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Effect of seed Treatment with *Trichoderma harzianum* and *Trichoderma asperellum* species for controlling *Fusarium* rot of common bean

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ABSRACT

Defense system of the plant against pathogen attach is the ultimate goal of any controlling process of the pathogen. Biological controls represent an interesting strategy to stimulate the defense system of the plant. In the present study we investigated the protective effects of Trichoderma harzianum (T-1) and Trichodermahar asperellum (T-2) for controlling Fusarium rot of bean. Common bean roots were treated with T. harzianum (T-1) and T. asperellum (T-2) individually and in combination with each other and planted in artificially infested soil with F. solani pathogen. Our findings indicated that prepared conidial suspensions either in water and 10% sugar solution effectively are able to reduce the colonization of the F. solani.

Key words: *Fusarium* rot, bean, *Trichoderma harzianum*, *Trichodermahar asperellum*, sugar suspensions.

INTRODUCTION

A number of soil-borne, fungal pathogens are widespread throughout common bean (*Phaseolus vulgaris* L.) production areas. One such pathogen is Fusarium root rot (caused by *Fusarium solani* (Mart.) Sacc. f.sp. *phaseoli* (Burk.) W.C. Snyder and H.M. Hans.) which infects and colonizes common bean roots[1]. Pathogen infection acts to reduce root density by killing roots and may attenuate the functional efficiency of the remaining infected roots. Seed yield losses from root rots in susceptible kidney beans can be greater than 50% [2].

The disease caused by this fungus is characterized by wilted plants, yellowed leaves, root rot and minimal or absent crop yield. The first symptoms of root rot in beans are narrow, long, red to brown lesions on the stems, and lengthwise cracks often develop. Lesions extend down the main taproot, which may shrivel, decay and die. The symptoms in some cases extend up the hypocotyl to the soil surface. Clusters of fibrous roots (lateral roots or adventitious roots) commonly develop above the shriveled taproot. Severe Fusarium root rot kills primary and secondary roots of beans, and most times only adventitious roots are visible [3].

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 have 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 [4].

One of the major biocontrol agents which reduce soilborne diseases of various crops include isolates of the fungus *Trichoderma* spp. [5]. *Trichoderma* spp. are free-living fungi that are highly interactive in root, soil and foliar environments. It has been known for many years that they produce a wide range of antibiotic substances and that they parasitize other fungi [6]. They can also compete with other microorganisms; for example, they compete for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil [7] and, more generally, compete with soil microorganisms for nutrients and/or space [8]. Furthermore, they inhibit or degrade pectinases and other enzymes that are essential for plant-pathogenic fungi, such as *Botrytis cinerea*, to penetrate leaf surfaces [9]. Other antagonist recovered from *Fusarium* wilt-suppressive soils, especially nonpathogenic *F. oxysporum*, have been used to reduce *Fusarium* wilt diseases of several different crops [10]. 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 [11].

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

MATERIALS AND METHODS

Seed pelleting

T. harzianum(T_1) and *T. asperellum*(T_2) that were growing on PSA plates were used for pelleting bean seeds. Conidial suspension of combination and alone *Trichoderma* spp., were prepared by adding 10 ml sterilized water to a 7 days old culture of biocontrol agents in a 9cm diam., Petri plate, and rubbing the surface with the help of a sterilized spatula. Three ml of conidial suspension was added to 10 gr of seeds in polyethylene bags. The bags were shaken well to provide a uniform coating. In another set, 10% sugar solution was used to make conidial suspension.

Greenhouse tests

For root dipping, each biomass, alone and in combination were prepared separately in different container containing an uncentrifuged fungal suspension ($6x10^8$) of both biocontrol fungi biomass except for 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. Disease was monitored for 6 to 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 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 was conducted in Iran County of Azerbaijan province in 2009-2010 growing season. All greenhouse experiments were performed twice with four replicates per treatment and arranged in a randomized complete block design. Disease incidence (%) was analyzed using an analysis of variance (ANOVA) and grouped by DUNCAN test.

RESULTS AND DISCUSSION

Comparison of means of different treatments showed that reduction in Fusarium root rot in the case of treatment of bean root with combination of two Trichoderma species was more than that treated with individual species. Spore suspension prepared in 10% sugar solution was more effective in reducing disease, compared with spore suspension prepared in sterilized water (table1 and table2). Comparatively better growth in plants when seeds were coated with conidial suspension in sugar solution supports the results of Fouzia and Saleem [12], Adekunle *et al*. [13] and Adekunle *et al*. [14].

Table 1. Effect of two Trichoderma species prepared in water suspension on reduction of Fusarium root rot of common bean

Antagonists	Conidia in sterilized water	Reduction (%) *
T. asperellum (T_2)	$6x10^8$	41.2^a
T. harzianum (T_1)	$6x10^8$	51.7^b
T. harzianum (T_1) +*T. asperellum*(T_2)	$(3x10^8)+(3x10^8)$	53.5^c

**values followed by different letters within a column differ significantly, P<0.05*

Table 2. Effect of two Trichoderma species prepared in 10% sugar suspension on reduction of Fusarium root rot of common bean

Antagonists	Conidia in 10% sugar	Reduction (%)*
T. asperellum (T_2)	$6x10^8$	42.9^a
T. harzianum (T_1)	6x108	53.4^b
T. harzianum (*T1*) +*T. asperellum*(T_2)	$(3x10^8)+(3x10^8)$	59.8^c

**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 [15]. 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. Colonization of the root surface and root tissues probably depends not only on the fungal strain but also on the plant species and plant cultivar.

CONCLUSION

Trichoderma species are among the most-promising biocontrol fungi against many fungal plant pathogens.*T. harzianum* and *Trichodermahar asperellum* have multiple mechanisms of action, including coparasitism via production of chitinases, ß-1-3 glucanases and ß-1-4 glucanases, antibiotics, competition, solubilization of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process [16, 17, 18, 19, 20, 21, 22].

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