



Native *Trichoderma* spp. as biocontrol agents against common beans anthracnose

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Abstract

Trichoderma spp. has emerged as a promising biocontrol agent among soil microbiota. This study evaluated the biocontrol potential of 32 native *Trichoderma* strains recovered from Araucaria Forest soils in southern Brazil against *Colletotrichum lindemuthianum* (races 475 and 73), the causal agent of anthracnose in common bean (*Phaseolus vulgaris* L.). Among these isolates, *T. hamatum* LMA RQ, *T. asperellum* LMA RS2, and *T. atroviride* LMA PH exhibited outstanding antagonistic performance both in vitro and in vivo. In dual-culture assays, *T. hamatum* LMA RQ and *T. asperellum* LMA RS2 inhibited pathogen growth by 80–88%, while volatile and non-volatile metabolites reduced fungal development by 65–82%. All three strains exhibited high fungicide compatibility ($\geq 85\%$) and maintained robust colony growth under chemical exposure. In the in vivo experiments (detached leaf, pod, and seed germination assays), these *Trichoderma* strains reduced anthracnose incidence by 60–85% and disease severity (AUDPC) by up to 70%, while sustaining 92–100% seed germination. Their potential modes of action include mycoparasitism, competitive interactions, and the production of secondary metabolites. Notably, the strains were applied as simple conidial suspensions without adjuvants, underscoring their potential for commercial formulation and use as sustainable alternatives to chemical fungicides. Overall, these findings highlight the key role of fungal bioagents in sustainable crop protection and reinforce the biotechnological relevance of *Trichoderma* spp. from native forest soils.

Keywords Biological control agent · Fentin hydroxide alternative · Spray application · *T. hamatum* · *T. asperellum* and *T. atroviride*

Introduction

Fertile soil, influenced by factors such as temperature, humidity, and pH, sustains plant life through intense microbial activity (Heinze et al. 2016). Microorganisms

decompose organic matter, recycle nutrients, and interact with plants, playing a crucial role in agricultural productivity (Wang et al. 2024). Plant roots harbor a diverse microbiota that includes fungi capable of promoting plant development (Beattie et al. 2024). Among these microorganisms, fungi of the genus *Trichoderma* stand out as widely recognized biocontrol agents (Kumar et al. 2023; Paredes et al. 2025). These organisms suppress plant diseases by inducing resistance, activating natural plant defenses, competing for nutrients and space, and producing antimicrobial compounds (Contreras-Cornejo et al. 2024). The symbiotic interaction between *Trichoderma* and plants has supported the development of sustainable agricultural practices for phytopathogen management and the advancement of bio-inputs (Kumari and Tapwal 2023).

Common bean (*Phaseolus vulgaris* L.) is a strategic crop that benefits from alternative disease control technologies (Figueira et al. 2020; Gutiérrez-Moreno et al. 2021). It represents one of the most important food legumes worldwide,

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with a global production of approximately 31 million tons in 2023 (FAOSTAT 2024). Brazil ranks among the top three producers, contributing nearly 3.2 million tons annually, about 10% of global output (CONAB 2025).

Beyond its nutritional relevance, common bean generates an estimated USD 2.1 billion per year for the Brazilian economy through domestic consumption, commercialization, and exports (CONAB 2025), underscoring its agro-economic and socioeconomic importance. However, yield is strongly challenged by diseases, particularly anthracnose caused by *Colletotrichum lindemuthianum* (Assunção et al. 2020; Santos and Furlong 2022).

Beyond anthracnose, common bean is affected by several other diseases, including rust, angular leaf spot, bacterial blight, and white mold, all of which can severely compromise productivity (Assunção et al. 2020; Santos and Furlong 2022). Among these, anthracnose caused by *C. lindemuthianum* is one of the most destructive, with potential yield losses reaching up to 100% under favorable temperatures (8–28 °C) and high-humidity conditions (Padder et al. 2017), which are frequently observed in southern Brazil (Da Silva et al. 2020). Although chemical fungicides are widely used to control these diseases, they present significant drawbacks, including environmental impacts and the risk of pathogen resistance (Ceresini et al. 2024). As an alternative, *Trichoderma*-based bio-inputs offer sustainable solutions aligned with modern agricultural demands (Sharma et al. 2021).

Despite the limited exploration of foliar application of *Trichoderma* spp. for anthracnose control in *P. vulgaris*, preliminary evidence suggests that this approach can delay symptom onset, indicating effectiveness potentially comparable to furrow or seed treatments (Sesan et al. 2020). Building upon this context, recent studies have shown that biocontrol agents vary considerably in their efficacy against anthracnose in common beans. Bacterial antagonists such as *Bacillus subtilis* and *Pseudomonas fluorescens* typically reduce disease incidence by 40–60% under greenhouse conditions (Compant et al. 2005; Fira et al. 2018). In contrast, several *Trichoderma* species, including *T. harzianum*, *T. asperellum*, and *T. atroviride*, frequently achieve inhibition levels exceeding 70% in vitro and significantly reduce disease severity in vivo (Carrero-Carrón et al. 2016). This comparative evidence highlights the superior and more consistent performance of *Trichoderma* spp. and reinforces their relevance as effective and ecologically sustainable bio-control agents for managing anthracnose in common bean.

However, existing studies have predominantly focused on species within the *T. harzianum* group, leaving significant gaps in understanding the efficacy of other *Trichoderma* species in this context (Ding et al. 2025). These gaps are particularly notable with respect to the performance

of native species against different races of *C. lindemuthianum*, especially under foliar application conditions. This is a crucial point, as a large portion of commercially available products are formulated from *T. harzianum* strains, whose efficacy may be limited by the pathogen's genetic variability and regional edaphoclimatic conditions.

This research gap is further reflected in the current market landscape. In Brazil, data from the Ministry of Agriculture, Livestock, and Supply (*Ministério da Agricultura e Pecuária* – MAPA), obtained through the official Bioinsumos application, indicate that approximately 97 *Trichoderma*-based products are registered for use as microbiological fungicides targeting various diseases. However, not all of these products are intended for anthracnose control. Among those registered for managing this disease in crops such as common bean, only 21 contain microorganisms in their formulation, and just three of these have *Trichoderma* as the active ingredient and are recommended for foliar application (MAPA 2025; Sambuichi et al. 2024).

Addressing this critical need, the present study aimed to evaluate the efficacy of different *Trichoderma* strains in controlling *C. lindemuthianum*. Specifically, the research seeks to validate the potential of *Trichoderma* native strains for the sustainable management of anthracnose in common bean, thereby contributing to the advancement of agricultural solutions aligned with contemporary socio-environmental demands.

Materials and methods

Isolation, cultivation, and maintenance of *Trichoderma* fungal strains

Thirty-two *Trichoderma* spp. strains from the microorganism collection of the Applied Microbiology Laboratory of Unicentro (LMA), at Ambiotec–UNICENTRO, Paraná, Brazil, were utilized, all originally isolated from soil. Of these, nineteen strains were newly obtained in the present study from rhizosphere soil of *Araucaria angustifolia* collected in Forest Ombrophilous Mixed (FOM) remnants distributed across municipalities in the States of Paraná, Santa Catarina, and São Paulo (Fig. 1). The remaining thirteen isolates were also of soil origin but had been recovered in previous LMA–UNICENTRO studies from native forest environments and subsequently preserved in the laboratory's fungal culture collection.

A. angustifolia was selected as the sampling host because its rhizosphere harbors a highly diverse and functionally specialized microbial community adapted to the distinctive climatic and edaphic conditions of southern Brazil. This long-lived species establishes stable associations with

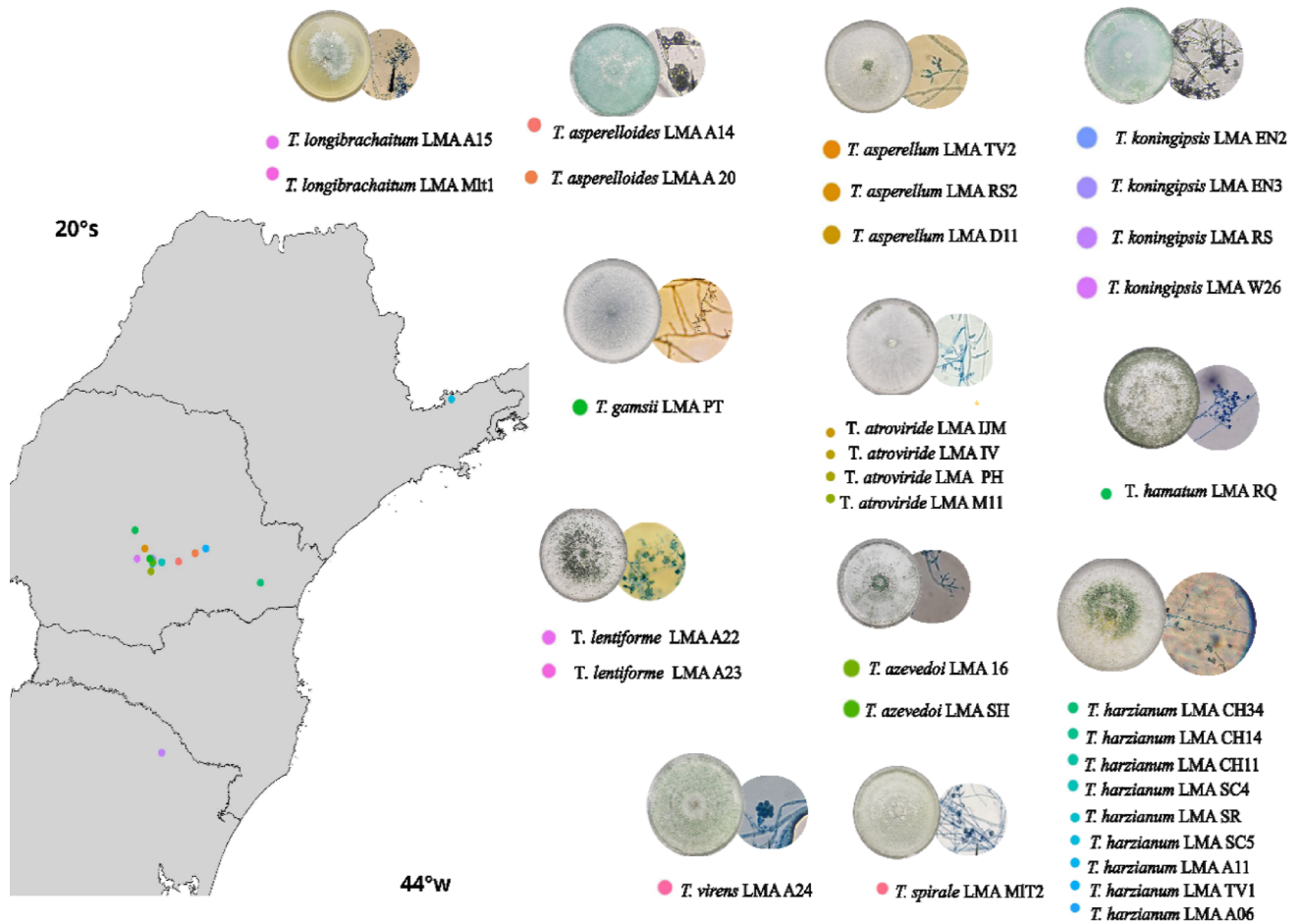


Fig. 1 Geographic regions for soil sampling near *Araucaria angustifolia* for the isolation of native *Trichoderma* spp. with biocontrol potential against *C. lindemuthianum*. The map includes state abbrevia-

tions (PR, SC, RS, SP) to indicate the sampling locations. The figure includes molecular identified *Trichoderma* strains and their respective morphological features

beneficial microorganisms (Souza et al. 2024; Zinn et al. 2024), making it a strategic model for bioprospecting functionally relevant fungal isolates aligned with the objectives of this study.

Soil samples were collected using sterile augers (0–10 cm depth), transported in insulated boxes, and stored at 4 °C until processing. Isolation was performed using serial dilutions plated on Martin medium supplemented with chloramphenicol (Martin 1950), followed by incubation in a Biochemical Oxygen Demand (BOD) chamber at 28 °C for 7–10 days. Typical colonies of the genus were transferred to Sabouraud agar to confirm purity. Subsequently, single-spore cultures were obtained on 2% (w/v) water agar to ensure morphological homogeneity prior to characterization.

Macroscopic traits (colony color, texture, pigment production, and concentric ring formation) were evaluated on potato dextrose agar (PDA) after 5–7 days of incubation at 28 ± 2 °C, following Siddiquee (2017). Microscopic characterization was performed under light microscopy at 400× magnification, assessing the structure and arrangement of

conidiophores, phialides, and conidia. Final morphological descriptions were compared with reference keys for *Trichoderma* species. All strains were preserved using the Castellani method (Castellani 1963) on PDA.

DNA extraction followed the CTAB method (Doyle 1991) with minor modifications. Molecular identification was performed in a previous study, detailed in the Master's dissertation of Selestrino (2024), which included sequencing and phylogenetic analyses of the ITS, *tef1*, and *rpb2* gene regions. The resulting sequences were deposited in the NCBI GenBank database under accession numbers ITS (PX590044–PX590074), *tef1* (PX578865–PX578899), and *rpb2* (PX578900–PX578918). These publicly available sequences allow independent verification of isolate identity.

Isolation and identification of the pathogen *C. lindemuthianum*

C. lindemuthianum strains used in this study included 17049_475 (LMA 475), obtained from the Paraná Rural

Development Institute (IDR-PR), and LMA_C25_73 (LMA C25), recovered from anthracnose-symptomatic bean pods collected in Ponta Grossa, Paraná, Brazil. Isolation was performed by excising lesions, surface-disinfecting the fragments, and cultivating them on PDA in a BOD incubator at 22 °C for 7–10 days.

Species identification was based on a polyphasic approach combining morphological, biological, and molecular analyses. Morphological identification of *C. lindemuthianum* relied on colony characteristics and microscopic features from 5-day-old cultures, including the formation of acervuli, presence and morphology of setae, and conidial shape and size. These traits were compared with reference descriptions in Sutton (1980), Ciampi-Guillardi et al. (2014), and Baroncelli et al. (2017). Pathogenicity assays on *Phaseolus vulgaris* confirmed the production of typical anthracnose lesions, supporting species-level identification. Internal transcribed spacer (ITS) sequencing was used to confirm genus placement.

Genomic DNA was extracted following the CTAB method (Doyle 1991) with minor modifications. Molecular amplification of the ITS rDNA region was performed using the primers ITS4 (5'-CTTGGTCATTTAGAGGAAGTA A-3') and ITS5 (5'-CTCACTAAGCCATTCAATCGG-3') (White et al. 1990). DNA was extracted from mycelium, purified, and sequenced using the Sanger chain-termination method. Sequence analyses were conducted in BioEdit, and species identity was confirmed through BLASTn comparison. The ITS sequences were deposited in GenBank under the accession numbers PX471960–PX471973.

General experimental design and replication strategy

Each assay was conducted with three independent biological replicates originating from separately handled experimental units. Technical replicates were not included because preliminary tests indicated minimal analytical variation, and the scale of the assays did not permit extensive technical repetition without compromising experimental uniformity.

Race characterization of *C. lindemuthianum*

The characterization of *C. lindemuthianum* races was performed using 12 differential cultivars as described by Pastor-Corrales (1995). Seedlings of common bean were inoculated with spore suspensions (10^6 conidia mL⁻¹) of isolates LMA C25 and LMA 475 and incubated in a B.O.D. chamber (SPLABOR SP-224/364) at 25 ± 2 °C with a controlled photoperiod for 7–10 days post-inoculation, when symptoms were assessed. Disease severity was scored on a

1–9 scale, in which scores of 1–3 indicated resistance and 4–9 indicated susceptibility.

Evaluation of antagonistic mechanisms of *Trichoderma* spp. against *C. lindemuthianum* in vitro

The antagonistic effect was evaluated using the paired culture method described by Dennis and Webster (1971). Discs of the pathogen (strains LMA 475 and LMA C25) and *Trichoderma* were placed on opposite sides of a 9-cm Petri dish, 7 cm apart, on PDA medium. The inhibition rate was calculated based on the colony diameters of the interacting organisms, according to the following equation:

$$\text{Inhibition rate} : \frac{R1 - R2}{R1} \times 100 \quad (1)$$

where R1 is the diameter of the control pathogen colony and R2 is the diameter of the pathogen colony in the presence of the antagonist. For inhibition assessments, the *C. lindemuthianum* strains LMA 475 and LMA C25 were used, with pathogenicity serving as the selection criterion.

The *Trichoderma* strains that exhibited the highest in vitro inhibitory effects in paired-culture assays were subsequently tested for antibiosis through the production of volatile and non-volatile metabolites, following the protocol of Dennis and Webster (1971). To evaluate the effects of volatile metabolites, discs of *C. lindemuthianum* were placed on one side of split PDA plates, while *Trichoderma* discs were positioned on the opposite side, allowing fungal inhibition mediated by volatile compounds to be assessed. For all analyses, a positive control (PC) consisting of 100% growth of *C. lindemuthianum* on PDA plates was included. Plates were incubated at 22–25 °C for 7–10 days.

For the non-volatile metabolite assay, filtrates were obtained from *Trichoderma* cultures grown in PDA for 7 days at 22–25 °C, passed through a 0.22 µm filter membrane, and incorporated into PDA at a proportion of 25% (v/v) filtrate to 75% (v/v) medium. The pathogen was then inoculated onto the surface of the treated medium, and colony growth was evaluated after 7–10 days of incubation in a BOD chamber at 22 °C.

Compatibility of *Trichoderma* with fungicides

The compatibility assay was performed using six *Trichoderma* isolates selected from the initial set of 32 strains based on their highest inhibitory performance in the preliminary in vitro assays. These isolates were grown on PDA supplemented with fungicide molecules. Fungicide stock solutions were first prepared at their recommended label concentrations and then incorporated into PDA at a proportion of 25%

(v/v) fungicide solution to 75% (v/v) medium. This mixing procedure resulted in a final concentration in the agar equivalent to the manufacturer's recommended dose, ensuring that the assay reflected the intended field-use concentration.

The selected fungicides correspond to active ingredients commonly used for anthracnose management in common bean crops in southern Brazil. The fungicides tested were identified by abbreviated commercial names followed by their active ingredients in parentheses: Zig (fluazinam), Uni (mancozeb), Til (propiconazole), Ap (thiophanate-methyl+fluazinam), Ell (azoxystrobin+benzovindiflupyr), Mer (fentin hydroxide), and Nat (trifloxystrobin+tebuconazole). All fungicides were applied at their respective label-recommended doses, and a control treatment without fungicide was also included. Discs (5 mm) of *Trichoderma* were incubated for 10 days at 25 °C, and mycelial growth was measured to calculate the percentage of growth inhibition for each fungicide. Petri dishes containing only PDA medium were inoculated with each strain and served as the control. The experimental design followed a completely randomized arrangement with four replications.

Competitiveness of *Trichoderma* strains in soil

The evaluation of soil competitiveness was performed using a modified agar disc diffusion method (Bauer et al. 1966), in which mycelial discs of *Trichoderma* were placed onto water-agar plates supplemented with soil. For this purpose, 5 g of soil from a cultivated area located within the experimental fields of the Agrarian Foundation for Agricultural Research (FAPA), in Guarapuava, Paraná, were used per Petri dish. Soil samples were homogenized separately with 2% (w/v) water agar and poured into Petri plates. After solidification, two 8-mm disks containing *Trichoderma* propagules, obtained from 5-day-old colonies grown on PDA at 25 °C, were placed on the agar surface. Plates were covered but not hermetically sealed to allow adequate gas exchange during incubation at 25 °C for five days.

Radial growth was measured from the edge of the inoculation disc to the outermost point of mycelial expansion along two perpendicular axes, and the mean radial growth was calculated. Water agar was used as a nutrient-poor medium to mimic soil-like conditions and evaluate competitive growth under nutrient limitation, whereas PDA served as a nutrient-rich control to determine the intrinsic growth potential of each isolate. Relative growth (%) was then calculated using the formula:

$$\text{Relative growth (\%)} = \left(\frac{\text{growth on water agar}}{\text{growth on PDA}} \right) \times 100,$$

which enabled direct comparison between nutrient-poor and nutrient-rich conditions.

Evaluation of anthracnose incidence in bean pods

Anthracnose incidence in bean pods was evaluated using the filter paper method adapted for Gerbox containers (MAPA 2009). Pods were disinfected with 1% sodium hypochlorite for 3 min, rinsed with sterile water, and placed in acrylic Gerbox containers containing moistened filter paper. Following disinfection, pods were inoculated with *C. lindemuthianum* using a manual sprayer (0.1 mL of a 10^6 spores mL⁻¹ suspension). After 48 h, *Trichoderma* spore suspensions (10^6 spores mL⁻¹) were applied with a manual sprayer calibrated to deliver 0.1 mL per pod. Two controls were included: a positive control (PC), consisting of pods inoculated with *C. lindemuthianum* LMA 475 but without *Trichoderma* application, and a commercial standard (*T. harzianum* ECO) applied at the same concentration as the other strains. Four pods were placed per container and incubated at 22 °C for seven days in the dark. Disease incidence was evaluated using a stereomicroscope, considering pods with typical anthracnose lesions confirmed by microscopy, and expressed as a percentage. The experimental design followed a completely randomized arrangement.

Detached leaf experiment

In the detached leaf experiment, trifoliate leaves of the cultivar IPR-Uirapuru were collected from 10-12-day-old seedlings at the first trifoliate stage and sprayed with treatments: (i) *Trichoderma* strains (as previously described); (ii) a positive control (leaves sprayed with distilled water and subsequently inoculated only with *C. lindemuthianum* LMA 475, designed as PC); (iii) a chemical control (fentin hydroxide - MPQ) and (iv) an untreated control (leaves sprayed with water and not inoculated with *C. lindemuthianum*). The leaves were incubated in gerbox containers at 20 °C with a 12-h photoperiod in a BOD chamber.

After 72 h, a suspension of *C. lindemuthianum* conidia (3.2×10^6 conidia mL⁻¹) was applied to the respective treatments, delivering 0.1 mL per leaf. Anthracnose severity was evaluated every two days over 24 days using the area under the disease progress curve (AUDPC, Shaner and Finney 1977). The experimental design followed a completely randomized arrangement.

Bean seed germination test

The paper roll incubation method (MAPA, 2009) was used to evaluate seed emergence, health, incidence of *C. lindemuthianum*, and the antagonistic capacity of *Trichoderma* strains in two common bean cultivars (IPR-Uirapuru and IAC-Veloz), under laboratory conditions. Seeds were inoculated with the following treatments: *T. koningiopsis* LMA

RS, *T. asperellum* LMA RS2, *T. harzianum* ECO (commercial standard), *T. hamatum* LMA RQ, *T. harzianum* LMA A06', *T. atroviride* LMA PH, and an untreated control (T0), consisting of seeds inoculated with *C. lindemuthianum* but without *Trichoderma* application. All treatments and the control were applied via spray, using a total of 10 mL of a spore suspension (3.2×10^6 conidia mL⁻¹) per paper roll. The experimental design was completely randomized, with six treatments (selected based on their highest inhibition activity in previous tests) and four replicates, with each roll containing of one paper roll containing 50 seeds.

After treatment, seeds were individually placed on two sterilized germination paper sheets (28 × 38 cm), moistened with autoclaved distilled water at 2.5 times the paper weight. After seed distribution, a third sheet was placed on top to cover them, forming a roll secured with an elastic band. The rolls were incubated in a BOD germination chamber at 20 °C ± 2, in the dark and under near-saturation humidity, for seven days. A total of 200 seeds were used per treatment, divided into four replicates of 50.

Evaluation was performed by individually examining all seeds, removing their tegument, and counting those exhibiting typical *C. lindemuthianum* infection symptoms (MAPA, 2009). Identification was conducted visually with the aid of tweezers, and incidence was expressed as a percentage. Seedling classification followed the International Rules for Seed Testing (ISTA 2023). Seedlings were considered normal when they exhibited a fully developed primary root, intact hypocotyl and epicotyl, well-formed cotyledons, and no necrosis, deformation, or pathogen-induced lesions. Only seedlings with structural integrity and the potential for continued healthy development were classified as normal.

Statistical analysis

All values are expressed as mean ± standard deviation (SD). All statistical analyses were conducted in R (version 4.3.1) using RStudio (version 2025.09.2). An analysis of variance (ANOVA) was first performed, and when significant, treatment means were grouped using the Scott–Knott test implemented in the 'ScottKnott' package (version 1.3.5). The R packages used included *openxlsx* for spreadsheet manipulation, *ggplot2* for data visualization, *dplyr* for data handling, and *patchwork* for combining graphs.

To support the selection of *Trichoderma* strains for in vivo assays, inhibition variables obtained from in vitro experiments were integrated: CPTI (dual culture inhibition), CVTI (volatile compounds), CNVTI (non-volatile compounds), CRESCf (growth in fungicides), and CRESCS (competitiveness in soil). These parameters represent complementary mechanisms relevant to biological control.

A Euclidean distance matrix was computed based on the quantitative responses of each strain, followed by hierarchical clustering using the UPGMA method. This approach follows biological clustering principles in which similarity among strains is inferred from their quantitative inhibition profiles (Grishin and Grishin 2002). Because all inhibition variables were expressed on the same scale (%), no data normalization was required prior to clustering.

To visualize overall performance and cluster relationships, a heatmap was generated representing the standardized values of each variable across strains. Color gradients illustrated the relative magnitude of inhibition or growth responses, enabling intuitive identification of high- and low-performance patterns. Cluster boundaries were overlaid on the heatmap to highlight groups exhibiting similar multitrait behavior.

This multivariate clustering and heatmap visualization strategy follows established approaches used for the integrative selection of promising biocontrol agents (Murtagh and Legendre 2014). Final strain selection for greenhouse evaluation was based on the high-performance cluster, prioritizing strains consistently exhibiting superior inhibition and competitiveness across variables.

Results

In vitro studies

Screening of *Trichoderma* strains for the control of *C. lindemuthianum*

To identify strains with the highest antagonistic capacity against *C. lindemuthianum*, 31 *Trichoderma* strains were evaluated, comprising both isolates from the LMA filamentous fungi collection and the 19 newly prospected strains obtained in this study. All strains are presented in Fig. 1, which provides their morphological characteristics, collection sites, and molecular identification, as reported by Selestrino (2024).

Evaluation of the antagonistic capacity of *Trichoderma* against *C. lindemuthianum* in vitro

Dual culture assay

All *Trichoderma* strains inhibited the growth of both *C. lindemuthianum* races evaluated (LMA 475 and LMA C25), and inhibition rates differed significantly among treatments ($p < 0.005$) (Table SI.1, Supplementary Information). For *C. lindemuthianum* LMA 475, *T. asperelloides* LMA A20 achieved an inhibition rate of $98.0 \pm 0.2\%$. For

C. lindemuthianum LMA C25, *T. koningiopsis* LMA EN3 achieved $93.0 \pm 3.7\%$, and *T. asperellum* LMA TV2 showed $91.0 \pm 2.1\%$ inhibition.

The lowest inhibition rates against *C. lindemuthianum* LMA 475 were observed for *T. atroviride* LMA IJM ($45.0 \pm 2.5\%$) and *T. spirale* LMA Mlt2 ($65.0 \pm 1.2\%$). For the pathogen LMA C25, the lowest inhibition rate was recorded for *T. spirale* LMA Mlt2 ($57.0 \pm 1.9\%$), although isolates like *T. atroviride* LMA M11 ($55.0 \pm 2.9\%$) and *T. azevedoi* LMA SH ($59.0 \pm 0.0\%$) were grouped within the same statistical category.

Production of volatile organic compounds (VOCs)

Trichoderma strains exhibiting inhibition rates above 60% in dual-culture assays were selected for evaluating volatile metabolite activity. Of the 32 initial strains, 26 met this criterion and were subsequently included in the assays for volatile and non-volatile metabolite production. Statistical analysis revealed a significant effect on inhibition rates against both *C. lindemuthianum* races (LMA 475 and LMA C25; $p=0.005$) (Table SI.2). For race LMA 475, inhibition by volatile metabolites ranged from $91.0 \pm 0.5\%$ to $65.0 \pm 0.0\%$, with *T. harzianum* LMA SC5, *T. lentiforme* LMA A22, *T. harzianum* LMA SC4, and *T. harzianum* A06' presenting the highest means according to the Scott–Knott test. In contrast, *T. asperelloides* LMA A20 ($69.0 \pm 3.7\%$), *T. asperelloides* LMA A14 ($68.0 \pm 1.0\%$), and *T. harzianum* ECO ($65.0 \pm 0.0\%$) exhibited the lowest inhibition levels. For the LMA C25 race, inhibition ranged from $90.0 \pm 1.8\%$ to $37.0 \pm 2.1\%$, with *T. lentiforme* LMA A23 showing the highest activity, whereas *T. azevedoi* LMA SH demonstrated the lowest inhibition rate.

Evaluation of inhibitory capacity by non-volatile organic compounds (N-VOCs)

To evaluate the inhibitory capacity by non-volatile organic compounds (N-VOCs), strains with inhibition rates greater than 60% in volatile compound production (VOC) were selected. The percentages of *C. lindemuthianum* (LMA 475 and LMA C25) growth inhibition by *Trichoderma* N-VOCs are presented in Fig. 2a and b. Analysis of variance revealed significant differences among the *Trichoderma* strains in N-VOC production ($p<0.0001$). Among the strains tested, those achieving complete inhibition (100%) through N-VOC production were *T. azevedoi* LMA SH, *T. atroviride* LMA PH, *T. hamatum* LMA RQ, and *T. harzianum* LMA A06, in addition to the commercial standard *T. harzianum* ECO. In contrast, *T. harzianum* LMA TV1 exhibited the lowest inhibition rate (72%).

Trichoderma strains exhibited distinct antagonistic mechanisms in vitro. In the dual-culture assay, the strains predominantly displayed mycoparasitism, evidenced by the overgrowth of *Trichoderma* mycelium over the *C. lindemuthianum* colony (Fig. 3a).

The antagonists showed potential mechanisms of pathogen suppression, including antibiosis and competitive inhibition (Fig. 3b–e). Strains including *T. lixii* LMA SR, *T. atroviride* LMA IJM, *T. harzianum* LMA CH14, *T. asperelloides* LMA D11, *T. harzianum* LMA SC4 and LMA SC5, *T. lentiforme* LMA A22, and *T. longibrachiatum* LMA Mlt1 exhibited potential antibiosis against *C. lindemuthianum* LMA 475 (Fig. 3b). For isolate LMA C25, the interaction pattern suggested competitive inhibition rather than antibiosis or mycoparasitism (Fig. 3c). *T. virens* LMA A24, *T. asperelloides* LMA A14, *T. asperellum* LMA RS2, and *T. harzianum* ECO also showed potential combined competition and antibiosis mechanisms.

Compatibility of *Trichoderma* strains with fungicides

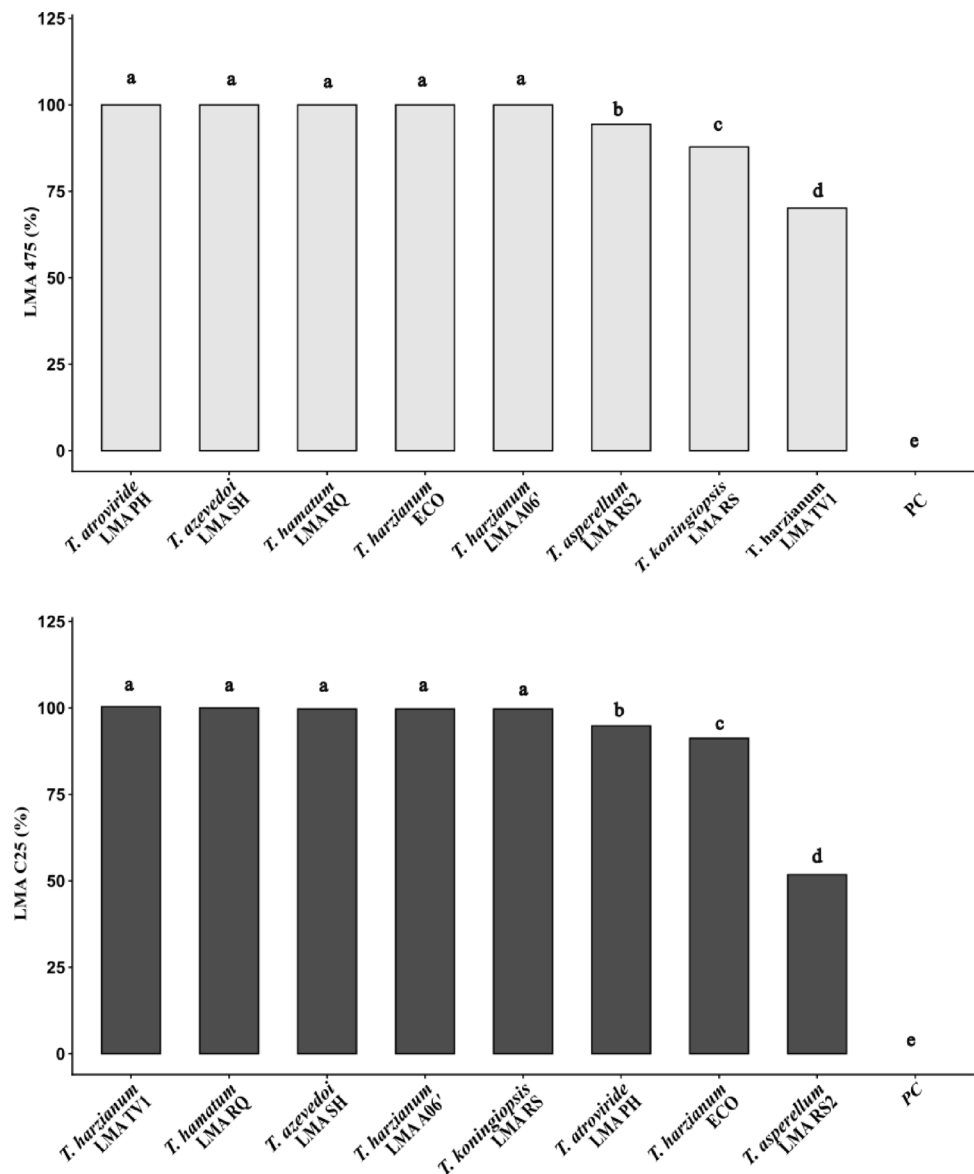
The compatibility of *Trichoderma* strains with fungicides applied at recommended doses for anthracnose management in common bean was evaluated. Only strains exhibiting more than 60% growth on the plate were included in the statistical analysis. Analysis of variance revealed statistically significant differences in *Trichoderma* growth in the presence of fungicides ($p<0.001$), and a significant fungicide $\times$ strain interaction ($p\text{-value}=0.0019$), indicating a differential responses among strains.

As illustrated in Fig. 4, *T. atroviride* LMA PH and *T. koningiopsis* LMA RS exhibited the highest growth rates, maintaining over 80% mycelial growth in the presence of propiconazole, fluazinam, and azoxystrobin+benzovindiflupyr. *T. hamatum* LMA RQ also demonstrated strong performance under chemical exposure, with growth rates above 70% in the presence of fluazinam, trifloxystrobin+tebuconazole, and mancozeb. In contrast, *T. harzianum* ECO showed markedly reduced growth, being completely or severely inhibited, especially under exposure to propiconazole, trifloxystrobin+tebuconazole, and fluazinam.

Competitiveness of *Trichoderma* strains in soil

The establishment and competitive capacity in soil of previously selected antagonists were evaluated (Fig. 5). The isolates *T. asperellum* LMA RS2 and *T. atroviride* LMA PH maintained high growth rates in the soil-water-agar medium ($\approx 85\%$). Their relative growth did not differ statistically from its growth on PDA ($p>0.05$), demonstrating robust competitive ability even under limited nutrient availability. The commercial standard, *T. harzianum* ECO, achieved

Fig. 2 Antagonistic potential of *Trichoderma* spp. against *C. lindemuthianum* strains LMA 475 and LMA C25 based on the production of non-volatile organic compounds (N-VOCs). Inhibition rates (%) were measured in vitro using culture filtrates incorporated into PDA medium. Different letters indicate statistically distinct groups ($p < 0.05$) according to the Scott–Knott test. Analyses were performed with three independent biological replicates per treatment



approximately 76%, similar to *T. azevedoi* LMA SH, with no significant differences between the two strains. Conversely, *T. harzianum* LMA TV1 exhibited relatively low growth rates, yet still performed better than the untreated control (PC).

Selection of *Trichoderma* strains for in vivo experiment.

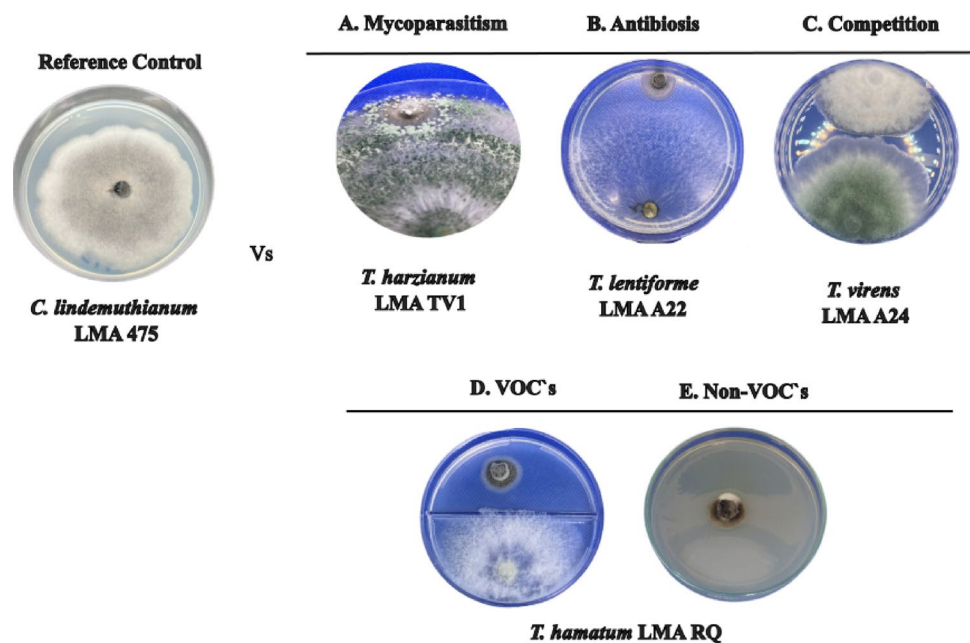
The performance of *Trichoderma* strains across the different *vitro* assays was integrated through a heatmap-based clustering analysis to identify consistent multitrait profiles (Fig. 6). The heatmap revealed three distinct performance clusters. Cluster 1 grouped the strains with the highest antifungal and competitiveness indices across all variables, exhibiting strong inhibition in dual and volatile assays, resilience to fungicides, and superior soil competitiveness. This cluster included *T. asperellum* LMA RS2, *T. atroviride* LMA PH, *T. azevedoi* LMA A16, *T. harzianum*

LMA A06, *T. hamatum* LMA RQ, and the commercial standard *T. harzianum* ECO. Cluster 2 contained *T. koningiopsis* LMA RS, which showed intermediate and variable inhibition responses across assays. Cluster 3 corresponded to the untreated control (T0), displaying low or negligible activity across all variables.

Color gradients in the heatmap enable immediate visualization of performance consistency, confirming that Cluster 1 strains maintained high responses across independent assays. To further illustrate strain proximity and group structure, a complementary Euclidean dendrogram and the full multivariate visualization panel are provided in the Supplementary Material (Fig. SI.3 A and SI.3B).

This integrative clustering pattern supported the selection of Cluster 1 strains for greenhouse evaluation, aligning with multitrait approaches previously applied in biocontrol

Fig. 3 Representative antagonistic mechanisms of *Trichoderma* spp. against *Colletotrichum lindemuthianum* observed in vitro: **a** mycoparasitism, **b** antibiosis, **c** competitive interaction, **d** inhibition by volatile organic compounds (VOCs), and **e** inhibition by non-volatile organic compounds (N-VOCs). The reference control (*C. lindemuthianum* LMA 475) is shown in the left panel. In each plate, the pathogen is positioned at the top and the *Trichoderma* at the bottom, illustrating the different antagonistic strategies expressed by the isolates



agent screening (Carrero-Carrón et al. 2016; Grishin and Grishin 2002).

In vivo experiments

Potential of *Trichoderma* in reducing anthracnose incidence in bean pods

Trichoderma strains were evaluated for their effectiveness in reducing anthracnose incidence in bean pods across different spore-suspension concentrations (10^4 , 10^6 , and 10^8 spores mL^{-1}). Analysis of variance revealed a statistically significant effect of the treatments on reducing disease incidence ($p < 0.0001$) compared to the positive control (PC). As shown in Fig. 7, all *Trichoderma* treatments, regardless of strain or concentration, significantly reduced disease incidence, demonstrating efficacy comparable to the commercial product *T. harzianum* ECO. Furthermore, according to the Scott-Knott test, all *Trichoderma* strains exhibited similar levels of disease reduction.

Evaluation of AUDPC in detached leaf experiment

The positive control (PC) exhibited the highest AUDPC value, indicating the greatest disease severity (Fig. 8). Among the *Trichoderma* strains, treatments with *T. harzianum* ECO and *T. atroviride* LMA PH also resulted in high AUDPC values, showing limited effectiveness comparable to the control. In contrast, the most effective disease suppression was observed with *T. azevedoi* LMA SH, *T. koningiopsis* LMA RS, *T. asperellum* LMA RS2, *T. harzianum* LMA A06, and *T. hamatum* LMA RQ. These strains yielded the

lowest AUDPC values, significantly reducing anthracnose severity compared to the positive control (PC), chemical treatment (MPQ), and other *Trichoderma* strains, thereby demonstrating superior efficacy in disease suppression.

Evaluation of germination and potential to reduce anthracnose incidence in bean seeds treated with *Trichoderma* spp

For the IPR-Uirapuru cultivar, *T. asperellum* LMA RS2, *T. hamatum* LMA RQ, *T. harzianum* ECO, and *T. koningiopsis* LMA RS were the most effective treatments in promoting seedling emergence, each reaching 100% or near-maximum emergence rates (Table 1). *T. hamatum* LMA RQ completely suppressed disease incidence (0.0%) and produced 100% normal seedlings, demonstrating superior disease control. *T. harzianum* ECO and *T. asperellum* LMA RS2 also resulted in low disease incidence (1.0% and 15.0%, respectively) and high percentages of normal seedlings compared with the control (97.0% and 85.0%, respectively) ($p < 0.05$).

For the IAC-Veloz cultivar, *T. koningiopsis* LMA RS and *T. harzianum* ECO showed the greatest reduction in disease incidence, with values of 0.0% and 1.0%, respectively, both of which significantly outperformed the control treatment (values shown in Table 1). Both strains also resulted in high seedling emergence (99.0%) and 99.0% normal seedlings, significantly outperforming the control treatment ($p < 0.05$). Other treatments, including *T. asperellum* LMA RS2, *T. atroviride* LMA PH, and *T. hamatum* LMA RQ, also performed significantly better than the control ($p < 0.05$), achieving 100% emergence and high proportions of normal seedlings (91–97%).

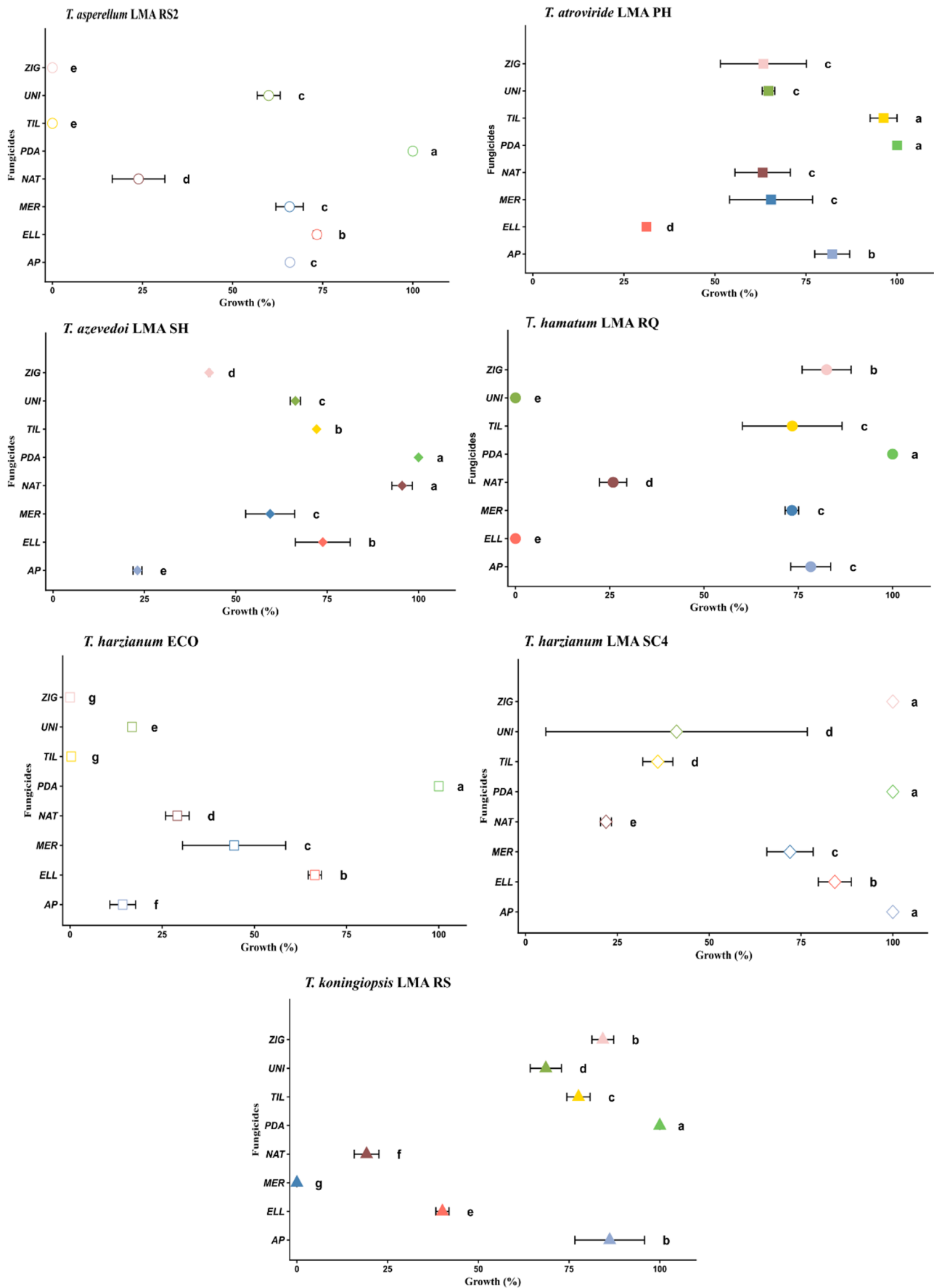


Fig. 4 Growth of *Trichoderma* strains in the presence of fungicides commonly used to manage anthracnose in common bean (*Phaseolus vulgaris*) cultivation in southern Brazil. Fungicide abbreviations: *Til* propiconazole, *Zig* fluazinam, *Uni* mancozeb, *Aph* thiophanate-methyl + fluazinam, *Ell* azoxystrobin + benzovindiflupyr, *Mer* fenton hydroxide, *Nat* trifloxystrobin + tebuconazole, *PDA* control. Different letters indicate statistically distinct groups ($p < 0.05$) according to the Scott–Knott test. Analyses were performed with three independent biological replicates per treatment

Discussion

The selection of biocontrol-effective *Trichoderma* spp. strains revealed distinct antagonistic profiles against *C. lindemuthianum*, with mycoparasitism and metabolite production emerging as key mechanisms that varied among species and experimental conditions. Among the antagonistic mechanisms observed, mycoparasitism predominated in strains like *T. asperelloides* LMA A20 and *T. harzianum* TV2. This finding aligns with Christmann et al. (2019), Gutiérrez-Moreno et al. (2021) and Poletto et al. (2024), who also reported *Trichoderma* spp. as highly effective in inhibiting the mycelial growth of *Colletotrichum* species through mycoparasitic mechanisms.

Mycoparasitism involves recognition, colonization, and enzymatic degradation of the pathogen's hyphae, ultimately leading to its suppression (Adas et al. 2024; Atanasova et al. 2013). Enzymes such as chitinases and cellulases produced by *Trichoderma* spp. play a significant role in this process by degrading the structural components of the pathogen's cell wall (Ghasemi et al. 2020). Notably, mycoparasitism is often complemented by competitive interactions, in which rapid mycelial growth and substrate colonization restrict the pathogen's access to nutrients and space (Collinge et al. 2022). In the present study, *T. asperellum* LMA RS2 and *T. atroviride* LMA PH demonstrated substrate colonization, indicating strong competitive and mycoparasitic potential (Table SI.1). Recent transcriptomic analyses have further reinforced the mechanistic basis of this behavior: *T. asperellum* and *T. atroviride* exhibit abundant expression of genes associated with mycoparasitism when interacting with phytopathogens (Atanasova et al. 2023; Ji et al. 2019).

In our study, mycoparasitism was inferred from the interaction patterns observed in dual-culture assays, including overgrowth and contact-associated growth behavior, which are widely recognized as indicators of parasitic activity by *Trichoderma* spp. against phytopathogenic fungi (Harman et al. 2004). Although direct microscopic evidence of hyphal degradation was not obtained, these interaction features are consistent with classical descriptions of mycoparasitic mechanisms, such as hyphal recognition, coiling, and enzymatic degradation reported in the literature (Atanasova et al. 2013; de Azevedo Silva et al. 2021; Ghasemi et al. 2020).

Another crucial mechanism involved in biocontrol is antibiosis, characterized by the production of secondary metabolites that directly inhibit pathogen development (de Medeiros et al. 2021). In this study, all tested strains produced VOCs, as indicated by the inhibition zones observed, which significantly inhibited *C. Lindemuthianum*. This finding is consistent with previous reports on the effectiveness of *T. atroviride* and *T. longibrachiatum* in controlling *Colletotrichum* spp. via volatile-mediated antibiosis (Ferreira et al. 2020; Kim et al. 2023; Stracquadanio et al. 2020). *Trichoderma* spp. are known to synthesize a diverse array of bioactive compounds, including gliotoxin, viridin, and trichodermin, as well as lytic enzymes that disrupt cell wall integrity, induce hyphal distortion, and inhibit spore germination (Awad et al. 2018). Among these compounds, VOCs have been shown to inhibit pathogen mycelial growth at a distance. Common VOCs include 6-pentyl-2-pyrene, acetoin, 2-methyl-1-propanol, and 3-methyl-1-butanol, many of which exhibit strong antimicrobial activity even at low concentrations by disrupting pathogen cell membrane integrity or inhibiting key metabolic enzymes (Elsherbiny et al. 2020). In addition, *Trichoderma* can emit gases such as ethylene and hydrogen cyanide, further enhancing antagonistic interactions (Salazar-Magallón et al. 2024).

N-VOCs also contribute to biocontrol, including organic acids, furan derivatives, lipids, and antibiotics such as gliotoxin and viridin (Salazar-Magallón et al. 2024). The antimicrobial activity of these metabolites includes suppression of spore germination, hyphal malformation, and degradation of pathogen structures (Dennis and Webster 1971). The effectiveness of these mechanisms under field conditions, however, depends on ecological traits such as environmental adaptability and the capacity of strains to deploy multiple antagonistic strategies (Sharma et al. 2022). The production of volatile (VOCs) and non-volatile metabolites associated with antibiosis has been reported as an important mechanism in *Trichoderma*-mediated inhibition of *Colletotrichum* species (Abdulkadir et al. 2022; Morais et al. 2022).

Comparative screens of native isolates have reported marked differences in whether mycoparasitism, competition or antibiosis predominates, depending on strain and assay conditions (Poletto et al. 2024). Such variability highlights that antagonistic mechanisms are not uniform across *Trichoderma* species or even among isolates of the same species. Taken together, these mechanisms form the basis of in vitro evaluations and provide insight into each strain's mode of action.

The evaluation of *Trichoderma* strains for anthracnose suppression in common bean revealed promising results in both detached-leaf assays and in vivo experiments. Previous studies have demonstrated that *Trichoderma* species belonging to the Harzianum clade are effective in controlling

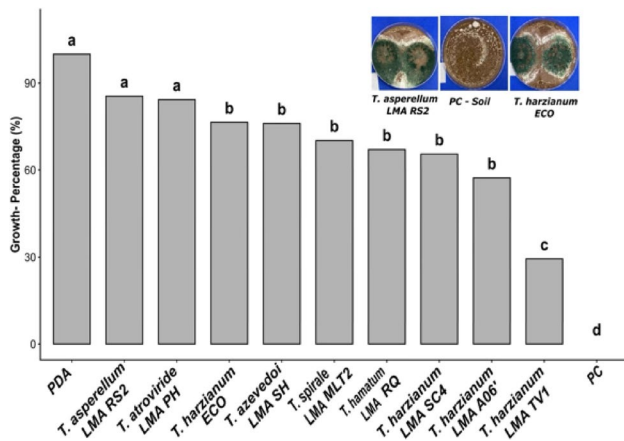


Fig. 5 Competitiveness of *Trichoderma* strains in soil, evaluated using a Petri plates assay. Strains were grown for five days at 25 °C on 2% water agar supplemented with soil. Relative growth (%) was calculated as (growth on water agar / growth on PDA × 100), allowing comparison of strain performance under nutrient-limited versus nutrient-rich conditions. Different letters indicate statistically distinct groups ($p < 0.05$) according to the Scott–Knott test. Analyses were performed with three independent biological replicates per treatment

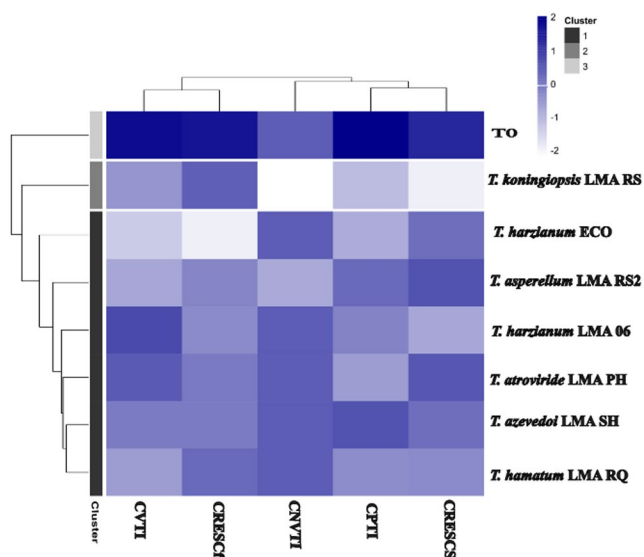


Fig. 6 Heatmap represents the performance of *Trichoderma* strains across multiple in vitro evaluations. Each cell indicates the standardized response value for a specific variable: CPTI – inhibition in dual culture; CVTI – inhibition by volatile compounds; CNVTI – inhibition by non-volatile compounds; CRESCf – growth in the presence of fungicides; and CRESCS – competitiveness in soil. Color intensity reflects relative performance (blue=low; yellow=high). Clustering was based on Euclidean distances among strains, using the UPGMA algorithm. Three main clusters were identified: Cluster 1 (high-performance group), Cluster 2 (intermediate performance), and Cluster 3 (low performance/control). The heatmap highlights consistent antifungal and growth-promoting behavior in Cluster 1 strains, which were subsequently selected for in vivo evaluation

fungal diseases across various crops, including anthracnose, both in vitro and in vivo (Conner et al. 2004; Mitrović et al. 2025; Padder and Sharma 2011). Notably, in the present study, *T. azevedoi* LMA SH and *T. koningiopsis* LMA RS significantly reduced disease severity, demonstrating superior efficacy compared to the standard chemical control (MPQ). *T. koningiopsis* has previously been reported as an effective biocontrol agent against other *Colletotrichum*-induced anthracnoses, showing the ability to reduce disease severity in chili pepper (Ruangwong et al. 2021). Given that *T. azevedoi* is a recently described species within the *Harzianum* clade, no published studies have yet evaluated its efficacy in reducing anthracnose incidence. Thus, the present findings provide one of the first indications that this species may possess significant biocontrol potential against anthracnose diseases.

Furthermore, *T. hamatum* LMA RQ and *T. asperellum* LMA RS2 resulted in the highest seedling emergence and vigor, reinforcing their potential as sustainable alternatives for disease management (Supplementary Table S1.1). While the effectiveness of *T. harzianum* and *T. asperellum* in anthracnose control is well documented (Cheruiyot 2023; Dailin et al. 2020), the outstanding performance of *T. hamatum* in this study represents a noteworthy advancement, given that this species has been comparatively underexplored as a biocontrol agent (Lodi et al. 2023).

In contrast, the commercial *T. harzianum* standard did not show superior performance compared to the untreated control under our experimental conditions. This outcome aligns with reports indicating that the efficacy of commercial *Trichoderma* formulations can vary depending on environmental conditions, pathogen strain specificity, application method, and host–microbe compatibility (Ceresini et al. 2024). Locally adapted strains often display enhanced performance due to greater ecological fitness and more efficient interactions with the target crop and soil microbiota (Woo et al. 2022), which may explain the superior outcomes obtained with the native strains evaluated in this study.

Interestingly, although all evaluated strains belong to the genus *Trichoderma*, they exhibited considerable variation in antagonistic potential, including among strains of the same species. This variability can be attributed to intra-specific genetic diversity (Singh et al. 2020), which can influence key functional traits such as metabolite profiles, enzyme activity, growth rate, and environmental adaptability (Burdon and Silk 1997). These findings underscore the importance of evaluating multiple strains during biocontrol screening rather than relying solely on species-level classification (Padder and Sharma 2011). Identifying strains with high sporulation, strong pathogen inhibition, and resilience to environmental stress is crucial for developing effective biofungicides.

Fig. 7 In vivo antagonistic activity of *Trichoderma* strains against anthracnose in bean pods at different spore concentrations (A: 10^4 , B: 10^6 , C: 10^8). Disease incidence was assessed after treatment and compared to the positive control (PC). Different letters indicate statistically distinct groups ($p < 0.05$) according to the Scott–Knott test. Analyses were performed with three independent biological replicates per treatment

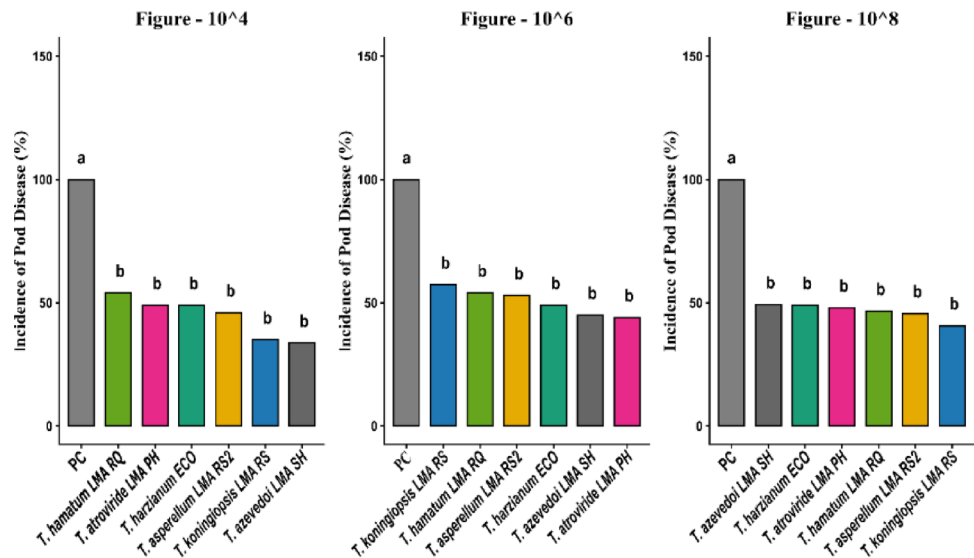
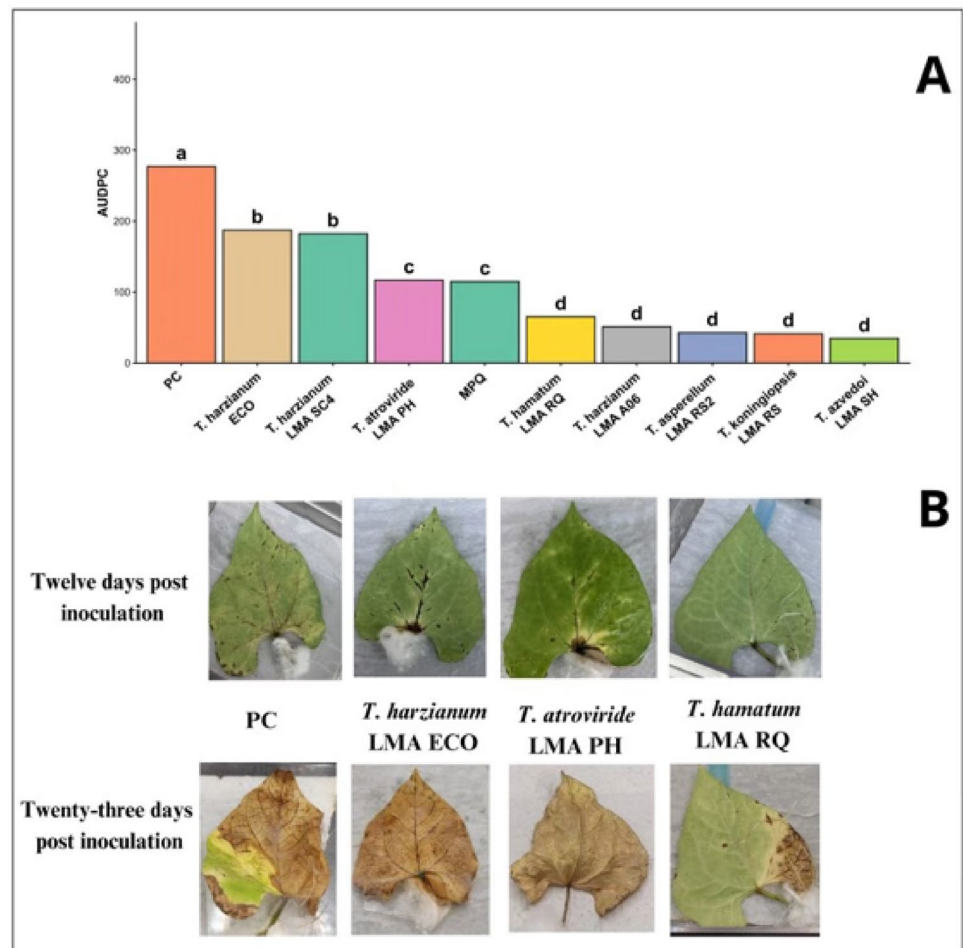


Fig. 8 In vivo antagonistic activity of *Trichoderma* strains against bean anthracnose caused by *C. lindemuthianum*. **A** Mean comparison of treatments based on disease severity (Scott–Knott test, $p < 0.05$); **B** Area under the disease progress curve (AUDPC) evaluated in the detached leaf assay. The positive control (PC) consisted of leads sprayed with distilled water but subsequently inoculated only with *C. lindemuthianum*. Different letters indicate statistically distinct groups ($p < 0.05$) according to the Scott–Knott test. Analyses were performed with three independent biological replicates per treatment



In vitro results demonstrated that *Trichoderma* strains were more effective in suppressing *C. lindemuthianum* race LMA 475 than LMA C25 (race 73), suggesting that the latter may be more aggressive or less susceptible to antagonistic activity. The differential efficacy observed among pathogen

racess aligns with the continuous emergence of new virulent races, a known trait of *C. lindemuthianum* (Nunes et al. 2021), driven by mutations, gene flow, and genetic recombination (Burdon and Silk 1997). Molecular analysis using ITS, ISSR and AFLP markers has revealed high genetic

Table 1 Evaluation of seed germination capacity and incidence of *C. lindemuthianum* in susceptible (IPR-Uirapuru) and tolerant (IAC-Veloz) common bean cultivars

IPR-Uirapuru			
Treatments	Incidence (%)	Emergence (%)	Normal seedlings (%)
PC (positive control)	100.0±0.0 ^a	91.0±1.4 ^a	0.0±0.0 ^b
<i>T. asperellum</i> LMA RS2	15.0±1.4 ^b	100.0±0.0 ^a	85.0±1.4 ^a
<i>T. atroviride</i> LMA PH	3.0±1.4 ^b	92.0±8.4 ^a	96.0±0.0 ^a
<i>T. hamatum</i> LMA RQ	0.0±0.0 ^b	100.0±0.0 ^a	100.0±0.0 ^a
<i>T. harzianum</i> ECO	1.0±1.4 ^b	96.0±0.0 ^a	97.0±1.4 ^a
<i>T. koningiopsis</i> LMA RS	0.0±0.0 ^b	97.0±1.4 ^a	3.0±1.4 ^b
T0 (untreated control)	100.0±0.0 ^a	5.0±1.4 ^b	0.0±0.0 ^b
IAC-Veloz			
Treatments	Incidence (%)	Emergence (%)	Normal seedlings (%)
PC (positive control)	100.0±0.0 ^a	90.0±0.0 ^b	14.0±1.98 ^b
<i>T. koningiopsis</i> LMA RS	0.0±0.0 ^b	99.0±1.4 ^a	99.0±1.4 ^a
<i>T. asperellum</i> LMA RS2	2.0±0.0 ^b	100.0±0.0 ^a	97.0±1.4 ^a
<i>T. atroviride</i> LMA PH	2.0±0.0 ^b	100.0±0.0 ^a	91.0±7.0 ^a
<i>T. hamatum</i> LMA RQ	3.0±1.4 ^b	100.0±0.0 ^a	97.0±1.4 ^a
<i>T. harzianum</i> ECO	1.0±1.4 ^b	99.0±1.4 ^a	99.0±1.4 ^a
T0 (untreated control)	100.0±0.0 ^a	5.0±4.2 ^b	0.0±0.0 ^b

Parameters assessed include disease incidence (%), seedling emergence (%), and percentage of normal seedlings (%). The positive control (PC) consisted of seeds sprayed with *C. lindemuthianum*, while the untreated control (T0) consisted of non-inoculated seeds, without either *C. lindemuthianum* or *Trichoderma* treatments. All values are expressed as mean±standard deviation (SD). Different letters indicate statistically distinct groups ($p<0.05$) according to the Scott–Knott test. Analyses were performed with three independent biological replicates per treatment

variability within *C. lindemuthianum* populations (Coêlho et al. 2016, 2020; Mahuku et al. 2002; Nunes et al. 2021), emphasizing the need for continuous race monitoring in major bean-producing regions such as Paraná. Understanding the predominant races in a given area is essential for selecting *Trichoderma* strains with the most effective and broad-spectrum antagonistic potential, thereby ensuring long-term biocontrol efficacy.

The efficacy of *Trichoderma* strains in controlling *C. lindemuthianum* can be significantly influenced by pathogen genetic variability, host cultivar, and its intrinsic resistance level (Pedro et al. 2012; Sinha et al. 2023; Wang et al. 2024). Different *Colletotrichum* strains can trigger distinct physiological responses in host plants, resulting in variations in defense signaling and recruitment of beneficial microorganisms (Zhu et al. 2024). Pathogen invasion can alter the expression of key defense-related enzymes such as peroxidase (POD), superoxide dismutase (SOD), and polyphenol oxidase (PPO), as well as signaling molecules like hydrogen peroxide (H₂O₂), nitric oxide (NO), jasmonic acid (JA), and salicylic acid (SA) (Sahu et al. 2022). These signals induce

the expression of pathogenesis-related proteins and modulate the rhizosphere microbiome, promoting the recruitment of beneficial microbes in response to biotic stress (Santoyo 2022).

Trichoderma exploits plant defense signaling to outcompete pathogens and induce systemic resistance, often providing more durable protection than chemical treatments (Zhu et al. 2024). However, studies have shown that different races of *C. lindemuthianum* exhibit variable susceptibility to the same *Trichoderma* strain, indicating that biocontrol efficacy is influenced by the genetic and biochemical compatibility between specific *Trichoderma* and *Colletotrichum* strains. Such variability can modulate or even impair the biocontrol mechanisms deployed by *Trichoderma*.

Our initial hypothesis — that *Trichoderma* strains would perform as effectively as chemical fungicides — was supported. In both in vitro and in vivo experiments, *T. hamatum* LMA RQ, *T. atroviride* LMA PH, and *T. asperellum* LMA RS2 performed as efficiently as the commercial biofungicide ECO and the chemical molecule Fentin hydroxide (MPQ), even when applied as simple spore suspensions without additives. These findings corroborate previous studies highlighting the versatility of *Trichoderma* as a biocontrol agent under various application strategies (Ashgar et al. 2024; Sameer 2019). Beyond the key characteristics already mentioned, effective biocontrol agents must also be compatible with fungicides commonly used to manage the target disease (Sameer 2019). Most *Trichoderma* strains demonstrated high compatibility with the tested fungicides at label-recommended concentrations. Although reduced compatibility was observed with a few specific active ingredients, these exceptions do not compromise the overall applicability of the strains. On the contrary, the consistency of their antagonistic performance reinforces their value as alternative or complementary tools in disease management. Altogether, the findings support the integration of these *Trichoderma* strains into biological control programs aimed at reducing chemical inputs.

Ultimately, validation under in vivo conditions is crucial for selecting biocontrol agents with real potential for managing anthracnose in beans. Comparative studies analyzing in vitro and in vivo performance against different races of *C. lindemuthianum* can further refine selection criteria and support future usage recommendations.

Conclusions

This study demonstrates the potential of *Trichoderma* strains from Araucaria Forest soils in southern Brazil as sustainable alternatives for managing common bean anthracnose. In vitro and in vivo assays revealed strains with distinct

mechanisms—mycoparasitism, antibiosis, and competition. Several strains, including *T. koningiopsis* LMA RS, *T. asperellum* LMA RS2, *T. hamatum* LMA RQ, and *T. atroviride* LMA PH, outperformed not only the chemical control but also the commercial biological product *T. harzianum* ECO, highlighting the potential of less commonly used *Trichoderma* species in anthracnose management. These findings support their incorporation into the development of bio-inputs for integrated disease management in bean cultivation. However, field and greenhouse trials are still required to validate their effectiveness under real cropping conditions.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s41348-026-01262-x>.

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