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Research Article

Screening of native isolates of *Trichoderma* spp. for ability to control cucumber damping-off caused by *Phytophthora drechsleri*

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Abstract: In this study, 41 isolates of the genus *Trichoderma* including six species of *Trichoderma arundinaceum, T. asperellum, T. atroviride, T. harzianum*, *T. longibrachiatum* and *T. virens* were isolated from soils of cucumber fields in Kermanshah province, and their efficacy to control *Phytophthora drechsleri,* the causal agent of cucumber damping off, was investigated in laboratory and greenhouse conditions. In direct confrontation between *Trichoderma* isolates and the pathogen, all isolates were able to promote, establish, and sporulate on pathogen mycelia. Through dual culture test, the most inhibitory effect on pathogen growth (62.89%) was recorded for *T. harzianum* (isolate T1). Volatile compounds of *T. harzianum* (isolate T7) had the greatest mycelial growth inhibition (46.59%) compared with control. Complete growth inhibition of the pathogen was recorded when the growth medium was supplemented with culture filtrate of *Trichoderma* isolates at concentration of 15% and 30%. At the concentrations of 15% and 30%, 18 isolates and 22 isolates completely inhibited the pathogen expansion respectively. In the greenhouse, the results of data analysis on the survival percent of plants in various treatments showed significant differences between the isolates of *Trichoderma* in terms of preventing cucumber damping off. In conclusion, *T. asperellum* showed the highest ability to control Phytophthora damping off and promoting cucumber growth which is a new record for Iran. This species can be an appropriate choice for biological control of the disease caused by *P. drechsleri* in cucumber.

Keywords: Antagonist, biological control, cucurbits, pathogen

Introduction

In Iran yield reductions of cucumber in the greenhouse and field conditions is largely due to the damping off disease caused by *Phytophthora* species (Alavi *et al*., 1982; Alavi and Saber, 1985). The disease causes symptoms such as seedless sowing, concavity in the crown and creating spots on the fruit, which are initially small and then develop to a broad reddish-brown area with unpleasant odor. Finally, the xylems, especially in the crown area, are destroyed and the plant dies while is fully wholesome (Elahinia, 1993). In the case of soil-borne plant pathogens with a wide host range and long survival in the soil, chemical control is not cost-effective and at the same time has adverse effects on human

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health, environment and beneficial soil microorganisms (Faheem Amin *et al*., 2010). Thus biological control of *P. drechsleri* Tucker by fungal antagonists can be essential for the management of the disease. Fungi of the genus *Trichoderma* are a very large group of microorganisms that play a significant role in the environment and use a variety of mechanisms to colonize various ecological niches. Several *Trichoderma* spp. positively affect plants by stimulating plant growth, and protecting them from fungal and bacterial pathogens. They are used in biological plant protection as biofungicides as well as in bioremediation (Błaszczyk *et al*., 2014). Research in recent years have shown that *Trichoderma* species are non-pathogenic and useful symbionts that are well suited against most of the fungal pathogens through the mechanisms of competition, mycroparasitism, antibiotic and enzyme production, stimulation of plant growth and induction of defensive responses in various plants (Howell, 2003 ; Benitez *et al*., 2004). Jamali *et al*. (2016) examined the inhibitory effects of 16 isolates of *Trichoderma* spp. on the growth of *P. drechsleri* in a dual culture test. Their results revealed that *Trichoderma* isolates had a variable effect on the growth and zoospore production of *P. drechsleri*. Two biological control agents encoded as *T. harzianum* Rifai-136 and *T. harzianum*-8279 exhibited the greatest inhibitory effect on radial growth of *P. drechsleri* at 20 and 30 percent concentrations of liquid extra-cellular secretions, respectively (Jamali *et al*., 2016). Several volatile metabolites including lactones, alcohols, terpene derivatives, and alpha-pyron derivatives have been obtained from *T. viride* Pers. under different culture conditions (Zeppa *et al*., 1991). Dickinson *et al*. (1995) introduced the substance 6-Phenyl-2-pyrone from the species *T. harzianum* that penetrates into the soil and acts as a poor gaseous disinfectant. Research results of Hernandez *et al*. (2011) on the antagonistic effects of 31 isolates of *Trichoderma,* from three species: *T. asperellum* Samuels, Liechfeldt & Nirenberg, *T. hamatum* (Bonord.) Bainier and *T. rossicum* Bissett, C. P. Kubicek & Szakacs from different regions of Mexico, on *P. capsici* Leonian, showed that volatile compounds were able to prevent the growth of the pathogen between four to 48 percent. Results of the effect of *Trichoderma* isolates on biological control of cucurbit damping off caused by *P. drechsleri* in greenhouse showed that the inhibitory effect of *T. harzianum* and *T. virens* Miller, Giddens & Foster was more than *T. viride* to inhibit the mycelial growth of *P. drechsleri* (Heidari Faroughi *et al*., 2004). The objective of this study was to investigate and screen different native species of *Trichoderma* against *P. drechsleri*, as well as define the role of these isolates on cucumber plant growth under laboratory and greenhouse conditions.

Materials and Methods

Provision of pathogen isolates and proving pathogenicity

In this research *P. drechsleri*, previously isolated from diseased cucumber plants, was received from Kermanshah Agricultural and Natural Resources Research and Education Center. To confirm the pathogenicity of the specimen, inoculation test was conducted using fungal material on 20-day old cucumber plants. Johnson grass [*Sorghum halepense* (L.) Pers.] leaves were used to prepare the inoculum. The leaves were cut to pieces about one cm placed in 250 ml flasks containing 100 ml of distilled water and autoclaved. Then, from the margin of three-day culture of *P. drechsleri* on CMA, five disks were transferred into the flask containing the cut leaves and incubated for one week at 25 ± 1 °C until the pathogen mycelia grew on the leaves. The Johnson grass leaves colonized with *P. drechsleri* in an approximate amount of five grams per kilogram of soil, were mixed with upper one-third of the potting soil 20 days after planting of cucumber seeds (Azizi *et al*., 2013).

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Sampling from cucumber fields

Samples were collected from 18 farms of cucumber in Kermanshah province. In each farm, on average, five samples of the rhizosphere soil of healthy cucumber plants were taken at a depth of 10 to 30 cm. After mixing, they were put in a nylon bag and transferred to the laboratory and kept at 4 °C.

Isolation of *Trichoderma* spp. from soil

Trichoderma selective medium (TSM) was prepared based on (Elad and Chet, 1983). On the basis of g/l: 3.0 of glucose monohydrate, 1.0 of NH_4NO_3, 0.9 of K_2HPO_4, 0.2 of $MgSO_4$.$7H_2O$, 0.15 KCl, 20 of agar (all chemicals from Merck, Germany) were mixed in one liter of distilled water. After autoclaving, g/l: 0.25 of Chloramphenicol (Biobsic, Canada), 0.2 of Pentachloronitrobenzene (Zigma-aldrich, Germany), 0.15 of Rose Bengal (Merck, Germany) and 0.2 of Captan (WP 50%, Giah corp., Iran) were added to this culture medium at about 45 °C.

To isolate *Trichoderma* spp., 10 g of each soil sample was poured in 10 cm diameter sterile petri dishes and, after preparing a water agar (WA) medium, an approximate volume of 20 ml was deposited on the soil sample and mixed with it. After solidification of the soil and agar mixture, 10 mm plugs were picked up and placed on the above mentioned culture medium.

Screening of *Trichoderma* isolates against *P. drechsleri* in laboratory conditions

Effect of *Trichoderma* isolates to parasitize pathogen mycelia

To perform this experiment, initially a sterile glass slide was placed inside a 10 cm sterile petri dish, and then 10-15 ml of Corn Meal Agar (CMA) (Quelab, Canada) was poured upon it, so that a thin layer of culture medium covered the slide. After solidification, 5 mm disks from the margin of three-day old colonies of *Trichoderma* isolates and *P. drechsleri* were cultured opposite each other. In the control treatment, *P. drechsleri* was the cultured opposite of 5 mm disc of culture medium without the antagonist. The Petri dishes were stored at 25 ± 1 °C in the dark. After 72 and 96 h, when the mycelia grew, the effect of *Trichoderma* isolates on the pathogen mycelia in terms of deformation, twisting and swelling was investigated under the microscope (Iraqi *et al*., 2008).

Effect of *Trichoderma* isolates on *P. drechsleri* growth in dual culture

This experiment was conducted to compare the nutritional competency of different isolates of *Trichoderma* with the pathogen, as well as to compare the ability of these isolates to inhibit the growth of the pathogen and their establishment and advance on the mycelia of *P. drechsleri*. For this purpose, a 5 mm disk from the margin of the 3 day culture of *P. drechsleri* and a 5mm disk from the margin of the 3 day culture of each *Trichoderma* isolate were placed on both sides of the petri dish containing CMA medium. For each isolate four replicates and for control treatment sterile medium culture was applied. Petri dishes were incubated at 25 ± 1 °C. The inhibition percentage of mycelial growth of *P. drechsleri* was calculated after 120 hours using the following equation (Iraqi *et al*., 2008).

$$\text{Percent inhibition of mycelial growth} = \frac{\text{mycelial growth in control} - \text{mycelial growth in treatment}}{\text{mycelial growth in control}} \times 100$$

The effect of volatile metabolites of *Trichoderma* spp. on inhibiting mycelial growth of *P. drechsleri*

In this part of the experiment, a five mm disc from the margin of the three-day culture of the *Trichoderma* isolates was placed in the middle of a Petri dish containing PDA medium. A 5mm diameter disc from a three-day culture of the pathogen was also placed in the middle of a Petri dish containing CMA

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medium. Then, the Petri dishes caps were removed under sterile conditions and the Petri dish containing *P. drechsleri* was inversely placed on the *Trichoderma* container. The joint place of Petri containers was well sealed with a parafilm strip so that the evacuation of the volatile compounds was prevented. In control, instead of *Trichoderma* isolates in Petri, PDA media without the antagonist was used. Petri dishes were stored in an incubator at 25 ± 1 °C, and the colony diameter was measured after 120 hours (Dennis and Webster, 1971). Then, the inhibitory percent of growth was measured using the above-mentioned equation (Iraqi *et al*., 2008).

The effect of culture filtrate of *Trichoderma* isolates on growth inhibition of *P. drechsleri*

For this purpose, initially inside each 250 ml flask, 150 ml of antibiotic-free Potato Dextrose Broth (PDB) (Quelab, Canada) culture medium was prepared and sterilized at a temperature of 121 °C for 20 min. After cooling the broth, each flask was inoculated with four 5 mm discs from the three-day culture of *Trichoderma* isolates. No *Trichoderma* was added in the flask for the control treatment. The flasks were placed on a shaker at 60 rpm for 12 days. Then, using filter syringes with an aperture diameter of 0.22 micron, filtration was performed. The PDA medium containing dilutions of 15% and 30% of the filtered extract were prepared and then 5 mm discs of *P. drechsleri* were placed on these Petri dishes and kept in the incubator at 25 ± 1 °C. After 24, 48 and 72 h, the diameter of *P. drechsleri* colony was measured and percent growth inhibition was calculated as mentioned above (Iraqi *et al*., 2008).

Greenhouse assay

Greenhouse assay was carried out in a completely randomized design pattern with three replications using *Trichoderma* isolates with promising results from laboratory tests.

***Trichoderma* spp. inoculum preparation**

Inoculated wheat seeds were used as *Trichoderma* inoculum according to the procedure described by Ayobi et al., 2010. At the sowing time, five grams of *Trichoderma* inoculum was added per kg of sterile soil (Heidari Faroughi *et al*., 2004).

***Phytophthora drechsleri* inoculum preparation**

Johnson grass leaves were used to prepare the inoculum. For this purpose, some Johnson grass leaves were cut to pieces about 1 cm and sterilized in an autoclave in 250 ml flasks containing 100 ml of distilled water. Then, from the margin of the three-day culture of *P. drechsleri* on CMA, five disks were transferred into the flask containing the cut leaves and incubated for one week at 25 ± 1 °C until the pathogen mycelia grew on the leaves. Five grams of the inoculum were mixed with upper one-third of the potting soil 20 days after planting of cucumber seeds (Azizi *et al*., 2013).

Preparing the pots and cultivating cucumber seeds

In order to prepare the soil in the pots, a 1:1 proportion of cultivation soil and sand was mixed and autoclaved at 75 °C for 12 hours. After cooling, the soil was poured into pots of 15 cm in diameter and six seeds were planted inside each of them. The pots were then kept at greenhouse temperature between 18-32 °C with relative humidity of 70% and 16:8 h of light and dark photoperiod.

Evaluation of the Effect of *Trichoderma* isolates in the presence or absence of *P. drechsleri*

Treatments were prepared as follows; Pots without both microorganisms (control-), *Phytophthora* inoculated Pots (Control +), Pots treated with selected *Trichoderma* isolates plus the pathogen, Pots treated with selected *Trichoderma* isolates. After inoculation of *P. drechsleri*, for better pathogen activity, the pots were flooded for

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48 hours and then irrigated normally. These pots were checked daily and the emergence and progression of the disease was recorded as yellowing, wilting and death of the plants (Browne *et al*., 1995). After observing the damping off symptoms in the control pots, the percent of alive plants were measured in different treatments and replications. Subsequently the dead plants were removed from the soil and washed under a gentle current of tap water. After surface sterilization some symptomatic pieces were cultured on selective culture media (CMA + PARPH) for assurance of the pathogen re-isolation (Ershad, 1992). In the experiment that was conducted to evaluate the effect of *Trichoderma* isolates on root and shoot growth, 8 weeks after cultivation the plants were removed from the soil and these organs were also separated and weighed.

Experimental design and statistical analysis

All laboratory and greenhouse tests were conducted in a completely randomized design. Statistical analysis was carried out by SAS-9.2 software. The mean comparison was also performed with Duncan's test at 5% ($p \leq 0.01$) level.

Results

Proof of pathogenicity

In the initial pathogenicity test of *P. drechsleri* on 20 day cucumber plants, symptoms of the disease appeared as wilting and damping off seven days after inoculation.

The isolated *Trichoderma* spp.

In this study, 41 isolates of *Trichoderma* belonging to 6 species viz *Trichoderma arundinaceum* Zafari, Gräfenhan & Samuels, *T. asperellum, T. atroviride* Bissett, *T. harzianum*, *T. longibrachiatum* Rifai and *T. virens* were identified based on the growth characteristics and color of the colony and microscopic specifications of the conidiophores, conidia, phyalides, chlamydospores and mycelia using valid identification keys (Bissett, 1991; Gams and Bissett, 2002; Samuels, 2004). The name, code number and location of the isolates are given in Table 1.

Laboratory experiments

Microscopic studies of thin layer of culture medium on the slides, revealed that mycelia of all the examined isolates of *Trichoderma* spp. had positive tropism to the pathogen and were able to parasitize it (Fig. 1). In dual culture experiment between *Trichoderma* isolates and *P. drechsleri*, there was significant difference among isolates to inhibit the growth of *P. drechsleri* at 1% probability level. These isolates after a period of 120 h could progress, colonize and sporulate on the pathogen colony. The most inhibitory effect on pathogen growth was found for *T. Harzianum* (isolate T1) recorded as 62.89% (Fig. 2).

Similarly, the statistical comparison of *Trichoderma* isolates in terms of the effect of volatile compounds on the pathogen showed that the difference between the isolates was significant at 1% probability level. Volatile compounds of *T. harzianum* (isolate T7) had the greatest effect on inhibiting mycelial growth as 46.59% compared with control (Fig. 3).

Results of the effect of culture filtrates of the antagonist isolates showed that *Trichoderma* isolates significantly differed in terms of the effect of these secretions on inhibiting mycelial growth of *P. drechsleri* in both concentrations of 15% and 30% at 1% probability level. At the concentration of 15%, 18 of the isolates inhibited growth of the pathogen by 100%, including 16 isolates of *T. harzianum*, one isolate of *T. virens* and one isolate of *T. asperellum* (Fig. 4). At the concentration of 30%, 22 isolates prevented the growth of the pathogen by 100%, including 17 isolates from *T. harzianum*, one isolate from *T. longibrachiatum*, two isolates from *T. virens*, one *T. arundinaceum* isolate, and one isolate from *T. asperellum* (Fig. 5).

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Table 1 The species of *Trichoderma* and their collecting locations.

Code number	Species	Township	District	Village
T1	*T. harzianum*	Kermanshah	Miandarband	Berenjan
T2	*T. longibrachiatum*	Kermanshah	Miandarband	Berenjan
T3	*T. harzianum*	Kermanshah	Baladarband	Malek khatabi
T4	*T. harzianum*	Kermanshah	Baladarband	Sarab niloofar
T5	*T. harzianum*	Kermanshah	Baladarband	Daeechi
T6	*T. harzianum*	Kermanshah	Baladarband	Sarab niloofar
T7	*T. harzianum*	Kermanshah	Baladarband	Chogha zard
T8	*T. harzianum*	Kermanshah	Miandarband	Varleh
T9	*T. harzianum*	Kermanshah	Miandarband	Gohar chogha
T10	*T. harzianum*	Kermanshah	Miandarband	Varleh
T11	*T. harzianum*	Kermanshah	Miandarband	Mahmood abad
T12	*T. longibrachiatum*	Sahneh	Dinevar	Mian rahan
T13	*T.harzianum*	Kermanshah	Miandarband	Yevan
T14	*T. asperellum*	Kermanshah	Baladarband	Malek khatabi
T15	*T. atroviridae*	Kermanshah	Baladarband	Malek khatabi
T16	*T. harzianum*	Kermanshah	Miandarband	Mahmood abad
T17	*T. longibrachiatum*	Sahneh	Dinevar	Mian rahan
T18	*T. harzianum*	Kermanshah	Miandarband	Berenjan
T19	*T. harzianum*	Kermanshah	Baladarband	Chogha kabood
T20	*T. harzianum*	Kermanshah	Miandarband	Gohar chgha
T21	*T. harzianum*	Kermanshah	Alahyarkhani	Ghazanchi
T22	*T. arundinaceum*	Kermanshah	Miandarband	Deh bagh
T23	*T. longibrachiatum*	Sahneh	Dinevar	Mian rahan
T24	*T. harzianum*	Kermanshah	Alahyarkhani	Shahrohk abad
T25	*T. harzianum*	Kermanshah	Alahyarkhani	Balekabood
T26	*T. harzianum*	Sahneh	Dinevar	Mian rahan
T27	*T. harzianum*	Kermanshah	Mahidasht	Ghomsheh
T28	*T. longibrachiatum*	Kermanshah	Miandarband	Deh bagh
T29	*T. longibrachiatum*	Kermanshah	Mahidasht	Ghomsheh
T30	*T. harzianum*	Kermanshah	Miandarband	Mehregan station
T31	*T. harzianum*	Kermanshah	Miandarband	Mehregan station
T32	*T. harzianum*	Kermanshah	Alahyarkhani	Ghazanchi
T33	*T. harzianum*	Kermanshah	Miandarband	Berenjan
T34	*T. harzianum*	Sahneh	Dinevar	Mian rahan
T35	*T. virens*	Kermanshah	Miandarband	Gohar chogha
T36	*T. virens*	Kermanshah	Alahyarkhani	Ghazanchi
T37	*T. longibrachiatum*	Kermanshah	Bala darband	Chogha kabood
T38	*T. harzianum*	Kermanshah	Mian darband	Mehregan station
T39	*T. harzianum*	Kermanshah	Mian darband	Berenjan
T40	*T. longibrachiatum*	Kermanshah	Mian darband	Berenjan
T41	*T. longibrachiatum*	Kermanshah	Mian darband	Khanom abad

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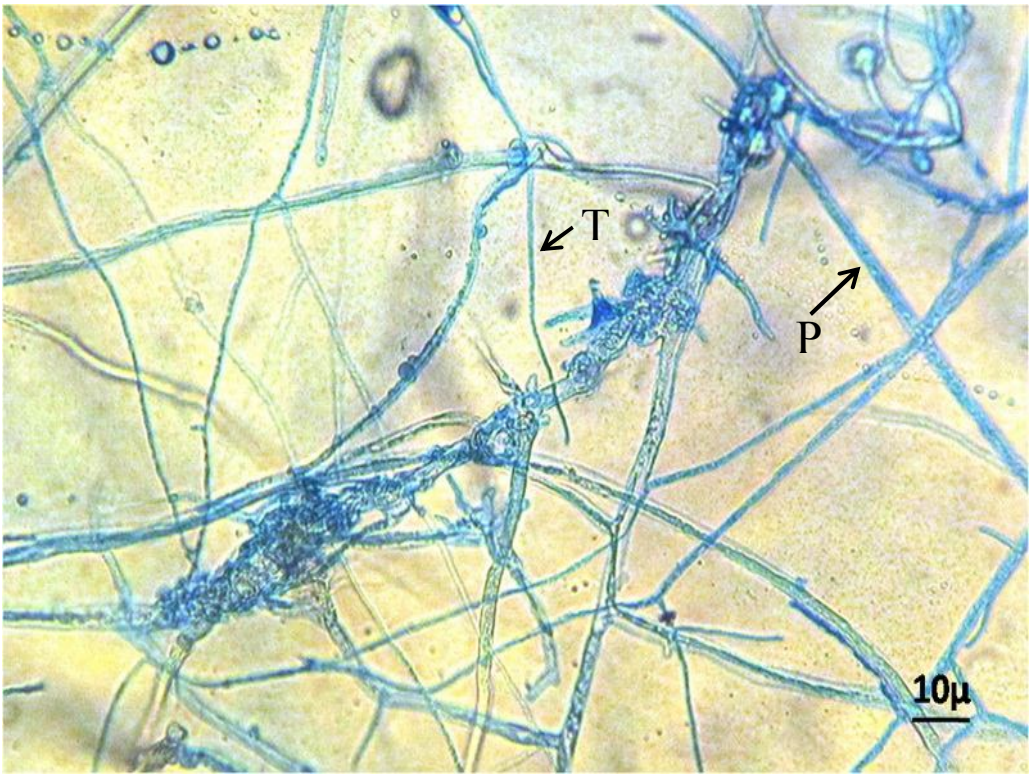

Figure 1 *Phytophthora drechsleri* mycelia parasitized by *Trichoderma harzianum* T7; T: Trichoderma, P: Phytophthora.

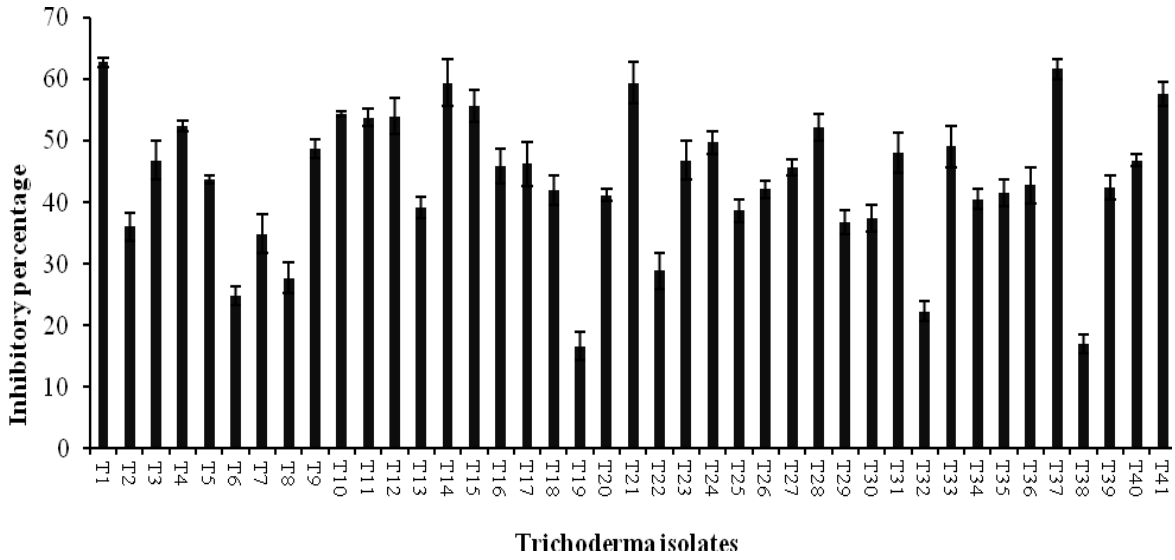

Figure 2 Comparison of inhibition percentage of various isolates of *Trichoderma* spp. in dual culture with *Phytophthora drechsleri*

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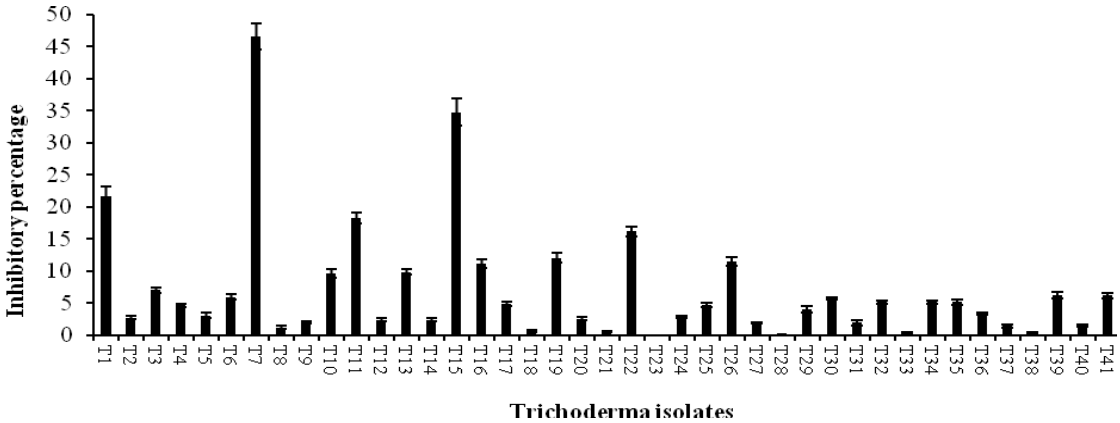

Figure 3 Comparison of inhibitory percentage of volatile compounds of various isolates of *Trichoderma* spp. on the mycelial growth of *Phytophthora drechsleri.*

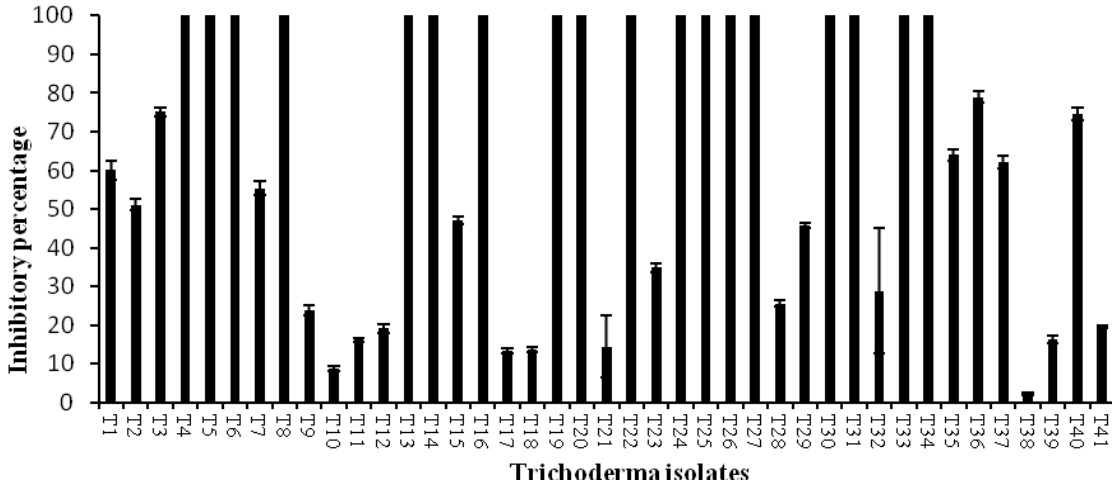

Figure 4 Comparison of inhibitory percentage of various isolates of *Trichoderma* spp. on the growth of *Phytophthora drechsleri* at 15% concentration of culture filtrate.

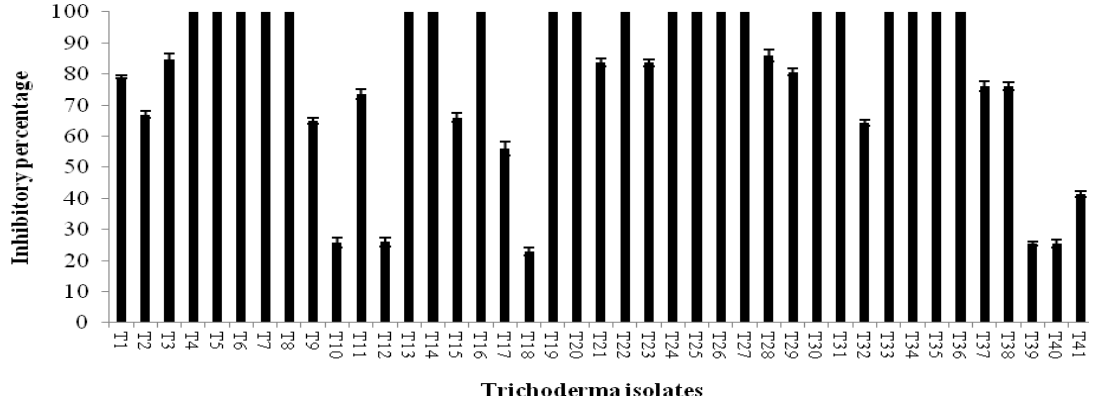

Figure 5 Comparison of inhibitory percentage of various isolates of *Trichoderma* spp. on the growth of *Phytophthora drechsleri* at 30% concentration of culture filtrate.

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Greenhouse experiments

In the greenhouse the results of data analysis on the survival percent of the plants in various treatments showed significant difference between the isolates of *Trichoderma* in terms of preventing cucumber damping off. Also, these isolates were grouped in different classes according to Duncan's comparative test. The most survival of cucumber plants was for *T. harzianum* T4 treatment but *T. harzianum* T7 and *T. asperellum* T14 did not differ statistically with it and were classified in the same group (Fig. 6). The isolate *T. longibrachiatum* T11, *T. harzianum* T12 and *T. harzianum* T32, showed the poorest results and were classified in the same group with the control exclusively inoculated with the pathogen (Fig. 6).

Results of data analysis of shoot fresh weight in the absence of the pathogen showed that the difference between the isolates on this parameter was not statistically significant, but this difference in the case of root fresh weight at 5% probability level was significant. The greatest shoot and root weight was measured for the treatment inoculated with *T. asperellum* T14 (Fig. 7-8).

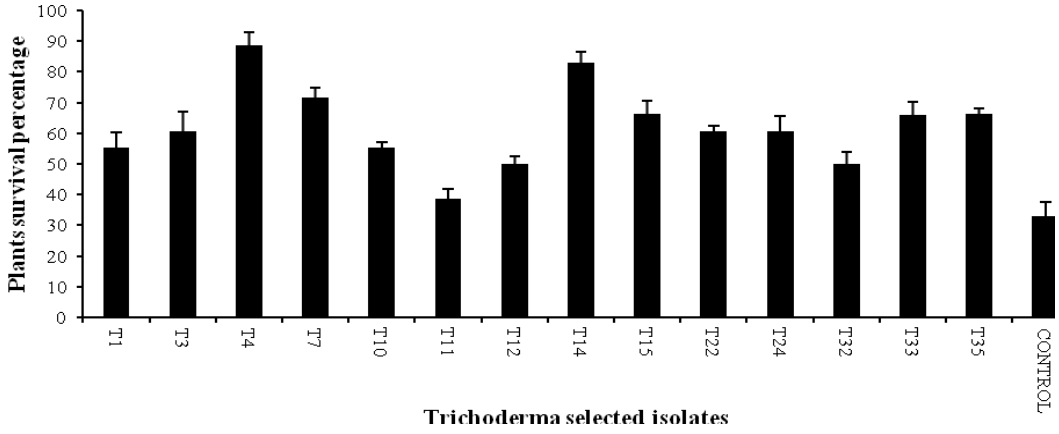

Figure 6 Effect of selected isolates of *Trichoderma* spp. on survival percent of cucumber plants inoculated with *Phytophthora drechsleri.*

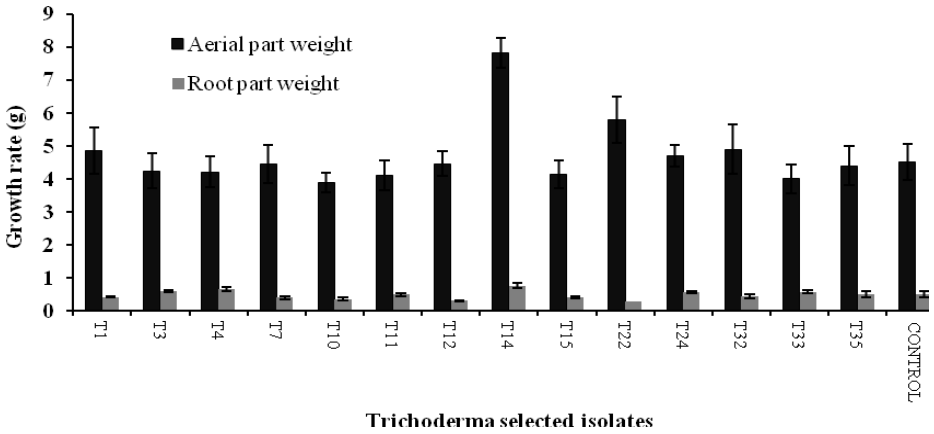

Figure 7 Comparison of the effect of selected isolates of *Trichoderma* spp. on enhancing aerial and root parts weight in the absence of *Phytophthora drechsleri.*

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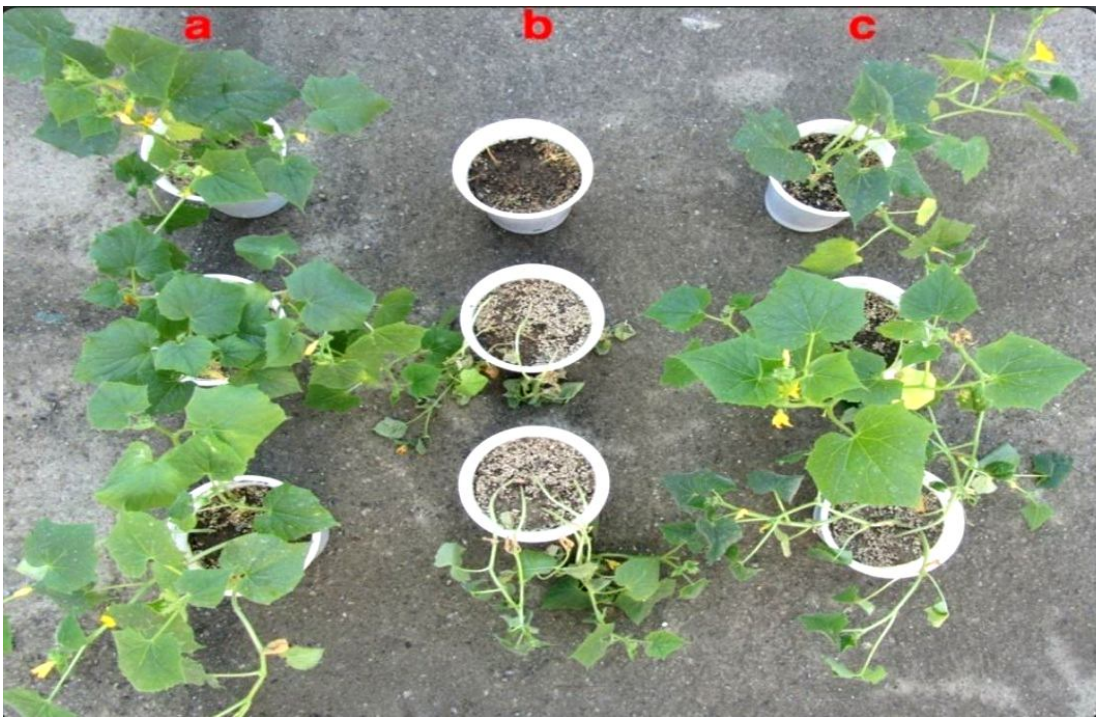

Figure 8 Effect of *Trichoderma asperellum* in controlling cucumber damping off caused by *Phytophthora drechsleri*; a: *P. drechsleri* + *T.asperellum*, b: *P. drechsleri*, c: Control.

Discussion

The main biocontrol mechanisms applied by *Trichoderma* spp. against fungal pathogens are mycoparasitism and antibiosis (Papavisas, 1985; Howell, 1998 & 2003). The process of mycoparasitism has several stages, which include identifying, attacking and subsequently infiltrating and killing the host. In this process, *Trichoderma* produces various enzymes such as chitinase, cellulase, glucanase and so on, which decompose the host cell wall and release oligomers from it (Kubicek *et al*., 2001; Woo *et al*., 2006). Among 16 isolates of *Trichoderma* spp. the highest activity of β-1, 3 glucanase and cellulase was observed in *T. harzianum* (Jamali *et al*., 2016). It is believed that *Trichoderma* spp. secrete degrading enzymes, and if an appropriate fungal host is around will beaffected by these enzymes. *Trichoderma* senses the host's presence through the molecules released from the decomposed host cells, and these released molecules activate a chain of expression of genes associated with mycoprasitism in *Trichoderma* (Harman *et al*., 2004; Lorito *et al*., 2006). In the microscopic examination of the contrast site between different isolates of *Trichoderma* spp. and *P. drechsleri*, it was found that all antagonistic isolates had positive traction to the pathogen mycelia. This tropism can be attributed to a series of chemicals available in the pathogen cell wall. Parasitic mechanisms of *Trichoderma* consist of chemotropism (Chet *et al*., 1981), detection of lectin in the pathogen cell wall (Inbar and Chet, 1992, 1994) and the formation of several structures such as appressorium, infiltrative organs and trapping rings for the pathogen (Elad *et al*., 1983). Non-volatile compounds of *Trichoderma* isolates have various effects on the pathogen. The differences have also been reported among the *Trichoderma* species and even between different isolates of a species in terms of the production of diffusible material inhibiting the fungal growth (Dennis and Webster, 1971). In a study performed by Moayedi and Mostowfizadeh-Ghalamfarsa (2010) among 85

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isolates of *Trichoderma* spp. from sugar beet fields in eight parts of Fars province various species and even various isolates of one species differed in their antagonistic capacities. *Trichoderma asperellum* Ksh2, *T. virens* DB6r, *T. virens* DB2, *T. virens* DB3 and *T. harzianum* MS3 gave the highest growth inhibition against *P. drechsleri* and *P. cryptogea*. Nonetheless in another study the results of greenhouse experiments on antagonistic potential of native *T. harzianum* isolates toward important strawberry pathogen *Verticillium dahliae* showed that there was no significant difference between *T. harzianum* treatments (Mirmajlessi *et al*., 2016). In the present study, it was observed that different isolates of *T. harzianum* have different effect on the pathogen. While T4: *T. harzianum* was the most effective isolate against the pathogen, T11: *T. harzianum* and T32: *T. harzianum* had the least effect on controlling the disease among the tested *T. harzianum* isolates and did not have any significant difference with the control treatment that was inoculated solely with *P. drechsleri*. In the present study two isolates of *T. virens* were investigated in which *in vitro* inhibitory effect on *P. drechsleri* was similar, however significant difference between *T. virens* isolates to inhibit the growth of *P. drechsleri* has been reported (Zavari *et al*. 2012). Nine isolates of *T. longibrachiatum* were investigated in this study most of which showed poor results in laboratory and greenhouse tests. So far, *T. longibrachiatum* has been used to control *H. avenae* (Zhang *et al*., 2014). In terms of the effect of *Trichoderma* isolates on increasing the growth parameters in greenhouse cucumber production, addition of *Trichoderma* inoculum as 3% w/w, in some isolates had increasing and in some others had a deterrent effect (Taqi Nasab, 2012). In this study among 14 isolates, which based on laboratory tests, were selected for greenhouse studies, isolate T14: *T. asperellum* had the greatest ability to control the cucumber damping off and increase plant growth (Fig. 8). Until now, there has been no study on the application of *T. asperellum* in controlling cucumber damping off caused by *P. drechsleri* in Iran. For the first time in this research, the ability of this species to control *Phytophthora* damping off in cucumber was approved. In the study performed by Segarra *et al*. 2013, *T. asperellum* was a useful biological alternative to chemicals for the control of *P. capsici* in pepper. *T. asperellum* T34 and etridiazole (Terrazole®) were compared for their ability to suppress *P. capsici* in pepper crop. T34 reduced disease in most of the assayed situations (up to 71% disease reduction), while etridiazole was effective only when applied at the same time as the pathogen (Segarra *et al*., 2013). Seven isolates of *T. asperellum* were consistent among replicated trials in eliminating recovery of *P. ramorum* from the exposed agar plugs and preventing leaf disk necrosis. Further testing of six *T. asperellum* isolates against two different *P. ramorum* isolates (A1 and A2 mating types) resulted in the same high level of mycoparasitic activity. Based on these results, specific *T. asperellum* isolates have the potential to remediate *P. ramorum* infested soil and have the potential to be developed into a commercially viable product (Widmer, 2014). Considering the function of *T. asperellum* for controlling *Phytophthora* damping off in cucumber and promoting the growth, it seems that this species can potentially be an appropriate option for use in biological control of *P. drechsleri* in cucumber crop.

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غربال جدایه‌های بومی .*Trichoderma* spp برای ارزیابی توانایی آنها در کنترل بوته‌میری خیار با عامل *Phytophthora drechsleri*

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چکیده: در این پژوهش ۴۱ جدایه *Trichoderma* از شش گونه *Trichoderma arundinaceum, T. asperellum, T. atroviride, T. harzianum, T. longibrachiatum, T. virens* از خاک مزارع خیار در استان کرمانشاه به‌دست آمد و تأثیر کنترل‌کنندگی آنها بر علیه *Phytophthora drechsleri* در شرایط آزمایشگاه و گلخانه بررسی شد. در تقابل مستقیم بین جدایه‌های *Trichoderma* و بیمارگر، تمام جدایه‌ها قادر به پیشروی، استقرار و اسپورزایی بر روی میسلیوم‌های بیمارگر بودند. در آزمایش کشت متقابل بین جدایه‌های *Trichoderma* و *P. drechsleri* بیش‌ترین تأثیر بازدارندگی بر روی رشد بیمارگر، توسط *T. harzianum* T1 به میزان ۶۲/۸۹ درصد در مقایسه با شاهد به‌دست آمد. ترکیبات فرّار *T. harzianum* T7 بیش‌ترین تأثیر را در ممانعت از رشد میسلیومی بیمارگر در مقایسه با شاهد به‌میزان ۴۹/۵۹ درصد داشت. توقف کامل رشد بیمارگر در زمانی که محیط کشت قارچ با عصاره فیلترشده جدایه‌های تریکودرما در غلظت‌های ۱۵ و ۳۰ درصد ترکیب شد، به‌دست آمد. در غلظت ۱۵ درصد ترکیبات فرّار ۱۸ جدایه و در غلظت ۳۰ درصد، ۲۲ جدایه از رشد بیمارگر به‌طور کامل ممانعت کردند. نتایج تجزیه داده‌ها مربوط به درصد بقای گیاهان در تیمارهای مختلف در گلخانه نشان‌دهنده تفاوت معنی‌دار بین جدایه‌های تریکودرما در مورد ممانعت از مرگ بوته‌های خیار بود. در مجموع *T. asperellum* بیش‌ترین توانایی را در کنترل بوته‌میری فیتوفتورایی و افزایش رشد خیار نشان داد که گزارش این قابلیت برای ایران جدید است. به این ترتیب می‌توان از این‌گونه به‌عنوان یک گزینه مناسب برای کنترل بیماری ناشی از *P. drechsleri* در خیار استفاده کرد.

واژگان کلیدی: آنتاگونیست، بیمارگر، کدوییان، کنترل بیولوژیکی

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