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Control *Fusarium* Rot of Bean by Combination of by *Trichoderma harzianum* and *Trichoderma asperellum* in Greenhouse Condition

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Abstract: In this study, the ability of biocontrol agents, *Trichoderma harzianum* and *T. aperellum* applied in combination and alone, which had been isolated from soil and root bean field were compared to control of *Fusarium solani*, these isolates had shown good control of *Fusarium solani in vitro* condition and bean roots were treated with *T. harzianum* individually and in combination and planted in artificially infested soil with pathogen *F. solani*. Although, all biocontrol agents applied individually reduced disease incidence, treatments as combination, except for *T. harzianum* (T-7) + *T. asperellum* (T-6) showed more protective effect combination of *T. harzianum* (T-7) + *T. asperellum* (T-3) isolate gave the best control (61.8) at greenhouse condition.

Key words: *Fusarium* rot, bean, combination, *Trichoderma*, control, greenhouse condition

INTRODUCTION

Fusarium solani is a highly destructive pathogen of field grown bean in cereal production areas. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot minimal or absent crop yield.

Many strategies to control this disease on bean have been investigated in the field. A promising strategy for the replacement of chemicals has been the implementation of biocontrol technology, used individually or as an integrated pest management component. The recent developments in the commercialization of biocontrol products has accelerated this approach. Biocontrol preparations of both fungi and bacteria have been applied to seeds, seedlings and planting media in several ways to reduce plant diseases in the field with various degrees of success (Alabouvette *et al*., 1993; Baker, 1990).

Two of the major biocontrol agents, which reduce soilborne diseases of various crops include isolates of the fungus *Trichoderma* sp. (Lumsden and Locke, 1989). Other antagonist, recovered from *Fusarium* wilt-suppressive soils, especially nonpathogenic *F. oxysporum*, have been used to reduce *Fusarium* wilt diseases of several different crops (Minuto *et al*., 1995).

The use of combinations of multiple antagonist organisms also may provide improved disease control over the use of single organisms. Multiple organisms may enhance the level and consistency of control by providing multiple mechanisms of action, a more stable rhizosphere community and effectiveness over a wider range of environmental conditions. In particular, combinations of fungi and bacteria may provide protection at different times or under different conditions and occupy different or complementary niches. Such combinations may overcome inconsistencies in the performance of individual isolates.

The objectives of this research were to evaluate the combined and alone effect of two biocontrol agents, *T. harzianum* and *T. asperellum* on *Fusarium* rot of bean in the greenhouse.

MATERIALS AND METHODS

Microbial cultures: The pathogenic fungi used in this study included 9 isolate of *Fusarium solani* isolates from roots of bean plant and identification (Nelson *et al*., 1983). Biocontrol agents studied were the fungus *Trichoderma harzianum* and the *T. asperellum* isolate with Elad medium from bean rhizospherehe and field soil during the preliminary study and identification (Gams and Bissett, 1998; Druzhinina *et al*., 2005). Screened antagonistic activity against each fungi and selected isolate showing antagonistic activity against pathogen tested, *T. harzianum* (T-7, T-3) and *T. asperellum* (T-6, T-3, T-5) was isolated and proliferated on Potato Dextrose Agar (PDA) medium (Elad and Chet, 1983).

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In this study, the effectiveness of different *Trichoderma harzianum* isolates in controlling bean *Fusarium* rot in comparison with *Trichoberma asperellum* alone and combination were compared.

Preparation of inocula: *F. solani, T. harzianum* and *T. asperellum* were cultured in moistened wheat bran-com mill (1:1 w w^{-1}) medium in flats for 3 weeks at 25°C. After incubation, the each inoculum was air-dried for 3 days, milled in a blender and sieved through a 3.36 mm screen and stored at 4°C. Inoculum viability of each fungi was determined by serial dilution on a peptone Pentachloroni-trobenzene (PCNB) medium for pathogen and water agar for *T. harzianum* and *T. asperellum*. There were approximately 1.6×10^6 and 1.8×10^7 colony forming units (cfu) per gram of inoculum of *F. solani* and *T. harzianum* T-7 and *T. asperellum*, respectively.

Greenhouse tests: For root dipping, each biomass, alone and in combination were prepared separately in different container containing an uncentrifuged fungal suspension (1.8×10^7) and 100.0 g L^{-1} of both biocontrol 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. Four control rows were planted with untreated bean transplants. 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. Experiment were conducted in Iran county of Azarbayjan Province in 2007-2008 growing season.

All greenhouse experiments were performed twice with four replicates per treatment and arranged in a randomized complete block design. Disease incidence (%) were analyzed using an Analysis of Variance (ANOVA) and grouped by Duncan test.

RESULTS AND DISCUSSION

Individual and combination of biocontrol organisms tested significantly reduced *Fusarium* rot of bean in greenhouse tests (Table 1). Reductions in disease incidence ranged from 26.3-61.8% relative to control. The most effective combinations were *T. harzianum* (T-7) + *T. asperellum* (T-3) and *T. harzianum* (T-7) reduced disease incidence by 61.8 and 51.5%, respectively. However, the level of disease control provided by *T. harzianum* (T-7) + *T. asperellum* (T-6) was not as good as than provided by the individual biocontrol organisms tested.

Table 1: Development of *Fusarium* rot in bean plants as affected by treatment with various combinations of biocontrol organism and alone

Treatments	Rot (%)*	Reduction (%)
Control	60.0	0.0
T. asperellum (T-6) + *T. harzianum* (T-7)	50.3	26.3
T. harzianum (T-7)	22.4	51.5
T. asperellum (T-3)	34.2	43.2
T. asperellum (T-3) + *T. harzianum* (T-7)	15.1	61.8
T. asperellum (T-5) + *T. harzianum* (T-7)	30.2	44.9
T. asperellum (T-6) + *T. harzianum* (T-4)	45.4	31.7

*Values followed by different letters within a column differ significantly, $p<0.05$

They can also compete for infection sites on the root and can trigger plant defense reactions, inducing systemic resistance (Benhamou *et al*., 2002). The competitive ability of a nonpathogenic 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 demonstrated that different strains have different capacities to colonize heat treated soil. 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 sp. are among the most-promising biocontrol fungi against many fungal plant pathogens. *T. harzianum* has multiple mechanisms of action, including my coparas itism via production of chitinases, β-1-3 glucanases and β-1-4 glucanases, antibiotics, competition, solubilization of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process (Elad *et al*., 1982; Sivan and Chet, 1989; Bailey and Lumsden, 1998; Altomare *et al*., 1999; Elad and Kapat, 1999; Harman, 2006). We suggested that nonpathogen *Fusarium* would be inhibited by these mechanisms mentioned above. Due to these possible antagonistic interactions, combination of nonpathogen *Fusarium* isolate and *T. harzianum* provided lower disease control than by individual nonpathogen *Fusarium*.

CONCLUSION

As a result, further research on the potential advantages of using combinations of these effective antagonists is needed. Although, only a limited number of potential biocontrol isolates could be tested in this study, it was obtained satisfactory results. In many studies, many nonpathogen *Fusarium* strains showed antagonistic potential as biological control a gents for the control of *Fusarium* wilt diseases.

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