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Inhibition of *Trichoderma* Species from Growth and Zoospore Production of *Phytophthora Drechsleri* and Their Effects on Hydrolytic Enzymes

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Abstract

Understanding the function of *Trichoderma* species in the control of *Phytophthora drechsleri* in pistachio orchards is very important. In this study, the effects of liquid extra-cellular secretions and volatile compounds secreted by 27 isolates of *Trichoderma harzianum*, *T. crassum*, *T. koningii*, *T.aureoviride*, *T. asperellum*, *T. brevicompactum*, *T.longibrachiatum* and *T. virens* were investigated on *Phytophthora drechsleri* growth and zoospore production. Due to cell wall combination of *P. drechsleri*, the ability of *Trichoderma* isolates in the production of β-1,3 glucanase and cellulase was evaluated in media with different carbon sources. The inhibitory effects of the 16 isolates of *Trichoderma* from growth of *P. drechsleri* were examined in a dual culture test. The results showed that *Trichoderma* isolates had a variable effect on the growth and zoospore production of *P. drechsleri*. *Trichoderma harzianum*-136 and *T. harzianum*-8279 revealed the highest inhibitory effect on radial growth of *P. drechsleri* in 20 and 30 percent concentrations of liquid extra-cellular secretions, respectively. Both isolates also showed the highest inhibitory effect on zoospore production of *P. drechsleri* in 10 percent concentration of extra-cellular liquid secretions. In the volatile compounds test, *T. harzianum*-8279 had the highest effect on the growth of *P. drechsleri*. In all *Trichoderma* isolates, the activity of β-1,3 glucanase was higher than cellulase activity. The enzyme production was also higher in the liquid medium containing the cell wall of *P. drechsleri* compared to glycerol as a carbon source. The highest activity of β-1,3 glucanase and cellulase was observed in *T. harzianum*-8279.

Keywords: β-1,3 glucanase, Cellulase, *Phytophthora* root rot disease, Pistachio, *Trichoderma*.

Introduction

Phytophthora root is one of the most important diseases of pistachio, which can cause economically considerable damages to the pistachio trees in favorable environmental conditions (Mohammadi, 2010). Several species of *Phytophthora*, including *P. pistaciae*, *P. drechsleri*, *P. citrophthora*, *P. nicotianae*, *P. cryptogea* and *P. parsiana*, have been reported as the causal agents of pistachio gummosis from Iran (Ashkan *et al.*, 1995, Aminaee and Ershad, 1991, Banihashemi, 1999, Fani *et al.*, 2004, Fattahi Ardekani *et al.*, 2000, Mirabolfathi and Ershad, 1986, Mirabolfathi *et al.*, 2004, Banihashemi, 1989, Miraboulfathi *et al.*, 2001, Mostowfizadeh -Ghalamfarsa *et al.*, 2008). To control the disease, several agricultural and chemical methods are used. The biological control of plant pathogens has been attended as an alternative disease management strategy due to its ability to provide environmentally-friendly disease control (Vigo *et al.*, 2000). Among the biocontrol agents, *Trichoderma* species have a special status. Mechanisms that have

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been proposed to explain how the fungus antagonizes its hosts are: competition, mycoparasitism and antibiosis (Ghisalberti and Rowland, 1993, Haran *et al.*, 1996a, Simon and Sivasithamparam, 1989) or a various combination of these (Chet, 1987; Papavizas, 1985). *Trichoderma* species have a high potential for reproduction and sporulation (Harman, 2006), competitive ability (Harman *et al.*, 2004) and saprophytic survival (Howell, 2003). Presence of high spectrum of antibiotics and enzymes in extra-cellular secretions of *Trichoderma* has been determined (Harman, 2006, Harman *et al.*, 2004, Howell, 2003). Chitinases and β-1,3 glucanases produced by some *Trichoderma* isolates are the key enzymes in the lysis of cell walls during their mycoparasitic action against plant pathogens (Cruz *et al.*, 1992,1995; Shen *et al.*, 1991).

Several species of *Trichoderma* have been isolated and identified from pistachio orchards, which can be used as suitable agents for biological control of pistachio pathogens (Haghdel *et al.*, 2009, Mirkhani *et al.*, 2013). Ayobi *et al.*, (2010) showed that culture filtrates of 12 isolates of *Trichoderma* can inhibit zoospore production of *Phytophthora sojae*. They also reported that the β-1,3 glucanase and cellulase are produced by *Trichoderma* isolates in a liquid medium (Ayobi *et al*., 2010). Volatile and non-volatile compounds of 12 isolates of *Trichoderma* prevented the radial growth of *Phytophthora capsici* in laboratory tests (Behboody *et al*, 2005). Fani *et al.*, (2013) reported that *T. harzianum* isolates can decrease radial growth of *Phytophthora melonis* by producing volatile and nonvolatile compounds (Fani *et al.*, 2013).

Materials and Methods

Preparation of Trichoderma isolates and Phytophthora drechsleri

In this research, 27 isolates of *T. harzianum*, *T. aureoviride*, *T. asperellum*, *T. koningii*, *T. longibrachiatum*, *T. virens*, *T. brevicompactum*, *T. crasum* were used. The isolates were previously isolated from pistachio orchards and identified by Haghdel *et al.*, (2009) and Mirkhani *et al.*, (2013). *Phytophthora drechsleri* had been isolated from infected pistachio tress by Mohammadi (2010).

Dual culture of Trichoderma isolates and Phytophthora drechsleri

The ability of *Trichoderma* isolates to prevent from radial growth of *P. drechsleri* was studied in Corn Meal Agar (CMA) by a dual culture method (Dennis and Webster, 1971).

Production of extra-cellular secretions by Trichoderma isolates

Extra-cellular secretions of *Trichoderma* isolates were prepared based on the Calistru *et al.*, (1997) method. Four plugs of 7 mm in diameter from 3-days-old colonies of *Trichoderma* isolates were added to flasks containing 100 ml of sterilized potato-dextrose broth and shaken for 10 days at 80 rpm. The mycelium was collected from liquid phase by filtration through Whatman no.1 filter paper, and the liquid was then centrifuged for 15 minutes at 5000 rpm. Culture filtrates were sterilized by using millipore filters (0.2 µm pore size) and then added at 5, 10, 20 and 30 % (V/V) to CMA. *Phytophthora drechsleri* was inoculated in the center of the agar plates and incubated at 27 ºC for four days. The CMA medium with no extra-cellular secretions was used as a control. This test was conducted in a completely randomized design with three replicates. Inhibition percent of *Trichoderma* isolates on radial growth of *P. drechsleri* was calculated based on the formula: $N= (A-B)/A \times 100$

Where N was the percentage of growth inhibition, A was the average of the diameter of the control colony and B was the average of the diameter of the treatment colony (Tapwal *et al.*, 2004).

To assess the extra-cellular secretions on zoospore production, seven disks of 5 mm in diameter from 3-days-old colonies of *P. drechseleri* were added to the petri plates containing 15 ml of 5 and 10% of sterilized extra-cellular secretions separately and kept under fluorescent light in room temperature for 48 hours. After sporangium production, the petri plates were

transferred to refrigerator with 5ºC temperature for 30 minutes and then kept at room temperature. Numbers of released zoospores were calculated using a hemacytometer. Sterilized distilled water was used as a control treatment. Inhibitory effect of extra-cellular liquid secretions on zoospore production was calculated using the above mentioned formula.

Production of volatile metabolites by Trichoderma isolates

This test was performed using Dennis and Webster's method (1971) by a little modification. Plugs of *Trichoderma* and *P. drechsleri* were separately placed in the center of 9 cm petri plates containing CMA media. Under sterile conditions, the lid of each *Trichoderma* plate was removed and replaced by a plate containing *P. drechsleri*. The two plates were completely taped together with parafilm and incubated at 27 ºC for 6 days. Inhibitory effect of the volatile compounds of *Trichoderma* isolates on radial growth of *P. drechsleri* was calculated using the previous formula.

Production of β-1,3 glucanase and cellulase by Trichoderma isolates using different carbon resources

The effect of two carbon sources, including cell wall of *P. drechseleri* and glycerol on production of β-1,3 glucanase and cellulose, was investigated. Lyophilized mycelium of *P. drechseleri* was prepared based on the El-Katanty method (El-Katanty *et al.,* 2001).

For each *Trichoderma* isolate, six flasks of 250 ml containing 50 ml of Hancock and Jones's liquid medium (Hancock and Jones, 1987) were prepared. In three flasks, 0.5% glycerol (V/V) was added and in others, 0.5% lyophilized cell wall of *P. drechsleri* was added as a carbon source. Spore suspension was prepared by adding 15 ml of Hancock and Jones's liquid to petri plates containing five day-old colonies of *Trichoderma.* The spore suspension was added to flasks and incubated at 25 ºC. After seven days, solid (mycelium mass) and liquid phases were separated using Whatman No.1 filter paper and liquid phase was centrifuged at 6000 rpm at 4ºC for 40 minutes. The prepared extract was lyophilized to increase the enzyme concentration and kept at -20ºC.

Activity of β-1,3 glucanase was assayed by measuring the amount of the glucose produced from laminarin (Yedidia *et al.,* 2000) and using the Jung and collaborators method (2005). The complete reaction mixture consisted of 100 µl of the prepared enzyme, 25 µl of 1% laminarin and 375 µl of 50 mM acetate sodium buffer (pH: 5). After incubation at 37ºC for one hour, 1.5 ml of 3-amino-5-nitrosalicylic acid (DNS) was added and then heated in boiling water for five minutes. The absorbance was immediately measured at 500 nm using spectrophotometer and the Pars Azmoon Glucose Measuring Kit. One unit of β-1,3 glucanase activity was defined as the amount of enzyme that released 1 µmol of glucose per hour (Jung *et al.,* 2005).

Cellulase activity was assayed by measuring the amount of the glucose produced from carboxymethyl cellulase (CMC) (Yedidia *et al.,* 2000) and using Jung collaborators method (2005). The reaction mixture contained 250 µl of the prepared enzyme and 250 µl of 1% CMC stock solution dissolved in 50 mM sodium acetate buffer (pH: 5). After incubation at 37 ºC for one hour, 1.5 ml of 3-amino-5-nitrosalicylic acid (DNS) was added and then heated in boiling water for five minutes and glucose was determined as already described. One unit of β-1,3 glucanase activity was defined as the amount of enzyme that release 1 µmol of glucose per hour (Jung *et al.,* 2005). After measuring protein concentration based on Bradford (1976), the specific activity of the enzymes (mg g^{-1} of protein) was measured.

Results

From 27 isolates belonging to eight species of *Trichoderma* in dual culture test, 16 isolates were able to prevent the radial growth of *P. drechsleri* (Fig. 1). These isolates consisted of *T. harzianum*-204, *T. harzianum*-4593, *T. harzianum*-8241, *T. harzianum*-8235,

T. harzianum-8279, *T. aureoviride*-117, *T. asperellum*-8219, *T. asperllum*-8210, *T. koningii*-8239, *T. koningii*-8210, *T. longibrachiatum*-105 and *T. crassum*-8222 from Rafsanjan, *T. harzianum*-136 from sirjan, *T. asperellum*-116 from Kerman and *T. virens*-79 from Shahre Babak.

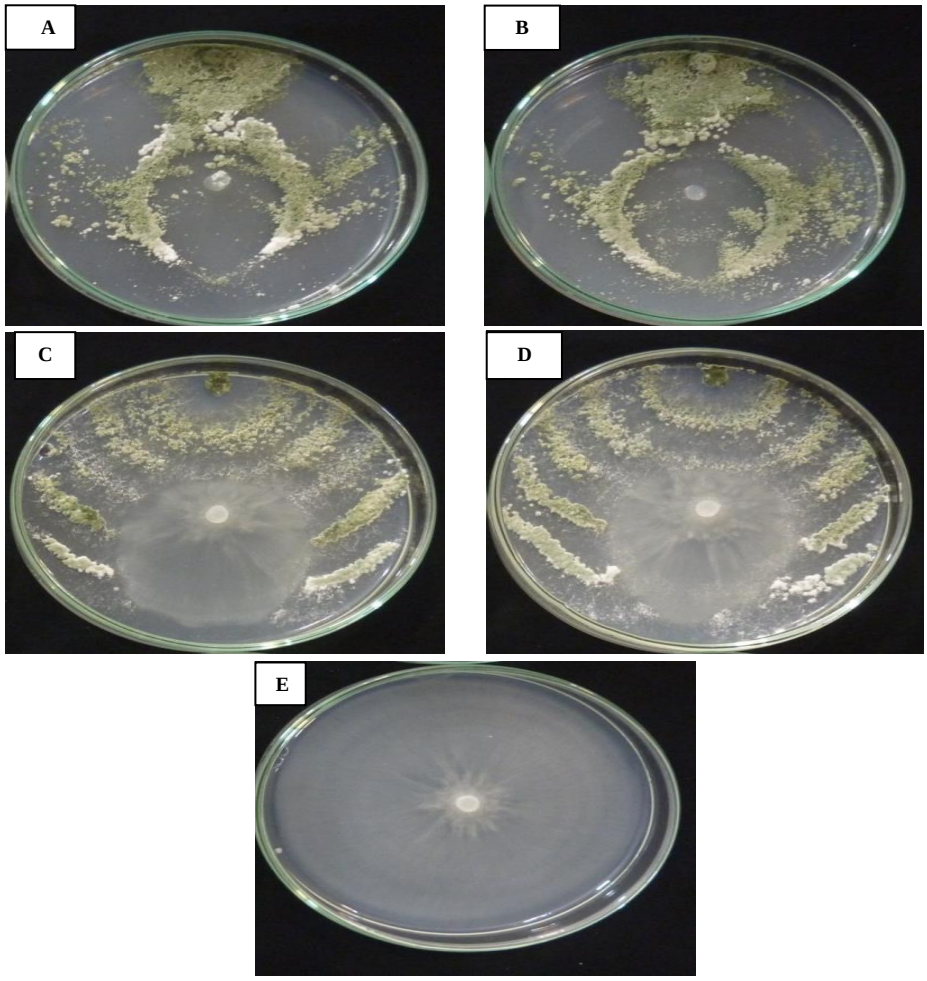

Fig. 1. Inhibition effect of *Trichoderma harzianum*-8279: A, *T. harzianum*-136: B, *T. aureoviride*-117: C and *T. asperellum*-8219: D from radial growth of *Phytophthora drechsleri* compared to the control: E.

Inhibitory effect of extra-cellular liquid secretion on radial growth of Phytophthora drechsleri

Extra-cellular liquid secretion of *Trichoderma* isolates reduced the radial growth of *P. drechsleri*. The results also showed that the inhibitory effects of the isolates of *Trichoderma* species from radial growth of *P. drechsleri* were variable (Fig. 2). In *T. harzianum* species, inhibitory effects varied from 23 percent in *T. harzianum*-204 species to 88 percent in *T. harzianum*-8279, and the isolates of this species were classified in four statistical groups. Two isolates of *T. aureoviride* with 12 and 43 percent inhibitory effects from the fungal growth were classified in two separate statistical groups. Also, three isolates of *T. asperellum* with 14, 20 and 29 percent, respectively, and two isolates of *T. koningii* with 52 and 62 percent inhibitory effects from the fungal growth were classified in separate statistical groups.

The highest inhibitory effect from growth of *P. drechsleri* was observed in 20 and 30 percent concentrations, which showed no significant difference. The lowest inhibitory effect was also observed in the 5% concentration, which was significantly different compared to the others.

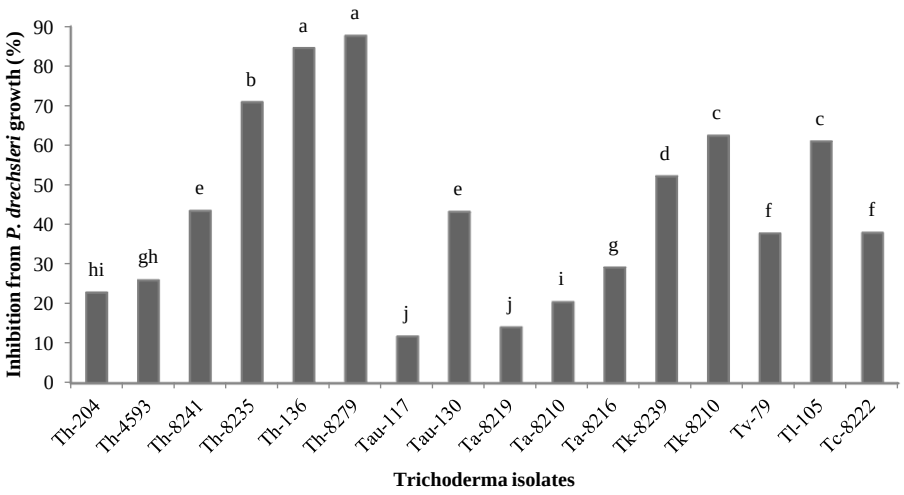

Fig. 2. Inhibitory effects of extra-cellular liquid secretions of *Trichoderma* isolates from radial growth of *Phytophthora drechsleri*. Means with the same letters are not significantly different according to the Duncan's multiple range tests ($p \leq 0.01$). The abbreviations are as following: (Th: *T. harzianum*, Tau: *T. aureoviride*, Ta: *T. asperellum*, Tk: *T. koningii*, Tv: *T. virens*, Tl: *T.longibrachiatum*, Tc: *T. crissum*).

Inhibitory effect of *T. harzianum*-8241, *T. harzianum*-8235, *T. aureoviride*-117, *T. asperellum*-8210, *T. koningii*-8239, *T. virens*-79 and *T. longibrachiatum*-105 in 30 percent concentration of extra-cellular secretions was significantly higher than the 20 percent concentration (Table 1).

Table 1. Inhibitory effect of 20 and 30 percent concentrations of extra-cellular liquid secretions on radial growth of *Phytophthora drechsleri*.

Trichoderma isolates	Concentration of extra-cellular liquid secretions				*Trichoderma* isolates	Concentration of extra-cellular liquid secretions			
	20%		30%			20%		30%	
T. harzianum-204	23	no	27.33	mn	*T. asperellum*-8219	26. 3	mn	28.33	lm
T. harzianum-4593	33.3	kl	34	k	*T. asperllum*-8210	40.7	j	45	i
T. harzianum-8241	53	h	60.67	fg	*T. asperellum*-116	62.3	fg	64	f
T. harzianum-8235	82. 7	de	88	c	*T. koningii*-8239	78.7	e	83.3	d
T. harzianum-136	97	a	96.67	a	*T. koningii*-8210	86.3	cd	90.7	bc
T. harzianum-8279	97. 7	a	97	a	*T. virens*-79	58	g	64.3	f
T. aureoviride-117	13	q	17.33	p	*T. longibrachiatum*-105	88	c	93.3	ab
T. aureoviride-130	18.7	op	23	no	*T. crassum*-8222	60.3	fg	61.3	fg

Means with the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.01$).

Inhibitory effect of extra-cellular liquid secretions on zoospore production of Phytophthora drechsleri

Ten percent concentration of extra-cellular liquid secretions showed a higher inhibitory effect from zoospore production of *P. drechsleri* than the 5 percent concentration, where in all isolates, these effects had significant differences in at the 1% level (Table 2).

Table 2. Inhibitory effect of 5 and 10 percent concentrations of extra-cellular liquid secretions from zoospore production of *Phytophthora drechsleri*.

Trichoderma isolates	Concentration of extra-cellular liquid secretions				*Trichoderma* isolates	Concentration of extra-cellular liquid secretions			
	5%		10%			5%		10%	
T. harzianum-204	10	mn	28	ijk	*T. asperellum*-8219	12	lmn	30	hij
T. harzianum-4593	12	lmn	30	hij	*T. asperllum*-8210	16	lmn	31	ghi
T. harzianum-8241	18	klm	43	efg	*T. asperellum*-116	18	klm	54	de
T. harzianum-8235	20	jkl	56	de	*T. koningii*-8239	36	fghi	78	bc
T. harzianum-136	42	efgh	99	a	*T. koningii*-8210	38	fghi	84	ab
T. harzianum-8279	45	ef	100	a	*T. virens*-79	27	ijk	73	bc
T. aureoviride-117	8	n	27	ijk	*T. longibrachiatum*-105	31	ghi	84	ab
T. aureoviride-130	30	hij	61	cd	*T. crassum*-8222	27	ijk	65	cd

Means with the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.01$).

Trichoderma harzianum-136 and *T. harzianum*-8279 isolates showed the highest inhibitory effect from zoospore production in 10 percent concentration of extra-cellular secretions, while *T. harzianum*-204, *T. harzianum*-4593, *T.aureoviride*-117, *T. asperellum*-8219 and *T. asperellum*-8210 isolates showed the lowest inhibitory effect from zoospore production by *P. drechsleri*.

Inhibitory effect of volatile metabolites of Trichoderma from radial growth of Phytophthora drechsleri

Volatile metabolites produced by *Trichoderma* isolates reduced the radial growth of *P. drechsleri* (Fig. 3). *Trichoderma harzianum* -8279 and *T. harzianum*-204 showed the highest (93%) and lowest (20%) inhibitory effect on radial growth of *P. drechsleri*, respectively. Inhibitory effect of volatile metabolites on radial growth of *P. drechsleri* varied in several isolates of *T. harzianum* from 20 to 93 percent, *T. asperellum* from 54 to 66 percent, *T. aureoviride* from 41 to 72 percent and*T. koningii* from 71 to 77 percent. In addition, *T. virens*-79, *T. longibrachiatum*-105 and *T. crassum*-8222 showed 53, 74 and 63 percent inhibitory effects on the radial growth of *P. drechsleri*, respectively (Fig. 3).

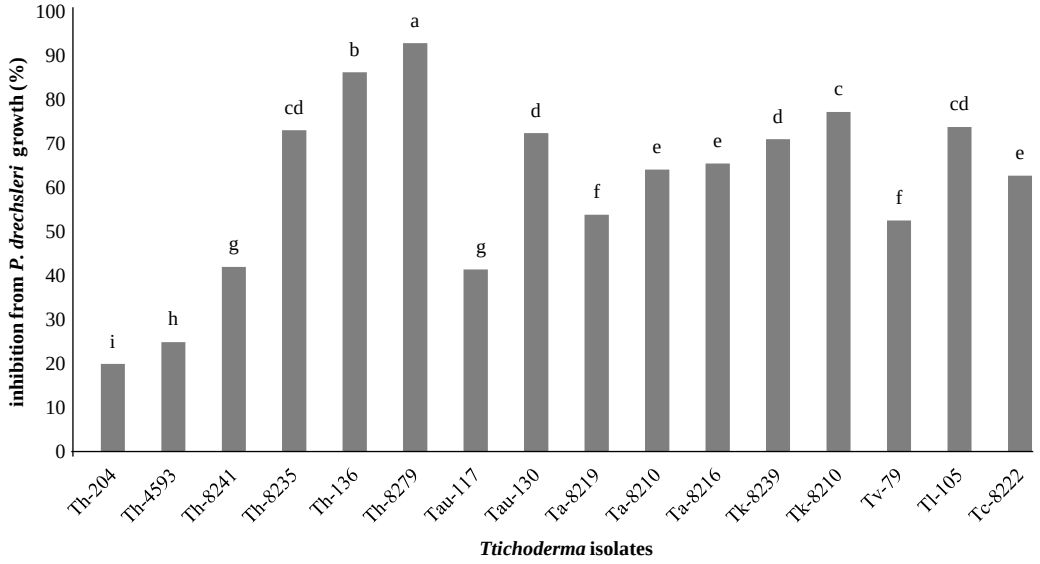

Fig. 3. Inhibitory effect of volatile compounds of *Trichoderma* isolates on radial growth of *Phytophthora drechsleri*. Means with the same letters are not significantly different according to Duncan's multiple range tests ($p \leq 0.01$). The abbreviations are as following: (Th: *T. harzianum,* Tau: *T. aureoviride,* Ta: *T. asperellum,* Tk: *T. koningii,* Tv: *T. virens,* Tl: *T.longibrachiatum,* Tc: *T. crissum*)

Production of β-1, 3 glucanase and cellulase in liquid media with different carbon sources

In all *Trichoderma* isolates, specific activity of β-1,3 glucanase was higher than the cellulase enzyme, which in some cases, the difference was about three times (Table 3). Activity of both enzymes in the medium containing cell wall of *P. drechsleri* was also about two times higher than glycerol. The most specific activity of β-1,3 glucanase (6 U/mg protein) was in *Trichoderma harzianum*-8279. Specific activity of β-1,3 glucanase in *T. aureoviride*-117 and *T. harzianum*-204 isolates was determined 1.36 and 1.43 (U /mg protein), respectively, that was the lowest specific activity. *Trichoderma harzianum*-8279 with 2.7 (U/mg protein) showed the highest specific activity of cellulose enzyme and *T. harzianum*-204, *T. harzianum*-4593, *T. asperellum*-8219 with 0.43, 0.5, 0.44 (U/mg protein) showed the lowest (Table 3). Isolates of each *Trichoderma* species showed variable specific activity of β-1,3 glucanase. Specific activity of β-1,3 glucanase enzyme in a medium containing cell wall of *Phytophthora* varied from 1.43 to 6 (U/mg protein) for *T. harzianum*, 1.59 to 3.03 for *T. asperellum*, 1.36 to 3.11 for *T. aureoviride* and 4.12 to 4.98 for *T. koningii*. In *T. virens*, *T. longibrachiatum* and *T. crassum* isolates, specific activity of β-1,3 glucanase was determined 3.49, 4.09 and 3.1 U /mg of protein, respectively. Specific activity of cellulase in *T. harzianum* varied from 0.43 to 2.7, in *T. asperellum* from 0.44 to 1.16 and in *T. aureoviride* from 0.25 to 1.5 U/mg of protein. In *T. koningii*, *T. virens*, *T. longibrachiatum* and *T. crassum*, specific activity of cellulase was determined 1.77, 1.26, 1.5 and 1.3 U/ mg of protein, respectively.

Table 3. Specific activity of β-1,3 glucanase and cellulase enzymes (U/mg protein) produced by *Trichoderma* isolates in media containing glycerol and cell wall of *Phytophthora drechsleri.*

Trichoderma isolates	B-1,3 glucanase				Cellulase			
	Cell wall		Glycerol		Cell wall		Glycerol	
control	0	r	0	r	0	p	0	p
T. harzianum-204	1.43	mn	0.93	q	0.43	kl	0.23	mno
T. harzianum-4593	1.66	l	1.13	op	0.5	jk	0.33	lm
T. harzianum-8241	2.86	hi	1.46	lmn	0.96	h	0.5	jk
T. harzianum-8235	3.53	e	3.03	K	1.37	de	0.62	ij
T.harzianum-136	5/44	b	3.42	ef	2.39	b	1.43	de
T. harzianum-8279	6	a	3.22	fg	2.7	a	1.46	de
T. aureoviride-117	1.36	n	1.02	pq	0.25	mn	0.12	o
T. aureoviride-130	3.11	gh	2.12	K	1.5	d	0.7	i
T. asperellum-8219	1.59	lm	0.85	q	0.44	kl	0.2	no
T. asperllum-8210	2.74	ij	1.26	no	0.96	gh	0.35	klm
T. asperellum-116	3.03	gh	1.66	l	1.16	fg	0.44	kl
T.koningii-8239	4.12	d	2.86	hi	1.77	c	0.63	ij
T. koningii-8210	4.98	c	3.06	gh	1.77	c	0.73	i
T. virens-79	3.49	ef	1.63	lm	1.26	ef	0.47	jkl
T. longibrachiatum-105	4.09	d	2.53	J	1.5	d	0.5	jk
T. crassum-8222	3.1	Gh	1.43	mn	1.3	def	0.49	jk

Means with the same letters are not significantly different according to Duncan's multiple range tests ($p \leq 0.01$).

Discussion

In extra-cellular liquid secretions and a volatile test, *Trichoderma* isolates reduced radial growth of *P. drechsleri*. These effects were variable among the *Trichoderma* isolates and species. *Trichoderma harzianum*-136 and *T.harzianum*-8279 in extra-cellular liquid secretions and *T. harzianum*-8279 in volatile test had the highest inhibitory effect from growth of *P. drechsleri*. Jamdar and colleagues (2013) showed that *Trichoderma* isolates have a variable effect on the radial growth of *Verticillium dahliae*. In the research, *T. harzianum* and *T. koningii* showed the highest effect on the growth of *V. dahliae* in extra-cellular liquid secretions and volatile compounds tests. Ayobi and co-workers (2010) indicated that two species of *T. virens* and *T. brevicompactum* had the highest inhibitory effect on zoospore production of *Phytophthora sojae*. Several isolates of *T. harzianum* also showed variable inhibitory effects on radial growth of *P. melonis* in extra-cellular liquid secretions and volatile compounds (Fani *et al.*, 2013). Different effects of the *Trichoderma* isolates on pathogens

growth has been reported by other researchers (Behboudi *et al.*, 2006, Zavari *et al.*, 2012). Complete inhibitory effect of *T. viride* (100%) and inhibitory effect equal to 60.74 percent in *T. koningii* on growth of *P.capsici* in 25 percent concentration of extra-cellular liquid secretions has been reported by Behboudi and colleagues (2006). They also showed that *T. virens* and *T. viride* had the highest and lowest inhibitory effect on mycelium growth of *P. capsici* in volatile compounds test, respectively (Behboudi *et al.*, 2006). The difference between *T. virens* isolates on the inhibitory effect on growth of *P. drechsleri* has been reported by Zavari and co-worker (2012). In this study, *T. virens*-401.4 with 58.23% was the most effective isolate for inhibition of mycelium growth of *P. drechsleri*, while *T. virens*-304 with 35.1% had the lowest inhibitory effect mycelium growth of *P. drechsleri* (Zavari *et al.*, 2012). Our results showed that although the inhibitory effect of *Trichoderma* isolates on radial growth of *P. drechsleri* had no significant difference between 20 and 30 percent concen-

tration of extra cellular liquid secretion, the inhibitory effect of *Trichoderma* isolates on pathogen growth was higher in the 30% concentration. Similar results have been reported by other researchers (Behboudi *et al.*, 2006, Ayobi *et al.*, 2010, Jamdar *et al.*, 2013).

The present study showed that using the cell wall of *P.drechsleri* as the main carbon source can increase specific activity of β-1,3 glucanase and cellulose compared with glycerol. Specific activity was also variable between *Trichoderma* isolates, which was in agreement with the results of Ridout and colleagues (1986). They showed that glucose and *Rhizoctonia solani* cell wall can influence β-1,3 glucanase and chitinase production by different isolates of *T. harzianum* and *T. viride* (Ridout *et al.*, 1986). Specific activity of β-1,3 glucanase had at least two fold cellulose in all *Trichoderma* isolates. The increase of significant activity of β-1,3 glucanase compared to cellulose in *T. asperellum* was reported by Tondje *et al* (2007). They also showed that the presence of a cell wall of *P. capsici* and *P. megakarya* can result in a five-fold increase in β-1,3 glucanase and cellulose activity compared with other carbon sources. Ayobi and co-workers (2010) also showed the difference between several species of *Trichoderma* based on producing β-1,3 glucanase and cellulase in addition to increase activity of these enzymes in presence of *P. sojae* cell wall in *Trichoderma* species. The difference between 30 *Trichoderma* isolates and*T. virens* for β-1,3 glucanase production has been reported by other researchers (Bahramsari *et al.*, 2005, Zavari *et al.*, 2012). *Trichoderma* species have been known as successful bio-control agents and use several mechanisms including of antibiosis (Howell, 2003), mycoparasitism (Ozbay and Newman, 2004), competition for food (Dinesh and Prateeksha, 2015) to control of plant pathogens. One of the important mechanisms in antagonistic activities of *Trichoderma* species is mycoparasitism, where the antagonist fungus produces extra-cellular hydrolytic enzymes to destroy the cell wall of plant pathogens (Lorito *et al.*, 1998, Haran *et al.* 1996a,b). Several studies have shown that there are many hydrolytic enzymes such as cellulase, glucanase, chitinase, protease and some antibiotics and growth inhibitory compounds in extra-cellular liquid secretions of *Trichoderma* isolates, which play an important role to bio-control plant diseases. (Dickinson *et al.*, 1995, Elad *et al.*, 1983, Hankock and Jones 1987, Lorito *et al.*, 1993, Papavizas 1985, Ridout *et al.*, 1986). Due to the presence of cellulose and β-glucan in *P. drechsleri* cell wall, it can be expected that *Trichoderma* species can hydrolyze the cell wall of the fungus and control *Phytophthora* disease by producing some enzymes such as β-1,3 glucanase and cellulase. In general, this research showed that extra-cellular liquid secretions and volatile metabolites of collected *Trichoderma* isolates from pistachio orchards have an inhibitory effect on radial growth and zoospore production of *P. drechsleri*. *T. harzianum*-8279 and *T. harzianum*-136 isolates were the most effective isolates for bio-control, and β-1,3 glucanase and cellulose are effective in the bio-control of *P. drechsleri*.

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