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A comparative study on different antagonistic mechanisms of *Talaromyces flavus* and *Trichoderma harzianum* in terms of growth inhibition on *Fusarium oxysporum* f. sp. *lycopersici*, causal agent of tomato wilt disease in laboratory conditions

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Tomato is one of the most important nutritional sources in the world. Tomato Fusariumwilt disease with the causal soil-born fungal agent of *Fusariumoxysporum* f. sp. *lycopersici* has extended in worldwide. Antagonistic fungi, *Trichodermaharzianum* and *Talaromycesflavus* have been known as antagonistic agents of Fusarium wilt disease. In this study, the effects of antagonistic mechanisms of *T.harzianum* and *T.flavus* on the causal agent of tomato Fusarium wilt were investigated. For isolation of antagonistic and pathogenic agents, infested soil and plant samples were collected from tomato fields in Shahrood and Varamin regions. The growth inhibitory percent of pathogenic agent colony by antagonistic mechanisms of *T. harzianum* and *T. flavus* including mycoparasitism, volatile and non-volatile compounds production were calculated separately. In this study, eight isolates of *F. oxysporum* were obtained from infected fields which FO-To-V-15 (isolated from Varamin) with maximum disease severity percent (40) was selected as the most virulent isolate for the rest of experiments. Twenty-seven*T. flavus* and nine *T. harzianum* isolates were isolated from soil samples. Among *T. flavus* isolates, maximum inhibitory percent (40.51) was mediated by TF-To-V-18. For this isolate, mycoparasitism mechanism played an important role in their antagonistic activity. However, among *T. harzianum* isolates, maximum inhibitory percent (31.37) mediated by TH-To-V-2. According to the obtained results, the most effective antagonistic mechanism for above mentioned isolates was mycoparasitism. For biological control of Fusarium wilt in tomato fields, the most effective of *T. harzianum* and *T. flavus* isolates were TH-TO-V-2 and TF-TO-V-18 separately.

Keywords: Biological control, Fusarium wilt, *Talaromycesflavus*,*Trichodermaharzianum*, tomato

INTRODUCTION

The cause of disease is *F. oxysporum* fungus that enters the plant through the roots and pervades throughout the plant through the vascular system. Fusarium wilt disease most expansion is in warm regions of world and in acid and sand soils. Disease cause will survive for a long time (over ten years) in the soil and on plant debris that this is because of existence of resistant spores (chlamydospores).

Several studies have shown that *T. flavus* has acted for greater impact on different pathogens with different mechanisms. For example, The most effective mechanism of this fungus For the Verticillium genus, Anti-Bios using non-volatile compounds including cell wall-degrading enzymes (chitinase, glucanase and cellulase) and glucose oxidase, whereas About Rhizoctonia genus both anti-bios and mycoparasitism , have Significant effect on the pathogen deterrence (Inglis and Kawchuk, 2002).

In other studies has Been observed that Even in the case of two species same genus fungi pathogenic, such as *S. sclrotiorum* and *S. rolfsii* , the *T. flavus* fungi through different uses of one kind mechanism, has effective deterrence on pathogens. In these studies have shown that the effects of *T. flavus* on *S.Seclertiorm* and *S.rolfsii* was respectively byhyperparasitism and Micro parasitism(Mc Laren et al., 1982; Madi et al., 1997).

In Iran, for the first time, was reported one strain of *T. flavus* fungus from a cotton field Located in the karkandehGolestan Province Research Center (Naraghi et al., 2003). Their research on this strain results showed that Under laboratory conditions Volatile and nonvolatile compounds in this fungi has reduced the growth of *V. dahliae* colony (Naraghi et al., 2003). Also the volatile and nonvolatile compounds of this antagonists factor causes biological control of greenhouse Potatoes, tomatoes and cucumbers Verticillium wilt disease in laboratory and greenhouse conditions (Naraghi et al., 2010a, b and c).

Also, the Trichoderma species particularly the *T. harzianum* species have Great ability in competition mechanisms (using place and different food supplies), micro parasitismand anti-bios; and are considered as the most important antagonists factor against the fungi plant pathogens (Naemi et al., 2010). Antagonistic effect of this fungi against Fusarium wilt pathogen of beans is proven in the Lab (*F. oxysporium* f.sp. *phaseoli*). Based on these researches, mentioned antagonist factor reduced the disease indices to the rate of 71.4 percent and its deterrence effect on growth of pathogens is far more than than any other antagonistic factors such as *penicillium citrinum*, *Aspergillus niger* and *Trichoderma viride* (Hand et al., 2011).

In conducting research in laboratory conditions in the context of *S. sclerotiorum* control mechanism, the mulberry twigs and buds drying up cause by different species of Trichoderma, showed that there are significant differences Between *T. harzianum* and other species In terms of reduction of colony growth and rate of demolanizatedsclerotia and the most effective mechanism of mentioned species, is production of volatile compounds. Trichoderma micro parasitismmechanisms against *R. solani* causes inhibitory of pathogen colony growth,rice sheath blight pathogen in different modes including fragmentation, chain contact, chain twisting and finally chain slipping (Naemi et al., 2010).

Brevibacillus brevis efficacy for Fusarium wilt control evaluated in the laboratory. Results showed a significant reduction in the disease indicator in Treatment Influenced by FOL and *B. brevis* Compared with infected control (Chandel et al., 2010). Assessment of the effect of the antagonistic micro parasitismmechanism by method of mutual cultivation and production non-volatile compounds of three strains of *Pseudomonas fluorescens*, *Bacillus cereus*, *Serratiamarcescens* and one isolate of *T. harzianum* on FOL growth inhibitory showed that in micro parasitismmechanism, maximum inhibitory percent has caused by *Serratia marcescens*, *Pseudomonas fluorescens* and *T. harzianum* strains (Karkachi et al., 2010).

MATERIALSANDMETHODS

Isolation of pathogenic agents from plant samples

To do this stage, Roots and stems samples of infected plants collected from tomatoes field were washed with town water and after removing the outer skin the samples vascular tissue four to five mm parts were surface sterilized for five minutes by commercial sodium hypochlorite 10% (NaOCl 5.25%). The parts after been washed with sterile distilled water and dried on filter paper for three

times have been putted in Petri dishes containing Potato Dextrose Agar (PDA) medium. Petri dishes at room temperature 28^0C for 10 to 14 days were incubated in fluorescent light to colonies strains of Fusarium were formed on the surface of the medium (Cai et al., 2003).

Isolation of pathogenic agents from soil samples

To pathogens isolation from soil, sampled to a depth of one cm from five random fields were examined. Then, one gram of each sample was added to 100 ml of sterile distilled water and the suspension was obtained by serial dilutions to 10^3 were prepared. One milliliter of each dilution was spread on selective medium (Komada medium) in a Petri dish. In this step,five replicates were used for each dilution. Then Petri dishes at 28^0C were incubated for 7 to 10 days in fluorescent light so that colonies Strains of Fusarium were appeared on the surface (Lemanczyk and Sadowski, 2000). Medium components used are as follows: One liter of distilled water, K2HPO4 g, KCL tepid, MgSO4.7H2O tepid, Fe-EDT One hundred grams, two grams of L-asparagine, D-galactose, 20 g and 15 g of agar (Komada, 1975).

Macroscopic and microscopic studies of *F. oxysporum*

After purification of each of the colonies obtained from single spore or hyphal tip (Booth, 1977), the detection and identification of Fusariumoxysporum on PDA and CLA were performed with the use of medium (Nelson et al., 1983).In this research macroscopic features Such as color, speed and How to colony growth, the presence or absence of aerial mycelia and also Microscopic features of fungi such as the size and shape of macro conidia formed in esporodokhium, the micro conidiophore, the apical and basal cell macro shape, Presence or absence of micro conidia, how to product micro conidia, Kind of fialid, Presence or absence of chlamydospores and the formation of that was studied (Booth, 1977; Summerell et al., 2003).

Pathogenicity test

This step, in order to confirm the specific form for tomato *F. oxysporum* (*F. oxysporum* f. sp. *lycopersici*) and prove its pathogenicity, based on the immersion of the roots of tomato two leaves seedlings in mentioned fungi conidian suspension with Concentration of $10^6 \times 2$ conidia per ml was performed and then inoculated seedlings were grown in greenhouse sterilized soil (Marlatt et al., 1996). Assessment In determining disease severity index was performed three weeks after inoculation (Mwangi et al., 2011).

Based on incidence, disease severity index for each plant According to Hao*et al*. (2005) is shown below with the numbers zero to five, disease severity percent was calculated

Percent disease severity percent=Number of grade ziro plants × 0+Number of grade one plants × 1+Number of grade two plants × 2+Number of grade three plants × 3+Number of grade four plants ×4+Number of grade five plants ×5/The total number of plants × 5

Zero = no evidence of disease

1= leaf chlorosis and wilting of 1 to 25% (the plant has one withered leaf)

2=leaf chlorosis and wilting in the 26 to 49 % (plants have two or three leaves wilted)

3=leaf chlorosis and wilting of 50 to 74 %(half of the leaves of a plant are withered)

4=leaf chlorosis and wilting in 75 to 100% (all the leaves of a plant withered)

5= dead plant or completely abolished

***T. flavus* and *T. harzianum* antagonistic fungi isolation from the soil of tomato**

At this stage, we collected soil from tomato farms in the Agricultural Experiment Station.Thus after removing the soil to a depth of five to eight centimeters, using a catheter with a diameter of two and a half centimeters, from depth of 25 cm in five random fields were sampled and mixed with eachothers . Then, at 20 to 24°C for four to six weeks and were kept and dry. In the next step, a suspension with a concentration of one gram of soil in 100 ml of sterile distilled water was prepared and one ml of the Petri dish content the specific culture medium of the fungus has spread and Petri dishes be placed at the temperature 27^0C for seven to ten days. For the *T. flavus* isolated according to Marios*et al*. (1984), the the specific culture medium T.F. was used (One liter of distilled water, 39 g of a commercial PDA, two

ml of 50% lactic acid, 100 mg of streptomycin sulfate, 50 mg chlorotetracycline, 50 mg chloramphenicol, four mg Pymarysinsuspension 2.5% and 0.05 g Rose Bengal) and*T. harzianum* isolated according to Koch (1989), the the specific culture medium TSM2 (one liter of distilled water, the gram K2HPO4, half a gram of MgSO4. 7H2O, 15 g peptone, 0.25 g chloramphenicol, 10 g Klrvpenta-nitro-benzene, 20 g agar and one ml of 50% lactic acid). To ensure the integrity of species, each of the isolates of *T. flavus* and *T. harzianum* appear on their specific medium, the PDA cultures, renew culture and its detection by macroscopic and microscopic studies were carried out.

Macroscopic and microscopic studies of strains *T. flavus* and *T. harzianum*

To identify colonies of *T. flavus* macroscopic and microscopic features of strains were studied. This way that strains that in terms of macroscopic its colonies after 10 days of storage at 0C 30 bright yellow halo around the green areas in the center were observed and also in terms of microscopic, have hyphae and form of asexual reproduction (conidia and conidiophore) similar to sexual Penicillium, were selected (Marois et al., 1984). In addition, In order to form the phase of sexual reproduction, this strains on PDA kept at 0C 30 for three weeks in an incubator and their sexual reproductive organs include ascogonium, antridium,ascocarp,ascus and ascospores were studied. *T. harzianum* colonies were identified based on macroscopic (color colonies) and microscopic (splits conidiophore, conidial shape and fialids) features (Siddiquee et al., 2009).

Study of the antagonistic mechanisms of *T. flavus* and *T. harzianum*

The antagonistic mechanisms of *T. flavus* and *T. harzianum*isolates against pathogens according to Wright *et al*. (1990) was studied as follows:
Diameter colony growth of pathogenic fungus on plants by antagonistic fungus -Pathogenic fungus colony growth diameter in control/ Pathogenic fungi colony growth diameter in control

Mico parasitism effect of antagonistic isolates on pathogen

For this study, on PDA in center of half of the petri dishes, pathogenic fungi and in the cross half, each of isolate wascultured separately. Four petri dishes for each strain were used as repetition. In the control treatment, Strains did not enter and just in center of half petri dishes pathogenic fungi were placed. Five days after culture at 25^0C the colonies growth condition were observed. Some cuttings from the impact of pathogenic fungi strains was prepared and how to contact or communicate between hyphae studied under the microscope. The average inhibitory percent of colony growth of pathogenic fungi by Mico parasitism mechanisms of every antagonistic isolate was calculated according to the following formula:

The comparison of the means related to pathogen colony growth inhibitory percent was conducted in form of CRD statistical signusing Duncan's multiple range tests.

Volatile components antagonistic effects on pathogens

In order to this study the strains were grown on PDA in petri dishes and were incubated for 36 h at 25^0C. After that time in center of another petri containing PDA, some pills with a diameter of 9 mm were placed from 5 days grown of fungi pathogenic. The pan lids was removed beside the flame and in sterile conditions And petri dishes containing culture media tablets contain pathogenic fungus mycelium upside down on each pan containing strains and completely sealed by adhesive tape. In the control treatment, the petri dishes containing isolates, none of the antagonistic strains was ordered. Treated Petri as the pan containing the cultivation strains located in below were located in incubated at 25^0C.After four days the measurement the grown of pathogenic fungi isolates diameter starts to 12 days. This experiment repeated four time for each isolates and the inhibitory percent for colony growth of pathogenic fungus by volatile compounds in each of Petri dish was calculated accordance with above- mentioned formula.

Figure 1: *Fusarium oxysporum* isolates obtained from different regions of Iran

Table 1: *Fusarium oxysporum* isolates obtained from tomato fields in Varamin and Shahrood

F. oxysporum isolates	Sample collected for isolation	Location of collection	*F. oxysporum* isolates	Sample collected for isolation	Location of collection
Fu-To-V-1	Tomato field soil	Varamin	Fu-To-V-12	Tomato field soil	Varamin
Fu-To-V-2	Tomato field soil	Varamin	Fu-To-V-13	Tomato field soil	Varamin
Fu-To-V-3	Tomato field soil	Varamin	Fu-To-V-14	Tomato field soil	Varamin
Fu-To-V-4	Tomato field soil	Varamin	Fu-To-V-15	Tomato field soil	Varamin
Fu-To-V-5	Tomato field soil	Varamin	Fu-To-V-16	Tomato field soil	Varamin
Fu-To-V-6	Tomato field soil	Varamin	Fu-To-V-17	Tomato field soil	Varamin
Fu-To-V-7	Tomato field soil	Varamin	Fu-To-V-18	Tomato field soil	Varamin
Fu-To-V-8	Tomato field soil	Varamin	Fu-To-Sh-19	Tomato stem	Shahrood
Fu-To-V-9	Tomato field soil	Varamin	Fu-To-Sh-20	Tomato stem	Shahrood
Fu-To-V-10	Tomato field soil	Varamin	Fu-To-Sh-21	Tomato stem	Shahrood
Fu-To-V-11	Tomato field soil	Varamin	Fu-To-Sh-22	Tomato stem	Shahrood

The comparison of the means related to pathogen colony growth inhibitory percent was conducted in form of CRD statistical sign using Duncan's multiple range tests.

Non-volatile compounds antagonistic effects on pathogens

To provide non-volatile compounds, the antagonists fungal isolates separately was grown in C'zapeckDox Broth liquid medium inside the Meyer flasks and shacking device with shaking at 50 per min in the laboratory for 10 days. Then, from this medium by using the filters 0.45 micrometer pore diameter and using vacuum was extracted. The extract was mixed with cooling PDA in ratio of 20% and then was poured into a Petri dish. In next step, pathogenic fungus was cultured on that. Then the cultured petri dishes placed at 25^0C and after 2 days, the measurement of pathogenic fungus colony growth diameter was carried out for 7 days. This experiment has repeated four time separately for each antagonistic solate. In control, filtered extract ZapeckDox Broth C was used in 20% ratio. Also in this step, inhibitory percent for colony growth of pathogenic fungus by non- volatile compounds in each of Petri dish was calculated accordance with above- mentioned formula. The comparison of the means related to pathogen colony growth inhibitory percent was conducted in form of CRD statistical sign using Duncan's multiple range tests.

RESULTS

Isolation of the causal agent of tomato Fusarium wilt

In macroscopic and microscopic studies carried out on 45 isolates, 22 isolates were identified as *F. oxysporum* (18 isolates from soil samples of tomatoes and 4 isolates from infected tomato stems) (Figure 1, Table 1).

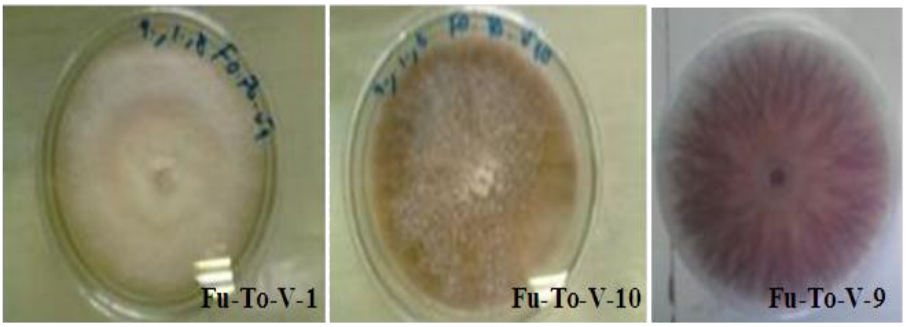

Figure 2: Colony color of Fu-To-V-1 and Fu-To-V-10 isolates from the front and colony color of Fu-To-V-9 isolate from behind on

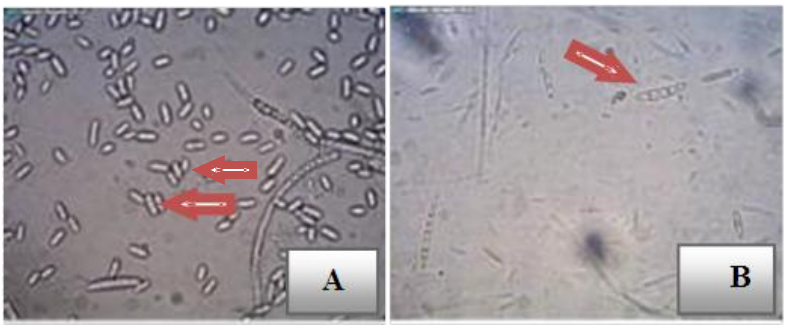

Figure 3: Micro-conidi (A) and macro-conidi 3-5 cell (B) of the Fu-To-V-15 isolates

Figure 4: Single conidi at the end of the conidiophore in Fu-To-V-15 isolate

Microscopic and macroscopic studies on *F. oxysporum*

In terms of macroscopic the color of colonies of Fusarium isolates on PDA from the front and back sides, respectively, of white or pinkish-white and pink-violet appears (Figure 2). In terms of microscopic, non-sexual spore spore types including micro conidi, macro conidi was observed (Figure 3). Micro conidis are often oval and usually one or two cells and macro conidis was three to five cells observed in large amounts (Figure 3). Also, single conidi at the end of the conidiophore was clearly seen (Figure 4).

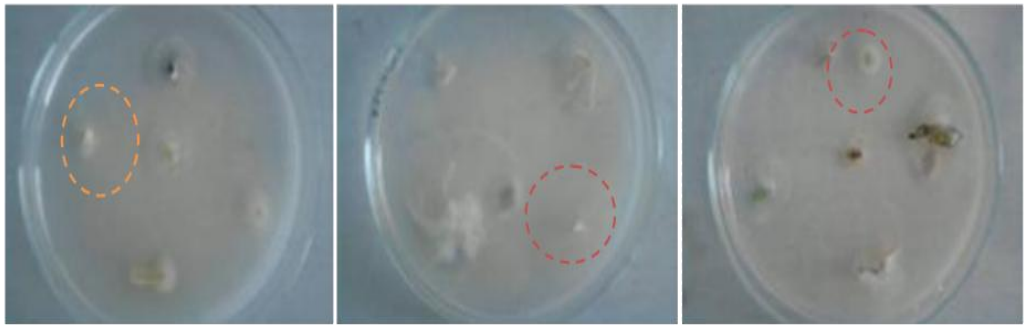

Figure 5: Different parts of the infected tomato plant (from left to right: leaves, roots and stems) cultured on PDA medium

Table 2: The effect of the different *Fusarium oxysporum* isolates on Fusarium wilt disease severity percent in tomato (α= 0.01)

***Fusarium oxysporum* isolates**	**Mean inhibitory percent of pathogenic agent colony growth (%)**
Fo-To-V-1	30.00c
Fo-To-V-2	0.00f
Fo-To-V-3	0.00f
Fo-To-V-7	6.60e
Fo-To-V-9	32.00b
Fo-To-V-15	40.00a
Fo-To-V-17	10.00d
Fo-To-V-18	8.00e
controll	0.00f

Chlamydospores have thick walls and are usually single or in pairs (one or two cells) but occasionally were observed in batch or in short chains.

In this study, Fu-To-V-1, Fu-To-V-2, Fu-To-V-3, Fu-To-V-7, Fu-To-V-9, Fu-To- V-15, Fu-To-V-17, and Fu-To-V-18 isolates were identified as *F. oxysporum* (Table 2- 4).

Pathogenicity test

Results of pathogenicity test revealed that eight isolates of *F. oxysporum* were pathogenic for the plant and caused Fusarium wilt symptoms. In addition, during isolation of the pathogen from infected plant samples, several fungal isolates from different parts of roots, stems and leaves were obtained. These isolates were identified as *F. oxysporum* after macroscopic and microscopic studies on them (figure 5).

In pathogenicity test, there were significant differences among treatments affected by different *F. oxysporum* isolates (Table 2). In among these treatments, maximum and minimum disease severity percent belonged to FO-To-V-15 and FO-To-V-2 respectively (Table 2). For greenhouse experiments, FO-To-V-15 was selected as an isolate with the highest activity for pathogenicity.

Isolation of *Talaromyces flavus* and *Trichoderma harzianum* antagonistic fungi

In this study, a total of 18 different fungal isolates of *T. flavus* and *T. harzianum* from soil related to

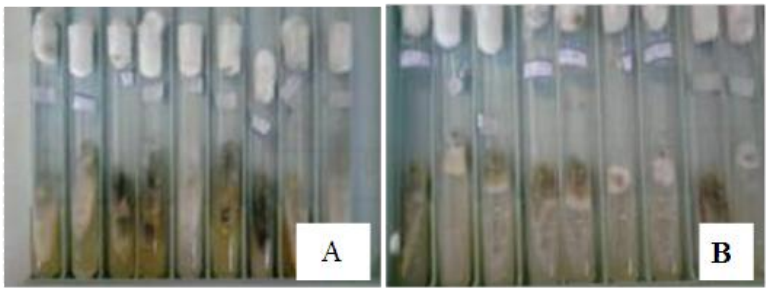

Figure 6: *Trichodrrma harzianum* isolates (A) and *Talaromyces flavus* isolates (B)

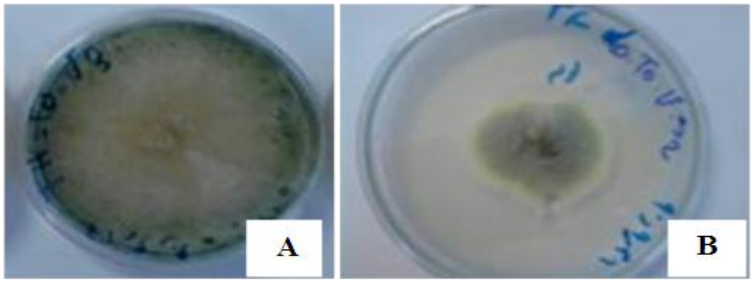

Figure 7: The color of the colonies of *Trichodrrma harzianum* (a) and *Talaromyces flavus* (b) on PDA medium

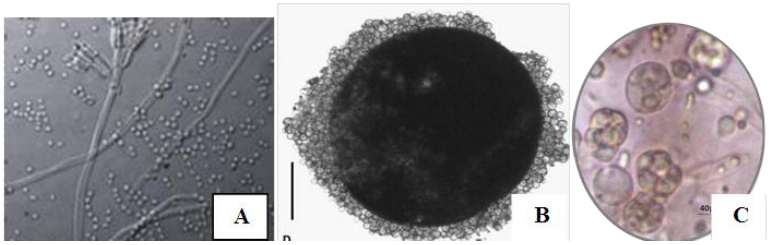

Figure 8: Asexual form (*Penicillium dangeardii*), sexual form (*Talaromyces flavus*): ascocarp and asci containing ascospores

areas cultivated tomato (Shahrood and Varamin), respectively (Figure 6). Nine isolates were belonged to T. harzianum and other nine isolates were related to Talaromyces flavus. All *T. harzianum* isolates and seven isolates of *T. flavus* were obtained from Varamin field soil. However two isolates of *T. flavus* obtained from Shahrood field soil.

Microscopic and macroscopic studies on of *T. flavus* and *T. harzianum* isolates

In terms of macroscopic, the color of the colonies of *T. flavus* isolates on general and specific culture media (TF and PDA), a bright yellow halo around the green areas in the center (Figure 7). The microscopic form of hyphae and asexual (conidia and conidiophore) were similar to the genus Penicillium. In sexual form of *T. flavus* isolates, ascogonium, asc and ascospore were observed (Figure 8). The colonies of *T. harzianum* isolates were initially yellowish green to dark green (Figure 7). In the microscopic study, *T. harzianum* isolates had conidiophores as whole, fialids as gave shape to the swollen and conidia were roughly spherical to oval shape (Figure 9).

Figure 9: *Trichodrrma harzianum*: conidiophores

Table 3: Grouping means of inhibitory percent for *Fusarium oxysporum* f. sp. *lycopersici* in mycoparasitism mechanism by *T. flavus* and *T. harzianum* isolates (α=0.01)

Treatment affected by *T. flavus* or *T. harzianum*	Inhibitory percent for colony growth of *F. oxysporum*	Treatment affected by *T. flavus* or *T. harzianum*	Inhibitory percent for colony growth of *F. oxysporum*
TH-To-V-1	56.11f	TF-To-V-19	27.77m
TH-To-V-2	58.33d	TF-To-V-20	35.00i
TH-To-V-3	56.11f	TF-To-V-21	33.33j
TH-To-V-4	55.55f	TF-To-V-22	30.55k
TH-To-V-5	58.33d	TF-To-V-23	30.55k
TH-To-V-6	61.11c	TF-To-V-24*	62.83b
TH-To-V-7	58.33d	TF-To-V-25*	15.04s
TH-To-V-8	58.33d	TF-To-V-26*	32.74j
TH-To-V-9	61.66c	TF-To-V-27*	16.81r
TF-To-V-15	33.33j	TF-To-V-28*	39.82g
TF-To-V-16	57.22e	TF-To-V-29*	64.60a
TF-To-Sh-17	27.77m	TF-To-V-30*	20.35q
TF-To-Sh-18	33.33j	TF-To-V-31*	19.46q
TF-To-V-32	22.12p	TF-To-U-36*	29.20l
TF-To-V-33	23.89o	TF-To-U-37*	25.66n
TF-To-U-34	22.12p	TF-To-U-38*	36.28h
TF-To-U-35	29.20l	Controll	0.00t

* The inhibitory percent of *F. oxysporum* colony growth by treatments related to isolates from 24 to 38 had been calculated in previous study (Naraghi et al., 2010c).

Antagonistic mechanisms of *T. flavus* and *T. harzianum*

Mycoparasitism mechanism

In mycoparasitism mechanism by *T. flavus* isolates, the range of inhibitory percent for pathogenic agent colony growth was from 15.04 to 60.64%. Maximum and minimum inhibitory percent were observed in treatments affected by TF-To-V-29 and TF-To-V-25 respectively (Table 3). *T. harzianum* isolates caused inhibitory growth for *F. oxysporum*. The range of inhibitory percent by *T. harzianum* mycoparasitism mechanism was form 55.55 to 61.66. In among *T. harz*ianum isolates, maximum and minimum inhibitory percent were observed in treatments affected by in TH-To-V-9 and TH-To-V-5 respectively (Table 3). In this mechanism, fragmentation and lysis were observed for the mycelium of the pathogenic agent (Figures 10 and 11).

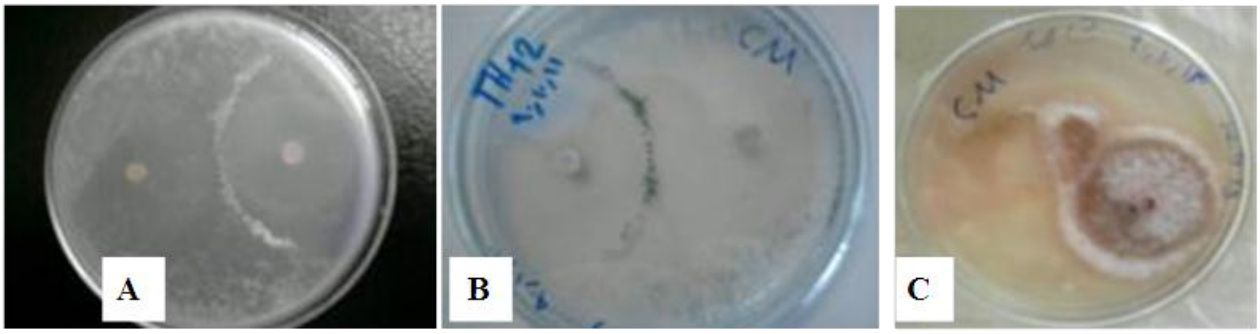

Figure 10: The effect of the micoparasitism mechanism of *Talaromyces flavus* (A) and *Trichoderma harzianum* (b) on the *Fosarium oxysporum* compared with control (C)

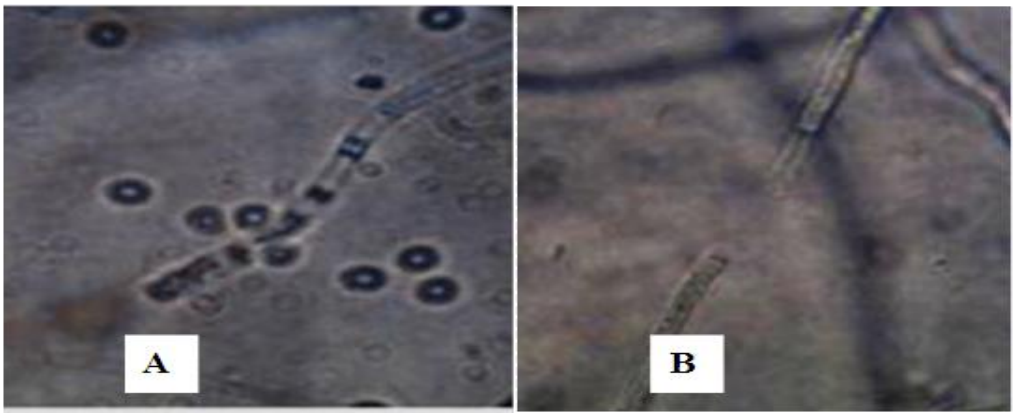

Figure 11: Fragmentation (A) and Lysis (B) in *Fusarium oxysporum* by mycoparasitism mechanism of *Trichoderma harzianum*

Volatile metabolites production mechanism

In volatile metabolites production mechanism by *T. flavus* isolates, the range of inhibitory percent for pathogenic agent colony growth was from 0 to 43.33%. Maximum and minimum inhibitory percent were observed in treatments affected by TF-To-V-33 and TF-To-V-16 respectively (Table 4). *T. harzianum* isolates caused inhibitory growth for *F. oxysporum*. The range of inhibitory percent by *T. harzianum* volatile metabolites production mechanism was form 3.57 to 14.28. In among *T. har*zianum isolates, maximum inhibitory percent were observed in treatments affected by in TH-To-V-1, TH-To-V-4 and TH-To-V-8 respectively (Table 4). However, minimum inhibitory percent were observed in treatments affected by TH-To-V-3, TH-To-V-5 and TH-To-V-9 (Table 4). In figure 12, the colony growth *F. oxysporum* in *T. flavus* treatment has been compared with control.

Non-volatile metabolites production mechanism

In non- volatile metabolites production mechanism by *T. flavus* isolates, the range of inhibitory percent for pathogenic agent colony growth was from 0 to 24.58%. Maximum inhibitory percent were observed in treatment affected by TF-To-V-24. However, minimum inhibitory percent occurredin treatments affected by TF-To-V-15, TF-To-V-16 and TF-To-V-16 (Table 5). *T. harzianum* isolates caused inhibitory growth for *F. oxysporum*. The range of inhibitory percent by *T. harzianum* non- volatile metabolites production

Table 4: Grouping means of inhibitory percent for *Fusarium oxysporum* f. sp. *lycopersici* in volatile metabolites production mechanism by *T. flavus* and *T. harzianum* isolates (α=0.01)

Treatment affected by *T. flavus* or *T. harzianum*	Inhibitory percent for colony growth of *F. oxysporum*	Treatment affected by *T. flavus* or *T. harzianum*	Inhibitory percent for colony growth of *F. oxysporum*
TH-To-V-1	14.28i	TF-To-V-19	14.28i
TH-To-V-2	12.85j	TF-To-V-20	5.00m
TH-To-V-3	3.57n	TF-To-V-21	3.57n
TH-To-V-4	14.28i	TF-To-V-22	3.57n
TH-To-V-5	3.57n	TF-To-V-23	25.00g
TH-To-V-6	6.14l	TF-To-V-24*	23.66h
TH-To-V-7	12.85j	TF-To-V-25*	11.66k
TH-To-V-8	14.28i	TF-To-V-26*	31.66d
TH-To-V-9	3.57n	TF-To-V-27*	23.33h
TF-To-V-15	3.57n	TF-To-V-28*	38.33b
TF-To-V-16	0.00o	TF-To-V-29*	31.66d
TF-To-Sh-17	12.85j	TF-To-V-30*	23.33h
TF-To-Sh-18	11.42k	TF-To-V-31*	25.00g
TF-To-V-32	14.28i	TF-To-U-36*	43.33a
TF-To-V-33	30.00e	TF-To-U-37*	35.00c
TF-To-U-34	28.33f	TF-To-U-38*	35.00c
TF-To-U-35	30.00e	Controll	0.00o

* The inhibitory percent of *F. oxysporum* colony growth by treatments related to isolates from 24 to 38 had been calculated in previous study (Naraghi et al., 2010c).

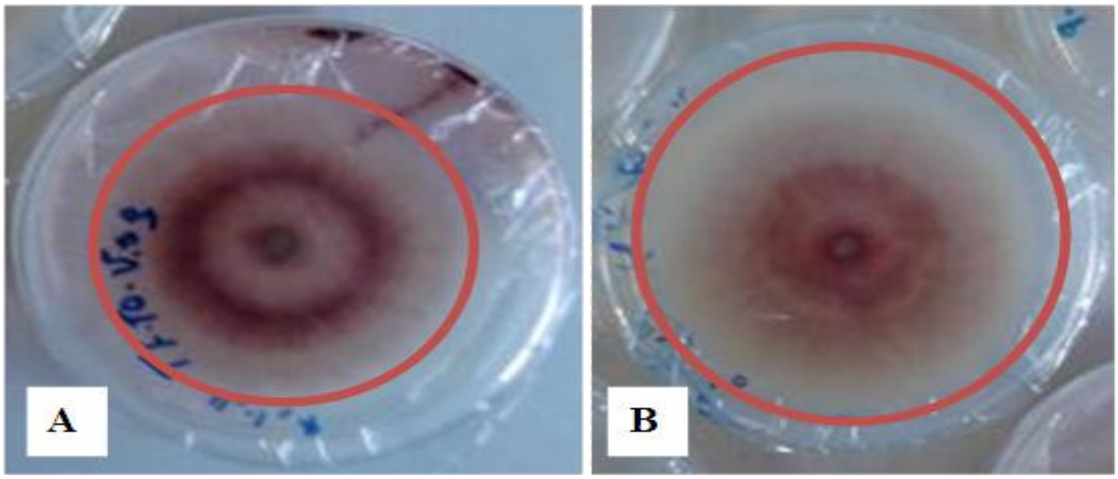

Figure 12: The comparison of the colony growth of *Fusarium oxysporum* in treatment affected by volatile metabolites production mechanism of *Talaromyces flavus* (A) and control (B)

mechanism was form 0 to 22.64. In among *T. harz*ianum isolates, maximum and minimum inhibitory percent were observed in treatments affected by in TH-To-V-2 and TH-To-V-5, respectively (Table 5). In figure13, the colony growth *F. oxysporum* in *T. flavus* treatment has been compared with control.

Table 5: Grouping means of inhibitory percent for *Fusarium oxysporum* f. sp. *lycopersici* in non-volatile metabolites production mechanism by *T. flavus* and *T. harzianum* isolates (α=0.01**)**

Treatment affected by *T. flavus* or *T. harzianum*	Inhibitory percent for colony growth of *F. oxysporum*	Treatment affected by *T. flavus* or *T. harzianum*	Inhibitory percent for colony growth of *F. oxysporum*
TH-To-V-1	17.83i	TF-To-V-19	22.34b
TH-To-V-2	22.64j	TF-To-V-20	3.68j
TH-To-V-3	17.83n	TF-To-V-21	0.00l
TH-To-V-4	11.76i	TF-To-V-22	2.34k
TH-To-V-5	0.00n	TF-To-V-23	24.58a
TH-To-V-6	6.20l	TF-To-V-24*	13.95f
TH-To-V-7	15.48j	TF-To-V-25*	2.32k
TH-To-V-8	18.26i	TF-To-V-26*	18.60d
TH-To-V-9	11.76n	TF-To-V-27*	18.60d
TF-To-V-15	0.00n	TF-To-V-28*	18.60d
TF-To-V-16	0.00o	TF-To-V-29*	2.32k
TF-To-Sh-17	5.29i	TF-To-V-30*	16.27e
TF-To-Sh-18	15.36e	TF-To-V-31*	9.30h
TF-To-V-32	0.00l	TF-To-U-36*	23.25b
TF-To-V-33	23.25b	TF-To-U-37*	16.27e
TF-To-U-34	13.95f	TF-To-U-38*	20.93c
TF-To-U-35	2.32k	Controll	0.00l

* The inhibitory percent of *F. oxysporum* colony growth by treatments related to isolates from 24 to 38 had been calculated in previous study (Naraghi et al., 2010c).

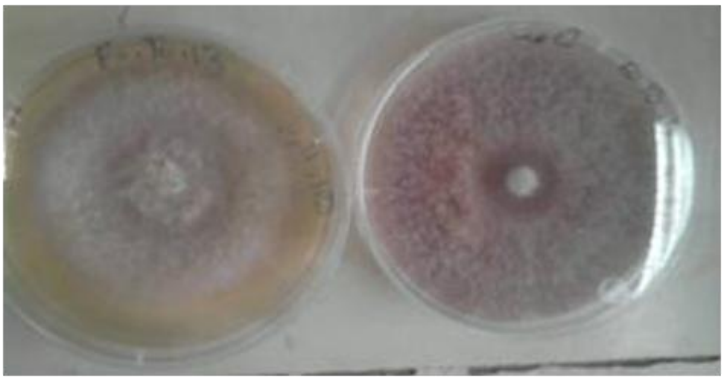

Figure 12: The comparison of the colony growth of *Fusarium oxysporum* in treatment affected by volatile metabolites production mechanism of *Talaromyces flavus* (A) and control (B)

DISCUSSION

Overall results of this study indicate that it may be possible to promote health and growth of sugar beet using Talaromyces and Trichoderma fungal antagonists. These fungal antagonists were capable of both disease suppression and promotion of growth and yield of sugar beet in the greenhouse as well as field conditions.

Trichoderma and Talaromyces have previously been used in the biological control of several plant diseases including cotton seedling damping-off, cucumber wilt, potato wilt and tomato wilt diseases (Howell, 2002; Naraghi et al., 2010a, b and c). Seedling damping-off which is one of the most important diseases of sugar beet around the world has recently been controlled by the application of different microbial antagonists

including fungi and bacteria (Shahiri Tabarestani, 2005; Shahraki et al., 2008; Jorjani et al., 2011).

In our greenhouse experiment, the treatments affected by isolates of *T. flavus* had significantly greater numbers of healthy seedlings in the case of soil artificially inoculation with Rhizoctonia or a combination of pathogenic factors compared to the inoculation with Fusarium, while a significant increase was observed in the number of healthy seedlings in soil artificially inoculated with Fusarium compared to the soil inoculated with Rhizoctonia or combination of pathogenic factors for treatments affected by isolates of *T. harzianum*. Therefore, it can be concluded that the effect of *T. flavus* on the reduction of sugar beet damping-off disease caused by *R. solani* or combination of pathogenic factors was higher than the case in which Fusarium was the only cause of this disease. However, the maximum effect of *T. harzianum* on the reduction of sugar beet damping-off disease occurred when its only cause was Fusarium. In this regard, the results of previous study by Nicoletti *et al*. (2009) also indicated that the effect of *T. flavus* on the inhibition of the growth *R.solani* was higher than other fungal pathogen of seedling damping off.

Furthermore, the results of our field studies in Karaj area indicated that the treatment, affected by isolate of *T. flavus* (T.F.K.3) had the maximum yield compared with other treatments and it had no significant difference with the treatment affected by isolate *T. harzianum* (T.H.K.1) (Table 4). According to the results of previous studies by Moayedi and Mostowfizadeh-Ghalamfarsa (2010) in the sugar beet field, the population of soil microorganisms after application of fungal antagonist, isolates of *T. harzianum* and *T. flavus* during the first crop year of this research led to the breeding population of these isolates especially *T. flavus*, so that they could maintain the effects of metabolites until the end of growth period.

Furthermore, according to the greenhouse and field experiments in our study, the difference between the antagonistic isolates of T. flavus or T. harzianum in terms of their capability to reduce the seedling damping-off disease can be caused by a wide range of activities and mechanisms including the production and secretion of different metabolites by these isolates which may be due to their genetic variation (Madi et al., 1992).

The results of the present study may have practical use in the promotion of the health and growth of sugar beet in the field conditions through a non-chemical and ecological friendly strategy which can result in the cultivation of healthy products and the protection of the agricultural environment and biological resources.

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