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Management of bacterial wilt of tomato through consortium of biocontrol agents

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Abstract
Bacterial wilt of tomato, caused by *Ralstonia solanacearum*, is a very serious disease of tomato which causes huge economic losses year after year. *R. solanacearum* is a phytopathogenic bacterium that colonizes the xylem vessels of host plants leading to a lethal wilt disease. Bacterial wilt disease is very difficult to manage through traditional disease management practices. In the present study, three biocontrol agents namely *Bacillus subtilis*, *Pseudomonas flourescens* and *Trichoderma viride*, either alone or in combination, were used to study their effect for the management of bacterial wilt disease in sick pots of pathogen. It was found that application of consortium of both *B. subtilis* and *T. viride* along with neem cake and molasses completely hold off the disease and no disease incidence was observed. It was followed by application of *Bacillus subtilis* as seedling dip with foliar spray after 7 days of transplanting with 35.55 per cent disease incidence compared to 82.22 per cent disease incidence in un-treated control. Maximum plant weight of 1.5 g observed with application of consortium of biocontrol agents before transplanting and application of *Bacillus subtilis* as seedling dip with foliar spray after 7 days of transplanting. However, maximum plant height of 19.0 cm was observed with application of consortium as drenching after five days of transplanting.

Keywords: Bacterial wilt, tomato, consortium, biocontrol agents

Introduction
Tomato (*Solanum lycopersicum* L.), a member of *Solanaceae* family, is one of the most important commercial vegetable grown in Himachal Pradesh. The agroclimatic conditions of Himachal Pradesh are ideally suited for the production of tomato as an off season vegetable crop since the fruits are available in the market at such a time (June to October) when it does not grow in the plains due to the unfavourable high temperature. Solan, Sirmour, Mandi and Kullu are main tomato-producing districts of state. Successful and economic cultivation of tomato is consistently being threatened by biotic factors such as fungal, bacterial and viral diseases. Among these diseases bacterial wilt of tomato incited by *Ralstonia solanacearum* (Smith) Yabuuchi *et al*. (Syn. *Pseudomonas solanacearum* E.F. Smith) is one of the most devastating disease. The disease results in rapid and fatal wilting symptoms in host plants and causes huge economic losses. However, attempts have been made to manage the disease through chemicals, soil amendments, use of resistant varieties and other cultural practices, yet satisfactory management of the disease is still lacking. Hence, the present investigation was carried out to manage the disease through a consortium of biocontrol agents.

Materials and Methods
Isolation and identification of bacterial pathogen
The samples showing the typical symptoms and presence of ooze and vascular discolouration were selected for isolation. The bacterial pathogen was isolated by following standard procedure of isolation (Dhingra and Sinclair, 1995) [7]. The pathogen was identified on the basis of morphological, cultural and biochemical characters (Buchbinder, 1957; Anonymous, 1957) [1, 2].

Preparation of inoculum
Bacterial cell suspension was prepared by dissolving 48 h old bacterial colonies raised on nutrient agar plates in 100 ml sterilized triphenyl tetrazolium chloride medium basic broth (Dhingra and Sinclair, 1995) [7]. It was put on shaker for 24-36 h until desired optical density of 0.3 (equivalent to 3.0 x 10^8 cfu/ml) was achieved (Kumar, 2003) [10].

Culture of biocontrol agents
Two bacterial biocontrol agents namely *Bacillus subtilis* and *Pseudomonas flourescens* and one fungal biocontrol agent namely *Trichoderma viride* were obtained from Department of

Plant Pathology and were revived on suitable media. *Bacillus subtilis* and *Pseudomonas fluorescens* were grown in nutrient broth and King's B broth (Dhingra and Sinclair, 2005), respectively in a 500 ml conical flask containing 250 ml of the medium and were placed on a rotary shaker for 48 hr until required concentration i.e. 0.3 O.D. ($3.0x10^8$ cfu/ml) was achieved. *Trichoderma viride* was grown in potato dextrose broth as a stationary culture at room temperature for seven days. The fungal mass was obtained and used.

Preparation of consortium of biocontrol agents

Neem cake and molasses were mixed in 2:1 ratio and water was added to maintain 20 per cent moisture level. *Bacillus subtilis* was inoculated to this mixture. After two days, *Trichoderma viride* was inoculated. After seven days, the consortium of biocontrol agents was evaluated against the bacterial wilt of tomato.

Evaluation of biocontrol agents against bacterial wilt of tomato

In order to study the effect of biocontrol agents on bacterial wilt disease development, an experiment was conducted under pot conditions in which, two bacterial biocontrol agents namely *Bacillus subtilis* and *Pseudomonas flourescens* and one fungal biocontrol agent namely *Trichoderma viride* were used.

Twenty five days old seedlings of tomato cultivar Solan Lalima were raised in plastic pots. The seedlings were uprooted and the soil particles adhering to it were removed. Biocontrol agents and consortium of biocontrol agents were applied as soil treatment or seedling dip treatment (Table 1) for the management of bacterial wilt of tomato. The roots were then dipped either in *Bacillus subtilis* or *Pseudomonas fluorescens* cell suspension for 30 minutes. These were then air dried for 1 h. Afterwards, challenge inoculation was done by dipping the roots in suspension of *Ralstonia solanacearum* for 30 minutes. Then the seedlings were transplanted in the pots containing sterilized soil. For soil application before transplanting, 20 ml suspension of antagonistic strains was poured into each pot 3 days before transplanting. Similarly, same amount of concentration was used for soil application after two days of transplanting. The pots were placed in a polyhouse at 30±2°C and experiment was conducted in a Completely Randomized Design (CRD). Each treatment was replicated thrice. The data on disease incidence, plant length and fresh plant weight (after uprooting) was recorded.

Results and Discussion

The bacterium was isolated from diseased plant samples on nutrient agar and was identified as *Ralstonia solanacearum* on the basis of biochemical characters.

The effect of biocontrol agents on management of bacterial wilt disease of tomato was assessed and it was found that application of all the treatments consisting of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride* and consortium of biocontrol agents to tomato seedling roots, leaves and soil, exhibited significantly high degree of reduction in incidence of bacterial wilt disease, which ranged from 2.71 to 100.00 per cent over untreated control (Table 1). None of the bio control agents had any adverse effect on plant growth. No incidence of bacterial wilt was observed in the pots with consortium application (Fig. 1). This means that the combination of both *B. subtilis* and *T. viride* along with neem cake and molasses was very effective in completely holding off the disease. This was followed by *B. subtilis* (seedling root dip + foliar spray after seven days of transplanting) and *B. subtilis* (seedling root dip) treatment that resulted in 35.55 and 44.44 per cent disease incidence, respectively. The two treatments namely *B. subtilis* (soil application before transplanting) and *P. fluorescens* (seedling root dip + foliar spray after seven days of transplanting) were also effective in controlling the disease and resulted in 48.88 per cent disease incidence. The next best order were of *T. viride* (soil application before transplanting) and *B. subtilis* (soil application after 2 days of transplanting) that resulted in 55.55 and 64.44 per cent disease incidence, respectively. *P. fluorescens* (seedling root dip) and *P. fluorescens* (soil application before transplanting) were effective in controlling the disease and resulted in 66.66 per cent disease incidence. *P. fluorescens* (soil application after 2 days of transplanting) treatment was least effective (79.99%) but superior to control (82.22%).

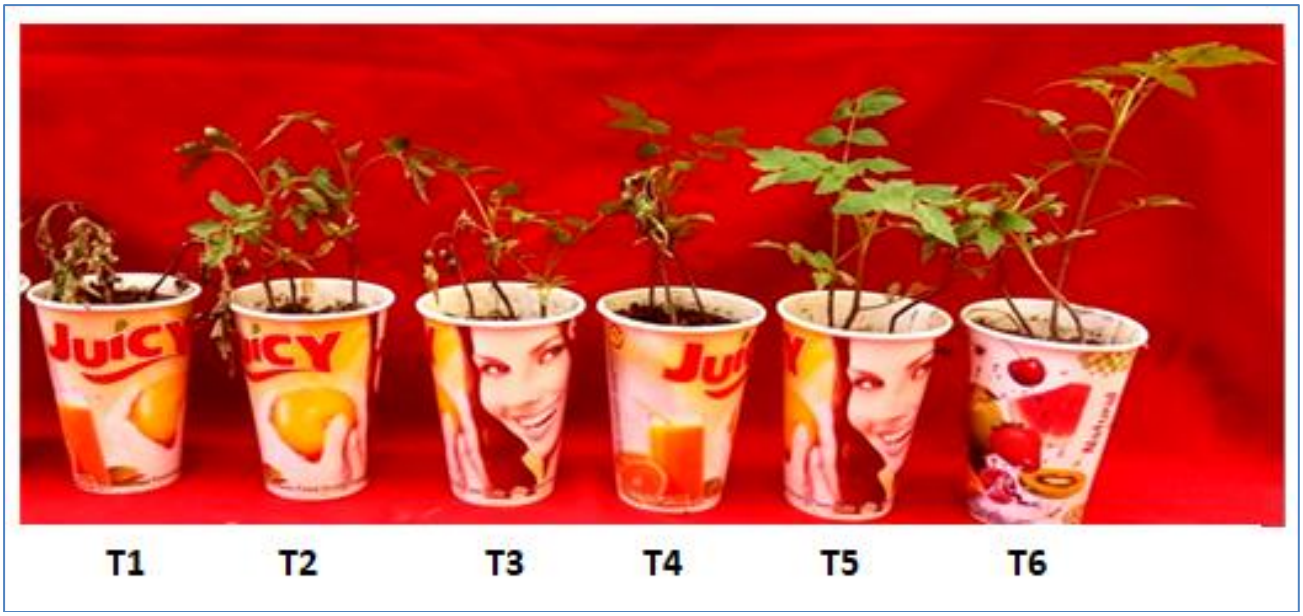
Data also revealed that all the treatments resulted in increase in fresh plant weight and plant height significantly as compared to un-treated control (Table1, Fig. 1). However, consortium application before transplanting and *B. subtilis* (seedling root dip + foliar spray after seven days of transplanting) resulted in highest increase in plant weight (1.5 g) followed by consortium application after transplanting, *Bacillus subtilis* (seedling root dip) and *Bacillus subtilis* (soil application before transplanting) treatment with 1.48 g, 1.47 g and 1.45 g plant weight, respectively (Table 1). While *Pseudomonas fluorescens* (foliar spray after 2 days of transplanting followed by second spray at 7 days interval) treatment resulted in minimum increase in plant weight (1.13 g) but superior to control (1.0 g). Analogically, consortium application resulted in highest increase in plant height (19.0 cm, 18.5 cm) followed by *B. subtilis* (seedling root dip + foliar spray after 7 days of transplanting) and *B. subtilis* (seedling root dip) treatment with 18.4 and 18.1 cm plant height, respectively. While *Pseudomonas fluorescens* (foliar spray after 2 days of transplanting followed by second spray at 7 days interval) treatment resulted in minimum increase in plant height (16.7cm) but superior to control (16.5 cm).

Table 1: Effect of biocontrol agents on bacterial wilt and plant growth parameters of tomato

Sr. No.	Biocontrol agent	Application type	Disease incidence (%)	Disease reduction (%)	Plant weight (g)	Plant height (cm)
1	Control	-	82.22 (65.76)	-	1.00	16.5
2	*Bacillus subtilis*	Seedling root dip	44.44 (41.78)	45.94	1.47	18.1
3	*Bacillus subtilis*	Seedling root dip + Foliar spray after 7 days of transplanting	35.55 (36.49)	56.76	1.50	18.4
4	*Bacillus subtilis*	Foliar spray after 2 days of transplanting followed by second spray at 7 days interval	77.77 (62.22)	5.41	1.20	16.8
5	*Bacillus subtilis*	Soil application before transplanting	48.88 (44.34)	40.55	1.45	17.9
6	*Bacillus subtilis*	Soil application after 2 days of transplanting	64.44 (53.38)	21.62	1.38	17.4

7	*Pseudomonas flourescens*	Seedling root dip	66.66 (55.00)	18.92	1.34	17.2
8	*Pseudomonas flourescens*	Seedling root dip + Foliar spray after 7 days of transplanting	48.88 (44.34)	40.55	1.40	17.5
9	*Pseudomonas flourescens*	Foliar spray after 2 days of transplanting followed by second spray at 7 days interval	73.33 (59.39)	10.81	1.13	16.7
10	*Pseudomonas flourescens*	Soil application before transplanting	66.66 (55.00)	18.92	1.34	17.2
11	*Pseudomonas flourescens*	Soil application after 2 days of transplanting	79.99 (63.94)	2.71	1.28	17.00
12	*Trichoderma viride*	Soil application before transplanting	55.55 (48.22)	32.43	1.40	17.6
13	Consortium of biocontrol agents	Soil application before transplanting	0.00 (0.00)	100.00	1.50	18.5
14	Consortium of biocontrol agents	Drenching of plants after 5 days of transplanting	0.00 (0.00)	100.00	1.48	19.0
$CD_{(0.05)}$			(10.69)		0.14	0.28

Figures in the parentheses are arc sine transformed values



T1: Inoculated control; T2: *Bacillus subtilis* - Seedling dip + Foliar spray after 7 days of transplanting; T3: *Pseudomonas flourescens* - Seedling dip + Foliar spray after 7 days of transplanting; T4: *Trichoderma viride* - Soil application before transplanting; T5: Biocontrol consortium - Soil application before transplanting; T6: Biocontrol consortium - Soil application after transplanting

Fig 1: Effect of biocontrol agents on bacterial wilt of tomato

Bacillus spp. is highly suitable for use in biological control because of their omnipresence in soils, high thermal tolerance, rapid growth in broth culture and ready formation of resistant spores. *Pseudomonas* spp., on the other hand, are metabolically very active, have high growth rate and aggressively colonize root systems (Burr *et al*., 1978) [4].

The results obtained in present study are in accordance with the report of Sivamani and Gnanamanickam *et al*. (1988) [12] and Karuna and Khan (1994) [8]. They reported that isolate of *Bacillus* sp. reduced wilt incidence by 74 per cent and two isolates of *P. fluorescens* decreased wilt incidence by 68 and 55 per cent in *Solanaceous* crops. Anuratha and Gnanamanickam (1990) [3] recorded reduction in wilt incidence of tomato to an extent of 95 per cent by using *P. fluorescens* and *Bacillus* sp.

The above mentioned results of *Trichoderma viride* application in tomato plants are in accordance with that of Kumar and Ganesan (2006) [9] who reported that *T. viride* can effectively control the bacterial wilt disease in tomato and results in 46.67 per cent disease incidence. Lemessa and Zeller (2007) [11] also reported that *B. subtilis* and *P. fluorescens* can reduce bacterial wilt disease incidence by 70 per cent and 63 per cent, respectively in tomato plants. These findings of our study are confirmed by Chen *et al*. (2014) [6] who revealed that *Bacillus subtilis* exhibited above 50 per cent biocontrol efficacy on tomato plants against the plant pathogen *R. solanacearum* under greenhouse conditions. Chakravarty and Kalita (2012) [5] reported the efficacy of *P. fluorescens* when applied as suspension in pot experiment by different methods *viz*. seed, root, soil, and their integration methods seed + root, root + soil, seed + soil and seed + root + soil. The per cent wilt incidence was found to be lowest (33.33%) in root + soil and seed + root + soil treatment of the antagonist.

Conclusion

The present study showed that application of all the treatments consisting of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride* and consortium of biocontrol agents to tomato seedlings exhibited significant reduction in incidence of bacterial wilt disease. All the treatments resulted in increase in fresh plant weight and plant height significantly as compared to control treatment. A consortium of *Bacillus subtilis* and *Trichoderma viride* in neem cake and molasses gave best result as it was able to completely hold off the disease as well as showed highest increase in plant height and resulted in 0 per cent disease incidence. Among three biocontrol agents used, *Bacillus subtilis* (seedling dip + foliar spray after 7 days of transplanting) treatment was most effective as it was able to reduce the disease by 56.76 per cent.

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