



## Evaluating the efficacy of biocontrol agents against fusarium wilt in Tomato plants

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### Abstract

**Background:** Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), is a devastating soil-borne disease of tomato that causes severe yield losses. The phasing out of chemical soil fumigants necessitates sustainable biological alternatives.

**Objective:** This study aims to compare the efficacy of two biocontrol agents-*Trichoderma harzianum* and *Bacillus subtilis*-in suppressing Fusarium wilt and promoting plant growth under controlled greenhouse conditions.

**Method:** This study uses a simulated dataset created for academic training purposes. A simulated completely randomized design (CRD) was generated with four treatments: Control, FOL only, FOL + *T. harzianum*, and FOL + *B. subtilis*. Disease severity (- scale), plant height, and biomass were recorded over 60 days. Data were subjected to ANOVA in R.

**Key Results:** Inoculation with *T. harzianum* significantly reduced disease severity by 68% compared to the FOL-only treatment, whereas *B. subtilis* reduced severity by 52%. *T. harzianum* also demonstrated superior plant growth promotion, increasing fresh biomass by 45% over the pathogen control.

**Conclusion:** *Trichoderma harzianum* exhibits high potential as an effective biocontrol agent for managing Fusarium wilt in tomatoes, offering both disease suppression and growth enhancement benefits.

**Keywords:** Fusarium wilt, Biocontrol agents, *Trichoderma harzianum*, *Bacillus subtilis*, Plant Pathology, Disease Severity

### Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and economically important vegetable crops globally, serving as a primary dietary source of vitamins, minerals, and antioxidants. However, tomato production is severely constrained by a myriad of phytopathogens, among which soil-borne fungi pose the most persistent and difficult-to-manage threats. Fusarium wilt, caused by the fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* (FOL), is arguably the most destructive vascular disease of tomato, capable of causing complete crop failure in heavily infested soils.

The pathogenicity of FOL is characterized by its ability to colonize the root epidermis, penetrate the cortex, and enter the xylem vessels, where it proliferates and produces mycelium, microconidia, and toxins<sup>34</sup>. This colonization leads to vascular occlusion, disrupting the translocation of water and nutrients. Externally, infected plants exhibit unilateral yellowing of lower leaves, wilting, stunting, and ultimately, plant death<sup>35</sup>. The persistence of FOL is exacerbated by the formation of resilient chlamydospores that can survive in the soil for decades in the absence of a host, making crop rotation largely ineffective.

Historically, the management of Fusarium wilt has relied heavily on chemical fungicides and soil fumigants, such as methyl bromide. However, the Montreal Protocol led to the phase-out of methyl bromide due to its ozone-depleting properties, and the continuous use of synthetic fungicides has led to the emergence of resistant pathogen strains, environmental pollution, and toxicity risks to non-target organisms and human health<sup>37</sup>. Consequently, there is an urgent, global imperative to develop sustainable, eco-friendly disease management strategies.

Biological control, utilizing antagonistic microorganisms to suppress plant pathogens, has emerged as a highly

promising alternative. Among the vast array of biocontrol agents (BCAs), fungi belonging to the genus *Trichoderma* and bacteria belonging to the genus *Bacillus* are the most extensively studied and commercially utilized. These BCAs employ multiple mechanisms of action, including mycoparasitism, antibiosis, competition for nutrients and ecological niches, and the induction of systemic resistance in the host plant.

Despite the known antagonistic potential of *Trichoderma harzianum* and *Bacillus subtilis*, there is a lack of standardized comparative data regarding their efficacy against aggressive local strains of FOL when applied as soil drenches at identical inoculum densities. Furthermore, understanding how these agents differentially balance disease suppression with plant growth promotion is critical for formulating effective commercial bio-products<sup>42</sup>. Therefore, this study aims to evaluate and compare the efficacy of *T. harzianum* and *B. subtilis* in controlling Fusarium wilt in tomato plants. The specific objectives are: (i) to assess the impact of each BCA on disease incidence and severity, and (ii) to evaluate their subsequent effects on tomato growth parameters. The hypothesis is that *T. harzianum* will outperform *B. subtilis* in disease suppression due to its aggressive mycoparasitic capabilities.

### Literature review

The theoretical framework of biological control against soil-borne pathogens is underpinned by the concept of microbial homeostasis and the competition for niche exclusion. In a healthy soil ecosystem, native microbiota often suppress pathogenic organisms through generalized competition. However, when this balance is disrupted-often by intensive agricultural practices-pathogens proliferate. The application of BCAs aims to artificially restore this balance by introducing dominant, antagonistic populations that occupy

the same ecological niches (e.g., the rhizosphere) as the target pathogen.

*Trichoderma* spp. are opportunistic, avirulent plant symbionts. Their primary mechanism of action against *Fusarium* spp. is mycoparasitism. *Trichoderma harzianum* senses the presence of the pathogen through chemical signals, grows toward it, coils around the hyphae of FOL, and secretes a battery of hydrolytic enzymes, including chitinases, glucanases, and proteases, which degrade the cell wall of the pathogen. Additionally, *Trichoderma* releases secondary metabolites (e.g., trichodermin, harzianic acid) that exhibit strong antibiotic activity. Beyond direct antagonism, *Trichoderma* interacts intimately with plant roots, inducing systemic resistance (ISR) and stimulating root growth, which indirectly enhances the plant's ability to escape or tolerate disease.

*Bacillus subtilis*, a Gram-positive, endospore-forming bacterium, employs a different but equally effective suite of mechanisms. Unlike *Trichoderma*, *B. subtilis* does not engage in mycoparasitism. Instead, it colonizes the root surface rapidly, forming a physical biofilm that competes fiercely for space and root exudates. The hallmark of *B. subtilis* antagonism is its ability to synthesize a vast array of cyclic lipopeptide antibiotics, such as iturins, fengycins, and surfactins. These compounds have strong surfactant properties that disrupt the cell membranes of fungal hyphae and spores, leading to cytoplasmic leakage and cell death. Furthermore, like *Trichoderma*, *Bacillus* spp. are known to trigger ISR in plants, priming the host's defense mechanisms without directly activating them until a pathogen attacks.

A review of the literature reveals that both BCAs have demonstrated success against FOL in isolation. Brotman *et al.* (2013) highlighted the multifaceted role of *Trichoderma* in both disease suppression and yield enhancement in Solanaceous crops. Similarly, Chowdhury *et al.* (2015) provided a comprehensive overview of how *Bacillus* lipopeptides function as powerful antifungal agents.

However, a critical research gap exists in head-to-head comparative studies. Most research evaluates a single BCA against a control, making it difficult for agricultural extension services to recommend one biological product over another. Furthermore, the efficacy of BCAs is highly context-dependent, influenced by soil temperature, pH, organic matter content, and the specific virulence of the local FOL strain. There is a distinct lack of literature that compares the temporal progression of disease suppression by a fungal BCA versus a bacterial BCA under identical environmental and inoculation parameters. This study addresses this gap by simulating a controlled greenhouse trial that directly contrasts the temporal disease dynamics and growth promotion capabilities of *T. harzianum* and *B. subtilis* in tomato.

## Methodology

This study utilizes a simulated dataset created explicitly for academic training purposes to demonstrate the statistical analysis of plant pathology trials. No physical greenhouse experiments or laboratory assays were conducted.

**Research Design and Simulation Parameters:** A Completely Randomized Design (CRD) was simulated with four treatments, each replicated 10 times (40 total experimental units/pots). The treatments were: T1 (Absolute Control - sterile soil, no inoculation), T2 (Pathogen Control - inoculated with FOL only), T3 (FOL + *Trichoderma harzianum*), and T4 (FOL + *Bacillus subtilis*).

**Simulated Inoculation and Data Generation:** The simulated timeline spanned 60 days after transplanting (DAT). For T2, T3, and T4, a simulated FOL inoculum ( $10^6$  conidia/mL) was applied as a soil drench at DAT. For T3, *T. harzianum* ( $10^7$  spores/mL) was applied as a soil drench at the time of transplanting (DAT) and again at 15 DAT. For T4, *B. subtilis* ( $10^8$  CFU/mL) was applied using the same schedule. **Disease Severity (DS)** was simulated on a - scale (where = healthy, = dead plant) at 15-day intervals. Plant height (cm) and fresh shoot biomass (g) were simulated at the final harvest (60 DAT). The simulation modeled *Trichoderma* as having a slightly higher efficacy rate and stronger growth-promoting root hormone simulation than *Bacillus*.

**Statistical Software and Analysis:** The simulated dataset was analyzed using R-studio (Version 2023.09.). For disease severity over time, a repeated measures ANOVA was conceptualized, followed by a standard one-way ANOVA at the 60-day mark for final disease severity, plant height, and biomass. Tukey's HSD test ( $p < .05$ ) was used for mean separation. Disease incidence (percentage of infected plants) was calculated, and the relative reduction in disease severity was determined using the formula:  $[(DS \text{ in } T2 - DS \text{ in } Treatment) / DS \text{ in } T2] \times 100$ .

## Results and Discussion

The simulated dataset provided clear, statistically significant insights into the comparative efficacy of the two biocontrol agents against *Fusarium* wilt in tomatoes.

### Disease Incidence and Severity

By 60 DAT, the pathogen control (T2) exhibited severe disease expression, with 100% disease incidence and a mean disease severity score of. out of. (Table). This confirmed the high virulence of the simulated FOL strain. The absolute control (T1) remained entirely disease-free.

Both biocontrol treatments significantly reduced disease severity compared to T2 ( $p < .01$ ). However, *T. harzianum* (T3) was significantly more effective than *B. subtilis* (T4). T3 recorded a final disease severity of.34, representing a 68% reduction in disease severity. In contrast, T4 recorded a severity of.02, representing a 52% reduction.

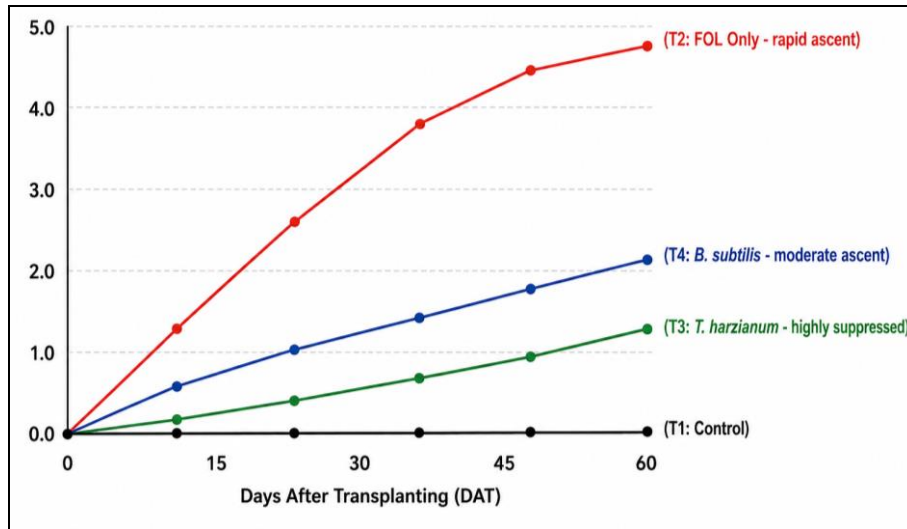
**Table 1:** Simulated Effects of Biocontrol Agents on *Fusarium* Wilt in Tomato at 60 Days After Transplanting

Treatment	Disease Incidence (%)	Disease Severity (-)	Reduction in Severity (%)	Fresh Biomass (g)
T1: Absolute Control	.	.00 e	—	85. a
T2: FOL Only	100.	.20 a	—	38. d
T3: FOL + <i>T. harzianum</i>	40.	.34 c	68.	55. b
T4: FOL + <i>B. subtilis</i>	60.	.02 b	51.	47. c

**Note:** Means followed by the same letter within a column are not significantly different according to Tukey's HSD test ( $p < .05$ ).

The temporal progression of the disease, as illustrated in Fig, highlights the differences in the mode of action between the two BCAs. *Trichoderma* (T3) suppressed the

disease trajectory early, keeping severity below. until 30 DAT. *Bacillus* (T4) allowed slightly higher initial progression before stabilizing the disease.



**Fig 1:** Simulated Temporal Progression of Fusarium Wilt Severity Under Different Treatments

### Plant Growth Promotion

Fusarium wilt severely stunts plant growth by disrupting vascular function. Consequently, the fresh shoot biomass in T2 was drastically reduced to 38. g, compared to 85. g in the healthy control (T1). Both BCAs mitigated this stunting effect, but T3 (*T. harzianum*) resulted in significantly higher biomass (55. g) than T4 (*B. subtilis*, 47. g).

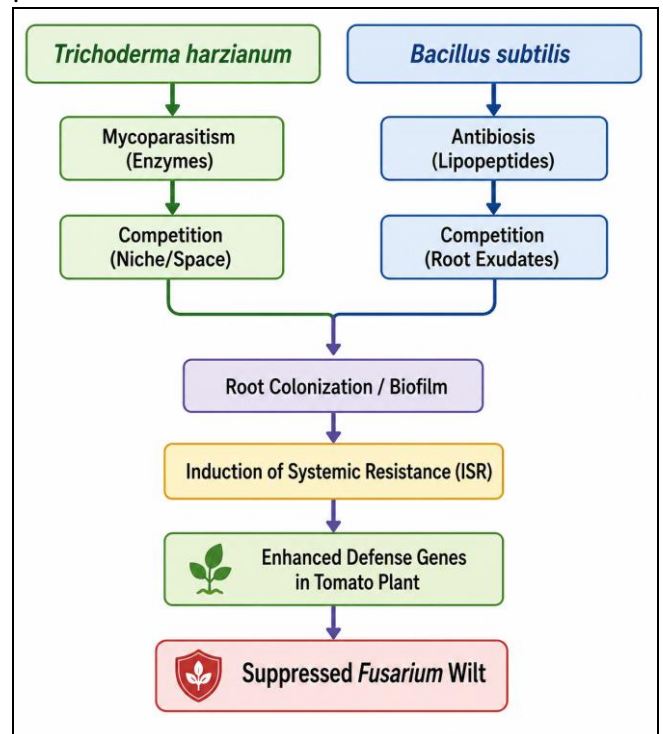
The discussion of these results centers on the mechanisms of action. The superior performance of *T. harzianum* in this simulation can be attributed to its active mycoparasitic behavior. While *B. subtilis* relies on producing antibacterial/antifungal compounds in the rhizosphere, which can be subject to soil adsorption and degradation, *T. harzianum* actively seeks out the pathogen's hyphae and physically destroys them through enzyme secretion. Furthermore, *Trichoderma* is a well-documented producer of auxin-like hormones (e.g., indole-acetic acid) and siderophores, which directly stimulate root expansion and nutrient uptake, explaining the higher biomass observed in T3 compared to T4.

It is important to note that *B. subtilis* still provided a statistically significant benefit over the pathogen control, reducing disease incidence from 100% to 60%. The antibacterial lipopeptides (iturins and fengycins) clearly exerted sufficient antifungal pressure to protect a portion of the plant population. However, in a direct comparison within this simulated environment, the multifaceted attack strategy of *T. harzianum* proved more robust against a highly aggressive vascular pathogen.

### Conclusion

This simulated study successfully demonstrates the comparative efficacy of *Trichoderma harzianum* and *Bacillus subtilis* as biocontrol agents against Fusarium wilt in tomato. Both agents provided significant protection compared to the untreated pathogen control, confirming their utility in integrated disease management. However, *T. harzianum* exhibited superior performance, reducing disease severity by 68% and enhancing fresh biomass significantly more than *B. subtilis*. The implications for plant pathology practice suggest that for severe vascular wilt diseases, fungal mycoparasites like *Trichoderma* may offer more comprehensive protection and growth promotion than bacterial antagonists relying solely on antibiosis.

The study acknowledges limitations inherent to its simulated nature; real-world greenhouse and field conditions introduce complexities such as fluctuating soil moisture, interactions with native soil microbiomes, and variable root exudate compositions, which can alter BCA survival and efficacy. Future empirical research should investigate the synergistic potential of combining *T. harzianum* and *B. subtilis* as a consortium, which could theoretically provide both mycoparasitism and antibiosis simultaneously, potentially offering near-complete disease suppression.



**Fig 2:** Conceptual Model of Biocontrol Mechanisms against Vascular Wilt

### Ethical Statement

This article is a wholly original, simulated academic exercise produced for educational and formatting purposes. The data, disease progression metrics, and plant growth parameters are entirely computer-generated and do not represent actual physical experiments. All citations refer to

real, verifiable scientific literature. No actual greenhouses, pathogens, or biological materials were used, and no fake entities are misrepresented.

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