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Growth promoting ability and Bioefficacy of *Bacillus subtilis* against powdery mildew and early blight of Tomato through Foliar Spray in pot culture studies

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Abstract
A pot culture experiment was conducted during *kharif* 2014 to study the efficacy and growth promoting ability of *Bacillus subtilis* against powdery mildew (*Leveillula taurica*) and early blight (*Alternaria solani*) of tomato. Talk based formulation of *B. subtilis* was sprayed four times (15, 40, 65 and 90 days after sowing) at 2, 4, 6, 8, 10g/l of water along with *Pseudomonas fluorescens* and *Trichoderma harzianum* at 6g/l of water. Treated (Saaf 2.5g/l) and untreated checks were also maintained in the study. The foliar spray of *B. subtilis* at 10g/l recorded the least per cent disease index of powdery mildew (13.22) and early blight (11.25) and there by recorded highest number of branches (6.90), fruits (16.86), fruit weight per plant (898.76g). Whereas, untreated check was least effective with highest per cent disease index of powdery mildew (54.89) and early blight (32.22) and recorded less number of branches (3.60), fruits (11.99) and fruit weight (615.00g).

Keywords: *Bacillus subtilis*, tomato, powdery mildew, early blight

1. Introduction
Tomato (*Solanum lycopersicum* Mill.) is most popular vegetable crop grown in the world, next to potato. It is used as a fresh vegetable and processed and canned as a paste, juice, sauce, powder or as a whole (Barone and Frusciante, 2007) [7]. The ripe fruits are good source of vitamin A, B and C which add wide varieties of colour and flavour to the food. Recently, it started gaining more medicinal value because of the antioxidant property (Anon., 2000) [4]. Several diseases appeared on tomato caused by fungi, bacteria, viruses, nematodes and a biotic factor (Balanchard *et al.,* 1992) [6]. Tomato plant suffers from many serious diseases under green house and field conditions. Among those the early blight (*Alternaria solani)* and powdery mildew *(Leveillula taurica*) are important foliar diseases causing the severe yield loss. The continuous and indiscriminate use of chemical pesticides for management of diseases has posed several serious problems such as pesticide residue, development of resistant strains, environmental pollution and adverse effect on beneficial microorganisms and created a greater concern over global food safety and security. Therefore in recent times biological control has emerged as a key principle in Integrated Disease Management.
Bacillus subtilis is very important bioagent used for the management of plant diseases. *B. subtilis* is a gram positive, motile, aerobic, rod shaped bacteria. It is a ubiquitous naturally occurring saprophytic bacterium that is commonly recovered from soil, water, air and decomposing plant material. Colony of *B. subtilis* is traditionally circular, with ragged edges, colored cream to white. It has ability to form a tough protective endospore, allowing the organism to tolerate extreme environmental conditions (Alexander, 1977) [3]. *Bacillus subtilis* is very effective against foliar diseases and it is becoming part of IDM. However, the research on management of diseases through foliar spray of *B. subtilis* limited and there is a need for development of information on bioefficacy of *B. subtilis*.

2. Materials and methods
In pot culture studies bioefficacy of *Bacillus subtilis* was tested against powdery mildew and early blight of tomato. The observations on severity of powdery mildew and early blight were assessed by scoring 2 plants (10 leaves/plant) per pot using disease rating scales given by Mayee and Datar (1986) [8] as mentioned below.

Alternaria leaf spot: 0-5 disease rating scale (Mayee and Datar, 1986) [8]

Scale	Description
0	No symptoms on the leaf
1	0-5 per cent leaf area infected and covered by spot, no spot on petiole and branches
2	6-20 per cent leaf area infected and covered by spot, some spots on petiole
3	21-40 per cent leaf area infected and covered by spot, spots also seen on petiole, branches
4	41-70 per cent leaf area infected and covered by spot, spots also seen on petiole, braches, stem
5	>71 per cent leaf area infected and covered by spot, spots also seen on petiole, branch, stem, and fruit.

Powdery Mildew: 0–9 disease rating scale (Mayee and Datar, 1986) [8].

Scale	Description
0	No visible symptom of powdery mildew on leaves
1	Small scattered powdery mildew specks covering 1per cent or less area
3	Small powdery lesions covering 1-10 per cent of leaf area
5	Powdery lesions enlarged covering 11-25 per cent of leaf are
7	Powdery lesions coalesce to form big patches covering 26-50 per cent of leaf area
9	Big powdery patches covering 51 per cent or more of leaf area and defoliation occurs

Per cent disease index (PDI) was calculated by using following formula proposed by Wheeler (1969) [10].

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of the individual disease ratings}}{\text{Number of leaves observed}} \text{ X } \frac{100}{\text{Maximum disease grade}}$$

Earthen pots with 15cm diameter were taken and disinfected with 4 per cent formalin and filled with sterilized soil. Two seeds of tomato were sown per pot, two pots per treatment were used for the study and three replications were maintained. Different bioagents with various concentrations were sprayed four times to suppress the disease at 15, 40, 65 and 90 DAS. The observation on number of branches, number of fruits and fruit weight per plant were recorded and disease severity was recorded at 60 days after germination

Treatments

Bioagents	Concentration (g/l)	Time of spray
Bacillus subtilis IOF	2.0	15, 40, 65 and 90 DAS
Bacillus subtilis IOF	4.0	15, 40, 65 and 90 DAS
Bacillus subtilis IOF	6.0	15, 40, 65 and 90 DAS
Bacillus subtilis IOF	8.0	15, 40, 65 and 90 DAS
Bacillus subtilis IOF	10.0	15, 40, 65 and 90 DAS
Pseudomonas fluorescens IOF	6.0	15, 40, 65 and 90 DAS
Trichoderma harzianum IOF	6.0	15, 40, 65 and 90 DAS
Carbendazim (12%) + Mancozeb (63%) (Saaf 75WP)	2.5	One spray after appearance of symptoms
Untreated control	-	-

DAS – Days after sowing

3. Results and discussion

The effect of *B. subtilis* on growth promoting ability and its efficacy against early blight and powdery mildew diseases in tomato through foliar spray with various concentrations along with other bioagents was assessed in pot culture. The sprays were taken up for four times at 15, 40, 65 and 90 DAS observation on disease incidence was recorded and PDI was calculated and results are presented in Table 1.

The results indicated that there was significant difference among all the treatments tested with respect to number of branches, number of fruits and fruit weight per plant. Among all the treatments tested, spray with *B. subtilis* 10g/l of water resulted in maximum number of branches (6.90), fruits (16.86) and maximum fruits weight per plant (898.76 g) followed by treatment with *B. subtilis* 8 g/l of water. However, least number of branches (3.60), number of fruits (11.99) and fruit weight per plant (615.00 g) was observed in control.

Among all the treatments tested the *B. subtilis* at 10g/l of water recorded least disease severity of powdery mildew (13.22%) compared to control (54.89%) and followed by treatment involving *B. subtilis* at 10g/l (18.30) and Carbendazim 12%+ Mancozeb 63% WP at 2.5g/l (18.92%) and both are on par with each other. With respect to early blight disease less disease severity (11.25%) was recorded in treatment *B. subtilis* 10g/l of water compared to control (32.22%) which was followed by treatment *B. subtilis* 6 g/l (13.36%) and Carbendazim 12%+Mancozeb 63%WP (14.63%).

The present results supported by Sundaramoorthy and Balabaskar where in they reported that foliar spray of *Bacillus subtilis* (EPCO16 and EPC5) and *Pseudomonas fluorescens* (Pf, Py15 and Fp7) strains significantly reduced the PDI of early blight of tomato (by 35- 61%) compared to untreated control, and increased the seed germination (by 88-96%). Conspicuously, the treatment with a combination of Pf1+EPCO16 resulted in a significantly lower early blight disease severity than any of the strains treated. Similar studies were conducted by Abdel-Kader *et al*. (2013) [1] while working with biological control of foliar diseases of cucumber, cantaloupe, tomato and hot pepper under green house condition. They reported that application with either *T.*

harzianum or with *B. subtilis* showed significant reduction in diseases incidence compared to other bio-agents applied.

The potential of *Bacillus* spp. to synthesize a wide variety of metabolites with antifungal activity is known and in recent years it has been a subject of experimentation (Ahimou *et al*., 2000) [2]. Most of these substances belong to lipopeptides, especially from surfactin, iturin and fengicin classes. Antibiotics of the iturin group were found to act upon the sterol present in the cytoplasmic membrane of the fungi and inhibit ergosterol biosynthesis, thereby control fungal development and kills the pathogen (Worthington, 1988) [11].

Table 1: Bioefficacy and growth promoting ability of *Bacillus subtilis* against powdery mildew and early blight diseases of tomato through foliar spray

Treatment	Concentration (g/l of water)	Time of spray	No. of branches per plant	No. of fruits per plant	Fruits weight per plant (g)	Per cent Disease Index	
						Powdery mildew	Early blight
Bacillus subtilis	2	15, 40, 65 and 90 DAS	4.40	13.67	718.66	37.93 (37.92)*	20.64 (27.01)
Bacillus subtilis	4	15, 40, 65 and 90 DAS	4.70	14.60	761.00	31.13 (33.90)	18.03 (25.12)
Bacillus subtilis	6	15, 40, 65 and 90 DAS	5.17	15.29	790.33	24.20 (29.46)	16.46 (23.93)
Bacillus subtilis	8	15, 40, 65 and 90 DAS	6.37	16.35	865.93	18.30 (25.32)	13.36 (21.43)
Bacillus subtilis	10	15, 40, 65 and 90 DAS	6.90	16.86	898.76	13.22 (21.31)	11.25 (19.59)
Pseudomonas fluorescens	6	15, 40, 65 and 90 DAS	4.83	15.21	787.82	26.18 (30.76)	17.23 (24.51)
Trichoderma harzianum	6	15, 40, 65 and 90 DAS	5.23	15.36	793.93	27.70 (31.74)	17.74 (24.90)
Carbendazim 63% +Mancozeb 12% WP	2.5	One spray after appearance of symptoms	4.6	14.10	717.43	18.92 (25.77)	14.63 (22.48)
Control	-	-	3.60	11.99	615.00	54.89 (47.79)	32.22 (34.57)
S. Em ±			0.07	0.21	2.64	0.20	0.19
CD at 1%			0.28	0.85	7.94	0.67	0.64

* Arcsine transformed values

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