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S. Umesha [a], R. Kavitha & H. S. Shetty

[a] Department of Applied Botany, Seed Pathology and Biotechnology, University of Mysore, Manasagangotri, Mysore, Karnataka, India

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Transmission of seed-borne infection of chilli by *Burkholderia solanacearum* and effect of biological seed treatment on disease incidence

S. UMESHA, R. KAVITHA, & H. S. SHETTY

Department of Applied Botany, Seed Pathology and Biotechnology, University of Mysore, Manasagangotri, Mysore, Karnataka, India

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Abstract
A survey of chilli fields in the state of Karnataka, India, showed the presence of bacterial wilt disease in important chilli growing regions. The disease incidence ranged from 26 – 32%. The pathogen was isolated from infected plant material and seeds. Infected plant material showed the release of milky white bacterial ooze. *Burkholderia solanacearum* was detected from chilli seeds by liquid assay and its identity was confirmed by biochemical tests, hypersensitive reaction and pathogenicity tests. Seed transmission of the pathogen up to 45% was observed in seeds artificially infested with the pathogen. Among different tissues of the seed, endosperm showed the presence of the pathogen. Biological seed treatment with antagonistic *Pseudomonas fluorescens* significantly ($p = 0.05$) improved the seed quality parameters under laboratory conditions and drastically reduced the bacterial wilt incidence under field conditions. Seed-borne nature, transmission and effect of *Pseudomonas fluorescens* in both the forms of pure culture and formulation on seed quality parameters and bacterial wilt incidence are discussed in the present work.

Keywords: Burkholderia solanacearum, *bacterial wilt, seed-borne nature, chilli,* Pseudomonas fluorescens, *seed quality*

Introduction

Chilli (*Capsicum annuum* L.) is an important, well-known commercial crop used both as a condiment or culinary supplement and as a vegetable. It is cultivated for fruits, flavoring vegetables, dry-chilli as spice and in medicines. India is the major producer and exporter of chilli, which is cultivated in an area of 9.65 lakh ha with the production of 10.75 lakh tones. This contributes 35.5% and 31.6% respectively to the total area and production from all spices (Anon 2004).

Solanaceous crops are prone to a number of bacterial diseases among which bacterial wilt caused by *Burkholderia solanacearum* Smith (=*Pseudomonas solanacearum* = *Ralstonia solanacearum*) is a very destructive harmful disease of the solanaceous family, resulting in complete loss of the crop (Kelman 1953; Hayward 1991). Bacterial wilt is also referred to as vascular wilt, Southern bacterial wilt, Solanaceous wilt, Southern bacterial blight, and brown

Correspondence: S. Umesha, Department of Applied Botany, Seed Pathology and Biotechnology, University of Mysore, Manasagangotri, Mysore 570 006, Karnataka, India. Tel: + 91 821 2515126. Fax: + 91 821 411467. E-mail: pmumesh@yahoo. com

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rot. The pathogen attacks economically important crops such as chilli, eggplant, tomato, tobacco and potato (Guo et al. 2004). The pathogen has been considered as an important quarantine organism in many countries. During infection, bacteria become motile and travel throughout the vascular system of the plant (Grey & Steck 2001). The pathogen is a devastating seed-, soil-borne, with a global distribution and an unusually wide host range (Salanoubat et al. 2002). Studies aimed at determining how bacteria can grow within a Eukaryotic host are providing a wealth of information on the molecular mechanisms of pathogenesis in addition to fundamental aspects of host-cell biology (Roy & Sansonetti 2004).

Sowing infected seeds can reduce germination, vigor and potential yield by transmitting pathogen from seed to plants. The most adverse effect of seed-borne pathogen is contaminating disease free areas. Thus seed-borne pathogens act as a primary source of inoculum for disease development. Although the level of seed-borne inoculum may be extremely low, the rate of its increase may be extremely high when combined with favorable epidemiological factors such as local agricultural practices (Neergaard 1979). Seed infection usually occurs during three distinct physiological phases in the seed production, seed development and seed maturation. The pathogen can be involved in all these stages of growth and can transmit from planted to the new crop, thus developing a systemic infection that can colonize the seed (McGee 1995). Nevertheless, a better knowledge of the mechanics of seed-transmission may lead to better methods of controlling diseases such as proper cultural practices affecting the microclimate or the edaphic factors, improvement of seed health testing procedures in field and laboratory (Neergaard 1979). Although bacterial diseases are difficult to control, various measures have been suggested to manage the disease of which clean seed, resistant varieties, use of biocontrol agents (Gnanamanickam et al. 1999), use of avirulent mutants of *B. solanacearum* (McLaughlin & Sequeira 1988) and the use of lime and bleaching powder (Ramkishun & Ramachand 1988) are important.

Pseudomonas fluorescens Trevisan Migula is one of the most important antagonists of *Fusarium* in cucumber (Liu et al. 1995); *Sclerospora graminicola* in pearl millet and *Xanthomonas oryzae* pv. *oryzae* in rice (Umesha et al. 1998; Vidhyasekaran et al. 2001). Many greenhouse studies (Kraus & Loper 1992) and a few field experiments have been conducted (Parke et al. 1991; Umesha et al. 1998) to show the efficiency of *P. fluorescens* in the management of many plant diseases.

The pathogen mostly perpetuates through soil and crop residue (Granada & Sequeira 1983). In crops such as tomato and eggplant, the pathogen is carried in seed (Shakya 1992). However, the seed-borne nature of this pathogen in chilli has not been established. Seed transmission of the pathogen is of significance for seed trade and in quarantine inspection for seed exchange. Since wilt symptoms were noticed in crops grown around the Mysore area, the present studies were taken up with the following objectives:

- A field survey in the major chilli-growing districts of the Karnataka state, India;
- Establishment of the seed-borne nature, transmission; and
- To determine the effect of biological seed treatment on bacterial wilt incidence under field conditions.

Materials and methods

Field survey for the incidence of Burkholderia solanacearum and collection of seed samples

A field survey was undertaken in the major chilli-growing districts of Karnataka during Kharif seasons. The plants were inspected at the nursery stage, after transplantation, flowering stage

and fruiting stage. Wilt incidence was estimated among randomly selected subplots (10 subplots hectare^{-1}, measuring m^2) and plants were diagnosed as infected on the basis of typical symptoms of bacterial wilt disease *viz*., yellowing, dwarfing, stunting and development of adventitious roots and discoloration of the vascular system from pale yellow to dark brown. The suspected fruits and plant parts were collected separately from the field and brought to the laboratory for identification and confirmation of the disease-causing organism.

Isolation of Burkholderia solanacearum

Isolations were made from the collected seeds and chilli plant parts. Stem and root portions were washed thoroughly with running tap water, cut open and observed for discoloration. The cut portions were immediately immersed in sterile distilled water and observed for the bacterial ooze. The ooze was streaked on Kelman's medium (Kelman 1954) and streaked plates were incubated at 28 $\pm$ 2°C for 24 to 48 h.

The seeds collected from the suspected plants were subjected to liquid assays as follows, one gram of the seeds were surface sterilized with 3% (v/v) sodium hypochlorite solution for 4 min and were thoroughly washed with sterile distilled water. Seeds were crushed in sterile pestle and mortar using 5 ml of phosphate buffered saline (pH 7.0) (PBS). The suspension was left for 20 min and serial dilutions were prepared and 0.05 ml aliquots were spread onto triplicate plates of Kelman's and Nutrient agar media with the help of drigalski's spatula. Plates were incubated at 28 $\pm$ 2°C for 24 to 48 h.

Bacterial colonies exhibiting specific morphological characters described for *Burkholderia solanacearum viz.,* pinkish white, mucoid, smooth, convex with entire margins were isolated on to Kelman's medium. Single cell colonies were further isolated on to nutrient agar slants and stored at 5°C for further studies.

Characterization of the pathogen

Characterization of the pathogen was carried out by subjecting the cultures to various biochemical tests like Gram's staining, Kovac's oxidase test (Kovac's 1956; Hildebrand & Schroth 1972), levan formation, gelatin hydrolysis, starch hydrolysis, arginine hydrolysis, lipase activity, (Lelliot & Stead 1987), nitrate reduction test (Fahy & Persley 1983). Pathogen was also subjected to tobacco hypersensitive reaction and pathogenicity test (Lelliot & Stead 1987). All these tests were conducted in four replicates and were repeated thrice.

Component plating

For the study of the location of the pathogen in seed, 100 seeds of three chilli cultivars *viz.*, *G4, Byadagi* and *Pusa jwala* were soaked in sterile distilled water for 10 minutes. Seed components viz., seed coat, endosperm and embryo were dissected using sterile forceps, blades and needles under aseptic conditions. These components were surface sterilized as mentioned earlier and blot dried. They were then placed on Kelman's medium and incubated at 28 $\pm$ 2°C for 24 to 48 h.

Effect of Burkholderia solanacearum on seed germination and seedling vigor

Seeds were artificially inoculated with the pathogen or sterile distilled water (SDW) by immersing the seeds in to the bacterial suspension (1×10^8 CFUml^{-1}) for 2 h. The optical density (OD) of the bacterial suspension was adjusted to 0.45 (A_{610} nm) to obtain 1×10^8

CFUml^{-1} with the help of a UV-Visible spectrophotometer (Hitachi U-2000, Japan) (Mortensen 1999). After 2 h, treated and untreated seeds were air-dried, plated on wet blotters and the germination test was carried out by standard blotter method (ISTA 2003). Vigour index was calculated by using the formula (Mean shoot length + mean root length) × percent germination (Abdul Baki & Anderson 1973). Experiments were conducted in four replicates of 100 seeds each and repeated thrice.

Seed transmission studies

Disease development from the naturally infected seeds

Transmission of *B. solanacearum* from seed to seedling was studied in three chilli cultivars *viz., G4, Byadagi* and *Pusa jwala*. Four hundred seeds from each cultivar, collected from diseased plants, were sown in the experimental plot. Plants were raised by following the normal agronomical practices recommended for commercial chilli cultivation. The plants were closely observed until the fruiting stage for the typical symptoms of bacterial wilt disease as previously explained. Infected plant parts were collected at every 15 days and subjected to direct plating method to observe the re-establishment of *B. solanacearum* in all the three chilli cultivars. Infected fruits from each variety were randomly picked from the plots to assess the impact of seed-transmission and percentage re-establishment of *B. solanacearum* in the seeds.

Mass multiplication of biological agent and preparation of formulation

The antagonistic strain of *Pseudomonas fluorescens* was isolated from the native soil from a farmer's field by serial dilution technique and maintained on King's B medium (KMB) (King et al. 1956). The identity of the organism was further confirmed by conducting various tests specific to *P. fluorescens* (Stainer et al. 1966). The 48-h old culture in KMB was centrifuged at 10,000 rpm for 10 min. The pellets were resuspended in sterile distilled water (SDW) and washing was repeated thrice. The washed bacterial pellet was made in to a turbid solution with SDW. The optical density (OD) of the solution was adjusted to 0.45 (A_{610} nm) to obtain 1×10^8 CFUml^{-1} with the help of a UV-Visible spectrophotometer (Hitachi U-2000, Japan) (Mortensen 1999). *P. fluorescens* formulation (28×10^7 CFUg^{-1}) was prepared by mixing 100 ml of bacterial suspension in 25g of purified talcum powder under sterile conditions. Carboxy methyl cellulose (2.5 g) was added to 250 g of formulation, shade dried, packed in polythene bags and stored under ambient conditions (Umesha et al. 1998).

Effect of P. fluorescens and its formulation on seed quality parameters under laboratory conditions and bacterial wilt incidence under field conditions

Seeds of the three chilli cultivars (collected from diseased plants) were treated with the pure culture of *P. fluorescens*. Seeds of all the three cultivars were shaken in pure culture (1×10^8 CFUml^{-1}) of antagonist suspension/SDW for 12 h. Seeds were treated with formulation of *P. fluorescens* (28×10^7 CFUg^{-1}) in the form of slurry treatment at the rate of 8 g Kg^{-1} and 10 g Kg^{-1} of seeds. After 12 h, treated and untreated seeds were air-dried plated on wet blotters and the germination test was carried out by standard blotter method (ISTA 2003). Vigour index was calculated by using the formula (mean shoot length + mean root length) × percent germination (Abdul Baki & Anderson 1973). The experiment was carried out with four replicates of 100 seeds each and repeated thrice.

The field trials were carried out with all the treatments namely seed treatment with pure culture and seed treatment with antagonist formulations at the rate of 8 g Kg^{-1} and 10 g Kg^{-1} of seeds. In all the field experiments, there were eight replicates in a randomized block design with about 25 – 30 plants in each replicate and each experiment was repeated in two consecutive seasons. Normal agronomical practices were followed which are recommended for commercial chilli cultivation. Plants were closely observed from the seventh day till fruiting stage for the typical symptoms of bacterial wilt disease as explained previously.

Statistical procedures

Data on percentages were transformed to arcsine and analysis of variance was carried out with transformed values. The means were compared for significance using Duncan's multiple range test (DMRT; $p = 0.05$).

Results

Among the chilli fields surveyed, the mean incidence of *B. solanacearum* in three cultivars (*Pusa jwala; Byadagi and G4*) ranged from 26 – 32%. The cultivar *Pusa jwala* in the Mysore area recorded 26%, the cultivar *Byadagi* in Chitradurga and Bellary region showed 32%. In the fields of Mandya, Bangalore and Dharwar, the cultivar G_4 recorded the incidence of 26% bacterial wilt disease. In all the fields surveyed, *Pusa jwala*, *Byadagi* and G_4 cultivars of chilli were being cultivated and the highest disease incidence from all the fields being considered.

Isolation and characterization of B. solanacearum

On Kelman's medium plates, the bacterial colonies were pinkish white, mucoid, smooth, complete, convex after 24 h. The bacterial colonies on the nutrient agar were creamish white, mucoid, circular, convex with entire margins. The bacterium was identified as *B. solanacearum*, which causes bacterial wilt disease in vegetables. Infected stem and root portions showed discoloration when cut open. Milky white bacterial ooze was observed when the plant material was placed in water (see Figure 1). Healthy plant material did not show any discoloration or bacterial ooze.

The characterization test results of the bacterium are as follows: On observation of Gram's staining preparation, the color of Safranin was observed on the bacterial cells indicating Gram negative nature of the bacterium. The development of purple color when the bacterial cells were mixed with 1% aqueous solution of N,N,N^1,N^1 tetra methyl- paraphenyl diamine dihydrochloride on the centre of Whatman filter paper no.1 within 10 sec indicated the positive result of the test bacterium for Kovac's oxidase test. On 5% sucrose nutrient agar, tested bacterial cells did not produce any white, dome shaped, mucoid, levan type of colonies, even after the 4th day of incubation indicating negative results for the levan formation test. For the gelatin hydrolysis test, the test isolates failed to liquefy (completely) the medium, even on the 4th day of incubation indicating weak reaction for gelatin hydrolysis. The bacterial isolates did not utilize the starch present in the medium, no clear zones of starch utilization were noticed, when Lugol's iodine solution was added to the medium indicating negative reaction. It was found that the color of the medium remained as it was on the third day of incubation indicating negative result for arginine dihydrolase test. After the seventh day of incubation, it was found that, there was no milky-white precipitate around the colonies, indicating the inability to hydrolyze the lipid tween. It was observed that the color of the medium was turned to black, after the addition of 3 – 4 drops of the solutions 1 and 2 (Starch

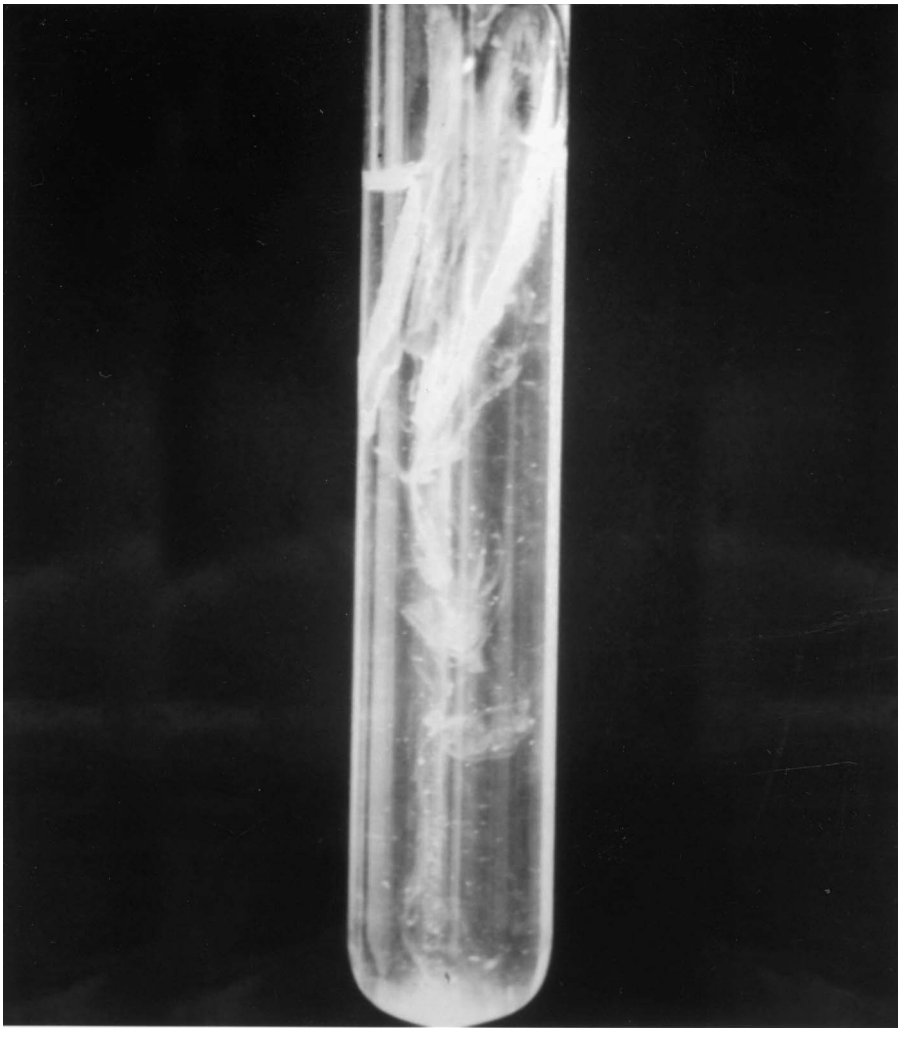

Figure 1. Bacterial ooze; Freshly cut portion of infected chilli stem showing the milky white bacterial ooze.

iodide solution and hydrochloric acid solution), indicating the positive test results for nitrate reduction test. Necrosis was observed on tobacco leaves, within 24 h of infiltration with bacterial cells. The leaf which was in filtered with avirulent isolate and SDW did not show any change in the leaf color. Plants inoculated with the pathogen for pathogenicity test showed wilting symptoms after 15 days, leaves turned yellow, with severe defoliation. Sterile distilled water dipped plants remain healthy (see Table I; Figure 2).

Component plating method

Among the different components of seeds tested from all the three cultivars, the mean incidence of *B. solanacearum* was more dominant in endosperm with 28%. The other parts of the seed, seed coat and embryo recorded meagre amount of pathogen (2%) in all the tested chilli cultivars.

Table I. Biochemical characterization tests of the pathogen *B. solanacearum*.

Biochemical tests	Results
Gram's staining	–
Kovac's oxidase test	+
Levan formation	–
Gelatin hydrolysis	w
Starch hydrolysis	–
Arginine dihydrolysis	–
Lipase activity	–
Nitrate reduction	+
Tobacco hypersensitive reaction	+
Pathogenicity test	+

All these tests were conducted in four replicates and were repeated thrice; '+' indicates positive reaction; '–' indicates negative reaction and 'w' indicates weak reaction.

Effect of B. solanacearum on chilli seed germination and seedling vigor

There was a significant ($p = 0.05$) change in seed germination of chilli seeds with and without *B. solanacearum* (see Table II). The seed germination in the *Pusa jwala* cultivar was decreased from 72% in untreated control to 41% when the seeds were treated with *B. solanacearum*. A similar trend was reflected in the other two cultivars of chilli. The mean shoot length and root lengths were also significantly ($p = 0.05$) reduced upon the pathogen treatment. This was reflected in the vigor index. The vigor index was significantly ($p = 0.05$) reduced from 626 in the control to 201 when seeds were treated with *B. solanacearum* with the *Pusa jwala* chilli cultivar. The same trend was recorded with the other two chilli cultivars (Table II).

Seed transmission of B. solanacearum from naturally infected seeds under field condition

Results from the transmission studies indicated that *B. solanacearum* is seed-borne and seed transmitted. Disease transmission studies showed varied symptoms of bacterial wilt disease under field conditions from the tested chilli cultivars. The disease incidence in the cultivar *Pusa jwala* showed 22%. The other two cultivars *Byadagi* and G_4 recorded 24 and 25% respectively. No disease symptoms could be observed during the initial stages. However, detection assay at every 15-day intervals showed the presence of the pathogen in root, stem and leaves. Typical symptoms of the bacterial wilt disease were observed at the later stages – flowering and fruiting stages of the chilli plants. *B. solanacearum* was not detected in healthy plants and from the seeds collected from such plants.

Effect of P. fluorescens and its formulation on seed germination and seedling vigor of chilli seeds under laboratory conditions

A significant ($p = 0.05$) improvement was recorded in seed germination when chilli seeds were treated with *P. fluorescens* pure culture and formulations (see Tables III and IV). Seed germination in the chilli cultivar *Pusa jwala* was increased from 66% in the untreated control to 81% and 78%, when the seeds were treated with pure culture and formulation of *P. fluorescens*. However, there was no difference between the two different dosages of formulations tested in the study (Tables III and IV). The mean root length and shoot lengths were also increased upon the treatment with *P. fluorescens* pure culture and

Figure 2. Effect of *Burkholderia solanaccarum* on the chilli plants 'a' – uninoculated control plant, 'b' – plants inoculated with B. solanaccarum.

formulation, which was reflected in the vigor index. The vigor index was increased from 502 in the untreated control to 802, and 702 when the seeds were treated with pure culture and formulation of *P. fluorescens* (Tables III and IV). Among the chilli cultivars tested, *P. fluorescens* improved the seed quality parameters in both pure culture and formulation (see Tables III and IV).

Table II. Effect of *B. solanacearum* on chilli seed germination and seedling vigor.

Chilli cultivars		Germination (%)	MSL (Cm)	MRL (Cm)	VI
Pusa jwala	Control	72 ± 0.98^{a}	2.8 ± 0.34	5.9 ± 0.49	626^{a}
	Inoculated	41 ± 0.86^{b}	1.7 ± 0.19	3.2 ± 0.58	201^{b}
Byadagi	Control	68 ± 0.69^{a}	3.1 ± 0.41	6.1 ± 0.94	626^{ab}
	Inoculated	36 ± 0.90^{b}	1.9 ± 0.28	3.8 ± 0.99	205^{b}
G_4	Control	70 ± 0.59^{a}	2.9 ± 0.42	5.7 ± 0.59	602^{ab}
	Inoculated	34 ± 0.61^{b}	1.7 ± 0.24	2.9 ± 0.69	157^{a}

Values are the means of three independent experiments ± S.E. of four replicates of 100 seeds each; MRL = Mean root length; MSL = Mean shoot length; VI = Vigor index; 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 III. Effect of *P. fluorescens* pure culture on chilli seed germination and seedling vigor.

Chilli cultivars	Treatment	Germination (%)	MSL (Cm)	MRL (Cm)	VI
Pusa jwala	Control	66 ± 0.19^{c}	2.5 ± 0.42^{a}	5.1 ± 0.72^{a}	502^{a}
	Treated	81 ± 0.31^{a}	3.1 ± 0.41^{b}	$6.8 \pm .69^{a}$	802^{b}
Byadagi	Control	61 ± 0.28^{b}	3.0 ± 0.82^{c}	5.8 ± 0.58^{b}	537^{a}
	Treated	79 ± 0.31^{b}	4.1 ± 0.49^{a}	6.5 ± 0.50^{b}	837^{b}
G_4	Control	58 ± 0.38^{c}	2.9 ± 0.79^{b}	5.1 ± 0.49^{a}	464^{a}
	Treated	73 ± 0.49^{a}	3.8 ± 0.81^{a}	6.2 ± 0.28^{a}	730^{b}

Values are the means of three independent experiments ± S.E. of four replicates of 100 seeds each; MRL = Mean root length; MSL = Mean shoot length; VI = Vigor index; 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 IV. Effect of *P. fluorescens* formulation on chilli seed germination and seedling vigor.

Chilli cultivars/treatment		Germination (%)	MSL (Cm)	MRL (Cm)	VI
Pusa jwala	Control	66 ± 0.19^{c}	2.5 ± 0.42^{b}	5.1 ± 0.72^{a}	502^{a}
Seed treatment	@8g Kg^{-1}	78 ± 0.49	2.9 ± 0.58^{c}	6.1 ± 0.98^{c}	702^{b}
	10g Kg^{-1}	78 ± 0.58	3.0 ± 0.85^{a}	6.0 ± 0.89^{a}	702^{b}
Byadagi	Control	61 ± 0.28^{b}	3.0 ± 0.82^{c}	5.8 ± 0.58^{b}	537^{a}
Seed treatment	@ 8g Kg^{-1}	76 ± 0.58^{c}	3.8 ± 0.28^{b}	6.2 ± 0.59^{b}	760^{b}
	10g Kg^{-1}	77 ± 0.69^{b}	3.9 ± 0.82^{a}	6.2 ± 0.95^{a}	778^{b}
G_4	Control	58 ± 0.38^{c}	2.9 ± 0.79^{b}	5.1 ± 0.49^{a}	464^{a}
Seed treatment	@ 8g Kg^{-1}	69 ± 0.79^{b}	3.5 ± 0.89^{a}	6.0 ± 0.28^{a}	656^{b}
	10g Kg^{-1}	69 ± 0.97^{b}	3.5 ± 0.89^{a}	6.1 ± 0.82^{a}	662^{b}

Values are the means of three independent experiments ± S.E. of four replicates of 100 seeds each; MRL = Mean root length; MSL = Mean shoot length; VI = Vigor index; The values in the column followed by the same letter(s) are not significantly different according to analysis of variance (DMRT; $p = 0.05$).

Effect of P. fluorescens and its formulation in the incidence of bacterial wilt under field condition

With the seed treatment, the chilli wilt incidence in the untreated *Pusa jwala* seeds was 48%, whereas it was significantly ($p = 0.05$) reduced to 18%, 23% and 22% respectively when the seeds were treated with *P. fluorescens* pure culture and formulation. In the cultivar *Byadagi,* the wilt incidence was reduced from 46% in the untreated control to 21% and 24%, when the seeds were treated with both culture and formulation of *P. fluorescens*. However, among the

two dosages of *P. fluorescens* formulations, there no significant ($p = 0.05$) difference was noticed. In the cultivar G_4, the wilt incidence was 54% in the untreated control, which was significantly ($p = 0.05$) reduced to 16%, 20% and 19%, when the seeds were treated with pure culture and formulation of *P. fluorescens* (see Figure 3).

Discussion

In the present studies, field survey analysis of the three consecutive seasons followed by laboratory analysis was undertaken to estimate the percentage incidence of bacterial wilt induced by *Burkholderia solanacearum*. The study also addresses the stage of disease susceptibility during development. Field survey analysis including symptoms observed, discolored stem and fruits indicated the percentage of disease incidence in chilli. The characterization of the pathogen was carried out using a host specificity, hypersensitivity test, and pathogenicity test by host-inoculation method and seed transmission aspects. The disease causes 20 – 22% yield loss in chilli crops (Khan et al. 1977). Khan et al. (1977) first reported the bacterial wilt of chilli caused by *B. solanacearum* in India. As soil-borne pathogen, it disperses through soil, from infected plant material and is transmitted on infested or infected seeds (Hayward 1964). The seed-borne nature of the pathogen serves as an important primary source of inoculum in the spread of the disease. Artificially inoculated and contaminated seeds serve as a primary source of inoculum (Moffett et al. 1981). But the details regarding naturally infected seeds, detection methods used, location of the pathogen, transmission of the pathogen, and control measures are not published in literature. In the present work, the disease incidence in different chilli cultivars, seed-borne nature, detection, characterization, location of the pathogen, its effect on seed quality and the effect of biological seed treatment on the disease incidence in the field conditions was studied.

The bacterial wilt can be easily distinguished from fungal wilt based on the brown discoloration of the stem at ground level and in preliminary ooze test, from the cut ends of the stem and root portion (see Figure 1) of the plant when placed in clean water. For detection of *B. solanacearum* Kelman's medium is more suitable than any other media, because the number of saprophytes were expressed in lesser percentages. This correlates with the view of Kelman (1954). For isolation and purification, the Kelman's medium was considered the best media (Kelman 1954). The location of the pathogen in the seed was investigated by plating different components of the seed on Kelman's medium. Pathogen was detected from the endosperm region only but not from other components of the seed. This indicates that the pathogen is internally seed-borne. The information on the location of the pathogen in different components would be valuable in understanding the mechanism of seed transmission and to design appropriate seed treatments for controlling the disease. In component plating, studies revealed that the pathogen invades the endosperm of the seed but not the seed coat, so it is considered as a systemic disease. It may be controlled by using systemic chemicals. The present investigation showed that, for the detection of pathogen from the seeds, liquid assay method can be used for routine use.

Further research is needed in transmission studies and disease management by production of a resistant cultivar to bacterial wilt to improve the production of chilli. The present investigation clearly revealed that *B. solanacearum* is both externally and internally seed-borne, which can carry the inoculum from the seed to plant as evidences from the present work. Agarwal and Sinclair (1977) reported that seed-borne pathogens can be transmitted either by infection of embryo, endosperm or by contamination of seed coat. The presence of *B. solanacearum* on or in the seed may provide an unsuspected and potentially dangerous source of infection. In the present studies, seed- transmission from naturally infected plants

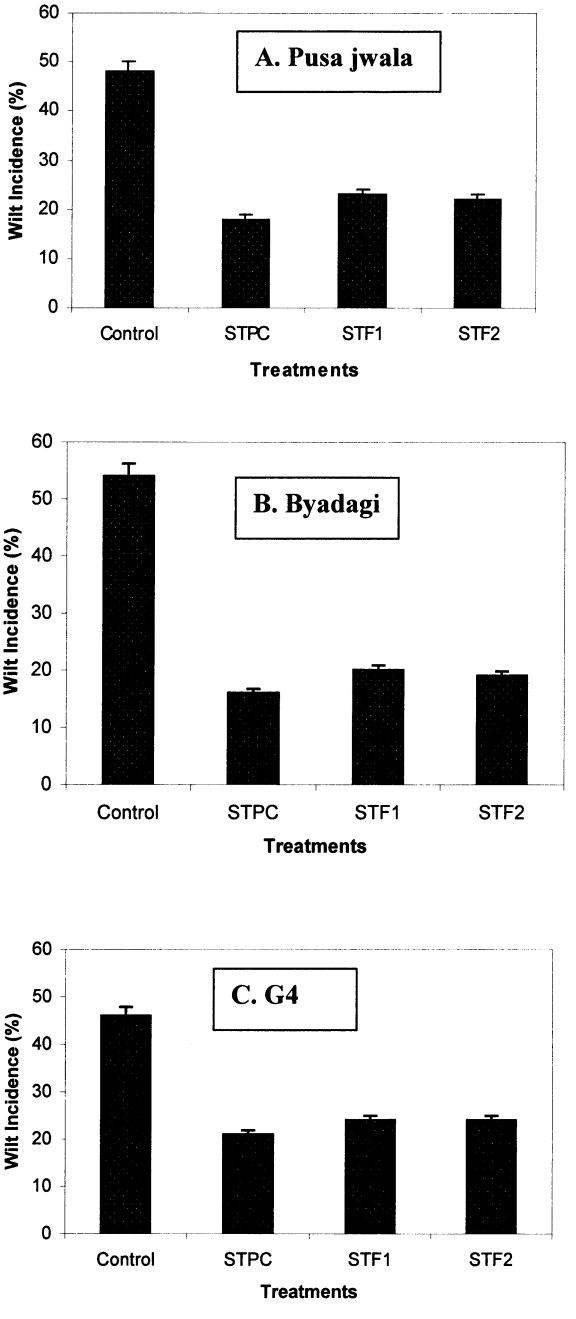

Figure 3. Effect of P. fluorescens and its formulations on the incidence of bacterial wilt under field conditions. The lines on each bar represents S.E. when subjected to Analysis of Variance (DMRT; P = 0.05). STPC = Seed treatment with pure culture; STF1 = Seed treatment with formulation @ 8 g Kg^{-1}; STF2 = Seed treatment with formulation @ 10 g Kg^{-1}.

under field conditions clearly indicated transmission of *B. solanacearum* from seed to seedling. Typical symptoms of the disease *viz.*, yellowing, dwarfing, stunting and development of adventitious roots, were noticed due to the severity of infection.

The results of the present study were compared with Palleroni's (1994) results and hence the pathogen was confirmed as *B. solanacearum*. Different methods of characterization of the pathogen, morphological characters on Kelman's medium, hypersensitive response on tobacco leaves, pathogenicity test results were in accordance with the results of Kelman (1954); the pathogen has adversely affected the chilli seed quality.

The bacterial wilt incidence was highly severe with an average incidence of 32% in the cultivar *Byadagi* in the Chitradurga and Bellary region. Since many non-related plant pathogens produce similar type symptoms, our studies strongly indicated that preliminary diagnosis of the bacterial ooze test proved to be a very efficient method in identifying bacterial disease symptoms.

Survival of *B. solanacearum* is affected by asymptomatic hosts. Various control strategies, including host-plant resistance, cropping systems (Dalal et al. 1999), transgenic resistant plant, soil amendments (Vincent & Mew 1998), integrated control and biological control have been developed (Guo et al. 2004). Reports from elsewhere indicate that five different strains of *Trichoderma* significantly reduced the incidence of *Pythium* in pea and increased the shoot/root ratio significantly, and also different isolates of *Pseudomonas* spp shown antifungal activity by means of releasing some metabolites which affected *Tuber borchii* mycelial growth. Potential biological agents used to control bacterial wilt of tomato include VAM, avirulent mutants of *B. solanacearum* and some naturally occurring antagonistic rhizobacteria (Guo et al. 2004). Additional information is required to understand the complex process of biological control of bacterial wilt.

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