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Microbe-Mediated Stress Mitigation and Growth Promotion in Early Blight Challenged Tomato (*Solanum lycopersicum* L.) Under Greenhouse Conditions

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Abstract

A greenhouse pot experiment was carried out to investigate the ability of the microorganisms to reduce stress and enhance growth of tomato (*Solanum lycopersicum* L.) cv. during the Rabi season 2023-24 under *Alternaria solani* challenge Kashi aman cultivar of tomato. The experiment was designed in a completely randomized design consisting of ten treatments with four replications (pathogen-only, untreated control and preventive applications of *Trichoderma asperellum*, two *Bacillus* strains (BN-5 and BS-9) and *Pseudomonas fluorescens* alone or in combination with the pathogen). Plant Height, Fruit Weight, Fruit Dimension, Fruit Thickness, Locule Number and Total Soluble Solids were significantly affected by all the treatments. *Trichoderma asperellum* was the only fungus that recorded the highest plant height (73.17 cm), fruit weight (95.37 g), equatorial diameter (4.27 cm), thickness (0.53 cm), number of locules (3.50) and total soluble solids (4.32%). In the pathogen challenged treatments, pathogen alone and pathogen + *T. asperellum* + *A. solani* had significantly higher plant height and fruit weight than other pathogen challenged treatments. The outcomes showed a beneficial effect of the selected microbial agents, especially *T. asperellum* and BN-5 on growth and fruit quality of tomato in greenhouse and their potential role in sustainable early blight stress management.

Introduction

Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop, whose productivity is often reduced due to fungal diseases and stress in protected and open cultivation systems. *Alternaria solani* (Ellis and Martin) Sorauer is an important foliar disease of tomato, which is responsible for reducing photosynthetic leaf area, plant vigour and marketable fruit production when humidity and temperature conditions are favourable (Hyder et al., 2024; Wilson and Arunachalam, 2024). The rising interest in residue-free production of vegetable crops has boosted the use of biological control agents and plant growth promoting microbes as alternatives and

supplements to chemical disease control. In addition to nutrient solubilization and production of siderophores, rhizobacteria and beneficial fungi can help plants perform a variety of functions including modulation of phytohormones, production of antifungal metabolites, competition for infection sites, and induced systemic resistance (ISR) (Bellotti et al., 2023; Hasan et al., 2024; Sun et al., 2024). *Trichoderma spp*. are among the most commonly studied biocontrol agents, characterized by their ability to mycoparasitize, secrete cell-wall degrading enzymes, and possess rhizosphere competence and stimulate host defence responses (Behiry et al., 2023; Hasna et al., 2025; Harman et al., 2004). Likewise, endospore-forming ability, rhizosphere persistence, production of lipopeptides and antibiotics and the capacity to induce resistance in host plants are widely used arguments that justify the use of *Bacillus* species (Dobrzyński et al., 2023; Karačić et al., 2024). *Pseudomonas fluorescens* is also known to produce fluorescent pigments and to be a competitive siderophore-producing organism and to be able to colonize and promote stress tolerance in tomato and other crops (Kukreti et al., 2024; Weller, 2007). Very recent studies have demonstrated the potential effects of microbial inoculants on tomato growth and stress tolerance when challenged by pathogens, with the extent of the response often being associated with the type of microbial and how it was applied, the plant genotype and the environment in which the plants are growing (Cochard et al., 2022; Hernández-Amador et al., 2024; Sarma and Deka, 2024). The present investigation was aimed at studying growth and fruit quality responses of tomato cv. Under greenhouse conditions, Kashi Aman to *Trichoderma asperellum*, two *Bacillus* strains and *Pseudomonas fluorescens* showed significant activity against *Alternaria solani*.

Materials and Methods

The experiment was conducted in the research greenhouse of the Department of Plant Pathology, ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India during the Rabi season of 2023-24. The experimental site was set up at the latitude of 25°10′ N and longitude 82°52′ E in the subtropical region of the Varanasi district at an elevation of 86 m above mean sea level. The weather of the region is mild during the rabi season with cool nights suitable for tomato cultivation. The weather during rabi season in the region is mild with cool nights, suitable for tomato cultivation. Seeds of tomato cv. Kashi Aman was collected from ICAR-IIVR, Varanasi. The seeds were sown in sterilized nursery trays and after four weeks, uniform seedlings were transferred into sterilized earthen pots (30 cm diameter). The loam soil and farmyard manure (FYM) were autoclaved and added to each pot in the ratio 3:1. Normal cultural practices were used for crop maintenance (irrigating, staking and nutrient management). The chemical pesticide was not used in the experiment to ensure the observed plant response was due to the introduced pathogen and biocontrol agents. The pot experiment in the greenhouse was designed in a completely randomized design of 10 treatments and 4 replications (Table

1). This treatment system enabled the comparison of single biocontrol agents, mixtures of biocontrol agents and pathogen challenged treatments with controls. The cultures of fungi were kept on potato dextrose agar slants and those of bacteria on King's B agar or nutrient agar slants at 25 ± 2°C with sub-culturing at regular intervals. *Alternaria solani* was cultured on potato dextrose agar, Trichoderma was isolated and multiplied on Trichoderma selective medium whereas Pseudomonas fluorescens was cultured on King's B medium, and pigment confirmation was done (Aneja, 2003; Elad et al., 1981; King et al., 1954). All media were sterilized using the autoclave at 121°C for 15 min and sterilized under aseptic conditions. *Alternaria solani* was isolated from naturally infected tomato leaves showing typical concentric brown lesions. The infected pieces of leaf were surface sterilized in 0.1% mercuric chloride for 1 minute, rinsed several times with sterile distilled water and then surface-plated on potato dextrose agar. The pathogen was purified by single-spore isolation and maintained on potato dextrose agar slants at 4°C. The pigmented, multicellular conidia with transverse and longitudinal septa are typical of A. solani (Ellis, 1971; Simmons, 2007). Pathogen inoculum was prepared by flooding 10-day old culture with sterile distilled water, gently scraping with a sterile glass rod and filtering through double layer cheesecloth. The conidial suspension was diluted to about 1×105 conidia mL-1 with Tween-20 (0.02%) added to aid the spreading on the leaf surface (Agrios, 2005). Plants at 5-6 leaves stage were sprayed till the runoff was attained during the late afternoon and high humidity was maintained for 48 h by covering with transparent polythene sheets. Non-pathogen controls were given sterile distilled water with Tween-20. Biocontrol agents used were two strains of Bacillus (BN-5 and BS-9) and *Pseudomonas fluorescens*, as well as the biocontrol fungus *Trichoderma asperellum*. *Trichoderma asperellum* was grown on potato dextrose agar for 7-10 days at 28°C and abundant sporulation was obtained. Conidia were collected in sterile distilled water with 0.1% Tween-20, filtered through sterile muslin cloth and counted using a haemocytometer at about 1×10^7 conidia mL^1 (Harman et al., 2004). The fungus was also mass multiplied on autoclaved sorghum grains for about 15 days, and mixed at the rate of 5g kg^{-1} soil at transplanting into pot soil for soil application. *Bacillus strains* BN-5 and BS-9 were cultured in nutrient broth at 30°C with shaking for 48 h, and *Pseudomonas fluorescens* was cultured in King's B broth at 28°C with shaking for 48 h. Bacterial cells were harvested, washed and adjusted to approximately 1×10^8 CFU mL^1 (OD of about 0.5 at 600 nm) and confirmed by serial dilution and plate counts (Kloepper et al., 2004; Weller, 2007). Accession numbers of the available strains were *Bacillus* sp. IIVRBN-5 (PQ686354), *Bacillus* sp. IIVRBS-9 (PQ686356) and Pseudomonas fluorescens OKC (JN128891). Early colonization of root and phyllosphere was promoted by the application of biocontrol agents before the pathogen challenge. Five days prior to pathogen application, the bacterial inoculants were

applied as a soil drench with 50 mL around the root zone. Furthermore, a foliar spray was done 3 days prior to pathogen application at the same rate (25 mL/plant). Steer clear of microbial suspensions in plants treated with sterile distilled water. Microbial preventive application was applied due to the ability to establish beneficial microbes early which would enhance competition, colonization and defence priming (Compant et al., 2005; Kukreti et al., 2024). The plant height (cm), average weight of two fruits (g), polar diameter (cm), equatorial diameter (cm), fruit thickness (cm), number of locules and total soluble solids (%) were recorded at the end of the experiment. Fruit size was determined with a vernier caliper and total soluble solids were measured with a hand refractometer. The data were analyzed using analysis of variance, and the means of the treatments were compared by critical difference at the 5% level. Data are expressed as means ± SD.

Table 1. Treatment details used in the greenhouse pot experiment.

Treatment	Description
T1	*A. solani* (pathogen only)
T2	*Trichoderma asperellum*
T3	*T. asperellum* + *A. solani*
T4	BN-5 (*Bacillus* sp.)
T5	BN-5 + *A. solani*
T6	BS-9 (Bacillus sp.)
T7	BS-9 + A. solani
T8	*Pseudomonas fluorescens*
T9	*P. fluorescens* + *A. solani*
T10	Untreated control

Result and Discussion

The treatments significantly influenced all recorded growth and fruit quality traits of tomato (Table 2; Figures 1-4). Plant height differed significantly among treatments ($F = 44.6$, $p < 0.01$), with the tallest plants recorded in T2 (73.17 ± 0.76 cm), followed by T3 (71.15 ± 0.90 cm) and T4 (70.31 ± 0.71 cm). In contrast, the untreated control T10 recorded the lowest plant height (60.56 ± 0.78 cm), and the pathogen-only treatment T1 recorded 63.07 ± 0.90 cm. The 20.8% increase in plant height in T2 over the untreated control indicates a strong growth-promoting response to *Trichoderma asperellum*. Such improvement may be associated with enhanced root activity, phytohormone modulation, nutrient mobilization and defence priming, as reported in recent work on microbial biostimulants and tomato-associated growth-promoting rhizobacteria (Cochard et al., 2022; Hernández-Amador et al., 2024; Sun et al., 2024).

Among pathogen-challenged treatments, T3 maintained the highest plant height and exceeded T1 by 12.8%, suggesting that *Trichoderma asperellum* supported vegetative growth even under *Alternaria solani* challenge. Similar growth retention under microbial protection has been associated with improved root colonization, antagonistic metabolite production and host resistance activation (Bellotti et al., 2023; Behiry et al., 2023; Hasna et al., 2025). *Bacillus*-based treatments also improved plant height relative to pathogen-only plants, especially T5 and T7, which is consistent with the recognized role of *Bacillus* spp. as spore-forming biocontrol and plant-growth-promoting agents (Dobrzyński et al., 2023; Karačić et al., 2024).

Average fruit weight varied highly significantly among treatments ($F = 198.1$, $p < 0.01$). T2 produced the heaviest fruits (95.37 ± 1.48 g), followed by T4 (90.50 ± 1.37 g) and T3 (88.13 ± 1.01 g). The untreated control T10 and pathogen-only T1 recorded 69.59 ± 0.60 g and 68.16 ± 1.00 g, respectively. The 39.9% increase in fruit weight in T2 over T1 and the 29.3% increase in T3 over T1 indicate that microbial inoculation contributed to better reproductive growth and assimilate partitioning. Recent tomato studies indicate that beneficial rhizobacteria and bacterial inoculants improve biomass accumulation, nutrient status and yield-related traits through direct and indirect plant growth-promoting mechanisms (Hyder et al., 2024; Hernández-Amador et al., 2024; Kukreti et al., 2024).

Fruit dimensional traits also responded significantly to treatments. Polar diameter and equatorial diameter recorded significant treatment effects ($F = 5.6$ and 6.2, respectively; $p < 0.01$). T2 recorded the largest polar diameter (4.07 ± 0.16 cm) and equatorial diameter (4.27 ± 0.25 cm). T4 also performed well for both traits, indicating that BN-5 may improve fruit expansion under non-pathogen conditions. Increased fruit diameter is usually linked with improved cell division, cell expansion and photosynthate supply during fruit development, processes that can be supported by microbial enhancement of nutrient availability and hormonal balance (Sarma and Deka, 2024; Sarti et al., 2024).

Fruit thickness and locule number differed significantly among treatments ($F = 5.4$ and 3.9, respectively; $p < 0.01$). The highest fruit thickness was observed in T2 (0.53 ± 0.06 cm), followed by T4 (0.40 ± 0.10 cm), whereas T1 and T10 recorded the lowest value (0.20 cm). Similarly, the maximum locule number was recorded in T2 (3.50 ± 0.50), followed by T4 (3.23 ± 0.25). These improvements suggest better fruit structural development in microbe-treated plants. The positive influence of beneficial microorganisms on reproductive development may arise from improved nutrition, root function and physiological stress regulation (Cochard et al., 2022; Hasan et al., 2024).

Total soluble solids also varied significantly ($F = 5.8$, $p < 0.01$). T2 showed the highest TSS (4.32 ± 0.18%), followed by T4 (3.88 ± 0.29%) and T6 (3.67 ± 0.42%). Higher TSS in microbe-treated plants

suggests better carbohydrate accumulation and fruit quality. In tomato, microbial inoculation may alter source-sink relations and nutrient uptake, which can improve fruit biochemical quality in addition to growth (Hernández-Amador et al., 2024; Sarti et al., 2024).

Overall, *Trichoderma asperellum* alone (T2) produced the strongest improvement across most growth and fruit quality traits, whereas *T. asperellum* + *A. solani* (T3) was the most effective among pathogen-challenged treatments for maintaining plant height and fruit weight. BN-5 also showed a strong positive response, particularly under non-pathogen conditions. The lower performance of the pathogen-only treatment compared with the leading microbial treatments supports the view that beneficial fungi and bacteria can reduce stress impact by promoting growth and improving physiological resilience rather than acting only through direct pathogen suppression (Bellotti et al., 2023; Hyder et al., 2024; Wilson and Arunachalam, 2024). The results therefore support preventive use of *Trichoderma asperellum* and selected *Bacillus* strains as sustainable components of tomato crop management under early blight-prone conditions.

Table 2. Effect of microbial treatments and *Alternaria solani* challenge on vegetative growth and fruit quality parameters in tomato.

Treatment	Plant height (cm)	Average weight of two fruits (g)	PD (cm)	ED (cm)	Thickness (cm)	Locules	TSS (%)
T1	63.07 ± 0.90e	68.16 ± 1.00h	3.65 ± 0.25bc	2.67 ± 0.51e	0.20 ± 0.00d	2.33 ± 0.58cd	3.08 ± 0.08d
T2	73.17 ± 0.76a	95.37 ± 1.48a	4.07 ± 0.16a	4.27 ± 0.25a	0.53 ± 0.06a	3.50 ± 0.50a	4.32 ± 0.18a
T3	71.15 ± 0.90b	88.13 ± 1.01c	3.60 ± 0.30bc	3.07 ± 0.21de	0.30 ± 0.10bcd	2.70 ± 0.26bcd	3.25 ± 0.15cd
T4	70.31 ± 0.71bc	90.50 ± 1.37b	3.80 ± 0.10ab	4.03 ± 0.70ab	0.40 ± 0.10b	3.23 ± 0.25ab	3.88 ± 0.29ab
T5	68.44 ± 0.88c	82.81 ± 1.08d	3.40 ± 0.10cd	3.03 ± 0.16de	0.33 ± 0.06bc	2.60 ± 0.10cd	3.25 ± 0.15cd
T6	69.20 ± 0.89bc	79.80 ± 1.06e	3.67 ± 0.15bc	3.83 ± 0.21abc	0.36 ± 0.04bc	2.80 ± 0.26bc	3.67 ± 0.42bc
T7	66.36 ± 0.82d	70.36 ± 0.67fg	3.23 ± 0.15d	3.40 ± 0.66bcd	0.27 ± 0.12cd	2.67 ± 0.58bcd	3.32 ± 0.28cd
T8	63.07 ± 2.01e	79.23 ± 0.74e	3.43 ± 0.32cd	3.57 ± 0.15bcd	0.33 ± 0.06bc	2.63 ± 0.32bcd	3.45 ± 0.52bcd
T9	61.16 ± 1.95ef	72.10 ± 2.03f	3.20 ± 0.10d	2.73 ± 0.15e	0.23 ± 0.06cd	2.13 ± 0.15d	3.20 ± 0.20cd
T10	60.56 ± 0.78f	69.59 ± 0.60gh	3.50 ± 0.10bcd	3.20 ± 0.10cde	0.20 ± 0.10d	2.30 ± 0.26cd	3.25 ± 0.15cd
F stat	44.6**	198.1**	5.6**	6.2**	5.4**	3.9**	5.8**
CD	1.97	2.00	0.33	0.64	0.13	0.62	0.47
MSE	1.34	1.39	0.04	0.14	0.01	0.13	0.08
SE(m)	0.67	0.68	0.11	0.22	0.04	0.21	0.16
SE(d)	0.94	0.96	0.16	0.31	0.06	0.30	0.22

CV (%)	1.7	1.5	5.4	11.1	24.1	13.5	7.9

Values are mean ± SD. Different letters within a column indicate significant differences at $p < 0.05$. ** indicates significance at $p < 0.01$. PD = polar diameter; ED = equatorial diameter; TSS = total soluble solids.

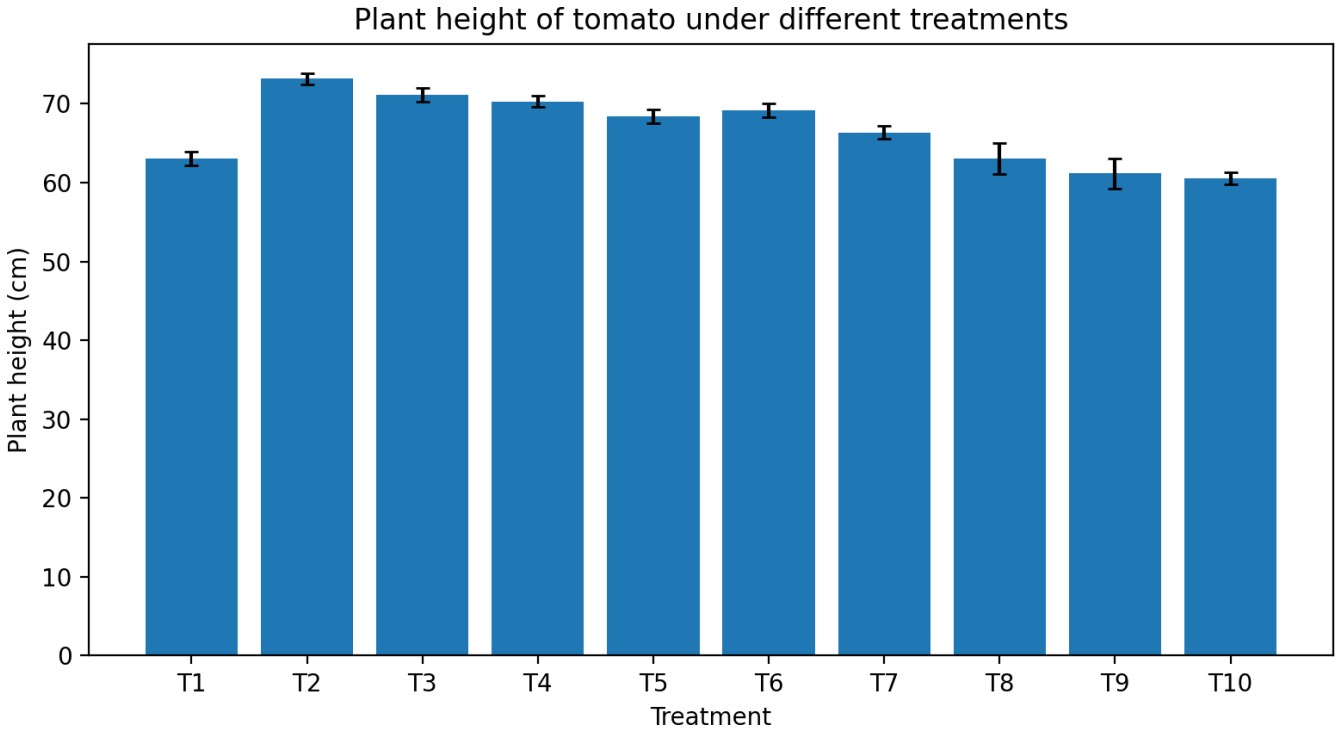

Figure 1. Plant height of tomato under different treatments. Values represent mean ± SD.

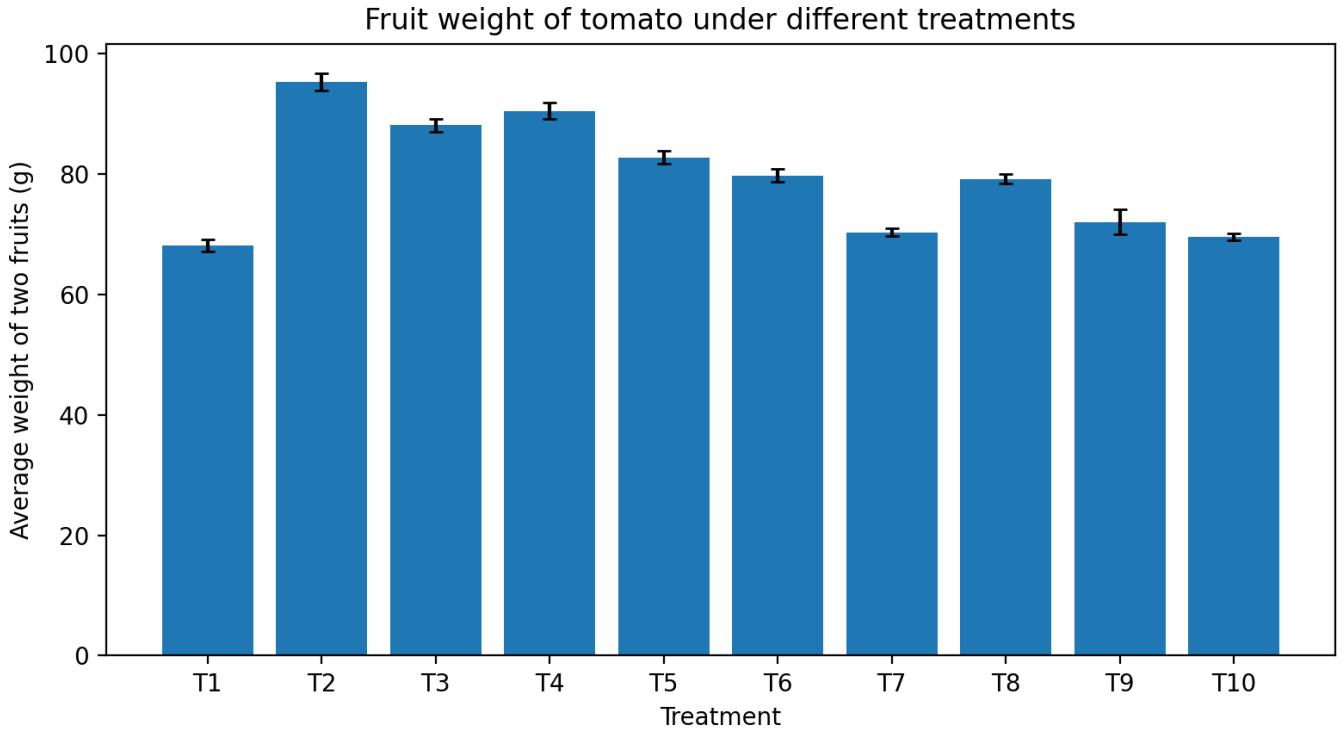

Figure 2. Average weight of two fruits under different treatments. Values represent mean ± SD.

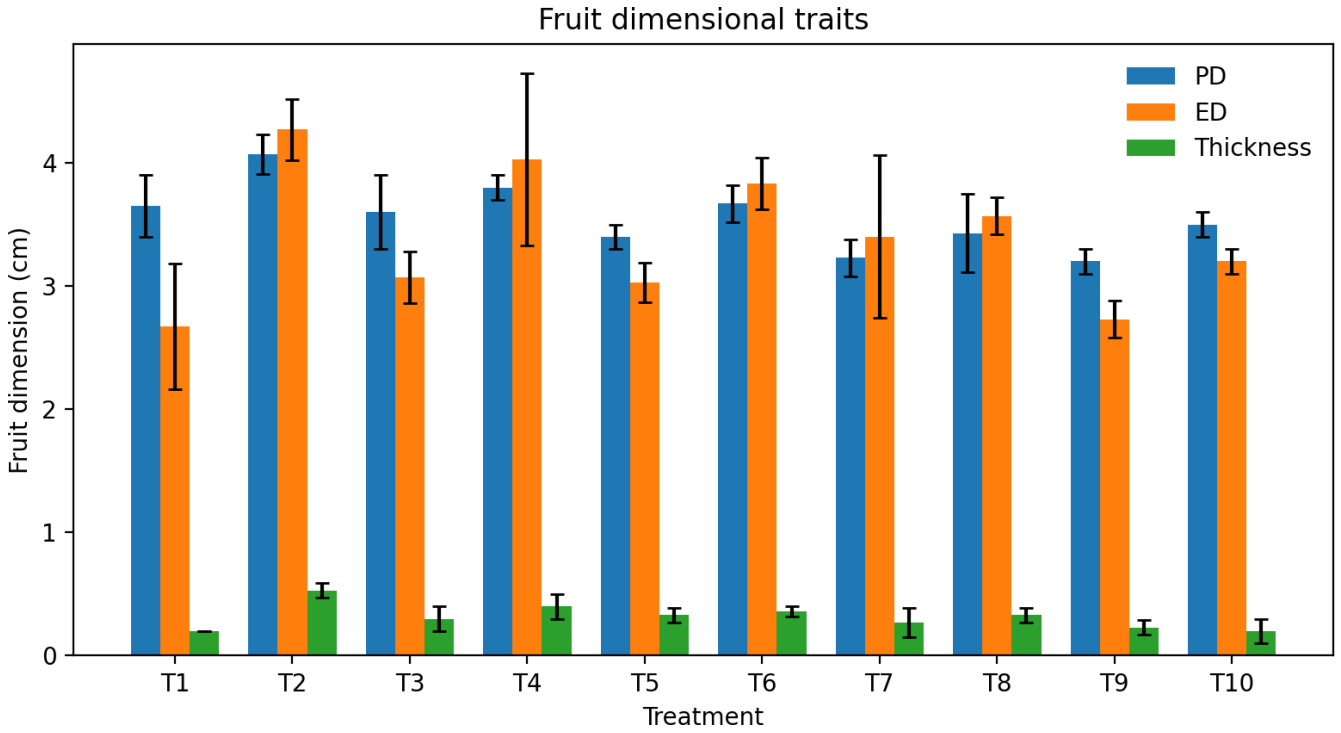

Figure 3. Fruit dimensional traits under different treatments. Values represent mean ± SD.

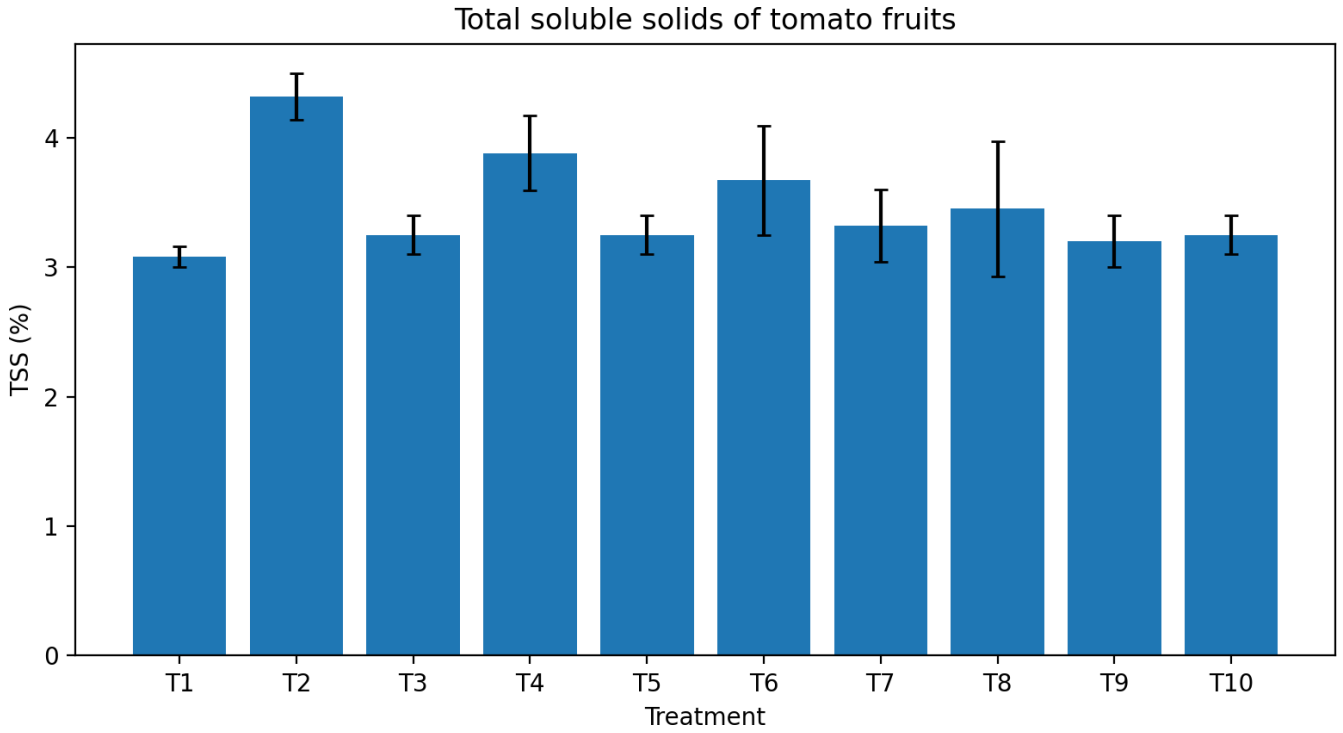

Figure 4. Total soluble solids of tomato fruits under different treatments. Values represent mean ± SD.

Conclusion

Preventive application of beneficial microbial agents improved growth and fruit quality of tomato cv. Kashi Aman under greenhouse conditions. *Trichoderma asperellum* was the best-performing treatment overall, while *T. asperellum* + *Alternaria solani* showed the strongest growth retention among pathogen-challenged treatments. The findings support the use of selected microbial agents as sustainable inputs for improving tomato performance under early blight-prone production systems.

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Conflict of interest

The authors declare no conflict of interest.

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Legends

Figure 1. Plant height of tomato under different treatments. Values represent mean ± SD.

Figure 2. Average weight of two fruits under different treatments. Values represent mean ± SD.

Figure 3. Fruit dimensional traits under different treatments. Values represent mean ± SD.

Figure 4. Total soluble solids of tomato fruits under different treatments. Values represent mean ± SD.