



Research Article

In Vitro and Screenhouse Evaluation of *Trichoderma harzianum* T22 and *Bacillus subtilis* BS01 for Management of Tomato Bacterial Wilt

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Abstract

Bacterial wilt, caused by the *Ralstonia solanacearum* species complex, severely constrains tomato production in warm production environments. This study evaluated the compatibility and efficacy of *Trichoderma harzianum* T22 and *Bacillus subtilis* BS01, applied individually or together, for suppression of tomato bacterial wilt. T22 and BS01 were compatible in dual culture. In the quantitative T22 antagonism assay, inhibition of *R. solanacearum* increased from $11.45 \pm 3.77\%$ at 24 h to $46.48 \pm 2.56\%$ at 96 h ($p < 0.001$). A screenhouse experiment comprised five treatments: pathogen + T22, pathogen + BS01, pathogen + T22 + BS01, pathogen-only control, and an uninoculated control. Treatment, assessment week, and their interaction influenced disease incidence and severity ($p < 0.05$). At the final assessment, the pathogen-only control recorded 94.4% incidence, 100.0% severity, and a severity area under the disease progress curve of 251.7 %-weeks. The combined treatment recorded the lowest numerical final incidence (33.3%) and severity (38.3%) among inoculated treatments, although it did not differ significantly from either single-agent treatment. Treatment also affected plant height ($p = 0.010$) and stem girth ($p = 0.002$). The combined treatment produced the greatest numerical plant height (117.4 cm) and stem girth (2.13 mm). Thus, individual and combined applications suppressed bacterial wilt under screenhouse conditions, but the combination did not demonstrate statistical superiority over the single agents. Field validation is required.

Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop for household nutrition, market supply, and cash income in smallholder and commercial production systems. In Tanzania, the crop is cultivated under rainfed and irrigated conditions, but its productivity is constrained

by several biotic and abiotic stresses, including bacterial wilt (Aloyce, 2020).

Bacterial wilt is caused by members of the *Ralstonia solanacearum* species complex and is among the most destructive diseases of tomato in tropical and subtropical environments. The pathogen colonises xylem tissues, disrupts water transport, persists in soil and water, and has

a broad host range that complicates eradication once an area becomes infested (Vailleau and Genin 2023; Wang *et al.* 2023).

Management approaches include sanitation, crop rotation, resistant cultivars or rootstocks, soil amendments, and chemical or synthetic products. Their effectiveness is often constrained by long pathogen persistence, broad host range, strain-dependent resistance, variable environmental performance, cost, and concerns regarding repeated chemical use (Wu *et al.*, 2023; Phiri *et al.*, 2024). Consequently, biologically based strategies are increasingly considered as components of integrated bacterial wilt management.

Biological control Agents (BCAs) may suppress plant pathogens through antibiosis, competition for nutrients and colonisation sites, production of hydrolytic enzymes, and activation of plant defence responses (Bonaterra *et al.*, 2022; Lahlali *et al.*, 2022; Ayaz *et al.*, 2023). *Trichoderma* and *Bacillus* species are particularly attractive because of their rhizosphere competence, antagonistic potential, plant growth-promoting effects, and suitability for formulation. Previous studies have demonstrated suppression of tomato bacterial wilt by *Trichoderma* isolates or metabolites and by antagonistic bacteria under laboratory, protected, and field conditions (Konappa *et al.* 2018; Elsayed *et al.*, 2020; Kariuki *et al.*, 2020; Guo *et al.*, 2021; Yan and Khan, 2021; Sun *et al.*, 2023).

Combining *Trichoderma* with beneficial bacteria may broaden functional complementarity, but outcomes depend on strain compatibility, inoculum ratio, formulation, rhizosphere establishment, and environmental conditions (Poveda and Eugui 2022; Kredics *et al.*, 2024). A *Trichoderma*–*Bacillus* combination has suppressed tomato bacterial wilt more effectively than either component in some systems (Zhou *et al.*, 2021), but such synergy cannot be assumed for other strains and products. We hypothesised that T22 and BS01 would each suppress bacterial wilt relative to the pathogen-only control and that their combined application would provide more consistent

disease suppression and growth maintenance than either agent alone. The study therefore assessed compatibility and T22-mediated antagonism in vitro and evaluated the individual and combined effects of T22 and BS01 on disease development and tomato growth under screenhouse conditions.

Materials and Methods

Study Site

Laboratory and screenhouse experiments were conducted at Sokoine University of Agriculture, Morogoro, Tanzania. Laboratory assays were performed in the Plant Pathology Laboratory, while the in-planta experiment was conducted in a university screenhouse under protected conditions.

Pathogen Isolate and Inoculum Preparation

A virulent isolate, TBWK10, historically designated as race 1, biovar 3 and phylotype I within the *Ralstonia solanacearum* species complex, was obtained from the World Vegetable Center, Arusha, Tanzania. The isolate was revived on nutrient agar and incubated at 28–30 °C for 72h. A 3% potassium hydroxide test was used to confirm a Gram-negative reaction, and pathogenicity was evaluated using tomato seedlings, potato tuber slices, and tobacco leaves (Fig. 1). Fluidal colonies were harvested from tetrazolium chloride medium and suspended in sterile distilled water to approximately 10⁸ colony-forming units (CFU) mL⁻¹.

Biocontrol Agents

Commercial formulations of *T. harzianum* T22 (Trium-G and Trium-P; Koppert Biological Systems) and *B. subtilis* BS01 were used as sources of the biocontrol agents. One gram of the T22 formulation and 1 mL of the BS01 formulation were separately suspended in 9 mL of sterile distilled water and serially diluted. T22 was plated on potato dextrose agar and incubated for 7d, whereas BS01 was plated on nutrient agar and incubated at 28 ± 2 °C for 48 h. Distinct colonies were subcultured on fresh medium and maintained at 4 °C until use.

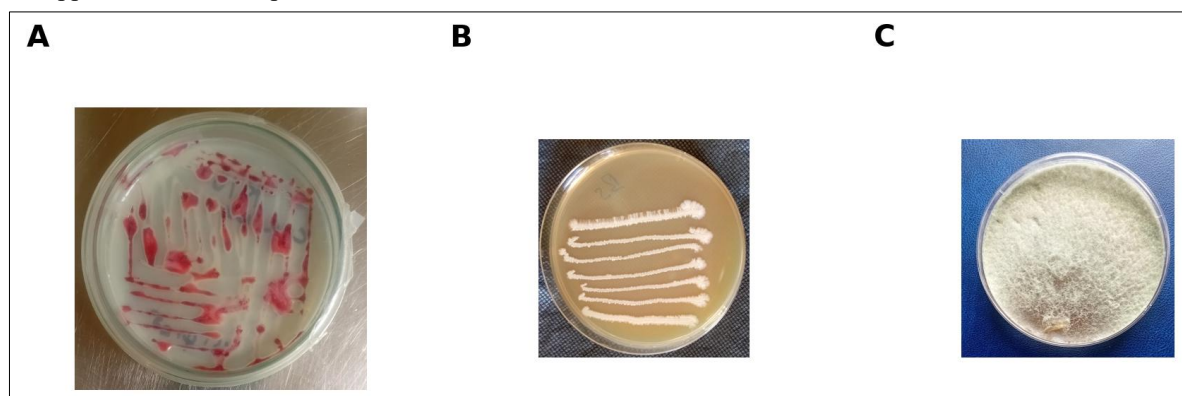


Fig. 1: Cultures used in the experiments: (A) *Ralstonia solanacearum* TBWK10 on tetrazolium chloride medium, (B) *Bacillus subtilis* BS01 on nutrient agar, and (C) *Trichoderma harzianum* T22 on potato dextrose agar.

In Vitro Compatibility and Antagonism Assays

Compatibility between *T. harzianum* T22 and *B. subtilis* BS01 was evaluated on potato dextrose agar using a dual-culture assay. A 24h BS01 culture was streaked on one side of a plate and incubated for 24h at 28 °C. A 5 mm mycelial disc taken from an actively growing T22 culture was then placed on the opposite side. Plates were incubated at 28 °C and examined for overlapping growth or a distinct inhibition zone between the agents.

Quantitative antagonism of *T. harzianum* T22 against *R. solanacearum* was evaluated in a completely randomised assay with three replications. A 5 mm T22 disc was placed 2 cm from the edge of a potato dextrose agar plate, and TBWK10 was streaked on the opposite side 24 h later. Pathogen growth in antagonist and control plates was measured at 24, 48, 72, and 96h. Percentage inhibition was calculated as:

$$\text{Inhibition (\%)} = \frac{(R_c - R_t)}{R_c} \times 100 \quad (\text{Eqn. 1})$$

where R_c is the radial growth of *R. solanacearum* in the control and R_t is the radial growth in the antagonist treatment.

Screenhouse Experimental Design and Treatments

The screenhouse experiment was arranged in a completely randomised design with five treatments and three replications. Each replicate comprised six plants distributed among 4 L pots containing 3 kg of sterilised soil, with two plants maintained per pot. Consequently, 18 plants were assessed per treatment. The treatments are defined in Table 1.

Table 1. Screenhouse treatments used to evaluate biological control of tomato bacterial wilt.

Code	Treatment	Purpose
T1	<i>R. solanacearum</i> + <i>T. harzianum</i> T22	Biocontrol agent applied before pathogen inoculation
T2	<i>R. solanacearum</i> + <i>B. subtilis</i> BS01	Biocontrol agent applied before pathogen inoculation
T3	<i>R. solanacearum</i> + <i>T. harzianum</i> T22 + <i>B. subtilis</i> BS01	Equal-volume mixture applied before pathogen inoculation
T4	<i>R. solanacearum</i> only	Pathogen-inoculated positive control
T5	Sterile distilled water	Uninoculated and untreated negative control

T22, *Trichoderma harzianum* T22; BS01, *Bacillus subtilis* BS01. Treatments T1–T4 received pathogen inoculum; T5 received sterile distilled water.

Plant Establishment and Inoculation

Seeds of the tomato hybrid Imara F1 were raised in seedling trays for 21d under screenhouse conditions and then transplanted. Suspensions of *B. subtilis* BS01 and *T. harzianum* T22 were adjusted to approximately 10^8 CFU or conidia mL^{-1} , respectively. At transplanting, 50 mL of the appropriate biocontrol suspension was applied per pot as a soil drench around the root zone. For T3, a total of 50 mL of a 1:1 mixture of the T22 and BS01 suspensions was applied. Twenty-four hours later, T1–T4 received 50 mL per pot of *R. solanacearum* suspension at approximately 10^8 CFU mL^{-1} around the stem base, while T5 received sterile distilled water.

Disease and Growth Assessments

Plants were assessed weekly for 6 weeks after inoculation. Disease incidence was calculated for each replicate as the percentage of plants showing wilt symptoms. Disease severity was rated on a five-point scale corresponding to 0, 25, 50, 75, and 100% wilt, where score 1 represented no symptoms and score 5 represented complete wilting or plant death. Severity scores were converted to percentage severity using $[(\text{score} - 1)/4] \times 100$. Plant height, number of fully expanded leaves, and stem girth were recorded at each assessment.

The area under the disease progress curve (AUDPC) was calculated separately for disease incidence and severity using the trapezoidal method of Campbell and Madden (1990):

$$\text{AUDPC} = \sum[(Y_i + Y_{i+1})/2](t_{i+1} - t_i), \text{ for } i = 1 \text{ to } n - 1 \quad (\text{Eqn. 2})$$

where Y_i and Y_{i+1} are consecutive observations, $t_{i+1} - t_i$ is the interval between assessments, and n is the number of assessments.

Statistical Analysis

Analyses were conducted in the R statistical environment using replicate-level data. In vitro inhibition was analysed by one-way analysis of variance (ANOVA), and means were separated using Tukey-adjusted comparisons at $\alpha = 0.05$. Repeated screenhouse measurements were analysed by repeated-measures ANOVA with treatment, assessment week, and treatment $\times$ week as fixed effects and replicate as the repeated subject. Final-assessment variables and AUDPC were analysed by one-way ANOVA followed by Tukey-adjusted comparisons. Results are reported as raw means $\pm$ standard errors, and statistical significance was evaluated at $p < 0.05$.

Results

Pathogen Identity and Pathogenicity

The potassium hydroxide test produced a Gram-negative reaction. Wilt symptoms developed within 48 h in the seedling pathogenicity assay, and 87.5% of inoculated

seedlings wilted within 5d, while uninoculated seedlings remained symptomless. The root-inoculation sequence and subsequent symptom development are shown in Fig. 2. Potato tuber slices inoculated with TBWK10 developed tissue softening and brown discolouration, while tobacco leaves infiltrated with the isolate developed chlorosis followed by necrosis; the corresponding sterile-water controls remained symptomless (Fig. 3). Together, these responses were consistent with a pathogenic *R. solanacearum* isolate.

Compatibility and In-Vitro Antagonism

T. harzianum T22 and *B. subtilis* BS01 showed overlapping growth in dual culture without a distinct inhibition zone, indicating compatibility under the in vitro conditions tested. Inhibition of *R. solanacearum* by T22 increased with incubation time ($F_{3,8} = 39.824$, $p < 0.001$). Mean inhibition increased from $11.45 \pm 3.77\%$ at 24 h to $34.25 \pm 1.58\%$ at 48 h and $45.75 \pm 1.89\%$ at 72h, before approaching a plateau at $46.48 \pm 2.56\%$ at 96 h (Fig. 4).

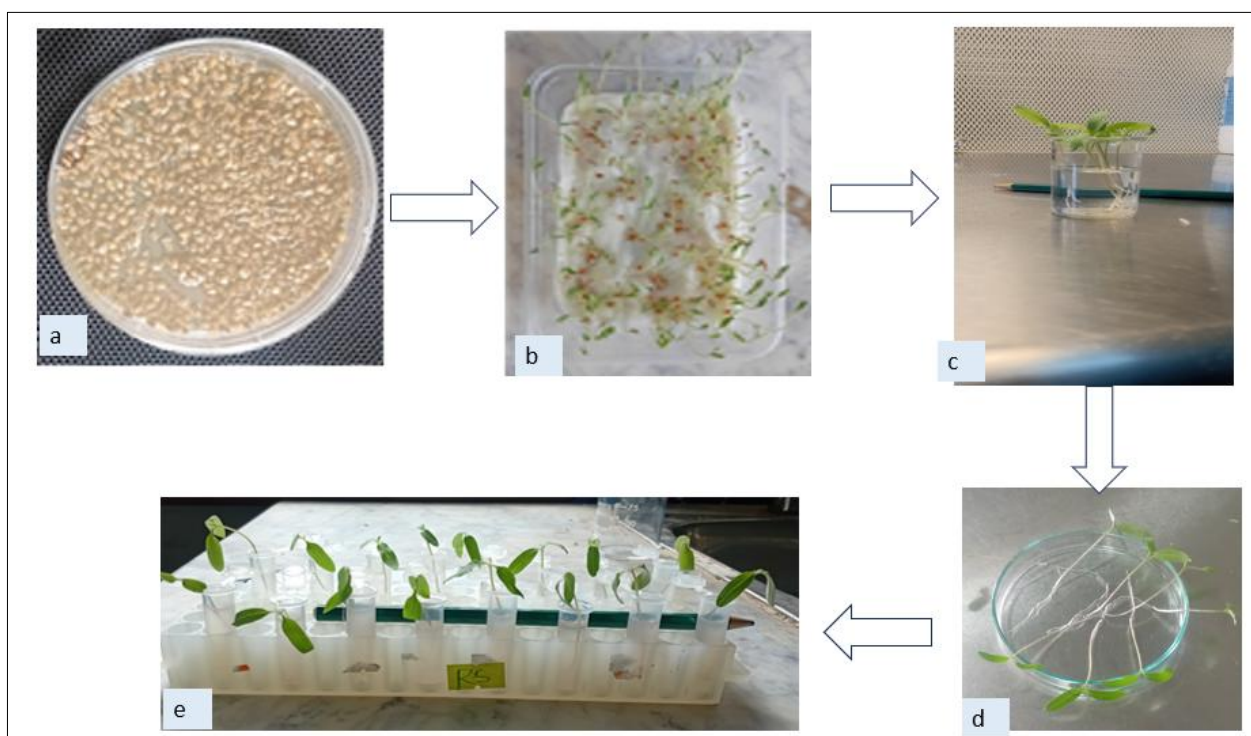


Fig. 2: Stepwise pathogenicity testing of *Ralstonia solanacearum* TBWK10 by root inoculation of tomato seedlings, showing seed preparation, germination, inoculation, and symptom development.

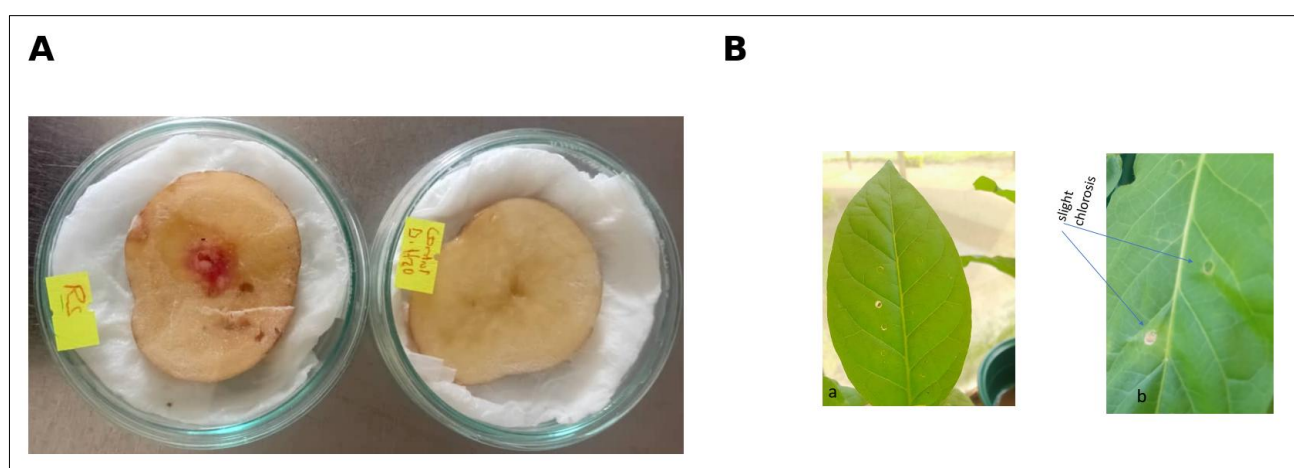


Fig. 3: Additional pathogenicity responses produced by *Ralstonia solanacearum* TBWK10: (A) tissue softening and brown discolouration on potato tuber tissue inoculated with TBWK10 compared with the sterile-water control, and (B) tobacco leaves infiltrated with sterile distilled water (left) or TBWK10 (right), showing chlorosis and necrosis after pathogen infiltration.

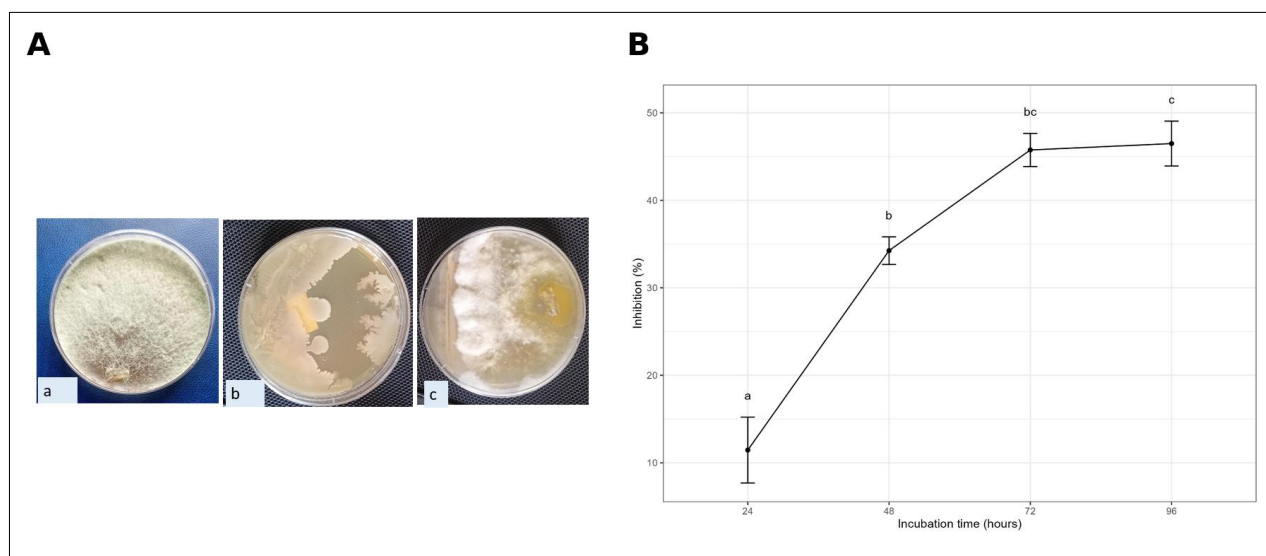


Fig. 4: In vitro evaluation of the biocontrol agents: (A) individual growth of *Trichoderma harzianum* T22 and *Bacillus subtilis* BS01 and their compatibility in dual culture, and (B) inhibition of *Ralstonia solanacearum* by T22 over 96 h. Error bars represent standard errors; different letters indicate significant differences among incubation times at $p < 0.05$.

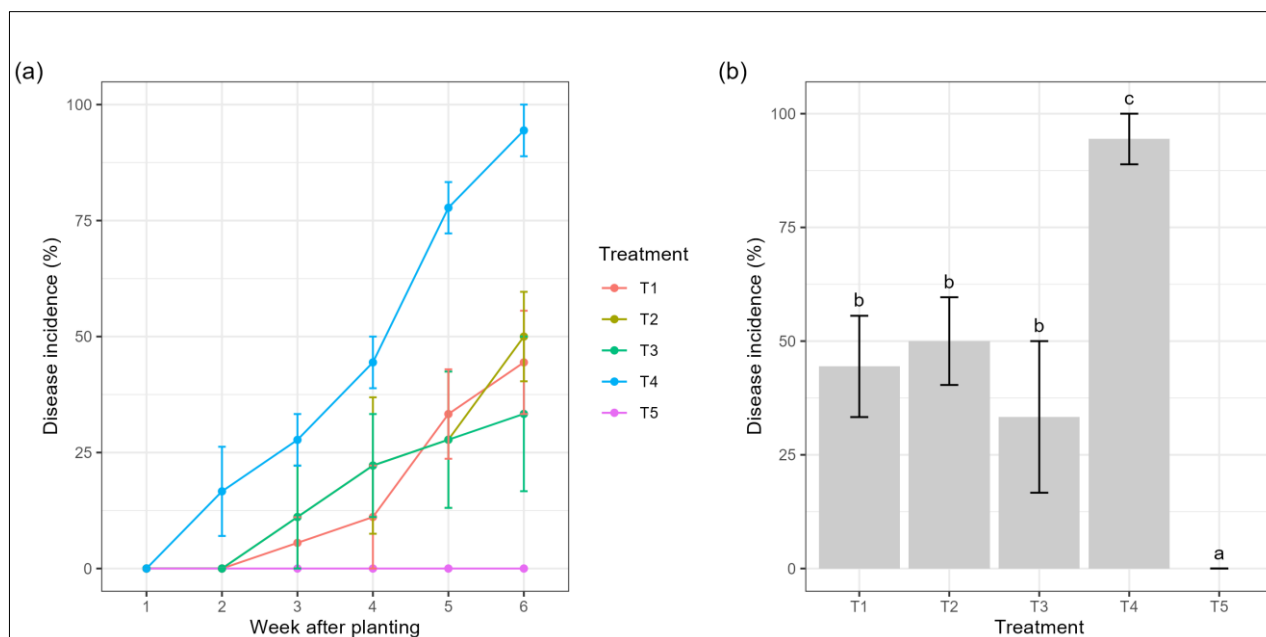


Fig. 5: Disease incidence under the screenhouse treatments: (A) temporal progression and (B) final assessment. Error bars represent standard errors; final means with different letters differ at $p < 0.05$.

Disease Incidence and Severity

Disease incidence was affected by treatment ($F_{4,10} = 7.784$, $p = 0.004$), week ($F_{5,50} = 36.986$, $p < 0.001$), and the treatment $\times$ week interaction ($F_{20,50} = 4.398$, $p < 0.001$; Table 4). No symptoms occurred during the first week. By week 2, disease had appeared in the pathogen-only control, while the biocontrol and uninoculated treatments remained symptomless. Incidence subsequently increased most rapidly in T4 and reached $94.43 \pm 5.57\%$ at the final assessment. Final incidence was lower in all biocontrol treatments, with the lowest numerical value recorded in T3 ($33.33 \pm 16.67\%$). T1, T2, and T3 did not differ

significantly from one another at the final assessment (Fig. 5; Table 2).

Disease severity was influenced by treatment ($F_{4,10} = 14.898$, $p < 0.001$), week ($F_{5,50} = 64.765$, $p < 0.001$), and their interaction ($F_{20,50} = 7.309$, $p < 0.001$; Table 4). Severity remained at 0% in all treatments during week 1. By the final assessment, T4 had reached 100%, whereas final severity was $50.00 \pm 0.00\%$ in T1, $66.67 \pm 16.67\%$ in T2, and $38.33 \pm 18.33\%$ in T3. The three biocontrol treatments did not differ significantly from one another but had lower final severity than the pathogen-only control (Fig. 6; Table 2).

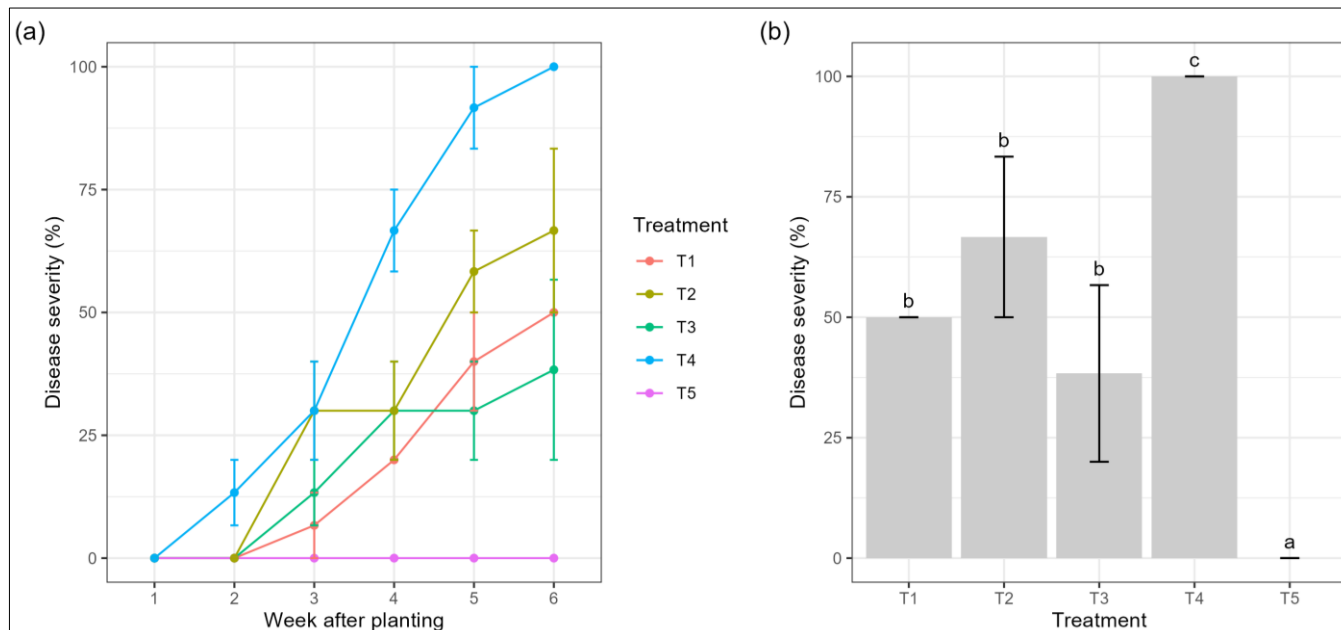


Fig. 6: Disease severity under the screenhouse treatments: (A) temporal progression and (B) final assessment. Error bars represent standard errors; final means with different letters differ at $p < 0.05$.

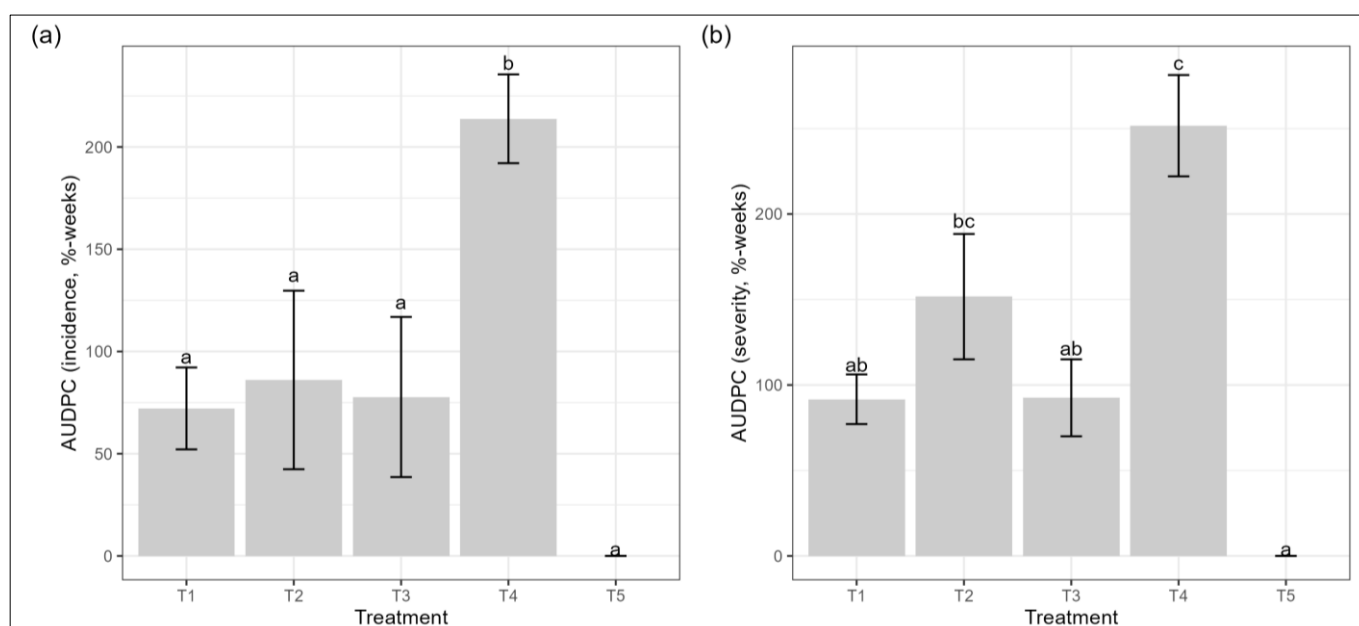


Fig. 7: Area under the disease progress curve (AUDPC) for bacterial wilt: (A) incidence and (B) severity. Error bars represent standard errors; bars with different letters differ significantly at $p < 0.05$.

Cumulative Disease Pressure

Cumulative disease pressure differed among treatments for incidence ($F_{4,10} = 11.605$, $p < 0.001$) and severity ($F_{4,10} = 14.571$, $p < 0.001$; Table 4). The pathogen-only control accumulated the greatest incidence and severity AUDPC values (213.78 ± 21.71 and 251.67 ± 29.63 %-weeks, respectively). Among inoculated biocontrol treatments, incidence AUDPC ranged from 72.15 ± 20.03 to 86.07 ± 43.66 %-weeks. Severity AUDPC was lowest numerically in T1 (91.67 ± 14.53 %-weeks) and was nearly identical in T3 (92.50 ± 22.50 %-weeks), while T2 recorded $151.67 \pm$

36.67 %-weeks. The uninoculated control remained at zero (Fig. 7; Table 2).

Plant Growth Responses

Plant height was affected by treatment ($F_{4,10} = 6.014$, $p = 0.010$), week ($F_{5,50} = 1028.892$, $p < 0.001$), and treatment $\times$ week ($F_{20,50} = 4.554$, $p < 0.001$; Table 4). At the final assessment, T4 produced the shortest plants (80.53 ± 1.44 cm). The greatest numerical height occurred in T3 (117.40 ± 2.96 cm), followed by T1, T2, and T5, but these four treatments did not differ significantly (Fig. 8; Table 3).

Leaf number increased with week ($F_{5,50} = 651.238$, $p < 0.001$), but neither treatment ($F_{4,10} = 1.793$, $p = 0.207$) nor treatment $\times$ week ($F_{20,50} = 1.151$, $p = 0.333$) was significant. Final leaf number ranged from 13.33 ± 0.33 in T4 to 15.00 in T2, T3, and T5 (Table 3). Stem girth was affected by treatment ($F_{4,10} = 9.700$, $p = 0.002$), week ($F_{5,50} = 171.569$,

$p < 0.001$), and treatment $\times$ week ($F_{20,50} = 1.907$, $p = 0.033$; (Table 4). The lowest final stem girth occurred in T4 (1.20 ± 0.12 mm), while T3 had the greatest numerical value (2.13 ± 0.12 mm). T1, T2, T3, and T5 did not differ significantly (Fig. 9; Table 3).

Table 2: Final disease incidence, disease severity, and area under the disease progress curve under the screenhouse treatments.

Treatment	Final incidence (%)	Final severity (%)	AUDPC incidence (%-weeks)	AUDPC severity (%-weeks)
T1	44.43 ± 11.13^b	50.00 ± 0.00^b	72.15 ± 20.03^a	91.67 ± 14.53^{ab}
T2	50.00 ± 9.64^b	66.67 ± 16.67^b	86.07 ± 43.66^a	151.67 ± 36.67^{bc}
T3	33.33 ± 16.67^b	38.33 ± 18.33^b	77.73 ± 39.16^a	92.50 ± 22.50^{ab}
T4	94.43 ± 5.57^c	100.00 ± 0.00^c	213.78 ± 21.71^b	251.67 ± 29.63^c
T5	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a

Values are means $\pm$ standard error ($n = 3$ replicates). Within a column, means followed by different superscript letters differ significantly at $p < 0.05$ according to Tukey-adjusted comparisons. AUDPC, area under the disease progress curve.

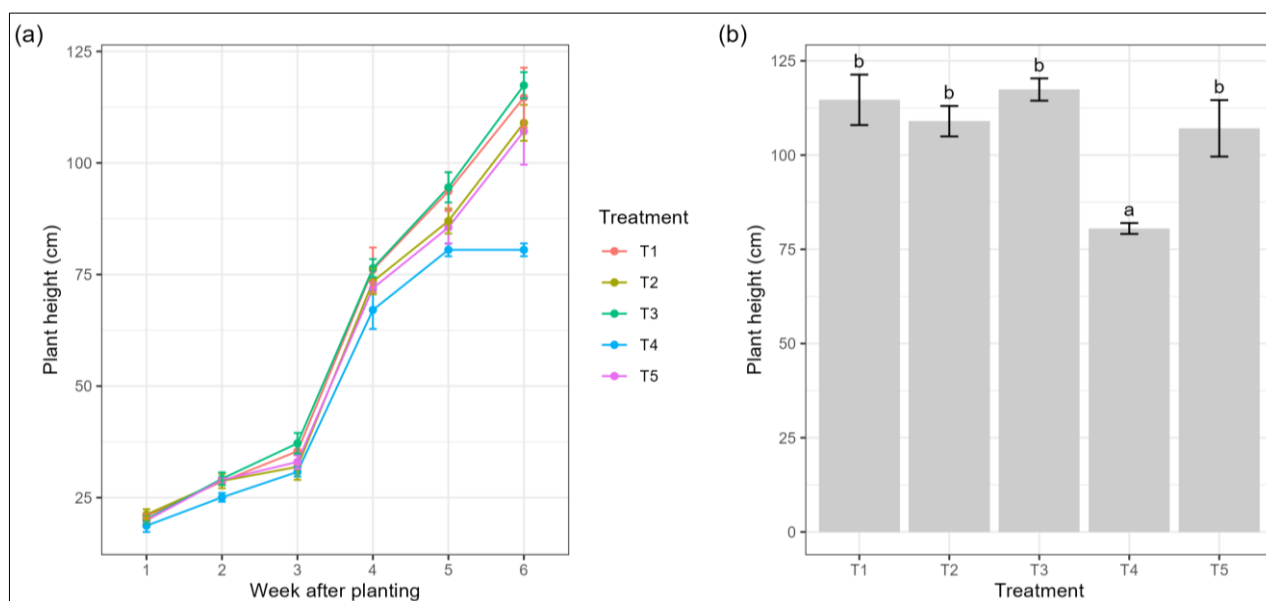


Fig. 8: Plant height under the screenhouse treatments: (A) temporal progression and (B) final assessment. Error bars represent standard errors; final means with different letters differ at $p < 0.05$.

Table 3. Final tomato growth responses under the screenhouse treatments.

Treatment	Plant height (cm)	Number of leaves per plant	Stem girth (mm)
T1	114.67 ± 6.69^b	14.67 ± 0.88^a	1.93 ± 0.18^b
T2	109.00 ± 4.04^b	15.00 ± 0.58^a	1.83 ± 0.22^b
T3	117.40 ± 2.96^b	15.00 ± 1.15^a	2.13 ± 0.12^b
T4	80.53 ± 1.44^a	13.33 ± 0.33^a	1.20 ± 0.12^a
T5	107.10 ± 7.49^b	15.00 ± 0.00^a	1.83 ± 0.15^b

Values are means $\pm$ standard error ($n = 3$ replicates). Within a column, means followed by different superscript letters differ significantly at $p < 0.05$ according to Tukey-adjusted comparisons.

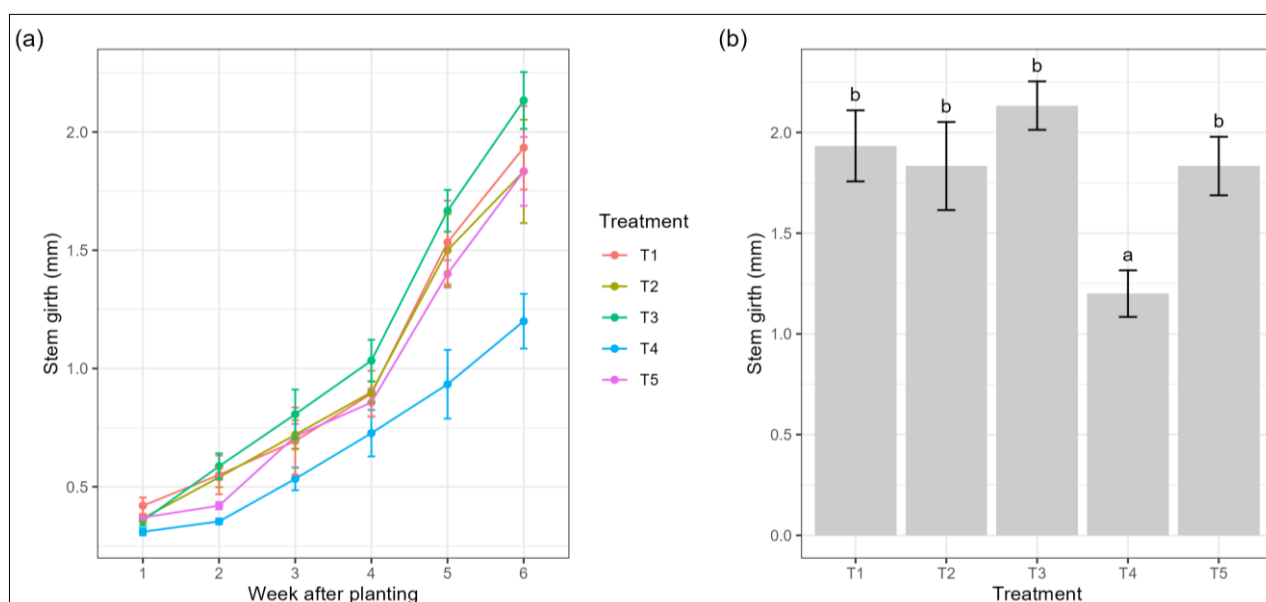


Fig. 9: Stem girth under the screenhouse treatments: (A) temporal progression and (B) final assessment. Error bars represent standard errors; final means with different letters differ at $p < 0.05$.

Table 4: Analysis of variance summary for disease and plant growth responses.

Response	Effect	df ₁	df ₂	F-value	p-value
Disease incidence	Treatment	4	10	7.784	0.004
Disease incidence	Week	5	50	36.986	<0.001
Disease incidence	Treatment × week	20	50	4.398	<0.001
Disease severity	Treatment	4	10	14.898	<0.001
Disease severity	Week	5	50	64.765	<0.001
Disease severity	Treatment × week	20	50	7.309	<0.001
AUDPC incidence	Treatment	4	10	11.605	<0.001
AUDPC severity	Treatment	4	10	14.571	<0.001
Plant height	Treatment	4	10	6.014	0.010
Plant height	Week	5	50	1028.892	<0.001
Plant height	Treatment × week	20	50	4.554	<0.001
Number of leaves	Treatment	4	10	1.793	0.207
Number of leaves	Week	5	50	651.238	<0.001
Number of leaves	Treatment × week	20	50	1.151	0.333
Stem girth	Treatment	4	10	9.700	0.002
Stem girth	Week	5	50	171.569	<0.001
Stem girth	Treatment × week	20	50	1.907	0.033

Repeated observations were analysed with treatment, week, and treatment × week as fixed effects and replicate as the repeated subject. AUDPC variables were analysed by one-way ANOVA. df₁ and df₂ are numerator and denominator degrees of freedom, respectively.

Discussion

The rapid and extensive wilt development in the pathogen-only control, together with the seedling, potato tuber, and tobacco responses, demonstrated that TBWK10 was aggressive under the experimental conditions. In contrast, all three biocontrol treatments reduced endpoint incidence, severity, and cumulative disease pressure while maintaining plant height and stem girth. Agreement among these disease and growth indicators strengthens the evidence for

biological suppression and avoids reliance on a single endpoint (Wu *et al.*, 2026).

The progressive increase in T22-mediated inhibition during the first 72h indicates that antagonism strengthened as the fungus established on the medium, followed by little additional inhibition between 72 and 96h. *Trichoderma* species may act through antimicrobial metabolites, nutrient and niche competition, hydrolytic enzymes, and stimulation of plant defence, although these mechanisms were not

measured directly in this study (Lahlali *et al.*, 2022; Ayaz *et al.*, 2023). The response agrees with reports that *Trichoderma asperellum* and *T. harzianum* isolates or their metabolites can inhibit *R. solanacearum* and suppress bacterial wilt in tomato (Konappa *et al.*, 2018; Guo *et al.*, 2021; Yan and Khan 2021). Because the quantitative assay evaluated T22, the in vitro results should not be interpreted as a direct comparison of T22 and BS01 antagonistic strength.

BS01 also reduced disease relative to the pathogen-only control, despite the absence of a parallel quantitative plate-inhibition series for this agent. *Bacillus* species can suppress disease through rhizosphere colonisation, antimicrobial compound production, resource competition, biofilm formation, and induction of plant resistance (Bonaterra *et al.*, 2022). The screenhouse response is consistent with suppression reported for *B. subtilis* R31 in tomato (Sun *et al.*, 2023), greenhouse evidence involving antagonistic *Bacillus* in the tomato rhizosphere (Elsayed *et al.*, 2020), and field evidence from East Africa showing that selected *Bacillus* and *Trichoderma* isolates can reduce bacterial wilt incidence and severity (Kariuki *et al.*, 2020).

The combined treatment produced the lowest numerical endpoint incidence and severity and the greatest numerical height and stem girth. However, it was not statistically superior to the individual agents, and its severity AUDPC was nearly identical to that of T22 alone. This contrasts with the synergistic response reported for a *T. virens*–*B. velezensis* combination against tomato bacterial wilt (Zhou *et al.*, 2021), but accords with evidence that multicomponent inoculants do not invariably outperform the best single strain. Their performance depends on compatibility, viable dose, inoculum ratio, formulation, spatial niche occupation, and persistence after application (Poveda and Eugui 2022; Kredics *et al.*, 2024). Equal-volume mixing also changes the dose of each component relative to its single-agent application; therefore, factorial dose–response experiments are needed before synergy or antagonism can be inferred.

Plant height and stem girth responded to the disease-management treatments, whereas leaf number did not. Bacterial wilt disrupts xylem function and water transport, making reduced structural growth in heavily diseased plants biologically plausible (Vailteau and Genin 2023; Wang *et al.*, 2023). The growth responses should be interpreted primarily as maintenance of plant vigour under reduced disease pressure because the experiment did not separate direct growth promotion from indirect benefits of disease suppression.

The study was limited to one tomato hybrid, one pathogen isolate, three experimental replicates, and one protected-environment experiment. The small number of replicates reduced precision, as reflected by broad standard errors for

some treatments, and the controlled environment cannot represent the pathogen, soil, and climate heterogeneity encountered in farmers' fields. Moreover, mechanisms of suppression, rhizosphere persistence, pathogen population density, yield, and economic return were not measured. The results should therefore be regarded as proof of concept rather than a field recommendation. Multi-location trials should evaluate dose, application timing, persistence, yield, and cost-effectiveness across cultivars and pathogen populations.

Conclusion

Trichoderma harzianum T22 and *Bacillus subtilis* BS01 were compatible in vitro and, when applied individually or in combination, reduced tomato bacterial wilt relative to the pathogen-only control under screenhouse conditions. The combined application produced the most favourable numerical endpoint disease and growth responses, but it was not statistically superior to either individual agent; T22 alone and the combination also produced nearly identical cumulative severity. These findings support field evaluation of both single-agent and combined applications, with explicit optimisation of viable dose, mixture ratio, application timing, persistence, yield response, and cost-effectiveness.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contribution

MMW conceived the study, conducted the experiments, curated and analysed the data, and drafted the manuscript. ROM and SMSM contributed to study design and interpretation, supervised the research, and critically revised the manuscript. All authors reviewed and approved the final version.

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Ethical Approval

Not applicable. The study involved plants and microbial cultures and did not involve human participants or vertebrate animals.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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