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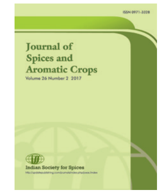

Bacillus subtilis, a potential biocontrol agent for the management of coriander wilt

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Abstract

Coriander is an important spice crop affected by wilt caused by *Fusarium oxysporum* f. sp. *corianderii.* Two trials were conducted to evaluate the efficacy of *Bacillus subtilis* for the management of wilt under glass house condition. The results of the trials indicated that, among the various treatments, seed treatment with talc formulation of *Bacillus subtilis* (10 g kg^{-1} of seed) along with soil application (2.5 kg ha^{-1}) as basal and top dressing at 30 and 45 days after sowing was effective in reducing the wilt incidence (14.59%) compared to control (32.68%).

Keywords: coriander, wilt, *Fusarium oxysporum* f. sp. *corianderii,* biocontrol, *Bacillus subtilis*

Coriander (*Coriandrum sativum* L.) one of the important seed spices belonging to the family Apiaceae is mainly cultivated in Rajasthan, Gujarat, Haryana, Punjab, Madhya Pradesh, Uttar Pradesh, Andhra Pradesh, Karnataka and Tamil Nadu.

Pests and diseases are the major constraints in the production of coriander. Coriander cultivation is affected by several diseases like wilt caused by *Fusarium oxysporum* f.sp. *corianderii* (Srivastava 1972), stem gall caused by *Protomyces macrosporus* (Das 1971), grain mould caused by *Helminthosporium* spp., *Fusarium* spp., *Curvularia* spp. and *Alternaria* spp. (Rajan *et al.* 1990) and powdery mildew (*Erisyphe polygoni*).

The occurrence of coriander wilt has been reported from few parts of India *viz*., Gwalior and Guna districts of Madhya Pradesh, Kota division of Rajasthan (ICAR report 1953) and Coimbatore district of Tamil Nadu (Prakasam *et al*. 1987). Among the diseases, wilt caused by *Fusarium oxysporum* f. sp. *corianderii* causes a yield loss of 10-60% (Prakasam *et al*. 1987). The infected plant exhibits drooping of leaves, the characteristic symptoms along with vascular discolouration.

It has been found that members of the genus *Bacillus* has been successfully used in controlling plant diseases in a wide variety of crops including rice (Peng *et al*. 2014), wheat (Liu *et al*. 2009), potato (Balabel *et al*. 2013), brinjal (Saha *et al*. 2012), and cucumber (Huang *et al*. 2012).

The present study was conducted to evaluate the effectiveness of *Bacillus subtilis* for the management of coriander wilt under glass house condition.

The wilt pathogen *Fusarium oxyspoum* f.sp. *corianderii* was isolated from infected plants and the different strains of *Bacillus subtilis* were isolated from rhizosphere of healthy coriander plants by following serial dilution technique in Nutrient Agar medium and some *Bacillus* strains were also obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University. The isolated *Bacillus* spp. were identified based on morphological characters and by molecular methods (sequencing). The *Bacillus* strains were tested for their inhibitory activity on mycelial growth of the pathogen by dual plate technique (Dennis & Webster 1971). The effective strain of *B. subtilis* (VB1) was selected based on their inhibitory activity on mycelial growth of the pathogen (data not presented).

A loopful of effective *B. subtilis* (VB1) was inoculated into sterilized Nutrient agar broth and incubated in a rotary shaker at 150 rpm for 72 h at room temperature (28 ± 2^0C). After 72 h, 400 mL of bacterial broth suspension (9 × 10^8 cfu mL^{-1}) was mixed with 1 kg of the talc powder (carrier material), 15 g calcium carbonate (to adjust pH to neutral) and 5 g CMC (adhesive) under sterile conditions. The mixture was shade dried and packed in polythene bags and kept at room temperature condition (Vidhyasekaran & Muthamilan 1995).

During *Kharif* & *Rabi* 2015, two glass house trials were conducted at TNAU orchard to test the efficacy of *B. subtilis* strain for the management of wilt disease. The virulent isolate (Isolate from Mettupalayam – Foc 1) of wilt pathogen was mass multiplied in sand maize medium and it was mixed with sterilized potting mixture at the ratio of 5% (w/w). Surface sterilized coriander seeds were treated with bacterial antagonist formulations (*B. subtilis* @10 g kg^{-1} of seeds, *Pseudomonas fluorescens* @10 g kg^{-1} of seeds) and sown in pathogen inoculated 30 cm diameter pots. Ten seeds (variety: CO 4) were sown pot^{-1} and three replications per treatment were maintained. Observations were recorded on germination percentage, plant height, disease incidence and seed yield.

The experiment was conducted in completely randomized block design and replicated thrice. The incidence of wilt was assessed using the formula:

Per cent Disease Incidence = [Number of infected plants / Total number of plants] × 100

The results of glass house trial conducted during *Kharif* 2015 revealed that, the germination percentage ranged from 74% to 89%. The observation on growth parameter *i.e.,* plant height was recorded on 60 DAS, which ranged from 46 to 53 cm.

The disease incidence was recorded on 45 and 60 days after sowing (DAS). The lowest wilt incidence (16.33%) was recorded in seed treatment combined with soil application of *B. subtilis* (VB1), while in control the incidence was 35.56% (Table 1).

The results of the *Rabi* trial indicated that, the lowest wilt incidence (12.85%) was recorded in seed treatment combined with soil application of *B.subtilis* (VB1), while in control it was 29.81% (Table 2).

The pooled mean of two trials indicated that, seed treatment with talc formulation @ 10 g kg^{-1} of seed and soil application @2.5 kg ha^{-1} significantly reduced the wilt incidence (14.59%), compared with control (32.68%), followed by seed treatment and soil application of *P. fluorescens* (16.24%). This treatment also recorded grain yield of 85.97 g pot^{-1} compared to control which recorded 48.67 g pot^{-1} (Table 3).

In the glass house studies, application of *B. subtilis* as seed treatment (10 g kg^{-1}) and soil application (2.5 kg ha^{-1}) as basal and top dressing at 30 and 45 DAS, was found to be effective in reducing wilt incidence in coriander under glass house condition.

Ashwini & Srividya (2014) studied the potentiality of *B.subtilis* as biocontrol agent for management of anthracnose disease of chilli caused by *Colletotrichum gloeosporioides.* Mezeal (2014) revealed that *B. subtilis* was effective

Table 1. Effect of *B. subtilis* strain VB1 on the incidence of wilt under glass house conditions (*Kharif* 2015)

Treatments	Germination (%)	Plant height (cm) 60 DAS*	Wilt incidence (%) 60 DAS*	Yield (g pot^{-1})
ST with *B. subtilis* VB1	86.17^{c}	48.35^{c}	23.12^{bcd}	70.69^{b}
SA with *B. subtilis* VB1 (Basal)	76.06^{h}	49.32^{b}	24.12^{bc}	74.81^{b}
ST + SA with *B. subtilis* VB1 (Basal)	88.49^{a}	52.97^{a}	18.48^{cd}	76.70^{ab}
ST + SA with *B. subtilis* VB1 (Basal & top dressing)	87.78^{b}	53.53^{a}	14.59^{e}	85.97^{a}
ST with *P. fluorescens* Pf1	77.00^{g}	47.18^{c}	22.71^{bc}	72.49^{b}
SA with *P. fluorescens* Pf1 (Basal)	85.68^{d}	49.28^{b}	22.27^{b}	73.24^{b}
ST + SA with *P. fluorescens* Pf1 (Basal)	84.21^{e}	53.85^{a}	18.22^{cd}	79.36^{ab}
ST + SA with *P. fluorescens* Pf1 (Basal & top dressing)	84.12^{e}	53.45^{a}	16.24^{de}	83.24^{a}
ST with carbendazim 0.1%	78.92^{f}	46.66^{d}	24.27^{bcd}	75.07^{b}
ST + SA with carbendazim 0.1%	85.01^{d}	49.23^{b}	21.22^{cd}	77.20^{ab}
Control	77.57^{g}	47.29^{c}	32.68^{a}	48.67^{c}

ST=Seed treatment @10 g kg^{-1} of seeds; SA=Soil application @2.5 kg ha^{-1}; *DAS=Days After Sowing

*Values are mean of three replications.

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 2. Effect of *B. subtilis* strain VB1 on the incidence of wilt under glass house conditions (*Rabi* 2015)

Treatments	Germination (%)	Plant height (cm) 60 DAS*	Wilt incidence (%) 60 DAS*	Yield (g pot^{-1})
ST with *B. subtilis* VB1	85.61^{c}	48.16^{c}	23.91^{bcd}	70.10^{b}
SA with *B. subtilis* VB1 (Basal)	78.12^{f}	48.98^{c}	21.80^{bc}	75.18^{b}
ST + SA with *B. subtilis* VB1 (Basal)	87.97^{a}	53.76^{b}	16.57^{cd}	76.83^{ab}
ST + SA with *B. subtilis* VB1 (Basal & top dressing)	87.78^{a}	53.81^{b}	12.85^{e}	83.58^{a}
ST with *P. fluorescens* Pf1	75.00^{g}	47.03^{d}	21.55^{bc}	72.10^{b}
SA with *P. fluorescens* Pf1 (Basal)	87.72^{a}	48.81^{c}	18.07^{b}	72.80^{b}
ST + SA with *P. fluorescens* Pf1 (Basal)	85.56^{c}	54.16^{a}	15.87^{cd}	79.85^{ab}
ST + SA with *P. fluorescens* Pf1 (Basal & top dressing)	82.12^{d}	54.10^{a}	13.16^{de}	80.80^{a}
ST with carbendazim 0.1%	79.18^{e}	46.78^{e}	25.98^{bcd}	74.98^{b}
ST + SA with carbendazim 0.1%	86.02^{b}	48.83^{c}	22.10^{cd}	75.85^{ab}
Control	79.14^{e}	47.03^{d}	29.81^{a}	46.89^{c}

ST=Seed treatment @10 g kg^{-1} of seeds; SA=Soil application @2.5 kg ha^{-1}; *DAS=Days After Sowing

*Values are mean of three replications.

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 3. Effect of *B. subtilis* strain VB1 on the incidence of wilt under glass house conditions (Pooled mean of 2 trials)

Treatments	Germination (%)	Plant height (cm) 60 DAS*	Wilt incidence (%) 60 DAS*	Yield (g pot^{-1})
ST with *B. subtilis* VB1	86.17^{c}	48.35^{c}	23.12^{bcd}	70.69^{b}
SA with *B. subtilis* VB1 (Basal)	76.06^{h}	49.32^{b}	24.12^{bc}	74.81^{b}
ST + SA with *B. subtilis* VB1 (Basal)	88.49^{a}	52.97^{a}	18.48^{cd}	76.70^{ab}
ST + SA with *B. subtilis* VB1 (Basal & top dressing)	87.78^{b}	53.53^{a}	14.59^{e}	85.97^{a}
ST with *P. fluorescens* Pf1	77.00^{g}	47.18^{c}	22.71^{bc}	72.49^{b}
SA with *P. fluorescens* Pf1 (Basal)	85.68^{d}	49.28^{b}	22.27^{b}	73.24^{b}
ST + SA with *P. fluorescens* Pf1 (Basal)	84.21^{e}	53.85^{a}	18.22^{cd}	79.36^{ab}
ST + SA with *P. fluorescens* Pf1(Basal & top dressing)	84.12^{e}	53.45^{a}	16.24^{de}	83.24^{a}
ST with carbendazim 0.1%	84.12^{e}	53.45^{a}	16.24^{de}	83.24^{a}
ST + SA with carbendazim 0.1%	85.01^{d}	49.23^{b}	21.22^{cd}	77.20^{ab}
Control	77.57^{g}	47.29^{c}	32.68^{a}	48.67^{c}

ST=Seed treatment @10 g kg^{-1} of seeds; SA=Soil application @2.5 kg ha^{-1}; *DAS=Days After Sowing

*Values are mean of three replications.

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

against *Rhizoctonia solani* and *F. oxysporum* causing wilt disease in tomato. Lin *et al*. (1990) reported that *B. subtilis* inhibited the growth of *Fusarium oxysporum* f. sp. *niveum* (watermelon wilt) under *in vitro* and pot culture studies.

In the present study, application of bio control agents at different stages of crop as seed treatment and soil application at 30 and 45 DAS, supported the population load of antagonistic organisms in the rhizosphere region, thereby suppressing the pathogen load and disease incidence.

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