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Induction of systemic resistance in tomato plants to *Fusarium oxysporum* f. sp. *lycopersici* causal agent of Fusarium wilt of tomato by non-pathogenic *F. oxysporum* under greenhouse conditions

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Abstract

The aim of the present research was to assess the relative importance of systemic induced resistance in the suppression of Fusarium wilt of tomato by non-pathogenic *Fusarium oxysporum* (Avr5). Effects of one isolate of nonpathogenic *F. oxysporum* (Avr5) were tested *in vitro* and *in vivo* for their efficacy in controlling Fusarium wilt of tomato. Two methods were applied including root-dip-inoculation and soil's infestation method with the *F. oxysporum* f. sp. *lycopersici* (FOL). Isolate of nonpathogenic *F. oxysporum in vitro* did not inhibit radial growth of FOL and had no significant effect on reduction growth of pathogen. At the same time *in vivo* nonpathogenic *F. oxysporum* (Avr5) with concentration of 0.1 ml conidial suspensions of 10^6 conidial/ml per gram soil (100 ml conidia per pot) significantly reduced incidence and severity of Fusarium wilt (62% reduction) compared to the control pathogen. *F. oxysporum* (Avr5) only colonized the epidermis of tomato's root but was not found in vessels of stems, while the FOL colonized inside of root cells and stems of inoculated plants. Plants inoculated with FOL showed disease symptoms after 3 weeks, whereas plants inoculated with *F. oxysporum* (Avr5) or a mixture of both fungi remained symptomless for 60 days. In order to induce systemic resistance in tomato plant, three

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bioassays including split-root, benomyl and cutting systems were used. Results of three bioassays indicated that *F. oxysporum* (Avr5) is able to induce resistance in tomato plants against FOL. Non-pathogenic *F. oxysporum* (Avr5) remained spatially separated from the pathogen in plants in the split root bioassay, but in the cutting and benomyl systems *F. oxysporum* (Avr5) and FOL were separated in time, suggesting that these effects were systemic in nature. It has been concluded that application of nonpathogenic *F. oxysporum* (Avr5) can be considered as a potential biocontrol agent in next studies.

Key words*: Fusarium oxysporum* f. sp. *lycopersici*, induced resistance, biological control, tomato.

چکیده

بیماری پژمردگی فوزاریومی‌گوجه‌فرنگی به وسیله قارچ *Fusarium oxysporum* f.sp. *lycopersici* ایجاد شده و یکی از بیماری‌های مهم آوندی گوجه‌فرنگی است که خسارت قابل توجهی به محصول گوجه‌فرنگی در مزرعه و گلخانه وارد می‌نماید. در این تحقیق امکان کنترل این بیماری توسط جدایه غیر بیماری‌زای *F. oxysporum* در شرایط آزمایشگاه و گلخانه مورد بررسی قرار گرفت. جدایه فوزاریوم غیر بیماری‌زا در شرایط آزمایشگاه با استفاده از روش کشت متقابل، هیچگونه تأثیری بر کاهش رشد شعاعی قارچ بیمارگر روی محیط کشت PDA نداشت. تأثیر جدایه مذکور در شرایط گلخانه با استفاده از دو روش مایه‌زنی ریشه (root dip) و آلودگی خاک مورد ارزیابی قرار گرفت. در روش اول، ریشه نشاءهای سه هفته‌ای گیاه گوجه‌فرنگی رقم Beliy naliv-241 حساس به نژاد یک به مدت نیم ساعت در سوسپانسیون 10^6 اسپور در میلی‌لیتر جدایه فوزاریوم غیر بیماری‌زا قرار داده شد و سپس در داخل گلدان نشاء گردیدند. بعد از یک هفته دوباره گیاهچه‌ها از خاک خارج و ریشه آنها با سوسپانسیون اسپور قارچ بیمارگر در غلظت و زمان مذکور مایه‌زنی و در خاک گلدان‌ها کشت و در شرایط گلخانه نگهداری شدند. در روش دوم بذور گوجه‌فرنگی رقم ذکر شده در خاکی که اسپور فوزاریوم غیر بیماری‌زا به ازای هر گرم خاک گلدان یک دهم میلی لیتر از سوسپانسیون اسپور 10^6 به آن اضافه شده بود کشت (به ازای هر گلدان یک لیتری ۱۰۰ میلی‌لیتر) و بعد از سه هفته به داخل خاک گلدان‌های آلوده به قارچ بیمارگر (یک دهم میلی‌لیتر از سوسپانسیون اسپور 10^6 در هر گرم خاک) منتقل شدند. آزمایش در قالب طرح کامل تصادفی با شش تکرار انجام

شد. از دو سری تیمار شاهد به ترتیب با آب مقطر سترون و بیمارگر استفاده شد. در هر دو روش علایم بیماری بعد از سه هفته در تیمار شاهد مشاهده گردید و جدایه فوزاریوم غیر بیماری‌زا شدت بیماری را به میزان ۶۲ درصد در مقایسه با شاهد کاهش داد. به منظور بررسی القای مقاومت در گیاه گوجه‌فرنگی به وسیله جدایه فوزاریوم غیر بیماری‌زا، سه آزمایش دو قسمت نمودن ریشه، استفاده از بنومیل و قطع ریشه استفاده شد، بطوریکه در روش اول بیمارگر و جدایه غیر بیماری‌زا از لحاظ مکانی و در دو روش دیگر از لحاظ زمانی با هم فاصله داشتند. در هر سه روش گیاهچه‌های سه هفته‌ای در داخل محلول غذایی پرورش و جدایه غیر بیماری‌زا و بیمارگر با فاصله زمانی به آن اضافه گردید. نتایج آزمایشات بعد از هشت هفته نشان داد که جدایه مذکور بطور متوسط شدت بیماری را به میزان ۷۵ درصد در مقایسه با شاهد کاهش داد. بطور کلی نتایج حاصل از سه آزمایش مشخص نمود که جدایه فوزاریوم غیر بیماری‌زا باعث القای مقاومت در گیاه گوجه‌فرنگی علیه بیماری پژمردگی فوزاریومی گوجه‌فرنگی می‌شود و می‌تواند به عنوان یک عامل بیوکنترل بالقوه در مدیریت این بیماری در بررسی‌های آتی لحاظ گردد*.

واژه‌های کلیدی: *Fusarium oxysporum* f. sp. *lycopersici*، القای مقاومت، کنترل بیولوژیک، گوجه‌فرنگی**.

Introduction

Fusarium oxysporum (Schlecht.) f. sp. *lycopersici* (Sacc.) Snyder et Hansen, is a fungal pathogen that causes wilt of tomato. Resistant cultivars have been the most effective means of controlling Fusarium wilt (Beckman, 1987; Akkopru and Demir, 2005). New races of the pathogen have appeared that overcome resistance in currently grown cultivars (Tello-Marquina and Lacasa, 1988). In addition, chemical control of the disease is not satisfactory, and biological control is an attractive alternative to the use of chemicals to control Fusarium

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wilt of tomato (De Cal *et al*., 1995).

The difficulty in controlling Fusarium wilt has stimulated the research in biological control independently from the recent concern for environmental protection (Fravel *et al*., 2003). Successful biological control systems commonly employ naturally occurring, antagonistic microorganisms that are able to reduce the activities of plant pathogens and may increase plant growth and health (Akkopru and Demir, 2005). In addition, some of these microorganisms induce resistance in host plants, which enhances the plant's ability to defend itself from pathogen attack (Shihido *et al*., 2005; El Hassni *et al*., 2007). The ability of nonpathogenic *F. oxysporum* and pathogenic *F. oxysporum* belonging to a different formae speciales than the pathogen, to induce plant resistance to fusarioses has been demonstrated in several studies (Jolanta *et al*., 2008; Bao and lazarovits, 2000; Larkin and Fravel, 1999). These authors found biocontrol of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *basilica* by a non-pathogenic *F. solani* strain CS-1.

Nonpathogenic strains of *Fusarium* spp. have suppressed soil borne diseases caused by pathogenic *Fusarium* spp. and *Verticillium dahliae* under greenhouse and field conditions (Reid *et al*., 2002; Forsyth *et al*., 2006; Jian *et al*., 2009; Malandraki *et al*., 2008; Iakovos *et al*., 2009). Biocontrol organisms including specific nonpathogenic isolates of *F. oxysporum* and *F. solani* collected from a Fusarium wilt-suppressive soil were the most effective antagonists, providing significant and consistent disease control (Larkin and Fravel, 1998). In recent years, the process of plant “immunisation” or induced resistance to diseases received increasing attention (Benhamou *et al.*, 1996 and 1994; Kuc, 1987). De Cal *et al*. (1997) reported that treatment of tomato plants with conidia of *Penicillium oxalicum* Durrie and Thom induced resistance against tomato wilt. As *P. oxalicum* induced morphological changes in all cultivars of tomato, and these changes led to decreased vascular damage caused by *F. oxysporum* f. sp. *lycopersici* (De Cal *et al*., 2000). Larkin and Fravel (1999) reported that isolates of nonpathogenic *Fusarium* spp. (CS-1, CS-20, and Fo47) induced systemic resistance in tomato and watermelon plants, but varied in their relative abilities to reduce disease. Inoculation of tomato with Fo47 caused suppression of Fusarium wilt in tomato plants, so that Fo47 induced resistance in tomato to Fusarium wilt (Fuchs *et al*, 1997; Larkin and Fravel, 1996). Moreover *F. oxysporum* f. sp. *dianthi* reduced disease symptoms caused by *F. oxysporum* f. sp. *lycopersici.* It may be concluded that *F. oxysporum* f. sp. *dianthi* is able to induce resistance in tomato plants against pathogen (Kroon *et al*, 1991). Systemic induced resistance can be detected by implementing challenge-inoculation at a later time and different

location on a plant (Stromberg, 1989). Results indicated that nonpathogenic Fusarium protected tomato plants from Fusarium wilt by several mechanisms consist of competition for sites on the root surface, for iron, for carbon sources and induced resistance (Fuchs *et al*., 1997). For example, nonpathogenic Fusarium strain Fo47 induced resistance to Fusarium wilt in tomato that resulted in physiological changes in the stems and leaves of tomato plants. These changes are commonly associated with induce systemic resistance, including enhanced activity of chitinase (Matta *et a*l., 1988), ß-1, 3-glucanase and ß-1, 4-glucosidase (Tamietti *et al*., 1993).

The major objective of present study was to evaluate possibility of suppression of Fusarium wilt of tomato by nonpathogenic *F. oxysporum* (Avr5) and whether nonpathogenic strain can induce resistance to Fusarium wilt in tomato plants.

Materials and Methods

Pathogen and biocontrol agent: *F.oxysporum* f. sp. *lycopersici* (FOL) race 1 (Race 1, 2, 3) was isolated from infected tomato seedlings. Race determination were conducted using root-dip inoculation with different tomato cultivars, Beliy naliv-241 (not resistant), Blagovest (resistant to race 1) and Benito (resistant to both races of 1 and 2). Nonpathogenic *F. oxysporum* (Avr5) was provided by Professor F.S. Djalilov (Phytopathology Dep. of Moscow Timiryazev Agricultural Academy) isolated from the rhizosphere of healthy tomato. Pathogen and biocontrol agent was maintained in sterile sand tubes at 4^{o}C and both were grown on potato-dextrose agar (PDA) in darkness at 22-25^{o}C for 2 weeks. Spores from 14-day-old cultures were removed gently from the surface of each plate culture by adding sterile distilled water. Suspension were filtered through five layers of sterile gauze (cotton) and suspended in sterile distilled water (SDW), and the density of the macro and microconida was determined with a haemocytometer and adjusted by dilution to the desired concentration (10^6 spores/ ml) for root inoculation. Spores were more than 90% microconida.

In vitro interaction between the tested fungi: The antagonistic effects of one isolate of nonpathogenic *F. oxysporum* (Avr5) was tested on Potato dextrose Agar (PDA) against FOL. The inoculum of FOL and nonpathogenic *F. oxysporum* (Avr5) consisted of mycelial plug from 7-days-old PDA cultures. To do that in each Petri dish, *F. oxysporum* (Avr5) and FOL were inoculated simultaneously (with five replications for each one). The two inoculates (4mm discs) were cut from the margins of young vigorously growing cultures and placed 4 cm apart at opposite points of the Petri dish. Control plates contained two mycelial plugs of

FOL. The plates were incubated in the dark at (22 ± 2°C). After 5 days, percentage inhibition of radial growth (PIRG) was determined as an estimate of the growth inhibition of FOL by the antagonist.

Glasshouse experiment:

Plant material. Tomato (*Lycopersicon esculentum* Mill.) seeds of cv. Beliy naliv-241 susceptible to races 1 and 2 of FOL at first were surface-disinfected in 1% hydrochloric acid for 30 min and rinsed repeatedly in sterile double-distilled water prior to sowing. Then seed sown in standardised soil sterllized (two times for 20 min at 121 °C with 24 intervals) mixed with sand (80: 20) and were grown in seedling plug trays (plug size 3.4 by 3.4 by 5 cm, 64 plugs). Trays maintained in a glasshouse at 22 to 28°C, 60-70% relative humidity, and 16 h light, 8 h darkness.

Bioassays for disease suppression and induced resistance in vivo:

Experiment 1: Soil infestation. Two tomato seeds of cultivar Beliy naliv-241 were planted in sterilized soil in seedline plug trays (Plug size 3.4 by 3.4 by 5 cm, 64 plugs). Then conidial suspension (0.1 ml conidial suspensions of 10^6 conidial/ml per gram soil) of isolate of *F. oxysporum* (Avr5) was added to each plug cell at the time of planting. Control plants were treated with sterile water. The plug tray, watered as needed, and maintained in the greenhouse. After 18 days, plugs containing the tomato plants were transplanted into 10-cm-diameter pots containing sterilized soil infested with the FOL (0.1 ml conidial suspensions of 10^6 conidial/ml per gram soil). Each treatment consisted of twelve replicates (six pots, two plants per pot). Control plants were treated only with pathogen (10^6 spores/g soil). After 35 days, disease was assayed as the total percentage of seedlings showing any symptoms of Fusarium wilt consist of yellowing and dropping of leaves, vascular discoloration, and height of a plant (Larkin *et al*., 1998).

Experiment 2: Root inoculation. Roots of three-week-old tomato plants (2-3 true-leaf stage) were slightly cut and inoculated by dipping the roots in conidial suspension (10^6 spores/ml) of isolate of *F. oxysporum* (Avr5) for 30 minutes and then were transplanted into 10-cm-diameter pots. After a week, the roots of these plants taken out of the pots, were recut and dipped in conidial suspension of FOL with a concentration of 10^6 spores/ml for 30 minutes and replanted in the same pots. Control plants were dipped in tap water and control pathogen (10^6 spores/ml for 30 minutes) dipped only in pathogen (without inoculation with *F. oxysporum* (Avr5) (Kroon *et al*., 1991).

Experiment 3: Bioassays for induced resistance. In order to diagnose and determine

the primary mechanism of action of *F. oxysporum* (Avr5) for protection of tomato against Fusarium wilt disease, three bioassays (split-root, benomyl and cutting systems) were used by method of Fuchs *et al*. (1997). Whereas in the split-root, *F. oxysporum* (Avr5) was separated spatially from FOL, in the cutting and benomyl systems those were separated in time.

Split-root and non-split system: This method performed in order to physically separate the antagonist and the pathogen. The 21- day- old seedlings were cut off at the base of the stem and the hypocotyls were split longitudinally in two parts with a sterile scalpel, to a length of approximately 3 cm. Each half of the hypocotyls was planted in a separate tube containing nutrient solution (macro and microelements 0.5 g/lit) for rooting. One week later, each part of the split hypocotyls was placed in a 100-ml tube filled with nutrient solution and *F. oxysporum* (Avr5) was added (60 ml) to one of the tubes (10^6 spors/ml). Ten days later, FOL was inoculated into the other tube (60 ml; 10^6 spors/ml). In the same way, the non-split seedlings were planted in tubes containing nutrient solution. One week later, *F. oxysporum* (Avr5) was added to tubes (60 ml; 10^6 spores/ml) and after 10 days, FOL was inoculated into the tubes in same concentration. In this bioassay percentage of plants with Fusarium wilt symptoms was scored every week (Grattidge and O' Brien, 1982). The experiments were ended 56 days after inoculation with pathogen. In order to exclude any direct contact between the antagonists and the pathogen in the split plants, the absence of nonpathogenic Fusarium on the root side infested with the pathogen, and in the stem was checked during microbial analyses performed at the end of the experiment. In order to this work, the presence of nonpathogenic Fusarium on the rhizoplane was evaluated after plating 100 µl of the rhizoplane suspensions on Special Nutrient Agar (SNA; Nirenberg, 1976). Three Petri dishes were plated per suspension-dilution. Also, the stems were surface-disinfected (flamed after dipping into a 95% ethanol solution) and cut into sections at 2, 5 and 10 cm above the split of the stem. Section were placed on SNA and incubated in 27^{oC}, for 72 h. The colonies of *F. oxysporum* strain Avr5 were discriminated from FOL ones on the basis of their different morphology.

Cutting-system: The 21- day- old seedlings were cut off 0.5 cm from the basal of stem, and the shoots were transplanted into plastic pots filled with autoclaved potting mix that infested with FOL (0.1 ml conidial suspensions of 10^6 conidial/ml per gram soil). *F. oxysporum* (Avr5) was added to the soil of pot prior to sowing and FOL (0.1 ml conidial suspensions of 10^6 conidial/ml per gram soil) was inoculated into the potting mix prior to replanting.

Benomyl system: 21-day-old tomato seedlings were dug from potting mix, and the roots were carefully washed under running tap water. The root system of the plants was placed for 7 days in nutrient solution that was either sterile or inoculated with *F. oxysporum* (Avr5). The roots were rinsed with double-distilled water, and the plants were transferred to 2- litter containers containing nutrient solution and 30 µg/ml of Benomyl (Fundazol WP, 500 g/l). Two days later, the roots were cut off 2 cm from the base of the hypocotyls and were placed in nutrient solution containing FOL. In all Bioassays, Disease severity was evaluated 56 days later. Three pots of each treatment kept in greenhouse for three months to see the disease improvement.

In the end of experiments, the fungi were reisolated from the inoculated plants by placing of the stem's segments on PDA supplemented with streptomycin (0.02%).

Disease assays: Disease index by FOL was assessed 56 days after inoculation by the method described in Grattidge and O' Brien (1982) using a scale from 0 to 4: 0, (0-24%) of leaves yellowed and wilted; 1, (25-49%) of leaves yellowed and wilted ; 2, (50-74%) of leaves yellowed and wilted; 3, (75-99%) of leaves yellowed and wilted; 4, (100%) dead plant.

Data analysis. All pathogenicity tests were conducted in completely randomised design. All data at first analyzed by least significant difference (LSD) testing. Duncan's multiple Range test was applied to compare means. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences).

Result and discussion

Nonpathogenic *F. oxysporum* (Avr5) *in vitro* did not inhibit radial growth of FOL and had no significant effect on reduction growth of pathogen (only 8.9 %), but it significantly reduced Fusarium wilt of tomato in greenhouse test (Table 1). Disease symptoms in plants that only inoculated with FOL after two weeks were apparent, whereas in plants inoculated with a combination of both fungi FOL and *F. oxysporum* (Avr5) remained symptom less for eight weeks, the duration of the experiment. Nonpathogenic *Fusarium oxysporum* (Avr5) at 10^6 spores/ml for plant root-inoculated and 0.1×10^6 spores/g soil for adding in soil reduced disease severity, respectively 3.7 and 2.1 times compared to the control FOL (Table 2).

Disease reduction by *F. oxysporum* (Avr5) leaded to increase of height of plant; so plant height was significantly greater than in the pathogen controls (2.2-3 times). All plants showed vessel discoloration except for the plants inoculated with *F. oxysporum* (Avr5) or with a combination of both fungi. No fungi could be isolated from stems of plants inoculated

with *F. oxysporum* (Avr5). Only FOL could be isolated from stems of inoculated plants with the same fungus.

Table 1. Percentage of growth inhibition of *F. o*. f. sp. *lycopersici* on PDA media after 120 hours of growth by *F. oxysporum* (Avr5)

Treatment	Mean of radial growth (mm)	% growth inhibition
Control Pathogen	45.0	-
Control *F. oxysporum* (Avr5)	40.0	-
FOL + *F. oxysporum* (Avr5)	41.0	8.9
LSD at 5 %	3.3	-

Data are means of 5 replicates. Significant differences are denoted by different letters within each column according to L.S.D test at 5%.

Table 2. Effect of nonpathogenic *F. oxysporum* (Avr5) on Fusarium wilt of tomato in a greenhouse test by methods of root dip and soil infestation

Treatments	Disease severity	
	Root dip inoculation (spores/ ml)	Soil infestation (spores/ g soil)
Control water	0	0
Control pathogen	3.4 [a]	2.1 [a]
FOL + Avr5	0.9 [b]	1.0 [b]
LSD at 5 %	0.4	0.3

Data are means of 6 replicates. Significant differences are denoted by different letters within each column according to L.S.D test at 5%.

Table 3. Effect of nonpathogenic *Fusarium oxysporum* (Avr5) on development of tomato Fusarium wilt symptoms in non-split and split plants method, 56 days after inoculation with *F. oxysporum* f. sp. *lycopersici* (FOL)

Treatment	Non-split plants		Split plants	
	Disease[x] severity	% reduction	Disease severity	% reduction
Control water	0.0 [a]	0	0.0 [a]	0
Control FOL	3.7 [b]	-	3.6 [b]	-
Control Avr5	0.0 [a]	0	0.0 [a]	0
Avr5[a] + FOL [b]	1.3 [c]	60.0	0.8 [c]	70.0
LSD at 5 %	1.8		1.3	

Data are means of 6 replicates. X, Disease severity (%wilt): 0, no symptoms; 1, <25% of leaves with symptoms; 2, 26-50% of leaves with symptoms; 3, 51-75% of leaves with symptoms; 4, 76-100% of leaves with symptoms**.**

Means in the column followed by different letters indicate significant differences among treatments at 0.05 according to Duncan's multiple range Test.

a, nonpathogenic Fusarium; b, *F. oxysporum* f. sp. *lycopersici*

Table 4. Effect of nonpathogenic *Fusarium oxysporum(* Avr5) on Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* (FOL) by three bioassays: split-root, cutting and Benomyl system

Fungal inoculation		Disease severity *		
Avr5	Pathogen	Split-root	Cutting	Benomyl
None	None	0 a	0 a	0 a
None	FOL	3.6 b	3.2 b	3.4 b
Avr5	FOL	0.8 c	0.5 c	0.4 c

Data are mean of six replicates. Within same column, means designated with the same letter are not significantly different (P= 0.05).

× Disease index: 0 = 0 - 24% of leaves yellowed; 1 = 25 - 49 % of leaves yellowed; 2 = 50 - 74 % of leaves yellowed; 3 = 75 - 99 % of leaves yellowed; 4 = Dead (100 %).

Results, obtained from artificial inoculation and in vivo, showed that *F. oxysporum* (Avr5) induced resistance in tomato plants against Fusarium wilt disease. In order to do this work several tests by method of Fuchs *et al*. (1997) were studied. In the split-root system, disease index were scored 56 days after the second inoculation and differences in disease index were statistically significant comparing with the controls (Fig. 1 and Table 3). FOL and *F. oxysporum* (Avr5) were separated throughout the experiment. After eight weeks, disease was not observed in the presence of FOL. In this method, Fusarium wilt was 15% (control pathogen 90%) and necrosis and vessel discoloration was not observed in tomato plant (Table 3 and 4). Also *F. oxysporum* (Avr5) was not found in stem of plants. Interestingly, *F. oxysporum* (Avr5) was a less effective biocontrol agent in the non-split-root than in the split-root systems (Fig. 2 and Table 3).

Cutting system method also showed significant effect of control of Fusarium wilt of tomato by *F. oxysporum* (Avr5), and it significantly reduced disease symptoms (Table 4). In addition, *F. oxysporum* (Avr5) was not isolated from stem sections of either healthy or diseased plants, indicating that this isolate did not invade the vascular tissue or more systemically within the plants. Treatment with biocontrol isolate *F. oxysporum* (Avr5) reduced wilt % 6.4 times comparing with the control FOL (80%) on tomato (Table 4).

Benomyl system method indicated that effect and disease index for short contact (7 days) of tomato root system with nonpathogenic strain was nearly equal to disease index for a long contact with *F. oxysporum* (Avr5). Indeed *F. oxysporum* (Avr5) reduced disease severity from 85 to 10% (Table 4). Also *F. oxysporum* (Avr5) could not be isolated from the roots and stem of tomato plants after the benomyl treatment.

In all three bioassays control plants (water treatments) and plants inoculated with nonpathogenic Fusarium stayed healthy. Plants inoculated with both *F. oxysporum* (Avr5) and pathogen showed less disease symptoms than with control pathogen (Table 3 and 4). All plants inoculated with pathogen became more seriously diseased, but also a delay in symptom development was observed in the plants that firstly inoculated with nonpathogenic Fusarium. Differences in disease index were statistically significant from 3 weeks after the second inoculation until eight weeks (Fig 1 and 2), the end of the experiment ($p = 0.05$).

We confirmed that nonpathogenic *F. oxysporum* (Avr5) is an effective agent for suppression of Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici*. The results obtained in the present study are in agreement with the results of other researchers (Kroon *et al*., 1991; Larkin and Fravel, 1999; Fuchs *et al*., 1997).

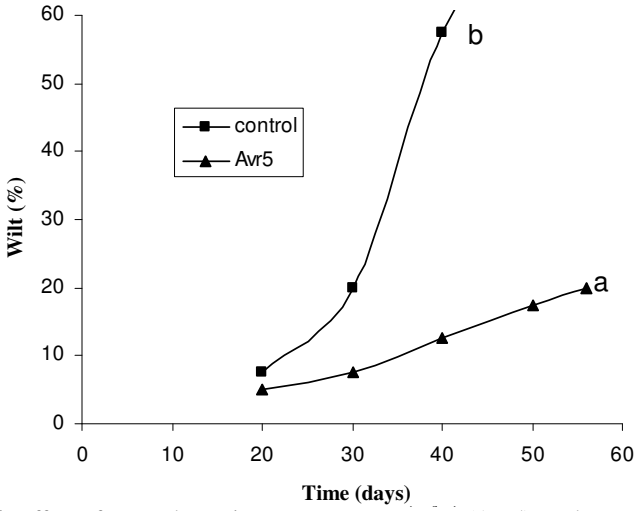

Fig. 1. Effect of nonpathogenic *Fuzarium oxysporum* (Avr5) on the tomato Fuzarinm wilt caused by *F. oxysporum* f. sp. *lycopersici* (FOL) in split plants

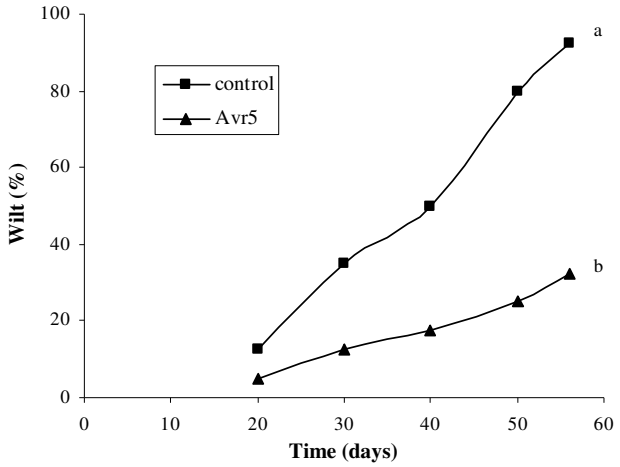

Fig. 2. Effect of nonpathogenic *Fuzarium oxysporum* (Avr5) on the tomato Fuzarinm wilt caused by *F. oxysporum* f. sp. *lycopersici* (FOL) in split after 56 days

Mean of wilt % recorded on day 56 and designated with the different letters (a and b) indicates significant difference according to Duncan's multiple range test at 5%

Protection of tomato against Fusarium wilt by application of nonpathogenic isolates *Fusarium* spp. has been studied in greenhouse and fields (Lemanceau and Alabouvette, 1991 and 1993). Also protection against Fusarium wilts by application of nonpathogenic strains of *F. oxysporum* or formae speciales not pathogenic to the challenged host is a well-studied phenomenon (Ogawa and Komada, 1984). Mandeel and Baker (1991) have shown that nonpathogenic strain of *F. oxysporum* can induce resistance to Fusarium wilt in cucumber and it also can induce resistance to Fusarium wilt in chickpea (Hervas *et al*., 1995) and tomato (Kroon *et al*., 1991). Our experiment shows that, strain *F. oxysporum* (Avr5) plays a very important role in reduction of disease severity and induction of systemic resistance in plant against Fusarium wilt of tomato without any direct contact with the pathogen, because in split plant, the absence of any direct contact between the pathogen and the biocontrol strains prevented any microbial antagonism. Kroon *et al*. (1991) reported that, *F. oxysporum* f. sp. *dianthi* is able to induce resistance against *F. oxysporum* f. sp. *lycopersici* in tomato plants. It was also observed that only *F. oxysporum* f. sp. *lycopersici* could be isolated from the stems of the plants which were inoculated with both fungi. This observation indicates that induction of resistance is accomplished in the roots or on the root surface. It was reported (De Cal *et al*., 1997) that there was an involvement of induced resistance in greenhouse experiments on the biocontrol of Fusarium tomato wilt by *Penicillium oxalicum*. Fuchs *et al*. (1997) and Larking and Fravel (1996) reported that nonpathogenic *F. oxysporum* isolate Fo47 induce resistance in tomato against Fusarium wilt of tomato, and inoculation of tomato with Fo47 resulted in physiological changes in the stems and leaves of tomato plants. Other authors have also shown induced resistance in other crops to several diseases with only one antagonist (Bargmann and Schonbeck, 1992; Gottstein and Kus, 1989; Kim *et al*., 1997).

The current work was carried out with *F. oxysporum*, a nonpathogenic strain with promising biocontrol ability under commercial greenhouse conditions (Alabouvette and Couteaudier, 1992). Indeed, a single inoculation of cv. Beliy naliv-241 tomato plants with *F. oxysporum* (Avr5) never resulted in disease symptoms, and the presence of FOL was necessary to cause Fusarium wilt. Therefore, three bioassays were developed in which nonpathogenic strain *F. oxysporum* (Avr5) and pathogenic isolate (FOL) were introduced separately in space (split-root) or time (benomyl and cutting systems). In these bioassays, a single inoculation with FOL resulted in incidence of Fusarium wilt between 80 and 90%.

F. oxysporum (Avr5) protected tomato from pathogen in all three bioassays. Interestingly, *F. oxysporum* (Avr5) was a less effective biocontrol agent in the split-root

system than in the two other systems. Overall, *F. oxysporum* (Avr5) reduced Fusarium wilt by 20 (split-root system) to 10% (benomyl system) of the disease index.

In this research, nonpathogenic strain *F. oxysporum* (Avr5) protected tomato plants from Fusarium wilt, despite not being in direct contact with the pathogen. This indicates that protection did not result from antagonism or competition between nonpathogenic Fusarium and pathogen, and suggests that strain *F. oxysporum* (Avr5) induced resistance to Fusarium wilt in tomato, because antagonists and pathogen remained spatially separated all through the experiment. Split-root system has been used successfully to investigate induced resistance (Liu *et al*., 1995; Mandel and Baker, 1991; Khan *et al*., 2004). This hypothesis is supported by the observation that a single inoculation with *F. oxysporum* (Avr5) resulted in physiological changes in the stems and leaves of tomato plants that are commonly associated with systemic induced resistance (Kuc, 1982). Although it is possible that *F. oxysporum* (Avr5) could have produced a substance transported within the plant that stimulated defence mechanisms in particular plant tissues or cell that leading to protection of the whole tomato plant. Van loon (1997) indicated that systemic induction of resistance has often been associated with the accumulation of PR–proteins in stems and leaves of Fo47-treated tomato plants. Nonpathogenic Fusarium strain Fo47 induce the synthesis of chitinase and increase glycosidase activities in tomato roots and leaves (Fuchs *et al.,* 1997; Matta *et al*., 1989).

It has been concluded that application of nonpathogenic *F. oxysporum* (Avr5) can be considered as a potential biocontrol agent for controlling tomato wilt disease, because it necessitates the exposure of tomato plants to *F. oxysporum* (Avr5) at an early stage for efficient induced resistance against subsequent attack by Fusarium wilt fungi.

Further work about the kind of defence reaction in induced resistance and about the mechanism of induction, however is needed to confirm this hypothesis.

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References

AKKOPRU, A. and D. DEMIR, 2005. Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF Glomus intraradices and some

Rhizobacteria. J. Phytopathology 153: 544-550.

ALABOUVETTE, C and Y. COUTEAUDIER, 1992. Biological control of Fusarium wilts with nonpathogenic fusaria. Pages 415-426 in: Biological control of plant Diseases: Progress and Challenges for the Future. E. C. Tjamos, G. C. Papavizas and R. J. Cook, eds. Pienum press. New York.

BAO, J. R., G. LAZAROVITS, 2001. Differential colonization of tomato roots by non pathogenic and pathogenic *Fusarium oxysporum* strains may influence fusarium wilt control. Biological Control 5: 449-456.

BARGMANN, C and F. SCHONOBECK, 1992. *Acremonium kiliense* as inducer of resistance to wilt diseases on tomatoes. J. Plant Dis. Prot 9: 266-272.

BECKMAN, C. H. 1987. The nature of wilt Diseases of plants. St. Paul, Minnesota. The American Phytopathology Society, APS press.

BENHAMOU, N., J. W. KLOEPPER, A. QUADT-HALLMAN and S. TUZUN, 1996. Induction of defence-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol 112: 919-929.

BENHAMOU, N., P. J. LAFONTAINE and M. NICOLE, 1994. Induction of systemic resistance to Fusarium crown and root rot in tomato plants by seed treatment with chitosan. Phytopathology 84: 1432-1444.

DE CAL, A., R. GARCIA-LEPE and P. MELGAREJO, 2000. Induced resistance by *Penicillium oxalicum* against *Fusarium oxysporum* f. sp. *lycopersici*: Histological studies of infected and induced tomato stems. Phytopathology 90: 260-268.

DE CAL, A., S. PASCUAL and P. MELGAREIO, 1995. Biological control of *Fusarium oxysporum* f. sp. *lycopersici*. Plant pathology 44: 909-917.

DE CAL, A., S, PASCUAL and P. MELGAREIO, 1997. Infectivity of Chlamydospores vs microconidia of *Fusarium oxysporum* f. sp. *lycopersici* on tomato. J. Phytopathol 145: 231-233.

EL HASSNI, M., A. EL HADRAMI, F. DAAYF, M. CHERIF, E. AIT BARKA and I. EL HADRAMI, 2007. Biological control of bayoud diseasese in date palm: selection of microorganisms inhibiting the causal agent and inducing defence reaction. Environ. Exp. Bot 59: 224-234.

FORSYTH, L. M., L. J. SMITH and A. B. AITKEN, 2006. Identification and characterization of non-pathogenic *Fusarium oxysporum* capable of increasing and decreasing Fusarium wilt severity. Mycological Research 110(8): 929-935.

FRAVEL, D., C. OLIVAIN and C. ALABOUVETTE, 2003. *Fusarium oxysporum* and its biocontrol. New Phytol 157: 493-502.

FUCHS, J. G., MOENNE-LOCOZ, Y. and DEFAGO, 1997. Nonpathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt in tomato. Plant Dis. 81: 492-496.

GOTTSTEIN, H. D and J. A. KUS, 1989. Induction of systemic resistance to anthracnose in cucumber by phosphates. Phytopathology, 79: 176-179.

GRATTIDGE, R. and R. G. O'BRIEN, 1982. Occurrence of third race of Fusarium wilt of tomatoes in Queensland. Plant Disease. 66: 165-166.

HERVAS, A., J. L. TRAPERO-CASAS and R. M. JIMENEZ-DIAZ, 1995. Induced resistance against Fusarium wilt of chickpea by nonpathogenic races of *Fusarium oxysporum* f. sp. *ciceris* and nonpathogenic isolates of *F. oxysporum*. Plant Dis 79: 1110-1116.

IAKOVOS, S. P., T. E. SOTIRIOS, A. S. IANNIS, C. IORDANIS and J. P. EPAMINONDAS, 2009. Mode of action of a non-pathogenic *Fusarium oxysporum* strain against *Verticillium dahliae* using Real time QPCR analysis and biomarker transformation. Biological Control 50(1): 30-36.

JIAN, R. B., R. F. DEBORAH, R. O. NICHOLE, L. GEORGE and V. B. PETER, 2009. Genetic analysis of pathogenic and non-pathogenic *Fusarium oxysporum* from tomat plants. Can. J. Microbiol 55(2): 117-125.

JOLANTA, J. S., K. EWA, W. KRYSTYNA, B. ANNA and L. ALEKSANDER, 2008. Colonization of root tissues and protection against Fusarium wilt of rye (*Secale cereale*) by non-pathogenic rhizosphere strains of *Fusarium culmorum*. Biological Control 45: 297-307.

KHAN, J., J. J. OOKA, S. A. MILLER, L. V. MADDEN and H. A. J. HOITINK, 2004. Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against Phytophthora crown rot and leaf blight. Plant Dis 88: 280-286.

KIM, D. S., R. J. COOK and D. M. WELLER, 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. Phytopathology 87: 551-558.

KROON, B. A., R. J. SCHEFFER and D. M. ELGERSMA, 1991. Induced resistance in tomato plant against Fusarium wilt invoked by *Fusarium oxysporum* f. sp. *dianthi*. Neth. J. PL. Path 97(6): 401-408.

KUC, J. 1982. Induced immunity to plant disease. Bioscience 32: 854-860.

KUC, J. 1987. Plant immunization and its applicability for disease control. Pages 225-272 in: Innovative Approaches to plant Disease control. I. Chet, ed. John Wiley & Sons, New York.

LARKIN, R. P. and D. R. FRAVEL, 1996. Ecological characteristics of biological control of Fusarium wilt of tomato using nonpathogenic *Fusarium* spp. (Abstr.) Phytopathology 86(suppl.): S9.

LARKIN, R. P. and D. R. FRAVEL, 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. Plant Dis 82: 1022-1028.

LARKIN, R. P. and D. R. FRAVEL, 1999. Mechanisms of action and dose-response relationships governing biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 89: 1152-1161.

LEMANCEAU, P. and C. ALABOUVETTE, 1991. Biological control of Fusarium diseases by *fluorescent Pseudomonas* and non-pathogenic Fusarium. Crop Prot 10: 279-286.

LEMANCEAU, P. and C. ALABOUVETTE, 1993. Suppression of *Fusarium* wilts by *Fluorescent Pseudomonads*. Mechanisms and application Biocontrol science and Technology 3: 219-234.

LIU, L., J. W. KLOEPPER and S. TUZUN, 1995. Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology 85: 697-698.

MALANDRAKI, I., S. E. TJAMOS, I. PANTELIDES and E. J. PAPLOMATAS, 2008. Thermal inactivation of compost suppressiveness implicates possible biological factors in disease management. Biological Control 44:180-187.

MANDEEL, Q. and R. BAKER, 1991. Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. Phytopathology 81: 462-469.

MATTA, A., I. A. GENTILE and L. FERRARIS, 1989. Induced resistance to Fusarium wilt diseases. In: Tjamos EC and Beckman CH (eds) Vascular wilt diseases of plants. Basic studies and control (pp. 175-196), NATO ASI Series, vol H28, Springer-Verlag, Berlin.

MATTA, A., I. A GENTILE and L. FERRARIS, 1988. Stimulation of ß-1,3-glucanase and chitinase by stresses that induce resistance to Fusarium wilt in tomato. Phytopathol. Mediterr 27: 45-50.

NIRENBERG, M. 1976. Unitreswuchugen uber die morphologische and biologische

differenzierung in der Fusarium section Liseola. Mitt. Biol. Bundesanstal and – Foratw. Berlin- dahlem 169: 117 pp.

OGAWA, K. and H. KOMADA, 1984. Biological control of Fusarium wilt of sweet potato with cross-protection by nonpathogenic *Fusarium oxysporum.* In Ecology and management of soilborn plant pathogens. Edited by Parker C. A., Rovira A. D., Moore K. J. The American Phytopathologycal Society, St. Paul, MN. Pp. 121-124.

REID, T. C., M. K. HAUSBECK and K. KIZILKAYA, 2002. Use of fungicides and biological control in the suppression of Fusarium crown and root rot of asparagus under greenhouse and growth chamber conditions. Plant Dis 86: 493-498.

SHISHIDO, M., C. MIWA, T. USAMI, Y. AMEMIYA and K. B. JOHNSIN, 2005. Biological control efficiency of Fusarium wilt of tomato by nonpathogenic *Fusarium oxysporum* Fo-B2 in different environments. Phytopathology 95: 1072-1080.

STROMBERG, A. 1989. Systemically induced resistance in Tomatoes, Tobacco and Potatoes. A Literature Review Vaxtskyddsrapporter, Jordbruk 55. Research Information centre, Swedish university of Agricultural Sciences, Uppsala.

TAMIETTI, G., L. FERRARIS, A. MATTA and I. ABBATTISTA GENTILE, 1993. Physiological response of tomato plants grown in Fusarium suppressive soil. J. Phytopathol 138: 66-67.

TELLO-MARQUINA, J. C. and A. LACASA, 1988. Evolution of races among *Fusarium oxysporum* f.sp. *lycopersici.* de-Sanidad-vegetal-Plagas 14: 335-341.

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