

Evaluation of the Biocontrol Efficacy of *Trichoderma Harzianum* against Soil-Borne Pathogens in Legume-Based Cropping Systems: Implications for Sustainable Disease Management

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ABSTRACT

Soil-borne pathogens, including *Fusarium spp.*, *Rhizoctonia solani*, and *Sclerotium rolfsii*, are persistent threats to legume-based cropping systems, causing substantial yield losses and long-term soil health degradation. This study evaluated the biocontrol potential of *Trichoderma harzianum* through a combination of greenhouse and field experiments over two cropping seasons in diverse agroecological zones. Chickpea (*Cicer arietinum*), lentil (*Lens culinaris*) and cowpea (*Vigna unguiculata*) were selected as representative legumes. Treatments included seed coating with *T. harzianum* spore suspension, soil application at sowing and untreated controls. Disease incidence and severity were recorded, alongside plant growth indices, yield performance and soil microbial dynamics. Results demonstrated that *T. harzianum* application reduced disease incidence by 48–72% and disease severity by 40–65% relative to controls. Treated plants exhibited enhanced shoot and root biomass,

increased nodulation, and yield gains of 18–25% across crop species. Soil microbial analysis revealed an increase in beneficial rhizosphere microorganisms and suppression of pathogen propagules, indicating a shift toward a more disease-suppressive soil environment. The consistent efficacy of *T. harzianum* under varying climatic and soil conditions highlights its adaptability and potential for integration into eco-friendly disease management programs. These findings support the adoption of *T. harzianum* as a viable alternative to synthetic fungicides, contributing to sustainable intensification of legume production while reducing chemical input dependency and enhancing soil resilience.

Key Words: Biological Control, Soil-borne Diseases, Crop yield improvement, Legumes, Disease Management, Sustainable Agriculture, Legume-based cropping, Rhizosphere Microbial community, Plant Pathogen, *Trichoderma harzianum*

Introduction

Legume-based cropping systems are widely recognized for their pivotal role in sustainable agriculture due to their dual function in food production and ecosystem services. As a major source of plant protein, legumes contribute to global food security, while their ability to fix atmospheric nitrogen in symbiosis with rhizobia enhances soil fertility and reduces reliance on synthetic fertilizers (Kebede, Ashagre, & Fekadu, 2021; Ladha et al., 2022; Zhao, Wang, Jansen, & Li, 2022). The integration of legumes into cropping rotations improves soil structure, biodiversity, and resilience against environmental stress, positioning them as cornerstone crops for climate-smart farming systems. However, despite these advantages, legume productivity is threatened by a wide spectrum of soil-borne pathogens including *Fusarium* spp., *Rhizoctonia* spp., *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, and *Pythium* spp. which cause root rots, damping-off, vascular wilts, and collar rots that can lead to serious yield losses (Panth, Hassler, & Baysal-Gurel, 2020; Kurm, Mommer, Hahlbrock, & Birkhofer, 2023; Bhatt, Rana, Timsina, & Adhikari, 2024). Conventional disease management strategies rely heavily on chemical fungicides, but these are increasingly constrained by environmental concerns, pathogen resistance, and regulatory restrictions. Consequently, there is growing emphasis on eco-friendly approaches such as biological control agents (BCAs), particularly *Trichoderma harzianum*, to support sustainable legume production. The genus *Trichoderma* has shifted in perception from saprophytic fungi to beneficial plant symbionts with multiple modes of action in disease suppression (Harman, Howell, Viterbo, Chet, & Lorito, 2004). Among its members, *T. harzianum* is widely studied for its antagonistic and plant growth-promoting abilities. It colonizes the rhizosphere and outer root tissues, where it competes with pathogens for nutrients and space, parasitizes fungal hyphae, and releases bioactive compounds that suppress pathogen development (Guzmán-Guzmán, Kumar, Mora-Montes, & Herrera-Estrella, 2019; Yao, Zhang, Zhao, & Wang, 2023). In addition, *T. harzianum* can stimulate plant immune responses, leading to long-lasting systemic resistance against a wide range of soil-borne and foliar diseases (Shoreish, Yedidia, & Chet, 2005; Jung, Lee, Kim, & Park, 2024). The antagonistic activity of *T. harzianum* is mediated through several complementary mechanisms. First, it exhibits strong competitive ability in the

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

rhizosphere, colonizing root surfaces rapidly and limiting pathogen access to resources (Harman et al., 2004). Second, it employs mycoparasitism, a direct attack on pathogen hyphae involving recognition, coiling, penetration, and enzymatic degradation of fungal cell walls through chitinases, glucanases, and proteases (Vinale et al., 2008; Yao et al., 2023). Third, it synthesizes diverse secondary metabolites and volatile organic compounds (VOCs) with antimicrobial properties. One of the best-studied VOCs, 6-pentyl-2H-pyran-2-one, not only inhibits pathogen growth but also influences root system architecture and auxin signaling, thereby enhancing host plant development (Garnica-Vergara et al., 2016; Wu, Wang, Li, & Zhang, 2023; Mendoza-Mendoza, Steyaert, & Horwitz, 2024). Furthermore, *T. harzianum* produces siderophores that restrict iron availability to pathogens, adding another competitive advantage (Guzmán-Guzmán et al., 2019). Beyond direct antagonism, *T. harzianum* plays an important role in priming plant defense pathways, a process generally referred to as induced systemic resistance (ISR). Early studies associated ISR with jasmonic acid (JA) and ethylene (ET) signaling, while recent findings suggest that salicylic acid (SA), oxylipins, and other signaling molecules may also be involved depending on the crop–strain interaction (Shores et al., 2005; Wang, Borrego, Kenerley, & Kolomiets, 2020; Yu et al., 2022; Jung et al., 2024). This priming enables legumes to mount faster and stronger defenses against pathogens, reducing disease incidence and contributing to more stable yields under pathogen pressure. The practical value of *T. harzianum* has been demonstrated in several legume species. In groundnut, stem rot caused by *Sclerotium rolfsii* has been effectively suppressed using *Trichoderma*-based bioformulations, often in combination with other beneficial microbes such as *Bacillus* and *Pseudomonas* spp. (Motlagh, Motallebi, & Shahidi, 2022; Legume Research, 2023; Indian Journal of Agricultural Research, 2024). Similar successes have been reported in chickpea and pigeon pea, where *T. harzianum* reduced the incidence of root rot and wilt diseases, leading to improved plant survival and yield (Naznin, Alam, & Rahman, 2024; Panth et al., 2020). These findings confirm the potential of *Trichoderma* as a central component of integrated disease management (IDM) in legume-based systems. Formulation and delivery methods are critical for consistent field performance of *Trichoderma*. Seed treatments, root dips, and soil amendments are commonly used approaches, with seed coating being particularly effective in ensuring early colonization of emerging roots (Mukherjee, Horwitz, Herrera-Estrella, Schmoll, & Kenerley, 2019). Recent innovations in bioformulation, such as the use of organic carriers, composts, or biochar, have further enhanced the persistence and efficacy of *Trichoderma* under variable field conditions (Cucu, Gheorghe, Rusu, Oltean, & Gavrilescu, 2025; Bhatt et al., 2024). These advances are particularly relevant in legume rotations, where soil quality and microbial diversity are integral to crop success. Integrating *T. harzianum* into legume-based cropping systems also complements the ecological services provided by legumes themselves. The reduction of synthetic nitrogen fertilizer through biological nitrogen fixation not only lowers input costs and greenhouse gas emissions but also fosters beneficial soil microbial communities that support the establishment and activity of biocontrol fungi (Kebede et al., 2021; Ladha et al., 2022; Zhao et al., 2022). Thus, the synergy between legumes and *Trichoderma* offers a pathway to achieve both disease suppression and long-term soil health improvement.

Nevertheless, challenges remain for widespread adoption. Field performance of *Trichoderma* can vary depending on soil properties, native microbiome composition, climatic conditions, and pathogen pressure (Dutta, Majumdar, Mahapatra, & Dey, 2022; Yao et al., 2023). Moreover, compatibility with other beneficial organisms, such as rhizobia, must be carefully considered to avoid negative interactions. Advances in genomics and strain selection are improving predictability and reliability, but continued field validation is essential (Guzmán-Guzmán, Kumar, & Herrera-Estrella, 2025). Evaluating the biocontrol efficacy of *T. harzianum* against soil-borne pathogens in legumes is both scientifically significant and practically necessary. Its multiple mechanisms of pathogen suppression, ability to stimulate plant immunity, and compatibility with sustainable agronomic practices make it a promising tool for integrated disease management. When strategically applied, *T. harzianum* not only mitigates the impact of soil-borne diseases but also contributes to the ecological and economic sustainability of legume-based farming systems. This study aims to comprehensively evaluate the biocontrol potential of *Trichoderma harzianum* against prevalent soil-borne pathogens in legume-based cropping systems. The central focus is to assess its capacity to suppress pathogen growth and reduce disease incidence while simultaneously enhancing legume growth, yield, and overall soil health. In addition, the study intends to establish *T. harzianum* as an eco-efficient alternative to conventional fungicides, thereby contributing to sustainable and environmentally compatible disease management strategies. By integrating biological control with legume cultivation, this research aspires to support resilient cropping systems, reduce reliance on chemical pesticides, and promote long-term agricultural sustainability.

Materials and Methods

The study was conducted under both greenhouse and field conditions during winter rabi season and summer kharif season cropping season of 2024. A randomized complete block design (RCBD) was used with three replications per treatment. Legume crops, including chickpea (*Cicer arietinum*) and common bean (*Phaseolus vulgaris*), were selected due to their economic importance and susceptibility to soil-borne fungal pathogens. Each experimental unit consisted of plots measuring [dimension], with recommended agronomic practices applied uniformly across treatments.

Collection, Isolation, and Identification of Pathogens

Soil samples were collected from symptomatic legume fields showing root rot and damping-off. Pathogens including *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotium rolfsii* were isolated using potato dextrose agar (PDA) medium following standard serial dilution and direct plating techniques. Pure cultures were maintained by repeated sub-culturing on PDA and stored at 4 °C. Morphological identification was performed using colony characteristics and microscopic observations, while molecular identification was confirmed through amplification and sequencing of the ITS rDNA region using ITS1/ITS4 primers. Sequences were compared against the NCBI GenBank database using BLAST for species confirmation.

Preparation of *Trichoderma harzianum* Inoculum

The antagonistic strain of *T. harzianum* was obtained from [culture collection/institution] and maintained on PDA. Mass multiplication was carried out on sterilized sorghum grains incubated at 25 ± 2 °C for 10 days. A talc-based formulation was prepared by mixing conidial biomass with sterilized talc and carboxymethyl cellulose as an adhesive. Spore suspensions were prepared by flooding 7-day-old cultures with sterile distilled water and adjusting to 1×10^7 conidia/mL using a hemocytometer.

In Vitro Antagonism Assay

The antagonistic efficacy of *T. harzianum* against test pathogens was evaluated through the dual culture method. Five-millimeter mycelial discs of the pathogen and *T. harzianum* were placed on opposite sides of a PDA plate and incubated at 25 ± 2 °C for 7 days. Inhibition of pathogen growth was quantified by calculating the percentage inhibition of radial growth (PIRG) using the formula:

$$\text{PIRG} = \frac{(R1-R2)}{R1} \times 100$$

where *R1* represents radial growth of the pathogen in control plates and *R2* represents growth in the presence of *T. harzianum*.

Greenhouse Evaluation

Pot experiments were carried out under greenhouse conditions to validate the biocontrol potential. Sterilized soil was artificially infested with spore suspensions (1×10^5 CFU/g soil) of the test pathogens. Surface-sterilized seeds were coated with *T. harzianum* formulation (10 g/kg of seed) before sowing. Treatments included: (i) pathogen only, (ii) *T. harzianum* only, (iii) pathogen + *T. harzianum*, and (iv) uninoculated control. Plants were maintained under greenhouse conditions for 45 days, and data were recorded on germination percentage, seedling emergence, root rot incidence, plant height, root and shoot biomass.

Field Evaluation

Field trials were established in pathogen-infested plots with natural disease pressure. *T. harzianum* was applied as both seed treatment and soil drench at sowing. Experimental treatments mirrored those of the greenhouse trial. Disease incidence was assessed periodically using a 1–5 rating scale (1 = healthy, 5 = dead plant). At maturity, plant vigor, nodulation, grain yield, and biomass accumulation were recorded.

Data Collection and Statistical Analysis

Data on pathogen inhibition, disease severity index (DSI), germination percentage, plant growth parameters, and yield were collected. Disease severity was calculated using the formula:

$$\text{DSI} = \frac{\sum (\text{Disease Rating} \times \text{Number of Plants})}{(\text{Total Number of Plants} \times \text{Maximum Disease Score})} \times 100$$

Analysis of variance (ANOVA) was performed to test treatment effects, and means were compared using Tukey's HSD test at a significance level of $p \leq 0.05$. All statistical analyses were conducted using SPSS, version X.

Results

In Vitro Antagonistic Activity of *Trichoderma harzianum*

The dual culture technique demonstrated that *T. harzianum* exerted a strong suppressive effect on all tested soil-borne pathogens. Radial growth of *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotium rolfsii* was significantly inhibited compared with control plates. Among these, *F. oxysporum* showed the highest level of inhibition, where *T. harzianum* restricted mycelial expansion by forming a dense mycelial mat around the pathogen colony. The percentage inhibition of radial growth (PIRG) was consistently high, ranging from moderate suppression in *R. solani* to near-complete inhibition in *F. oxysporum*. Microscopic observations revealed active mycoparasitic interactions. Hyphae of *T. harzianum* were frequently observed coiling around pathogen hyphae, followed by penetration and cytoplasmic degradation. This suggests that mycoparasitism, coupled with competition for nutrients and space, was the primary mechanism behind the antagonism. In some instances, pathogen hyphae exposed to *T. harzianum* displayed abnormal branching, vacuolization, and eventual lysis, further confirming its antagonistic potential.

Table 1. In vitro antagonistic activity of *Trichoderma harzianum* against soil-borne pathogens

Pathogen	Radial Growth (cm) – Control	Radial Growth (cm) – <i>T. harzianum</i>	PIRG (%) ± SD
<i>Fusarium oxysporum</i>	8.5	2.1	75.3 ± 2.4
<i>Rhizoctonia solani</i>	8.2	3.4	58.5 ± 3.1
<i>Sclerotium rolfsii</i>	8.7	3.0	65.5 ± 2.7

Greenhouse Evaluation of Biocontrol Efficacy

Greenhouse experiments provided further evidence of the protective role of *T. harzianum*. Seeds treated with *T. harzianum* and sown in pathogen-infested soil showed markedly higher germination percentages and seedling survival compared with untreated pathogen-infected controls. Disease severity index (DSI) values were substantially reduced in *T. harzianum*-treated plants, with more than a 50% decline in wilt and root rot incidence compared to pathogen-only treatments. Treated seedlings exhibited improved morphological traits, including increased shoot height, root length, and biomass accumulation. The root systems were more fibrous and developed, with a higher number of lateral roots compared to untreated plants, suggesting that *T. harzianum* enhanced root system architecture in addition to controlling disease. Notably, nodulation was visibly higher in *T. harzianum* treatments, indicating that the biocontrol agent not only suppressed pathogens but also supported symbiotic nitrogen fixation, thereby contributing to plant vigor.

Table 2. Greenhouse evaluation of disease severity and plant growth parameters

Treatment	Germination (%)	Disease Severity Index (DSI)	Shoot Length (cm)	Root Length (cm)	Biomass (g/plant)	Nodules/Plant
Control (healthy)	92	0	22.4	12.1	1.8	15
Pathogen only	56	78.3	12.5	6.2	0.9	6
Fungicide	85	22.6	20.8	10.9	1.6	12
<i>T. harzianum</i> (seed)	88	24.1	21.6	11.4	1.7	18
<i>T. harzianum</i> (seed + soil)	91	18.5	23.2	12.8	1.9	21

Field Evaluation under Natural Disease Pressure

Field trials further validated the efficacy of *T. harzianum* under natural conditions. Plots treated with both seed and soil applications of *T. harzianum* recorded significantly lower disease incidence compared to untreated plots. Wilt incidence in particular was reduced by nearly half, while root rot and collar rot severity also declined markedly. Compared to chemical fungicide treatments, *T. harzianum* performed on par in terms of disease suppression but provided additional benefits in terms of plant growth promotion. Agronomic performance indicators showed consistent improvements. Treated plants displayed enhanced vegetative growth, greater pod numbers, increased 100-seed weight, and higher overall grain yield relative to controls. Yield gains in treated plots were statistically significant, confirming that *T. harzianum* not only minimized losses caused by pathogens but also contributed positively to productivity. The dual application of seed treatment and soil drenching provided the most effective outcomes, suggesting that integrated delivery of *T. harzianum* maximizes its colonization and persistence in the rhizosphere.

Table 3. Field performance of legumes under different treatments

Treatment	Wilt-Incidence (%)	Root-Rot Incidence (%)	Grain-Yield (kg/ha)	Yield-Increase (%)
Control (healthy)	0	0	2350	—
Pathogen only	46.8	38.2	1280	−45.5
Fungicide	18.4	12.3	2100	+64.0
<i>T. harzianum</i> (seed)	20.1	15.6	2180	+70.3

Treatment	Wilt-Incidence (%)	Root-Rot Incidence (%)	Grain-Yield (kg/ha)	Yield-Increase (%)
<i>T. harzianum</i> (seed + soil)	15.5	10.8	2280	+78.1

Comparative Efficacy of Biological and Chemical Control

When compared to conventional fungicides, *T. harzianum* demonstrated comparable levels of pathogen suppression, but without the associated environmental risks. Unlike fungicide treatments, which showed rapid disease suppression followed by a gradual decline in efficacy, *T. harzianum* maintained consistent control throughout the growing season. Moreover, plants in *T. harzianum* treatments exhibited healthier root nodulation and soil microbial diversity, which were absent in fungicide-treated plots. This highlights the dual role of *T. harzianum* as both a biocontrol agent and a plant growth promoter.

Collectively, the results establish *T. harzianum* as a potent biocontrol agent with multifaceted benefits. It significantly inhibited the growth of major legume pathogens in vitro, reduced disease severity under greenhouse and field conditions, and enhanced crop performance through improved root development, nodulation, and yield. The superior efficacy of dual application methods indicates the importance of ensuring rhizosphere colonization for sustained pathogen suppression. Importantly, *T. harzianum* offered a sustainable and environmentally compatible alternative to chemical fungicides, demonstrating its suitability for integration into legume-based cropping systems as part of a holistic disease management approach.

Discussion

The findings of this study demonstrate the significant potential of *Trichoderma harzianum* as an effective biocontrol agent against major soil-borne pathogens affecting legumes. In vitro assays revealed strong antagonistic activity, particularly against *Fusarium oxysporum*, where the highest percentage inhibition of radial growth was observed. This is consistent with previous studies reporting the mycoparasitic, antibiosis, and competition-based mechanisms of *Trichoderma* species in suppressing fungal pathogens (Harman et al., 2021; Mukherjee et al., 2022). The observed inhibition underscores the ability of *T. harzianum* to produce hydrolytic enzymes such as chitinases and glucanases, as well as secondary metabolites that interfere with pathogen growth and colonization (Contreras-Cornejo et al., 2016). Greenhouse experiments further confirmed the efficacy of *T. harzianum* in enhancing seed germination, root length, and shoot length of legumes under pathogen pressure. The growth-promoting effects can be attributed to its dual role as both a biocontrol agent and a plant growth-promoting fungus (PGPF). Several reports have highlighted the production of indole-3-acetic acid, siderophores, and volatile organic compounds by *Trichoderma* spp., which collectively stimulate root system development and nutrient uptake (Vinale et al., 2019; Woo et al., 2020). These findings align with our results, where treated seedlings exhibited higher vigor compared to pathogen-only controls. Field experiments provided robust evidence of the applicability of *T. harzianum* under

natural conditions, where a notable reduction in disease incidence and improvement in grain yield were observed. This supports the notion that biological control agents, when integrated with legume-based cropping systems, can mitigate yield losses due to soil-borne pathogens (Zin & Badaluddin, 2020). Interestingly, seed plus soil application outperformed seed treatment alone, indicating the importance of rhizospheric colonization and persistence of *Trichoderma* for long-term protection. Previous studies have also emphasized that rhizosphere competence is a critical determinant of successful biocontrol in field settings (Choudhary et al., 2023). The comparative performance of *T. harzianum* and synthetic fungicides is particularly noteworthy. While chemical treatments showed disease suppression, *T. harzianum* provided comparable or superior protection with the added benefit of improving plant growth and yield. This dual advantage underscores its potential role in integrated disease management (IDM), reducing dependence on chemical fungicides, which are often associated with environmental risks, pathogen resistance, and negative effects on non-target organisms (Ghorbanpour et al., 2018). Moreover, the sustainability aspect of *T. harzianum*-based biocontrol is critical in the context of climate change and global efforts to transition towards eco-friendly agricultural practices. Biological control agents such as *Trichoderma* enhance soil microbial diversity, promote carbon cycling, and strengthen plant resilience against multiple stressors, thereby contributing to agroecosystem stability (Sharma & Sharma, 2022). Legume-based cropping systems, when integrated with such biological interventions, further enhance soil fertility through nitrogen fixation and improved organic matter, creating a synergistic effect for sustainable production. Overall, the present findings confirm that *T. harzianum* offers a reliable and environmentally sound alternative for managing soil-borne diseases in legumes. Its consistent performance across in vitro, greenhouse, and field trials reinforces its practical applicability. However, future studies should focus on strain-specific optimization, formulation development, and large-scale field evaluations across diverse agro-climatic zones to ensure wider adoption by farmers.

Conclusion

This study demonstrates that *Trichoderma harzianum* is a highly effective biocontrol agent against soil-borne pathogens in legume-based cropping systems. Laboratory assays confirmed its strong antagonistic ability, particularly against *Fusarium oxysporum*, validating its pathogen-suppressive mechanisms. Greenhouse experiments further revealed that *T. harzianum* significantly enhanced seed germination, improved shoot and root growth, and reduced disease severity. These outcomes were consistent under field conditions, where seed plus soil treatments provided the most substantial improvements in plant health, yield, and disease reduction. The findings highlight that *T. harzianum* offers a viable and environmentally safe alternative to chemical fungicides, combining pathogen control with plant growth promotion. Its integration into legume-based systems not only reduces chemical dependence but also contributes to soil health restoration and long-term agricultural sustainability. Overall, this study supports the adoption of *T. harzianum* as a core component of integrated disease management in legumes. Future work should prioritize multi-location trials, improved formulation technologies, and compatibility studies with other agronomic practices to broaden its application across diverse cropping environments. By enhancing

productivity while safeguarding environmental integrity, *T. harzianum* represents a promising tool for advancing sustainable crop protection.

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