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Determination of the *In vitro* Effect of *Trichoderma harzianum* on Phytopathogenic Strains of *Fusarium oxysporum*

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Abstract

Fusarium oxysporum is a well-known soil-borne fungi and it is difficult to control their pathogenic strains by conventional strategies. The cultures of two strains of *Trichoderma harzianum* (T16 and T23) were examined in laboratory conditions and with pot experiments for the control of pathogenic strains of *Fusarium oxysporum* f. sp. *melongenae* (Fomg), *Fusarium oxysporum* f. sp. *lycopersici* (Fol), *Fusarium oxysporum* f. sp. *niveum* (Fon) and *F. oxysporum* f. sp. *melonis* (Fom). The T16 and T23 strains showed significant inhibition of mycelial growth in the pathogenic strains of *F. oxysporum* and the maximum inhibition were recorded when the *T. harzianum* strain T16 was used (72.69%). Both *T. harzianum* strains produced volatile and non-volatile metabolites that inhibited growth of *F. oxysporum* strains on PDA medium. *In vitro* colonization study demonstrated the root-colonizing ability of these antagonists. The interaction between *T. harzianum* isolates (T16 and T23) and pathogenic *F. oxysporum* hyphae showed no overgrowth, hyphal coiling, cell wall degradation or any hyphal penetration around any of the tested *F. oxysporum* hyphae. Pre-treatment of soil with T16 significantly reduced the severity of *Fusarium* wilt disease. The disease severity in control plants reached to 90-95% whereas those of the T16-Fomg and T16-Fol treated seedlings of eggplants were 37.74% and 47.12%, respectively, on the 21st day. In this study, while both *T. harzianum* isolates had a considerable antagonistic effect on the tested pathogens, T16 was found to be more successful than T23. The strong repressive effect of *T. harzianum* (T16) towards pathogenic *Fusarium oxsporum* can be applied in biological control of these pathogens.

Keywords: antagonist, biological control, disease severity, pathogenic strains

Introduction

Soil-borne fungi have a wide host range and persist for longer periods in soil by means of resistant resting spores. The plant diseases caused by such fungi are among the most difficult to control (Nelson *et al*., 1983; Yücel *et al*., 2007). Within these species, *Fusarium oxysporum* Schlechtend.:Fr. is one of the most important soil-inhabiting fungi and consists of both pathogenic and nonpathogenic strains (Gordon and Martyn, 1997). Individual pathogenic strains are known to be phylogenetically diverse and have a high degree of host specificity within *F. oxysporum*; they are generally known as species complexes which are assigned to intraspecific groups, called *formae speciales* (f. sp.) (Gordon and Martyn, 1997; Kistler, 2001). The usage of chemical compounds is a widely applied method to control soil-borne diseases, but these have adverse effects on the environments such as like affecting the beneficial functions of microorganisms living in the soil and root ecosystem (Harman *et al*., 2004). Biological control has been advanced as an eco-friendly alternative to synthetic fungicides, and remarkable success has been achieved by utilizing antagonistic microorganisms. For example, *Trichoderma* species are common saprophytic fungi found in the soil and many studies have focused on their ability to reduce the incidence of the disease caused by plant pathogenic fungi (Elad, 2000; Freeman *et al*., 2004; Dubey *et al*., 2007). The *Trichoderma* species are useful avirulent plant symbionts that play an important role especially in controlling soil-borne fungal pathogens. Commercial biological products based on the *Trichoderma* species are manufactured and marketed worldwide as biofungicide, plant biostimulant and soil fertilizers for use on a wide range of crops. The use of these products for the management of diseases is eco-friendly, economical and also practical for improving soil health.

There are many biological control strategies for the control of soil-borne diseases. Among the potential biocontrol agents in the rhizosphere, several strains of the genus *Trichoderma* are reported to be effective in controlling a variety of fungal plant diseases (Menziez, 1993; Chet and Inbar, 1994; Freeman *et al*., 2004).

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Trichoderma species are filamentous soil antagonist fungi known to be an efficient biocontrol agent against a range of diseases in many economically important crops (Alabouvette *et al*., 2009; Carvalho *et al*., 2014). A number of *Trichoderma*-based commercial biofungicides which are of great importance as sources of enzymes, antibiotics and plant growth promoters have been developed. *Trichoderma* strains inhibit the infections caused by plant pathogens using different biocontrol mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion (Papavizas and Lumsden, 1980; Elad, 2000; Dubey *et al*., 2007; Hajieghrari *et al*., 2008; Poovendran *et al*., 2011). Recent studies have indicated that certain strains of *Trichoderma* can enhance plant growth and crop productivity, and can also induce systemic and localized resistance to several plant pathogens (Harman *et al*., 2004; Tran, 2010). Root colonization with *Trichoderma* strains can increase levels of defense-related plant enzymes, including various peroxidases, chitinases, β-1-3 glucanases, formation of callose-enriched wall appositions at sites of fungal penetration and pathogenesis-related proteins (Yedidia *et al*., 1999; Harman *et al*., 2004).

In the present study, the biological potential of *Trichoderma harzianum* isolates (T16 and T23) were evaluated with in *in vitro* experiments against four different Fusarium wilt pathogens (*F. oxysporum* f. sp. *melongenae*, Fomg; *F. oxysporum* f. sp. *lycopersici,* Fol; *F. oxysporum* f. sp. *niveum,* Fon and *F. oxysporum* f. sp. *melonis*, Fom) and their role in the control of Fusarium wilt disease in eggplant, tomato, watermelon and melon were determined with pot experiments. The results obtained from this work provides clues also for further field biocontrol studies.

Materials and Methods

Isolates

T. harzianum isolates (T16 and T23) were kindly provided from the culture collection of the Institute for Phytomedicine at the University of Hohenheim, Germany and these were used in this study. The *F. oxysporum* f. sp. *melongenae*, *F. oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *lycopersici* isolates selected for this study were obtained from the collection of *Fusarium* spp. in Erciyes University, Agriculture Faculty, Department of Plant Protection Mycology Laboratory, Kayseri-Turkey. These isolates, which were isolated from the host plants (eggplant, watermelon, melon and tomato) and highly virulent isolates were used in this study. The isolates were maintained on a potato dextrose agar (PDA) medium and stored at 4°C for further use.

Plant material and growth conditions

Eggplant (*Solanum melongena* L. cv. 'Kemer'), tomato (*Lycopersicon esculentum* Mill. cv. 'Hazera'), watermelon (*Citrullus vulgaris* cv. 'Crimson sweet') and melon (*Cucumis melo* cv. 'Kirkagac') seeds were surface sterilized with 1% sodium hypochlorite (v/v) for 30 min, sown in a soil mixture containing sand-perlite-peat compost (1:1:2) and kept in a growth chamber (temperature: 25 °C, relative humidity: 80-90%, 12-h photoperiod, 50 to 60 Klux m^{-2}). Four-week-old plants were transplanted to pots of the mixture containing the ingredients given above.

Dual culture technique

Two strains of *T. harzianum* (T16, T23) with pathogenic *F. oxysporum* strains were studied in a dual culture assay on PDA medium in 90 mm Petri plates as described by Altinok (2009). The experiment was arranged as a completely randomized design with ten replicates in a factorial arrangement of 2 x 4 (two *T. harzianum* strains and four *F. oxysporum* strains). The inoculated plates were incubated at 25 ± 1 °C until the end of the incubation period. The radial growth of the pathogen *F. oxysporum* isolates was measured on the 3^{rd}, 5^{th} and 7^{th} days after inoculation (DAI). The inhibition percent in the mycelial development of the pathogen fungus was calculated by the formula: $R_I=(C-T)/C$ x 100; Where R_I is the inhibition percentage of the radial mycelial growth, *C* is the radial growth of the pathogen in the control (mm), and *T* is the radial growth of the pathogen in dual culture (Hajieghrari *et al*., 2008). The results were subjected to one-way ANOVA. Then the means were separated by Duncan's multiple range test to see the individual differences between the *Fusarium* isolates.

Effects of volatile and non-volatile metabolites

The effect of the volatile metabolites produced by *T. harzianum* on the mycelial growth of pathogenic strains of *F. oxysporum* was determined with the method described by Dennis and Webster (1971a). The effect of the non-volatile metabolites of the two isolates of *T. harzianum* on the mycelial growth of *F. oxysporum* strains was studied as described by Dennis and Webster (1971b). The filtrate was mixed together with molten PDA medium (10% w/v). After solidification, the Petri plates were inoculated with discs of the pathogen *F. oxysporum* strains and then incubated at 25±1 °C. There were 10 replicates for each treatment. Percent inhibition of pathogen radial growth was calculated as described above.

In vitro root colonization

The colonization of tested plants by *Trichoderma* species were studied using a method reported by Montealegre *et al.* (2003). For each treatment, 20 surface sterilized seeds were transferred to a sterile moist chamber. A one milliliter aliquot of each inoculum was added to the seeds in the moist chamber and the plates were incubated at room temperature for 1 h to allow binding of the antagonist fungi to the seed coat. Control seeds were inoculated with sterile distilled water. *T. harzianum* treated seeds and the control were incubated at 30 °C for five days in the dark for root development. One centimeter of root from each treatment was aseptically excised, then transferred to $MgSO_4$ (0.1 M) solution, and diluted serially. From each dilution, a 0.1 ml aliquot was plated on PDA media and the plates were incubated at 30 °C for colony counts. The amount of fungal colonization in the root tissues was calculated as colony forming units per cm root (cfu cm $root^{-1}$).

Slide culture method

A clean slide was placed in a 9 cm diameter Petri dish and autoclaved. Then a small amount of molten water agar was dropped onto it this and quickly spread over the slide to make a thin agar film. The inoculum of *T. harzianum* (10^8 conidia ml^{-1}) and the pathogen (10^6 conidia ml^{-1}) were placed on the slide, one cm apart from each other. A few ml of sterile water was added to the Petri dish to prevent drying and was incubated at 25 ± 1 °C for five days. After incubation, the area where the hyphae of *T. harzianum* met the hyphae of the pathogen was observed under a light microscope (Zeiss Axiostar Plus) at 400X, in bright field mode. Three different fields within the observed area were examined for each slide.

Pot experiment

In this experiment, 50 ml conidial suspension of *Trichoderma* isolates was drenched into the soil in each pot. Preparation of *T. harzianum* (T16 and T23) spore suspensions: one ml spore suspension (10^8 spore ml^{-1}) of 7-day-old *T. harzianum* cultured on PDA was used as the inoculum (Yedidia *et al.*, 1999). The cfu numbers were determined by plating serial dilutions of conidial preparations of T16 and T23 onto PDA. Viability percentages were determined by comparing cfu with total conidia. One week later, eggplant, tomato, watermelon and melon seedlings were artificially inoculated with *F. oxysporum* conidia (1×10^6 conidia ml^{-1}) by the root-dip method. Non-inoculated seedlings served as negative control. The positive control plants were inoculated with only the pathogen inoculum and were treated with sterile distilled water instead of *T. harzianum* suspension. The inoculated plants were grown in the growth chamber at the conditions stated above. The experiment was conducted as a 2 x 4 factorial arrangement of treatments in a completely randomized design with three replicates. The symptoms of the disease were recorded on the 7th, 14th and 21st days after the exhibition of the first symptoms in infected plants with a *Fusarium* yellow scale of 0 to 4 (Altinok and Can, 2010). The plants were evaluated individually and a mean percent of disease severity was calculated for each assessment day based on the scale values according to the Townsend-Heuberger formula. The data were subjected to two-way ANOVA to identify interactions between the two factors. The differences among *Fusarium* isolates were also tested with one-way ANOVA for each of the *Trichoderma* strains, then the means were separated by Duncan's multiple range test ($P \leq 0.05$) contained in the SPSS v.16 software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Dual culture technique

In the dual culture experiments, T16 and T23 showed a significant inhibitory effect on the mycelial growth of pathogenic strains of *F. oxysporum* when compared to the control. *T. harzianum* (T16) grew much faster on PDA than the tested pathogens under the same culture conditions. The maximum inhibition was recorded when the *T. harzianum strain* T16 was used (72.69%). The study concluded that T16 was more efficient than strain T23 in inhibiting colony growth of the pathogen *F. oxysporum* isolates (Table 1).

Effects of volatile and non-volatile metabolites

The efficiency of the volatile metabolites on the mycelial growth of the pathogenic strains of *Fusarium oxysporum* varied from 45.03 to 62.71. While Fomg was found to be the most susceptible to volatile inhibitors produced by *T. harzianum* strain T16, the minimum inhibition percentage was in Fom, which was paired with T23 (Table 2).

The maximum inhibition of Fomg radial growth was observed with the non-volatile metabolites of *T. harzianum* strain T16. Fom showed the minimum inhibition percentage of 49.61% and 44.92% by the non-volatile inhibitors of T16 and T23, respectively (Table 2).

In vitro root colonization

The *in vitro* root colonization study demonstrated that antagonist isolates were effective as root colonizers. The maximum count of the viable conidia was obtained from the eggplant, tomato, watermelon and melon roots after five days of germination. T16 and T23 strains were detected as successful colonizers in eggplant seeds (11.83 - 12.25 cfu/cm root x 10^5) and tomato seeds (10.54 - 8.36 cfu cm $root^{-1}$ x 10^5, respectively (Fig. 1).

Slide culture method

Hyphal interaction between the antagonist (*T. harzianum*) and tested pathogenic strains of the *F. oxysporum* was examined five days after incubation. Hyphal penetration or overgrowth was not observed on pathogenic *F. oxysporum* hyphae by *T. harzianum* strains. Although close contact occurred between *T. harzianum* (T16) and pathogen hyphae, no massive coiling, cell wall degradation or disintegrating structures were observed around any of the pathogen hyphae in three different microscopic fields.

Pot experiment

Prior to pot experiments, the viability of the conidia of *T. harzianum* strains was tested. The viability was 88% and 85% for T16 and T23, respectively. Both strains of *T. harzianum* successfully suppressed Fusarium wilt on tested plants. Initial symptoms appeared as yellowing of the older leaves on Fomg, Fol, Fon, Fom-treated and (+) control plants (only pathogen inoculated) seven days after inoculation. The systemic progress of the disease in the (+) control plants rapidly increased in time and by 21 DAI showed severe wilting. Browning areas were observed in the xylem of all infected plants, although the progress of the disease was much slower than that of positive control plants. Non-inoculated seedlings (negative control plants) showed no symptoms and appeared healthy throughout the course of the experiment. The soil applications of *T. harzianum* resulted in up to 50% reduction in Fusarium wilt disease progression in all tested seedlings at 21st day, compared to the control. The effects of T16 and T23 treatments on the 7th, 14th and 21st days after inoculation with the pathogen are presented in Fig. 2 and 3.

At the end of the experiment, the mean disease severity in positive control plants reached to 90-95% whereas in the T16-Fomg and T23-Fomg treated plants it was 37.74% and 50.12%, respectively. The maximum level of disease control was obtained from T16 treatment and the statistical differences between the pathogenic *F. oxysporum* isolates were found to be significant, $F(3,36) = 4.84, P = 0.006$.

The main effects of *Trichoderma* and *Fusarium* isolates were both found to be significant (F=37.67, F=8.12) while no interaction was observed between these factors (F=0.62). In adittion T16 was more effective in suppressing Fomg, on all scoring days (Fig. 2). T23, on the other hand, showed no difference in the virulence of the *Fusarium* isolates until 21st day after inoculation. On the same day, the effect of T23 was significantly higher on Fomg-treated plants than in other *Fusarium* treatments. As seen in Fig. 2 and 3, the results of *T. harzianum* strains were similar at the end of the experiment (21 DAI). Therefore, the effects of T16 and T23 strains were compared for each of *Fusarium* isolates. No significant difference was found between T16 and T23, on Fol; *Fusarium oxysporum* f. sp. *lycopersici* (F (1,18) = 3.82, P = 0.066). The disease suppression ability of T23 was significantly higher than that of T16 on Fom=*F. oxysporum* f. sp. *melonis* [F(1,18) = 3.37, P = 0.021], Fomg=*Fusarium oxysporum* f. sp. *melongenae* [F(1,18) = 24.27, $P < 0.001$] and Fon=*Fusarium oxysporum* f. sp. *niveum* [F(1,18) = 18.01, $P < 0.001$].

The dual culture method as described by many researchers has been widely used in antagonistic studies (Dennis and Webster, 1971a; Bell *et al.*, 1982; Küçük and Kivanç, 2004). In this study, the results of the dual culture experiment revealed the rapid colonization of the growth medium by T16 and T23. Both isolates evaluated were effective in controlling colony growth of the tested pathogenic *F. oxysporum*. The volatile and non-volatile metabolites also showed an effective performance on inhibiting the mycelial growth of the pathogens. Similar findings have been reported by Küçük and Kivanç (2004), Idris *et al.* (2007), Hajieghrari *et al.* (2008), and Perveen and Bokhari (2012).The substantial functions associated with *Trichoderma* species are their ability to produce a number of antibiotics as well as some cell wall degrading enzymes like chitinase and glucanase hydrolytic enzymes which are closely related to mycoparasitism (Elad, 2000). The volatile metabolites produced by antagonists may diffuse easily and inhibit the growth of soil-borne pathogens *in vitro* and even in soil (Dennis and Webster, 1971b). The *in vitro* root colonizing ability of these antagonists was found to be successful. Similar results were reported by Al-Jedabi (2009), after four days of germination, in that the cell counts obtained from the roots increased and the maximum count was achieved by *T. harzianum*. Microscopic studies showed no overgrowth, the hyphal penetration, or hyphal coiling (hyperparasitism) of isolates T16 and T23 around hyphae of pathogenic *F. oxysporum* strains, suggesting that mycoparasitism was not a major mechanism for the observed inhibitory effects. The pathogen and *T. harzianum* hyphae did not interfere with each other in dual cultures and this was explained by the production of extracellular mycolytic enzymes by *T. harzianum* (Calistru *et al.*, 1997; El-Katatny *et al.*, 2006). El-Katatny *et al.* (2006) suggested that the extracellular mycolytic enzymes secreted by *Trichoderma* might play an important role in antibiosis against pathogenic *F. oxysporum*. On the other hand, fungitoxic metabolite secretion by *Trichoderma* might not be the primary mechanism in biocontrol, instead it could be through the competition or parasitism of pathogen hyphae (Mukherjee and Raghu, 1997). The production of yellow pigment in an overlapped area of two colonies and mycelial coiling were also reported by other researchers (Dennis and Webster, 1971c; Küçük and Kivanç, 2004).

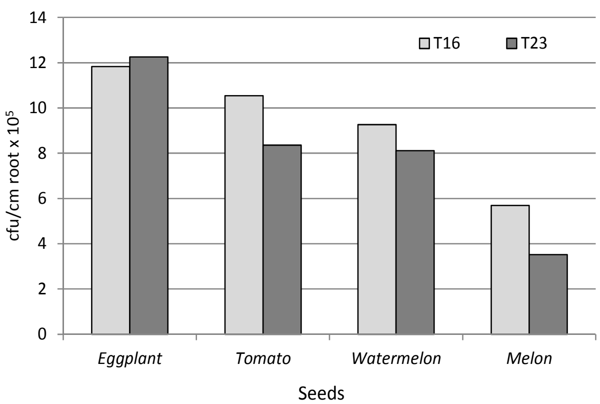

Fig. 1. *In vitro* root colonization of eggplant, tomato, watermelon and melon seeds by antagonistic *Trichoderma harzianum* strains T16 and T23

Table 1. Mycelial growth inhibition of pathogenic *Fusarium oxysporum* by *T. harzianum* isolates in dual culture

Isolates	Mean radial growth (mm)			Inhibition (%)	
	T16	T23	Control	T16	T23
Fomg**	24.50±1.1	26.70±1.8	89.70±2.3	A72.69a*	B70.23a
Fol	36.30±1.5	43.10±1.3	83.20±1.9	A64.40c	B57.83b
Fon	26.70±1.4	37.50±1.2	81.00±2.5	A67.04b	B53.70c
Fom	30.90±0.9	36.06±0.7	86.80±2.2	A56.37d	B48.20d

*Means within columns followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05). Means starting with the same letter within lines are not significantly different at the 0.05 level using Tukey's HSD test. Values represent mean ± SD of ten replicates. Measurements of radial growth were taken 7 days after inoculation.

**Fomg=*Fusarium oxysporum* f. sp. *melongenae*, Fol=*Fusarium oxysporum* f. sp. *lycopersici*, Fon=*Fusarium oxysporum* f. sp. *niveum* and Fom=*F. oxysporum* f. sp. *melonis*.

Table 2. The effect of volatile and non-volatile metabolites by *T. harzianum* on pathogenic strains *Fusarium oxysporum* mycelial growth (mm)

Isolates	Volatile compound				Non-volatile compound			
	Mean radial growth (mm)		Inhibition (%)		Mean radial growth (mm)		Inhibition(%)	
	T16	T23	T16	T23	T16	T23	T16	T23
Fomg**	33.30±1.1	40.10±1.2	A62.71a*	B55.13a	31.80±0.9	34.60±1.1	A64.67a	B61.56a
Fol	38.50±0.9	41.80±1.9	A54.55ab	B50.65b	45.10±0.5	49.30±1.3	A60.48b	B56.59b
Fon	40.50±1.3	45.60±2.0	A52.96b	B47.04c	36.70±1.0	40.20±1.6	A57.23b	B53.15b
Fom	43.50±0.5	39.80±1.8	A50.85b	B45.03d	34.50±0.7	37.90±0.8	A49.61a	B44.92c

*Means within columns followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05). Means starting with the same letter within lines are not significantly different at the 0.05 level using Tukey's HSD test. Values represent mean ± SD of ten replicates. Measurements of radial growth were taken 7 days after inoculation.

**Fomg=*Fusarium oxysporum* f. sp. *melongenae*, Fol=Fusarium oxysporum f. sp. lycopersici, Fon=*Fusarium oxysporum* f. sp. *niveum* and Fom=*F. oxysporum* f. sp. *melonis*.

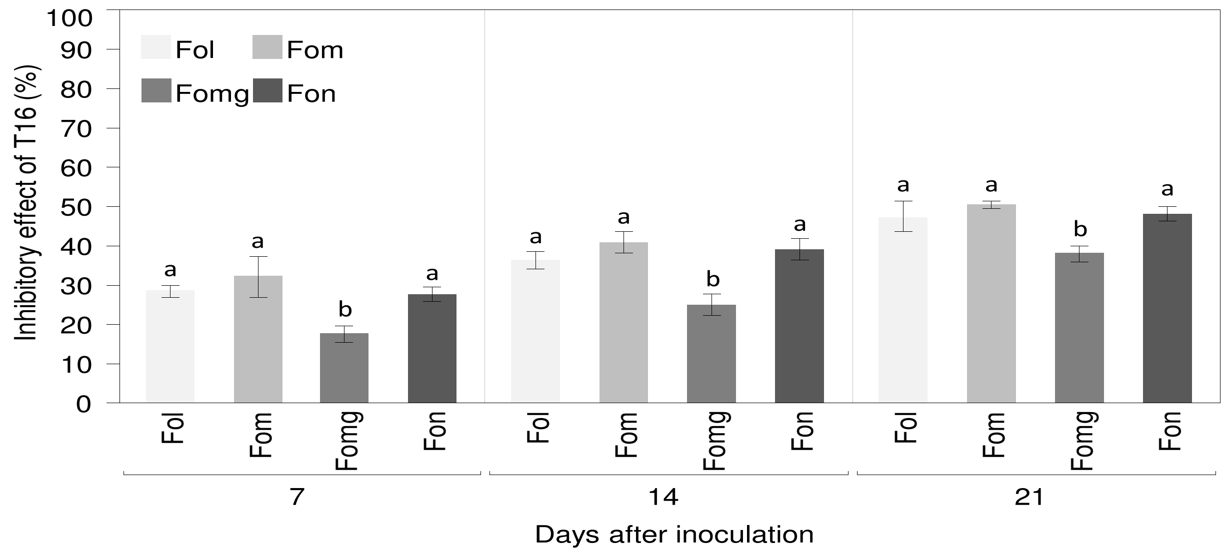

Fig. 2. The inhibitory effect of soil application of *T. harzianum* (T16) against Fusarium wilt diseases caused by Fomg=*Fusarium oxysporum* f. sp. *melongenae*, Fol=*Fusarium oxysporum* f. sp. *lycopersici*, Fon=*Fusarium oxysporum* f. sp. *niveum* and Fom=*F. oxysporum* f. sp. *melonis*.
The seedlings were scored on 7th, 14th and 21st days after pathogen inoculation, using 0-4 scale. Inhibitory effect was calculated by Abbott's formula.
The same letters above means for each scoring day are not significantly different according to Duncan's Multiple Range Test (P ≤ 0.05).
Error bars indicate ±1 standard error of the mean.

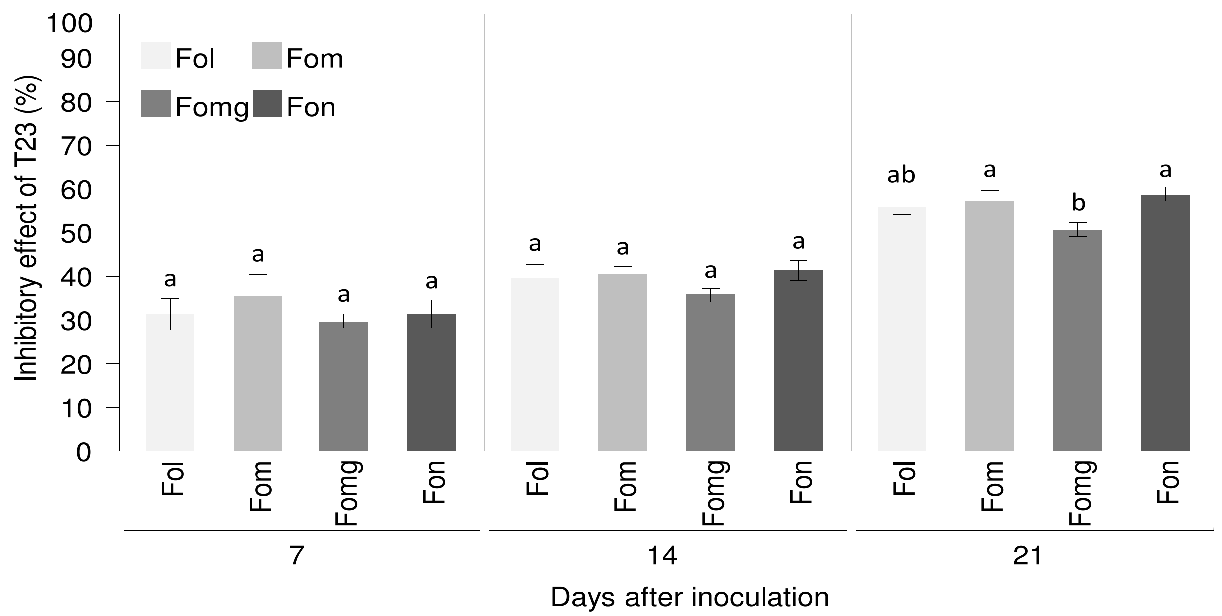

Fig. 3. The inhibitory effect of soil application of *T. harzianum* (T23) against Fusarium wilt diseases caused by Fomg=*Fusarium oxysporum* f. sp. *melongenae*, Fol=*Fusarium oxysporum* f. sp. *lycopersici*, Fon=*Fusarium oxysporum* f. sp. *niveum* and Fom=*F. oxysporum* f. sp. *melonis*.
The seedlings were scored on 7th, 14th and 21st days after pathogen inoculation, using 0-4 scale. Inhibitory effect was calculated by Abbott's formula.
The same letters above means for each scoring day are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).
Error bars indicate ± 1 standard error of the mean.

The inoculation of the eggplant, tomato, watermelon and melon seedlings with pathogenic *F. oxysporum* caused symptoms similar to natural infections. The results showed that strains T16 and T23 were effective in reducing disease severity of Fusarium wilts. When *T. harzianum* was applied as seed coating, crown and root rot incidence was reduced by up to 80% in greenhouse conditions (Sivan *et al.,* 1987). *T. harzianum* reduced the incidence of Fusarium crown and root rot in tomatoes (Van Steekelenburg, 1991; Ozbay *et al.,* 2004). These results clearly indicate that the two *T. harzianum* strains exhibited strong ability to suppress pathogenic *Fusarium* species of different crops in pot experiments.

Conclusions

The results of the antibiosis, the root colonization study and the artificial inoculation experiments indicated that *T. harzianum* strains T16 and T23 have potential as biocontrol agents of Fusarium wilt disease in major crop plants (eggplant, tomato, watermelon and melon). Further detailed studies should be directed at determining the role of antifungal metabolites and extracellular mycolytic enzymes produced by *T. harzianum* strains as Fusarium wilt agents. These strains should also be tested for their performance against Fusarium wilt diseases by greenhouse and/or field plot experiments.

References

Al-Jedabi AA (2009). Biological control of Fusarium root-rot of sorghum. Research Journal of Agriculture and Biological Sciences 5(4):465-473.

Alabouvette C, Olivain C, Migheli Q, Steinberg C (2009). Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. New Phytologist 184:529-544.

Altinok HH (2009). *In vitro* production of fumonisin B_1 and B_2 by *Fusarium moniliforme* and the biocontrol activity of *Trichoderma harzianum*. Annals of Microbiology 59(3):509-516.

Altinok HH, Can C (2010). Characterization of *Fusarium oxysporum* f. sp. *melongenae* isolates from eggplant in Turkey by pathogenicity, VCG and RAPD analysis. Phytoparasitica 38:149-157.

Bell DK, Wells HD, Markham CR (1982). *In vitro* antagonism of *Trichoderma* species against six fungal pathogens. Phytopathology 72:379-382.

Carvalho DDC, Junior ML, Martins I, Inglis PW, Mello SCM (2014). Biological control of *Fusarium oxysporum* f. sp. *phaseoli* by *Trichoderma harzianum* and its use of common bean seed treament. Tropical Plant Pathology 39(5):384-391.

Calistru C, McLean M, Berjak P (1997). *In vitro* studies on the potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by *Trichoderma harzianum* species. Mycopathologia 137:115-124.

Chet I, Inbar J (1994). Biological control of fungal pathogens. Applied Biochemistry and Biotechnology 48:37-43.

Dennis C, Webster J (1971a). Antagonistic properties of species groups of *Trichoderma* II, production of volatile antibiotics. Transactions of the British Mycological Society 57:41-47.

Dennis C, Webster J (1971b). Antagonistic properties of species groups of *Trichoderma* I, production of non-volatile antibiotics. Transactions of the British Mycological Society 57:25-39.

Dennis C, Webster J (1971c). Antagonistic properties of species groups of *Trichoderma* III, hyphae interaction. Transactions of the British Mycological Society 57:363-369.

Dubey SC, Suresh M, Singh B (2007). Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp. *ciceris* for integrated management of chickpea wilt. Biological Control 40:118-127.

Elad Y (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection 19:709-714.

El-Katatny MH, Abdelzaher HMA, Shoulkamy MA (2006). Antagonistic actions of *Pythium oligandrum* and *Trichoderma harzianum* against phytopathogenic fungi (*Fusarium oxysporum* and *Pythium ultimum* var. *ultimum*). Archives of Phytopathology and Plant Protection 39(4):289-301.

Freeman S, Minz D, Kolesnik I, Barbul O, Zreibil A, Maymon M, Nitzani Y, Kirshner B, Rav-David D, Bilu A, Dag A, Shafir S, Elad Y (2004). *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea*, and survival in strawberry. European Journal of Plant Pathology 110:361-370.

Gordon TR, Martyn RD (1997). The evolutionary biology of *Fusarium oxysporum*. Annual Review of Phytopathology 35:111-128.

Hajieghrari B, Torabi-Giglou M, Mohammadi MR, Davari M (2008). Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. African Journal of Biotechnology 7 (8):967-972.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004). *Trichoderma* species- opportunistic, avirulent plant symbionts. Nature Reviews Microbiology 2:43-56.

Idris HA, Labuschagne N, Korsten L (2007). Screening rhizobacteria for biological control of Fusarium root and crown rot of sorghum in Ethiopia. Biological Control 40(1):97-106.

Kistler HC (2001). Evolution of host specificity in *Fusarium oxysporum* In: Summerel BA, Leslie JF, Backhouse D, Bryden WL, Burgess LW (Eds). *Fusarium*. Paul E Nelson Memorial Symposium. APS Pres, St Paul, Minn pp 70-82.

Küçük Ç, Kivanç M (2004). *In vitro* antifungal activity of strains of *Trichoderma harzianum*. Turkish Journal of Biology 28:111-115.

Menzies JG (1993). A strain of *Trichoderma viride* pathogenic to germinating seedlings of cucumber, pepper and tomato. Plant Pathology 42:784-791.

Montealegre JR, Reyes R, Perez LM, Herrera R, Silva P, Besoain XA (2003). Selection of bio-antagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Electronic Journal of Biotechnology 6:115-127.

Mukherjee PK, Raghu K (1997). Effect of temperature on antagonistic and biocontrol potential of *Trichoderma* sp. on *Sclerotium rolfsii*. Mycopathologia 139:151-155.

Nelson AJ, Toussoun TA, Marasas WFO (1983). *Fusarium* Species. University Park and London, The Pennsylvania State University Press.

Ozbay N, Newman, SE, Brown WM (2004). Evaluation of *Trichoderma harzianum* strains to control crown and root rot of greenhouse fresh market tomatoes. Acta Horticulturae 635:79-85.

Perveen K, Bokhari NA (2012). Antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. African Journal of Microbiology Research 6(13):3348-3353.

Papavizas GC, Lumsden RD (1980). Biological control soil borne fungal propagules. Annual Review of Phytopathology 18:389-413.

Poovendran P, Kalaigandhi V, Parivuguna V (2011). *In vitro* study of antagonistic effect of *Trichoderma* sp., on tea plant pathogen, *Phomopsis theae*. Archives of Applied Science Research 3(4):352-358.

Sivakumar D, Wilson Wijeratnam RS, Wijesundera RLC, Marikar FMT, Abeyesekere M (2000). Antagonistic effect of *Trichoderma harzianum* on postharvest pathogens of rambutan (*Nephelium lappaceum*). Phytoparasitica 28(3):240-247.

Sivan A, Ucko O, Chet I (1987). Biological control of Fusarium rot of tomato by *Trichoderma harzianum* under field conditions. Plant Disease 71:587-592.

Tran NH (2010). Using *Trichoderma* species for biological control of plant pathogens in Vietnam. Journal of ISSAAS 16 (1):17-21.

Van Steekelenburg NAM (1991). Effect of *Trichoderma harzianum* on incidence of Fusarium crown and root rot in rockwool-grown tomatoes. In: Beemster ABR, Bollen GJ, Gerlagh M, Ruissen MA, Schipper B, Tempel A (Eds). Biotic Interactions and Soil-borne Diseases. Elsevier, Amsterdam pp 199-205.

Yedidia I, Benhamou N, Chet I (1999). Induction of defense responses in cucumber (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Applied and Environmental Microbiology 65:1061-1070.

Yucel S, Ozarslandan A, Colak A, Ay T, Can C (2007). Effect of solarization and fumigant applications on soilborne pathogens and root-knot nematodes in greenhouse grown tomato in Turkey. Phytoparasitica 35:450-456.