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Role of plant growth promoting microbes in the control of fungal foliar diseases of tomato under protected cultivation

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Abstract

Background: This study was conducted to assess the effectiveness of plant growth-promoting microbes on the foliar disease of organic tomatoes under protected cultivation. The biological control agents, viz., *Trichoderma harzianum* (*T. harzianum*), *Bacillus subtilis* (*B. subtilis*), and *Pseudomonas fluorescens* (*P. fluorescens*), used seed treatment, seedling dip, and spray separately in sequence with the plot size of (2.5 × 1 m) and the spacing of (45 × 60 cm). The observations on percentage disease yield and plant growth parameters were assessed, and economics was calculated. Tukey's HSD at $p < 0.05$ was used to compare all means among treatments.

Results: The results showed that seed treatment with *B. subtilis* at 5 g, followed by a seedling dip with *B. subtilis* at 10 g l⁻¹ and spray with *B. subtilis* at 5 g l⁻¹ four times at 15 days intervals significantly ($p < 0.05$) reduced the tomato diseases early blight, septoria leaf spot, and powdery mildew (T2). These results were comparable to seed treated with *P. fluorescens* at 5 g l⁻¹, followed by a seedling dipping with *P. fluorescens* at 10 g l⁻¹ and spraying with *P. fluorescens* at 10 g l⁻¹ four times at 15-day intervals (T3). Except for T1, plant parameters, yields, net returns, and benefits were significantly higher ($p < 0.05$) than the untreated control (T8).

Conclusion: The applications of *B. subtilis* and *P. fluorescens* as plant growth-promoting microbes had a considerable scope for managing fungal foliar (multiple) diseases and achieving sustainable yields under the protected cultivation of organically grown tomatoes.

Keywords: Biological control, Fungal foliar diseases, Protected condition, Tomato

Background

Tomato cultivation in open fields is challenging throughout the year due to unpredictable weather conditions and plant diseases that affect the development and quality of the produce (Arushi and Banyal 2018). Tomatoes grown in protected settings produce larger yields, extend the harvest season, improve quality, and increase production stability, resulting in peak performance and reliable off-season supply (Pooja and Hakkim 2017). The

protected cultivation of crops enhances the success of biological control agents with greater precision than the field crops. Increased education and extension of proven, ready-to-use biological control options are vital strategies to accelerate adoption (Atalla et al. 2020). There are reports (Kabdwal et al. 2019) of tomato loss in the field health risks associated with agrochemicals and how these issues could be addressed by adopting organic protected cultivation. There exists scope for growing organic vegetables under protected structures. Tomatoes are affected by many plant pathogens, such as bacterial, fungal, nematode, viral diseases, and abiotic factors. Among the most prevalent fungal foliar infections, early blight, septoria leaf spot, gray leaf spot, and powdery mildew are the ones that cause havoc across growing regions globally (Sokhi

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et al. 1991). The cultivation of tomatoes using biocontrol agents was very effective in developing environmentally safe, long-lasting, and effective I.P.M. programs to manage tomato foliar diseases (Kaur et al. 2016).

Tomato cultivation in a polyhouse is a well-known technology, and so is the organic production of tomatoes. However, most studies were carried out separately, either as organic production of tomatoes or tomatoes grown under protected structures. While our examinations conducted during 2017–2018 and 2018–2019 was the combination of organic cultivation of tomato under polyhouse conditions, which draws the attention of growers who wishes to practice organic tomato production under polyhouse conditions. The sequential use of microbes alone to control multiple fungal diseases of tomatoes requires the attention of growers and biopesticide industries. In the era of organic agriculture, biopesticides have a promising role in managing various diseases and influencing plant growth-promoting parameters. Thus, growing pesticide-free tomatoes under polyhouse conditions will be a bio-based technology in augmenting farmers' income and benefiting plant, soil, and human health. This study was conducted to assess the effectiveness of plant growth-promoting microbes on the foliar disease of organic tomatoes under protected cultivation.

Methods

A polyhouse experiment was carried out at the Institute of Organic Farming Bio-Resource Farm, Agricultural Sciences University, Dharwad, for two consecutive years, 2018 and 2019. The experimental site was an organically certified farm, and soil fertility was maintained by the application of Farmyard manure (350 kg acre⁻¹), vermicompost (200 kg acre⁻¹), and Neem cake (100 kg acre⁻¹) based on soil test results.

Seed treatment (ST), seedbed preparation, and sowing

Moderately susceptible indeterminate tomato hybrid Sarpan-8 seed was taken for the study. The talc-based formulations of B.C.A.s were used for the present study. The seeds treated with B.C.A.s @ 5 g kg⁻¹ were sown to a depth of 0.5 cm in the primary seedbed. Seeds were germinated in 4–6 days, and the seedlings were ready for transplanting by 25 days after sowing. Seedlings were dipped in various solutions of *Trichoderma harzianum* (*T. harzianum*), *Bacillus subtilis* (*B. subtilis*), and *Pseudomonas fluorescens* (*P. fluorescens*) *Trichoderma harzianum* (*T. harzianum*), *Bacillus subtilis* (*B. subtilis*), and *Pseudomonas fluorescens* (*P. fluorescens*) @ 10 g l⁻¹ of water and shade dried for 20 min before transplanting. The soil area inside the polyhouse was thoroughly dug to 30 cm. All the weeds, stones, stubbles, etc., were removed entirely. The soil was irrigated

three to four times, ploughed again, and brought to a fine tilth. Raised beds of 30 cm in height and 100 cm in width were prepared with a walking space of 60 cm among beds. The beds were incorporated with F.Y.M. @ 2.5 t ha⁻¹, vermicompost @ 2.00 t ha⁻¹, neem cake @ 2.00 t ha⁻¹, and rock phosphate @ 1.00 t ha⁻¹. These were added to soil at different intervals of crop growth. Additionally, phosphate solubilizing bacteria (PSB) (100 g^{-unit}) like *T. harzianum* (accession no. NAIMCC-F-03328) (2 × 10⁶) and *P. fluorescens* (accession no. NAIMCC-B-01981) (2 × 10⁶), *Purpureocillium lilacinum* (accession no. IOF Dharwad) at (2 × 10⁶) and *Metarhizium anisopliae* (IOF Dharwad) (2 × 10⁶) @ 2 kg t⁻¹ of organic matter were added to soil at 15 days before transplanting. At the center of the bed, two inline dripper laterals were placed. Inline dripper lateral had an emitting point for every 30 cm interval with a discharge of 2 l hr⁻¹. Paired row system of planting with a zigzag manner was followed to have more aeration and space among plants.

Experimental design

The experiment consisted of 8 treatments, viz., T1 (ST with *T. harzianum* @ 5 g kg⁻¹ followed by (Fb) seedling dip with *T. harzianum* 10 g l⁻¹ spray with *T. harzianum* 4 times at 15 days interval), T2 (ST with *B. subtilis* @ 5 g kg⁻¹ Fb seedling dip spray with *B. subtilis* 4 times 10 g l⁻¹ at 15 days interval), T3 (ST with *P. fluorescens* @ 5 g kg⁻¹ Fb seedling dip, spray with *P. fluorescens* 10 g l⁻¹ four times at 15 days interval), T4 (ST with *T. harzianum* @ 10 g l⁻¹ Fb spray with *T. harzianum* @ 10 g l⁻¹ four times), T5 (ST with *T. harzianum* @ 5 g kg⁻¹ Fb spray with *B. subtilis* @ 10 g l⁻¹ four times), T6 (ST with *T. harzianum* @ 5 g kg⁻¹ Fb spray with *P. fluorescens* @ 10 g l⁻¹ four times), T7 (Recom. Check (Sulphur) @ 3 g l⁻¹) and T8 (Untreated Control). A split-plot with three replications with a plot size of (2.5 × 1 m) and (45 × 60 cm) spacing at a distance of 45 cm among the rows and 60 cm within a row on a one-meter wide bed was followed.

Assessment of diseases

The diseases like early blight were recorded on a 5-point scale, while powdery mildew and septoria leaf spot were recorded on a 10-point scale as given by Mayee et al. (1985).

Early blight 0–5 scale

Grade	Description
0	No symptom on a leaf

Grade	Description
1	0–5% Leaf area infected and covered by spot, no spot on petiole and branches
2	6–20% Leaf area infected and covered by spots, some spots on the petiole
3	21–40% Leaf area infected and covered by spot, spots also seen on the petiole, branches
4	41–70% Leaf area infected and covered by spot spots are also seen on the petiole, branches, and stem
5	>71% Leaf area infected and covered by spots, spots also seen on petiole, branch, stem, fruits

Powdery mildew 0–9 scale

Grade	Description
0	No symptom on a leaf
1	Tiny powdery specks on the leaves cover 1% or less area
3	Powder lesions are small and scattered, covering 1–10% of the leaf area
5	Powdery patches are big and scattered, covering 11–25% of the leaf area
7	Powdery patches are prominent, coalescing covering 26–50% of the leaf area
9	Powdery growth covers 51% or more of the leaf area. Leaf turn yellow and dry up

Septoria leaf spot 0–9 scale

Grade	Description
0	No symptom on a leaf
1	Tiny irregular spots covering less than 1% of the Leaf area
3	Small irregular, brown spots covering 1–10% of the leaf area
5	Small lesions, but enlarged with a dark brown margin with a gray center covering 11–25% of the leaf area
7	Enlarged lesions with a dark brown margin with a gray center covering 26–50% of the leaf area
9	Lesions coalesce to form irregular dark brown to black patches covering 51% or more leaf area

Percentage disease index (P.D.I.) was calculated by using the formula (Mayee et al. 1985).

$$\text{Per cent disease index (PDI)s} = \frac{\text{Sum of individual rating}}{\text{No. of leaves examined} \times \text{Maximum disease rating}} \times 100$$

The first spray was immediately given after the appearance of early blight and septoria leaf spot. Subsequent sprays were presented at an interval of 15 days. Four sprays were given @10 g l⁻¹ to manage the fungal foliar diseases. The early blight symptoms appeared firstly (30 days after transplanting) and symptoms of powdery

mildew and septoria leaf spot disease appeared later (75 days after transplanting). Sulphur was recommended for powdery mildew of tomato and was certified in the organic cultivation of vegetables. Hence it was used as a treated check. An untreated check was maintained for comparison.

Characterization of antagonistic microorganisms used in the study

HCN (hydrogen cyanide) production by Bakker and Schippers (1987), siderophore production by Schwyn and Neilands and chitinase production by Agrawal and Kotasthane (2012) were assessed by using standard protocol with slight modifications. After incubation for a week at 30 °C, HCN production potential was assessed based on colour change of filter paper from deep yellow to orange and finally orange brown to dark brown. Orange halos produced around the colonies indicated the production of siderophores. Colour change from yellow to purple around the colony indicated chitinase production.

Observations on plant, yield, and cost parameters

Observations on plant height, number of fruits per cluster, number of pickings, yield per plot, and cost economics were recorded at 120 days.

Statistical analysis

Statistical analysis was carried out per the standard procedures (Panse and Sukhatme 1954). Data analysis was done using R (R Core Team 2018). The differences among the means were analyzed by two-way ANOVA with mean separation by Tukey's HSD at $p < 0.05$ to determine whether there were significant differences among the treatments (T_1 – T_8) and their interactions. A significant level was set at the probability level of * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ for all the analyses.

Results

Early blight

During Kharif 2017–2018 (Table 1), the disease incidence treated with T_3 and T_7 was recorded as 14.80 and

16.28%, respectively, compared to other treatments. After the third spray, the disease incidences at T_2 (19.24%), T_3 (19.98%), followed by T_5 (28.86%), T_6 (27.38%), and T_7 (25.16%) were found to be significantly ($p < 0.05$) lower than the T_8 (40.70%) against early blight. During Kharif 2018–2019 (Table 2), the incidence after the

Table 1 Management of Foliar fungal diseases on Tomato under protected conditions during Kharif 2017–2018

Treatments	Treatment details	Foliar diseases of tomato (percentage disease index) %										Yield
		Early blight		Powdery mildew		Septoria leaf spot		Yield		Yield		
		Before spray	After I spray	After II spray	After III spray	After IV spray	After III spray	After IV spray	After III spray	After IV spray	After III spray	
T1	ST with <i>Trichoderma harzianum</i> @ 5 g kg ⁻¹ Fb seedling dip with <i>T. harzianum</i> 10 g l ⁻¹ spray with <i>T. harzianum</i> 4 times at 15 days interval	6.66	27.38	22.2	38.48	39.22	27.38	14.06	25.9	51.48		
T2	ST with <i>Bacillus subtilis</i> @ 5 g kg ⁻¹ Fb seedling dip with <i>B. subtilis</i> 10 g l ⁻¹ spray with <i>B. subtilis</i> 4 times 10 g l ⁻¹ at 15 days interval	14.80	23.68	21.46	19.24	18.50	15.54	13.32	15.54	60.05		
T3	ST with <i>Pseudomonas fluorescens</i> @ 5 g kg ⁻¹ Fb seedling dip with <i>P. fluorescens</i> 10 g l ⁻¹ spray with <i>P. fluorescens</i> 10 g l ⁻¹ 4 times at 15 days interval	14.06	14.8	19.98	19.98	20.72	17.76	16.28	17.76	61.57		
T4	ST with <i>Trichoderma harzianum</i> @ 10 g kg ⁻¹ Fb spray with <i>T. harzianum</i> @ 10 g l ⁻¹ 4 times at 15 days interval	8.88	31.82	21.46	32.5	34.78	22.2	23.68	22.94	50.28		
T5	ST with <i>Trichoderma harzianum</i> @ 5 g kg ⁻¹ Fb spray with <i>B. subtilis</i> @ 10 g l ⁻¹ 4 times at 15 days interval	11.10	32.56	19.24	28.86	24.42	19.24	14.06	17.02	56.73		
T6	ST with <i>Trichoderma harzianum</i> @ 5 g kg ⁻¹ Fb spray with <i>P. fluorescens</i> @ 10 g l ⁻¹ 4 times at 15 days interval	11.84	34.78	17.02	27.38	25.16	24.42	17.76	13.32	59.47		
T7	Recom. Check (Sulphur) @ 3 g l ⁻¹	13.32	16.28	22.2	25.16	32.94	26.29	13.32	23.76	56.70		
T8	Untreated control	13.32	22.94	30.34	40.70	45.14	29.6	14.80	28.86	43.13		
	S.Em±	0.83	1.57	1.80	1.88	1.63	1.83	0.80	1.55	2.16		
	CD (5%)	2.53	4.80	5.50	5.70	4.94	5.55	2.42	4.70	6.55		
	CV (%)	7.26	9.04	11.30	10.06	8.80	11.41	5.91	10.24	15.05		

Production of HCN, Siderophore and Chitinase

SEM standard error of mean, CD confidence distribution, CV coefficient of variation

Table 2 Management of Foliar fungal diseases on Tomato under protected conditions during Kharif 2018–2019

Treatments	Treatment details	Foliar diseases of tomato (percentage disease index)							
		Early blight					Powdery mildew	Septoria leaf spot	Yield t/ha
		Before spray	After I spray	After II spray	After III spray	After IV spray			
T1	ST with <i>Trichoderma harzianum</i> @ 5 g kg ⁻¹ Fb seedling dip with <i>T. harzianum</i> @ 10 g l ⁻¹ -spray with <i>T. harzianum</i> 4 times at 15 days interval	13.12	22.46	28.10	38.40	40.10	19.23	32.40	48.51
T2	ST with <i>Bacillus subtilis</i> @ 5 g kg ⁻¹ seedling dip, spray with <i>B. subtilis</i> 4 times 10 g l ⁻¹ at 15 days interval	15.20	22.18	24.10	22.10	22.31	12.10	23.16	58.61
T3	ST with <i>Pseudomonas fluorescens</i> @ 5 g kg ⁻¹ Fb seedling dip with <i>P. fluorescens</i> @ 10 g l ⁻¹ 4 times at 15 days interval	15.81	23.90	25.16	24.10	22.60	13.19	26.40	57.84
T4	ST with <i>Trichoderma harzianum</i> @ 5 g kg ⁻¹ Fb spray with <i>T. harzianum</i> @ 10 g l ⁻¹ 4 times	12.84	21.18	23.40	27.31	34.42	20.19	32.10	50.16
T5	ST with <i>Trichoderma harzianum</i> @ 5 g kg ⁻¹ spray with <i>B. subtilis</i> @ 10 g l ⁻¹ 4 times	13.45	26.29	31.18	28.10	25.13	16.21	24.78	53.40
T6	ST with <i>Trichoderma harzianum</i> @ 5 g kg ⁻¹ spray with <i>P. fluorescens</i> @ 10 g l ⁻¹ 4 times	13.29	23.16	29.19	27.33	26.15	17.23	24.12	54.34
T7	Recom. Check (Sulphur) @ 3 g l ⁻¹	15.32	29.74	32.10	34.19	39.41	19.13	36.16	51.10
T8	Untreated Control	14.98	26.19	36.30	48.45	53.15	23.61	41.42	39.22
	S.Em+	0.94	1.89	1.80	2.10	1.74	1.93	1.55	1.85
	CD (5%)	2.82	5.60	5.50	6.27	5.27	5.52	4.50	5.41
	CV (%)	9.30	10.24	11.30	12.06	9.80	12.41	10.20	8.98

S.Em standard error of mean, CD confidence distribution, CV coefficient of variation

first spray was found to be ranged from 21.18% (T4) to 26.19% (T8). After the second spray, the diseases in T2 (24.10%), T3 (25.16%), and T4 (23.40%) were comparatively lower than the untreated control T8 (36.30%). After three times spray, T2 (22.10%), T3 (24.10%), T4 (27.31%), T5 (28.10%), and T6 (27.33%), the disease incidence was found significantly ($p < 0.05$) lower or at par with each other than the untreated control T8 (48.45%). At the end of the fourth spray, the most negligible early blight incidence was recorded in the treatments T2 (22.31%), T3 (22.60%), T5 (25.13%), and T6 (26.15%), and the highest was in untreated control T8 (53.15%).

Powdery mildew

During the Kharif 2017–2018 (Table 1), the low disease incidences ranged from 15.54 to 24.42% in all the treatments except in T1 (27.38%) and T7 (26.29%), compared to T8 (29.60%), which were assessed only after the fourth spray. The disease incidences during Kharif 2018–2019 (Table 2), T2 (12.10%), and T3 (13.19%) treatments were compared and statistically superior vis-à-vis untreated control T8 (23.61%).

Septoria leaf spot

The disease incidence during the Kharif 2017–2018 (Table 1), the septoria leafspot incidences ranged from 13.32% (T2) to 14.80% (T8) after the third spray. The lowest septoria leaf spot diseases were recorded in the T2 (23.16%), T3 (26.40%), T5 (24.78%), and T6 (24.12%), compared to control (41.42%) during the Kharif, 2018–2019. The pooled data of both the years also showed that the early blight incidences were significantly lower ($p < 0.05$) in T2 (21.41%) and T3 (20.66%), and the powdery mildew incidences in T2 (13.82%) and T3 (15.48%) and septoria leaf spot incidences in T2 (19.35%) and T3 (22.08%) had also followed similar trend.

The biocontrol agents viz., *T. harzianum* and *B. subtilis* had shown positive results for the production of HCN by changing the colour of filter paper that was pre-added with picric acid from light yellow to orange (Fig. 1a). *P. fluorescens* tested negative for the HCN production as it didn't change the colour of filter paper from yellow to orange. *P. fluorescens* and *B. subtilis* produced yellow halos around the colony confirming the siderophore production (Fig. 1b). *T. harzianum* showed a negative result for the siderophore production as it did not produce yellow halos around the mycelial growth in the CAS (Chrome Azuroil S) media. *T. harzianum* was found to be positive for the test. It was observed by a change in media colour from pinkish-red to purple. *P. fluorescens* and *B. subtilis* have shown negative for the chitinase test as the colour of the media did not change (Fig. 1c).

Plant height (Fig. 2)

The plant height varied between 20.3 and 35.7 cm. The maximum height was recorded in T3 (35.7 cm), which was found on par with 32.7 cm (T4), followed by T2 (31.7 cm), T5 (32.0 cm), and T6 (30.0 cm), and the minimum height was recorded in T8 (20.3 cm).

Fruits per cluster (Fig. 2)

The highest numbered of fruits per cluster were recorded in T3 (6.33) and T2 (5.67), followed by T4 (5.00), T5 (5.33), and T6 (4.33), and the lowest in the T8 (2.33).

Several pickings (Fig. 2)

The maximum pickings were recorded in T3 (12.00), T4 (11.67), T2 (11.33), and T5 (11.33). The minimum pickings were recorded in T8 (7.33).

Yield

During Kharif 2017–2018, the pooled mean values ranged from 43.13 t ha⁻¹ to 61.57 t ha⁻¹ (Table 1). The highest was in the T2 (60.05 t ha⁻¹), T3 (61.57 t ha⁻¹), and T6 (59.47 t ha⁻¹), and the lowest in T8 (43.13 t ha⁻¹). A similar trend was recorded during the subsequent year, Kharif 2018–2019 (Table 2), with the highest yields in T2 (58.61 t ha⁻¹), T3 (57. t ha⁻¹), followed by T6 (54.34 t ha⁻¹), and T7 (51.10 t ha⁻¹) and the lowest in untreated control T8 (39.22 t ha⁻¹). The pooled analysis of the yield data also revealed that the maximum yields were recorded in T2 (58.93 t ha⁻¹) and T3 (59.92 t ha⁻¹), followed by T5 (55.07 t ha⁻¹) and T6 (56.41 t ha⁻¹) and the minimum in yield in untreated control T8 (40.68 t ha⁻¹).

Cost–benefit ratio (Fig. 3)

The cost of cultivation in all the treatments ranged from Rs. 2, 60,000 ha⁻¹ (T8) to a maximum of Rs. 2, 78,960 ha⁻¹ (T3). The maximum gross return was obtained in T3 (Rs. 8, 98,800 ha⁻¹), followed by T2 (Rs.8, 83,950 ha⁻¹) and the minimum in T8 (Rs.6, 10,200 ha⁻¹). The highest net returns were recorded in T3 (Rs. 6, 19,840 ha⁻¹) and T2 (Rs. 6, 06,250 ha⁻¹) and the lowest in T8 (Rs. 3, 50, 200 ha⁻¹). The highest benefit: cost ratios of 3.22:1 and 3.18:1 were obtained in T3 and T2 and the lowest was in T8 (2.34:1).

Discussion

This application of biocontrol agents (viz., *P. fluorescens*, *B. subtilis*, and *T. harzianum*) revealed promising effectiveness against tomato fungal foliar disease. It was found that the fungal bioagent, *T. harzianum* was more effective than the bacterial antagonists *P. fluorescens* and *B. subtilis*

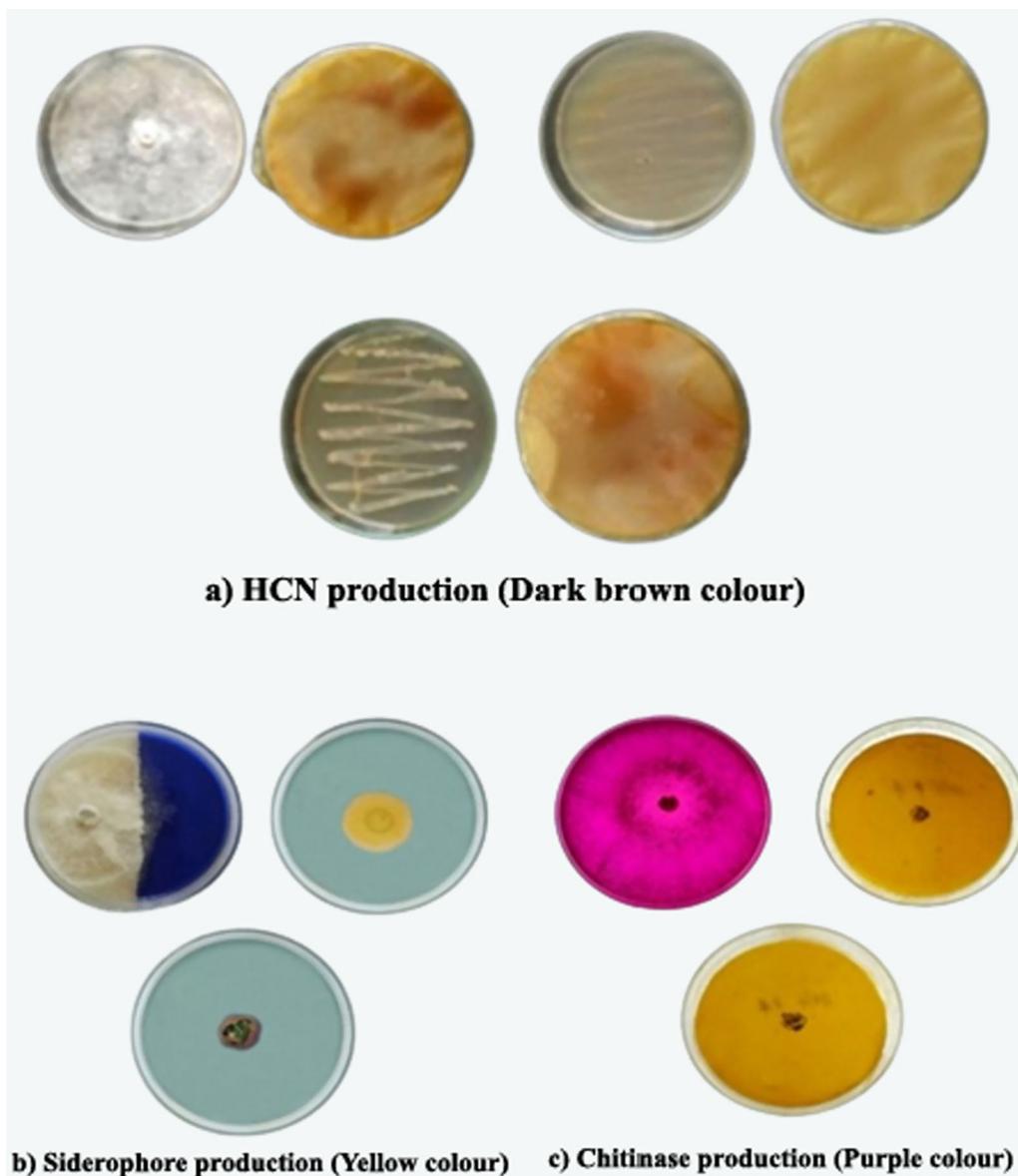


Fig. 1 a–c Characterization of antagonistic microorganisms for the production of HCN, Siderophore and Chitinase by *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*

(Basamma and Kulkarni 2017). Promotions of plant growth (more plant height and more fruit per cluster and picking) often demonstrated the effectiveness of applying these bioagents. Net income and benefit: cost ratios were also found maximum in *P. fluorescens*, *B. subtilis*, and *T. harzianum* treated plots (T7) compared to the untreated control (T8). These results agreed with the studies conducted on cucumbers under protected cultivation (Joshi et al. 2010). Many strains of *P. fluorescens* enhanced plant growth promotion and reduced the severity of various diseases. PGPR (Plant Growth Promoting Rhizobacteria)

are highly diverse biocontrol agents, and their effects can occur via local antagonism to soil-borne pathogens or by induction of systemic resistance against pathogens throughout the entire plant (Compant et al. 2005). Among the evaluated biocontrol agents, *T. harzianum* and *B. subtilis* showed positive results, for the production of HCN by changing the colour of filter paper from yellow to orange. HCN production by the antagonists was reported by other workers as well (Kaiser et al. 2018). The HCN is known to inhibit electron transport, disrupting the energy supply to the cells, may act as toxic to

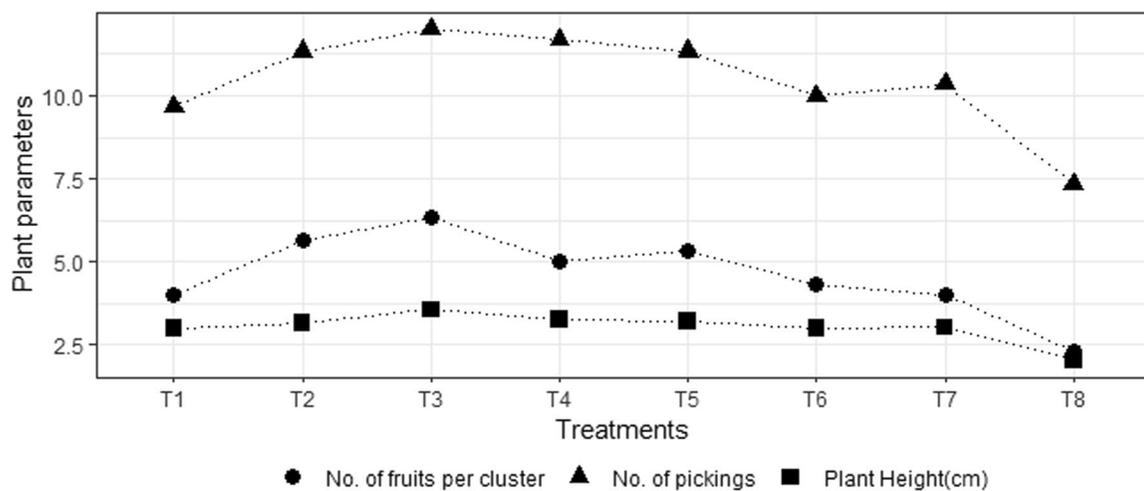


Fig. 2 Pooled analysis of various treatments and their influence on plant parameters (Kharif 2017–2018 and 2018–2019). As compared to control T8, all the treatments have significant impact ($p < 0.05$) on the plant parameters (plant height, number of fruits per cluster and number of pickings). This figure shows that as compare to other treatments, plant parameters are comparatively higher ($p < 0.05$) with the treatment T3 and provided a maximum yield followed by the treatments T2, T5 and T6

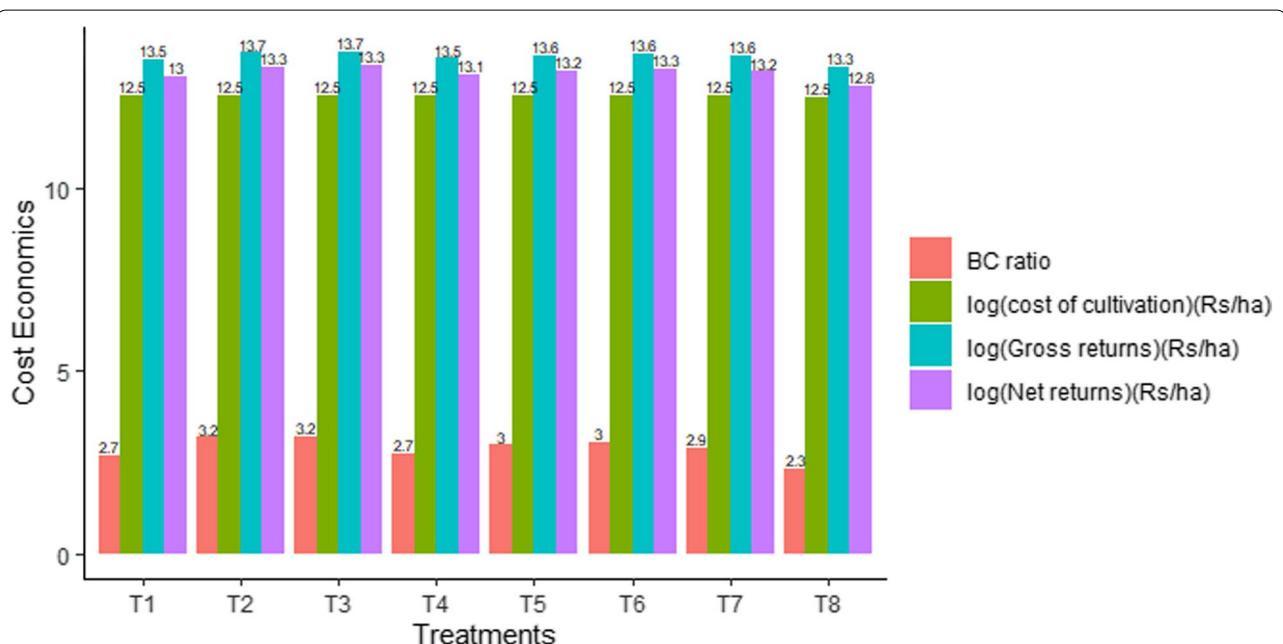


Fig. 3 Cost economics of management of fungal foliar diseases of tomato in a polyhouse condition (T1-Seed treatment, seedling dip and spray with *T. harzianum*; T2-Seed treatment, seedling dip and spray with *B. subtilis*; T3-Seed treatment, seedling dip and spray with *P. fluorescens*; T4-Seed treatment and spray with *T. harzianum*; T5-Seed treatment with *T. harzianum* and spray with *B. subtilis*; T6-Seed treatment with *T. harzianum* and spray with *P. fluorescens*; T7-Spray with Sulphur and T8-Untreated control)

pathogen and ultimately leads to the death of pathogens thus reducing plant disease (Knowles, 1976). *P. fluorescens* and *B. subtilis* showed positive results for the siderophore production by developing yellow halos around the colony in the CAS agar medium. Siderophores are

iron-chelating ligands secreted extracellularly by antagonists under iron liming conditions. They increase the solubility of ferric ions (Fe^{3+}), making them available to plants and preventing pathogens from acquiring iron (Loper and Buyer 1991).

Trichoderma harzianum showed positive for the production of chitinase by changing media colour to purple. Chitinase is the enzyme that breaks the cell wall of pathogens, thus suppressing the pathogens. These findings are supported by Khatri et al. 2017. Several substances produced by antagonistic rhizobacteria have been related to pathogen control and indirectly promoted growth in many plants, such as siderophores and antibiotics (Tariq et al. 2017). Rhizobacteria belonging to the genera, *Pseudomonas* and *Bacillus* are well known for their antagonistic effects and ability to trigger induced systemic resistance (Jogaiah et al. 2018). After sequential application of *P. fluorescens* and *B. subtilis* reduced the disease incidence significantly ($p < 0.05$), enhanced flowering, and resulted in more pickings. A similar trend was reported in term yield in tomatoes (Kabdwal et al. 2019). Similarly, BCAs viz., *T. harzianum*, *T. longibrachiatum*, *T. atroviride* have proved potential antagonism against *Alternaria solani* (Imran et al. 2022) in different combinations in open field trials. Seedling treatment and foliar spray for growth promotion and disease control in tomatoes proved efficacy. *A. solani* and *S. lycopersici* causing the tomato early blight and septoria leaf spot under both *in-vitro* and polyhouse conditions resulted in reduced disease incidence, improved yields, and overall net yields. Usage of plant growth-promoting microbes increased plant height, which helped the plants bear more flowers and produce more fruits.

Conclusions

The present study on the organic cultivation of tomatoes with special regard to the use of PGPRs as biopesticides under protected cultivation showed significant reduction in disease incidence, enhanced flowering, and resulted in more pickings. To manage fungal foliar diseases of tomato in a cost-effective manner, a set of standardized experimental design and seed treatment, followed by sequential spray at 15-day intervals were required.

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Author contributions

GMH conceptualized and conducted research. LHM carried out soil analysis and nutrient requirement of the crop, MNS carried out CFU count of microbes used and their confirmation, GMH and BKC carried out statistical analysis, writing the manuscript, referencing setting, and editing of the manuscript. All authors read, revised, and approved the final manuscript.

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The authors declare no competing interests.

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