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Biological Control of *Fusarium* wilt of Tomato (*Solanum lycopersicum*) by *Trichoderma spp.* as Antagonist Fungi

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ABSTRACT: *Fusarium* spp. (*F. solani* and *F. oxysporum*) are the important soil-borne pathogens and infects wide variety of hosts. The effects of *Trichoderma harzianum*, *T. asperellum*, and *T. virens* on the wilt disease complex of tomato (*Solanum lycopersicum*) caused by *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* were investigated under greenhouse conditions. Tomato cultivar inoculated with F. oxysporum f. sp. ciceri and R. solani, showed greater wilt incidence, chlorosis of leaves and induced vascular discoloration in roots. Soil administration with biocontrol agents adjusted the severity of wilt in roots, substantially (P<0.01). The disease control was highest with a combination of *T. harzianum*, *T. asperellum*, and *T. virens* (80-87%) followed by binary combination of Trichoderma. spp. (79%-82%), while the lowest control was done with *T. viride* (65%). It is concluded that *T. harzianum*, *T. asperellum* and *T. viride* could control pathogen attacks in tomato and it can be considered as an applicable strategy in control measures against pathogens.

Keywords: Tomato, Disease management, *Trichoderma* spp., *Fusarium oxysporum* f. sp. ciceri, *Rhizoctonia solani*

INTRODUCTION

Tomato *(Solanum lycopersicum* L.) is a major contributor to the fruit vegetable diet of humans. It is cultivated in essentially all countries either in fields or in protected culture. Its many varieties are now widely grown, sometimes in greenhouses in cooler climates. It is one of the most important vegetable crops in Iran and other countries (Abd-El Kareem *et al.,* 2006). *Fusarium* root and stem rot is regarded as one of the most devastating diseases in cucurbits affecting cultivations in many countries around the world (Pavlou and Vakalounakis, 2005). *Fusarium oxysporum* is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, causes major economic losses by inducing necrosis and wilting symptoms in many crop plants (Cotxarrera, *et al.,* 2002). Several diseases are known to limit worldwide production of tomato, of which *Fusarium solani* f. sp. *Eumartii* and *Fusarium oxysporum* f. sp. *ciceris* (*Fusarium* wilt) is one of the most important. Management of *Fusarium* wilt has been primarily through development of resistant cultivars as part of an integrated management approach. *Fusarium solani* strain (*F. solani* (Mart.) Sacc. f. sp. *Eumartii* (*C. carpenter*) (W.C. Snyder & H.N. Hans.) is a worldwide soil-borne fungus attacking a wide range of host plants including citrus (Sherbakoff, 1953) with a great overall impact on productivity. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot minimal or absent crop yield (Nemec *et al.,* 1996). *Fusarium* wilt of tomato caused by *Fusarium oxysporum f. sp. Lycopersici* (FOL) is the major limiting factor in the production of tomato. The disease causes great losses, especially on the susceptible varieties of tomato especially when soil and air temperature are rather high during the warm season (Agrios, 1997, Mandal *et al.,* 2009). The interaction between *F. oxysporum* and *F. solani* causes a root-rot disease complex that severely damages this important crop (Klotz, 1973).

Controlling such diseases mainly depend on fungicides treatments (Rauf, 2000). However, fungicidal applications cause hazards to human health and increase environmental pollution. Therefore, alternatives, eco-friendly approach treatments for control of plant diseases are needed (Rojo *et al.,* 2007). The biological control is the best alternative especially against soil borne pathogens. Biological control of pathogens, i.e., the total or partial destruction of pathogen populations by other organisms, occurs routinely in nature. Among the various antagonists used for the management of plant diseases, *Trichoderma* spp. plays a vital role. Recently, it was suggested that, trichoderma affects induced systemic resistance mechanism in plants against pathogens (Haggag and Amin, 2001, Prasad *et al.,* 2002, Hibar *et al.,* 2007, Jayalakshmi *et al.,* 2009). Among the various isolates of *Trichoderma*, *T. Asperellum*, *T. harzianum*, *T. virens*, *T. viride*, and *T. hamatum* are used against the management of various diseases of crop plants especially with soil borne pathogens. These filamentous fungi are very common in nature, with high population densities in soil and plant litters (Samuels, 1996). Many studies have proved the potential of *Trichoderma* spp. as biological agents antagonistic to several plant pathogens (Sivan and Chet, 1993, Naseby *et al.,* 2000, Tondje *et al.,* 2007, Houssien, *et al.,* 2010) and use of *Trichoderma* spp. on banana (Thangavelu 2004) arbuscular mycorrhiza (AM) on banana (Jaizme-Vega *et al.,* 1998). and soil amendment of Lettuce on cucumber have also been reported.

In the presented study, a promising strategy for bio control disease agents such as *F. solani* and *F. oxysporum* that exposed to tomato field has been implemented by three *Trichoderma* spp., *T. Asperellum, T. harzianum* and *T. virens*.

MATERIALS AND METHOD

A. Source of Fungi and tomato filed

Pathogenic fungal isolates, *F. solani* (Mart.) Sacc. f. sp. *Eumartii* and *Fusarium oxysporum* f. sp. *Lycopersici* were isolated from tomato roots according to method described by Nelson *et al*. (1983). Non-pathogenic fungal isolates (*Trichoderma* spp.), *T. harzianum, T. Asperellum* and *T. virens* were obtained from tomato rhizosphere and field soil during the preliminary study according to methods described by Elad and Chet (1983) and Harman (2006).

B. Pathogenic and Trichoderma Fungi Inoculants

Both *Fusarium spp.* was subcultured on Potato Dextrose Agar at 25± 1°C. 10 ml of each of *Fusarium spp.* culture suspension (10^7cfu/ ml) was added to soil of each field. *Trichoderma* species were used: *T. harzianum* (T-100) *T. asperellum* and *T. viride*. Cultures were maintained on PDA medium and stored at 4°C for further use. Concerning to the *Trichoderma*, three types of treatments were used alone or in combination; so that soil treatment by adding 10 mL from all or each of *Trichoderma spp.* for selected tomato fields from a concentration of 10^8cfu/ ml were added one week before *Fusarium* inoculation.

C. Field soil infestation and treating

For root dipping, each biomass, alone and in combination were prepared separately in different container containing an un-centrifuged fungal suspension (1.8×10^7) and 100.0 g L^{-1} of both bio control fungi biomass except for pathogen *Fusarium*. Before the transplanting, roots of transplants were dipped into each biomass and then transplanted to field soil artificially infested with pathogen. Twenty tomato fields (treatments) designed for soil infecting by *F. solani* (Fs) and *F. oxysporum* (Fo) and treating by *T. harzianum* (T1), *T. Asperellum* (T2), *T. virens* (T3), T1 + T2, T1 + T3, T2 + T3, T1 + T2 + T3 accompanying with control group in order to control *Fusarium* rot of tomato by *Trichoberma spp.* alone and in combination. Soil was artificially infested with pathogen fungi grown on moistened PDA at rate of 100 gm^{-2} soil. Each treatment consisted of four replicate rows of 10 plants row^{-1}. Disease was monitored for 6-8 weeks and assayed as the total percentage of plants showing any wilt symptoms due to the pathogen (yellowing and dropping of leaves, vascular discoloration, wilting) and calculated as percentage of disease incidence (Table 1).

Table 1: Root discoloration and percentage of disease incidence in tomato fields treated with bio agents *(Trichoderma harzianum, T. asperellum* and *T. virens)* against *Fusarium* wilt caused by *Fusarium oxysporum* and *Fusarium solani.*

Treatments[1, 2]	External yellowing and dropping	Root discoloration %	disease incidence %
Control	0.50^{g}	0.25^{g}	0^{h}
T1	0.50^{g}	0.00^{g}	0^{h}
T2	0.75^{g}	0.25^{g}	0^{h}
T3	0.75^{g}	0.25^{g}	0^{h}
Fo	90.75^{a}	59.00^{a}	95.00^{a}
Fs	84.00^{a}	57.75^{a}	94.25^{a}
Fo + T1	21.05^{b}	27.75^{b}	21.50^{bc}
Fs + T1	19.50^{cd}	14.50^{e}	20.50^{c}
Fo + T2	20.25^{cd}	18.50^{dc}	19.75^{de}
Fs + T2	17.50^{ed}	17.50^{dc}	19.25^{de}
Fo + T3	20.25^{b}	18.00^{c}	23.50^{b}
Fs + T3	18.50^{cb}	17.00^{dc}	20.75^{bc}
Fo + (T1+T2)	12.00^{f}	12.75^{f}	15.25^{de}
Fs + (T1+T2)	11.25^{f}	13.50^{ef}	14.00^{de}
Fo + (T1+T3)	13.00^{ef}	11.75^{de}	12.75^{e}
Fs + (T1+T3)	11.75^{f}	12.50^{ef}	14.50^{fg}
Fo + (T2+T3)	12.50^{f}	10.75^{fg}	13.50^{d}
Fs + (T2+T3)	11.75^{f}	11.00^{ef}	12.75^{e}
Fo + (T1+T2+T3)	9.50^{f}	9.50^{f}	12.00^{e}
Fs + (T1+T2+T3)	9.25^{f}	6.75^{f}	10.25^{f}
SEM	0.725	0.620	0.733
P value	**	**	**

[a-h,] Values in the same row and variables with no common superscript differ significantly. [1] Values represent the means of twelve observations per treatment and standard error of mean (SEM). [2] Fs = *F.solani*, Fo = *F.oxysporum*, T1 = *T.harzianum*, T2 = *T.asperellum*, T3 = *T.virens*; [3] *: $P<0.05$, **: $P<0.01$, **: $P<0.001$.

Stem sections of wilted plants were surface-disinfested in 0.5% sodium hypochlorite and plated on PCNB medium to confirm the presence of the wilt pathogen. Stem sections of asymptomatic plants were also plated at the conclusion of the experiment to evaluate potential pathogen infection. All experiments were performed twice with four replicates per treatment and arranged in a randomized complete block design. Disease incidences (%) were analyzed using an Analysis of Variance (ANOVA) and grouped by Duncan test.

RESULTS

Antifungal activity of *Trichoderma spp.* against *Fusarium spp.* in a nutritional competition is shown in in Fig. 1. The active principles of algae are responsible for antifungal activity. It is clear that the algae *Trichoderma spp.* has the potential to control the fungal pathogen *Fusarium spp.* which causes the fungal disease to a larger extent.

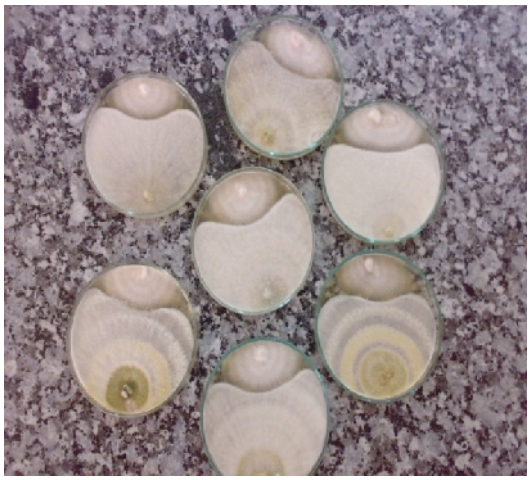

Fig. 1. Antifungal activity of *Trichoderma* sp. against *Fusarium sp.* in a nutritional competition.

Table 2: percentage of disease incidence and efficacy % in chickpea fields treated with bioagents *(Trichoderma harzianum, T. asperellum* and *T. virens)* against *Fusarium* wilt caused by *Fusarium oxysporum* and *Fusarium solani.*

Treatments	% Disease incidence	% Efficacy
Control	0	100
T1	0	100
T2	0	100
T3	0	100
Fo	100[d]	0
Fs	100[d]	0
Fo + T1	25[b]	79
Fs + T1	29[a]	75
Fo + T2	27[b]	74
Fs + T2	23[b]	77
Fo + T3	35[c]	65
Fs + T3	32[c]	68
Fo + (T1+T2)	21[a]	79
Fs + (T1+T2)	18[a]	82
Fo + (T1+T3)	20[a]	80
Fs + (T1+T3)	16[a]	80
Fo + (T2+T3)	18[a]	82
Fs + (T2+T3)	19[b]	81
Fo + (T1+T2+T3)	15[a]	85
Fs + (T1+T2+T3)	14[a]	87

FS = *Fusarium solani*, FO = *Fusarium oxysporum*, T1 = *Trichoderma harzianum*, T2 = *Trichoderma Asperellum*, T3 = *Trichoderma virens*

In the present study tomato roots were planted in *Fusarium* pre-infested soil and then treated with one or mix of Trichoderma spp. (T1, T2, T3, T1 + T2, T1 + T3, T2 + T3, T1 + T2 + T3) for detecting the best bio-control treatment against pathogens. The *F. solani*-infested fields treated with mix trio of *Trichoderma spp.* (T1 + T2 + T3), showed the lowest external yellowing and dropping**,** root discoloration and disease incidence (9.25%, 6.50% and 10.25%, respectively). *Trichoberma spp.* bio control of tomato fields exposed to pathogens significantly reduced *Fusarium* rot in field (Table 1). *F. solani* infested groups that treated with *Trichoberma spp.* caused to a significant decline in disease incidence, hence detected a well-control results. The best bio control agents efficacy was related to tomato fields controlled by ≥ 2 *Trichoberma spp.* (Table 2). The alone treatments showed a lower control especially in compared to T1 + T2 + T3 groups; however, tomato roots rot well-controlled by *T. harzianum* (Fo + T1 = 79% and Fs + T1 = 80%) in compared with other *T. asperellum* (T2) and *T. virens* (T3). The lowest control was observed in fields infested to both *Fusarium spp.* treated with *T. virens* (T3).

The disease control was highest with a combination of *T. harzianum*, *T. asperellum*, and *T. virens* (80-87%) followed by binary combination of *Trichoderma. spp*. (79%-82%), while the lowest control was done with *T. viride* (65%).

DISCUSSION

As shown in Table 1, all applied treatments of *Trichoderma* either separately or in combination could control against *F. oxysporum* and *Fusarium solani* and wilt and root rot of tomato fields. Plants treated one week before inoculation with the pathogen, appeared healthy and with no wilting or root rot symptoms. Wilt can be observed within 25 days of sowing into infected soil (Nene *et al.*,1978). Wilting may also occur in adult plants up until the reproductive and podding stage. Drooping of the petioles, rachis and leaflets in the upper part of the plant, together with the pale green colour of the foliage, are the most common symptoms. Often within 2 to 3 days the entire plant is affected (Haware *et al.*, 1986). Lower leaves also become chlorotic. When uprooted before completely dried, affected plants show no external root discolouration. However, internal discolouration may be seen extending up towards the stem. Internal discolouration is due to infection of the xylem tissues of the root and stem. Transverse sections of the infected root examined under the microscope show the presence of hyphae and spores of the fungus in the xylem. This is a diagnostic feature of *Fusarium* wilt. In certain ctomato cultivars typical symptoms may not develop. Instead, there is a yellowing and drying of the lower leaves, and a stunting of the plant. Roots will show internal discolouration.

Based on above-mentioned information related to *Fusarium* wilt and rot of tomato that was sourced from the National Diagnostic Protocol, it was revealed that disease incidence percentage (%) in the presented study was significantly reduced by each controlling agents compared with the infected controls (Fo and Fs) which was 0% (Table 1). All treatments had a higher efficacy in inducing resistance; however, in *Fusarium*-infected and subsequently Trichoderma-treated fields, disease incidence percentages was highly significantly reduced by applying all *Trichoderma spp.* in controlling infected tomato fields and therefore, a better protection against disease incidence was observed. However, symptoms in *F. spp.*-infected groups that treated with all three of *T. spp.* (*T. harzianum*, *T. Asperellum* and *T. virens*) were observed with almost negligible differences compared with two or one of *T. spp.* with the exception of Fusarium-infected groups that separately treated with *T. virens* (T3), so that differences were high compared to other treated groups (12 to 22 % disease incidence). Our results are agreement with other findings (Amsellem *et al.,* 1999, Benhamou and Chet, 1993, Yedidia, *et al.*, 2000, Sivan and Chet, 1986).

In present study, the better efficacy was observed in treatments including *T. harzianum* (Fig. 1). Therefore, combination of *Trichoderma* spp. provided better disease control than alone isolates against *Fusarium*.

Trichoderma spp., are free-living fungi that are common in soil and root ecosystems (Thangavelu *et al.*, 2004). They are highly interactive in root, soil and foliar environments. They produce or release a variety of compounds that induce localized or systemic resistance responses in plants. This fungal biocontrol agent has long been recognized as biological agents, for the control of plant disease and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients. It can be efficiently used as spores (especially, conidia), which are more tolerant to adverse environmental conditions during product formulation and field use, in contrast to their mycelial and chlamydospore forms as microbial propagules (Amsellem *et al.*, 1999). However, the presence of a mycelial mass is also a key component for the production of antagonistic metabolites (Benhamou and Chet, 1993, Yedidia *et al.*,2000). Several reports indicate that *Trichoderma* species can effectively suppress Fusarium wilt pathogens (Sivan *et al.*, 1986). *T. harzianum* has multiple mechanisms of action, including coparasitism via production of chitinases, β-1-3 glucanases and β-1-4 glucanases, antibiotics, competition, solubilisation of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process (Altomare, *et al.*,1999, Elad and Kapat, 1999).

Raghuchander *et al.* (1999) reported that *T. viride* and *P. fluorescens* were equally effective in reducing the wilt incidence. Inoculation of potted abaca plants with *Trichoderma viride* and yeast showed 81.76% and 82.52% reduction of wilt disease severity respectively in the antagonist treated plants.

There are several reports demonstrating control of a wide range of plant pathogens including *Fusarium* spp. by *Trichoderma* spp. by elicitation of induced systemic or localized resistance which occur due to the interaction of bioactive molecules such as proteins avr-like proteins and cell wall fragments released by the action of extracellular enzymes during mycoparasitic reaction. Thangavelu and Musataffa, reported that the application of *T. viride* NRCB1 as rice chaffy grain formulation and challenge inoculation with *Foc* in cv. Rasthali resulted in the induction of defense related enzymes such as Peroxidase and Penylalanine Ammonia lyase (PAL) and also the total phenolic content significantly higher (>50%) as compared to control and *Foc* alone inoculated banana plants and the induction was maximum at 4-6th day after treatment. They suggested that this increased activities of these lytic enzymes and thus increased content of phenols in the *T. viride* applied plants might have induced resistance against *Foc* by either making physical barrier stronger or chemically impervious to the hydrolytic enzymes produced by the pathogen (Thangavelu and Mustaffa, 2010).

CONCLUSION

From the results of presented study it is concluded that, although all bio control agents applied individually reduced disease incidence, synthetic treatments including *T. harzianum, T. Asperellum* and *T. virens* were showed more protective effect for bio control tomato field exposed to *F. solani* and *F. oxysporum*.

It is widely known that environmental parameters such as abiotic (soil type, soil temperature, soil pH and water potential) and biotic (plant species and variety, and microbial activity of the soil) factors as well as other factors such as method and timing of applications may have influence on the biological control efficacy of *Trichoderma* isolates. Therefore, it is important that *Trichoderma* bio control potential in field should be further evaluated. Although several biocontrol agents including botanicals have been tried against Fusarium wilt disease, still this lethal disease could not be controlled completely. Besides most of the biocontrol experiments were conducted either under lab condition or green house conditions and only in few cases, field experiments were conducted.

In addition, mixture of bioagents of different genera or mixture of fungal and bacterial bioagents along with or without fungicides or botanicals have to be tried to improve the level and extent of disease control under different environmental and soil conditions.

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