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

Gurudatt M. Hegde1,3*, Lokanath H. Malligawad1, M. N. Sreenivasa1 and Bimal K. Chetri2

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...
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 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⁻unite like T. harzianum (accession no. NAICCC-F-03328) (2 × 10⁶) and P. fluorescens (accession no. NAICCC-B-01981) (2 × 10⁶), Purpureocillium lilacinum (accession no. IOF Dharward) at (2 × 10⁶) and Metarhizium anisoplae (IOF Dharward) (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).

| 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 spots, spots also seen on the petiole, branches
4 41–70% Leaf area infected and covered by spots, 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).

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

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 transplating) 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 Neillands 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 \( (T1–T8) \) 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 T3 and T7 was recorded as 14.80 and 16.28%, respectively, compared to other treatments. After the third spray, the disease incidences at T2 (19.24%), T3 (19.98%), followed by T5 (28.86%), T6 (27.38%), and T7 (25.16%) were found to be significantly \( (p < 0.05) \) lower than the T8 (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) % | Production of HCN, Siderophore and Chitinase |
|------------|-------------------|------------------------------------------------------|---------------------------------------------|
|            |                   | Early blight                                         | Powdery mildew | Septoria leaf spot | Yield |
|            |                   | Before spray | After I spray | After II spray | After III spray | After IV spray | After III spray | After IV spray | SEM ± | CD (5%) | CV (%) |
| T1         | ST with *Trichoderma harzianum* @ 5 g kg⁻¹ Fb seedling dip with *T. harzianum* 10 g l⁻¹ spray with *T. harzianum* 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 *Bacillus subtilis* @ 5 g kg⁻¹ Fb seedling dip with *B. subtilis* 10 g l⁻¹ spray with *B. subtilis* 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 *Pseudomonas fluorescens* @ 5 g kg⁻¹ Fb seedling dip with *P. fluorescens* 10 g l⁻¹ spray with *P. fluorescens* 10 g l⁻¹ at 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 *Trichoderma harzianum* @ 10 g kg⁻¹ Fb spray with *T. harzianum* @ 10 g l⁻¹ 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 *Trichoderma harzianum* @ 5 g kg⁻¹ Fb spray with *B. subtilis* @ 10 g l⁻¹ at 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 *Trichoderma harzianum* @ 5 g kg⁻¹ Fb spray with *P. fluorescens* @ 10 g l⁻¹ at 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 |
|            |                   | SEM ± | 0.83 | 1.57 | 1.80 | 1.88 | 1.63 | 1.83 | 0.80 | 1.55 | 2.16 |
|            |                   | CD (%) | 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
| Treatments | Treatment details | Foliar diseases of tomato (percentage disease index) | Powdery mildew | Septoria leaf spot | Yield t/ha |
|------------|-------------------|---------------------------------------------------|----------------|-------------------|------------|
|            |                   | Before spray | After I spray | After II spray | After III spray | After IV spray | After IV spray |
| T1         | ST with *Trichoderma harzianum* @ 5 g kg⁻¹ Fb seedling dip with *T. harzianum* @ 10 g l⁻¹ -spray with *T. harzianum* 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 *Bacillus subtilis* @ 5 g kg⁻¹ seedling dip, spray with *B. subtilis* 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 *Pseudomonas fluorescens* @ 5 g kg⁻¹ Fb seedling dip with *P. fluorescens* @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 *Trichoderma harzianum* @ 5 g kg⁻¹ Fb spray with *T. harzianum* @ 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 *Trichoderma harzianum* @ 5 g kg⁻¹ spray with *B. subtilis* @ 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 *Trichoderma harzianum* @ 5 g kg⁻¹ spray with *P. fluorescens* @ 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 |
|            | SEm | 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 |

SEm 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 (24.40%) 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%).

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), T2 (12.10%), T3 (13.19%) treatments were compared and statistically superior vis-à-vis untreated control T8 (23.61%).

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.
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 \textit{P. fluorescens}, \textit{B. subtilis}, and \textit{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 \textit{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, \textit{T. harzianum} and \textit{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 (Qaiser et al. 2018). The HCN is known to inhibit electron transport, disrupting the energy supply to the cells, may act as toxic to
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 limiting 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).
... 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 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.

Acknowledgements
The authors are grateful to the Director of Research, University of Agricultural Sciences, Dharwad, for providing the financial assistance to experiment with protected cultivation. Supporting studies conducted at the Institute of Organic Farming is duly acknowledged.

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 BKRC carried out statistical analysis, writing the manuscript, referencing setting, and editing of the manuscript. All authors read, revised, and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
Not applicable.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
1 Institute of Organic Farming, University of Agricultural Sciences, Dharwad, Karnataka 580 005, India. 2 Department of Environment and Life Science, Sherubtse College, Royal University of Bhutan, Kanglung, Trashigang 42002, Bhutan. 3 AICRP on Wheat and Barley, Main Agricultural Research Station, University of Agricultural Sciences, Yettinagudda Campus, Krishinagar, Dharwad, Karnataka state 580 005, India.

Received: 16 June 2022 Accepted: 24 August 2022
Published online: 03 September 2022

References
Agrawal T, Kotasthane AS (2012) Chitinolytic assay of indigenous Trichoderma isolates collected from different geographical locations of Chhattisgarh in Central India. Springerplus 1:73. https://doi.org/10.1186/2193-1801-1-73
Arushi AB, Banyal DK (2018) Evaluation of IDM components for management of tomato powdery mildew under protected cultivation. Int J Curr Microbiol App Sci 7:21–31
Atalla SMM, Ahmed NE, Awad Hm, El Gamal NG, El Shamy AR (2020) Statistical optimization of xylanase production, using different agricultural wastes by Aspergillus oryzae MN894021, as a biological control of faba bean root diseases. Egypt J Biol Pest Control 30:125. https://doi.org/10.1186/s41938-020-00323-z
Bakker AW, Schippers B (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp-mediated plant growth-stimulation. Soil Biol Biochem 19:451–457. https://doi.org/10.1016/0038-0717(87)90037-X
Basamma RH, Kulkarni S (2017) Growth promoting ability and bioefficacy of Bacillus subtilis against powdery mildew and early blight of Tomato through Foliar Spray in pot culture studies. J Pharmacogn Phytochem 6:244–246
Comptan S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959
Imran M, Abo-Elyour KAM, Moussa MA, Saad MM (2022) Screening and biocontrol evaluation of indigenous native Trichoderma spp. against early blight disease and their field assessment to alleviate natural infection. Egypt J Biol Pest Control 32:1–10. https://doi.org/10.1186/s41938-022-00544-4
Jogaiah S, Abdelrahman M, Tran LSP, Ito Si (2018) Different mechanisms of Trichoderma virens-mediated resistance in tomato against Fusarium wilt involve the jasmonic and salicylic acid pathways. Mol Plant Pathol 19:870–882. https://doi.org/10.1111/mpp.12571
Joshi BB, Bhatt RP, Bahukhandi D (2010) Antagonistic and plant growth activity of Trichoderma isolates of Western Himalayas. J Environ Biol 31:921–928
Kabdwal BC, Sharma R, Tewari R, Tewari AK, Singh RP, Dandona JK (2019) Field efficacy of different combinations of Trichoderma harzianum, Pseudomonas fluorescens, and arbuscular mycorrhiza fungus against the major diseases of tomato in Uttarakhand (India). Egypt J Biol Pest Control 29:1–10. https://doi.org/10.1186/s41938-018-0103-7
Kaur R, Joshi N, Virk JS, Sharma S (2016) Evaluation of Pseudomonas fluorescens for the management of tomato early blight disease and fruit boer. J Environ Biol 37:869–872
Khatri DK, Tiwari DN, Bariya HS (2017) Chitinolytic efficacy and secretion of cell wall degrading enzymes from Trichoderma spp. in response to phyto-pathological fungi. J Appl Biol Biotechnol 5:1-Notice
Knowles CJ (1976) Microorganisms and cyanide. Bacteriol Rev 40:652–680
Loper JE, Buyer JS (1991) Siderophores in microbial interactions on plant surfaces. Mol Plant Microbe Interact 4:3–13
Mayee CD, Mayee CD, Datar V V (1985) Phytopathometry (Technical Bulletin No. 1). Marathwada Agril. Univ. Parbhani
Panse VG, Sukhatme PV (1954) Statistical methods for agricultural workers. Stat methods Agric Work
Pooja BG, Hakkim AVM (2017) Comparative study of tomato under polyhouse and rainshelter conditions. Int J Eng Sci Comput 7:15317–15319
Qaiser J, Seong LY, Deok JH, Young KK (2018) Effect of plant growth-promoting bacteria Bacillus amyloliquefaciens Y1 on soil properties, pepper seedling growth, rhizosphere bacterial flora and soil enzymes. Plant Prot Sci 54:129–137
R Core Team Rf (2018) R: a language and environment for statistical computing
Sokhi SS, Munshi GD, Grewal RK, et al (1991) Current fungal disease problems of important vegetables in India. 10:20–30
Tariq M, Noman M, Ahmed T, Hameed A, Manzoor N, Zafar M et al (2017) Antagonistic features displayed by plant growth promoting rhizobacteria (PGPR): a review. J Plant Sci Phytopathol 1:38–43

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.