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Full Length Research Paper

Bio controlling two pathogens of chickpea *Fusarium solani and Fusarium oxysporum* by different combinations of *Trichoderma harzianum, Trichoderma asperellum* and *Trichoderma virens* under field condition

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The capability of *Trichoderma* (T.) *harzianum* (T1), *T. asperellum* (T2) and *T. virens* (T3) as bio control agents for two pathogens of chickpea roots, *Fusarium solani* and *Fusarium oxysporum* were evaluated alone or in combination under field and greenhouse conditions. Chickpea planted in artificial soil infested with pathogens *F. solani* and *F. oxysporum were* treated with T1, T2, T3, T1+T2, T1+T3, T2+T3, T1+T2+T3. Our results indicated that the best biocontrol treatment occurred in groups infested with *F. solani* and treated with T1+T2+T3 (84%), followed by groups exposed to *F. oxysporum* with the same treatment (83%). The least effective group was roots infested with both *Fusarium* spp. and treated with *T. virens* (T3). It is concluded that although all bio control agents that were applied individually reduced disease incidence, synthetic treatments showed a more protective effect as a biocontrol for chickpea fields exposed to *F. solani* and *F. oxysporum* under greenhouse condition.

Key words: *Fusarium solani*, *Fusarium oxysporum, Trichoderma*, biocontrol, chickpea.

INTRODUCTION

Fusarium is an important and insidious disease which attacks chickpea, bean, wheat, barley and other grains worldwide, especially in humid and semi-humid areas (Schroeder and Christensen, 1963). *Fusarium* species can also grow in postharvest if grain is not dried properly.
Fusarium solani is a worldwide soil-borne fungus that attacks a wide range of host plants including citrus (Sherbakoff, 1953), with a great overall negative impact on productivity. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot, and minimal or absent crop yield (Nemec, 1976). Studies conducted by Menge and Nemec (1997) showed that the most common citrus rootstocks are susceptible to dry root rot caused by *F. solani*, and their results are consistant with other field observations (Klotz, 1973).
F. oxysporum is a ubiquitous soil inhabitant that has the ability to exist as saprophytes, and can degrade lignins (Sutherland et al., 1983) and complex carbohydrates (Snyder and Hansen, 1940) associated with soil debris. They are also pervasive plant endophytes that can colonize plant roots (Gordon et al., 1989) and may even protect plants or be the basis of disease suppression (Larkin et al., 1993). Although the predominant role of these fungi in soils may be as harmless or even beneficial plant endophytes or soil saprophytes, many strains within the *F. oxysporum* complex are pathogenic to plants, especially in agricultural settings.
In many regions of the world, chickpea (*Cicer arietinum* L.) is a popular vegetable and chief source of protein in the human diet. During chickpea cultivation problems

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have occurred that were connected to diseases. which could reduce yield and crop quality. Chickpea is susceptible to *Fusarium* root rot strain (*F. solani* (Mart.) Sacc. f. sp. *eumartii* (C. Carpenter) (W.C. Snyder & H.N. Hans.) and *Fusarium* wilt strain (*F. oxysporum* Schlechtend.: Fr. f. sp. *ciceris* (Padwick) Matuo and K. Sato). The interaction between *F. oxysporum* and *F. solani* causes a root-rot disease complex that severely damages this important crop (Klotz, 1973).

A biological control is the best alternative especially against soil borne pathogens, compared to chemical controls. Biological control of pathogens, i.e., the total or partial destruction of pathogen populations by other organisms, occurs routinely in nature (Agrios, 2004). Among the various antagonists used for the management of plant diseases, *Trichoderma* spp. plays a vital role. Among the various isolates of *Trichoderma*, *T. asperellum, T. harzianum, T. virens, T. viride,* and *T. hamatum* are used for 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). Teleomorphs of *Trichoderma* are species of the ascomycetes genus *Hypocrea*. 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 many strategies to control this disease on chickpea have been investigated in the field (Sarwar et al., 2005; Akhtar and Siddiqui, 2007; Chérif et al., 2007; Jayalakshmi et al., 2009).

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

MATERIALS AND METHODS

Source of Pathogenic and Non-pathogenic Fungi

Pathogenic fungal isolates, *F. solani* (Mart.) Sacc. f. sp. *eumartii* and *Fusarium oxysporum* f. sp. *lycopersici* were isolated from chickpea 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 chickpea rhizosphere and field soil during the preliminary study according to methods described by Elad and Chet (1983) and Harman (2006). The experiment to evaluate potential pathogen infection. Experiment were conducted in Iran county of Azarbayjan province in 2009-20011.

Field Tests

Both *Fusarium* spp. were subcultured on Potato Dextrose Agar (PDA) at 25± 1°C. 10 ml of each *Fusarium* spp. culture suspension (10^7cfu/ ml) was added as the soil treatment for each field. *Trichoderma* species that were used included *T. harzianum* (T-100) *T. asperellum* and *T. viride*. Cultures were maintained on PDA medium and stored at 4°C for further use.

Seven types of *Trichoderma* treatments were used either alone or in various combinations. Soils treated by adding 10 ml of all or each of *Trichoderma* spp. for selected chickpea fields were inoculated with a concentration of 10^8cfu/ ml one week before the *Fusarium* inoculation.

Greenhouse Tests

Our greenhouse test was conducted in summer 2008, in East Azerbaijan Province, Iran. 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 greenhouse soil artificially infested with pathogen. Twenty chickpea 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 chickpea by *Trichoderma spp.* alone and in combination.

Greenhouse soil was artificially infested with pathogen fungi grown on moistened wheat bran-corn mill at rate of 100 g m^{-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). 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 greenhouse 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 AND DISCUSSION

Chickpea planted in artificial soil infested with *F. solani* and *F. oxysporum* were treated with T1 (*T. harzianum*), T2 (*T. asperellum*), T3 (*T. virens*), T1+T2; T1+T3; T2+T3; T1+T2+T3 for detecting the best bio control treatment (alone or in combination) against each pathogen agents

Table 1. Percentage of disease incidence (DI) and efficacy % in chickpea roots and 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	19[b]	81
Fs + T1	18[a]	82
Fo + T2	21[b]	79
Fs + T2	20[b]	80
Fo + T3	35[c]	65
Fs + T3	32[c]	68
Fo + (T1+T2)	17[a]	83
Fs + (T1+T2)	18[a]	82
Fo + (T1+T3)	17[a]	83
Fs + (T1+T3)	18[a]	82
Fo + (T2+T3)	18[a]	82
Fs + (T2+T3)	19[b]	81
Fo + (T1+T2+T3)	17[a]	83
Fs + (T1+T2+T3)	16[a]	84

*The experiment was conducted completely randomized design and means were compared using LSD $_{1\%}$ test.

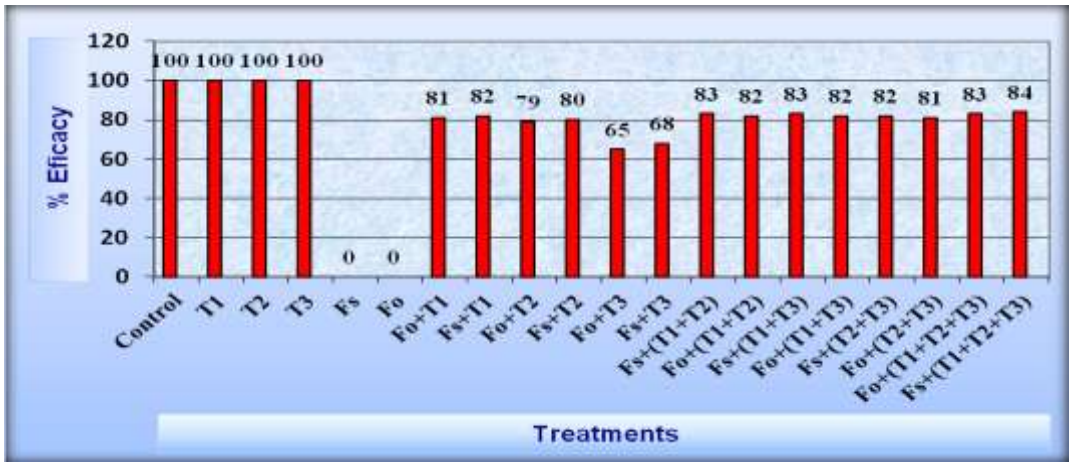

Figure 1. Percentage of efficacy in chickpea fields treated with bio agents (*Trichoderma* spp.) against *Fusarium* wilt caused by *Fusarium oxysporum* and *Fusarium solani.* FS= *Fusarium solani*; FO= *Fusarium oxysporum*; T1= *Trichoderma harzianum*; T2= *Trichoderma asperellum*; T3= *Trichoderma virens.*

in an *in vitro* condition.

Trichoderma spp. bio control of chickpea fields exposed to pathogens significantly reduced *Fusarium* rot in greenhouse conditions (Table 1).

F. solani infested groups treated with *Trichoberma* spp. caused a significant decline in disease incidence. The best bio control was related to chickpea fields controlled by ≥ 2 *Trichoberma* spp. (82 – 84% effective). The alone

treatment by each of *T.* spp. showed a lower result especially compared to T1+T2+T3 groups; however, chickpea root rot was well-controlled by *T. harzianum* (Fo+T1=81% and Fs+T1=82%, Figure 1) compared to other *Trichoberma* spp. (*T. asperellum*, T2 and *T. virens*, T3). The lowest result was observed in roots infested with both *Fusarium* spp. and treated with *T. virens* (T3).

provided better disease control than alone isolates against *Fusarium*.

Effects on Disease Incidence

The resistance was evident as a reduction in disease incidence compared with the infected control. As shown in Table 1, all applied treatments of *Trichoderma* either separately or in combination protected chickpea against *Fusarium solani* and *F. oxysporum*. Plants treated one week before inoculation with the pathogen, appeared healthy and with no wilting symptoms. Disease incidence percentage (%DI) was significantly reduced (0%) compared to the infected controls (Fo and Fs) (Figure 1). But in infected fields treated with *Trichoderma* spp. the %DI was highly significantly reduced by applying more controlling agents (*Trichoderma* spp.) to infected chickpea fields, and thus a better protection against disease incidence was observed. However, symptoms in *F.* spp.-infected groups 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 *Trichoberma* spp. (1 to 4% DI), with the exception of *F.* spp.-infected groups that were separately treated with *T. virens* (T3), so that differences were high compared to other treated groups (14 to 19 % DI). Thus, all treatments had a high efficacy in inducing resistance. Our results are in harmony with other research results (Haggag and Amin, 2001; Prasad et al., 2002; Mayer, 2006; Chérif et al., 2007; Jayalakshmi et al., 2009).

The competitive ability of a non-pathogenic strain partly determines its capacity to establish in soil and in the plant rhizosphere and is probably involved in its capability to colonize the root surface. Different strains have different capacities to colonize heat treated soil (Benhamou et al., 2002). In addition, saprophytic colonization of soil depends not only on the fungal strain but also on biotic and abiotic soil characteristics. Colonisation of the root surface and root tissues probably depends not only on the fungal strain but also on the plant species and plant cultivar.

Trichoderma spp. are among the most-promising bio control agents that can be used against many fungal plant pathogens. *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 (Sivan and Chet, 1993; Altomare et al., 1999; Elad and Kapat, 1999; Harman, 2006). Hence, the better efficacy was observed in treatments including *T. harzianum* (Figure 1). Therefore, combination of *Trichoderma* spp.

CONCLUSION

From the results of present 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 chickpea field exposed to *F.solani* and *F.oxysporum* under greenhouse condition. This study showed that species *T. harzianum* and *T. asperellum* have great potential to control chickpea *Fusarium* disease. Findings also showed that a combination of three species of antagonist can be effective *in Fusarium* diseases control. These isolates decreased the disease severity between 83 to 84 percent in the probability level of 1%. Treatment Fs + (T1+T2+T3) was the most effective one in reducing disease severity in plants.

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