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## Control of Fusarium Wilt of Tomato by Combination of Fluorescent *Pseudomonas*, Non-pathogen *Fusarium* and *Trichoderma harzianum* T-22 in Greenhouse Conditions

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**Abstract:** Ability of biocontrol agents, fluorescent *Pseudomonas*, non-pathogen *Fusarium* strain and *Trichoderma harzianum* T-22 applied in combination and alone, to control of *Fusarium oxysporum* f. sp. *lycopersici* was studied in the greenhouse. Tomato roots were treated with biomass of fluorescent *Pseudomonas* strain from rhizosphere, non-pathogen *Fusarium* sp. and *Trichoderma harzianum* T-22 individually and in combination and planted in artificially infested soil with pathogen *F. oxysporum* f. sp. *lycopersici*. Although all biocontrol agents applied individually reduced disease incidence, treatments as combination, except for nonpathogen *Fusarium* strain + *T. harzianum* T-22, showed more protective effect. Combination of *T. harzianum* T-22 + fluorescent *Pseudomonas* isolate gave the best control (70.2%).

**Key words:** *Fusarium* wilt, tomato, fluorescent *Pseudomonas*, *Trichoderma*, nonpathogen *Fusarium*

### INTRODUCTION

*Fusarium oxysporum* f. sp. *lycopersici* is a highly destructive pathogen of both greenhouse and field grown tomatoes in warm vegetable production areas. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal or absent crop yield. Many strategies to control this disease on tomato 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 (Lumsden *et al.*, 1996; Fravel *et al.*, 2003). Biocontrol preparations of both fungi and bacteria have been applied to seeds, seedlings, and planting media in several ways to reduce tomato diseases in the field with various degrees of success. Two of the major biocontrol agents which reduce soilborne diseases of various crops include isolates of the bacterium fluorescent *Pseudomonas* spp. and the fungus *Trichoderma* spp. (Lumsden and Locke, 1989; Bowers and Parke, 1993; Howell and Stipanovic, 1995. 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; Larkin *et al.*, 1996; Nel *et al.*, 2006). 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 three biocontrol agents, *T. harzianum* T-22, nonpathogen *Fusarium* and fluorescent *Pseudomonas* on *Fusarium* wilt of tomato in the greenhouse.

### MATERIALS AND METHODS

**Microbial cultures:** The pathogenic fungi used in this study included isolate of *Fusarium oxysporum* f. sp. *lycopersici* Snyder and Hansen. Biocontrol agents studied were the fungus *Trichoderma harzianum* T-22 and the bacterium fluorescent *Pseudomonas* isolate. The pathogenic fungi were provided by Demir, S., Van Y.Y. University, Agriculture Faculty, Department of Plant Protection. The antagonistic bacterium and non-pathogen *Fusarium* were isolated from tomato rhizosphere during the before preliminary study. Fluorescent *Pseudomonas* isolates were screened antagonistic activity against each fungi and selected isolate showing antagonistic activity against pathogen tested, not to *T. harzianum*. *T. harzianum* strain T-22 was isolated from commercial biopreparat (T-22 Planter Box) and proliferated on Potato Dextrose Agar medium.

**Preparation of inocula:** *F. oxysporum* f. sp. *lycopersici* and *T. harzianum* T-22 were cultured in moistened wheat bran-corn 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 (Mao *et al.*, 1997). Inoculum viability of each fungi was determined by serial dilution on a peptone pentachloronitrobenzene (PCNB) medium for pathogen and water agar for *T. harzianum*. There were approximately  $1.6 \times 10^6$  and  $1.8 \times 10^7$  colony forming units (cfu) per gram of inoculum of *F. oxysporum* f. sp. *lycopersici* and *T. harzianum* T-22, respectively. The fluorescent *Pseudomonas* isolate was grown on 9 cm diameter petri plates containing 10 mL King-B. Plates were incubated in the dark for one week at  $25 \pm 2^\circ\text{C}$ . when the antagonistic bacterium would be inoculated to plant roots, culture grown on the medium were scraped from the surface of the plates and adjusted  $1 \times 10^9$  cfu mL<sup>-1</sup> by serial dilution.

**Greenhouse tests:** For root dipping, each biomass, alone and in combination were prepared separately in different container containing an uncentrifuged bacterial suspension ( $1.8 \times 10^7$ ) and 100.0 g L<sup>-1</sup> 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 tomato transplants. Greenhouse soil was artificially infested with pathogen fungi grown on moistened wheat bran-corn mill at rate of 100 g m<sup>-2</sup> 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 were conducted in Fethiye county of Mugla province in 2005-2006 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* wilt of tomato in

greenhouse tests (Table 1). Reductions in disease incidence ranged from 30 to 70.2% relative to control. The most effective combinations were non-pathogen *Fusarium* with fluorescent *Pseudomonas* sp and *T. harzianum* T-22 with fluorescent *Pseudomonas* sp and reduced disease incidence by 70.2 and 60.8%, respectively. However, the level of disease control provided by non-pathogen *Fusarium* + *T. harzianum* T-22 was not as good as than provided by the individual biocontrol organisms tested. When compared to individual effect, non-pathogen *Fusarium* and fluorescent *Pseudomonas* sp. were more affective than *T. harzianum* T-22. These results showed that non-pathogen *Fusarium* isolates would be important in reducing *Fusarium* wilt disease. Larkin and Fravel (1998) reported that specific nonpathogenic isolates of *Fusarium oxysporum* and *F. solani* collected from a *Fusarium*-wilt suppressive soil were the most effective antagonists and provided significant and consistent disease control (50 to 80% reduction of diseases incidence) in several repeated tests. In addition, isolates of *Gliocladium virens*, *T. hamatum*, *Pseudomonas fluorescens* and *Burkholderia cepacia*, also significantly reduced *Fusarium* wilt compared to disease controls (30 to 65% reduction), but were not as consistently effective as the nonpathogenic *Fusarium* isolates.

The most important result from our research was the improved biocontrol performance when nonpathogen *Fusarium* isolate was used in combination with a fluorescent *Pseudomonas* strain. Park *et al.* (1988) and Leeman *et al.* (1995, 1996) have also reported a similar enhanced response with combinations of *Fusarium* and *Pseudomonas* spp. in cucumber and radish. In addition, Fuchs *et al.* (1999) and Alabouvette *et al.* (1993) reported improved biocontrol performance when *Fusarium* isolate Fo47 was used in combination with a fluorescent *Pseudomonas* strain (C7). The nonpathogenic *F. oxysporum* strains show several modes of action contributing to their biocontrol capacity. They are able to compete for nutrients in the soil, affecting the rate of chlamydospore germination of the pathogen.

Table 1: Development of *Fusarium* wilt in tomato plants as affected by treatment with various combinations of biocontrol organisms and alone

Treatments	Wilt (%)*	Reduction (%)
Control	58.0a	0.0
Non-pathogen <i>Fusarium</i>	24.36d	58.0
<i>Trichoderma harzianum</i> T-22	33.64c	42.0
Fluorescent <i>Pseudomonas</i> sp.	31.55c	45.5
Non-pathogen <i>Fusarium</i> + <i>Trichoderma harzianum</i> T-22	40.60b	30.0
Non-pathogen <i>Fusarium</i> + Fluorescent <i>Pseudomonas</i> sp.	17.28e	70.2
<i>Trichoderma harzianum</i> T-22 + Fluorescent <i>Pseudomonas</i> sp.	22.73d	60.8

\*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. Nagao *et al.* (1990) demonstrated that different strains have different capacities to colonize heat treated soil. In addition, Saprophytic colonisation 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.

Benhamou and Garand (2001) determined enzymatic changes in the plant inoculated with nonpathogen *Fusarium*, often leading to induction of the physical barriers. Tamietti *et al.* (1993) found increased activity of several plant enzymes related to plant defense reactions in tomato plants transplanted in sterilized soil infested with a strain of nonpathogenic *F. oxysporum*. Fuchs *et al.* (1997) attributed the biocontrol activity of the nonpathogenic strain Fo47 to induced resistance in tomato, correlated with an increased activity of chitinase, 1-3 glucanase and 1-4 glucosidase. Duijff *et al.* (1998) showed that the nonpathogenic strain Fo47 although not very effective in inducing systemic resistance in tomato induced an increase of PR proteins. Recorbet *et al.* (1998) showed an overall increased activity of constitutive glycosidase isoforms in response to infection by *F. o. f. sp. lycopersici* that did not occur in roots colonized with nonpathogenic strains. Finally, when the main mode of action of a nonpathogenic strain is induction of systemic resistance, it is obvious that this phenomenon implies the physiological state of the plant and fluctuating environmental conditions may affect the ability of the plant to express its resistance to the pathogen, induced by the nonpathogenic *F. oxysporum*. The other mechanism involves competition for carbon provided by both *F. oxysporum* and fluorescent *Pseudomonas* and competition for iron provided by the bacteria. Fluorescent *Pseudomonas* strains are an important components of the rhizosphere and exclusive the pathogens from rhizosphere via several factors, such as toxins, antibiotics and siderophores (Thomashow and Weller, 1996). Lemanceau *et al.* (1992, 1993) suggested that the presence of the siderophore produced by *P. fluorescens* increases the intensity of the antagonism provided by Fo47, making the pathogen more sensitive to glucose competition by the antagonistic *Fusarium*. However, in our experiment, it was not search this response. Duijff *et al.* (1998) reported that the suppression of *Fusarium* wilt by *P. fluorescens* WCS417 was due to systemic induced resistance without

any detection of the pathogenesis-related proteins tested. In contrast, the suppression achieved by nonpathogenic *F. oxysporum* Fo47 appeared to be mainly ascribed to microbial antagonism but also to a lesser extent to systemic induced resistance. Duijff *et al.* (1998) reported that suppression of *Fusarium* wilt by *P. luorescent* WCS417 was due to systemic induced resistance. In present sturdy, it was thought that the reason of highly suppressive effect of nonpathogen *Fusarium* and fluorescens *Pseudomonas* in combination could be due to synergist effects of all biocontrol mechanisms.

*Trichoderma* species are among the most-promising biocontrol fungi against many fungal plant pathogens. *T. harzianum* has multiple mechanisms of action, including mycoparasitism via production of chitinases,  $\beta$ -1-3 glucanases and  $\beta$ -1-4 glucanases, antibiotics, competition, solubilization of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process (Elat *et al.*, 1982; Sivan and Chet, 1989; Lorito *et al.*, 1996; Bailey and Lumsden, 1998; Altomare *et al.*, 1999; Elad and Kapat, 1999; Hanson and Howell, 2004; 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* T-22 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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