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BIOEFFICACY OF *Bacillus subtilis* AGAINST FOLIAR FUNGAL DISEASES OF TOMATO

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Abstract

Pot culture experiment was conducted during kharif 2014 to study the efficacy and growth promoting ability of Bacillus subtilis against powdery mildew (Leviellula taurica) and early blight (Alternaria solani) of tomato. In pot culture experiment B. subtilis tested for its bioefficacy against early blight and powdery mildew diseases of tomato through different methods viz., seed treatment, seedling dip, foliar spray and soil application method. In seed treatment method, treatment involving B. subtilis at 8 g/kg of seed was most superior in promoting the growth and reducing the disease severity compared to other treatments. In seedling dip method, B. subtilis at 15 g/l of water recorded maximum number of branches, fruits and fruit weight per plant followed by B. subtilis at 10g/l when compared to other treatments. The results of present investigation revealed that foliar spray of B. subtilis at 10g/l of water sprayed for four times at 15,40, 65and 90 DAS recorded the less disease severity and also promote growth of the plants. In soil application method, combined application of FYM (50 g) + B. subtilis (4.00 g)/ pot was the best followed by neem cake (25 g) + B. subtilis (4.0 g)/ pot when compared to other treatments. Among all the methods foliar spray method was the most effective in reducing the disease severity of powdery mildew and early blight whereas, soil application of bioagent with organic amendments had increased growth of plant.

Key words: Bacillus subtilis, Tomato, Powdery mildew, Early blight

I. 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). 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). Several diseases appeared on tomato caused by fungi, bacteria, viruses, nematodes and a biotic factor (Balanchard *et al.,* 1992). 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). *Bacillus subtilis* is very effective against foliar diseases and it is becoming part of IDM. However, the research on management of diseases through use of *B. subtilis* limited and there is a need for development of information on bioefficacy of *B. subtilis* against foliar diseases.

II. 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 (alternaria leaf spot) were assessed by scoring 2 plants (10 leaves/plant) per pot using disease rating scales given by Mayee and Datar (1986) as mentioned below.

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

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).

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).

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

The bioefficacy of *B. subtilis* tested against early blight and powdery mildew disease of tomato by different application methods *viz.,* seed treatment, seedling dip and soil application methods. In all the methods sprayed the spores of *Leveillula taurica* and *Alternaria solani* (10^{-6}/ml) sprayed at 30 DAS and disease severity was recorded after 60 and 90 days after germination.

Seed treatment method

The pots with 15 cm diameter were taken and disinfected with 4 per cent formalin and filled with sterilised soil. Tomato seeds were coated uniformly with 4, 6 and 8 g of *Bacillus subtilis* talc based commercial formulation powder (containing 2 x 10^8 cfu / g) per kg of seeds obtained from IOF (Institute of Organic Farming), AC Dharwad and kept for 15 min for drying. Similar procedure was followed to treat the seeds with other organisms concentrations and chemical Carbendazim (Bavistin 50WP) and ten seeds of tomato were sown in each pot and number of seeds germinated were recorded after 7-8 days. Observation on shoot length, root length was recorded 30 days after germination. Two plants per pot were retained and two pots were maintained for each treatment and three replication were maintained. Inoculum of *Leveillula taurica* and *Alternaria solani* (10^{-6} /ml) were sprayed at 30 DAS and observation on number of branches, number of fruits and fruit weight per plant were recorded and disease severity was recorded 60 days after germination.

Treatments

Bioagents	Concentration (g/kg seed)
Bacillus subtilis IOF	4.0
Bacillus subtilis IOF	6.0
Bacillus subtilis IOF	8.0
Pseudomonas fluorescens IOF	6.0
Trichoderma harzianum IOF	4.0
Carbendazin 50WP	2.0
Control	-

Seedling dip method

Earthen pots with 15cm diameter were taken and disinfected with 4 per cent formalin and filled with sterilized soil. Thirty days old seedlings were dipped in solution of different organisms and their concentrations for 15 minutes. Two seedlings were planted in each pot and

two pots per replication were used for the study and three replications were maintained for each treatment.

Treatments

Bioagents	Concentation (g/l of water)
Bacillus subtilis IOF	5.0
Bacillus subtilis IOF	10.0
Bacillus subtilis IOF	15.0
Pseudomonas fluorescens IOF	5.0
Pseudomonas fluorescens IOF	10.0
Trichoderma harzianum IOF	5.0
Trichoderma harzianum IOF	10.0
Carbendazim 50WP	2.0
Control	

Observation on number of branches, number of fruits and fruit weight per plant were recorded and disease severity was recorded at 30days after transplanting.

Foliar spray method

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.

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

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

Studies with bioagent as a soil application

Earthen pots with 15cm diameter were taken and disinfected with 4% formalin and filled with sterilised soil. Different bioagents, manures and their combinations (as per the treatment details) were added to soil. Two seeds of tomato were sown in each pot, two pots were used for each treatment and three replications were maintained. Observation on number of branches, number of fruits and fruit weight per plant were recorded at appropriate growth stages and disease severity was recorded at 60 days after germination.

Treatments

Treatments	Concentration (g/pot)
T_1 - *Bacillus subtilis* IOF	1.00
T_2 -*Bacillus subtilis* IOF	2.00
T_2 -*Bacillus subtilis* IOF	4.00
T_3 -Neem cake alone	25.00
T_5 -Neem cake + T1	25.00+1.00
T_6 -Neem cake +T2	25.00+2.00
T_7 -Neem cake +T3	25.00+4.00
T_8 -FYM alone	50.00
T_9 -FYM + T1	50.00+1.00
T_{10} -FYM + T2	50.00+2.00
T_{11} -FYM + T3	50.00+4.00
T_{12}-Carbendazim12%+Mancozeb 63% WP (Saaf 75WP)	3.00
T_{13} – Control	-

III. RESULTS AND DISCUSSION

The pot culture studies indicated that the efficacy of the talc based formulation of *B. subtilis* was the most effective in enhancing the plant growth and higher antagonistic activity against early blight and powdery pathogens in tomato. Results of the experiments also revealed significant variation in plant growth characters, *viz.,* number of branches, number of fruits and fruit weight per plant when treated with different concentrations of *B. subtilis*.

The pot culture studies in glass house were conducted by various methods such as seed treatment, seedling dip method, foliar spray and soil application. Seed treatment with *B. subtilis* at 8 g/kg resulted in maximum of 90 per cent germination against 63.33 per cent in control. Treatments involving *B. subtilis* 6g/kg and *Pseudomonas fluorescens* 6g/kg were also good in enhancing the germination upto 76.67 per cent. The treatment involving *B. subtilis* 8g/ Kg of seed also recorded increased shoot length (22.93 cm), root length (5.57cm), more number of fruits (16.67), branches (6.07), and fruit weight per plant (834.50) followed by treatment *B. subtilis* 6 g/kg of seed. Powdery mildew disease severity was less in treatments involving *B. subtilis* at 8 g/kg (37.17%) and Carbendazim at 2g/kg (37.53%) compared to control (40.60%) followed by *B. subtilis* at 6g/kg of seed (40.19%). With respect to early blight *B. subtilis* 6g/kg of

seed recoded the less disease severity (26.53%) compared to the control (36.08%) and followed by *B. subtilis* at 6g/kg of seed (30.36%) (Table 1).

Many workers reported management of various diseases by using *B. subtilis* strains as a seed treatment material (Munasultan 2012 and Abdel-Kader *et al*., 2013). Similar studies were conducted by Sundermoorthy and Balabasker (2012) in which they studied the efficacy of *B. subtilis* (EPCO16 and EPC5 strains) and *Pseudomonas fluorescens* (Pf, Py15 and Fp7 strains) against *Alternaria solani* in tomato as seed treatment and found significant improvement in plant growth characters as well as reduction in disease severity.

The results of the pot culture experiment with seedling dip indicated that tomato seedlings dipped in *B. subtilis* talc based formulation at 15 g/l concentration recorded the maximum number of fruits (16.26), branches (6.77) and fruit weight per plant (861.54 g) and reduced the early blight (24.07%) and powdery mildew (37.47%) disease severity compared to control (35.13% early blight and 52.15% powdery mildew) (Table 2). The results of the present study are in tune with findings of Schisler *et al*., 2004 indicating growth promoting ability and disease suppression by *B. subtilis.* Anusha *et al.* also reported the seedling dip of tomato seedlings with the bioagent *Paecilomyces lilacinus* improved the plant growth parameters.

In the present investigation tomato plants were sprayed with talc based formulation of *B. subtilis* at different concentrations to evaluate its efficacy against powdery mildew and early blight diseases. *B. subtilis* at 10g/l sprayed at 15, 40, 65 and 90 DAS recorded less disease severity of powdery mildew (13.22%) and early blight (11.25%) and also maximum number of fruits (16.86), branches (6.90) and fruit weight per plant (898.76g) compare control and other treatments (Table 3). Similar studies were conducted by Abdel-Kader *et al*. (2012) 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). 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).

In the present study it was evident from results that plant growth parameters such as number of branches, number of fruits and fruit weight per plant increased when *B. subtilis* was applied along with organic amendments such as FYM and Neem cake to soil. Apart from disease management increase in number of branches, number of fruits and fruit weight per plant was significantly higher in *B. subtilis* treated plants than the plants in untreated control (Table 4). Similar results were recorded by Ebtsam *et al.* (2009) working with *Trichoderma viride* and *Bacillus subtilis* as biocontrol agents gainst *Fusarium solani* in tomato. They reported that soil inoculation with *B. subtilis* recorded higher yield than that treated by *T. viride*. The less disease severity of early blight and powdery mildew was recorded in tomato seedlings grown in soil applied with FYM + *B. subtilis* and Neem cake +*B. subtilis*. The pot experiments conducted in

the present investigation also revealed a significant increase in plant growth parameters, *viz.* number of branches, fruits, fruit weight per plant in tomato treated with *B. subtilis.* Niknejad *et al.* (2000) and Zaghloul *et al*. (2007) reported that application of selected antagonists (*B. subtilis* and *T. harzianum*) either individually or in combination has significantly increased the number of fruits per plant, weight of fruits and the total yield of tomato fruits supporting the findings of present study. The promotion of tomato growth parameters by *B. subtilis* may be due to their abilities to produce phytohormones, vitamins and solublizing minerals besides, their role in direct inhibition of pathogen growth (Morsy, 2005 and Zaghloul *et al.,* 2007).

IV. CONCLUSION

In pot culture experiment *Bacillus subtilis* tested for its bioefficacy against early blight and powdery mildew diseases of tomato through different methods *viz.,* seed treatment, seedling dip, foliar spray and soil application method. In seed treatment method, treatment involving *B. subtilis* at 8 g/kg of seed was most superior in promoting the growth and reducing the disease severity compared to other treatments. In seedling dip method, *B. subtilis* at 15 g/l of water recorded maximum number of branches, fruits and fruit weight per plant followed by *B. subtilis* at 10g/l when compared to other treatments. The results of present investication revealed that foliar spray of *B. subtilis* at 10g/l of water sprayed for four times at 15,40, 65and 90 DAS recorded the less disease severity and also promote growth of the plants. In Soil application method, combined application of FYM (50 g) + *B. subtilis* (4.00 g)/ pot was the best followed by Neem cake (25 g) + *B. subtilis* (4.0 g)/ pot when compared to other treatments. Among all the methods foliar spray method was the most effective in reducing the disease severity of powdery mildew and early blight whereas, soil application of bioagent with organic amendments had increased growth of plant.

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