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Bacterial wilt of tomato in Karnataka and its management by *Pseudomonas fluorescens*

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Abstract Field surveys undertaken in major tomato growing districts of the Karnataka state, located in southern part of India, revealed a high incidence of bacterial wilt caused by *Ralstonia solanacearum* and it is one of the most destructive bacterial diseases of economically important crops. Across all the tomato cultivars under evaluation, the disease incidence in plants ranged from 9% to 39% whereas the incidence in seeds ranged from 4% to 18%. The effects of tomato seed treatments with *Pseudomonas fluorescens* in the control of bacterial wilt under greenhouse conditions revealed that the treatments protected plants against soil-borne infections of the bacterial wilt organism. Seed treatment with antagonistic *P. fluorescens* strain significantly improved the quality of seed germination and seedling vigour. The disease incidence was significantly reduced in plants raised from *P. fluorescens* treated seeds followed by challenge inoculation with *R. solanacearum.* Periodic field surveys for the incidence of bacterial wilt of tomato could be recommended to monitor the populations of the bacterial wilt pathogen. Workable measures are presented that could lead to the reduction of the prevalence of this serious disease in affected fields of the small farm-holders.

Keywords Bacterial wilt · Tomato · *Ralstonia solanacearum* · Management · *Pseudomonas fluorescens*

Introduction

Bacterial wilt caused by the soil-borne plant pathogen *Ralstonia solanacearum* (Smith 1896) is one of the most devastating bacterial plant diseases in the tropical and subtropical regions of the world. *Ralstonia solanacearum* gained its importance in the world due to its destructive nature, wide host range and geographical distribution. It affects a wide range of economically important crops such as tomato, potato, eggplant, chilli and non-solanaceous crops such as banana and groundnut in India. The bacterial wilt symptoms in tomato are characterised by initial wilting of upper leaves and within a few days followed by complete wilting of the plants. The

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vascular tissues of the infected stem have brown discoloration and, if the stem is cut crosswise, white or yellowish bacterial ooze may be visible.

Management of bacterial wilt in tomato and in other crops has been difficult. The disease still threatens commercial tomato production, even though integrated management including cultural practices, crop rotation and use of resistant cultivars provides limited success. Some bacterial wilt resistant cultivars have been developed from the Asian Vegetable Research and Development Center (AVRDC); however, their resistance is restricted to locations, climate, and strains of the pathogen and soil characteristics. Even if the pathogen population is suppressed by crop rotation with non-host plants, it can survive in weed hosts, weakening the effect of crop rotation. Chemicals may be an effective method in controlling many bacterial diseases, but these chemicals potentially cause negative impact on plant growth or yield. Moreover, antibiotics such as streptomycin, ampicillin, tetracycline and penicillin hardly showed any effect; in fact, streptomycin application increased the incidence of bacterial wilt in Egypt (Farag et al. 1986).

Biological control has become known to have a high impact on the management of soil-borne plant pathogens. Biological control makes management of diseases less dependent on the use of high-risk chemicals and it is environmentally friendly. Fluorescent pseudomonads are among the most effective rhizosphere bacteria used to suppress diseases caused by soil-borne plant pathogens. These bacteria can antagonise soil-borne pathogens through various direct and indirect modes of action i.e. directly through production of antimicrobial substances, competition for space, nutrients and indirectly through induction of systemic resistance. The use of fluorescent pseudomonads in controlling soil-borne plant diseases has been well documented. Efforts have also been made to use bacterial antagonists in the management of bacterial wilt of tomato (Ciampi-Panno et al. 1989).

Pseudomonas fluorescens is one of the most important biocontrol agents against certain seed and soil-borne plant pathogens. Positive results were achieved with *P. fluorescens*, which controlled bacterial wilt and also bacterial blight on potato in both field and laboratory trials (Ciampi-Panno et al. 1989). *P. fluorescens* was also used as a biocontrol agent to manage bacterial wilt of tobacco (Liu et al. 1999), *Fusarium* wilt in radish (Leeman et al. 1995), cucumber (Liu et al. 1999), *Sclerospora graminicola* in pearl millet (Umesha et al. 1998), *Xanthomonas oryzae* pv. *oryzae* in rice (Vidhyasekaran et al. 2001), *Eucalyptus* wilt (Ran et al. 2005), *R. solanacearum* in Chilli (Umesha et al. 2005) and *Clavibacter michiganensis* ssp. *michiganensis* (Umesha 2006). *P. fluorescens* also improved seed quality under laboratory conditions and drastically reduced bacterial spot disease in field conditions (Kavitha and Umesha 2007). Attempts have also been made under greenhouse studies and field conditions alike to show the efficacy of *P. fluorescens* in the management of plant diseases (Jayashree et al. 2000; Vidhyasekaran et al. 2001; Umesha 2006; Kavitha and Umesha 2007).

Ralstonia solanacearum mostly persists through soil and crop residues (Granada and Sequeira 1983). In crops such as tomato and eggplant, the pathogen is carried in seed (Shakya 1993). Tomato (*Solanum lycopersicum* Mill.) is considered as one of the most widely grown vegetable crops in the world. In India, it is grown over an area of 0.497 m ha (mh) with a production of 17.35 million tones (mt) and with the productivity of 8.63 mt h^{-1} (FAO 2006). Since wilt symptoms were noticed in tomato crops grown around Mysore and Bangalore area, the present studies were conducted with the following objectives: To survey fields in major tomato growing districts of the Karnataka state for bacterial wilt and to determine the effect of biological seed treatment on bacterial wilt incidence under greenhouse conditions.

Materials and methods

Field surveys

Field surveys were conducted to determine the prevalence of bacterial wilt in tomato field plants and in seed samples from the major tomato growing districts of Karnataka, India. A survey was conducted during September 2005, March 2006 and October 2007 around Bangalore, Doddaballapur, Chikkaballapur, Kolar and Mysore districts. The tomato plants in the 31 fields were inspected at the nursery stage, after transplanting, at flowering stage and at the fruiting stage. Wilt incidence was estimated among the randomly selected subplots (10 subplots ha^{-1},

measuring 1 m^2). The total number of healthy and wilted plants was counted in a 1 m^2 area and percent wilt incidence was recorded. Information on the cultivars grown in the area and related field history was gathered from the farmers. The plants were observed for the typical symptoms of bacterial wilt viz., leaf yellowing, wilting and vascular browning.

Detection of populations of *R. solanacearum* in the leaves and seeds of tomato plants

The suspected plant material, soil samples and fruits were collected from the field survey, brought to the laboratory and tested for the presence of bacterium. Prior to isolation of the target pathogen from the diseased plants, observations were conducted for bacterial ooze secreted from sections of plant parts. Collected plant materials were surface sterilization with 70% ethyl alcohol followed by three repeated washings with distilled water and blot-dried. Then the plant parts ($\approx$0.5–1 cm) were plated onto 2, 3, 5 Triphenyl tetrazolium chloride (Kelman's TZC agar) medium (glucose 10 g, peptone 10 g, casein hydrolysate 1 g, agar 18 g, distilled water 1,000 ml, 5 ml of TZC solution filter sterilized was added to the autoclaved medium to give final concentration of 0.005%) (Kelman 1954). The virulent (white fluidal irregular colonies with pink centre) colonies of *R. solanacearum* were recorded from the inoculated plates. The seed samples of 20 different tomato cultivars procured from local seed agencies were subjected to screening in the laboratory followed by direct plating method onto TZC agar medium. Collected seed samples were plated directly onto the TZC agar medium after surface sterilization with 70% ethyl alcohol followed by three repeated washings with distilled water and blot-dried. Plates were incubated at 28 $\pm$ 2°C for 24–48 h. White fluidal colonies with pink centres around the pieces of plant material and seeds were observed, sub-cultured onto the TZC media, and suspected colonies were subjected to different biochemical, physiological, hypersensitive and pathogenicity tests for confirmation of the identity of the pathogen. Isolated bacteria were then streaked onto nutrient agar (NA) media and 24–48 h old cultures were used as and when required. Experiments were conducted with four replicates of 100 pieces/seeds each and repeated in three consecutive seasons.

Characterisation of the bacterial wilt pathogen was carried out by subjecting the isolated bacterial colonies to various biochemical tests; Gram's staining, KOH solubility test, Kovacs' oxidase test (Kovacs 1956; Hildebrand and Senroth 1972), levan formation, gelatin hydrolysis, starch hydrolysis, nitrate reduction (Fahy and Persley 1983) and arginine dihydrolase test (Lelliott and Stead 1987). The strains were also subjected to the hypersensitive reaction test (HR) in tobacco (*Nicotiana tobaccum*) plants (Carlton et al. 1998) and the pathogenicity test (Lelliott and Stead 1987) with the susceptible (cv. Quality). Each test was conducted with four replicates and repeated twice.

Preparation of bacterial pathogen inoculum

Inoculum of *R. solanacearum* (Isolate DABBV1) was prepared by growing cells of the bacterium on Kelman's TZC agar medium for 48 h at 30°C. The bacterial cells were harvested in sterile distilled water by centrifugation (UniCen, 15 DR, Herolab GmbH, Germany) at 12,000 rpm for 10 min. The pellet was resuspended in distilled water and bacterial suspension was adjusted to 0.45 at A_{610} nm using UV–visible spectrophotometer to obtain the concentration of 1×10^8 cfu ml^{-1} (Hitachi U-2000, Japan) (Ran et al. 2005).

Isolation of antagonistic bacteria and preparation of bacterial inoculum

The antagonistic strain of *P. fluorescens* was isolated from native soil (Red loamy) obtained from farmers' fields of the Bangalore, Kolar, Mysore and Mandya districts of the Karnataka state. Soil samples were serially diluted and bacteria were isolated from soil microcosms by adding approximately 1 g of soil to 9 ml of sterile distilled water. The solution was vortexed, allowed to settle for at least 20 min, and then vortexed again. The bacterial fraction was collected from the supernatant following centrifugation at 10,000 rpm and aliquots from serially diluted soil extracts were plated onto King's B medium agar plates (KMB) (King et al. 1956). The isolated *P. fluorescens* strains were further evaluated for their antagonistic activity by the dual culture technique. The identity of the isolate based on inhibition zone with the best antagonistic activity was confirmed by performing various tests specific to *P. fluorescens*

(Stainer et al. 1966). The 48-h old cultures grown on King's medium B broth were centrifuged at 10,000 rpm for 10 min using bench top centrifuge. Inoculum was prepared by adjusting the bacterial concentration with sterile distilled water to 1×10^8 cfu ml^{-1} at A_{610} nm (OD = 0.45) using UV–visible spectrophotometer (Mortensen 1999).

Effects of seed inoculation with *R. solanacearum*

The effect of *R. solanacearum* strains isolated from tomato seeds on seed germination and vigour of seedlings was evaluated under laboratory conditions. Healthy seeds of the three tomato cultivars Golden, Rasi and Quality obtained from local seed agencies were treated with *R. solanacearum* pure culture suspensions at the rate of 1×10^8 cfu ml^{-1} and shaken continuously for 12 h. Seeds treated with distilled water in a similar manner served as negative controls. The treated and untreated seeds were air-dried, plated on wet blotters and subjected to germination tests (ISTA 2003). The vigour index was calculated using the formula (Mean root length + Mean shoot length) × percent germination (Abdul Baki and Anderson 1973). The experiment was carried out with four replicates of 100 seeds each and repeated twice.

Effect of *P. fluorescens* on tomato seed quality

Seeds of the three tomato cultivars Golden, Rasi and Quality procured from local seed agencies were treated with *P. fluorescens* isolate DABBV4 pure culture suspensions at the rate of 1×10^8 cfu ml^{-1} and shaken continuously for 12 h. Distilled water treated seed samples served as negative control. After 12 h, treated and untreated seeds were air-dried, plated on wet blotters and germination rates and vigor index were determined as explained above. The experiment was carried out with four replicates of 100 seeds each and repeated twice.

Effect of *P. fluorescens* on bacterial wilt incidence

Individual tomato seed samples (400 seeds) from the twenty cultivars were treated with a suspension of *P. fluorescens* isolate DABBV4 (1×10^8 cfu ml^{-1}) for 12 h under shaking. Another set of tomato seed samples from each variety were soaked in distilled water, which served as control and seeds were sown into pots (9 cm diameter) containing soil, sand and farmyard manure at 2:1:1 proportion. The pots were maintained under greenhouse conditions where day/night cycle of 16/8 h and 28/30°C and 65% relative humidity was maintained. Fifteen day-old seedlings were challenge inoculated with a bacterial suspension of *R. solanacearum* by the soil-soak method (Tans-Kersten et al. 2001). Plants were closely observed for symptoms of bacterial wilt following the inoculations with the bacterial pathogen. The disease incidence was recorded up to 30 days after pathogen inoculation. Bacterial wilt was readily diagnosed by macroscopic exudation of bacterial ooze from a cut surface from the infected stem sections or by the bacterial streaming from vascular tissue observations under the microscope. Sections of plant leaflets showing symptoms were surface sterilization with 70% ethyl alcohol followed by three repeated washings with distilled water and blot-dried and plated onto TZC agar medium plates. The isolated bacteria showing morphological characteristics of the virulent strains of *R. solanacearum* were further characterised by various biochemical, physiological, HR and pathogenicity tests (Carlton et al. 1998; Tans-Kersten et al. 2001). The experiments were conducted in three replicates and were repeated thrice.

Statistical analysis

Statistical significance was measured by using the data of the percentage of affected plants and transformed to arcsine square root equivalents prior to a two-way analysis of variance (ANOVA). Means were separated using Duncan's multiple range test (DMRT; $P = 0.05$) using SPSS software.

Results

Field survey

The farmers' fields surveyed in different districts of Karnataka, India for bacterial wilt incidence of tomato are depicted in Fig. 1. In the farmers' fields surveyed, Allrounder and Avinash were the most popularly grown tomato cultivars in all seasons. The mean bacterial wilt incidence ranged up to 39%.

Fig. 1 Karnataka state map showing different districts and the location of the current studies are marked (▨). Field survey for the bacterial wilt incidence was carried out for three consecutive seasons

Disease incidence was 34% in Mysore and up to 9% in Mandya district with Allrounder cultivar in all the seasons. The incidence of bacterial wilt was up to 24% and 39% in Avinash and Allrounder cultivars in Bangalore and Kolar districts in all the surveyed growing seasons.

Detection of populations of *R. solanacearum*

Colonies of virulent strains of *R. solanacearum* that were white fluidal with pink centres were commonly recovered from leaves, stems and soil onto TZC agar medium. The bacterial wilt pathogen was commonly isolated from the infected plant parts and seeds tested. None of the seeds of the tomato cultivars tested appeared to be free from *R. solanacearum*. The incidence ranged from 4% to 18% across all the tomato cultivars. Cultivar Golden recorded a minimum (4%) incidence, the cultivars Rasi and Quality recorded 12% and 18% of *R. solanacearum* incidence, respectively, (Table 1).

When the stem and roots of plants showing bacterial wilt symptoms were cut open, brown discoloration was observed in the vascular system. Milky white bacterial ooze was observed when plant material was placed in water. Healthy plant material did not show any sign of discoloration or bacterial ooze. The results of physiological and biochemical tests used in the identification of the pathogen are shown in Table 2. Necrosis was observed in tobacco plants, within 24 h of infiltration with bacterial cells, whereas sterile distilled water in filtered leaf regions did not show any change in the leaf colour, which served as a control. Tomato plants inoculated with the suspected *R. solanacearum* isolates showed bacterial wilt symptoms. Control plants inoculated with sterile distilled water did not show any symptoms (Table 2). No attempts were made for further characterisation of the strains.

Table 1 Screening of seeds of tomato cultivars grown in districts of the Karnataka state for the presence of *Ralstonia solanacearum*

Tomato cultivars	Incidence of *R. solanacearum* (%)*
Golden	4 ± 0.5^{h}
Nautican	6 ± 0.3^{h}
ECL	8 ± 0.4^{gh}
Ujwala	11 ± 0.5^{def}
Lakshmi	12 ± 0.5^{cde}
Higeo	14 ± 0.6^{bcd}
Arunodaya	18 ± 0.7^{a}
OK	10 ± 0.5^{efg}
Indosem	9 ± 0.6^{fg}
Leadbeter	11 ± 0.4^{def}
PHS	13 ± 0.8^{cde}
Sree chakra	15 ± 0.5^{b}
Rasi	12 ± 0.4^{cde}
Allrounder	16 ± 0.5^{ab}
Ashoka	8 ± 0.7^{gh}
Malini	10 ± 0.5^{efg}
Sulthan	12 ± 0.2^{cde}
Avinash	11 ± 0.3^{def}
Solar	17 ± 0.6^{a}
Quality	18 ± 0.5^{a}

* Results of direct plating method, tomato seeds were surface sterilized and plated on TZC agar media and incubated at 28 ± 2°C for 48 h. Seeds showing white fluidal colonies with pink centre appearance were screened. Values are the means ± SE of four replicates of 100 seeds each and repeated thrice. The values in the column followed by the same letter(s) are not significantly different according to analysis of variance (DMRT; $P = 0.05$)

Table 2 Characterisation tests of *Ralstonia solanacearum*

Biochemical tests	Results
Gram's staining	−
KOH solubility	+
Kovacs' oxidase test	+
Levan formation	−
Gelatin hydrolysis	W
Starch hydrolysis	−
Nitrate reduction	+
Arginine dihydrolase	−
Tobacco hypersensitive reaction	+
Pathogenicity test	+

All tests were conducted in four replicates and were repeated thrice; '+' indicates positive reaction; '−' indicates negative reaction and 'W' indicates weak reaction

Effect of *R. solanacearum* on tomato seed quality

There was a reduction in the seed germination of tomato seeds upon *R. solanacearum* inoculation. Seed germination in cultivar Golden was reduced significantly ($F = 407.64$, $df = 5, 12$, $P < 0.0001$) from 72% in the untreated control to 40% when the seeds were treated with *R. solanacearum*. A similar trend was reflected in the other two tomato cultivars. The mean shoot and root lengths were also significantly ($F = 9.98$, $df = 5, 12$, $P < 0.001$ and $F = 32.40$, $df = 5, 12$, $P < 0.0001$, respectively) reduced upon pathogen treatment, which was reflected in the vigour index. The vigour index was significantly ($F = 80.18$, $df = 5, 12$, $P < 0.0001$) reduced from 705 in control to 196 when seeds were treated to *R. solanacearum* in the Golden cultivar. The same trend was noticed with the other two tomato cultivars (Table 3).

Effect of *P. fluorescens* on tomato seed quality

There was an improvement in seed germination when tomato seeds were treated with *P. fluorescens* pure culture suspensions (Table 4). Seed germination in the cultivar Golden increased significantly ($F = 42.11$, $df = 5, 12$, $P < 0.0001$) from 78% in the untreated control to 89% when the seeds were treated with *P. fluorescens*. The mean shoot and root lengths were also increased significantly ($F = 33.80$, $df = 5, 12$, $P < 0.0001$ and $F = 10.90$, $df = 5, 12$, $P < 0.0001$, respectively) with *P. fluorescens* treatment. The vigor index was increased significantly ($F = 55.46$, $df = 5, 12$, $P < 0.0001$) from 748 in the untreated control to 1,237 when the seeds were treated with *P. fluorescens* in the Golden cultivar (Table 4). Among all the cultivars tested, seed treatments with *P. fluorescens* improved the seed quality parameters (Table 4).

Effect of *P. fluorescens* on bacterial wilt incidence

Bacterial wilt incidence was reduced significantly ($F = 622.92$, $df = 39, 80$, $P < 0.0001$) in all 20 tomato cultivars tested, in plants raised from seeds treated with *P. fluorescens* followed by challenge inoculation with *R. solanacearum*. The bacterial wilt incidence in the untreated control ranging from 12%

Table 3 Effects of *Ralstonia solanacearum* on tomato seed germination and vigour of seedlings

Tomato cultivars	Germination (%)	MSL (cm)	MRL (cm)	VI
Golden				
Control	72 ± 0.57^{b}	3.9 ± 0.31^{a}	5.9 ± 0.23^{ab}	705 ± 1.45^{a}
Treated	40 ± 0.57^{d}	1.7 ± 0.28^{c}	3.2 ± 0.28^{c}	196 ± 2.60^{c}
Rasi				
Control	79 ± 1.15^{a}	2.8 ± 0.17^{b}	6.4 ± 0.23^{a}	726 ± 42.43^{a}
Treated	44 ± 1.15^{c}	1.8 ± 0.23^{c}	3.0 ± 0.28^{c}	211 ± 8.08^{c}
Quality				
Control	71 ± 0.88^{b}	2.1 ± 0.28^{bc}	5.4 ± 0.34^{b}	532 ± 51.38^{b}
Treated	34 ± 1.15^{e}	1.7 ± 0.34^{c}	2.9 ± 0.28^{c}	156 ± 26.85^{c}

Values are the means (±SE) of three independent experiments of four replicates of 100 seeds each. MRL, mean root length ($F = 32.40$, $df = 5, 12$, $P < 0.0001$); MSL = mean shoot length ($F = 9.98$, $df = 5, 12$, $P < 0.001$); VI, vigor index ($F = 80.18$, $df = 5, 12$, $P < 0.0001$). The values in the column followed by the same letter(s) are not significantly different according to analysis of variance (DMRT)

Table 4 Effect of *Pseudomonas fluorescens* treatments on the germination of tomato seed and seedling vigour

Tomato cultivars	Germination (%)	MSL (cm)	MRL (cm)	VI
Golden				
Control	78 ± 1.15^{cd}	3.5 ± 0.28^{c}	6.1 ± 0.28^{bcd}	748 ± 34^{d}
Treated	89 ± 0.57^{a}	6.1 ± 0.23^{a}	7.8 ± 0.17^{a}	$1{,}237 \pm 13^{a}$
Rasi				
Control	76 ± 1.15^{d}	2.5 ± 0.28^{d}	5.5 ± 0.28^{cd}	608 ± 9^{e}
Treated	85 ± 1.15^{b}	5.7 ± 0.34^{ab}	6.8 ± 0.34^{b}	$1{,}062 \pm 44^{b}$
Quality				
Control	70 ± 1.15^{e}	2.1 ± 0.28^{d}	5.2 ± 0.28^{d}	511 ± 48^{e}
Treated	81 ± 0.88^{c}	5.5 ± 0.28^{ab}	6.2 ± 0.28^{bc}	947 ± 49^{c}

Values are the means (±SE) of three independent experiments of four replicates of 100 seeds each. MRL, mean root length ($F = 10.90$, $df = 5, 12$, $P < 0.0001$); MSL, mean shoot length ($F = 33.80$, $df = 5, 12$, $P < 0.0001$); VI, vigor index ($F = 55.46$, $df = 5, 12$, $P < 0.0001$). The values in the column followed by the same letter(s) are not significantly different according to analysis of variance (DMRT)

to 85% was reduced to 2–30% in the treated cultivars. In the Golden cultivar the disease incidence was reduced from 12% to 2%. In the cultivars Rasi and Quality, a reductions from 52% and 85% to 20% and 30%, respectively were recorded (Fig. 2).

Discussion

Field survey in the three consecutive seasons revealed the widespread nature and high incidence of bacterial wilt in the major tomato growing regions of Karnataka. The bacterial wilt pathogen *R. solanacearum* was commonly associated to diseased plants and seeds as well as to soil samples taken in the affected fields. The high incidence of bacterial wilt indicates that bacterial wilt is a recurrent problem in the Karnataka state and that all popular varieties appeared to be susceptible to the disease. The incidence of the disease could in fact be higher as no attempts were made to isolate the pathogen from plants without symptoms. Assessment of the presence of *R. solanacearum* in plants merely by scoring of disease symptoms and CFUs often gives only a superficial picture of the actual invasive properties of this organism. Latency of infection has been reported in tomato (Graham and Lloyd 1978). The consequence of symptomless invasion of tomato plants by *R. solanacearum* is, without doubt, an important means for the survival of the pathogen resulting in the

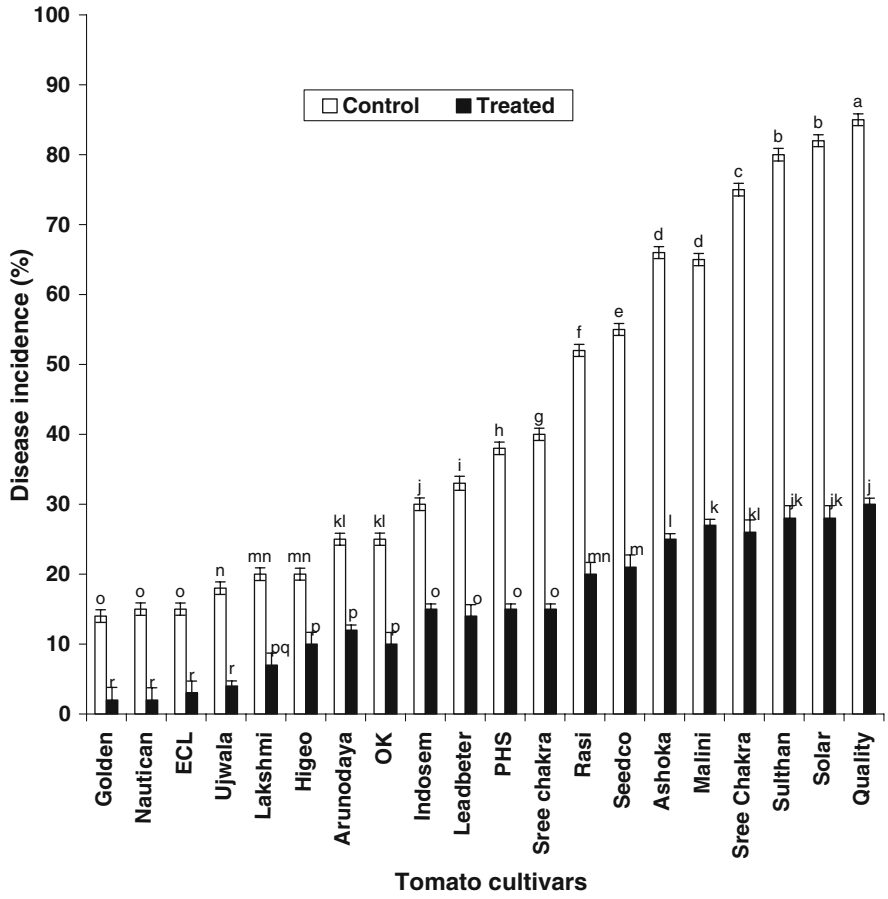

Fig. 2 Effect of seed treatments with *Pseudomonas fluorescens* on bacterial wilt incidence in tomato under greenhouse conditions. Values are the means of five replicates of all cultivars. The lines on each bar represents ± SE and the values in the bars followed by the same letter(s) are not significantly different according to analysis of variance (DMRT; $F = 622.92$, $df = 39, 80$, $P < 0.0001$)

potential for infestation of soil and other plants. Bacterial wilt was easily distinguished from fungal wilt based on the brown discoloration of the vascular tissues, and in the profuse bacterial ooze observed when cut sections of the stems were placed in clear water. The morphological characteristics of the bacteria on TZC agar medium, physiological, biochemical characterization results, hypersensitive response in tobacco plant leaves and pathogenicity test results confirmed the identity of the pathogen as *R. solanacearum* (Kelman 1954). In the present study, *R. solanacearum* was easily detected by the direct plating method. TZC agar medium proved to be useful also in the detection of *R. solanacearum* from tomato plants and soil (Kelman 1954). *R. solanacearum* on or in the seed may provide to be a potentially dangerous source of inoculum and play a role in the perpetuation of bacterial wilt in tomato crop production of small farm holders in the districts of Karnataka in India.

Various control strategies, including host-plant resistance, soil amendments, transgenics, resistant cultivars, integrated control and biological control have been developed. Management of plant diseases by biological control has been well documented (Sunaina et al. 1997; Anuratha and Gnanamanickam 1990). *Pseudomonas fluorescens* has emerged as an important biocontrol agent in the management of soil-borne plant pathogens. Many experiments carried out in laboratory, growth chamber and field conditions in the evaluation of the efficacy of *P. fluorescens* to control bacterial and other pathogens diseases have been conducted (Angela et al. 1992; Paul and Sarma 2006; Kuarabachew et al. 2007; Reddy et al. 2008). *P. fluorescens* controls the plant diseases directly or indirectly through different modes of action. Induction of systemic resistance by *P. fluorescens* has earlier been reported by several researches. It has been demonstrated to induce systemic resistance to a variety of diseases including

wilt diseases, bacterial (Vidhyasekaran et al. 2001), viral (Maurhofer et al. 1994), and fungal diseases (Benhamou et al. 1996). *P. fluorescens* has also been effective against early leaf blight and leaf spot in the tomato plant (Anand et al. 2007).

In order to simulate the infectious process and possible control measures in the present study, the pathogen was applied to soil, whereas the biocontrol strain were administered via seed inoculation. The rhizosphere of tomato plants appeared to be a favourable niche for both the pathogen and the potential biocontrol strain (Van Overbeek et al. 2002) and this was demonstrated by the observation of disease symptoms in the inoculated plants and the effect in the reduction of bacterial wilt symptoms in plants raised from seeds inoculated with *P. fluorescens*. *R. solanacearum* seed treatments reduced the germination and vigour of plants while seed treatments with *P. fluorescens* significantly enhanced the seed quality parameters. *P. fluorescens* may have played an important role in stimulating the plant growth by supplying nutrients (Compant et al. 2005) or by production of phytohormones (Lugtenberg et al. 1991). *Pseudomonas fluorescens* significantly reduced the bacterial wilt incidence in all the tomato cultivars under greenhouse conditions which can mimic the field conditions. When the 20 tomato cultivars were tested against bacterial wilt the cultivar Golden was found significantly resistant to bacterial wilt infection. The rate of infection is further reduced significantly after treatment with *P. fluorescens* in comparision with other cultivars. The cultivar Quality was found highly susceptible for bacterial wilt but the level of infection was significantly reduced after *P. fluorescens* treatment. These results indicate that *P. fluorescens* may have increased host resistance to bacterial wilt of tomato hence it has the potential to be used for management of bacterial wilt in tomato production. *P. fluorescens* might be inducing systemic resistance or antagonism against *R. solanacearum*. The present study revealed the widespread nature and the prevalence of bacterial wilt in the major tomato growing districts of Karnataka state. In the present studies attempts were made to report the incidence of bacterial wilt in the Karnataka state and use of *P. fluorescens* as a biocontrol agent against bacterial wilt of tomato. Earlier studies reported the prevalence of bacterial canker and bacterial spot in the major tomato growing districts of Karnataka (Umesha 2006; Kavitha and Umesha 2007). In the present study bacterial wilt incidence ranged up to 39% when compared to the incidences of bacterial canker and spot which were up to 48% and 50%. The symptoms of these three bacterial diseases are distinct from one another but there was a confusing symptomatology between canker and spot diseases. Bacterial wilt symptoms include yellowing, drooping and wilting of plants. Bacterial canker symptoms include vascular wilt, leaf spots and fruit spots whereas bacterial spot symptoms include small water-soaked lesions, which become necrotic. Although the fruit symptoms of bacterial spot appear similar to those of bacterial canker, the differentiation can be made by the persistent halo around the spots of the fruits in the case of bacterial canker. All the three bacterial diseases show milky white bacterial ooze when the freshly cut plant material was placed in water. All the three pathogens are over season in soil and crop residue. These three bacterial diseases are very destructive and cause heavy yield losses. Therefore the management of these bacterial diseases is very essential for sustainable tomato production. Though the bacterial diseases are difficult to control, various measures have been suggested to manage them. Among these management strategies biological control using *P. fluorescens* is one of the effective control measures to manage the bacterial diseases. The bacterial wilt incidence reduced from 85% in the untreated control to 30% after treatment with *P. fluorescens*. Similar results were reported in the management of bacterial canker and the bacterial spot diseases of tomato with *P. fluorescens* (Umesha 2006; Kavitha and Umesha 2007).

Anith et al. (2004) conducted greenhouse experiments to study the effect of plant growth promoting rhizobacteria on bacterial wilt incidence in susceptible tomato cultivars. Van Peer et al. (1991) showed that bacterisation of carnation roots with *P. fluorescens* reduced *Fusarium* wilt. The combined use of the foliar biological control agent and PGPR was reported to provide a significant control of bacterial speck and spot of tomato in field trials (Ji et al. 2006). Further evaluations could be made on the efficacy of these treatments in other plant diseases of tomato. Various formulations of fluorescent pseudomonads are available in the market in India that has proven to be efficient in the control of bacterial and fungal pathogens of various crops (Vidhyasekaran et al.

1997; Krishnamurthy and Gnanamanickam 1998; Nandakumar et al. 2001).

The present work suggests that periodic field survey for the incidence of bacterial wilt of tomato will be necessary to understand the progression of wilt disease in newer tomato cultivars. Furthermore, evaluations of their resistance to this serious bacterial disease should also be conducted. A successful control of bacterial wilt could be obtained by using *P. fluorescens* as a biocontrol agent, which is a potential alternative to the use of chemicals in combination with other control measures and when crop rotation practices are not feasible. Future studies are needed to understand the mode of action and the complex process of biological control of bacterial wilt. More knowledge on the ecological behaviour of *R. solanacearum* and its antagonists is required to develop sound procedures for its control and eradication in infested fields.

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