Biological control of Verticillium wilt on cotton by use of fluorescent *Pseudomonas* spp. under field conditions

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Abstract

Thirty-two *Verticillium dahliae* (VD) isolates from cotton and 59 fluorescent *Pseudomonas* strains from cotton and weed rhizosphere were collected in the main cotton growing areas of Aydin province of Turkey. Fifteen *Pseudomonas* spp. strains having *in vitro* inhibitory effect (>40%) to VD were tested by injection with the conidia of the most virulent strain of VD into the stem of wilt-susceptible (Sayar 314) and wilt-tolerant (Acala Maxxa) cultivars of cotton grown from bacterized seeds in greenhouse conditions. Fourteen days after inoculation, disease severity was assessed with a 0 to 4 rating scale. Four *Pseudomonas* strains isolated from *Xanthium strumarium* (FP22), *Portulaca* sp. (FP23), *Gossypium hirsitum* (FP30) and *Convolvulus arvensis* (FP35) were significantly reduced symptom expression in artificially inoculated plants. Seed bacterization with four fluorescent *Pseudomonas* isolates and *Serratia plymutica* (HRO-C48) resulted in significant reduction in AUDPC both susceptible (Sayar 314) and tolerant (Acala Maxxa) cultivar compared to the non-bacterized control in 2005 and 2006 field trials. Seed cotton yield was not significantly influenced by the cultivar and its interaction with seed treatments (except 2005 trials) due to the late first-symptom appearance after approximately 1300 degree-days in both years. However, the growth parameters such as plant height, number of nodes on main stem and NAWF (nodes above white flower) were significantly higher in seed bacterized plants compared to the untreated control. Our results indicate that seed treatment of cotton plants with the *Pseudomonas* spp. strains and the known strain of *S. plymuthica* can help in the biocontrol of non-defoliating VD in cotton field.

Keywords *Verticillium,* Cotton, Fluorescent *Pseudomonas*, *Serratia plymutica*, Biological control

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1. Introduction

Cotton is a high value crop grown in the western and southern parts of Turkey. In recent years, a substantial increase in organic cotton production has been observed worldwide and Turkey is the first country ranked in the top ten organic cotton producing countries in the world[1]. With 12,507 metric tons of organic cotton production in 2007, Aydin province is the second-largest organic cotton producer in the country[2]. Verticillium wilt, caused by the soil inhabiting fungus *Verticillium dahliae* (Kleb.) is one of the most important diseases responsible for great economic losses in many crops (Tjamos 2000). The disease was first discovered in Manisa province of Turkey in 1941 (Iyriboz 1941) and since then has become widespread in the main cotton growing areas of Turkey (Karaca et al. 1971; Esentepe 1979; Dervis and Bicici 2005). Verticillium wilt disease are difficult to control due to the long viability of the resting structures (microsclerotia), the broad host range of the pathogen and the inability of fungicides to affect the pathogen once the enter the xylem (Fradin and Thomma 2006). Therefore, the most effective control of Verticillium wilt of cotton is the integrated management approaches covering both growing adapted resistant cultivars and using cultural and management practices known to reduce disease severity (El-Zik 1985). However, unavailability of commercially acceptable resistant cotton cultivars, high cost and difficulty in large-scale application of soil solarization and fumigation to reduce microsclerotia in soil makes it necessary to consider other control methods. The use of biological control agents has been increasing worldwide and has been a promising alternative to control soil borne diseases in sustainable and organic agriculture. One of the recent approaches to control Verticillium wilt disease is the rhizobacteria-mediated biological control. Previous studies have shown that antagonistic bacteria can be successfully used to suppress *V. dahliae* ad planta (Leben et al. 1987; Safiyazov et al. 1995; Zhengjun et al. 1996; Berg et al. 2001; Tjamos et al. 2004; Çubukçu and Benlioglu, 2007). A registered biopesticide (RhizoStar®) strain, *Serratia plymutica* (HRO-C48) isolated from the rhizosphere of oilseed rape, successfully controlled Verticillium wilt of strawberry and also increased the yield according to the greenhouse and field trials (Kurze et al. 2001). Recently Mercado-Blanco (2004) suggested that root treatments of olive plants with selected *P.fluorescens* isolates significantly delayed of the onset of Verticillium wilt symptoms and reduced the final disease incidence and severity by 31-82% and 73-96%, respectively, compared with the non-treated controls. The objective of this research was to determine whether fluorescent *Pseudomonas* isolated from cotton and weed rhizosphere could be effective in controlling Verticillium wilt in field.

2. Materials And Methods

2.1. Fungal isolates and pathogenicity tests

Diseased plant samples were collected from random cotton fields representing in 5 different main cotton-growing regions (Aydın Merkez, Çine, Koçarlı, Nazilli and Söke) of Aydin province from July through September in 2004. A total of 32 *V. dahliae* strains were isolated by using potato dextrose agar (PDA) amended with 0.5 g/L of streptomycine sulphate, and identified by the formation of microsclerotia and conidal structures. All *V. dahliae* isolates were sub-cultured on PDA, purified and stored in 30% glycerol at -76°C. Pathogenicity teste were conducted on VD susceptible cotton plants (cv Sayar 314) at six-true leaf stage in 10 cm diameter plastic pots

[1] Organic Cotton Farm and Fiber Report, 2007, Organic Exchange publications, www.organicexchange.org
[2] Organic Agriculture Statistics 2007, Ministry of Agriculture and Rural Affairs, www.tarim.gov.tr

containing a autoclaved soil-sand-peat (1:1:1) mixture. For inoculums preparation, VD isolates were grown on PDA at 24°C in dark for ten days. Conidia were washed once with sterile water and diluted to a concentration of 3 x 10^7 conidia/ml. Plants were wound inoculated by puncturing the stem on the first internodes above the soil line with a 22 gauge needle through a 5-µl drop of the conidial suspension. Plants were incubated at 25± 2°C with a 14-h photoperiod and fertilized once a week with liquid fertilizer (Sheffer 16-8-24 N-P-K). Controls were inoculated with sterile water. Each treatment consisted of 3 plants. Fourteen days after inoculation, disease severity was assessed for each plant on a 0 to 4 rating scale according to the percentage of foliage affected by acropetal chlorosis, necrosis, wilt, and/or defoliation (0=healthy plant, 1=1 to 33%, 2=34-66%, 3=67-97%, 4=dead plant) (Bejarano-Alcazar et al. 1995).

2.2. Bacterial isolates and preliminary screen

Plant rhizosphere samples were collected from the same cotton fields during the same period. Bacteria were isolated from the rhizosphere of symptomless cotton plants and weeds (*Chenapodium album, Solanum nigrum, Xanthium strumarium*, *Datura stramonium*, *Portulaca* sp., *Sinapis* sp., *Convolvulus arvensis,* and *Malva sylvestris*) known to be host of VD (Thanassoullopoulos et al. 1981). The plants were uprooted with rhizosphere soil and transported to the laboratory in polyethylene bags where they were thoroughly washed with tap water. Approximately 5 g root tissue excised from each plant samples, and surface sterilized for 3 min in NaClO (0.5% available chlorine) and rinsed thoroughly in sterile distilled water. Root samples were shaked in 95 ml of sterile 0.05 M phosphate buffer (pH=7.2) for 1 hour at room temperature. Samples were serially diluted with phosphate buffer and plated onto King's medium B agar-KB (King et al. 1954) amended with cycloheximide (100 ppm) and incubated at 24°C for 48 h. Single fluorescent colonies were purified and stored in 30% glycerol at -76°C until needed.

Fifteen out of 59 bacterial isolates were selected for *in vivo* biological control and growth promotion tests after screening for their ability to produce antifungal substances against VD by a dual-culture *in vitro* assay on PDA plates. Each plate was inoculated with four droplets of 10 µl bacterial suspension (at a conc. of 10^8 cell/ml) symmetrically placed on four sites at equal distances (2 cm) from the center of plate. After 24 hours incubation at 24 °C, a single 5-mm-diameter mycelial disc was placed in the center. As a control, a disc of *V. dahliae* was grown on a PDA plate. The radius of each fungal colony was measured after incubating 10 d at 24° C in darkness and the relative growth inhibition was expressed as [(treatment-control)/control*100]. The isolates caused significant inhibition were also examined for hypersensitive reaction on White Burley tobacco leaves.

2.3. Biological control of Verticillium wilt in the greenhouse

The effects of fifteen fluorescent pseudomonas isolates against Verticillium wilt were tested in growth room on two cultivars of cotton plants, one which is a VD sensitive local variety cv Sayar 314 (Göre et al. 2008) and the other which is a VD tolerant Upland cultivar Acala Maxxa (Bölek et al. 2005). Acid delinted cotton seeds were treated with antagonistic bacteria after surface disinfestation with 1% NaOCl for 1 min and followed by three washes in sterile 0.05 M phosphate buffer (PB, pH:7.4). The antagonistic bacteria were grown for 24 h at 25 °C on Tryptic soy broth (Difco) and pelleted by centrifugation (5000 g, 5 min, 4°C). After washing with PB, the pellets were resuspended in 1.5% carboxy-methyl-cellulose (CMC) solution. Seeds were soaked for 30 min in a bacterial suspension (100 mg bacteria and 1 ml of %1.5 CMC solution per 20 seeds) or in the same volume of CMC without bacteria (the control treatment)

and then dried under a laminar flow hood (Quadt-Hallmann at al. 1997). The seed bacterization procedure resulted in mean bacterial concentrations of 10^8 cfu/seed as determined by dilution plating after 48 h. The bacterized and control seeds were planted into each 10 cm diameter plastic pots (400 ml) containing an autoclaved soil-sand-peat (1:1:1) mixture and each treatment were replicated five times. Plant growth conditions, inoculations and evaluations were performed as above described pathogenicity tests.

2.4. Field trials

The experiments were conducted to determine the ability of *Pseudomonas* spp. isolates (FP22, FP23, FP30 and FP35) and *Serratia plymuthica* (strain HRO-C48, kindly provided by Gabriel Berg, University of Rostock, Germany) to suppress verticillium wilt symptoms and to increase cotton yield in field conditions. During 2005 and 2006, the experiments were carried out in a naturally infested field with non-defoliant pathotype of *V. dahliae* (inoculum density=10-14 p/g soil), which has been repeatedly used for cotton breeding field trials at Nazilli Cotton Research Institute since 1972. In experimental field, the soil texture was clay-sandy, pH=7.85, 1.94% organic matter, 18.41% lime and 0.11% salt, 603.7 ppm K_2O and 6.9 ppm P_2O_5. Each year 60 kg of N and 60 kg of P_2O_5 per hectare were incorporated with a harrow before cotton planting. The same amount of nitrogen was also applied in between rows just before flowering, and two sprays were made to control cotton pests like aphids and red spider mites.

Treatments were applied in plots 0.7 m wide (two rows) by 12 m long randomized complete block design (at 2 m distance between blocks) with four replicates. The delinted seeds of Sayar-314 and Acala Maxxa were bacterized with each of 5 bacterial strains as described previously. The number of living bacteria per seed for each treatment was determined by dilution plating after 48 h from seed bacterization procedure. The mean number of bacteria ranged from $5x10^6$ to $1.0x10^7$ cfu/seed in 2005, and from $1.1x10^7$ to $1.4x10^7$ cfu/seed in 2006 treatments.

Verticillium wilt in each sampled plot was assessed three times each year during the period beginning on early September and ending mid October, in the 5-10%, 50-60% and 75% of the bolls open stage. At each recording date, each individual plant was examined for the foliar symptoms of verticillium wilt and the diseasse severity was estimated for each plant by using the same scale as previously described and the incidence (%) of infected plants was then determined for each plot. A disease severity index (DSI) was calculated for each plot by: (mean severity X incidence%) / maximum severity rating. For DSI, the area under the DSI progress curve (AUDPC) was calculated by trapezoidal integration (between day 0 and final disease assessment day) for each plot (Campbell and Madden, 1990), and standardized (relative) AUDPC values were calculated for comparison of the results between two years.

Growths parameters, such as number of main stem nodes, NAWF (nodes above white flower) and plant height were also recorded on randomly ten selected plants from each experimental plot. Number of nodes and NAFW were determined 2 or 3 days before the first disease rating at the 5-10 % bolls open stage (8 September 2005 and 9 September 2006). When counting the number of nodes, the cotyledonal node was counted as “0”. To determine NAWF, the number of nodes down from the terminal (terminal is 0) to the first white flower was counted. Plant height was measured one week after the last disease rating at the 75% of bolls open stage. At the end of the growing season (7 November 2005 and 21 November 2006), seed cotton yield was determined by hand harvesting from each plot.

Maximum and minimum air temperatures were obtained from data logger (Hobo, Onset Computer, USA) located in the experimental field during the vegetation period of cotton. Physiological time was determined as an accumulation of temperatures above 11.9°C (the

developmental threshold temperature for cotton growth) and expressed in units of Celsius degree-day. Degree-days were calculated by using a computer program that integrated the area under sine curves passing through the daily maximum and minimum air temperatures and above the developmental threshold (Gutierrez et al. 1975).

3. Results

A total of 32 *V. dahliae* isolates were obtained from main cotton growing towns (Aydın Merkez, Çine, Koçarlı, Nazilli and Söke,) of Aydın province. Among the isolates of *V. dahliae* two collected from Nazilli and Söke (VD-11 and VD-12) caused more than 70% disease severity (disease index=3) on cotton plants (cv Sayar 314). The most virulent *V. dahliae* isolate, VD-11 was used for further bioassays. Fifty nine fluorescent *Pseudomonas* isolates were isolated from roots, 18 from cotton, 5 from each of following weeds: *Chenopodium album, Solanum nigrum, Xanthium strumarium*, *Datura stramonium*, *Portulaca* sp., *Sinapis* sp., *Convolvulus arvensis,* and *Malva sylvestris*, and 1 from *Echinochloa colonum*. Fifteen out of fifty nine isolates showed high *in vitro* inhibitory activity (>40%) toward *V.dahliae* (VD-11) in dual culture test on PDA plates (Table 1). The *in vivo* effect of 15 fluorescent *Pseudomonas* isolates on *V. dahliae* (VD-11) in susceptible and tolerant cotton varieties is overviewed in Table 2. None of fifteen selected isolates induced a hypersensitive response on tobacco leaves.

Table 1. Effects of Fluorescent *Pseudomonas* on *in vitro* inhibition of *V.dahliae* hyphal growth and development of Verticillium wilt on two cotton cultivars

Isolate	Origin	*In vitro* inhibition (%)	Disease index[y]	
			Sayar 314	Acala maxxa
FP1	*Chenopodium album*	48.5	3.0 bc	3.0 bc
FP5	*Portulaca sp.*	56.0	3.0 bc	3.0 bc
FP11	*Portulaca sp.*	50.0	3.0 bc	3.0 bc
FP12	*Gossypium hirsitum* (cv BA 119)	43.9	3.3 cd	3.0 bc
FP15	*Solanum nigrum*	53.0	3.0 bc	3.0 bc
FP18	*Gossypium hirsitum* (cv Carmen)	45.0	3.7 de	3.0 bc
FP21	*Solanum nigrum*	53.0	3.3 cd	3.0 bc
FP22	*Xanthium strumarium*	48.5	3.0 bc	2.0 a
FP23	*Portulaca* sp.	43.9	3.0 bc	2.0 a
FP25	*Chenopodium album*	43.9	3.0 bc	3.0 bc
FP29	*Portulaca sp.*	53.0	3.0 bc	3.0 bc
FP30	*Gossypium hirsitum* (cv Carmen)	43.9	2.7 b	2.0 a
FP35	*Convolvulus arvensis*	56.0	2.0 a	2.0 a
FP39	*Sinapis* sp.	50.0	3.7 de	3.0 bc
FP53	*Gossypium hirsitum* (cv Giza 45)	40.1	3.7 de	3.0 bc
Control		00.0	4.0 e	3.0 bc
Interaction cultivar x isolate			***	

[y]Disease index was assessed 14 days after inoculation by using 0-to-4 rating scale; In each column, mean values followed by the same letter are not significantly different according to Fisher's protected least significant differences at P=0.05.

*** Significant at the 0.0001 probability level

Based on statistical analysis of *in vivo* assay data, the effect of fluorescent *Pseudomonas* isolates under test on Verticillium wilt changed dependent on two cotton cultivars. The treatment of seeds with isolates FP22, FP23, FP30 and FP35 were significantly reduced symptom expression in artificially inoculated VD tolerant cotton plants (cv Acala Maxxa) while only two isolates FP30 and FP35 significantly reduced the symptom development in VD susceptible plants (cv Sayar 314). Thus, these four isolates were used for field trials.

3.1. Field Trials

Evidence of Verticillium wilt was apparent in all experimental plots in both 2005 and 2006. First symptoms consisting chlorotic areas between the main veins of the lower leaves appeared after 90-94 days when the physiological time reached to 1291/1326 degree-days in 2005 and 1324 in 2006 (Table 2).

Table 2. Some growth parameters of cotton and cumulative degree-days after sawing in 2005 and 2006

Events	Days after planting		Physiological time-Degree days	
	2005	2006	2005	2006
	Sayar/A.Maxxa	Sayar/A.Maxxa	Sayar/A.Maxxa	Sayar/A.Maxxa
First square	49/51	51/53	554/583	667/706
First bloom	68/70	70/72	859/894	978/1013
First symptom on leaves	92/94	90	1291/1326	1324
Recording number of nodes	127	123	1843	1871
Recording NAWF	128	124	1857	1886
5-10 % bolls open stage (I Count)	131	127	1895	1928
50-60 % bolls open stage (II Count)	146	140	2086	2079
75 % bolls open stage (III Count)	156	149	2184	2187
Measuring Plant height	160	160	2217	2292
Harvest	187	196	2337	2428

Seed bacterization with four fluorescent *Pseudomonas* isolates and *Serratia plymutica* (HRO-C48) resulted in significant reduction in AUDPC both susceptible (Sayar 314) and tolerant (Acala Maxxa) cultivar compared to the non-bacterized control in 2005 and 2006 (Table 3). There was a significant interaction ($P<0.0001$) between treatments and cultivar, and the tolerant cultivar had significantly lower AUDPC values then the susceptible one in both experimental years.

In both years, growth parameters (plant height, number of nodes and NAWF) and yield (except 2006) were significantly affected by the treatments under disease pressure, but the cultivar x treatments interaction was not statistically significant (Table 4). When recorded about 33 days after from the first wilting symptoms appeared on leaves, the number of nodes on main stem was significantly higher for the treatments (FP22, FP23, FP35 in 2005 and FP30, FP22, HRO-C48 in 2006) compared to the untreated control. Similarly, the plants in HRO-C48, FP22, and FP30 treated plots in 2005, and in FP22, FP23, FP35, and HRO-C48 treated plots had significantly higher heights compared to untreated control when they were measured 26 days before harvest. NAWF data, recorded 5 weeks later from the first symptom appearance, indicated that the mean values were slightly higher than NAWF=5 which was generally accepted as physiological cut out (Bourland et al., 2001). However, the mean NAWF values for all treatments were significantly higher than those of untreated control while there was no significant difference between two cultivars in 2005 and 2006. Plants were hand harvested at 2337 and 2438 degree-days from sowing in 2005 and 2006, respectively. Seed cotton yield significantly increased by seed bacterization compared to untreated control, but no statistical differences between cultivars observed in 2005. However, there was no statistical differences occurred among any of the treatments (including untreated controls) in 2006.

Table 3. Effects of Fluorescent *Pseudomonas* on Verticillium wilt on two cotton cultivars in 2005 and 2006

		Relative AUDPC[y]	
Cultivar	Treatments	Year	
		2005	2006
Sayar 314			
	FP22	4.50 cd	4.33 d
	FP23	4.54 c	4.36 cd
	FP30	5.09 bc	4.91 bc
	FP35	5.57 b	5.36 b
	HRO-C48	5.46 b	5.26 b
	Control	9.18 a	8.84 a
Acala Maxxa			
	FP22	2.31 e	2.65 ef
	FP23	2.31 e	2.82 ef
	FP30	2.29 e	2.68 ef
	FP35	2.76 e	2.58 f
	HRO-C48	2.34 e	2.57 f
	Control	3.88 d	3.22 e
ANOVA			
Treatments		***	***
Cultivar		***	***
Treatments x Cultivar		***	***

[y]Area under disease severity index (DSI) progress curve, DSI was calculated for each plot by: (mean severity x incidence%) / maximum severity rating after assessing at the 5-10% (12/9/2005 and 2006), 50-60% (28/9/2005 and 26/9/2006) and 75% (7/10/2005 and 5/10/2006) of the bolls open stage. Relative AUDPC were obtained by dividing AUDPC values by total observation days in 2005 and 2006. In each column, mean values followed by the same letter are not significantly different according to Fisher's protected least significant difference at P=0.05.
*** Significant at the 0.0001 probability level

Table 4. Effects of seed bacterization on cotton growth parameters and seed cotton yield in 2005 and 2006

Cultivar	Treatments	2005				2006			
		Plant Height (cm)	Number of nodes	NAWF	Yield kg/ha	Plant Height (cm)	Number of nodes	NAWF	Yield kg/ha
Sayar 314		77.6 a	10.6	5.78	3838	90.5 a	10.3	5.81	3265
Acala Maxxa		69.7 b	10.6	5.77	3736	80.4 b	10.5	5.89	3147
	FP22	76.2 ab	11.0 a	5.87 a	3964 a	87.0 a	10.6 ab	5.87 a	3196
	FP23	71.6 bc	10.7 ab	6.01 a	3853 a	86.2 a	10.3 b	5.87 a	3222
	FP30	75.8 ab	10.4 bc	5.87 a	3829 a	84.7 ab	10.8 a	5.87 a	3226
	FP35	72.4 abc	10.9 ab	5.89 a	3811 a	87.7 a	10.3 b	5.94 a	3195
	HRO-C48	77.1 a	10.5 abc	5.80 a	3897 a	85.4 a	10.4 ab	6.05 a	3267
	Control	69.1 c	10.0 c	5.20 b	3376 b	81.7 b	09.8 c	5.49 b	3130
ANOVA									
Cultivar		***	NS	NS	NS	***	NS	NS	NS
Treatments		*	**	***	***	**	***	***	NS
Cultivar x treatments		NS	NS	NS	NS	NS	NS	NS	NS

In each column, mean values followed by the same letter are not significantly different according to Fisher's protected least significant difference.
* Significant at the 0.05, ** at the 0.001, ***at the 0.0001 probability level, NS not signuficant

4. Discussion

Pseudomonads are the most diverse and ecologically significant group of bacteria in the planet (Spiers et al. 2000). These bacteria have considerable potential for element cycling and the degradation of biogenic and xenobiotic pollutants (Timmis 2002), bioremediation (Dejonghe et al. 2001), biocatalysis (Schmid et al. 2001), as well as biocontrol agents in plant protection (Walsh et al, 2001; Haas and Defago, 2005). As so mentioned, our potential biocontrol *Pseudomonas* strains FP22, FP23, FP30 and FP35 were isolated from *Xanthium strumarium*, *Portulaca* sp. *Gossypium hirsitum* (cv Carmen) and *Convolvulus arvensis,* respectively. Berg et al. (2001) developed a screening approach to select three potential biocontrol candidates *Pseudomonas putida* BE2 (strawberry rhizosphere), *P. chlororaphis* K15 (potato rhizosphere) and *Serratia plymuthica* R12 (oilseed rape rhizosphere) to control Verticillium wilt caused by VD. Four out of 15 fluorescent *Pseudomonas* strains originated from different plants rhizosphere significantly reduced symptom expression under greenhouse conditions. This reduction occurred even when the pathogen inoculated directly into the stems of cotton plants grown from bacterized seeds. During natural infection, infectious hyphae emerging from microsclerotia directly penetrates through the roots (El-zik 1985); thus the bacteria would be expected to influence the infection of VD penetrating the roots. Therefore, many researchers conducted *in vivo* trials with rhizobacteria by using artificially inoculated soil with microsclerotia (Leben et al. 1987; Berg et al. 2001; Mercado-Blanco et al. 2004; Tjamos et al. 2004) on cotton and other crops. However, Chen et al. (1995) reported that six endophytic bacteria (including two *Pseudomonas putida* strain) reduced the disease severity on cotton bacterized at the 7-day seedling stage when inoculated by stem injection with microconidia of *Fusarium oxysporum* f. sp *vasinfectum.* Furthermore, the authors reported that two of the five strains have limited movement abilitiy within the stem, not exceeding 5.0 cm, 14 days after bacterization, and they suggested that induced systemic resistance might be an important factor in biological control with endophytes. There are several known mechanisms by which fluorescent *Pseudomonas* could control soil-borne pathogens (Haas and Defago, 2005). Further studies could expand our knowledge on how our pseudomonads strains could control Verticillium wilt of cotton.

During 2005 and 2006 trial, seed bacterization with four fluorescent *Pseudomonas* strains and HRO-C48 significantly reduced Verticillium wilt symptoms of both wilt-susceptible and wilt-tolerant cotton cultivars under field conditions. HRO-C48 (*Serratia plymuthica*), a rhizosphere-associated bacterium originally isolated from oilseed rape, was applied to protect strawberry roots against VD and become a registered product named RhizoStar® (Müller and Berg 2008).

Although AUDPC (DSI) values were significantly influenced by seed bacterization, cultivar and their interaction in both years, this effect was slightly reflected in the seed cotton yield responses. Only in 2005 trials were the seed cotton yield significantly higher than the untreated control. The overall data indicated that seed cotton yield was not significantly influenced by the cultivar and its interaction with seed treatments. The major effect of Verticillium wilt on cotton plants was the inhibition of plant growth and development. Pulman and DeVay (1982) reported that cotton lint reductions due to the Verticillium wilt were small when foliar symptoms after mid August (approximately 2500 degree days F° = 1387 degree days C°). Similarly, Bejarano-Alcazar et al., (1997) concluded that the greatest yield reduction was observed in plants showing symptoms before opening of first flowers (about 650 degree-days after sowing) and the effect of wilt epidemics on yield was small or nil for plants that developed symptoms after opening of the first balls (1400-1500 degree-days after sawing). Lower yield reduction in our field trials could be

attributed to the first symptom appearance after 1291/1326 and 1324 degree-days in 2005 and 2006 respectively. However, the growth parameters such as plant height, number of nodes on main stem and NAWF were significantly higher in seed bacterized plants compared to the untreated control. Pulman and De Vay (1982) found that similar reductions in plant height during 1976 to 1980 trials and concluded that the earlier foliar symptoms appeared, the greater was the reduction in plant height.

Our study demonstrates the potential of some fluorescent *Pseudomonas* from weed and cotton rhizosphere and a known strain of *S. plymuthica* (HRO-C48) as effective biocontrol agents against non-defoliating VD in cotton field. As far as our knowledge this is the first study in our country to show biological control of Verticillium wilt of cotton under field conditions. Organic cotton production tends to expand in order to meet the growing market demand, and become a viable and convincing alternative to conventional cotton production in cotton producing countries as well as Turkey. However, field trials in combination with different cotton cultivars to biologically control defoliating pathotype of VD which has been recently reported in cotton growing areas of Aegean region in Turkey (Göre, 2007), efficient formulation procedure on the effect of our bacteria as in *S. plymuthica* (Müller & Berg, 2008), as well as studies on the mechanisms underlying disease suppression are in the main goals of future research.

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