Karaj (right).

Table 3. Inhibitory effect of volatile metabolites from *Trichoderma* species on mycelial growth of *Rhizoctonia solani* on potato dextrose agar after 72 hours at 25°C

Trichoderma species (1)	Isolate	Mean colony diameter of *R. solani* (2) mm)	% Decrease in mycelial growth (3)
T. viride	Shahriar	14.7	84 e
T. viride	Institute	23.5	74 d
T. harzianum	Ahwaz	35.0	61 b
T. harzianum	Institute	33.5	63 c
T. koningii	Arts and Science Research Organization	33.0	63 c
Control		90.0	0 a

(1) The PDA dishes were seeded with *Trichoderma* species 48 hours in advance of seeding with *R. solani*
(2) Four replicates per treatment used.
(3) Numbers in column followed by the same letter are not significantly different at $\alpha = 0.05$, LSD= 1.154.

Table 4. Inhibitory effect of volatile metabolites from the Ahwaz isolate of *Trichoderma harzianum* on mycelial growth of *Phytophthora drechsleri* after six days on potato dextrose agar at 25°C

Replication	Colony diameter of *P. drechsleri* (1) (mm)	Colony diameter of *P. drechsleri* after 48 hours (2) (mm)
1	65	10
2	67	14
3	54	15
4	64	11
5	55	12
Mean	61	12.4
Control	90	90
%Decrease in mycelial growth	32.2	86.5

(1) Both *T. harzianum* and *P. drechsleri* were grown on a Petri dish at the same time.
(2) The Petri dishes were seeded with *T. harzianum* 48 hours in advance of *P. drechsleri*.

T. harzianum hyphae grew parallel to that of *P. drechsleri* and produced appendages on the pathogen hyphae, but no coiling occurred.

Table 5 shows the inhibitory effect of volatile metabolites produced by different isolates of *Trichoderma* spp. on mycelial growth rate of *Colletotrichum coccodes*. *T. harzianum* isolates Institute and Ahwaz caused inhibition of *C. coccodes* mycelial growth by 44%, whereas growth inhibition by *T. viride* isolates Institute and Shahriar was 27 and 38%, respectively. On the other hand, non-volatile extracts from *T. viride* isolates Institute and Shahriar reduced the growth rate of *C. coccodes* by 18 and 55%, respectively, compared to only 3.5 and 5% inhibition by *T. harzianum* isolates Ahwaz and Institute (Table 6). The inhibitory effect of *Trichoderma* isolates on germination of surface-sterilized and non-sterilized pseudosclerotia of *C. coccodes* is shown in Table 7. Whereas *T. viride* isolates caused around 70% inhibition of surface-sterilized pseudosclerotia, *T. harzianum* isolates reduced germination by 95%. However, no significant difference was observed between the two *Trichoderma* spp. inhibiting the germination of nonsurface-sterilized pseudosclerotia.

The inhibitory effect of non-volatile metabolites from different isolates of *Trichoderma* spp. on mycelial growth of *Phytophthora erythroseptica* Pethybr. is shown in Table 8. *T. viride* isolate Shahriar inhibited the growth of *P. erythroseptica* by 92%, whereas the inhibition by *T. harzianum* isolates was between 0-2.5%. When agar disks from *T. harzianum* and *T. viride* culture plates were transferred to a PDA medium containing 100 ppm benomyl, a fungicide inhibitory to the mycelial growth of *Trichoderma* spp, the growth of *P. erythroseptica* was inhibited by these antagonists. On the other hand, volatile metabolites produced by *T. viride* isolate Shahriar and *T. koningii* ASRI isolate caused 30% inhibition of *P. erythroseptica* as compared to 3 and 17% growth inhibition by *T. harzianum* isolate Ahwaz and *T. viride* isolate Institute, respectively. After one week, *P. erythroseptica* mycelium was completely overgrown and lysed by *T. harzianum* isolates Ahwaz and Institute (Table 9).

Non-volatile compounds produced by *T. harzianum* isolates Ahwaz and Karaj caused around 18% inhibition of spore germination of *Fusarium solani* isol. chickpea-Karaj (Table 10). The inhibitory effect of non-volatile compounds seems to be concentration-dependent. As shown in Figure 4, non-volatile metabolites produced by *T. viride* isolate Shahriar caused a maximum inhibition of *Fusarium solani* mycelial growth, at the highest concentration.

Discussion

As the results in this study demonstrate, isolates of *Trichoderma* spp. inhibited the mycelial growth and spore germination of soil-borne pathogenic fungi *R solani, C. coccodes, Phytophthora* spp. and *F. solani* to different degrees. These phytopathogenic fungi have wide host ranges according to studies by Chesters and Hornby [4] on *C. coccodes* and Ershad [7] in Iran.

Non-volatile metabolites produced by *T. viride* seem to be much more effective in inhibiting fungal growth than those produced by *T. harzianum*. The growth inhibitions caused by non-volatile metabolites from *T. viride* were 92% (*P. erythroseptica*), 70% (*R. solani*), 65-73% (*C. coccodes* pseudosclerotia) and 18-46% (*C. coccodes* mycelium), and those caused by *T. harzianum* were 94% (*C. coccodes* pseudosclerotia), 19% (*F. solani*), 2-5% (*R. solani* and *C. coccodes*) and 1-2% (*P. erythroseptica*). These findings suggest that phytopathogenic fungi are much more sensitive to the non-volatile compounds produced by different isolates of *T. viride* than to those produced by *T. harzianum*. On the other hand, volatile metabolites produced by *T. harzianum* appear to be much more effective in inhibiting fungal growth than its non-volatile metabolites. Volatile metabolites from *T. harzianum* caused growth inhibitions of 86% (*P. drechsleri*), 43% (*C. coccodes*) and 3-17% (*P. erythroseptica*). Volatile metabolites from *T. viride* isolates also caused similar levels of inhibition: 84% (*R. solani*), 28% (*C. coccodes*) and 3-30% (*P. erythroseptica*). Volatile compounds from *T. koningii* inhibited the growth of *P. erythroseptica* by 30%.

These results are consistent with those obtained by Munnecke *et al.* [10] with *Armillariella mellea* (Vohl: Fr.) P. kumm, the causal agent of root rot of trees, in that *Trichoderma* spp. colonized the pathogenic fungus. Okhovvat and Karampour [11] also showed the effectiveness of fungal antagonism as a biocontrol strategy to suppress the growth of *F. solani*, the causal agent of chickpea black root rot. Papavizas [12] demonstrated that *Trichoderma* spp., in addition to the production of toxins such as viridin, trichodermin and gliotoxin, synthesized hydrolytic enzymes, such as chitinase, cellulase and β-1, 3-glucanase and other non-volatile metabolites capable of inhibiting the growth of fungal pathogens. It is thus possible that similar types of antogonistic mechanisms exist for *Trichoderma* spp. in our study. *Trichoderma*-produced volatile and non-volatile compounds can be very effective against rhizospheric fungal pathogens depending on the nature of their antibiosis. In practice, wheat bran can be impregnated with intact *Trichoderma* or

Table 5. Inhibitory effect of volatile metabolites from different isolates of three *Trichoderma* species on mycelial growth rate of *Colletotrichum coccodes* after 13 days on potato dextrose agar at 25°C

Trichoderma species	Isolate	%Mycelial growth rate	%Growth inhibition
T. koningii	The fungal collection of the Arts & Science Research Organization, Tehran	105 (1)	-4a
T. viride	Institute	73	27.4b
T. viride	Shahriar	64	38 b
T. harzianum	Institute	57	43.3b
T. harzianum	Ahwaz	56	44b
Control	–	100	0a

(1) This isolate increased the mycelial growth rate of the pathogen.
* Treatments followed by the same letters are not significantly different at $\alpha = 0.01$.

Table 6. Inhibitory effect of non-volatile extract of *Trichoderma* isolates on mycelial growth of *Colletotrichum coccodes* seven days after incubation at 25°C

Trichoderma species	Source of isolate	Volume of non-volatile extract added (a)	*C. coccodes* mycelial growth (b)	Percent inhibition mycelial growth of *C. coccodes*
T. harzianum	Institute	20	54.25	2.2 ab
//	Institute	33	53.57	3.5 ab
//	Ahwaz	20	54	2.7 ab
//	Ahwaz	33	52.75	5 b
//	Shahriar	20	30	46 d
//	Shahriar	33	25	55 e
//	Institute	20	45.5	18 c
//	Institute	33	45.5	18 c
Control	—	20	55.5	0 a
//	—	33	55.5	0 a

(a) Non-volatile extract of *Trichoderma* isolates added to 100 ml of potato dextrose agar (PDA)
(b) Mean growth (mm) of four culture plates seven days after incubation.

Table 7. Inhibitory effect of *Trichoderma* isolates on surface-sterilized (S) or non-sterilized (NS) pseudosclerotia of *Colletotrichum coccodes*

Trichoderma species	Isolate	%Pseudosclerotia (a)				Reduction in germination of pseudosclerotia by *Trichoderma*	
		Growth *Trichoderma*		*C. coccodes*			
		NS	S	NS	S	NS	S
T. viride	Shahriar	95	0	5	22	89	73 b
T. viride	Institute	96	0	4	29	90	65 b
T. harzianum	Institute	100	98	0	0	94	94 a
T. harzianum	Ahwaz	100	92	0	0	94	95 a

(a) There are five replications in each treatment, each with 20 pseudosclerotia plated on potato dextrose agar (PDA). Hyperparasitism period by *Trichoderma* isolates 21 days and five days on PDA.
(b) Numbers in column followed by the same letter are not significantly different at $\alpha = 1\%$.

Table 8. Inhibitory effect of non-volatile metabolites from different isolates of two *Trichoderma* species on mycelial growth rate of *Phytophthora erythroseptica* after seven days on potato dextrose agar (amended with 100 ppm of benomyl) at 25°C

Trichoderma species	Isolate	%Dilution of extract	Mean colony diameter (mm) of *P. erthroseptica*	%Decrease in mycelial growth (a)
T. harzianum	Institute	20	9.36	1.02 ab
//	//	33	9.50	0 a
//	Ahwaz	20	9.38	0.9 ab
//	//	33	9.27	2.4 b
T. viride	Shahriar	20	0.70	91.5 d
//	//	33	0.70	92.6 d
//	Edible fungus	20	8.06	14.9 c
//	// //	33	8.13	14.4 c
Control	Extract of	20	9.47	0 a
//	Davet medium	33	9.50	0 a

(a) Numbers in the column followed by the same letter are not significantly different at $\alpha = 0.01$. Each treatment contained four replicates.

Table 9. Inhibitory effect of volatile metabolites of five *Trichoderma* species on mycelial growth of *Phytophthora erythroseptica* on potato dextrose agar at 25°C (a)

Trichoderma species	Isolate	Mean colony diameter (mm) (a)	%Reduction in mycelial growth (b)
T. viride	Shahriar	50.3	30.0 b
T. harzianum	Ahwaz	69.8	3.0 a
T. harzianum	Institute	59.3	17.6 ab
T. viride	Institute	59.8	17.0 ab
T. koningii	Arts and Science Research Institute	50.8	29.4 b
Control	PDA	72.0	0 a

(a) Growth mean of four culture plates per *Trichoderma* species
(b) Numbers in the column followed by the same letter are not significantly different at $\alpha = 0.01$.

Table 10. Inhibitory effect of non-volatile compounds produced by *Trichoderma* species on spore germination of *Fusarium solani* isol. chickpea from Karaj.

Treatment (a)	Isolate	Mean of spore germination (b)	%Inhibition of spore germination (c)
Control (Davet's medium PDA)		90.3	0 a
T. harzianum	Ahwaz	73.0	19.2 b
T. harzianum	Karaj	74.0	18.1 b

(a) 30% dilution: 6 ml Davet mixed with 14 ml PDA containing *Trichoderma* species.
(b) X= average number of three culture plates showing spore germination.
(c) % Inhibition of spore germination= $\frac{\overline{XC} - \overline{XT}}{\overline{XC}} \times 100$ [11] $\alpha = 0.01$ LSD= 5.42.

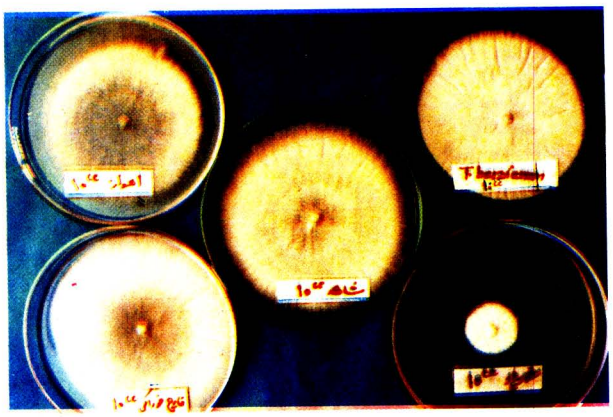

Figure 4. Inhibitory effect of different concentrations of non volatile compounds produced by *Trichoderma viride* isol. Shahriar on mycelial growth of *Fusarium solani*.
Top, from left to right: 10%, 5%, and control.
Bottom, from left to right: 15% 20% and 25%.

its metabolic compounds and then applied to the soil as a biocontrol agent. This has been used on *R. solani* in both greenhouse and field experiments [3]. The volatile compounds may penetrate through the free spaces in soil, thereby disinfecting the plant pathogenic fungi as fumigants do.

Kaiser and Hannan [8] reported that seed treatment with conidia of *Penicillium oxalicum* Currie and Thom significantly reduced seed rot and pre-emergence damping-off of chickpea, caused by *Pythium ultimum* Trow, in two naturally infested soils in eastern Washington state, USA. Papavizas [12] showed that *T. harzianum* together with the fungicide pentachloronitrobenzene, α methyl bromide, could be used to control diseases caused by *Rhizoctonia* in vegetables, tomatoes and strawberries. This author suggested that the use of fungicides such as thiram, methalaxyl, prosimidon and vinchlozline have no adverse effect on *Trichoderma*, whereas benzimidazole does.

In this study, we believe that benomyl may inhibit the seed rot caused by *R. solani* and *Fusarium* spp. as a seed treatment, but it may have no effect on *Phytophthora* spp. and thus can be used together with antagonistic fungi in an integrated control program.

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