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Bioagent mediated suppression of early blight in tomato caused by *Alternaria solani* under greenhouse conditions

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Abstract

Early blight caused by *Alternaria solani* is a major constraint to tomato productivity and requires ecologically safer disease management options. A greenhouse experiment was conducted on tomato cv. Kashi Aman to evaluate the suppressive potential of *Trichoderma asperellum*, *Bacillus* sp. BV-4, *Bacillus* sp. BV-7 and *Pseudomonas fluorescens* against *A. solani*. Percent disease index (PDI) was recorded at 0, 7, 14 and 21 days after inoculation. The pathogen-only treatment reached 94.67% PDI at 21 days, indicating severe disease development. Among the pathogen-challenged bioagent treatments, *T. asperellum* + *A. solani* recorded the lowest PDI (16.00%) and the highest disease reduction (83.10%) over the pathogen control. *Bacillus* sp. BV-4 + *A. solani* reduced disease by 74.65%, followed by *Bacillus* sp. BV-7 + *A. solani* (63.38%) and *P. fluorescens* + *A. solani* (57.75%). Bioagent-only treatments maintained PDI values below 2.00%, indicating biological safety under the tested conditions. These findings identify *T. asperellum* and *Bacillus* sp. BV-4 as promising bioagents for integrated early blight management in tomato.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops and is highly vulnerable to foliar diseases under warm and humid production systems. Early blight, caused mainly by *Alternaria solani*, produces characteristic concentric necrotic lesions, accelerates defoliation and reduces photosynthetic surface area, thereby affecting yield and fruit quality. The pathogen survives on infected debris and can produce repeated cycles of infection during favourable weather, making

chemical-only disease management difficult and less sustainable (Agrios, 2005; Chaerani and Voorrips, 2006).

Beneficial microorganisms have emerged as practical components of integrated disease management because they can restrict pathogen development while improving the defensive readiness of the host plant. Trichoderma species suppress fungal pathogens through mycoparasitism, competition, antibiosis and plant defense priming, while *Bacillus* and Pseudomonas species act through antimicrobial metabolites, lipopeptides, siderophores and induced systemic resistance (Harman et al., 2004; Compant et al., 2005; Haas and Defago, 2005; Kloepper et al., 2004).

Although several bioagents are reported to improve plant health, their relative performance under a defined tomato-*Alternaria solani* challenge must be quantified over time. The present study was therefore designed to compare selected fungal and bacterial bioagents for their ability to slow disease progression and reduce final PDI in tomato cv. Kashi Aman under greenhouse conditions.

Materials and methods

The experiment was conducted under greenhouse conditions during the Rabi season using tomato cv. Kashi Aman. Healthy four-week-old seedlings were transplanted into sterilized earthen pots containing autoclaved loam soil and farmyard manure in a 3:1 ratio. The treatment set included *A. solani* alone, individual bioagents, and pathogen-bioagent combinations: T1, *A. solani*; T2, *T. asperellum*; T3, *T. asperellum* + *A. solani*; T4, *Bacillus* sp. BV-4; T5, BV-4 + *A. solani*; T6, *Bacillus* sp. BV-7; T7, BV-7 + *A. solani*; T8, *P. fluorescens*; and T9, *P. fluorescens* + *A. solani*.

Alternaria solani was isolated from symptomatic tomato leaves on potato dextrose agar, purified through single-spore isolation and maintained on PDA slants. Ten-day-old cultures were flooded with sterile distilled water, gently scraped, filtered through double-layered cheesecloth and adjusted to approximately 1 x 10^5 conidia mL^{-1} with the aid of a hemocytometer. Tween-20 (0.02%, v/v) was added for uniform leaf coverage, and plants were sprayed to runoff at the 5-6 leaf stage. After inoculation, plants were maintained at high humidity for 48 h to favour disease establishment.

Trichoderma asperellum was cultured on PDA at 28°C for 7-10 days and the conidial suspension was adjusted to approximately 1 x 10^7 conidia mL^{-1}. *Bacillus* sp. BV-4, *Bacillus* sp. BV-7 and *Pseudomonas fluorescens* were grown in broth culture and adjusted to approximately 1 x 10^8 CFU mL^{-1}. Each microbial inoculant was applied as a soil drench five days before pathogen inoculation, and a supporting foliar spray was applied three days before pathogen challenge.

Disease severity was recorded at 0, 7, 14 and 21 days after pathogen inoculation using visual disease scoring. Percent disease index was calculated as:

PDI = [sum of numerical ratings / (number of plants observed x maximum disease grade)] x 100

Final disease reduction over the pathogen-only control was calculated at 21 days as: **Reduction (%) = [(PDI of T_1 - PDI of treatment) / PDI of T_1] x 100.**

Treatment means were interpreted using sequential tables and graphs; different disease classes were compared based on final PDI and percent reduction.

Disease severity increased progressively in the pathogen-only treatment, while all bioagent treatments restricted disease development to varying degrees (Table 1 and Fig. 1). At day 0, no symptoms were observed in any treatment, confirming a uniform disease-free baseline. By day 7, T1 already reached 36.00% PDI, which increased to 78.00% at day 14 and 94.67% at day 21. This steep rise confirmed the aggressive progression of early blight in tomato plants that did not receive microbial protection.

Result

Table 1. Percent disease index of tomato plants in response to bioagents and Alternaria solani challenge.

Treatment	Treatment description	Day 0	Day 7	Day 14	Day 21
T1	*A. solani* (pathogen only)	0	36	78	94.67
T2	*Trichoderma asperellum*	0	0.67	1.33	2
T3	*T. asperellum* + *A. solani*	0	3.33	9.33	16
T4	Bacillus sp. BV-4	0	0.67	1	1.21
T5	Bacillus sp. BV-4 + A. solani	0	7.33	13.33	24
T6	Bacillus sp. BV-7	0	0.67	1.33	1.33
T7	Bacillus sp. BV-7 + *A. solani*	0	11.33	19.33	34.67
T8	*Pseudomonas fluorescens*	0	0.67	1.33	1.37
T9	*P. fluorescens* + *A. solani*	0	15.33	24	40

Note: PDI = percent disease index. Values represent disease progression at 0, 7, 14 and 21 days after inoculation.

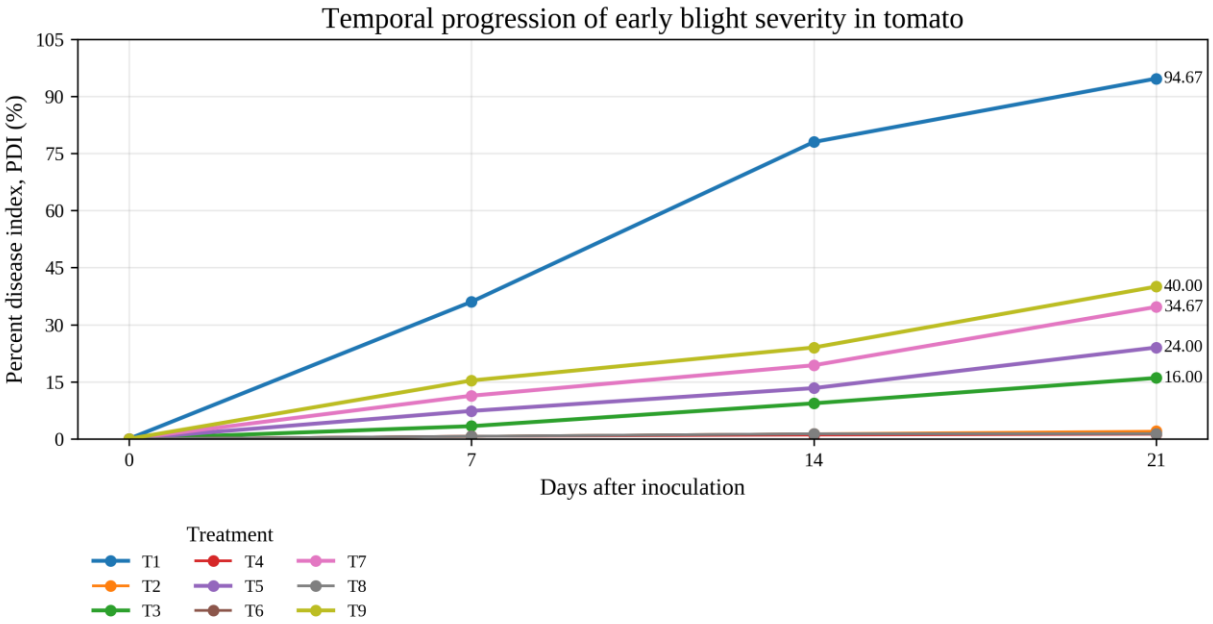

Fig. 1. Temporal progression of early blight severity in tomato plants in response to microbial bioagents and *Alternaria solani* challenge.

Among pathogen-challenged bioagent treatments, T3 showed the strongest suppression, with only 16.00% PDI at day 21. This represented an 83.10% reduction compared with the pathogen-only control (Table 2 and Fig. 2). T5 also showed strong protection with 24.00% PDI and 74.65% reduction. T7 and T9 showed intermediate protection, recording 34.67% and 40.00% PDI, equivalent to 63.38% and 57.75% reduction, respectively. Bioagent-only treatments (T2, T4, T6 and T8) remained at or below 2.00% PDI, indicating that the microbial inoculants did not induce disease-like injury and were safe for tomato plants under the tested conditions.

Table 2. Disease reduction in pathogen-challenged tomato treatments at 21 days after inoculation.

Treatment	PDI at Day 21 (%)	Reduction over T1 (%)	Relative efficacy
T1 (*A. solani* (pathogen only))	94.67	0.00	Pathogen control
T3 (*T. asperellum* + *A. solani*)	16.00	83.10	Very high
T5 (Bacillus sp. BV-4 + *A. solani*)	24.00	74.65	High
T7 (Bacillus sp. BV-7 + *A. solani*)	34.67	63.38	High

T9 (*P. fluorescens* + *A. solani*)	40.00	57.75	Moderate

Note: Reduction was calculated against T1, the pathogen-only treatment.

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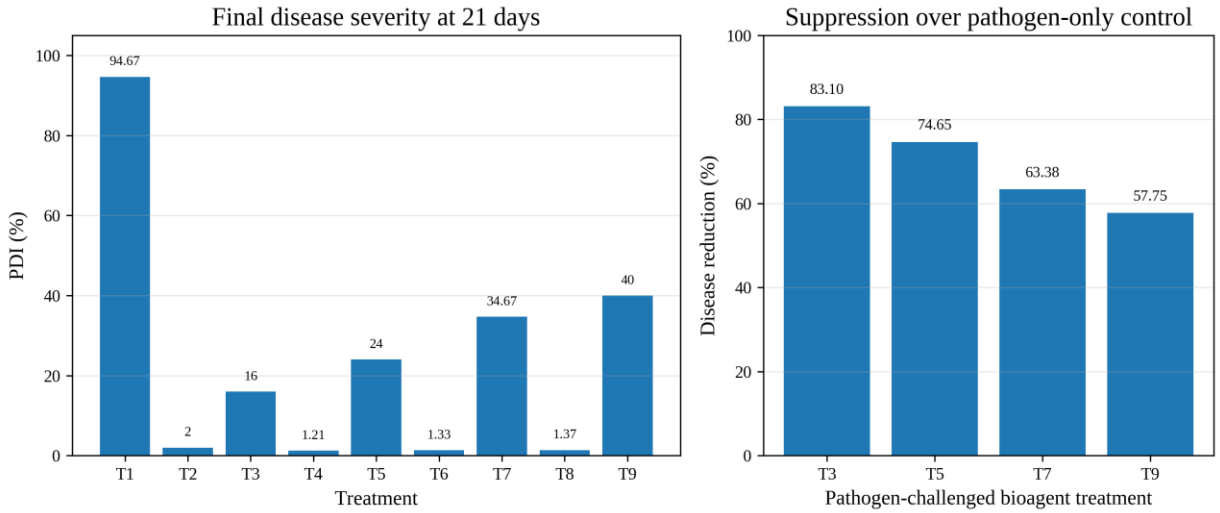

Fig. 2. Final percent disease index and disease reduction at 21 days after Alternaria solani inoculation.

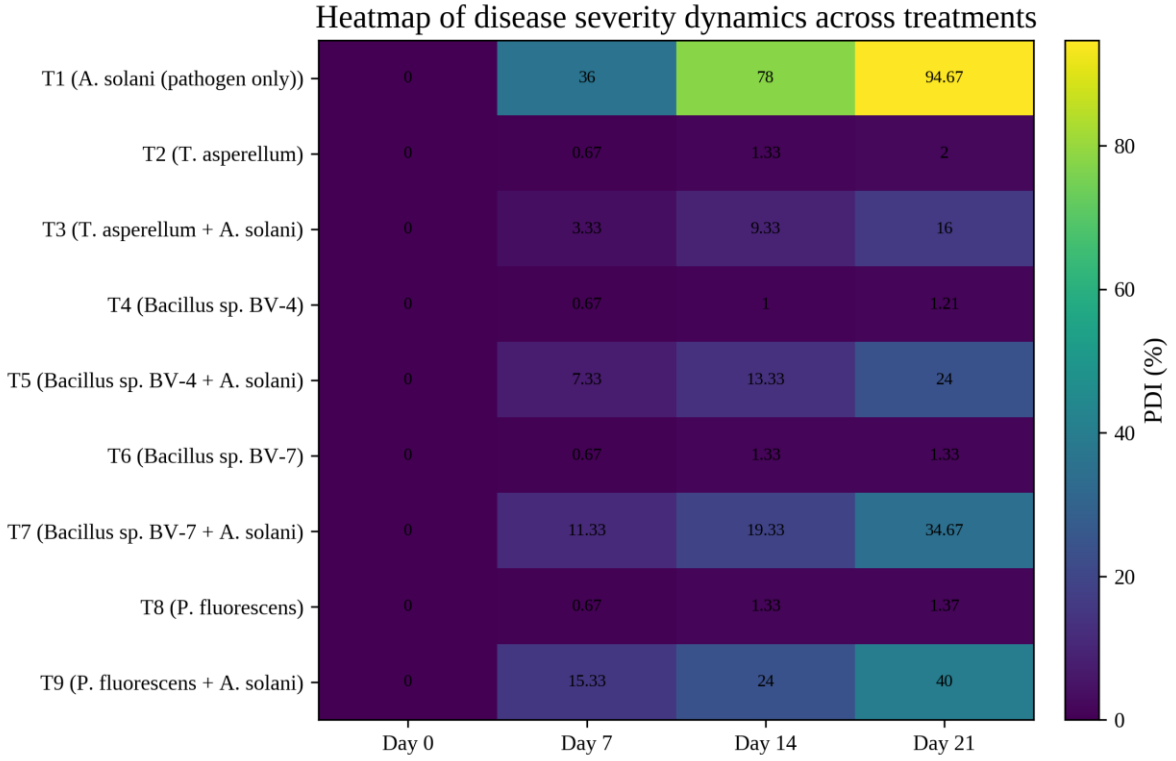

Fig. 3. Heatmap showing the temporal intensity of percent disease index across tomato treatments.

Discussion

The rapid rise of PDI in T1 confirmed that *Alternaria solani* caused severe disease pressure in the absence of a protective bioagent. The pattern is consistent with the polycyclic nature of early blight, where repeated spore production and lesion expansion can quickly intensify foliar damage under favourable humidity and temperature (Agrios, 2005; Chaerani and Voorrips, 2006). The very low PDI recorded in bioagent-only treatments also indicated that the tested microorganisms were not phytotoxic and could be used as preventive biological inputs.

Trichoderma asperellum gave the highest disease suppression when applied before pathogen challenge. This response may be attributed to several complementary mechanisms, including competition for infection sites, mycoparasitism, secretion of cell-wall-degrading enzymes, production of inhibitory metabolites and priming of host defense pathways. These mechanisms are well aligned with earlier reports describing Trichoderma as an avirulent plant symbiont capable of both direct antagonism and systemic activation of host resistance (Harman et al., 2004; Vinale et al., 2008; Shoresh et al., 2010).

The strong protection by *Bacillus* sp. BV-4 suggests that bacterial bioagents also contributed substantially to early blight suppression. *Bacillus* species are known for stable rhizosphere colonization and the production of antifungal lipopeptides such as surfactins, iturins and fengycins, which can affect pathogen membranes and also trigger defense signalling in host plants. The lower PDI in BV-4 compared with BV-7 indicates strain-specific differences in antagonistic capacity, colonization fitness or defense-eliciting potential, a common feature among plant growth-promoting *Bacillus* strains (Kloepper et al., 2004; Ongena and Jacques, 2008; Borriss, 2011).

Pseudomonas fluorescens reduced disease compared with the pathogen control, although its suppression level was lower than that of *Trichoderma asperellum* and *Bacillus* sp. BV-4. Fluorescent pseudomonads can inhibit pathogens through siderophore-mediated iron competition, production of antibiotics and volatile metabolites, and induction of systemic resistance in the plant. The moderate efficacy of *P. fluorescens* in this experiment may reflect differences in survival on the phyllosphere, competition in the rhizosphere or timing of defense activation relative to Alternaria infection (Haas and Defago, 2005; Weller, 2007; Lugtenberg and Kamilova, 2009).

Overall, the temporal PDI trend shows that effective biocontrol was not only visible at the final observation but occurred throughout disease development. This is important because early suppression of lesion formation can reduce secondary inoculum and delay epidemic build-up. The reduction hierarchy observed in this experiment, *T. asperellum* > *Bacillus* sp. BV-4 > *Bacillus* sp. BV-7 > *P. fluorescens*, supports the use of carefully selected microbial agents as part of an integrated early blight management strategy rather than relying solely on chemical fungicides. Similar ISR-oriented interpretations have been proposed for

beneficial microbes in crop disease management (Ramamoorthy et al., 2001; Choudhary et al., 2007; Pieterse et al., 2014; Olanrewaju et al., 2017).

Conclusion

The study demonstrated that preventive application of microbial bioagents can markedly reduce early blight severity in tomato under greenhouse conditions. *Trichoderma asperellum* was the most effective treatment, reducing final PDI by 83.10% over the pathogen-only control, followed by *Bacillus* sp. BV-4 with 74.65% reduction. These bioagents should be prioritized for further multi-season validation, formulation development and integration with cultural practices for sustainable tomato disease management.

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Conflict of interest

The authors declare no conflict of interest.

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