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Efficacy of Combination of Delivery systems with *Bacillus subtilis* on the Incidence of Damping-off caused by *Pythium aphanidermatum* (Edson) Fitz. and Growth and yield of tomato

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ABSTRACT
Tomato (Solanum lycopersicum L. syn: Lycopersicon esculentum Mill.) is a commercial and widely grown vegetable crop of both tropics and sub-tropics. The present study was undertaken to investigate the efficacy of the native isolate of Bacillus subtilis for managing damping - off disease under pot and field conditions. The results revealed that the combination of delivery systems viz., Seed treatment with B. subtilis @ 15.0 g/Kg of seeds and soil application @ 2.5 kg/ha plus FYM recorded the minimum pre and post emergency damping-off incidence under pot and field conditions. Also, the same treatment significantly increased the plant growth and yield of tomato in pot and field conditions.
Key words: *Bacillus subtilis, damping - off disease, Solanum lycopersicum, management.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important, commercial and widely grown vegetable crops of both tropics and sub-tropics. In India, it is grown throughout the year for its edible fruits, which can be consumed either raw (or) cooked and in the form of various processed products like juice, ketchup, sauce and pickles. It is also a good source of vitamin A & C, minerals like iron and phosphorus. Tomato is being grown in kitchen garden, commercial fields and economically exploited in green houses or controlled environmental conditions. China ranks first in the world with an area of 14.5 lakh hectares and 31 million tones of production per annum [3]. Though, India occupies second position in the world with respect to area of tomato, it occupies only fifth place in terms of production.

Tomato crop is affected by several fungal, bacterial and viral diseases. Among these damping-off of tomato seedlings caused by several species of *Pythium* is very common all over the world. Besides, it occurs both in tropical and temperate climates and in almost every glass house and green house conditions [6]. The management of *Pythium* is very difficult due to its wide host range, soil borne nature and prolonged survival of propagules in the soil. Though management of *Pythium*, the causal agent of damping - off was a difficult task, attempts were made to manage the same using biological agents and organic amendments.

The development of resistant variety is more ideal to suit the management strategy but it is not practicable due to occurrence of several races of soil borne pathogens. In fact, resistant varieties against important soil borne pathogens are lacking. In this context use of biological agents and organic amendments are increasingly capturing the attention of scientists as an alternative strategy for disease management which is also ecology conscious and environment friendly. Keeping the above points in view, an attempt has been made to investigate the management of damping-off disease using the native isolate of *B.subtilis*.

MATERIALS AND METHODS

Effect of seed treatment with different doses of *B. subtilis* on the incidence of damping-off and growth of tomato seedlings (Pot culture)

Sterilized soil (1.0 kg) was mixed with the pathogen inoculum @ 100g (multiplied on sand maize medium) and filled in 15 x 30 cm dia. earthen pots. Surface sterilized tomato seeds (Var. PKM 1) were

separately treated with the talc based formulation of the antagonist. The treatment schedule followed is mentioned

Treatment schedule

T_1 - Seed treatment with *B.subtilis* @ 5 g/kg of seed
T_2 - Seed treatment with *B.subtilis* @ 10 g/kg of seed
T_3 - Seed treatment with *B.subtilis* @ 12.5 g/kg of seed
T_4 - Seed treatment with *B.subtilis* @ 15g/kg of seed
T_5 - Seed treatment with Metalaxyl @ 2 g/kg of seed
T_6 - Control

The experiment was conducted in a randomized black design and replicated thrice. The treated seeds were sown in pathogen inoculated soil @ 50 seeds per pot and irrigated daily. Pathogen alone inoculated pots served as control and metalaxyl @ 2 g/kg of seed was used for comparison. The observation 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 14th day after sowing.

Effect of soil application with different doses of *B. subtilis* on the incidence of damping-off and growth of tomato seedlings (Pot culture)

Sterilized soil (1.0kg) was mixed with the pathogen inoculum @ 100g (multiplied on sand maize medium) and filled in 15 x 30 cm dia. earthen pots. Talc based formulation of the antagonist was applied to the soil 10 days before sowing. The treatment schedule followed is mentioned below.

Treatment schedule

T_1 - Soil application of *B. subtilis* @ 1.5 Kg/ha.
T_2 - Soil application of *B. subtilis* @ 2.0 Kg/ha.
T_3 - Soil application of *B. subtilis* @ 2.5 Kg/ha.
T_4 - Soil application of *B. subtilis* @ 3.0 Kg/ha.
T_5 - Soil drenching of Metalaxyl (@ 0.1%)
T_6 - Control

Soil drenching with metalaxyl @ 0.1% was used for comparison and pathogen alone inoculated pots served as control. The experiment was conducted in a randomized blaok design and replicated thrice. The treated seeds were sown in pathogen inoculated soil @ 50 seeds per pot and irrigated daily. 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 14th day after sowing. The shoot length and root length (cm) of the plants were recorded at 25 days after sowing. In order to find out the population of pathogen, the soil samples were collected at 0, 10, 20, and 30 days after sowing.

Disease incidence

The incidence of damping -off was calculated using the formula.

$$\text{Percent Disease incidence (Pre-emergence)} = \frac{\text{Number of seeds germinated}}{\text{Total no of seeds sowing}} \times 100$$

$$\text{Percent Disease incidence (Post-emergence)} = \frac{\text{Number of seeds affected}}{\text{Total no of seeds germinated}} \times 100$$

Effect of combination of delivery systems of *B. subtilis* on the incidence of tomato damping-off under pot culture

Sterilized soil was mixed with the pathogen inoculum @100g (multiplied on sand maize medium) and filled in 15x 30 cm dia. earthen pots. Talc based formulations of the antagonist were applied to the soil 10 days before sowing. The treatment schedule followed is mentioned below

Treatment schedule

T_1 - Seed treatment (ST) with *B. subtilis* @15 g.
T_2 - Soil application (SA) *B. subtilis* @ 2.5 Kg/ha
T_3 - FYM @ 10t/ha
T_4 - T_1+T_3
T_5 - T_2+T_3
T_6 - T_1+T_2
T_7 - T_1+T_2+T_3
T_8 - Metalaxyl (Seed treatment @ 2 gm/kg of seed and soil drenching @ 0.1 %)
T_9 - control

Seed treatment (@2 gm/kg of seed) and soil drenching (0.1 %) with metalaxyl was used for comparison and pathogen alone inoculated pots served as control. The experiment was conducted with three replications in a randomized block design. The seeds were sown in pathogen inoculated soil at the rate of

50 seeds per pot and irrigated daily. The observations on the incidence of pre and post-emergence damping-off were recorded as above. The shoot length and root length (cm) of the plants were recorded at 25 days after sowing.

Field trial

Field experiments were laid to find out the effect of different treatment combinations on tomato damping-off, biometrics and yield.

Variety	: PKM 1
Design	: RBD
Replication	: 3
Plot size	: 1 x 1m^2 (Nursery)
Treatment	: 9
Place	: Department of Plant Pathology, Faculty of Agriculture, Annamalai nagar

The treatment schedule discussed earlier chapter.

Effect of seed treatment with different doses of *B. subtilis* on the incidence of tomato damping-off under pot culture

An experiment was conducted with six treatments to test the efficacy of antagonists under pot culture and results are given in table 1. The minimum pre-emergence damping-off (06.69%) and post-emergence damping-off (07.82%) was recorded in the treatment of *B. subtilis* @ 15 g/kg of seed. This treatment showed 79.41 and 79.55 per cent reduction in pre and post-emergence damping-off respectively over control. This was followed by the dosage level with 12.5 g/kg of seed of *B. subtilis* which was on par with the dosage level of 15g/kg of seed. The maximum pre-emergence damping-off (26.12%) and post-emergence damping-off of (35.45%) was recorded in the treatment with *B. subtilis* 5.0 g/kg of seed dosage level.

It is in agreement with the result reported by Papavizas [11] who reported that damping-off of peas caused by *P. ultimum* was controlled by seed treatment with *B. subtilis*. Alagu [2] reported that damping-off of musk melon was controlled by seed treatment with *B. subtilis*. Zaidi *et al.* [14] indicated that the application of *P. fluorescens* effectively checked the pre and post-emergence damping-off of tomato caused by *P. aphanidermatum*. Similarly, Aravind [4] reported that *B. subtilis* was found to be the most effective in reducing the pre and post emergence damping-off of tomato. Further, the present results are in accordance with the findings of Muthu kumar [9] and Dewa Nagurah Suprapta [7]. The seed treatment method of *B. subtilis* would have helped in better colonization of the antagonist in the rhizosphere which would have resulted in enhanced disease suppression.

Effect of soil application with different doses of *B. subtilis* on the incidence and growth of tomato damping-off under pot culture

The results regarding the effect of soil application of *B. subtilis* on the damping-off incidence was given in the table 2. Soil application of *B. subtilis* @ 3.0 kg/ha recorded a reduction of 77.94 per cent pre-emergence and 80.59 per cent post-emergence damping-off disease incidence over control and was on par with *B. subtilis* @ 2.5 kg/ha and soil drenching with Metalaxyl (0.1%). The maximum damping off incidence was observed in control. Similar such efficacy of the soil application method of delivery system against damping off disease was reported by earlier worker [9]. The *B. subtilis* applied either through seed or soil application has resulted in quicker proliferations leading to faster establishment of the antagonist in the rhizosphere. This might be the reason for the increased activity of *B. subtilis*. In addition to this by their high rhizosphere competence leading to improved root health might have protected tomato seedlings against pre and post emergence damping off. Such type of faster establishment has been reported by Dewa Nagurah Suprapta, [7] and Abdlwareth *et al*. [1]. Also, the addition of antagonists to soil may have suppressed the growth and competitive saprophytic ability of *P. aphanidermatum* resulting in reduction of inoculum in soil [8].

Effect of combined application of *B. subtilis* and FYM on the incidence of tomato damping-off and biometrics of tomato under Pot and field conditions

The results (Table 3,4 & 5) of the pot trial showed that the pre and post-emergence damping-off disease incidence was effectively controlled by the combined application of *B. subtilis* as (ST) @15 g/Kg of seeds + *B. subtilis* (SA) @ 2.5 Kg/ha + FYM (T_7) recorded the least disease incidence accounting for 86.49 % and 89.11 % decrease in the incidence of damping off over control respectively and increase the germination percentage (95.5%), plant height (84.50 cm) and yield (1.50 kg/plant). This was followed by T_8 and T_6 in the decreasing order of merit. The maximum disease incidence and minimum growth and yield were recorded in control (T_9) (30.50 % and 54.50 % respectively; germination percentage (23.8%), plant height (5.62 cm) and yield (0.35 kg/plant). A similar trend of findings was also observed in the field experiments conducted at Dept. Plant Pathology, Faculty of Agriculture, Annamalai University, Annmalai nagar (Table, 5).

The results revealed that different plant colonization pattern and different mechanism of disease suppression elicited by *B. subtilis* might have offered greater protection to the plants against the damping-off disease. Someya *et al.* (2000) recorded reduced damping-off incidence with *S. marcescens* under green house conditions. Similarly plots treated with strain *B. cereus* resulted in reduced root rot incidence and increased the plant growth of groundnut [12]. Muthukumar [9] reported that chilli seeds treated with bacterial strain, *P. fluorescens* resulted in reduced the damping-off incidence and improved the germination percentage, plant growth and yield of chilli.

The disease suppression observed in the present study might be due to the activity of the antibiotic production [10]. Integration of FYM along with *B. subtilis* further reduced the damping-off incidence and enhanced the germination percentage, plant growth and yield in pot and field condition. Application of *P. fluorescens* along with FYM significantly reduced the damping off incidence in sugarbeet and tomato [5, 6]. Also, FYM could have served as an ideal food base for the antagonist to survive and establish in the rhizosphere which could have been attributed as the reason for the enhanced suppression of *Pythium* and biometrics of tomato.

Table 1. Effect of Seed treatment with different doses of *B. subtilis* on the incidence of tomato damping-off under pot culture

Tr. No	Different doses of *B. subtilis* (g)	% incidence of damping - off			
		Pre -emergence (%)	Per cent decrease over control	Post -emergency (%)	Per cent decrease over control
1	5	26.12(30.73)	19.63	35.45(36.54)	7.51
2	10	15.16(22.91)	53.35	25.33(30.21)	33.91
3	12.5	07.05(15.34)	78.46	8.45(16.41)	79.15
4	15	06.69(14.98)	79.41	7.82(16.23)	79.55
5	Metalaxyl @2.0g/Kg. of seed	07.75(16.16)	76.15	8.43(16.87)	78.00
6	Control	32.50(34.75)	-	38.33(38.25)	-
	SEd CD (p=0.05)	0.19 0.42	-	0.56 1.33	-

Data in parentheses indicate arcsine transformed values

Table 2. Effect of Soil application with different doses of *B. subtilis* on the incidence of tomato damping-off under pot culture

Tr.No	Different doses of *B. Subtilis* (kg)	% incidence of damping - off			
		Pre -emergence (%)	Per cent decrease over control	Post -emergency (%)	Per cent decrease over control
1.	1.5	25.00(30.00)	28.57	31.33(34.01)	24.01
2.	2.0	14.32(22.23)	59.08	23.42(28.94)	43.19
3.	2.5	7.82(16.23)	77.65	8.15(16.58)	80.23
4.	3.0	7.72(16.13)	77.94	7.72(16.42)	80.59
5.	Metalaxyl @ (0.1%)	7.76(16.17)	77.82	8.12(16.55)	80.30
6	Control	35.00(36.27)	-	41.23(39.94)	-
	SEd CD (p=0.05)	0.71 1.45	-	0.82 1.66	-

Data in parentheses indicate arcsine transformed values

Table 3. Effect of combined application of *B. subtilis* on the incidence of tomato damping - off under pot culture

Tr. No	Treatments	% incidence of damping - off			
		Pre- emergence (%)	Per cent decrease over control	Post -emergency (%)	Per cent decrease over control
1	Seed treatment (ST) @ 15g	8.21(16.65)	73.08	9.45(17.90)	82.66
2	Soil application (SA) @ 2.5kg	8.10(16.53)	73.44	9.13(17.58)	83.24

3	FYM	25.32(30.21)	16.98	32.42(34.70)	40.51
4.	ST+ FYM	7.46(15.85)	75.54	10.43(18.84)	80.83
5	SA+ FYM	6.00(14.17)	80.32	8.33(16.77)	84.71
6	ST+ SA	5.10(13.05)	83.27	7.25(15.62)	86.69
7	ST + SA+ FYM	4.12(11.71)	86.49	5.93(14.09)	89.11
8	Metalaxyl (0.1%)	6.00(14.17)	80.32	10.33(18.74)	81.04
9	Control	30.50(33.52)	-	54.50(47.58)	-
	SEd CD (p=0.05)	0.01 0.03	-	0.03 0.08	-

Data in parentheses indicate arcsine transformed values

Table 4. Effect of combined application of *B. subtilis* on the growth and yield of tomato seedlings under pot culture

T.No	Treatments	Germination (%)	Plant height (cm)	Fruit yield/ plant (Kg)
1.	Seed treatment (ST) @ 15g	53.6(47.06)	62.43	0.67
2.	Soil application (SA) @ 2.5kg	55.9(48.38)	65.53	0.70
3.	FYM	40.3(39.40)	52.06	0.50
4.	ST+ FYM	65.2(53.84)	71.43	0.75
5.	SA+ FYM	73.8(59.21)	73.50	0.80
6.	ST+ SA	86.7(68.61)	78.50	1.00
7.	ST + SA+ FYM	95.5(77.75)	84.50	1.50
8.	Metalaxyl (0.1%)	94.3(76.18)	80.33	1.30
9.	Control	23.8(29.19)	5.62	0.35
	SEd CD (p=0.05)	0.48 0.97	0.05 0.11	0.01 0.03

Data in parentheses indicate arcsine transformed values

Table 5. Effect of combined application of *B. subtilis* on the incidence tomato damping-off under field condition

Tr.No	Treatments	% incidence of damping - off			
		Pre - emergence (%)	Per cent decrease over control	Post-emergence (%)	Per cent decrease over control
1	Seed treatment (ST) @ 15g	14.52(22.39)	54.83	16.31(23.81)	66.11
2	Soil application (SA) @ 2.5kg	13.25(21.34)	58.76	15.13(22.89)	68.56
3	FYM	28.33(32.15)	11.88	40.12(39.30)	16.64
4	ST+ FYM	11.45(19.77)	64.38	19.25(26.02)	60.00
5	SA+ FYM	10.12(18.54)	68.52	18.12(25.19)	62.35
6	ST+ SA	09.32(17.77)	71.01	16.35(23.85)	66.02
7	ST + SA+ FYM	08.67(17.12)	73.03	14.45(22.34)	69.97
8	Metalaxyl (0.1%)	10.21(18.63)	68.24	15.25(22.98)	68.31
9	Control	32.15(34.54)	--	48.13(43.92)	--
	SEd CD (p=0.05)	0.20 0.42	-	0.46 0.94	-

Data in parentheses indicate arcsine transformed values

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