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The Ability of Combination of *Trichoderma Harzianum* and *Trichoderma Asperellum* Isolates in Controlling *Fusarium* Rot of Chickpea in Comparison with the Effects of *Trichoderma Harzianum*

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Abstract: In this investigation, the ability of biocontrol agents, *Trichoderma harzianum* and *T. aperellum* applied in combination and alone, which had been isolated from soil and root chickpea field were compared to control of *Fusarium solani.* These isolates had shown good control of *Fusarium solani* in *in-vitro* condition. Chickpea 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-4) + *T. asperellum* (T-6) showed more protective effect. Combination of *T. harzianum* (T-7) + *T. asperellum* (T-5) isolate gave the best control (51.29%) at greenhouse condition.

Key words: *Fusarium* rot, chickpea, combination, *Trichoderma*, control.

1. Introduction

Fusarium solani is a highly destructive pathogen of field grown chickpea 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 chickpea 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 [1, 2].

Two of the major biocontrol agents which reduce soilborne diseases of various crops include isolates of the fungus *Trichoderma* spp. [3]. Other antagonists recovered from *Fusarium* wilt-suppressivesoils, especially nonpathogenic *F. oxysporum*, have been used to reduce *Fusarium* wilt diseases of several different crops [4].

The use of combinations of multiple antagonist organisms also may provide improved disease control over the use of single organisms [5]. 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 [6]. In particular, combinations of fungi and bacteria may

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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 chickpea in the greenhouse.

2. Materials and Methods

2.1 Microbial Cultures

The pathogenic fungi used in this study included 9 isolate of *Fusarium solani* isolates from roots of chickpea plant and identification [7]. Biocontrol agents studied were the fungus *Trichoderma harzianum* and the *T. asperellum* isolate with Elad medium from chickpea rhizospherehe and field soil during the preliminary study and identification [8, 9] 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 medium [10].

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

2.2 Preparation of Inocula

F. solani, *T. harzianum* and *T. asperellum* were cultured in moistened wheat bran-com mill (1:1, w/w) medium in flats for three weeks at 25 °C. After incubation, the each inoculum was air-dried for three days, milled in a blender and sieved through a 3.36 mm screen and stored at 4 °C. Inoculum viability of each fungus was determined by serialdilution on a peptone pentachloronitrobenzene (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.

2.3 Greenhouse Tests

For root dipping, each biomass, aloneand in combination were prepared separately in different container containing an uncentrifuged fungal suspension (1.8×10^7) and 100.0 $g \cdot L^{-1}$ 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 chickpea transplants. Greenhouse soil was artificially infested with pathogen fungi grown on moistened wheat bran-corn mill at rate of 100 $g \cdot m^{-2}$ soil. Each treatment consisted of four replicate rows of ten 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 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 was conducted in Iran county of Azerbaijan province in 2007-2008 growing season [3, 11].

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.

3. Results and Discussion

Individual and combination of biocontrol organismstested significantly reduced Fusarium rot of chickpea in greenhouse tests (Table 1). Reductions in disease incidence ranged from 18.8 to 61.8% relative to control. The most effective combinations were *T. harzianum* (T-7) + *T. asperellum* (T-5) and

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

Treatment	Rot (%)	Reduction (%)*
Control	62.0	0.0
T. asperellum (T-6)+*T. harzianum* (T-7)	50.3	18.8
T. harzianum (T-7)	28.3	61.4
T. asperellum (T-3)	36.2	44.8
T. asperellum (T-3)+*T. harzianum* (T-7)	35.1	46.9
T. asperellum (T-5)+*T. harzianum* (T-7)	30.2	51.29
T. asperellum (T-6)+*T. harzianum* (T-4)	45.4	26.3

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

T. harzianum (T-7) reduced disease incidence by 51.29 and 63%, 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.

They can also compete for infection sites on the root and can trigger plant defense reactions, inducing systemic resistance [12].

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. It is 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 species 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, 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 [13-16].

4. Conclusions

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*.

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 agents for the control of *Fusarium* wilt diseases.

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