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Biocontrol assessment of *Trichoderma* species on tomato crops infested by *Curvularia Spicifera*: toward sustainable farming systems

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This study provides the first comprehensive evaluation of the efficacy of three *Trichoderma* species (*Trichoderma longibrachiatum*, *Trichoderma harzianum*, and *Trichoderma asperellum*) in controlling *Curvularia spicifera* on tomato plants under both *in vitro* and *in vivo* conditions. Laboratory-based experiments assays, including direct and indirect confrontation, application of culture filtrates, and inhibition of spore germination, demonstrated significant antagonistic activity by the *Trichoderma* species. These treatments markedly reduced the mycelial growth (<2.63 cm), mycelial growth rate (<1.28 mm/h), and spore germination (<0.40) of *C. spicifera*, with *T. longibrachiatum* exhibiting the strongest antagonistic effect. The efficacy of three *Trichoderma* spp. and salicylic acid was evaluated under greenhouse conditions. Greenhouse trials further confirmed that *T. longibrachiatum* (2.83) significantly reduced disease severity compared to the control inoculated with *C. spicifera* (5.50) at 90 days post-inoculation (dpi). Biochemical analysis revealed an increase in enzyme activity and total protein content in the leaves and roots of *Trichoderma*-treated plants, with values of 10.09 and 10.44 mg g^{-1}, respectively. These changes reflect an induced defense response. Specifically, *T. longibrachiatum* consistently induced higher activities of catalase (74.58 and 73.1 µmol H_2O_2 mg $protein^{-1}$, respectively), peroxidase (5.35 and 54.91 µmol mg^{-1} min^{-1}, respectively), ascorbate peroxidase (54.91 and 60.29 µmol mg^{-1} min^{-1}, respectively), and polyphenol oxidase (14.07 and 9.37 units mg^{-1} min^{-1}, respectively) in tomato leaves and roots at 90 dpi. Furthermore, *T. longibrachiatum* significantly enhanced chlorophyll content and other agronomic traits, including root and shoot biomass, fruit yield, and overall plant growth. These findings suggest that *T. longibrachiatum* is a promising biocontrol agent against *C. spicifera* in tomato plants, promoting both plant growth and the activation of defense mechanisms.

KEYWORDS

antifungal activity, biotic stress, biocontrol, *Solanum lycopersicum*, biostimulant

1 Introduction

Food security remains one of the most pressing challenges facing humanity today. According to the Food and Agriculture Organization (FAO), food security encompasses not only access to food but also its nutritional quality and safety. To address this challenge, ecological farming systems have gained increasing attention in recent years (Muhie, 2022). These systems integrate a range of environmentally sustainable agricultural practices, including the cultivation of high-yielding and stress-resistant crop varieties, integrated pest and disease management, the application of biological fertilizers, and other agroecological approaches that collectively support both productivity and food safety (Temirbekova et al., 2021; Rebouh et al., 2019; Kherif et al., 2021).

Among these practices, integrated pest and disease management (IPDM) is of particular importance, as it seeks to replace or reduce chemical inputs with effective biological alternatives (Deguine et al., 2021; Rebouh et al., 2020). However, the current efficacy of IPDM still requires substantial improvement to match the performance of conventional chemical-based methods (Williams et al., 2005). Therefore, the development and implementation of novel biological agents for the control of pests and diseases is both timely and of high scientific and practical relevance.

Tomato (*Solanum lycopersicum* L.) is among the most valuable crops worldwide, representing a plant of important nutritional and economic interest for agricultural systems (Simoglou et al., 2024). However, tomato crops are often threatened by a wide variety of fungal pathogens, among which one of the most aggressive agents is the fungus *C. spicifera*, causing leaf spot disease (Cui et al., 2020; Baral et al., 2022). This pathogen is highly virulent, causing foliar symptoms such as leaf spot and blight that result in significant yield losses, lower quality fruits, and higher production costs for farmers (Manzar et al., 2022; Rabaaoui et al., 2022). The impact of *C. spicifera* on plants is further enhanced by it's the preference for warm, and usually humid conditions, which are typically characteristic of many tomato-producing regions (Connally et al., 2022).

The chemical management of *C. spicifera* and similar phytopathogens has traditionally depended on the widespread use of synthetic fungicides. While these chemical agents can offer rapid and effective suppression of disease symptoms, their long-term application presents several critical challenges. Continuous and excessive use of fungicides contributes to environmental contamination, including the accumulation of toxic residues in soil and water bodies (Manjarres-Lopez et al., 2021), which disrupts ecological balance and negatively impacts soil microbiota (Wang et al., 2025). Furthermore, the selective pressure exerted by repeated fungicide applications accelerates the evolution of fungicide-resistant strains of pathogens, rendering these chemicals progressively less effective (Ishii, 2006). In addition to ecological concerns, there are increasing apprehensions regarding human health and the safety of non-target organisms exposed to fungicide residues through food chains or environmental contact (Tao et al., 2020; Pathak et al., 2022).

As a result of these growing concerns, biological control has emerged as a promising and sustainable alternative for plant disease management. Among various strategies, the use of antagonistic microorganisms such as *Trichoderma* spp., *Bacillus* spp., and *Pseudomonas fluorescens* has received particular attention due to their capacity to inhibit plant pathogens through mechanisms like mycoparasitism, competition, production of antimicrobial compounds, and induction of host plant resistance (Ojha and Chatterjee, 2011; Güçlü and Özer, 2022; Riera et al., 2023). These biocontrol agents offer a safer, more ecologically harmonious approach, aligning with the principles of integrated pest management and sustainable agriculture (Al-Shuaibi et al., 2024; Köhl et al., 2019).

Among these microorganisms, *Trichoderma* species have emerged as effective biocontrol agents against various tomato diseases, particularly those caused by soil-borne pathogens like *Fusarium oxysporum* f. sp. *lycopersici* (Jamil, 2021), *F. solani* (Shams et al., 2023), and *Phytophthora nicotianae* (Dini et al., 2021). It has been reported that species belonging to *Trichoderma* spp. employ various mechanisms for the biocontrol of plant pathogens, including competition for nutrients and space, production of antifungal metabolites, direct mycoparasitism, and the induction of systemic resistance in host plants (Behiry et al., 2023; Chávez-Avilés et al., 2024; Hernández et al., 2024; Huang et al., 2024). These attributes make *Trichoderma* spp. highly effective in controlling fungal pathogens, while promoting plant growth and enhancing overall crop health (Mahmoud et al., 2021). However, despite their potential for managing many plant diseases, the efficacy of *Trichoderma* spp. against *C. spicifera* has been scarcely explored in previous research. Furthermore, salicylic acid's effectiveness extends to direct antifungal actions, contributing to plant resistance against a spectrum of fungal pathogens. There is limited research investigating the efficacy of *Trichoderma* spp. against *C. spicifera* under *in vitro* conditions, and to date, no studies have evaluated their effectiveness under *in vivo* conditions (Rao et al., 2020). This knowledge gap need for focused research efforts to evaluate the potential of *Trichoderma* spp. as a biocontrol agent against this pathogen.

Given the demonstrated efficacy of *Trichoderma* spp. in controlling *C. spicifera* and other phytopathogens under in vitro conditions, we hypothesize that these species may also suppress *C. spicifera* under field conditions, while concurrently improving tomato yield and quality. Thus, the present study investigated the ability of *Trichoderma* spp. to control *C. spicifera* in tomato plants both in vitro and in vivo conditions, with the aim of developing an effective, sustainable, and ecologically safe approach for disease management. Additionally, the study determined the mode of action through which *Trichoderma* spp. antagonize *C. spicifera*, suppress disease, and promote plant growth, with a view to establishing a complete understanding of its biocontrol potentials. The successful application of *Trichoderma* spp. in controlling *C. spicifera* could contribute to improved tomato yields, improved fruit quality, and enhanced economic stability for farmers. The study finally stands in line with the rise of sustainable agriculture worldwide, offering durable practice to one of the big challenges in tomato cultivation while supporting more general objectives of food security and environmental conservation (Bouanaka et al., 2021; Dourou and La Porta, 2023; Ferreira et al., 2024). Therefore, the aim of this study was to evaluate the antagonistic and antifungal potential of three *Trichoderma* species against *Curvularia spicifera*, a pathogen associated with gray mold in tomato. In addition, the study assessed the effectiveness of *Trichoderma* spp. and salicylic acid under greenhouse conditions to manage disease severity and promote plant health.

2 Materials and methods

2.1 Fungal strains

Three *Trichoderma* species isolates, *T. longibrachiatum* (Tr1), *T. harzianum* (Tr2), and *T. asperellum* (Tr3), obtained from the Plant

Protection and Biological Sciences laboratory at the Regional Center of Agricultural Research of Sidi Bouzid, Tunisia. *Trichoderma* species were isolated previously from the rhizosphere of tomato plants. The three isolates (Tr1, Tr2, and Tr3) were submitted to GenBank and assigned under the accession numbers: OP799680, OP799678, OP799679, respectively (Hajji-Hedfi et al., 2023a).

The phytopathogen fungus, *C. spicifiera* was isolated from tomato fruits exhibiting symptoms of gray mold disease and maintained on Potato Dextrose Agar (PDA) medium at 25 ± 2°C for subsequent tests. Macroscopic and microscopic observation were performed to identify the fungi using a colony appearance and morphological keys as described by Ellis (1971) and Sivanesan (1987) for the *Curvularia* genus. Pure fungal cultures were preserved in 20% glycerol and then stored at −20°C.

2.2 Molecular identification

To confirm pathogen species identity, molecular identification was performed. DNA extraction was performed according to the method described by White et al. (1990), and using the universal primers ITS1 (5′-TCCGTAGGTGAACCT TGCGG-3′) and ITS4 (5′-TCC TCCGCTTATTGATATGC-3′). PCR cycling conditions were as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 1 min, and then a final extension at 72°C for 10 min, according to Glass and Donaldson (1995). All PCR products were separated by electrophoresis on a 1.5% agarose gel, stained with SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, United States), and visualized under UV illumination. Three positive amplified PCR products were excised from the gel, and subsequently purified and sequenced by Applied Biosystems (Bedford, MA, United States).

2.3 Sequence alignment and phylogenetic analysis

The obtained sequences of the pathogen were edited and quality checked by analyzing the chromatogram peaks using BioEdit 7.2.5 software (Hall, 1999). The identity and similarity of sequences were checked by Blast in the NCBI database (Madden et al., 1996), and then were aligned and compared with reference sequences, using the MEGA V.7 software (Kumar et al., 2016). Phylogenetic trees were constructed using a Maximum Likelihood (ML) method. Consensus sequence of the pathogen was deposited in GenBank under accession numbers: PQ892128.

2.4 *In vitro* evaluation of *Trichoderma* species against *Curvularia spicifera*

2.4.1 Antagonistic interaction

A dual culture assay on potato dextrose agar (PDA) plates was used to examine the antagonistic interaction between *Trichoderma* spp. and the *C. spicifera* pathogen. Two agar plugs, each with a diameter of 0.5 cm, were prepared: one containing *Trichoderma* spp. and the other containing the *C. spicifera* pathogen. Both plugs were taken from 4-day-old cultures. These plugs were placed on opposite sides of a single 9-cm diameter PDA plate, maintaining 2 cm from the plate edge toward the center for the antagonist plug and a distance of 5 cm between the two plugs. A control plate was included, containing only a PDA plug on one side and the *C. spicifera* plug on the opposite side (Hajji-Hedfi et al., 2023a).

Antagonistic interactions between *Trichoderma* spp. and *C. spicifera* were also investigated also using indirect confrontation. Disks (5 mm diameter) of both fungi were placed on separate Petri dishes containing PDA medium. The dishes were then superimposed, with *Trichoderma* spp. on the bottom and *C. spicifera* on the top. Parafilm was used to seal the junction between the dishes and prevent the loss of volatile compounds. A control plate was prepared with a blank PDA plug on one side and a *C. spicifera* plug on the other (Bouanaka et al., 2021). Three replicates (five plates/replicate) were conducted for each individual treatment, and the plates were incubated at 25 ± 2°C for 7 days.

2.4.2 Antifungal activity

The antifungal activity of Tr1, Tr2, and Tr3 filtrates against the mycelial growth of *C. spicifera* was assessed *in vitro* using a culture filtrate method. Mycelia plugs of *Trichoderma* spp. were cultured in potato dextrose broth (PDB) for 4 days, then filtered to obtain culture filtrates. These filtrates were incorporated into molten PDA medium at three concentrations (C1: 60%; C2: 80%; C3: 100%) and inoculated with *C. spicifera* (Hajji-Hedfi et al., 2023b).

Three replicates of each treatment were conducted, with each replicate containing five plates. All plates were maintained at 28 ± 2°C for a week. After incubation, the percentage of inhibition (PI) of *C. spicifera* radial growth was determined using the formula presented by Abdelmoteleb et al. (2023); as follows:

$$PI(\%) = (1 - C_n / C_0) \times 100$$

Where C_0 is the radial growth diameter of the pathogen, C_n is the pathogen colony's radial growth in the presence of the antagonist fungus.

2.4.3 Mycelial growth

Mycelial growth was also measured daily in cm, for 7 days post-incubation following Hajji-Hedfi et al. (2024). The mycelial growth rate (MGR) of *C. spicifera* was calculated using the formula reported by Hajji-Hedfi et al. (2023b) as follows:

$$MGR(mm/h) = [D_1 / Te_1] + [(D_2 - D_1) / Te_2] + [(D_3 - D_2) / Te_3] + \ldots [(D_n - D_n - 1) / Te_n]$$

The formula considers the radial growth diameter of the fungus over a period of 7 days (D) and the corresponding incubation time (Te). The MGR is determined by summing the incremental changes in diameter divided by the respective incubation time intervals.

A series of microtubes were prepared containing 200 µL of *Trichoderma* strains (10^6 spores/ml) and 200 µL of *C. spicifera* (10^6 spores/ml), suspended in 1 mL of sterile distilled water containing 5% glucose. The spore counts were standardized using a hemocytometer. These microtubes were incubated at 25°C for 24 h. After incubation, the inhibition of spore germination was assessed

microscopically using a Malassez cell. The number of germinated and non-germinated spores was recorded. The percentage of germinated spores (%SG) was calculated using the formula:

$$\%SG = \left((SG) / (SG + SNG) \right) x\, 100$$

Where SG is the number of germinated spores and SNG is the number of non-germinated spores (Benslim et al., 2016).

2.5 *In vivo*, greenhouse evaluation of *Trichoderma* spp. against *Curvularia spicifera*

2.5.1 Disease severity assessment

The experiment was conducted to investigate the potential of *Trichoderma* spp. to manage gray mold in tomato plants. Tomato seeds (cv. Firenze) were provided from a certified nursery. Thirty-day-old seedlings were treated by root-dipping in *Trichoderma* spp. (10^6 spores/ml) conidial suspensions for 30 min. Subsequently, the seedlings were inoculated with *C. spicifera* conidial suspension (10^6 spores/ml). The experiment included three replicates of 10 plants each. A randomized complete block design was used to evaluate the effects of three experimental treatments (Tr1, Tr2, and Tr3) and salicylic acid (by root-dipping) on plant response to *C. spicifera* infection. Each experimental block contained two control groups: a positive control inoculated only with *C. spicifera*, and a negative control treated with sterile distilled water. Seedlings were subjected to six treatments: T1 (Tr1 + *C. spicifera*), T2 (Tr2 + *C. spicifera*), T3 (Tr3 + *C. spicifera*), T4 (salicylic acid (SA 1%) + *C. spicifera*), T5 (*C. spicifera* only), and T6 (untreated). After treatment, plants were incubated in a greenhouse at 25°C for a duration of 90 days. To enhance data reliability, the entire experiment was replicated twice.

Disease assessment was conducted at 5, 10, 20, 30, 60, and 90 days after inoculation (dpi), utilizing a disease severity scale. A 0–6 scale was employed to evaluate fruit rot symptoms, as outlined by Hajji-Hedfi et al. (2023b). Scores on this scale corresponded to the extent of leaf surface covered by lesions: 0 (no lesions), 1 (1–5% leaf surface), 2 (6–10% leaf surface), 3 (11–20% leaf surface), 4 (21–35% leaf surface), 5 (36–50% leaf surface), and 6 (51–100% leaf surface) (AbdElfatah et al., 2021).

2.5.2 Enzymatic activities and defense marker

To investigate the biochemical effects of pre-treating tomato plants with *Trichoderma* spp. and salicylic acid, enzyme activities in root and leaf samples were measured. Five samples were collected per treatment and block at 7, 30, 60, and 90 days post-inoculation (dpi). Enzyme analyses included catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), polyphenol oxidase (PPO), and total protein content (TPC). Root and leaf samples were immediately flash-frozen in liquid nitrogen to prevent enzyme degradation. Subsequently, the samples were homogenized in a chilled phosphate buffer containing EDTA. The homogenate was centrifuged, and the supernatant was used for enzyme activity assays.

CAT activity was measured by monitoring the decrease in absorbance at 240 nm, following the method of Hajji-Hedfi et al. (2023a). POX activity was assayed using the protocol described by Reddy et al. (1995). APX activity was determined according to the method of Hajji-Hedfi et al. (2024). PPO activity was assessed based on the procedure outlined by Mayer et al. (1965), by measuring the increase in absorbance at 408 nm. TPC was quantified using the Bradford (1976) method.

Chlorophyll content was measured using a portable fluorometer (OS1p; NH 03051-United States). A Minolta SPAD-502 meter was used for non-destructive assessment of leaf chlorophyll content in tomato plants. This instrument measures the transmittance of the tomato leaf to determine the relative amount of chlorophyll present. The resulting dimensionless SPAD units are directly proportional to the chlorophyll content. Readings were recorded at 7, 30, 60, and 90 dpi, as detailed by Almansoori et al. (2021). Agronomic measurements, including fresh and dry weights of roots and aerial parts, as well as the number of fruits, leaves, flowers, and branches, were recorded, along with plant and root lengths, at 30, 60, and 90 dpi.

2.6 Statistical analyses

ANOVA one way was conducted in SPSS version 20.0 statistical software (SPSS, SAS Institute, United States) to assess differences among treatment groups. Normality and homogeneity assumptions were verified before proceeding. Duncan's Multiple Range Test was used to identify significant differences ($p \leq 0.05$) among treatment means. Post-hoc test allowed for detailed comparisons of treatment effects and identified variations in measured parameters across *Trichoderma* spp. and control groups.

3 Results

3.1 *In vitro*, antifungal activities of *Trichoderma* spp. against *Curvularia spicifera*

Over the seven assessment days, the growth of *C. spicifera* under direct confrontation with *Trichoderma* spp. remained lower compared to the control. The mycelial growth for the control was 5.34 cm, while that of Tr1/CS was 2.58 cm, Tr2/CS was 2.74 cm, and Tr3/CS was 2.94 cm at 7 days of incubation. Although all *Trichoderma* species inhibited *C. spicifera*, their strengths were not identical. *T. longibrachiatum* was the strongest inhibitor, resulting in the lowest *C. spicifera* growth across most incubation time (Figure 1a). Figure 1b presented the temporal variation of mycelial growth of *C. spicifera* during indirect confrontation with the three same species of *Trichoderma*. As observed in direct confrontation methods, the indirect one also reveals an inhibitory effect of *Trichoderma* spp. on *C. spicifera* growth, though their magnitude of inhibition from this approach seems a little reduced compared to the results shown in the direct method. Furthermore, *C. spicifera* growth remained generally lower in the presence of *T. longibrachiatum* (0.77–2.63 cm at J1 and J7, respectively) compared to the positive control (1.70–5.23 cm at J1 and J7, respectively) (Figure 1b).

Though all three species of *Trichoderma* are inhibiting growth of *C. spicifera* in comparison to positive control (2.58 mm/h),

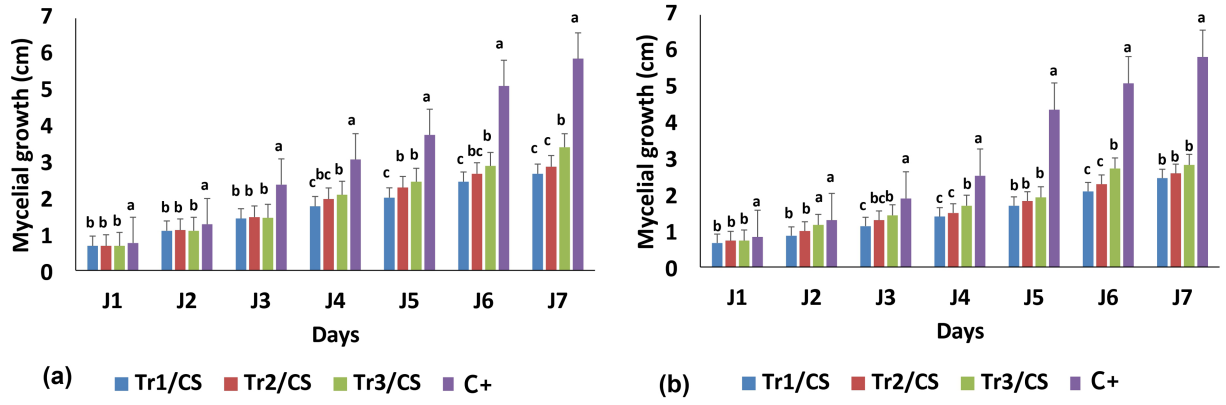

FIGURE 1
Temporal variation of *Curvularia spicifera* (CS) mycelial growth in response to *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) using direct (a) and indirect (b) confrontation methods. Small letters are used to compare different treatments. Different letters above bars indicate statistically significant differences within the experiments ($p \leq 0.5$) according to Duncan's multiple range tests. Bars without letters are not significantly different.

T. *longibrachiatum* exhibited the most effect with 1.28 mm/h, followed by *T. harzianum* (1.37 mm/h) and *T. asperellum* (1.43 mm/h) (Figure 2a). The results obtained revealed the effect of volatile compound produced by *Trichoderma* species on *C. spicifera*. Where the volatile compounds of *Trichoderma* treatments were exhibiting an inhibitory effect, a reduction in growth rate for *C. spicifera* was observed. Among all treatments, *T. longibrachiatum* expressed the strongest inhibition with a growth rate of 1.33 mm/h (Figure 2b).

3.2 Impact of *Trichoderma* spp. filtrates on mycelial growth

Table 1 points out the temporal variation in mycelial growth of *C. spicifera* under different concentrations of filtrates from three species of *Trichoderma* during the 7-day incubation period. In this study, all *Trichoderma* treatments significantly inhibited the mycelial growth of *C. spicifera* compared to the control ($p < 0.01$). This indicates that across all assessed time points and concentrations, the mycelial growth values were considerably lower in the *Trichoderma* treated groups. Mycelial growth in the positive control reached 5.97 cm on 7th day, while the growth in *Trichoderma* treatments ranged from 1.20 cm (Tr3/C3) to 1.47 cm (Tr2/C1), depending on the species and concentrations (Table 1). In the same context, Figure 3 illustrated the mycelial growth rate of *C. spicifera* at different filtrate concentrations of three species of *Trichoderma*, showing significant variation among them. The lower concentration of *Trichoderma* filtrates as 60% are associated with higher mycelial growth rates which compared to the higher concentrations, 80 and 100%, indicating that lower concentrations are less inhibitory. Among the *Trichoderma* species, *T. longibrachiatum* confirmed the most inhibitory effect at higher concentrations, as indicated by the lower growth rates at 80 and 100% (0.80 and 0.79 mm/h, respectively; Figure 3).

The results indicate that spore germination of *C. spicifera* is highly influenced by the presence of *Trichoderma* spp. Generally, the number of germinated spores was significantly lower in the treatments with *Trichoderma* filtrates compared to the positive control (17.60), confirming an inhibitory effect. Among the *Trichoderma* species, *T. longibrachiatum* revealed the highest inhibitory activity, by reducing the spore germination at (0.40), which is the lowest number of germinated spores. *T. harzianum* (2.20) and *T. asperellum* (4.40) also exhibited inhibitory effects (Figure 4).

3.3 Impact of *Trichoderma* species under greenhouse conditions

3.3.1 Disease severity assessment

The results of the preventative treatments using three *Trichoderma* species and salicylic acid against *C. spicifera* on tomato plants under greenhouse conditions are presented in Figure 5. At 5 dpi, none of the disease severity treatments exceeded 0 compared to the highly significant positive control (0.67). This result continued at 10 dpi, where all the treatments and the negative control maintained a disease severity of between 0 and 0.5, which is significantly lower than the positive control (1.67). At 90 dpi, during the final assessment, the disease severity reached its maximum in all treatments and the value ranged between 2.83 (*T. longibrachiatum* and salicylic acid) and 3.67 (*T. harzianum*) (positive control = 5.50; negative control = 0; Figure 5).

3.3.2 Enzymatic activities and stress markers

3.3.2.1 Catalase activity

Table 2 presents the catalase activity in tomato leaves and roots, subjected to the mentioned treatments above. Catalase activity,is ann important indicator of oxidative stress and marker for evaluating the effectiveness of preventive treatments against *C. spicifera*, varied significantly among treatments and sampling times. At 7 dpi, salicylic acid had the highest catalase activity of 52.04 µmol H_2O_2 mg protein^{-1}, followed by *T. asperellum* with 49.72 µmol H_2O_2 mg protein^{-1}, while the lowest was *T. harzianum* with 22.98 µmol H_2O_2 mg protein^{-1}. At 30 dpi, the highest activity was recorded in the positive control (51.64 µmol H_2O_2 mg protein-1), followed by salicylic acid (48.4 µmol H_2O_2 mg protein^{-1}), and *T. harzianum* (44.96 µmol H_2O_2 mg protein^{-1}). At 60 dpi, *T. longibrachiatum*

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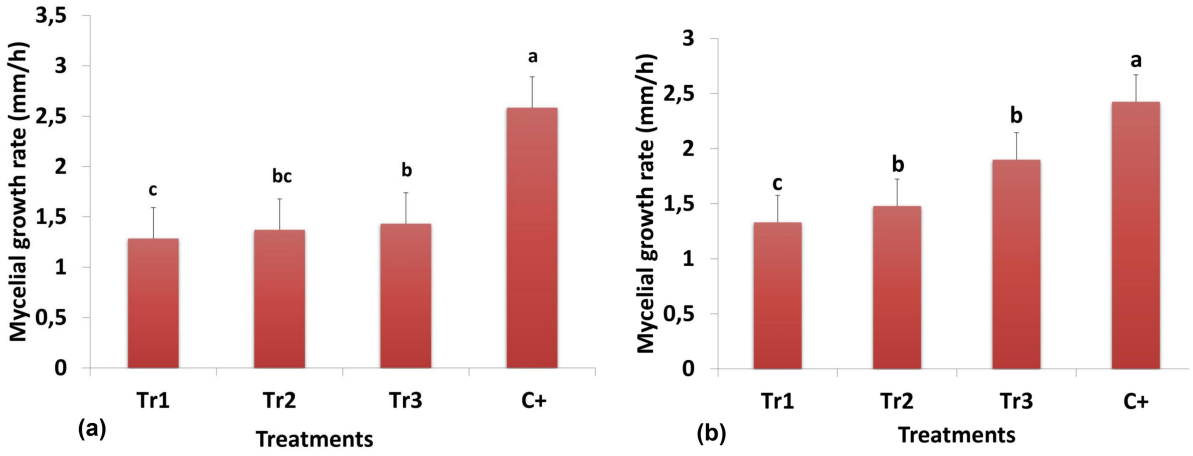

FIGURE 2
Mycelial growth rate of *Curvularia spicifera* in response to *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) after 7 days of incubation according to the direct (a) and indirect (b) confrontation methods. Small letters are used to compare different treatments. Different letters above the bars indicate statistically significant differences within the experiments ($p \leq 0.5$) according to Duncan's multiple range tests. Bars without letters are not significantly different.

TABLE 1 Temporal variation of *Curvularia spicifera* (CS) mycelial growth (cm) in response to different concentrations (C1: 60%, C2: 80%, and C3: 100%) of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) filtrates during 7 days of incubation (J1, J2, J3, J4, J5, J6, and J7).

Treatments	Concentrations	J1	J2	J3	J4	J5	J6	J7
Tr1/CS	C1	0.63 ± 0.05b[a]	0.73 ± 0.06b	0.87 ± 0.05bc	1 ± 0.10b	1.13 ± 0.06b	1.23 ± 0.05b	1.40 ± 0.10b
	C2	0.63 ± 0.06b	0.70 ± 0.10b	0.80 ± 0.1c	0.97 ± 0.12b	1.03 ± 0.11b	1.17 ± 0.05b	1.33 ± 0.05b
	C3	0.67 ± 0.06b	0.70 ± 0.01b	0.77 ± 0.01c	0.93 ± 0.06b	1.03 ± 0.05b	1.13 ± 0.06b	1.23 ± 0.05b
Tr2/CS	C1	0.70 ± 0.10b	0.77 ± 0.06b	0.83 ± 0.06bc	0.97 ± 0.11b	1.10 ± 0.10b	1.27 ± 0.06b	1.47 ± 0.05b
	C2	0.70 ± 0.10b	0.77 ± 0.10b	0.83 ± 0.05bc	0.93 ± 0.10b	1.10 ± 0.10b	1.23 ± 0.06b	1.37 ± 0.05b
	C3	0.73 ± 0.15b	0.80 ± 0.05b	0.83 ± 0.06bc	0.97 ± 0.10b	1.13 ± 0.06b	1.17 ± 0.06b	1.27 ± 0.06b
Tr3/CS	C1	0.83 ± 0.06b	0.87 ± 0.06b	0.90 ± 0.01bc	1.10 ± 0.11b	1.13 ± 0.06b	1.27 ± 0.05b	1.37 ± 0.06b
	C2	0.77 ± 0.05b	0.83 ± 0.12b	0.90 ± 0.01bc	1 ± 0.10b	1.13 ± 0.06b	1.23 ± 0.05b	1.33 ± 0.05b
	C3	0.77 ± 0.15b	0.83 ± 0.11b	1 ± 0.10b	1 ± 0.10b	1.07 ± 0.05b	1.17 ± 0.05b	1.20 ± 0.01b
C+		1.67 ± 0.21a	2.13 ± 0.21a	2.60 ± 0.2a	3.20 ± 0.26a	3.73 ± 0.15a	4.63 ± 0.47a	5.97 ± 0.45a
p-value[b]		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. [b]Probabilities associated with individual *F* tests.

demonstrated the highest activity (57.42 µmol H_2O_2 mg protein^{-1}), significantly surpassing the other treatments, including positive control (51.76 µmol H_2O_2 mg protein^{-1}) and *T. harzianum* (50.8 µmol H_2O_2 mg protein-1). By 90 dpi, *T. longibrachiatum* maintained the highest activity (74.58 µmol H_2O_2 mg protein^{-1}), followed by *T. harzianum* (70.18 µmol H_2O_2 mg protein^{-1}) and positive control (59.70 µmol H_2O_2 mg protein^{-1}). Notably, *T. asperellum* and salicylic acid showed a decline in activity over time, with the lowest values at 90 dpi (28.18 and 23.52 µmol H_2O_2 mg protein^{-1}, respectively) (Table 2). In tomato roots, at 7 dpi, the highest catalase activity was recorded in salicylic acid (5.24 µmol H_2O_2 mg protein^{-1}) and the lowest in *T. asperellum* (1.02 µmol H_2O_2 mg protein^{-1}). At 30 dpi, salicylic acid again showed the highest activity of 66.1 µmol H_2O_2 mg protein^{-1}, followed by *T. longibrachiatum*, which accounted 44.7 µmol H_2O_2 mg protein^{-1}. At 60 dpi, *T. longibrachiatum* maintained the highest activity of 67.6 µmol H_2O_2 mg protein^{-1}, significantly higher than positive control (58.02 µmol H_2O_2 mg protein^{-1}) and other treatments. At 90 dpi, *T. longibrachiatum* continued to show the highest activity of 73.1 µmol H_2O_2 mg protein^{-1}, followed by *T. harzianum* with 60.64 µmol H_2O_2 mg protein^{-1}. In contrast, at later stages of *T. asperellum* and salicylic acid exhibited low catalase activity, with *T. asperellum* recording the lowest values at 90 dpi (1.36 µmol H_2O_2 mg protein^{-1}) (Table 2).

3.3.2.2 Peroxidase activity

Results mentioned in Table 3 revealed that peroxidase activity varied significantly in treatments, sampling times, and different plant tissues. In tomato leaves, the highest peroxidase activity was recorded in *T. longibrachiatum* at 7 dpi, accounted 4.34 units mg^{-1} min^{-1} and followed by 90 dpi (5.35 units mg^{-1} min^{-1}), which reflects a strong and sustained induction of plant defense-related mechanisms. On the other hand, *T. asperellum* showed moderate activity at all-time points,

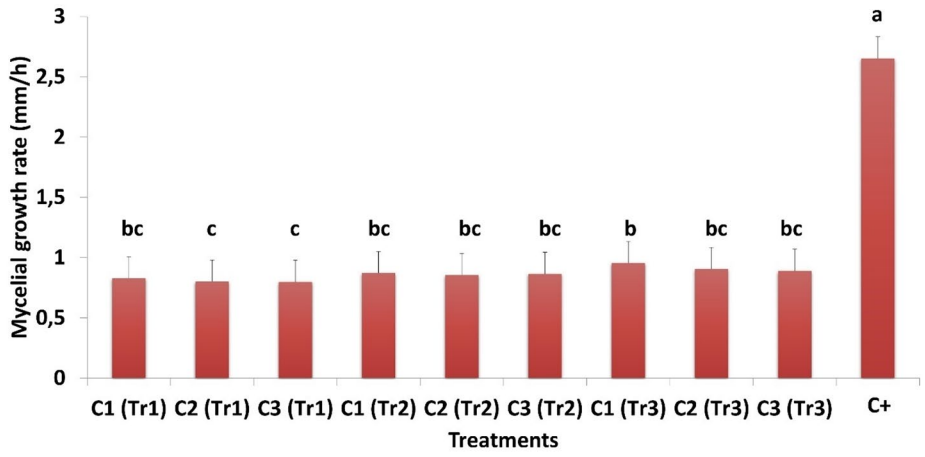

FIGURE 3
Mycelial growth rate of *Curvularia spicifera* in response to different concentrations (C1: 60%, C2: 80%, and C3: 100%) of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) filtrates. Small letters are used to compare different treatments. Different letters above bars indicate statistically significant differences within the experiments ($p \leq 0.5$) according to Duncan's multiple range tests. Bars without letters are not significantly different.

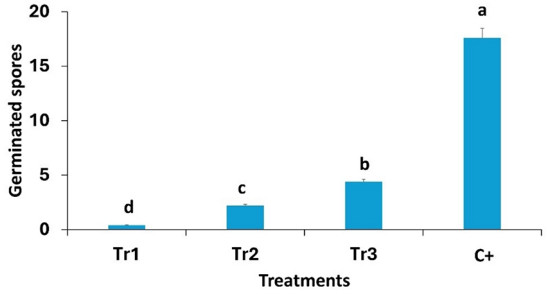

FIGURE 4
Effect of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) on the number of germinated spores of *Curvularia spicifera* after 24 h of incubation. Small letters are used to compare different treatments. Different letters above bars indicate statistically significant differences within the experiments ($p \leq 0.5$) according to Duncan's multiple range tests. Bars without letters are not significantly different.

and the value levels ranged from 3.36 to 1.52 units mg^{-1} min^{-1} (at 7 and 90 dpi, respectively). Salicylic acid was moderate at the beginning (4.17 units mg^{-1} min^{-1} at 7 dpi) but declined significantly by 90 dpi to a value as low as 2.07 units mg^{-1} min^{-1}, indicating a short-term effect. *T. harzianum* showed intermediate activity, which reached a maximum at 60 dpi (4.37 units mg^{-1} min^{-1}), but by 90 dpi, the activity declined to 4.77 units mg^{-1} min^{-1} (Table 3). As well roots, *T. longibrachiatum* showed the highest peroxidase activity (4.67 to 5.24 units mg^{-1} min^{-1} from 7 to 90 dpi). The enzymatic activity was also relatively high for *T. harzianum*, though it was lower in comparison with *T. longibrachiatum* activity, constituting 4.78 units mg-1 min-1 at 90 dpi. The lowest activity found to *T. asperellum* with values varied from 3.47 and 2.23-units mg^{-1} min^{-1} (at 7 and 90 dpi, respectively), evidencing its minimal efficiency in the root tissues. Salicylic acid showed a moderate activity that decreased along time, from 4.18 units mg^{-1} min^{-1} at 7 dpi to 2.57 units mg^{-1} min^{-1} at 90 dpi, as previously observed in leaves (Table 3).

3.3.2.3 Ascorbate peroxidase activity

Ascorbate peroxidase activity in tomato leaves was the highest under *T. longibrachiatum* treatment ranging from 45.21 to 54.91 µmol mg^{-1} min^{-1} at 7 and 90 dpi, respectively. In term of potential activity, *T. longibrachiatum* was followed by *T. harzianum*, which showed relatively high ascorbate peroxidase activity, with values rising from 42.82 to 50.96 µmol mg^{-1} min^{-1} at 7 and 90 dpi. Howevere, a less ascorbate peroxidase activity was recorded in *T. asperellum* and salicylic acid. Their activity declined with increase in days, starting from 21.38 to 18.03 µmol mg^{-1} min^{-1} at 7 and 90 dpi for *T. asperellum* and 38.58 to 18.16 µmol mg^{-1} min^{-1} at 7 and 90 dpi for salicylic acid (Table 4). In the roots, a similar patterns were revealed, where the maximum ascorbate peroxidase activity was recorded in *T. longibrachiatum*, increasing from 27.01 to 60.29 µmol mg^{-1} min^{-1} at 7 and 90 dpi. Compared to negative control, an increase from 26.82 µmol mg^{-1} min^{-1} at 7 dpi to 47.89 µmol mg^{-1} min^{-1} at 90 dpi was revealed, whereas positive control, a significant drop from 19.22 µmol mg^{-1} min^{-1} at 7 dpi to 9.20 µmol mg^{-1} min^{-1} at 90 dpi (Table 4).

3.3.2.4 Polyphenol activity

Trichoderma longibrachiatum consistently exhibited the highest polyphenol oxidase activity in tomato leaves at all the sampling periods, which varied between 9.89 and 14.07 units mg^{-1} min^{-1} at 7 and 90 dpi, respectively. These activities were significantly important than those the other applied treatments. *T. harzianum* and *T. asperellum* increased the activity of polyphenol oxidase over the controls, but activities were less than those of *T. longibrachiatum*. Salicylic acid showed a moderate polyphenol oxidase activity at 7 and 30 dpi (5.48 and 10.10 units mg^{-1} min^{-1}, respectively), but decreased considerably at 90 dpi (2.22 units mg^{-1} min^{-1}) (Table 5). In the same context, in tomato roots, *T. longibrachiatum* yielded the maximum polyphenol oxidase activity with increasing values over time (2.83 to 9.37 units mg^{-1} min^{-1} at 7 and 90 dpi, respectively). Tomato plants treated with *T. asperellum* showed the lowest enzyme activity, where the polyphenol oxidase activity reduced significantly at 60 dpi

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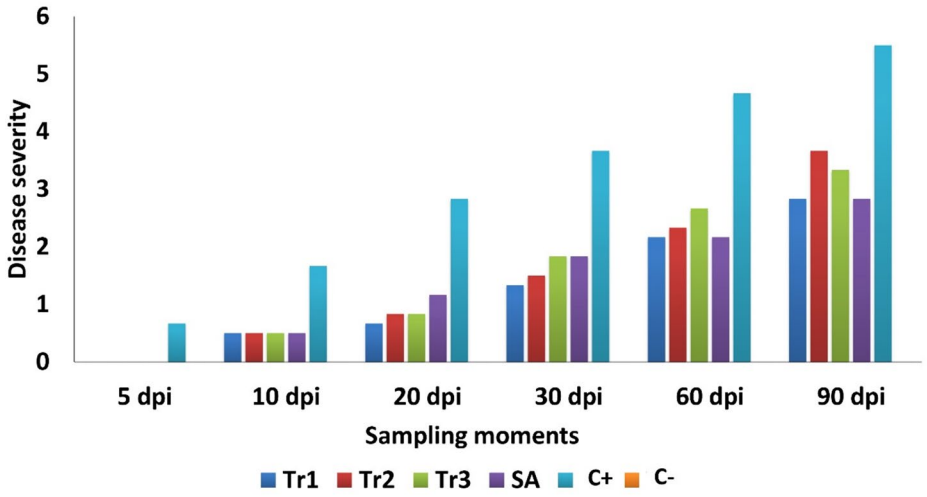

FIGURE 5
Effect of preventive treatments of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) and salicylic acid (SA) on the disease severity in tomato plants inoculated with *Curvularia spicifera* at six sampling moments [days after pathogen inoculation (dpi)] under experimental greenhouse conditions.

TABLE 2 Effect of preventive treatments of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) and salicylic acid (SA) on the catalase activity (μmol H_2O_2 mg protein^{-1}) in tomato leaves and roots inoculated with *Curvularia spicifera* at four sampling moments [7, 30, 60, and 90 days after pathogen inoculation (dpi)] under experimental greenhouse conditions.

Treatments	7 dpi	30 dpi	60 dpi	90 dpi
Tomato leaves				
Tr1	26.22 ± 0.11e[a]	39.46 ± 0.09f	57.42 ± 0.06a	74.58 ± 0.18a
Tr2	22.98 ± 0.12f	44.96 ± 0.15c	50.8 ± 0.12c	70.18 ± 0.18b
Tr3	49.72 ± 0.24b	40.14 ± 0.12e	31.4 ± 0.15e	28.18 ± 0.30e
SA	52.04 ± 0.09a	48.4 ± 0.39b	30.84 ± 0.12f	23.52 ± 0.18f
C+	28.76 ± 0.09d	51.64 ± 0.19a	51.76 ± 0.15b	59.7 ± 0.18c
C-	34.36 ± 0.09c	44.38 ± 0.27d	49.5 ± 0.06d	56.2 ± 0.27d
p-value	<0.01	<0.01	<0.01	<0.01
Tomato roots				
Tr1	3.48 ± 0.31ab	44.7 ± 0.06b	67.6 ± 2.10a	73.1 ± 0.18a
Tr2	2.92 ± 0.091abc	34.34 ± 0.091c	13.28 ± 0.09d	60.64 ± 0.12b
Tr3	1.02 ± 0.12c	1.82 ± 0.15f	1.48 ± 0.16f	1.36 ± 0.51f
sa	5.24 ± 0.15a	66.1 ± 0.09a	19.68 ± 0.22c	25.66 ± 0.21c
C+	2.54 ± 0.21bc	4.94 ± 0.15e	58.02 ± 0.16b	13.86 ± 0.12d
C-	4.1 ± 0.03ab	6.68 ± 0.41d	9.62 ± 0.18e	11.3 ± 0.21e
p-value[b]	<0.05	<0.01	<0.01	<0.01

[a]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. [b]Probabilities associated with individual *F* tests.

(1.82 units mg^{-1} min^{-1}) and persisted to be low at 90 dpi (3.24 units mg^{-1} min^{-1}) (Table 5).

3.3.2.5 Total protein

Table 6 documented the influence of preventive treatments with three species of *Trichoderma* spp. and salicylic acid on the total protein content in tomato leaves and roots inoculated with *C. spicifera*. The total protein content in the leaves varied significantly among treatments and sampling periods. At 30 dpi, *T. longibrachiatum* showed the highest protein content (11.23 mg g^{-1}). At 60 dpi, negative control revealed the highest protein content (11.28 mg g^{-1}) followed by *T. longibrachiatum* (11.12 mg g^{-1}). At 90 dpi, salicylic acid (10.35 mg g^{-1}), *T. longibrachiatum* (10.09 mg g^{-1}) once again contained the highest protein content in tomato leaves (Table 6). Protein content in the roots of tomato also differed greatly during treatment and sampling time. At 7 dpi, positive control (11.46 mg g^{-1}) exhibited maximum protein content followed by salicylic acid (10.67 mg g^{-1}) and *T. asperellum* (10.12 mg g^{-1}). At 90 dpi, positive control exhibited a remarkable rise in protein content (14.53 mg g^{-1}) that was far higher than all treatments, indicating its ability to maintain protein synthesis over time. *T. longibrachiatum* (10.44 mg g^{-1}) and negative control (10.39 mg g^{-1}) followed, while *T. asperellum* (9.42 mg g^{-1}) showed the lowest protein content (Table 6).

TABLE 3 Effect of preventive treatments of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) and salicylic acid (SA) on the peroxydase activity (units mg^{-1} min^{-1}) in tomato leaves and roots inoculated with *Curvularia spicifera* at four sampling moments [7, 30, 60, and 90 days after pathogen inoculation (dpi)] under experimental greenhouse conditions.

Treatments	7 dpi	30 dpi	60 dpi	90 dpi
Tomato leaves				
Tr1	4.34 ± 0.01a[a]	4.71 ± 0.06a	3.62 ± 0.009c	5.35 ± 0.009a
Tr2	3.62 ± 0.09d	3.99 ± 0.009d	4.37 ± 0.01b	4.77 ± 0.01c
Tr3	3.36 ± 0.06e	3.62 ± 0.01e	2.50 ± 0.08f	1.52 ± 0.01f
SA	4.17 ± 0.06b	4.40 ± 0.009b	2.93 ± 0.009e	2.07 ± 0.01e
C+	3.63 ± 0.06d	3.34 ± 0.07f	3.00 ± 0.08d	2.55 ± 0.09d
C-	4.11 ± 0.06c	4.30 ± 0.01c	4.70 ± 0.12a	4.96 ± 0.03b
p-value	<0.01	<0.01	<0.01	<0.01
Tomato roots				
Tr1	4.67 ± 0.07a	4.81 ± 0.08a	5.05 ± 0.01a	5.24 ± 0.09a
Tr2	4 ± 0.01c	4.38 ± 0.08c	4.59 ± 0.01b	4.78 ± 0.06b
Tr3	3.47 ± 0.01f	3.71 ± 0.01e	2.81 ± 0.03f	2.23 ± 0.06f
SA	4.18 ± 0.07b	4.52 ± 0.01b	2.93 ± 0.06d	2.57 ± 0.01e
C+	3.67 ± 0.1e	3.31 ± 0.07f	2.84 ± 0.06e	2.60 ± 0.01d
C-	3.74 ± 0.06d	4.09 ± 0.01d	4.26 ± 0.09c	4.57 ± 0.01c
p-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. [b]Probabilities associated with individual *F* tests.

TABLE 4 Effect of preventive treatments of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) and salicylic acid (SA) on the ascorbate peroxidase activity (μmol mg^{-1} min^{-1}) in tomato leaves and roots inoculated with *Curvularia spicifera* at four sampling moments [7, 30, 60, and 90 days after pathogen inoculation (dpi)] under experimental greenhouse conditions.

Treatments	7 dpi	30 dpi	60 dpi	90 dpi
Tomato leaves				
Tr1	45.21 ± 0.08a[a]	48.7 ± 0.16a	51.16 ± 0.11a	54.91 ± 0.05a
Tr2	42.82 ± 0.15b	43.57 ± 0.10b	46.22 ± 0.08b	50.96 ± 0.13b
Tr3	21.38 ± 0.07f	32.8 ± 0.24d	25.62 ± 0.12f	18.03 ± 0.10e
SA	38.58 ± 0.20c	41.63 ± 0.13c	26.58 ± 0.11e	18.16 ± 0.13e
C+	23.01 ± 0.11d	32.91 ± 0.12d	33.27 ± 0.03d	30.24 ± 0.05d
C-	22.71 ± 0.08e	31.69 ± 0.08e	33.86 ± 0.10c	35.43 ± 0.07c
p-value	<0.01	<0.01	<0.01	<0.01
Tomato roots				
Tr1	27.01 ± 0.11a	33.42 ± 0.12a	49.22 ± 0.07a	60.29 ± 0.06a
Tr2	19.21 ± 0.08c	29.13 ± 0.10d	37.31 ± 0.08b	40.19 ± 0.08c
Tr3	13.99 ± 0.10d	25.02 ± 0.08e	17.69 ± 0.12e	21.29 ± 0.11d
SA	24.62 ± 0.08b	32.47 ± 0.10b	23.07 ± 0.10d	15.29 ± 0.11e
C+	19.22 ± 0.19c	17.36 ± 0.05f	13.21 ± 0.15f	9.20 ± 0.12f
C-	26.82 ± 0.08a	31.37 ± 0.13c	36.87 ± 0.10c	47.89 ± 0.19b
p-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. [b]Probabilities associated with individual *F* tests.

3.3.3 Impact of *Trichoderma* spp. on plant growth promoting parameters

Statistical analysis showed a highly significant difference ($p < 0.01$) between treatments and sampling moments. The highest chlorophyll content was observed in the tomato plants treated with salicylic acid (38.77) and *T. longibrachiatum* (38.69), which were both significantly higher than the other applied treatments at 7 dpi, compared to control. At 30 dpi, chlorophyll content was boosted in most of the treatments, with salicylic acid (46.44) and *T. longibrachiatum* (46.31) showing the highest values, followed by *T. harzianum* (45.88) and *T. asperellum* (44.81), which were all significantly higher than the positive control (36.19), indicating long term protective effect of the treatments. At 60

TABLE 5 Effect of preventive treatments of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) and salicylic acid (SA) on the polyphenol oxidase activity (units mg^{-1} min^{-1}) in tomato leaves and roots inoculated with *Curvularia spicifera* at four sampling moments [7, 30, 60, and 90 days after pathogen inoculation (dpi)] under experimental greenhouse conditions.

Treatments	7 dpi	30 dpi	60 dpi	90 dpi
Tomato leaves				
Tr1	9.89 ± 0.02a[a]	11.05 ± 0.03a	10.08 ± 0.05a	14.07 ± 0.04a
Tr2	7.71 ± 0.04c	9.55 ± 0.02c	7.19 ± 0.03c	12.89 ± 0.02b
Tr3	7.83 ± 0.02b	8.67 ± 0.02d	5.25 ± 0.04e	11.13 ± 0.03c
SA	5.48 ± 0.03d	10.10 ± 0.03b	6.32 ± 0.04d	2.22 ± 0.06e
C+	4.74 ± 0.02f	4.29 ± 0.04f	4.18 ± 0.01f	4.09 ± 0.04d
C-	5.35 ± 0.04e	6.52 ± 0.04e	9.55 ± 0.02b	11.19 ± 0.03c
p-value	<0.01	<0.01	<0.01	<0.01
Tomato roots				
Tr1	2.83 ± 0.01a	4.23 ± 0.02a	6.33 ± 0.06a	9.37 ± 0.05a
Tr2	2.03 ± 0.02d	2.21 ± 0.03d	4.75 ± 0.04c	5.75 ± 0.05b
Tr3	2.16 ± 0.01c	1.99 ± 0.02e	1.82 ± 0.04e	3.24 ± 0.03d
SA	2.84 ± 0.02a	3.89 ± 0.03b	5.45 ± 0.04b	1.49 ± 0.03e
C+	2.74 ± 0.02b	3.85 ± 0.04b	0.99 ± 0.01f	0.75 ± 0.03f
C-	1.54 ± 0.02e	2.93 ± 0.04c	4.21 ± 0.04d	5.37 ± 0.05c
p-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. [b]Probabilities associated with individual *F* tests.

TABLE 6 Effect of preventive treatments of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) and salicylic acid (SA) on the total protein content (mg g^{-1}) in tomato leaves and roots inoculated with *Curvularia spicifera* at four sampling moments [7, 30, 60, and 90 days after pathogen inoculation (dpi)] under experimental greenhouse conditions.

Treatments	7 dpi	30 dpi	60 dpi	90 dpi
Tomato leaves				
Tr1	0.22 ± 0.03e[a]	11.23 ± 0.05a	11.12 ± 0.03b	10.09 ± 0.05c
Tr2	0.40 ± 0.03d	10.95 ± 0.05c	10.98 ± 0.05c	9.75 ± 0.05e
Tr3	1.51 ± 0.05b	10.56 ± 0.05e	10.78 ± 0.04d	10.07 ± 0.05c
SA	0.68 ± 0.03c	10.91 ± 0.06c	10.43 ± 0.07e	10.35 ± 0.05b
C+	0.39 ± 0.05d	11.06 ± 0.03b	11.03 ± 0.05c	9.90 ± 0.05d
C-	9.12 ± 0.09a	10.73 ± 0.03d	11.28 ± 0.02a	11.57 ± 0.05a
p-value	<0.01	<0.01	<0.01	<0.01
Tomato roots				
Tr1	9.88 ± 0.03d	10.90 ± 0.03c	11.49 ± 0.05a	10.44 ± 0.05b
Tr2	6.86 ± 0.09f	10.57 ± 0.08e	10.94 ± 0.03d	10.22 ± 0.07c
Tr3	10.12 ± 0.07c	10.78 ± 0.07d	10.21 ± 0.07e	9.42 ± 0.09e
SA	10.67 ± 0.07b	10.10 ± 0.07f	11.05 ± 0.07c	9.82 ± 0.08d
C+	11.46 ± 0.03a	12.43 ± 0.07a	10.99 ± 0.03 cd	14.53 ± 0.05a
C-	9.54 ± 0.03e	11.16 ± 0.03b	11.16 ± 0.02b	10.39 ± 0.03b
p-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$. [b]Probabilities associated with individual *F* tests.

dpi, salicylic acid (50.77) still maintained the highest chlorophyll content, outperforming significantly all the other treatments and controls. *T. longibrachiatum* (42.92) and *T. harzianum* (43.43) had comparatively more chlorophyll, while *T. asperellum* (35.68) showed a notable reduction, and it was nearly approaching the value observed in positive control (36.20). Salicylic acid (56.74) maintained the maximum content of chlorophyll at 90 dpi and exceeded that of all other controls and treatments. *T. longibrachiatum* (42.50) and *T. harzianum* (39.68) continued to decline but were still much higher in comparison to the positive control (30.60) (Table 7). The growth responses of the tomato plants varied significantly between treatments ($p < 0.01$) and was most enhanced in *Trichoderma*-treated plants at 90

dpi. The preventative application of *T. longibrachiatum* against *C. spicifera* significantly increased growth over all the other treatments. Specifically, *T. longibrachiatum* recorded 29 cm root length, 46 cm plant length, 13 leaves per plant, 2 fruits per plant, 8 flowers per plant, and 12 ramifications per plant at 90 dpi. This treatment also recorded the highest fresh weight of aerial (25.87 g) and root (15.24 g) parts, and dry weight of aerial (3.25 g) and root (3.17 g) parts. Plants inoculated with the pathogen only, however, recorded a notable decrease in all the growth parameters measured (Figure 6).

4 Discussion

Plant disease management is a critical component of sustainable crop production, and the use of biological control agents (BCAs), such as *Trichoderma* spp., represents a promising strategy (Becker et al., 2025). *Trichoderma* species are known to employ multiple modes of action against plant pathogens, including mycoparasitism (direct infection and lysis of the pathogen), antibiosis (production of inhibitory secondary metabolites), competition for nutrients and space, enzymatic degradation of pathogen cell walls (López-López et al., 2022), and the induction of host plant defense mechanisms (Gouit et al., 2024), potentially mediated through the emission of volatile organic compounds (Rubio et al., 2023).

In addition, *Trichoderma* spp. are capable of upregulating plant defense-related genes, thereby enhancing the plant's resistance to subsequent infections. These fungi have demonstrated strong antagonistic activity against a wide spectrum of phytopathogenic fungi—including *C. spicifera*—under diverse environmental conditions, confirming their broad-spectrum potential as effective BCAs (Baral et al., 2022; Manzar et al., 2022; Becker et al., 2025).

In vitro assays demonstrated the strong inhibitory effects of *T.longibrachiatum* on the mycelial growth and spore germination of *C. spicifera*, indicating a high level of antagonistic activity. These findings are consistent with previous studies by Hajji-Hedfi et al. (2023a, 2023b), which reported the production of both volatile and non-volatile bioactive metabolites by *Trichoderma* spp. as key mechanisms of pathogen suppression. *Trichoderma* spp. are known to synthesize a wide array of volatile secondary metabolites, including ethylene, hydrogen cyanide, various aldehydes, and ketones. These compounds have been shown to play a pivotal role in the biological control of numerous plant pathogens (Khan et al., 2020). Extensive research has confirmed that such volatile organic compounds VOCs, particularly those produced by *Trichoderma* spp., possess strong antifungal properties. They can effectively inhibit the growth of several economically important pathogenic fungi, including *Aspergillus* spp. and *Fusarium* spp., and in many cases, their inhibitory potential exceeds that of classical mycoparasitism (Awad-Allah et al., 2022; Modrzewska et al., 2022; Ren et al., 2022; Rebouh et al., 2022; Napolitano et al., 2024; Becker et al., 2025). Moreover, VOCs emitted by *Trichoderma* spp. have also been shown to suppress the growth of other phytopathogens, such as *Rhizoctonia* spp. and *Pythium* spp., further highlighting their broad-spectrum biocontrol potential (Behiry et al., 2023; Al-Shuaibi et al., 2024). These findings collectively underscore the role of *T. longibrachiatum* as an effective biological control agent, capable of reducing fungal pathogen viability through multiple biochemical mechanisms, with volatile metabolite production being a major contributing factor.

Trichoderma spp. have a very advanced mechanism of action against phytopathogenic fungi, including cell wall-degrading enzymes and antibiotic biosynthesis (Becker et al., 2025). In addition to the reported chitinolytic activity for the degradation of pathogen cell walls, *Trichoderma* spp. biosynthesize antibiotics that directly inhibit growth of the pathogenic mycelial growth (Rubio et al., 2023). Main extracellular enzymes such as endochitinases, β-1,3-glucanases, and proteases play an important role in *Trichoderma* mycoparasitism and bring about extensive disruption of structural integrity of pathogenic fungal cell walls (Suriani Ribeiro et al., 2019; Tomah et al., 2023). Our findings are consistent with these studies, as disease severity was significantly reduced in all treatments involving *Trichoderma* strains.

Recent studies have demonstrated that *Trichoderma* spp. can alleviate the negative impacts of both biotic and abiotic stress in plants by modulating reactive oxygen species (ROS) and enhancing the activity of antioxidant enzymes (Fu et al., 2017; Chen et al., 2019; Cornejo-Ríos et al., 2021). In our study, all tested *Trichoderma* species significantly increased enzymatic activity in plants, with *T. longibrachiatum* exhibiting the highest levels among them. These findings highlight species-specific variation in the capacity of *Trichoderma* to induce plant defense responses. Indeed, outcomes often vary depending on the *Trichoderma* species or isolate used and the target phytopathogen. For example, Almaghasla et al. (2023) reported that *T. asperellum* strain

TABLE 7 Effect of preventive treatments of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) and salicylic acid (SA) on the chlorophyll content in tomato leaves inoculated with *Curvularia spicifera* at four sampling moments [7, 30, 60, and 90 days after pathogen inoculation (dpi)] under experimental greenhouse conditions.

Treatments	7 dpi	30 dpi	60 dpi	90 dpi
Tr1	38.69 ± 1.43a[a]	46.31 ± 1.33b	42.92 ± 1.21b	42.50 ± 1.13c
Tr2	34.83 ± 1.96b	45.88 ± 1.99b	43.43 ± 1.05b	39.68 ± 1.31c
Tr3	35.44 ± 1.10b	44.81 ± 1.38b	35.68 ± 1.89c	36.28 ± 1.79 cd
SA	38.77 ± 1.13a	46.44 ± 1.06b	50.77 ± 2.10a	56.74 ± 1.35a
C+	32.94 ± 1.32b	36.19 ± 1.71c	36.20 ± 2.17c	30.60 ± 1.95d
C-	34.60 ± 1.89b	52.30 ± 1.32a	51.20 ± 1.32a	49.74 ± 1.58b
p-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Duncan's Multiple Range Test, values followed by different superscripts are significantly different at $p \leq 0.05$ [b]Probabilities associated with individual *F* tests.

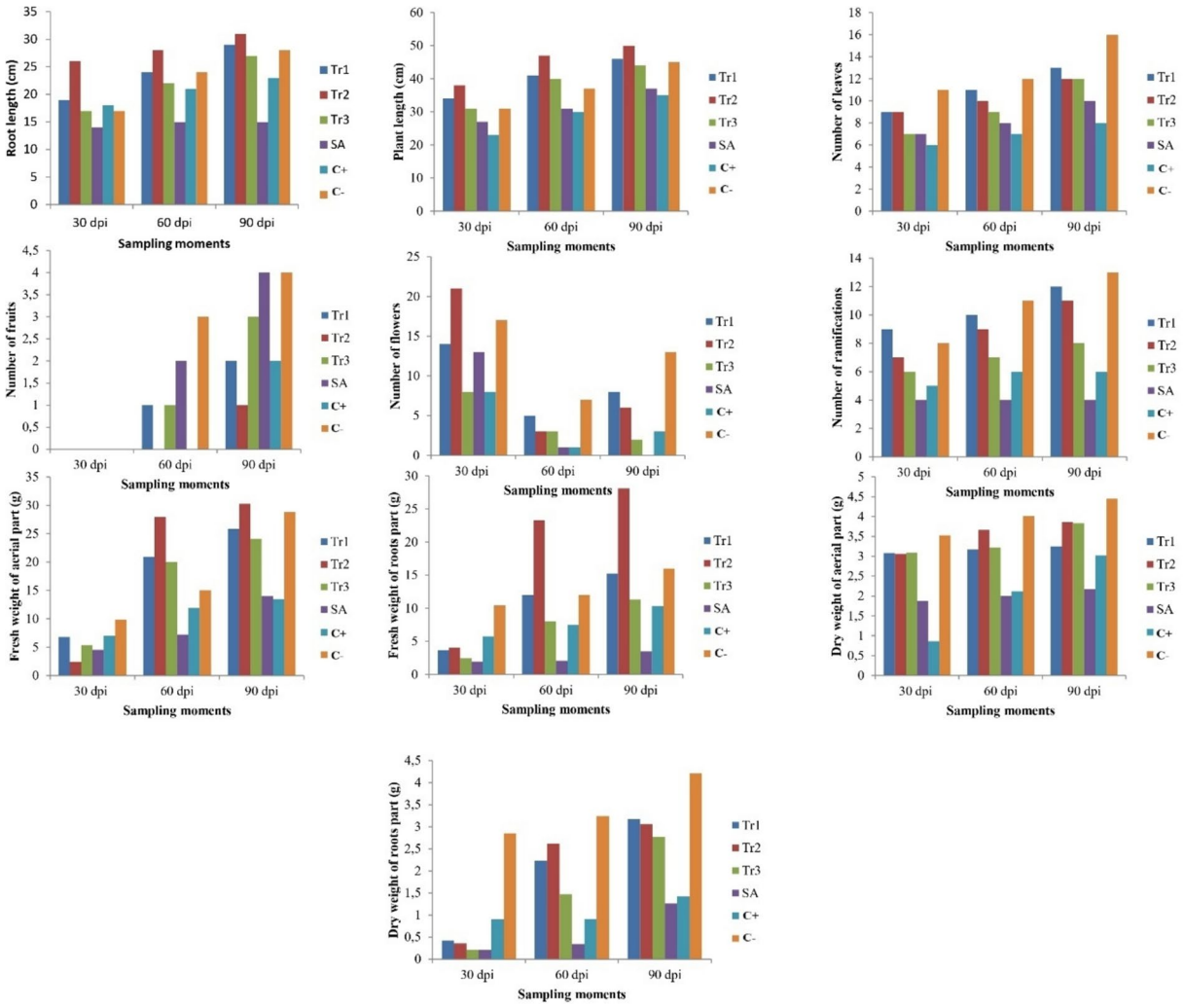

FIGURE 6
Effect of preventive treatments of *Trichoderma* spp. (Tr1: *T. longibrachiatum*, Tr2: *T. harzianum*, and Tr3: *T. asperellum*) and salicylic acid (SA) on the agronomic parameters of tomato plants inoculated with *Curvularia spicifera* at three sampling moments (30, 60, and 90 dpi) under experimental greenhouse conditions.

KSATR11 exhibited the weakest antagonistic activity against *Rhizoctonia solani* in cucumber when compared to six other *Trichoderma* species. Similarly, Yao et al. (2023) reported that *T. asperellum* and *T. harzianum* exhibited varying degrees of inhibitory activity against 29 plant pathogenic fungi across 18 genera. This finding highlights the broad-spectrum biocontrol potential of *Trichoderma* spp. and reinforces the concept of strain-specific effectiveness. The observed variability in antagonistic activity suggests that the efficacy of *Trichoderma* is not consistent across species or even strains, but rather depends on the specific interaction between the isolate and the target pathogen. These results underscore the importance of screening and selecting the most effective *Trichoderma* strains for particular pathogens and crops. Furthermore, they support the use of diverse *Trichoderma* isolates in biocontrol programs to enhance effectiveness across a wide range of plant diseases (Yao et al., 2023). This research was mainly directed toward the beneficial effects of *Trichoderma* spp. as a biocontrol agent, but it is also crucial to use salicylic acid in co-application and investigate its possible effect on the results achieved. Salicylic acid, a significant plant hormone, has a special role in triggering systemic acquired resistance against a range of phytopathogens by usually inducing defense-related genes and enabling the synthesis of antimicrobial compounds (Decsi et al., 2025).

The effectiveness of *T. longibrachiatum* compared to *T. harzianum* and *T. asperellum* in biocontrol applications is typically attributed to several unique biological traits and metabolic processes (Cao et al., 2025). *T. longibrachiatum* can grow in a variety of environmental conditions (Bint-e-Zahira et al., 2024). Its effectiveness often results from an effective complement of extracellular enzymes, especially cell wall-degrading enzymes. These enzymes are directly involved in mycoparasitism by degrading the cell walls of phytopathogenic fungi, thereby inhibiting or lysing them (Zhu et al., 2022; Díaz-García et al., 2024). Although other *Trichoderma* spp. also produce these enzymes, some strains of *T. longibrachiatum* can

produce them to a larger degree for a longer duration, or in a more synergistic combination (Boamah et al., 2025). Besides, *T. longibrachiatum* is a rich source of numerous secondary metabolites such as peptaibols, polyketides, pyrones, terpenes, and diketopiperazine-type compounds, some of which have been shown to display potent antimicrobial or antifungal activity (Yu et al., 2023; Li et al., 2025). These molecules can act by directly inhibiting the growth of the pathogen, disrupting its development, or triggering systemic resistance in the plant, thus creating several layers of defense (Caracciolo et al., 2023).

The finding that *Trichoderma* treatments significantly enhanced tomato plant growth aligns with a substantial body of research demonstrating the growth-promoting effects of *Trichoderma* spp. on tomato plants. For instance, a study by Mwangi et al. (2011) reported that inoculation with *T. harzianum* improved various growth parameters of tomato seedlings, including shoot length, root length, and dry biomass, compared to untreated controls. Similarly, Fontenelle et al. (2011) observed that 12 out of 28 *Trichoderma* isolates promoted an increase in dry matter mass of tomato seedlings by over 100%, indicating a significant enhancement in plant growth.

Moreover, a study by Palacios-Torres et al. (2019) found that application of *Trichoderma* spp. resulted in increased tomato yields and fruit quality compared to untreated controls, further supporting the role of *Trichoderma* in promoting tomato plant growth. These studies collectively underscore the potential of *Trichoderma* spp. as effective biostimulants for enhancing tomato plant growth.

The variability in growth responses among different *Trichoderma* species, suggests that the efficacy of *Trichoderma* treatments can be influenced by several factors, including the specific strain used and the genetic background of the host plant. Therefore, selecting appropriate *Trichoderma* strains tailored to specific tomato cultivars and environmental conditions is crucial for maximizing growth promotion and disease suppression.

5 Conclusion

This study demonstrates the potential of *Trichoderma* species, particularly *T. longibrachiatum*, as effective biocontrol agents against *C. spicifera* in tomato plants. Both *in vitro* and greenhouse experiments confirmed the strong antagonistic effects of *T. longibrachiatum*, which significantly reduced pathogen growth and disease severity. Additionally, treated plants exhibited enhanced biochemical defenses, including increased antioxidant enzyme activities, elevated protein content, improved chlorophyll levels, and better agronomic performance. These results highlight *T. longibrachiatum* as a promising, eco-friendly alternative to chemical control methods for managing *C. spicifera*, one of the most harmful pathogens of tomato, offering the dual benefits of disease suppression and plant growth promotion. Further field studies are recommended to validate these findings under diverse environmental conditions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

LH-H: Conceptualization, Data curation, Formal analysis, Validation, Writing – original draft, Writing – review & editing. AR: Investigation, Software, Writing – original draft. TW: Formal analysis, Resources, Writing – original draft. AU: Investigation, Software, Writing – original draft. NR: Conceptualization, Data curation, Formal analysis, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

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