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Full Length Research Paper

Antagonistic action of *Trichoderma harzianum* and *Trichoderma viride* against *Fusarium solani* causing root rot of tomato

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Trichoderma* spp. are fungi that are present in substantial numbers in nearly all agricultural soils. They received attention mainly due to their importance in biological control of soil born plant pathogens. They are used in reasonably large quantities in plant agriculture both for disease control and increased yield. The antagonistic potential of *Trichoderma harzianum* and *Trichoderma viride* were evaluated in the present study against *Fusarium solani* casual agent of root rot of tomato. *In vitro* test showed that *T. harzianum* exhibited higher antagonist activity than *T. viride* against *F. solani.* Dual culture and volatile metabolites assays showed that *T. harzianum* was more effective in suppressing the growth (51.4 and 38.1%) of pathogen. Culture filtrates of *T. harzianum* and *T. viride* caused reduction in the growth of *F. solani* by 21.3 and 17.3%, respectively. Under pot conditions, tomato plants treated with *T. harzianum* showed 117.5 and 138.9% increase in plant height and dry weight, respectively compared to the control. *T. harzianum* caused 55.5% reduction in disease incidence while *T. viride* reduced root rot by 41.1%. The results obtained here clearly indicate that *T. harzianum* is more effective antagonist against *F. solani* as compared to *T. viride.

Key words: *Fusarium solani*, *Trichoderma harzianum, Trichoderma viride,* root rot, *Lycopersicon esculentum*, biological control.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is considered as one of the most important vegetable crops in Saudi Arabia. According to FAOSTAT (2012), in Saudi Arabia the production of tomato was 489800 tons in the year 2010, which include production from traditional farms and greenhouses. Tomato plants are subjected to be attack by several soil-borne fungal pathogens, which cause serious diseases such as root rot and wilt (Vawdrey and Petersone, 1988; El-Mougy, 1995; Srinon et al., 2006; Morsy et al., 2009; Alwathnani and Perveen, 2012). *Fusarium solani* (Mart.) Appel and Wollenw is widely found in soil and constitutes one of the most important phytopathogens in agriculture. Symptoms include stunted growth with varying degrees of chlorosis, mottling and necrotic spotting on young foliage and cortical rot of tap root or discoloration of stele of the main lateral roots. In severely affected plants, the tap roots completely girdled and the crown was also rotted. Although plants are not often killed by the disease but it reduces the yield (Vawdrey and Petersone, 1988).

In Saudi Arabia, the tomato production practices involve the use of chemicals in the form of pesticides, fungicides and/or fertilizer (Al-Kahtani and Hebicha, 1997). Biological control is an alternative to the use of chemical pesticides. A variety of soil microorganisms have demonstrated activity in the control of various soil borne pathogens. Of the fungi used for control of soil borne pathogens, species of *Trichoderma* have received the most attention. *Trichoderma* spp. have evolved numerous mechanisms that are involved in attacking

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other fungi. These mechanisms include competition for space and nutrients (Elad et al., 1999), mycoparasitism (Haran et al., 1996; Lorito et al., 1996), production of inhibitory compounds (Sivasithamparam and Ghisalberti, 1998), inactivation of the pathogen's enzymes (Roco and Perez, 2001) and induced plant resistance (Yedidia et al., 2000). Today, more than 50 different *Trichoderma*-based agriculture products are registered in many countries in the five continents, and sold and applied to protect and improve yield of vegetables, ornamentals and fruit trees (Lorito, 2005). *Trichoderma* is completely safe and throughout 55 years of research, there has never been a recorded adverse reaction on humans and livestock (Ozbay and Newman, 2004).

The objective of this study was to evaluate the efficacy of existing biocontrol species of *Trichoderma harzianum* (Rifai) and *Trichoderma viride* (Pers.) against *Fusarium solani in vitro* and under greenhouse conditions.

MATERIALS AND METHODS

Isolation and pathogenicity test of *F. solani*

F. solani was isolated from naturally infected tomato plants grown on a commercial scale in a greenhouse. Root and crown sections were surface sterilized in sodium hypochlorite solution and transferred to plates of potato-dextrose agar (PDA) amended with streptomycin sulphate. Based on microscopic studies, the pathogen was identified as *F. solani* on the basis of presence, shape and size of macro- and micro-conidia (Leslie and Summerel, 2006).

The pathogenicity test was conducted by inoculating 20 healthy seedlings of two weeks old tomato plants with suspension of *F. solani* (1×10^7 CFU/ml) isolated from infected plants, the roots were inoculated by following the root-dip method. Inoculated seedlings were transplanted singly into steam-sterilized peat moss soil and sand mixed in a ratio of 5:1 respectively. Seedlings inoculated with sterilized water (1 ml per plant) served as control. After four weeks, a number of plants showed wilting and extensive root rot. *F. solani* was re-isolated from the root lesions of plants inoculated with the pathogen, while control plants remained asymptomatic. The pathogenicity test was conducted twice.

In vitro assay of *T. harzianum* and *T. viride* against *F. solani*

T. harzianum and *T. viride* were procured from the Botany and Microbiology Department, King Saud University, Riyadh. Stock cultures of *F. solani* and test fungi were maintained on PDA slants and stored at 4°C in refrigerator.

For dual culture assay, mycelial blocks of 5 mm diameter were cut from the active margin of seven days old colonies of antagonists and *F. solani*. A block of pathogen and one of the antagonists were placed 3 cm apart on PDA medium. Control of the test fungi were prepared by inoculation separately on a side of the Petri plates containing PDA medium.

The effect of volatile metabolites from *T. harzianum* and *T. viride* against *F. solani* was tested in the assemblage described by Dennis and Webster (1971). Two bottoms of Petri dishes containing PDA were individually inoculated with a disc of pathogen and antagonist, and bottoms were adjusted and attached by tape. The control was devoid of antagonist.

The efficacy of culture filtrates of *T. harzianum* and *T. viride* against *F. solani* was assessed by inoculating three discs (5 mm diameter) of *Trichoderma* spp. cut from the active margin of seven days old colonies into 100 ml sterilized potato dextrose broth and incubated at 25±2°C without shaking for 10 days. After the incubation period the culture broth was filtered through Whatman filter paper no.1 and re-filtered through Millipore membrane filter (0.45 µ) to obtain cell-free culture filtrates. Culture filtrates (4 ml) of each *Trichoderma* sp. was poured in sterilized Petri dish followed by pouring 16 ml of PDA, so as to increase the concentration to 20%. In control, sterilized water was added to PDA instead of culture filtrate. Mycelia discs of the *F. solani* (5 mm in diameter) obtained from actively growing colonies were placed in the center of the solidified agar plates. The Petri dishes were incubated at 25±2°C for four days and later the percent inhibition in the radial colony growth compared to the control was calculated.

The assays of dual culture interaction, volatile metabolites and inhibitory effect of culture filtrate were conducted in triplicates in randomized block design and repeated twice. The percent inhibition of mycelia growth of the pathogens was calculated using following formula (Singh et al., 2002):

$$I = (C-T/C) \times 100$$

Where, I = inhibition (%), C = colony diameter in control plate and T = colony diameter in treated plate.

Effect of *Trichoderma* spp. on inhibition of *F. solani* and growth of tomato plant under pot conditions

Bioefficacy of the *T. harzianum* and *T. viride* against *F. solani* was evaluated under pot conditions. For pot trial, the method described by Perveen et al. (2007) with slight modifications was used. Pots of 9 cm diameter were surface sterilized with 1% sodium hypochlorite and filled with 100 g of autoclaved mixture (5:1) of peat moss soil and sand. Single seedling (two weeks old) of tomato plant grown in sterilized soil was transplanted in each pot. Four plates of *F. solani* culture grown on PDA were scraped with a sterilized spatula and mixed with sterilized distilled water to obtain 1.0×10^6 CFU/ml spore suspension. *F. solani* spore suspension (10 ml) was added around the plant root by removing soil. A spore suspension [3% (v/v) of *T. harzianum* and *T. viride*] was mixed with the pathogen infested soil separately. Pots inoculated with only distilled water served as uninoculated control, while *F. solani* alone inoculated pots served as inoculated control. Six replicates were prepared for each treatment. Pots were arranged in glass house on a rack in randomized block design. Plants were irrigated with sterile water, as per requirement and uprooted after one month to measure the height, dry weight and calculate the disease incidence. The disease incidence was calculated by measuring the infected portion in relation to total length of roots (Perveen et al., 2007) and graded on the scale of 0 to 3 where, 0 = 0 to 25% severity, 1 = 26 to 50%, 2 = 51 to 75% and 3 = 76 to 100%.

Statistical analysis

Data were analyzed by least significant difference (L.S.D.) test at probability of 0.05 to identify significant effect of a treatment. Duncan Multiple Range Test was used to evaluate the significant differences between treatments ($P \leq 0.05$). Analysis of variance (ANOVA) analysis was done with the SPSS statistics software.

RESULTS

Results of dual culture assay, volatile metabolites and culture filtrate of *Trichoderma* spp. indicate that the tested

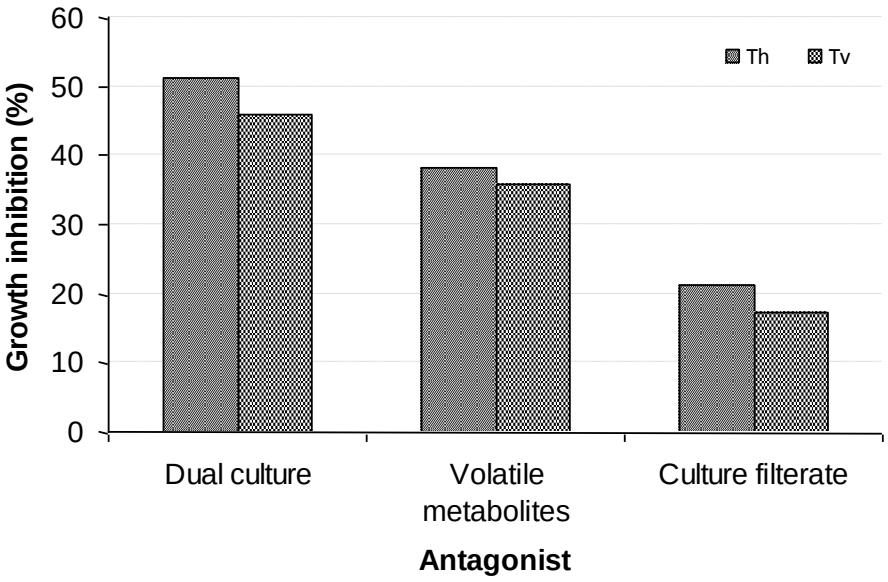

Figure 1. Antagonistic activity of *T. harzianum* and *T. viride* against *F. solani* evaluated by dual culture assay, volatile metabolites and culture filtrate. Each value is an average of six replicates. Th, *T. harzianum*; Tv, *T. viride*.

Table 1. Effect of *T. harzianum and T. viride* on height, dry weight and disease incidence of tomato plants inoculated with *F. solani* under pot conditions.

Treatment	Plant height (cm)	Plant dry weight (g)	Disease incidence
C	14.52^{a}	2.45^{a}	0.00^{a}
Fs	6.52^{b}	1.06^{b}	1.80^{b}
Fs + Tv	12.68^{c}	2.42^{a}	1.06^{c}
Fs+ Th	14.18^{d}	2.58^{a}	0.80^{d}

Each value is an average of six replicates. Data followed by different letters in the column are significantly different (P≤ 0.05) according to Duncan's multiple range test. C, Uninoculated control; Fs, *F. solani;* Tv, *T. viride;* Th, *T. harzianum.* Disease incidence graded on 0 to 3 scale where, 0 = 0 to 25% severity, 1 = 26 to 50%, 2 = 51 to 75% and 3 = 76 to 100%.

Trichoderma spp. were capable of inhibiting the growth of *F. solani* (Figure 1). The results of dual culture showed that *T. harzianum* reduced the mycelia growth of *F. solani* by 51.4% whereas *T. viride* inhibited the growth by 46.0%. Volatile metabolites from *T. harzianum* caused 38.1% growth inhibition of the pathogen while *T. viride* reduced the growth of *F. solani* by 35.8%. The culture filtrates of *T. harzianum* and *T. viride* caused appereciable reduction (21.3 & 17.3%, respectively) in the growth of *F. solani* however none were fungicidal to the pathogen.

Inoculation with F. *solani* caused both reduction in plant height (55.1%) and their dry weight (56.7%). In this study we have observed that plants inoculated with *T. harzianum and T. viride* showed significant (p≤0.05) plant growth promotion as well as increase in dry weight of plant (Table 1). Moreover, it was noted that *T. harzianum* was more effective in promoting the growth (117.5%) as well as in causing the increase in dry weight of plants (138.9%) as compared to *T. viride*. In addition *T. harzianum* reduced the disease incidence by 55.5% while *T. viride* caused 41.1% reduction in disease severity as compared to *F. solani* alone inoculated plants.

DISCUSSION

F. solani is a common pathogen of tomato in several regions of the world. It has been reported to cause wilt, pre- and post-emergence damping-off, root and crown rot of mature tomato plants and fruit rot**.** In Israel, *F. solani* is the principal cause of wilt in tomatoes resistant to *F. oxysporum* f.sp. *lycopersici* (Vawdrey and Petersone, 1988).

Trichoderma species were effective in controlling *F. solani*, the cause of root rot of tomato in both laboratory and pot conditions. *Trichoderma* spp. are common saprophytic fungi which is found in almost any soil and rhizosphere flora. The ability of some species of *Trichoderma* to antagonize and parasitize other fungi has made them effective biocontrol agents against range of plant pathogens (Elad, 2000; Freeman et al., 2004; Ashrafizadeh et al., 2005; Dubey and Suresh, 2007) although some have been occasionally recorded as plant pathogens (Menzies, 1993).

The dual culture interaction and volatile components results revealed that *Trichoderma* spp. caused appreciable inhibition of mycelia growth of *F. solani*. Previous studies have demonstrated that before mycelia of fungi interact, *Trichoderma* sp. produces low quantities of extracellular exochitinases (Kullnig et al., 2000; Brunner et al., 2003). The diffusion of these enzymes dissolves cell fragments of host cells. These cell fragments in turn induce the production of further enzymes that trigger a cascade of physiological changes, stimulating rapid and directed growth of *Trichoderma* spp. (Zeininger et al., 1999).

Also the culture filtrate of *T. harzianum* and *T. viride* caused inhibition of *F. solani,* however none were fungicidal. It is well known that *Trichoderma* produces a number of antibiotics such as trichodernin, trichodermol, harzianum A and harzianolide (Howell, 1998; Kucuk and Kivanc, 2004) as well as some cell wall degrading enzymes such as chitinases, glucanases that break down polysaccharides, chitins and glucanase, thereby destroying cell wall integrity (Lorito et al., 1996; Harman and Kubicek, 1998; Kubicek et al., 2001; Woo et al., 2006). The degree of effectiveness of these metabolites varies according to the nature, quality, quantity of antibiotics and inhibitory substances secreted by the antagonists (Harman and Kubicek, 1998). The further chemical investigation using gas chromatography mass spectrometry (GC-MS) for the presence of antifungal metabolites might indicate its potential as a source of novel and useful antifungal antibiotics.

Different isolates of *Trichoderma* have different combative ability for pathogen; their indirect effects may also vary. Therefore one of the most interesting aspects of biology is the study of the mechanisms employed by biocontrol agents to affect disease control. Possible mechanisms of antagonism employed by *Trichoderma* spp. includes nutrient and niche competitions, antibiosis by producing volatile components and non-volatile antibiotics that are inhibitory against a range of soil borne fungi, as well as parasitism. Also synergism between different forms of action modes occurs as the natural condition for the biocontrol of fungal pathogens. Many of these concepts have been extensively covered in recent reviews (Harman et al., 2004; Woo et al., 2006; Lorito et al., 2010; Druzhinina et al., 2011).

The observed increase in plant growth after inoculation with antagonist may be through modification of rooting system (Chao et al., 1986; Ahmad and Baker, 1987). Biological control may be safe, effective and eco friendly method for plant disease management. *Trichoderma* species added to soil or applied as seed treatment grow readily along with the developing root system of the treated plant (Harman, 2006; Howell et al., 2000). *Trichoderma* can parasitize fungal pathogens and produce antibiotics, in addition, the fungus have many positive effects on plant growth, yield, nutrient uptake, fertilizer utilization efficiency, rate of seed germination and systemic resistance to plant diseases (Harman et al., 2004; Harman, 2006). Cole and Zvenyika (1988) proposed that *F. solani* infection of tobacco plants could be controlled by incorporating *T. harzianum* into disease management procedures. A study carried by Yedidia et al. (1999) reported that *T. harzianum* inoculation improved the uptake of nutrients by the plant at a very early growth stage.

In the present communication, we have observed excellent antagonistic activity of *T. harzianum* against *F. solani in vitro* as well as in pot conditions. Therefore, the study establishes the potential use of *T. harzianum* for controlling root rot caused by *F. solani*. Though, further studies are necessary to test the efficacy of the biocontrol agent under field conditions and in different management practices.

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