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# The Biological Control of Fusarium Wilt on Carnation with Fluorescent Pseudomonads

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## ABSTRACT

*Fusarium oxysporum* f.sp. *dianthi* (*Fod*) causes severe wilting on carnations in Turkey. In order to control of carnation vascular wilt disease, 311 fluorescent Pseudomonads were isolated from the roots of carnation plants and the bacterial strains were tested for *in vitro* antagonism with *Fod* in plate tests. Fifty nine strains were evaluated and five strains found to suppress Fusarium wilt of carnation in pot bioassays. Antagonistic bacterial strains S53 and S54 were identified as *Pseudomonas putida*; I20, U34 and U73 as *Pseudomonas fluorescens*.

In the *in vivo* tests, carnation of cv Falcon seedlings were treated with the antagonistic bacteria in the greenhouse during the transplanting into pathogen infested soil. The S53, S54, I20 and U34 strains and combination of two bacteria (I20+S54) significantly reduced *Fusarium* wilt of carnation (*Fod*) and increased the healthy plant percentage.

These results provide a promising effect of the antagonistic activity of fluorescent Pseudomonads in controlling wilt disease caused by *F. oxysporum* f.sp. *dianthi.*

**Key words:** Biological control, *Fusarium oxysporum* f. sp. *dianthi*, carnation, fluorescent pseudomonads, Fusarium wilt

## INTRODUCTION

Carnation (*Dianthus caryophyllus* L.) is the most produced cut flower with a percentage of 50% in Turkey. The Aegean region comes the first among the other regions with a share 39,1 % in this total area and 35.5% of total cut flower production area in Izmir (Ertan *et al*., 1993). Fusarium wilt caused by *F. oxysporum* f. sp. *dianthi* has been a destructive disease of carnation and at certain times has been a limiting factor in production.

Several methods have been applied for control of carnation cultivars against to wilt disease although it is difficult to find an effective method. Great efforts have been made to control the wilt diseases. Selection for resistance is considered as a control measure to reduce the disease severity. Eight physiologically races were reported for *F.oxysporum* f. sp. *dianthi*. The most common one is race 2 (Baayen *et al*., 1988). There is a difficulty for breeding the resistant cultivars because of the pathogen can easily differentiate new pathotypes (Garibaldi and Gullino, 1984). Currently available control measures include cutting free of pathogen and fumigation of the soil usually minimizes the disease but it is not effective in preventing the disease (Ben-Yephet *et al*., 1994).

Controlling Fusarium wilt with antagonistic microorganisms have been reported in many studies (de Boer *et al*., 2003; Leeman *et al*., 1996; Duijff *et al*., 1998; Carver *et al*., 1996). In recent years, various root colonizing pseudomonads have been shown to be potential biological control agents in different plant pathogen systems. Fluorescent pseudomonads have been applied to the rhizosphere soil for biological control of carnation wilt (Duijff *et al*., 1993, 1994; Lemanceau *et al*., 1992).

Several mechanisms, competition for iron (Duijff *et al*., 1993; Bora *et al*., 1994), production of antibiosis (Bangera *et al*., 1996; Maurhofer *et al*., 1995) and induced systemic resistance (Haas and Defago, 2005) have been investigated in the disease suppression by various strains of fluorescent pseudomonads.

Research to improve alternative ways to control the disease has been conducted worldwide.

The objective of the current study was to determine the biological control activity of fluorescent pseudomonads against *Fusarium* wilt of carnation.

## MATERIALS and METHODS

### Bacterial strains from carnation rhizosphere

Bacteria were isolated in production season of 2001 from the rhizosphere of carnation plants on the greenhouses of Izmir province. The soil was removed the root systems of the carnations in order to isolate the fluorescent pseudomonads. Two grams of root materials were cut into small pieces with a razor blade and shaken at 100 rpm in 200 ml sterile water for one hour at 25 ºC and then the suspensions were plated (100 µg $ml^{-1}$) on King's B agar (KB) (King *et al.,* 1954) selective for fluorescent pseudomonads. The plates were incubated at 25 ºC for 48 h. Cultures of bacterial cells grown in King's B agar were supplemented with cycloheximide (100 µg $ml^{-1}$), ampicillin (50 µg $ml^{-1}$) and chloramphenicol (12.5 µg $ml^{-1}$) (Thomas *et al*., 1998).

### Pathogen and bacteria

*Fusarium oxysporum* f.sp. *dianthi* race 2 was provided by Dr. Ben-Yephet (ARO the Volcani Center, Israel). It was grown on Potato Dextrose Agar (PDA) and maintained at 25 ºC in a growth cabinet.

For identification to the biotype level of *Pseudomonas* spp. as described by Fahy and Hayward, 1983, the following tests were examined: production of levan, oxidase and arginin, growth in sucrose, trehalose and sorbitol.

To group the strains related with similar strains they were analyzed for fatty acid profiles by MIDI, Newark, USA (Sherlock Microbial Identification system Version 4.02).

**Plant material**

The six commercial carnation cultivars of Farida, Opera, White liberty, Casper, Judith and Falcon were tested for their wilt reaction in pot conditions. The cultivars were selected based on the severity of the symptoms. In cultivar response in the pathogenicity tests was found Falcon cv as a succeptible variety.

***In vitro* antagonism**

The plate test screening for *in vitro* antagonism against the pathogen was performed by spot inoculating the bacterial suspensions on the KB agar plate at four locations (Geels and Shippers, 1983). The plates were incubated at 25 ºC for 48 h.

The conidial suspension of *Fod* was prepared from Potato Dextrose Agar (PDA) cultures that were grown for 10 days at 25 ºC. The conidia were harvested and the suspension was filtered through sterilized cheesecloth to remove the mycelia fragments (Duijff *et al*., 1994). The conidial densities of the suspension of $1\times10^6$ conidia $ml^{-1}$ were determined by direct observation on a haemocytometer and adjusted by dilution. Conidia suspension was atomized over the bacteria inoculated King's B medium (KB) agar plates that had been incubated for 48 h. The plates were incubated of mycelia growth after 5 days. The inhibition of the pathogen was calculated by using a scale of 0-5 given by Geels and Shippers (1983). (Scale 0=no inhibition and 5= 41 and 50 mm). All in vitro tests were performed twice.

Production of siderophores was determined by the method of Geels and Schippers (1983). Strains were grown on KB agar plates supplemented with 80µl $FeCl_3$. Pseudomonads were determined antagonism based on the production of siderophores.

**Inhibition of Fusarium wilts disease in carnation**

The selected strains of pseudomonads were tested for fungal antagonism against *Fod* on carnation seedling in the pot experiments. Bacteria were grown in 250 ml Erlenmeyer containing 100 ml of sterilized King's B broth medium were placed on a rotary shaker at room temperature for 48 h at 90 rpm. The cells were harvested by centrifugation for 20 minutes at 2795xg and the pellet was suspended in 0.01 M $MgSO_4$. Cell suspension of bacteria was adjusted to $1\times10^9$ CFU $ml^{-1}$ (Bora et al., 1994). The inoculums' of *Fod* race 2 was grown in wheat bran culture consisting of 135 g of bran was placed and then inoculated with PDA plugs of *Fod*. After 10-15 days of incubation at 25 ºC, the inoculum was air dried and screened to small pieces (Turhan, 1992).

Rooted cuttings of carnation (*D. caryophyllus* cultivar Falcon) susceptible to *Fod* obtained from Aktif Tarim, Antalya were used for pot and greenhouse experiments.

**Pot bioassays**

The rooted cuttings of carnation were transplanted in 25-cm-diameter pots (3 liter) with peat, sand and soil with manure (1:1:1) after sterilized by formaldehyde (40%). Three seedlings were planted per pot and each pot was infested per one liter with four grams of inoculums' grown in bran culture after mixing to uniform distribution of the inoculums. The soil around the seedling was inoculated by pouring a 30 ml of suspension of bacterial cells of 59 isolates ($1\times10^9$ cells $ml^{-1}$) after the pots were incubated for one day in the growth chamber. The pots were incubated for one day in the growth chamber (25 ºC, 16 h of light and 8 h of darkness) for eight weeks.

Disease incidence recorded at weekly intervals after transplanting. The wilt development on each carnation plant grown in the growth chamber was rated by using the following scale (Ben-Yephet *et al*., 1993): 0, no symptoms; 1, 25% of leaves wilted; 2, 26-50% of leaves wilted 3, 51-75% of leaves with wilted 4, 76-100% completely wilted. The seedling treated with water served as a negative control and other carnation plants treated with *Fod* served as a positive control. Each of experiments had four replicates.

**Greenhouse bioassay**

The greenhouse soil (108 $m^{-2}$) that contain sand, soil and manure (1:1:1) was sterilized with formaldehyde (40%) prior to infestation and covered 4 days.

The inoculum was prepared by growing the pathogen on sterile wheat bran as described previously. The greenhouse was divided into 36 plots consisting of a 1 meter wide and 1 meter long of each (Ben-Yephet *et al*., 1993) (Fig.3).

The inoculation was carried out by mixing the wheat bran to 800 g / $m^2$ of the fungal culture to the 200 liter soil of the bed. 36 rooted cuttings of cv "Falcon" were planted in six rows. Antagonistic bacteria selected from the pot tests (U34, U73, S53, S54 and I20) were applied to the carnation plants during the transplanting one day after the artificial inoculums of the pathogen. Bacteria were inoculated either in combination or separately. The density of the bacterial suspension used for the inoculation was $1\times10^9$ cells $ml^{-1}$. Then 30 ml of the bacterial suspension poured onto the soil around each of transplanting carnation plants. The plants treated pathogen, benomyl (Benlate 50%; 100g/100l) and water alone kept as control.

The number of wilted plants of each plot was counted at each week intervals for fifteen weeks from April to August 2003. Disease incidence was assessed by visual scale used in the pot tests. The fertilization program was set as monthly and the fertilizers were applied as daily doses in drip irrigated system.

### Data analysis

All statistical analysis were performed using SPSS for Windows 11 observations were submitted to one way analysis of variance (ANOVA) followed by Duncan's multiple range test to separate mean ($P \leq 0.01$).

## RESULTS AND DISCUSSION

### *In vitro* antagonism between fluorescent pseudomonads and *Fod*

*In vitro* antagonism assays were conducted to evaluate the antagonistic properties of some of the pseudomonads strains. Three hundred and eleven fluorescent pseudomonads were screened for antagonistic activity on KB plates against *Fod.* The *in vitro* test results demonstrated that fifty nine strains isolated from the roots of carnation had significant antagonistic effect on the mycelia growth of pathogen (Table1).

**Table 1.** Inhibition of the growth of *Fod* by strains of fluorescent pseudomonads on KB medium and effect of $Fe^{3}$ on the antagonistic strains activity

| Number of the strains | fluorescent pseudomonas strains | Inhibition zones of *Fod* (mm) | Siderophore effect on the antagonistic strains |
|---|---|---|---|
| 8 | U32,U81,U86,U101,I3,I19,I22,S53 | 25.0[a] | ++[c] |
| 4 | U72,I15,I21,I73 | 26.0 | ++ |
| 7 | U14,U23,U27,U157,S1,S11,S55 | 27.0 | ++ |
| 3 | U3,I48,G1 | 28.0 | ++ |
| 11 | U7,U34,U35,U42,U62,U71,I28 I68,S31,S33,S36 | 29.0 | ++ |
| 6 | U68,U73,I64,S53,S51,S54 | 30.0 | ++ |
| 4 | I76,S39,S50,S56 | 31.0 | ++ |
| 3 | U69,I11,S32 | 32.0 | ++ |
| 4 | U2,I49,I72,S37 | 33.0 | ++ |
| 5 | U4,U66,I55,I60,I61 | 34.0 | ++ |
| 3 | I20,I31,S38 | 35.0 | -,++ |
| 1 | I69 | 38.0 | ++ |
| 59 | | | |

[a] means of three replication, each replication contain three plates
[b]Clear inhibition zone was observed in presence of $Fe^{+3}$ on King's B agar medium
[c]Siderofore effect positive for all strains

### Disease suppression in carnation seedlings in pot tests

Three rooted cuttings cv "Falcon" were transplanted to the pathogen infested pot soil. In pot experiment test in the growth chamber of 59 Pseudomonas strains isolated from carnation roots disease symptoms were evaluated with a visual scale at weekly intervals (Table 2). Treatment of carnation roots with five strains resulted in a significant increase in the healthy plants compared with the untreated control after 8 weeks of growth in soil infested with *Fod* (Fig. 1).

**Table 2.** Effects of Pseudomonas strains on *Fod* incidence on pot assay in growth chamber

| no | isolate | Dis.incidence[a] | no | isolate | Dis.severity | no | isolate | Dis.incidence |
|---|---|---|---|---|---|---|---|---|
| 1 | U2 | 58,34 abcd | 21 | U101 | 35,42 abcd | 41 | I73 | 37,51 abcd |
| 2 | U3 | 43,75 abcd | 22 | U157 | 70,84 cd | 42 | I76 | 50,00 abcd |
| 3 | U4 | 68,76 abcd | 23 | I3 | 54,17 abcd | 43 | S1 | 41,67 abcd |
| 4 | U7 | 60,42 abcd | 24 | I11 | 50,00 abcd | 44 | S11 | 33,34 abcd |
| 5 | U14 | 60,42 abcd | 25 | I15 | 56,25 abcd | 45 | S31 | 56,26 bcd |
| 6 | U23 | 39,59 abcd | 26 | I19 | 75,00 d | 46 | S32 | 45,84 abcd |
| 7 | U27 | 45,84 abcd | 27 | I20 | 25,00 abc | 47 | S33 | 25,00 abcd |
| 8 | U32 | 54,17 abcd | 28 | I21 | 47,92 abcd | 48 | S35 | 50,00 abcd |
| 9 | U34 | 16,67 ab | 29 | I22 | 33,34 abcd | 49 | S36 | 72,92 cd |
| 10 | U35 | 62,51 abcd | 30 | I28 | 52,09 abcd | 50 | S37 | 56,25 abcd |
| 11 | U42 | 62,51 abcd | 31 | I31 | 35,42 abcd | 51 | S38 | 33,34 abcd |
| 12 | U62 | 52,09 abcd | 32 | I48 | 47,92 abcd | 52 | S39 | 41,67 abcd |
| 13 | U66 | 50,00 abcd | 33 | I49 | 31,26 abcd | 53 | S50 | 33,34 abcd |
| 14 | U68 | 35,42 abcd | 34 | I55 | 31,26 abcd | 54 | S51 | 27,09 abcd |
| 15 | U69 | 45,84 abcd | 35 | I60 | 37,50 abcd | 55 | S53 | 18,75 abc |
| 16 | U71 | 50,00 abcd | 36 | I61 | 33,34 abcd | 56 | S54 | 12,51 a |
| 17 | U72 | 47,93 abcd | 37 | I64 | 58,34 abcd | 57 | S55 | 33,34 abcd |
| 18 | U73 | 22,92 abcd | 38 | I68 | 43,75 abcd | 58 | S56 | 22,92 abc |
| 19 | U81 | 66,67 abcd | 39 | I69 | 60,42 abcd | 59 | G1 | 43,75 abcd |
| 20 | U86 | 37,50 abcd | 40 | I72 | 47,92 abcd | | | |
| | K(+) | 55,22 | | | | | | |
| | K(-) | 14,59 ab | | | | | | |

[a] Means of four replication, each replication contain three plants of observation. Mean followed by common letters are significantly differed at Duncan Multiple range Test (P≤0.01) level

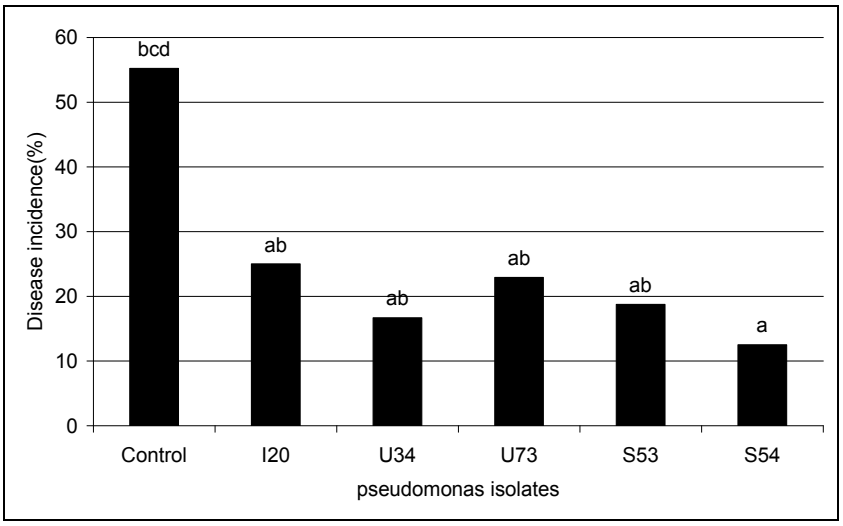


**Fig.1.** Effects of root inoculation with Pseudomonads strains on incidence of disease in pot bioassays with *Fod*-infested soil. Mean of four replications, each replication contain three plants of observation. Mean followed by common letters are significantly differed at Duncan Multiple range Test (P≤0.01) level

**Bioassay in greenhouse**

Selected five bacterial strains (U34, U73, S53, S54 and I20) that had been shown to inhibit Fusarium wilt in pots were tested for their availability to suppress the disease in the greenhouse trials. Two bacterial strains (I20 and S53) were also tested in combination in the plots. The wilt symptoms were recorded at fifteen days after inoculation. After that the first examination the wilt was increased up to 70 days after inoculation depending on the relative humidity (40-50%) and the temperature of 30 ºC. At the end of the fifteen week a mortality rate of 77% was recorded for plants inoculated with *Fod* alone. The disease incidence in the infested control plots increased with time.

The effects of biocontrol agents on suppression of disease incidence in plot tests are shown in Fig.2. *P. putida* (S53 and S54), *P. fluorescens* (I20, U34) and co-inoculation of two bacteria (S54 and I20) reduced the disease incidence ranged by 28.09% and 46.96% at 15 weeks after transplanting. Reductions of disease incidence by five bacteria were found significant at the end of experiment. However two bacteria used in combination (S54+I20) was not found more effective than bacteria achieved alone (Fig.2). Disease incidence of strains (S53 and S54) was initially increased but thereafter maintained a stable density at the 7th and 8th week and even gradually decreased with time (Fig. 4) during the growing period.

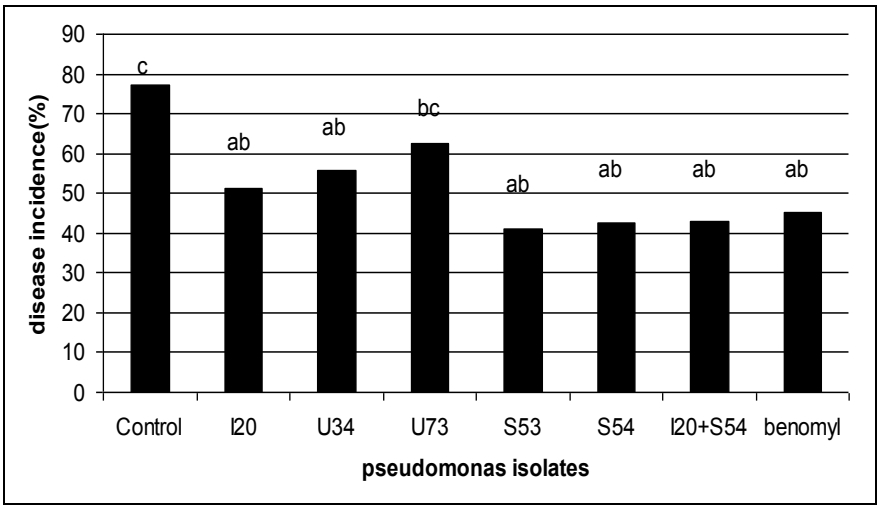


**Fig.2.** Effects of Pseudomonas strains on *Fod* incidence on greenhouse grown carnation 15 weeks after infestation with pathogen. Mean of four replication, each replication contain 36 plants of observation. Mean followed by common letters are significantly differed at Duncan's Multipl range test ($P \leq 0.01$) level

**Characterization of *Pseudomonas* spp.**

Three bacterial strains were identified as *P. fluorescens* Biotype III (I20, U34 and U73) and two strains as *P. putida* (S53 and S54) by biochemical and fatty acid analysis (Table 3)

**Table 3.** Identification and properties of fluorescent Pseudomonads strains from carnation rhizosphere

| Isolate number | Characteristics | | | | | | Identification |
|---|---|---|---|---|---|---|---|
| | Lev | Oxi | Arg | Suc | Tre | Sor | |
| I20 | - | + | + | - | + | - | *Pseudomonas fluorescens* Biotype III |
| U34 | - | + | + | - | + | - | *Pseudomonas fluorescens* Biotype III |
| U73 | - | + | + | - | + | - | *Pseudomonas fluorescens* Biotype III |
| S53 | - | + | + | - | - | - | *Pseudomonas putida* |
| S54 | - | + | + | - | - | - | *Pseudomonas putida* |

Strains were examined for levan formation (Lev), Oxidase (Oxi), Arginin (Arg) and growth on Trehalose (Tre), Sucrose (Suc) and Sorbitol (Sor). Identification of strains was based on MIDI test. (MIDI, Newark, DE, USA), MIDI (Sherlock Microbial Identification System Version 4.02).

In this research, the antagonistic bacteria were investigated against Fusarium wilt caused by *Fod* of carnation *in vitro* and *in vivo* assays. The selection of resistant cultivars, maintain the sanitation and planting the disease free cuttings is the important way to control the disease.

Research to improve the biocontrol studies with beneficial Pseudomonas strains is receiving increased attention. The results of the earlier studies showed that fluorescent *Pseudomonas* spp. contribute to reduce the disease by siderophore and antibiotic mediated suppression of *Fusarium* wilt disease (Duijff *et al*., 1993; Lemanceau *et al*., 1992; deBoer *et al.*, 2003; Duijff *et al*., 1999).

In the present study, *in vitro* selection of the efficient strains of Pseudomonas was the criterion used in this study in assessing their rhizosphere colonization in greenhouse. The efficient bacteria were selected for suppression of the wilt disease in carnation which inhibited mycelia growth of *Fod* in vitro (Geels and Shippers, 1983).

They scored positive on KB medium which is indicative of siderophore production and were fluorescent on KB plates under UV light (366 nm) thereby confirming that they were fluorescent pseudomonads. All of them have also fluorescent properties on medium supplemented with $FeCl_3$ (80 µl $ml^{-1}$).

A total of 311 strains of *Pseudomonas* spp. were tested in KB agar plates. The growth inhibition revealed that 59 of 311 strains were capable in a clear inhibition zone surrounding the spotted colony. To determine the siderophore effects of the strains were performed on KB supplemented with 80 µl $ml^{-1}$ $FeCl_3$. More than half of the bacterial strains were strongly inhibited the mycelium of the pathogen.

Inhibition of mycelia growth of *Fod* by *Pseudomonas* strains on agar plates based on competition for iron. *P. fluorescens* (I20) was the only non siderophore effect in the inhibition of *Fod* on agar plates (Table 1).

Competition for iron can be involved in interactions between Pseudomonads and Fusarium wilt was demonstrated by (Duijff *et al*. 1993; deBoer *et al*. 2003; Van Peer *et al*.1990; Leeman *et al*.1996).

In pot assays in which seedling inoculated in a suspension of the antagonistic bacteria after transplanting in to the pathogen infested soil. The effective antagonists were selected among with the averagely 10% strains tested reducing disease incidence by 50-80%. A total of 5 of 59 Pseudomonads bacteria significantly reduced the *Fod* of carnation in pot bioassays (Figure 1).

The antagonistic strains (S53 and S54) were identified as *P. putida* and also U34, I20 and U73 as *P. fluorescens* by the fatty acid and biochemical identification tests. *P.putida* controls *Fusarium* wilts on carnation (Duijff *et al.,* 1994, 1999; Xu *et al*., 1987), radish (deBoer *et al*., 2003) and *P.fluorescens* controls on carnation (Duijff *et al*., 1998), radish (Leeman *et al*., 1996; deBoer *et al*., 1999), tomato (Larkin *et al*., 1998) by means of antibiosis or competition for iron.

Five potentially antagonistic bacteria were screened for their disease suppression against Fusarium wilt of carnation using the pot bioassays. Of the *Pseudomonas* spp tested strains I20, U34, U73, S53 and S54 significantly suppressed *Fusarium* wilt in the pot bioassays (Figure 1).

In suppression of Fusarium wilt bioassay in greenhouse, the disease was reduced by *P. putida* (S53 and S54) and *P. fluorescens* (I20 and U34) strains. For *P.fluorescens* (I20) and *P.putida* (S54) there was no difference in wilt suppression between the individual inoculations of the antagonists (Figure 2).

Disease progression was initially low (Fig 3) but, with the increase in the daily temperatures which is over 30ºC, it became apparent by $7^{th}$ week. Between 7 to 15 weeks, disease in carnation treated with *P. putida* strains was more stable than in plants treated with the other antagonistic bacteria (*P. fluorescens*).

Disease severity was significantly reduced by the bacterial strains of *P fluorescens* (U34, I20), inoculation of two bacteria (S54 and I20), *P.putida* (S53 and S54) and they reduced disease severity by 28.09%, 33.48%,44.27%,45.16 and 46.96 % respectively (Fig 3).

Suppression of *Fusarium* wilt of carnation was enhanced with *P.putida* strains which suppress disease by iron competition (Lemanceau *et al*., 1992). Of all strains tested; two *P.putida* strains appear to possess potential for controlling Fusarium wilt. As previously demonstrated by Duijff *et al*., (1993) enhanced suppression of Fusarium wilt of carnation was obtained by *P. putida* strains. Strains with more than one suppression mechanism such as competition for iron, antibiosis appears to the effective to control Fusarium wilt in carnation.

Under greenhouse conditions, the application of biocontrol bacteria has given promising results in various plant productions (Lemanceau *et al*., 1992; Duijff *et al*., 1998; deBoer *et al.,* 1999; Natsch *et al*., 1997; Olivain *et al*., 2004; Haas and Defago, 2005).

**Fig 3.** Greenhouse biassay of *Fod* and antagonistic bacteria

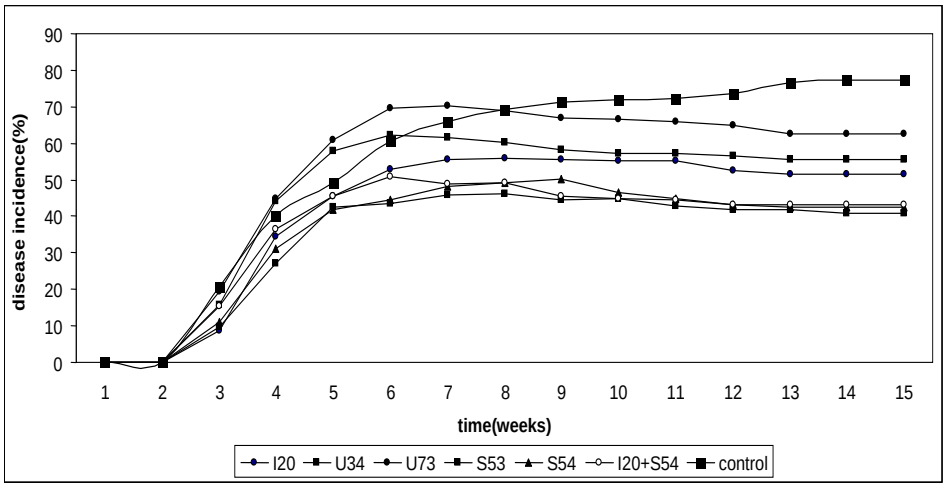


**Fig 4.** Disease incidence of carnation seedlings inoculated with *Fod* alone; *P. putida* (isolate S53 and S54); *P. fluorescens* (isolate I20, U34, U73) and *P. putida* + *P. fluorescens* (isolate I20 and S54) under greenhouse conditions

In conclusion, Fusarium wilt is the most severe disease of carnations in all production areas including Turkey. Eight physiological races or pathotypes have been described for *F.oxysporum* f.sp. *dianthi* on different cultivars of carnation (Baayen et

al., 1997; Migheli et al., 1998, Chioceehetti et al., 1999; Ben Yephet et al., 1993). Some studies by Sezgin et al (1984) and Ozer and Soran (1989) have demonstrated that *F. oxysporum* can be pathogenic on carnations. But the pathogenic races of Fusarium wilt in carnation have not been reported in Turkey. This study was designed to evaluate the interactions between the fusarium wilt pathogen (*Fod* race 2) and antagonistic bacteria for disease suppression. More studies are needed on the pathogenic characterization of the carnation wilt disease and biocontrol possibilities.

The selection for *P. putida* and *P. fluorescens* strains confirm a fungal antagonism and usefulness of fluorescent Pseudomonads is promising for future application of these bacteria in biological control of the carnation wilt disease.

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## ÖZET

### FLUORESCENT PSEUDOMONAS'LAR ILE KARANFİL FUSARIUM SOLGUNLUĞUNUN BIYOLOJIK SAVAŞIMI

Karanfil solgunluğu etmeni *Fusarium oxysporum* f.sp. *dianthi*, Türkiye'de karanfillerde görülen en önemli hastalıklardan birisidir.

Bu hastalığın önlenebilmesi için bitki köklerinden 311 Fluorescent Pseudomonas izole edilmiş ve bakterilerin antagonistik özellikleri *in vitro* testler ile incelenmiştir. Değerlendirmeye alınan elli dokuz bakteriyel izolattan 5'inin saksı testleri sonucunda Fusarium solgunluğunu baskıladıkları ortaya konmuştur. Antagonistik bakterilerden S53 ve S54; *Pseudomonas putida*, I20, U73 ve U34 ise *P. fluorescens* olarak tanılanmıştır.

*In vivo* testlerde kullanılan "Falcon" çeşidi karanfil fidelerinin kökleri, antagonistik bakteriler ile bulaştırılmış ve seradaki patojen bulaştırılmış toprağa şaşırtılmıştır. S53, S54, I20 ve U34 kodlu bakteriler ile iki bakteri kombinasyonu (I20+S54), Fusarium solgunluğunu (*Fod*) önemli ölçüde engellemiş ve sağlıklı bitki oranını arttırmıştır.

Sonuçlar, *Fod'*nin neden olduğu karanfil solgunluğu ile savaşımda antagonistik etki gösteren Fluoresent Pseudomonas'ların ümitvar olduğunu göstermiştir.

**Anahtar kelimeler:** Biyolojik savaş, karanfil, *Fusarium oxysporum* f.sp. *dianthi*, Fluorescent Pseudomanas, Fusarium solgunluğu

## LITERATURE CITED

BAAYEN,R.P., D.M.ELGERSMA., J.F., DEMMINK and L.D. SPARNAAIJ. 1988. Differences in Pathogenesis Observed Among Susceptible Interactions of Carnatıon With Four Races of *Fusarium oxysporum* f.sp. *dianth*i. **Neth. J. Plant Pathol**. 94: 81-94.

BAAYEN,R.P.,F. van DREVEN, M.C.KRIJGER and C.WAALWIJK. 1997. Genetic Diversity in *F.oxysporum* f.sp. *dianthi* and *F.redolens* f.sp. *dianthi*. **Eur. J. Pl. Pathol.** 103: 395-

BANGERA, M.G. and L.S. THOMASHOW. 1996. Characterization of a Genomic Locus Required for Synthesis of the Antibiotic 2,4-Diacetylphloroglucinol by the Biological Control Agent *Pseudomonas fluorescens* Q2-87. **Mol. Plant Microbe Int.** 9:83-90.

BEN-YEPHET, Y., D. SHTIENBERG, M. REUVENI and Y. MOR. 1993. Response of Carnation Cultivars to *Fusarium oxysporum* f.sp.*dianthi* in the Field. **Neth. J. Plant Path.** 99:3-12.

BEN-YEPHET, Y., M. REUVENI, Y. SZMULEWICH and Y. MOR. 1994. Effects of Methyl Bromide on the Control of *Fod* Propagules in Carnation Greenhouse Soils on Inoculum Iincrease After one Growth Ccycle of Carnation. **Crop Prot.** V: 13 N: 5: 357-361.

BORA,T., M.YILDIZ.and H.OZAKTAN.,1994. Effect of Fluorescent Pseudomonads on Fusarium Wilt of Watermelon. **J.Turk. Phytopath**., 23: 19-25.

CARVER, C.E., D. PITT and D.J. RHODES. 1996. Aetiology and Biological Control of *Fusarium* Wilt of Pinks (*Dianthus caryophyllus*) Using *Trichoderma aureoviridae*. **Plant Pathol.** 45: 618-630.

CHIOCCHETTI,A., I. BERNARDO., M.J.DABOUSSI, A: GARIBALDI and. L. GULLINO.1999. Detection of *F. oxysporum* f.sp. *dianthi* in Carnation Tissue by PCR Amplification of Transposan Insertions. **Phytopathology**. 89: 1169-1175.

DE BOER, M., I. VAN DER SLUIS, L.C. VAN LOON and P.A.H.M. BAKKER. 1999. Combining fluorescent *Pseudomonas spp*. Strains to Enhance Suppression of Fusarium Wilt of Radish. **Eur. J. Plant Pathol.** 105: 201-210.

DE BOER, M., P. BOM, F. KINDT, J.J.B. KEURENTJES, I. VAN DER SLUIS, L.C. VAN LOON and P.A.H.M. BAKKER. 2003. Control of *Fusarium* Wilt of Radish by Combining *Pseudomonas putida* Strains That Have Different Disease-Suppressive Mechanisms. **Phytopathology.** 93**:**626-632.

DUIJFF, B.J., J.W. MEIER, P. BAKKER and B. SCHIPPERS. 1993. Siderophore Mediated Competition for Iron and Induced Resistance in the Suppression of *Fusarium* Wilt of Carnation by Fluorescent Pseudomonas spp. **Neth. J. Plant Pathol.** 99 (5-6):277-289.

DUIJFF, B., A. PETER, H.M. BAKKER and B. SCHIPPERS. 1994. Suppression of *Fusarium* Wilt at Carnation by *Pseudomonas putida* WCS358 At Different Levels of Disease Incidence and Iron Availability. **Biocontrol Sci. Techn.** 4: 279-288.

DUIJFF, B.J., D. POUHAIR, C. OLIVAIN, C. ALABOUVETTE and P. L EMANCEAU. 1998. Implication of Systemic Induced Resistance in the Suppression of *Fusarium* Wilt of Tomato by *Pseudomonas fluorescens* WCS417r and by Nonpathogenic *Fusarium oxysporum* Fo47. **Eur. J. Plant Pathol.** 104:903-910.

DUIJFF, B.J., G. RECORBET, P.A.H.M. BAKKER, J.E. LOPER and P. LEMANCEAU. 1999. Microbial Antagonism at the Root Level in the Suppression of *Fusarium* Wilt by the Combination of Nonpathogenic *Fusarium oxysporum* Fo47 and *Pseudomonas putida* WCS358. **Phytopathology.** 89:1073-1079.

ERTAN, N., O, KARAGÜZEL,S.KOSTAK,K.GÜRCAN and A. ÖZÇELİK.1993. Kesme Çiçek raporu, VII. Beş Yıllık Kalkınma Planı, Süs Bitkileri Grubu Özel İhtisas Komisyonu. Atatürk Bahçe Kültürleri Araştırma Enstitüsü, Yalova.

FAHY, P. C. and A. C. HAYWARD. 1983. Media and Methods for Isolation and Diagnostic Tests. pp. 337-378. *In*: Fahy, P. C. and Persley, G. J. (Eds.). Plant Bacterial Diseases, A Diagnostic Guide. Academic Press, Pub., Sydney, Australia.

GEELS, F. P. and B. SCHIPPERS. 1983. Selection of Antagonistic Fluorescent *Pseudomonas* spp. and Their Root Colonization and Persistence Following Treatment of Seed Potatoes. **Phytopathol. Z.** 108: 193-206.

GARIBALDI, A. and M.L. GULLINO. 1984. Integrated Control of Fusarium Wilt of Carnation In Italy.Med.Fac.Landbouww. Rijksuniv.Gent. 49/2a: 357-362.

HAAS, D. and G.DEFAGO. 2005. Biological Control of Soil-Borne Pathogens by Fluorescent Pseudomonads. **www.Nature.Com/Reviews/Micro**.

KING, E.O., M.K. WARD and D.E.RANEY.1954. Two Simple Media for the Demonstration of Pyocyanin and Fluorescin. **J. of Lab. Clin.Med.** 44: 301-307.

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.

LEEMAN, M., F.M. DEN OUDEN, J.A. VAN PELT., C. CORNELISSEN and A. MATAMALA-GARROS. 1996. Suppression of Fusarium Wilt of Radish by Co-Inoculation of Fluorescent *Pseudomonas spp.* and Root-Colonizing Fungi. **Eur.J. Plant Pathol.** 102: 21-31.

LEMANCEAU, P. A., H. M. BAKKER, W. J. DEKOGEL, C. ALABOUVETTE and B. SCHIPPERS. 1992. Effects of Pseudobactin 358 Production by *P. putida* WCS358 on Suppression of Fusarium Wilt of Carnations by Non Pathogenic *Fusarium oxysporum* Fo47. **Appl. Environ. Microb.** 58**:** 2978-2882.

MAURHOFER, M., C. KEEL, D. HAAS and G. DEFAGO. 1995. Influence of Plant Species on Disease Suppression by *Pseudomonas Fluorescens* Strain CHAO With Enhanced Antibiotic Production. **Plant Pathol.** 44: 40-50.

MIGHELI., Q., E.BRIATORE. and A.GARIBALDI. 1998. Use of Random Amplified Polymorphic DNA (RAPD) to Identıty Races 1,2,4 and 8 of *Fusarium oxysporum* f.sp. *dianthi* in Italy. **Eur. J.of Plant. Pathol.** 104: 49-57.

NATSCH, A., C. KEEL, N. HEBECKER, E. LAASIK and G. DEFAGO. 1997. Influence of Biocontrol Strains *Pseudomonas Fluorescens* CHAO and Its Antibiotic Overproducing Derivative on the Diversity of Resident Root Colonizing Pseudomonads. **FEMS Microbiol Ecol.** 23: 341-352.

OLIVAIN, C., C. ALABOUVETTE and C. STEINBERG. 2004. Production of A Mixed Inoculum of *Fusarium oxysporum* Fo47 and *Pseudomonas fluorescens* C7 to Control *Fusarium* Diseases. **Biocontrol Sci. Techn.**, 14, 3: 227-238.

OZER,N. and H.SORAN.1989.Istanbul ve Çevresinde Bazı Kesme Çiçek Türlerinde Görülen Fusarium Türlerinin Tespiti, Dağılışları, Morfolojik Özellikleri ve Patojenisiteleri Üzerinde Araştırmalar. **Bitki Koruma Bülteni.** 29 (3-4):195-209.

SEZGİN, E., A. KARCILIOĞLU, M. ESENTEPE and E. ONAN. 1984. Ege Bölgesinde Ticari Amaçla Yetiştirilen Süs Bitkilerinde Görülen Hastalık, Zararlı, Yabancı Otlar ve Bunlarla Savaşım Olanaklarının Saptanması Üzerinde Araştırmalar. İzm. Bölg. Zir. Müc. Arş. Enst. Proje A- 105- D23/1. Proje Nihai Raporu (1979-1984).

THOMAS, F.C., C.A. WOENG. G.V. BLOMBERG. A.J. VAN DER, K.M.G.M. VAN DER DRIFT, J. SCHRIPSEMA, B. KRON, R.J. SCHEFFER, C. KEEL, P. A.H.M. BAKKER, H.V. TICHY, F.J. DE BRUIJN, J.E. THOMAS-OATES and B.J.J. LUGTENBERG. 1998. Biocontrol by Phenazine-1-Carboximide Producing *Pseudomonas chlorophis* PCL 1391 of Tomato Root Rot Caused by *Fusarium oxysporum radicis lycopersici*. **Mol. Plant. Microbe.** In. V, 11, No: 11, 1069-1077.

TURHAN, G. 1992. Unterdrückrung Des *Rhizoctonia*-Befalls Durch Einen Neuen Mycoparasiten, *Stacybotrys elegans* (Pidopl.). W. Gams. Mitt. Biol. **Bundesanst. Land-Forst-Wirtsch.**, Berlin-Dahlem, 283:380.

XU, T. R, H.VAN PEER, H. RATTING and B. SCHIPPERS. 1987. The Potential Use of Fluorescent Pseudomonads in the Protection of Carnations Against *Fusarium* Wilt in Hydroponics. **Acta Horticulture**. 216: 93-100.

VAN PEER, R., A.J. VAN KUIK, H. RATTINK and B. SCHIPPERS. 1990. Control of *Fusarium* Wilt in Carnation Grown on Rockwool by *Pseudomonas* sp. Strains WCS417r And By Fe-EDDHA. **Neth. J. Plant Pathol**. 96: 119-132.