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Isolation of endophytic bacteria for biological control of wilt pathogens

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ABSTRACT: **Twentyfive endophytic bacteria were isolated from internal tissues of root and stem portions of chickpea, sunflower, niger, chilli and capsicum plants. The endophytes were screened in dual culture on Potato Dextrose Agar (PDA) and Tryptic Soya Agar (TSA) against *Fusarium oxysporum* f. sp. *ciceri*, *Fusarium udum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Ten isolates exhibited inhibition of the pathogens. Maximum percent inhibition (37.93) of *F. oxysporum* f. sp. *ciceri* was obtained on PDA with *B. subtilis* (PDBCEN 3). On TSA percent inhibition was maximum (52.21) with isolate PDBCEN-7. Testing against *F. udum* in dual culture test revealed that *Pseudomonas* sp. (PDBCEN 8) showed maximum (40.45%) inhibition on PDA. *Pseudomonas* sp. (PDBCEN-2) was highly effective on TSA and showed maximum (56.9%) inhibition zone. Against *R.* solani, maximum inhibition (44.96%) was recorded with endophyte PDBCEN 7. On TSA all the ten endophytic bacteria were effective in restricting the growth of test fungus. Percent inhibition of *S. rolfsii* was maximum (40.93%) with *Pseudomonas* sp. (PDBCEN 6) on PDA. On TSA percent inhibition was maximum (46.73%) with *P. fluorescens* (PDBCEN 1). The endophytic isolates were able to promote better growth of chickpea but the vigour index varied between the isolates. We could not correlate high pathogen inhibition under *in vitro* with high vigour index.**

KEY WORDS: Biological control, endophytic bacteria, wilt pathogens

Fusarium oxysporum incited vascular wilt in crops reduces the yield by 80 to 90 percent. Among other species the vascular-wilt caused by *Fusarium oxysporum* Schlecht f. sp. *ciceri* (Padwick) of chickpea, *F. udum* of pigeonpea and *F. oxysporum* f. sp. *lycopersici* in tomato are very serious in India. Other soil and seed borne diseases caused by *Macrophomina phaseolina, Sclerotium rolfsii, Rhizoctonia solani* and *Botrytis cinerea* rank next to wilts and are usually associated with wilt complex. No precise information on losses caused by Fusarium wilt in chickpea is available. A rough estimate indicates that losses may be around 10 to 15 percent each year. In severe epidemics, crop losses may go as high as 60-70 percent. Damage is up to 61 percent at seedling stage and 43 percent at flowering stage (Haware and Nene, 1980).

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Combinations of systemic and non-systemic fungicides like Carbendazim, Thiram, Captan and Dithane M45 are used to combat the wilt pathogens. The present focus is on the reduction in use of harmful chemicals and to look for safer means of controlling the wilt pathogens. Biological control using microorganisms namely *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride*, and *T. harzianum* as seed or soil treatments are now being advocated (Rangeshwaran and Prasad, 2000a, Rangeshwaran and Prasad, 2000b; Vidhyasekaran *et al*., 1997).

Recent studies indicate that endophytic bacteria colonizing the internal tissues of plants show promise in conferring systemic resistance to plants (Sturz *et al*., 2000). The endophytic bacteria are present in various plant parts such as seeds, ovules, leaves, stem and fruits (Fisher *et al*., 1992).

The internal tissues of plants provide a uniform and safe environment when compared to the rhizosphere and phylloplane where the introduced bacterial population must compete for nutrients and also endure temperature changes and exposure to UV rays. These advantages envisage the use of endophytic bacteria for more successful biological control of plant diseases (Sturz and Christie, 1995; Rajappan and Ramaraj, 1999; Nejed and Johnson, 2000).

We undertook a study to know the biological control potential of endophytic bacteria isolated from root and stem portions of different plant samples.

MATERIALS AND METHODS

Endophytic bacteria were isolated from healthy roots and stem of chickpea, sunflower, mustard, niger and bell pepper in rural Bangalore, where root-rot, wilt and other soil-borne disease were prevalent.

Isolation of endophytic bacteria

Whole plants were manually uprooted and brought to laboratory. Root and stem sections (2-3cm long) were made using a sterile scalpel. For younger plants (14 days) root samples were taken just below the soil line and 5-10cm below the soil line were taken for older plants (21days). Stem sections were taken 1-2cm above the soil line in younger plants and 10cm above the soil line in older plants. Stem samples were first weighed and surface sterilized with hydrogen peroxide (20%) for 10 minutes and rinsed four times with 0.02M potassium phosphate buffer (pH 7.0). Root samples were surface disinfected with sodium hypochlorite (1.05%) and washed in four changes of 0.02M phosphate buffer solution. Measured quantity of 0.1-ml aliquot from the final buffer wash was removed and transferred in 9.9ml Tryptic-Soya broth to serve as sterile check. Samples were discarded, if growth was detected in the sterile check within 48 hours. Selected samples were triturated in 9.9ml of buffer in a sterile pestle and mortar. The triturate was serially diluted in potassium phosphate buffer solution and plated on Tryptic-Soya Agar (TSA). Representatives of colony morphology were transferred to fresh TSA plates as pure cultures (McInroy and Kloepper, 1995).

Isolation of pathogen

Test pathogens, viz., *Fusarium oxysporum* f. sp. *ciceri*, *F. udum* and *Rhizoctonia solani* were obtained from the culture collection of Project Directorate of Biological Control (PDBC) pure cultured and maintained on Potato Dextrose Agar medium.

Identification of bacterial endophytes

Initial Identification was carried out as per Bergey's Manual of Determinative & Systemic Bacteriology, 9th Edition, 1994.

Preservation of bacterial endophytes

The bacterium was grown up to log phase, diluted with fresh culture media containing 30 percent glycerol so as to get a final glycerol concentration of 15 percent. One to 10ml of the suspension was distributed into screwcap cryovials and frozen at –20 °C.

Multiplication of bacterial endophytes

Culture of individual isolate to be tested, were grown on TSA for at least 3 days. A loop full of this culture was then transferred to flasks containing 150ml of Kings B Broth and incubated at room temperature on an orbital shaker for 24 hours at 200rpm. Bacterial cells were harvested by centrifuging at 7000rpm for 10 minutes and re-suspended in 10 nM $MgSO_4$. The separated cells were used for assay of endophytes.

Seed bacterization with endophytes

The surface sterilized seeds of chickpea were steeped in endophytic bacterial suspension containing CMC (1%) and dried. The treated seeds were examined for the colony forming unit (cfu) on TSA plates, after 48 hours of incubation at 25°C. The coated seeds of chickpea carried ($1x10^7$) cfu per seed.

***In vitro* bioassay**

Dual Agar Test

Isolates of endophytic bacteria were tested for their efficacy against *Fusarium oxysporum* f.sp. *ciceri*, *F. udum*, *Sclerotium rolfsii*, and *Rhizoctonia solani* under dual-agar test by streaking endophytic bacteria in a 4cm line on one side of the plate containing 15ml of either TSA or PDA. Mycelial plugs of all pathogen listed above (5mm in diam) were placed at opposite side of the medium. Each treatment was replicated thrice and repeated once. Plates were incubated at 25°C and measured zone of inhibition was calculated by taking into account the differential growth of the pathogen in pathogen check and treated.

Roll-Towel Test

A roll towel method (ISTA, 1976) regularly used for seed vigour testing was used for testing bioefficacy of endophytes. Healthy seeds were first surface sterilized in sodium hypochlorite (1.05%) followed by three changes/washing in sterile water and then inoculated with the bacterial isolate as previously described. After air-drying, the seeds were again dipped in mycelial suspension *of F. oxysporum* f. sp. *ciceri* which was replicated in potato dextrose broth. Three replicates of 25 seeds each were randomly counted and placed in coarse blotter paper sheets and covered with a moistened blotter and rolled. Three such rolls were kept on a butter paper sheet and rolled as a single bundle and incubated in a growth chamber at 25 C with 80 percent relative humidity for 8 days. After incubation, germination percent was noted along with root and shoot length and vigour index was calculated. Vigour Index was calculated by multiplying percent plant stand with sum of shoot and root length.

RESULTS AND DISCUSSION

Twenty-five endophytic bacteria were isolated from internal tissues of root and stem portions of chickpea, sunflower, niger, chilli and capsicum plants. All the 25 isolates were screened for *in vitro* antagonism in dual culture on PDA and TSA against *Fusarium oxysporum* f. sp. *ciceri*, *F. udum*, *R. solani* and *S. rolfsii*. Based on inhibition zones observed ten isolates were selected for further studies. Eight of the ten endophytes were identified which included *Pseudomonas fluorescens* (PDBCEN 1), *Pseudomonas* sp. (PDBCEN 2, 4, 6, 8 and 10), *Bacillus subtilis* (PDBCEN 3) and *Bacillus* sp. (PDBCEN9).

Inhibition of *F. oxysporum* f. sp. *ciceri* varied in both the media tested (Table 1). Maximum inhibition (37.93%) was obtained on PDA with *B. subtilis* (PDBCEN 3) and minimum (21.64%) with endophyte (PDBCEN 5). On TSA, inhibition was maximum (52.21%) with isolate PDBCEN 7 and minimum (20.94%) with *B. subtilis* (PDBCEN 3). Maximum pathogen growth (45.23 sq. mm) was recorded on PDA with PDBCEN 5, whereas minimum (35.86 sq. mm) was noticed in dual culture with *B. subtilis* (PDBCEN 3). Radial growth on TSA was lesser than on PDA. The bacterial growth was also recorded and on TSA, the maximum growth (8.06 sq. mm) was exhibited by *Pseudomonas* sp. (EN 2) and minimum (22.9 sq. mm) was seen with endophyte (PDBCEN 5). On PDA, bacterial growth was maximum (4.2 sq. mm) with *Pseudomonas* sp. (PDBCEN 2) whereas, it was least (1sq. mm) with *B.*

subtilis (PDBCEN 3) and *Bacillus* sp. (PDBCEN 9). Bacterial growth was more on TSA than on PDA.

Table 1. Screening of endophytic bacterial isolates against *Fusarium oxysporum* f. sp. *ciceri* under dual culture in different media

Endophytic bacterial isolate	Percent growth inhibition on PDA	Percent growth inhibition on TSA	Fungal radial growth (sq. mm) on PDA	Fungal radial growth (sq. mm) on TSA	Bacterial growth (sq. mm) on PDA	Bacterial growth (sq.mm) on TSA
P. fluorescens (PDBCEN 1)	30.32 (32.44)	44.97 (38.38)	40.20	26.40	1.80	2.90
Pseudomonas sp. (PDBCEN 2)	26.02 (31.12)	35.76 (48.98)	42.70	30.66	1.93	8.06
Bacillus subtilis (PDBCEN 3)	37.93 (31.59)	20.94 (38.15)	35.86	37.86	1.73	5.50
Pseudomonas sp (PDBCEN 4)	29.05 (30.52)	35.89 (34.60)	40.93	30.60	2.20	5.20
Endophyte (PDBCEN 5)	21.64 (36.36)	52.22 (41.50)	45.23	22.90	4.20	6.76
Pseudomonas sp. (PDBCEN 6)	22.46 (25.21)	43.39 (43.03)	44.70	26.83	3.30	5.23
Endophyte (PDBCEN 7)	35.88 (27.98)	49.11 (47.77)	37.00	24.33	2.36	6.40
Pseudomonas sp. (PDBCEN 8)	24.10 (39.47)	34.42 (41.88)	43.80	30.03	2.10	5.70
Bacillus sp. (PDBCEN 9)	34.12 (28.20)	39.79 (40.50)	38.03	28.70	1.73	5.10
Pseudomonas sp. (PDBCEN 10)	29.75 (36.90)	48.28 (39.29)	40.60	24.86	2.13	2.45
Control	0	0	57.75	47.93	0	0
CD (P=0.05)	3.47	8.14	1.92	4.51	0.32	0.76

Figures in parentheses are angular transformed values.

Table 2. Screening of endophytic bacteria against *Fusarium udum* in dual culture in different media

Endophytic bacterial isolate	Percent growth inhibition on PDA	Percent growth inhibition on TSA	Fungal radial growth on PDA (sq. mm)	Fungal radial growth on TSA (sq. mm)	Bacterial growth on PDA (sq. mm)	Bacterial growth on TSA (sq. mm)
P. fluorescens (PDBCEN 1)	29.02 (34.62)	38.55 (42.42)	36.33	29.43	1.06	2.80
Pseudomonas sp. (PDBCEN 2)	27.91 (30.32)	56.90 (58.45)	35.53	20.63	4.26	5.63
Bacillus Subtilis (PDBCEN 3)	28.44 (36.65)	39.15 (40.46)	36.20	28.93	0.60	1.73
Pseudomonas sp (PDBCEN 4)	25.81 (30.86)	32.30 (38.86)	38.10	32.73	1.83	2.16
Endophyte (PDBCEN 5)	35.95 (40.20)	44.17 (48.50)	40.86	26.50	3.43	4.36
Pseudomonas sp. (PDBCEN 6)	18.29 (20.34)	46.55 (50.52)	42.03	24.26	3.00	3.40
Endophyte (PDBCEN 7)	22.07 (25.54)	16.56 (20.26)	40.20	21.53	2.63	3.10
Pseudomonas sp. (PDBCEN 8)	40.45 (45.40)	25.00 (32.30)	30.40	26.33	0.90	1.90
Bacillus sp. (PDBCEN 9)	23.02 (25.60)	42.24 (45.60)	36.36	27.46	0.33	0.96
Pseudomonas sp. (PDBCEN 10)	36.66 (42.20)	40.28 (56.00)	33.03	24.00	1.00	1.16
Control	0.00	0.00	51.46	48.00	0.00	0.00
CD (P=0.05)	3.11	7.29	2.50	5.85	0.32	0.75

Figures in parentheses are angular transformed values.

Observation on the screening of endophytic bacteria against *Fusarium udum* in dual culture (Table 2) revealed that *Pseudomonas* sp. (PDBCEN 8) showed maximum (40.45%) inhibition on PDA. The rest of the isolates exhibited comparatively low inhibition of *F. udum* on PDA. *Pseudomonas* sp.

(PDBCEN 2) was highly effective on TSA and showed maximum (56.9%) inhibition zone, whereas, endophytic bacterium (PDBCEN 7) was least effective (16.56 %). *Pseudomonas* sp. (PDBCEN 6) allowed maximum fungal growth (42.03 sq. mm), but minimum growth (30.40 sq. mm) was recorded with *Pseudomonas* sp. (PDBCEN 8) on PDA. Differential radial growth pattern of *F. udum* was observed on TSA, maximum radial growth of the pathogen (32.73 sq. mm) was recorded with *Pseudomonas* sp. (PDBCEN 4) and minimum (20.63 sq. mm) with the test isolate *Pseudomonas* sp. (PDBCEN 2). In dual agar test on PDA growth of *Pseudomonas* sp. (PDBCEN 2) was maximum (4.26 sq. mm) whereas, it was least (1 sq. mm) with *Pseudomonas* sp. (PDBCEN 10). Endophytic bacterial growth was again more on TSA than on PDA.

Results on screening of endophytic bacteria against *Rhizoctonia solani* under dual culture test are presented in Table 3. Data revealed that maximum inhibition (44.96%) was recorded with endophyte PDBCEN 7 and minimum (16.79%) with *P. fluorescens* (PDBCEN 1) on PDA. Inhibition of *R. solani* was more on TSA and all the ten endophytic bacteria were effective in restricting the growth of test fungus and maximum (72.57%) growth inhibition was observed with *Pseudomonas* sp. (PDBCEN 1) and minimum (18.66%) with *P. fluorescens* (PDBCEN 10).

Results presented in Table 4 reveal that percent inhibition of *S. rolfsii* was maximum (40.93%) with *Pseudomonas* sp. (PDBCEN 6) on PDA and minimum 25.6% with *Pseudomonas* sp. (PDBCEN 4). On TSA, inhibition was maximum (46.73%) with *P. fluorescens* (PDBCEN 1) and

Table 3. Screening of Endophytic bacteria against *Rhizoctonia solani* under dual culture in different media

Endophytic bacterial isolate	Percent growth inhibition on PDA	Percent growth inhibition on TSA	Fungal radial growth on PDA (sq. mm)	Fungal radial growth on TSA (sq. mm)	Bacterial growth on PDA (sq. mm)	Bacterial growth on TSA (sq. mm)
P. fluorescens (PDBCEN 1)	16.79 (33.38)	47.31 (41.88)	66.96	17.80	1.07	3.53
Pseudomonas sp. (PDBCEN 2)	31.64 (30.57)	72.57 (36.58)	55.16	11.43	5.46	7.10
Bacillus subtilis (PDBCEN 3)	20.37 (37.94)	53.21 (27.14)	64.10	14.40	0.63	3.33
Pseudomonas sp (PDBCEN 4)	24.83 (32.60)	44.10 (36.81)	60.50	21.70	1.76	5.30
Endophyte (PDBCEN 5)	33.36 (27.49)	41.38 (46.27)	53.60	19.03	5.33	6.36
Pseudomonas sp. (PDBCEN 6)	36.38 (27.86)	46.53 (41.20)	51.56	28.00	2.26	4.30
Endophyte (PDBCEN 7)	44.96 (36.57)	54.86 (44.49)	44.33	14.73	2.96	4.96
Pseudomonas sp. (PDBCEN 8)	24.94 (29.20)	34.66 (37.57)	69.43	27.86	0.36	4.83
Bacillus sp. (PDBCEN 9)	20.70 (35.62)	43.06 (39.00)	55.60	20.06	0.20	1.46
Pseudomonas sp. (PDBCEN 10)	20.73 (32.99)	18.66 (41.01)	63.66	30.73	3.10	2.76
Control	0.00	0.00	80.46	37.66	0.00	0.00
CD (P=0.05)	2.34	5.50	2.78	6.51	0.37	0.86

Figures in parentheses are angular transformed values.

Table 4. Screening of various endophytic bacterial isolate against *Sclerotium rolfsii* under dual culture in different media

Endophytic bacterial isolate	Percent growth inhibition on PDA	Percent growth inhibition on TSA	Fungal radial growth on PDA (sq. mm)	Fungal radial growth on TSA (sq. mm)	Bacterial growth on PDA (sq. mm)	Bacterial growth on TSA (sq. mm)
P. fluorescens (PDBCEN 1)	33.23 (35.20)	46.93 (43.23)	39.73	32.20	1.53	2.53
Pseudomonas sp. (PDBCEN 2)	35.00 (36.27)	38.26 (43.23)	43.70	39.76	4.20	4.70
Bacillus subtilis (PDBCEN 3)	32.96 (35.04)	37.13 (38.15)	36.00	31.76	1.66	2.80
Pseudomonas sp (PDBCEN 4)	25.60 (30.32)	32.06 (37.54)	40.30	34.26	1.86	2.93
Endophyte (PDBCEN 5)	35.20 (36.39)	38.33 (34.47)	37.00	30.26	3.43	4.76
Pseudomonas sp. (PDBCEN 6)	40.93 (39.78)	48.16 (38.20)	38.40	31.02	3.93	4.93
Endophyte (PDBCEN 7)	38.06 (38.09)	41.66 (43.97)	38.30	33.20	3.36	4.40
Pseudomonas sp. (PDBCEN 8)	32.83 (34.94)	40.60 (40.20)	32.83	27.60	2.93	3.36
Bacillus sp. (PDBCEN 9)	38.70 (38.47)	44.80 (39.55)	28.36	22.76	2.00	3.16
Pseudomonas sp. (PDBCEN 10)	30.00 (33.20)	36.10 (42.10)	30.20	26.53	3.20	3.53
Control	0.00	0.00	57.20	48.85	0.00	0.00
CD (P=0.05)	0.65	0.57	9.03	7.90	0.92	0.65

Figures in parentheses are angular transformed values.

minimum (32.06%) with *Pseudomonas* sp. (PDBCEN 4). Maximum fungal growth (43.73%) was recorded on PDA with *Pseudomonas* sp. (PDBCEN 4) and least 28.36sq.mm was recorded with *Bacillus* sp. (PDBCEN 9). On TSA, maximum (39.76%) fungal growth was noticed with endophyte *Pseudomonas* sp. (PDBCEN 2).

Misaghi and Donndelinger (1990) isolated endophytic bacteria from two cultivars of cotton and showed that the endophytes were present in seeds and various tissues of the plants during all stages of development. Hallman *et al.* (1997) defined bacterial endophytes as bacteria living in plant tissues that do not visibly harm the plant but rather could be beneficial. A relatively low proportion of candidate antagonists identified from *in vitro* tests show activity in preventing seedling disease and the degree of protection varies from complete to low (Linderman, 1993).

Variation in inhibitory affect on PDA and rapid growth of bacterial antagonists on TSA strongly suggests that competition for nutrition is the major mode of varying action of bacterial isolates in the inhibition of test against pathogens apart from production of antimicrobial compounds (Sivakumar and Subramaniam, 1999). Pleben *et al.* (1995) reported that endophyte *P. fluorescens* (isolate no. 14) isolated from bean after stringent surface disinfection inhibited growth of *S. rolfsii* and *R. solani* and *B. subtilis* isolated from onion inhibited *R. solani* and *Pythium ultimum*. The antagonistic activity of *Pseudomonas* sp. and *Bacillus* sp. as observed in the present study is consistent with the findings of Nejed and Johnson (2000), Sturz and Christie (1995) and Rajappan and Ramaraj (1999).

The biometrics of chickpea growth was recorded at 7th day on roll towel test wherein the endophytes were challenged with *F. oxysporum* f. sp. *ciceri* as seed treatment. The fungicide treated seeds recorded highest vigour index (947.5) whereas, lowest (485.1) was with *Bacillus* sp. (EN 9) and in control (161.1) (Table 5). Maximum germination percent (82.6%) was recorded with

Table 5. Evaluation of selected endophytic bacteria on chickpea plant growth

Endophytic bacterial isolate	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
P. fluorescens (PDBCEN 1)	55.6 (49.8)	6.5	4.3	600.5
Pseudomonas sp. (PDBCEN 2)	58.9 (50.20	5.2	3.5	512.4
Bacillus subtilis (PDBCEN 3)	61.6 (51.2)	3.8	4.2	492.8
Pseudomonas sp (PDBCEN 4)	50.1 (46.1)	6.2	4.4	531.1
Endophyte (PDBCEN 5)	59.8 (51.2)	4.0	3.0	418.6
Pseudomonas sp. (PDBCEN 6)	61.4 (52.1)	4.8	4.7	583.3
Endophyte (PDBCEN 7)	56.1 (49.6)	3.7	3.5	404.1
Pseudomonas sp. (PDBCEN 8)	61.3 (52.1)	4.8	4.1	545.7
Bacillus sp. (PDBCEN 9)	66.0 (52.8)	3.0	2.9	389.4
Pseudomonas sp. (PDBCEN 10)	58.9 (50.2)	5.1	3.5	506.5
Control	40.8 (39.9)	2.7	2.1	196.1
Fungicide	75.2 (61.2)	6.7	5.9	947.5
CD (P=0.05)	1.98	0.48	0.3	7.03

Figures in parentheses are angular transformed values.

Bacillus subtilis (EN 9) and lowest seed germination (40.8%) was noticed in check. Maximum root length 6.2cm was recorded with *Pseudomonas* sp. (EN 3) and maximum shoot length 4.7cm was recorded with *P. fluorescens* (EN 6). Nejed and Johnson (2000) showed that endophytic bacteria were able to suppress the development of the wilt pathogen *F. oxysporum* f. sp. *lycopersici.* In the present study endophytic isolates were able to promote better growth of chickpea. The vigour index also varied. We could not correlate high pathogen inhibition under *in vitro* with high vigour index.

Shushmitha and Gaikwad (1995) isolated endophytic bacteria from healthy seeds of pigeonpea. Antagonistic bacteria showed no adverse effect on pigeonpea seed germination. Seeds coated with the antagonists germinated better than untreated seeds and produced longer root and shoot when sown in either wilt infested or sterilized soil. Vidhyasekaran *et al.* (1997) obtained effective control of pigeonpea wilt caused by *F. udum* using talc-based formulation of *P. fluorescens*. Our findings were consistent with the findings of Zhengqing *et al.* (1999), wherein they reported the successful control of cotton wilt (*Verticillium dahliae*) and better cotton seed germination by endophytic bacteria isolated from cotton tissues. Recently, Manoranjitham *et al.* (1999) reported that application of *Trichoderma viride* and *P. fluorescens* either individually or in combination, highly reduced the pre and post emergence damping off of chilli and increased the root length; shoot length and dry matter production of chilli seedlings.

In this study, all the ten endophytic bacteria inhibited the growth of *F. oxysporum* f. sp. *ciceri, F. udum, R. solani* and *S. rolfsii* in dual culture test in both the media. The level of inhibition however, varied among isolates. An overall analysis of data obtained with seed inoculation studies has shown

that prior seed inoculation with *F. oxysporum* f. sp. *ciceri* and subsequent treatment with antagonists is not effective in inhibiting the pathogen. Hence seeds have to be first treated with the antagonist so as to allow it to colonize the plant first.

Endophytic bacteria residing in roots and stems of plants play a role in disease suppression. Some of them are also plant growth promoters. The capacity of these endophytic bacteria to protect plants from disease by merely treating seeds is indicative of their root/plant colonizing ability, and seed treatment with endophytes prior to sowing is recommended for biocontrol of wilt diseases. Moreover, treatment of seeds with endophytes prior to sowing should be advocated to farmers and this will go a long way in strengthening biocontrol approaches to combat plant diseases.

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