



## Efficacy of endophytic *Pseudomonas fluorescens* (Trevisan) migula against chilli damping-off.

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### ABSTRACT

Nine bacterial endophytes were isolated from stem and root portions of chillies and tested for their efficacy against *Pythium aphanidermatum* (Edson) Fitzp. inciting chilli damping-off under glasshouse condition. Out of these nine bacterial endophytes, EBC 5, EBC 7 and EBC 6 recorded the minimum mycelial growth (28.00, 30.66 and 33.33 mm, respectively) with maximum inhibition zone of (12.33, 11.66 and 11.08 mm, respectively) of pathogen over control. In the present study, chilli seeds treated with these endophytes in combination (EBC 5 and EBC 6) recorded the lowest incidence of pre and post-emergence damping-off (9.10 and 12.33 per cent, respectively) at seven and 14 days after sowing when compared to individual treatment. This was followed by seed treatment with EBC 5 and EBC 7 in combination. The combination (EBC 5 and EBC 6) treatment also increased the germination percentage, shoot length and root length of chilli plants significantly (87.66%, 13.89 and 4.0 cm, respectively). Further, this writer concluded that the combination of endophytes were more effective in controlling disease when compared to individual treatments.

### INTRODUCTION

Chilli is a universal spice of India. In India it is cultivated over an area of 9.15 lakh ha with an annual production of 10.18 lakh tonnes of dry chilli (Anonymous, 2007). This crop is being affected by several fungal, bacterial and viral diseases. Among the fungal disease, damping-off incited by *Pythium aphanidermatum* (Edson) Fitzp. is very common and cause & serious loss in chilli production. It cause & 60 per cent mortality of the seedlings both in nursery and main field (Manoranjitham *et al.*, 2000).

In recent years the focus has shifted to the control of insect pests and diseases using bio-control agents, which are a safe and promising alternative to synthetic pesticides. There is some evidence that endophytes can contribute to the control of plant disease (Klopper *et al.*, 1992; Ramesh *et al.*, 2009). In India, limited work has been done on the isolation of endophytic bacteria *viz.*, *Pseudomonas fluorescens* (Trevisan) Migula and *Bacillus subtilis* (Ehrenberg.) from stem and roots of chilli seedlings (Muthukumar, 2008); *Bacillus sp.*, *P. fluorescens* and *Erwinia herbicola* (Dye.) from chickpea (Rangeshwaran *et al.*, 2008). 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; Nejed and Johnson,

2000; Rong-lin He *et al.* 2009). In this study the biological control potential of endophytic bacteria isolated from stem and root portions of chilli seedlings.

### MATHODOLOGY

#### Seed material, pathogen and endophytic bacterial isolates

The chilli cultivar Coimbatore-1 (Co-1), obtained from the Department of Olericulture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India. Bacterial endophytes were isolated from stem and root portions of chilli plants. Isolation was done as per the method described by Rajendran *et al.* (2006). Totally 9 isolates were obtained from chilli plants and designated as EBC 1-4 from stem and EBC 5-9 from root (Endophytic Bacteria of Chilli).

#### Identification of effective endophytic bacteria

The effective endophytic bacterial isolates were subjected to various bio-chemical tests like gram staining, motility, starch hydrolysis, gelatin hydrolysis, fluorescent pigment nitrate reduction and KOH solubility test (Karuna Vishunavat and Kolte, 2005). Based on the dual culture technique the effective endophytic bacterial isolates were used for further studies.

#### Isolation of pathogen and *in vitro* assay

Soil samples were collected from nursery area of Annamalainagar. The unit size of soil samples was 1kg. The collected sample was filled in 15 x 30 cm diameter

earthen pots. Earthen pots were sown with chilli seeds. Approximately 150 seeds were sown uniformly in pots. The pots were watered and maintained at 80 per cent water holding capacity using gravimetric method. The pots were maintained in greenhouse at  $28 \pm 2^\circ\text{C}$  with 12 hour of light and 12 hour of darkness. After 10 days, seedlings expressing the symptoms from pots were collected on a tissue paper separately and the pathogens associated with diseased samples were isolated by tissue segment method on potato dextrose agar medium. Later the isolate was purified in plain agar medium by single hyphal tip method (Rangaswami, 1958). The purified isolate was identified as *Pythium aphanidermatum* (Reference no. 1495.07) at National Centre of Fungal Taxonomy (NCFT), Indian Agricultural Research Institute (IARI), New Delhi, India. The antagonistic activity of bio-control agents against *P. aphanidermatum* was tested by dual culture technique (Dennis and Webster, 1971) using PDA (Potato Dextrose Agar) medium. The per cent inhibition of mycelial growth was calculated according to Nakkeeran *et al.* (2006).

Preparation of bacterial inoculum was done by the method suggested by Rajendran *et al.* (2006). The method suggested by Vidhyasekaran *et al.* (1996) was followed for the seed bacterization was done by the method suggested by Rajendran *et al.* (2006).

#### Glasshouse study

Sterilized soil (1.0 kg) was mixed with the pathogen inoculum @ 100 g (multiplied on sand maize medium) and filled in 15 x 30 cm diameter earthen pots. Surface sterilized chilli seeds were separately treated with the talc-based formulation of the antagonists. The experiment was conducted in a randomized block design and replicated thrice. The treated seeds were sown in pathogen inoculated soil @ 150 seeds per pot and irrigated daily. Pathogen alone inoculated pots served as control. The observations on the incidence of pre-emergence damping-off was recorded on seventh day of sowing and the incidence of post-emergence damping-off was recorded on 14<sup>th</sup> day after sowing. The shoot length and root length (cm) of the plants were recorded at 25 days after sowing. The seedling vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973).

#### Statistical Analysis

The data generated were transformed into arcsine values for statistical scrutiny, wherever necessary (Gomez and Gomez, 1984). The experiments were subjected to statistical scrutiny following the method of Gomez and Gomez (1984).

#### RESULTS

The results presented in the Table 1 revealed varying degree of antagonism by the endophytic bacterial isolates against *P. aphanidermatum*. Among the isolates tested, EBC 5 recorded the maximum inhibition zone of 12.33mm with a minimum of 28.00mm mycelial growth of *P. aphanidermatum* accounting for 68.88 per cent of the mycelial growth inhibition over control. This was followed by EBC 7 and EBC 6 in combination. The other isolates were less effective in inhibiting the mycelial growth of *P. aphanidermatum* under *in vitro*.

The data presented in the Table 2 revealed that seed treatment with antagonists either alone or in combination showed significant influence on the incidence of chilli damping-off. Among the various treatments with antagonists, seed treatment with EBC 5 and EBC 6 in combination recorded minimum pre and post-emergence damping-off of 9.10 and 12.33 per cent, respectively, which was on par with metalaxyl treatment. It was followed by seed treatment with EB 5 and EB 7 recorded 10.33 and 12.70 per cent of pre and post-emergence damping-off, whereas, seed treatment with individual bacteria was not highly effective in reducing the incidence of chilli damping-off. The maximum disease incidence of pre and post-emergence damping-off was observed in control.

Among the treatment with antagonist, endophytic bacteria in combination (Table 3) recorded maximum germination percentage of 87.66 per cent and increased the shoot length of 13.89 cm, root length of 4.00 cm and vigour index of 1221.59, respectively. It was on a par with metalaxyl treatment. This was followed by seed treatment with EBC 5

**Table 1.** *In vitro* inhibition of mycelial growth of *P. aphanidermatum* by endophytic bacterial chilli (EBC) isolates

Isolates	Mycelial growth (mm)	Inhibition zone (mm)	Per cent inhibition over control
EBC 1	41.60	9.00	53.77
EBC 2	39.66	9.66	55.93
EBC 3	45.33	8.53	49.63
EBC 4	46.00	8.23	48.88
EBC 5	28.00	12.33	68.88
EBC 6	33.33	11.08	62.96
EBC 7	30.66	11.66	65.93
EBC 8	42.33	8.66	52.96
EBC 9	37.80	10.00	58.00
Control	90.00	-	-
Sed	0.49	0.03	-
CD (p = 0.05)	0.98	0.09	-

**Table 2.** Effect of seed treatment with effective endophytic *P. fluorescens* on the incidence of chilli damping-off under pot culture

Treatments	% incidence of damping-off	
	Pre emergence	Post-emergence
Seed treatment with EBC 5	15.13 (23.67)	15.64 (23.29)
Seed treatment with EBC 6	17.63 (23.28)	18.35 (22.26)
Seed treatment with EBC 7	16.69 (20.82)	17.33 (25.34)
Seed treatment with EBC 5 and EBC 6	9.10 (17.55)	12.33 (20.55)
Seed treatment with EBC 6 and EBC 7	13.00 (21.13)	15.00 (25.10)
Seed treatment with EBC 5 and EBC 7	10.33 (18.49)	12.70 (20.82)
Seed treatment with Metalaxyl (0.1%)	9.00 (21.419)	12.40 (27.55)
Control	30.66 (33.62)	34.00 (35.66)
SEd	0.50	0.12
CD (p = 0.05)	1.10	0.31

and EBC 7 in combination. Germination percentage, shoot length, root length and vigour index was the least in control.

**DISCUSSION**

The present study revealed that EBC 5 was highly inhibitory to the growth of pathogen when compared to other isolates tested. Similarly, Muthukumar (2008) observed that the endophytic bacteria stem isolate-5 significantly reduced the colony growth of *P. aphanidermatum*. Muthukumar and Bhaskaran (2007) revealed that among the 12 isolates of *P. fluorescens* tested, *P. fluorescens* 3 and 4 were highly effective in inhibiting the mycelial growth of *Pythium* sp. Nakkeeran *et al.* (2006) reported that *P. chlororaphis* strain PA23 and *B. subtilis* strain BSCBE4 showed the maximum inhibitory effect on the mycelial growth of *P. aphanidermatum* causing chilli damping-off. Rangeshwaran *et al.*

(2002) reported that endophytic *Pseudomonas* sp. (PDBCEN 8) showed maximum inhibition of *Fusarium udum* (Pigeon pea). The same author also reported that the endophytic *Pseudomonas* sp. (PDBCEN 3) showed maximum inhibition against tomato wilt caused by *F. oxysporum* f.sp. *lycopersici*. The mycoparasitic potential of *Pseudomonas* spp. is well documented by earlier workers (Anitha and Tripathi, 2001; Bhowmik *et al.*, 2002). Several other workers have also reported that the production of antifungal compounds by fluorescent pseudomonads that were responsible for suppression of plant pathogenic fungi (Padmodaya, 1994; Gupta *et al.*, 2001). This indicates that *P. fluorescens* produced antifungal compound in different concentration. Parasitism is one of the major mechanisms involved in the biological control of plant pathogens by *P. fluorescens*. Several cell wall degrading enzymes such as chitinase and  $\alpha$ -1, 3-glucanase are involved in this process and they are capable of degrading chitin and  $\alpha$ -1,3-glucan respectively, the major components of fungal cell walls. Hence in the present study demonstrated that the mycelial growth inhibition occurred due to production of appropriate deleterious antifungal metabolites produced by *P. fluorescens* which caused inhibition of *P. aphanidermatum*.

The present study also indicated that endophytic bacteria in combination (EBC 5 and EBC 6) recorded less disease incidence when compared to individual treatment. The combined treatment also increased the germination percentage and plant growth. The ability of bio-control agents as seed coating is probably associated with the ability to grow and sporulate on germinating seed and ultimately colonize in the rhizosphere which hinder the pathogen to establish in germinating seeds and thereby reduce damping-off (Hazarika *et al.*, 2000). Similarly, Harris *et al.* (1994) reported that chilli seeds treated with *P. fluorescens* reduced the damping-off of *Capsicum* and increased shoot length. Enhanced plant growth by the

**Table 3.** Effect of seed treatment with effective endophytic *P. fluorescens* on the growth of chilli seedlings under pot culture

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
Seed treatment with EBC 5	80.66 (63.91)	11.01	3.30	891.36
Seed treatment with EBC 6	74.33 (58.26)	9.93	2.80	740.89
Seed treatment with EBC 7	76.00 (60.66)	10.20	2.90	778.10
Seed treatment with EBC 5 and EBC 6	87.66 (68.86)	13.89	4.00	1221.59
Seed treatment with EBC 6 and EBC 7	84.33 (66.68)	11.09	3.58	938.79
Seed treatment with EBC 5 and EBC 7	85.66 (68.57)	12.09	3.70	1039.32
Seed treatment with Metalaxyl (0.1%)	87.00 (58.05)	9.53	2.90	832.01
Control	62.00 (51.94)	9.43	2.10	586.76
SEd	0.43	0.03	0.22	-
CD (p= 0.05)	0.89	0.07	0.45	-

siderophore producing strains of fluorescent pseudomonads was reported by John Davison (1988) and Gnanamanickam *et al.* (1992). Seed bacterization with plant growth promoting isolates of *P. fluorescens* enhanced the plant growth parameters and yield attributes of peanut. Besides the content of the nitrogen and phosphorus in the soil was also enhanced significantly (Dey *et al.*, 2004). *Pseudomonas fluorescens* was found better in increasing the germination percentage, dry weight, leaf area and chlorophyll content of mungbean (Dutta *et al.*, 2005). *P. fluorescens* might have stimulated the plant growth by improving uptake of minerals into the host plants particularly phosphate (Kloepper *et al.*, 1980), siderophore mediated iron uptake (Jurkevitch *et al.*, 1988), association with nitrogen fixation (Hong *et al.*, 1991), production of IAA (Dubeikovsky *et al.*, 1993), promotion of mycorrhizal function (Garbaye, 1994), regulating ethylene production in roots (Glick, 1995) and solubilizing nutrients such as phosphorus (Whitelaw, 2000). Bhowmik *et al.* (2002) reported that seed bacterisation with one of the endophytes (Endo PR 8) reduced damping-off disease of cotton caused by *R. solani* and *S. rolfsii*. Ziedan (2006) revealed that bacterial treatment of peanut seeds before sowing (soaking of bacterial suspensions) resulted in reduced *Aspergillus niger* and *F. oxysporum* colonization over peanut seed at 30 days after harvesting.

Moreover, a better root system (increased root length) in seeds treated with *P. fluorescens* as observation in the present study might have tolerated or escaped from root infection facilitating an active absorption of nutrients thereby promoting the plant growth and health. Thus, the results of the present study and the earlier reports have confirmed that the growth promoting substances and plant growth hormones *viz.*, IAA, auxins, cytokinins and gibberellic acid etc., produced by *P. fluorescens* were responsible for the increased plant growth. Further, the growth hormones and metabolites produced by these antagonists in combination have exerted synergism in promoting the plant growth and suppression of pathogen.

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