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Indigenous Endophytic *Trichoderma* and *Streptomyces* Suppress Potato Late Blight Through Direct Antagonism and Modulation of Host Defense Responses

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Highlights

What are the main findings?

- Indigenous endophytic *Trichoderma* and *Streptomyces* suppressed *Phytophthora infestans* through antagonism and host defense modulation.
- Selected isolates reduced late blight severity and maintained stable efficacy across two growing seasons.

What is the implication of the main finding?

- Indigenous bioagents show potential as sustainable alternatives to synthetic fungicides.
- Integrated mechanistic and field validation supports their application in potato late blight management.

Abstract

Background: Late blight, caused by *Phytophthora infestans*, remains one of the most destructive diseases limiting potato production worldwide. Methods: This study evaluated the biocontrol potential of indigenous endophytic *Trichoderma* and *Streptomyces* isolates using an integrated approach involving antagonistic assays, molecular identification, microscopy, biochemical characterization, multivariate analyses, and two-season field trials. Results: Among 39 endophytic isolates recovered from healthy potato tissues, eight *Trichoderma* and two *Streptomyces* strains exhibited strong antagonistic activity, suppressing pathogen mycelial growth by up to 82.6%. Molecular analyses identified the most effective isolates as *Trichoderma atrobrunneum*, *Streptomyces globisporus*, and *Streptomyces parvus*. Microscopic observations revealed multiple antagonistic mechanisms, including hyphal coiling, penetration, deformation, and destruction of *P. infestans* hyphae and sporangia. Under field conditions, selected bioagents consistently reduced disease incidence and severity across two growing seasons; *Trichoderma* isolate T4 showed the strongest efficacy, reducing disease incidence to 3.00% and severity to 2.18%. Bioagent treatments also alleviated pathogen-induced oxidative stress, reducing malondialdehyde to 62.00 nmol g^{-1} and modulating antioxidant enzyme activities. Principal component and heatmap analyses confirmed stable performance of top-performing treatments across seasons. Conclusions: These findings

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indicate that indigenous endophytic *Trichoderma* and *Streptomyces* suppress potato late blight through direct antagonism and modulation of host defense responses, highlighting their potential as sustainable components of integrated disease management strategies.

Keywords: late blight; *Phytophthora infestans*; potato; *Trichoderma*; *Streptomyces*; biological control

1. Introduction

Potato (*Solanum tuberosum* L.), a member of the Solanaceae family, is among the most widely cultivated staple crops worldwide and is grown in over 150 countries. In Egypt alone, approximately 738,848 acres were harvested during the 2023/2024 season, producing nearly 9.6 million tons (CAPMAS) [1]. This substantial production highlights the economic importance of potato cultivation for food security and national and international markets. However, potato productivity is constrained by numerous diseases, including late blight, early blight, powdery scab, and wart disease, all of which contribute to significant yield losses. Among these, late blight, caused by the oomycete *Phytophthora infestans*, remains one of the most devastating diseases, affecting all growth stages and capable of causing complete crop failure under favorable environmental conditions [2,3].

Late blight has historically been recognized as one of the most destructive diseases in potato production, with profound socioeconomic impacts exemplified by the Irish Potato Famine. The pathogen's complex biology, including both sexual and asexual reproduction and the formation of persistent oospores, facilitates rapid disease development and long-term survival across diverse environments. Symptoms typically include extensive foliar necrosis and tuber rot during both field growth and postharvest storage, reflecting the pathogen's capacity to colonize multiple plant tissues and disrupt physiological processes) [4]. Consequently, effective management of late blight remains challenging because of the pathogen's aggressive infection cycle and the intricate molecular interactions underlying host–pathogen relationships [5].

Conventional late blight management has relied heavily on repeated applications of synthetic fungicides. Although these treatments can effectively reduce disease severity in the short term, their prolonged and intensive use has contributed to the emergence of fungicide-resistant *P. infestans* populations, increasing both application frequency and dosage requirements [6]. Moreover, excessive fungicide and fertilizer inputs may disrupt soil microbial communities, alter physicochemical properties, and reduce long-term soil productivity and plant health [7]. These limitations underscore the urgent need for sustainable and ecologically sound disease management strategies aligned with integrated pest management (IPM) principles.

Biological control, which exploits beneficial microorganisms to suppress pathogens and enhance host resilience, has emerged as a promising alternative to chemical-based approaches. Beneficial microorganisms may inhibit pathogens directly through antibiosis, competition, and mycoparasitism or indirectly through modulation of host defense responses and induction of systemic resistance. By combining pathogen suppression with enhanced plant defense mechanisms, biological control can improve crop resilience while minimizing environmental risks, thereby contributing to sustainable agricultural production systems [8].

Among beneficial microorganisms, indigenous endophytic species are particularly attractive biocontrol agents because of their adaptation to local agroecological conditions, ability to colonize host tissues asymptomatically, and potential to enhance stress tolerance. *Trichoderma* species are well recognized for their strong antagonistic activity, mycopar-

asitism, production of hydrolytic enzymes, and ability to stimulate plant growth and defense responses. Likewise, *Streptomyces* species produce diverse bioactive metabolites and have demonstrated substantial anti-oomycete activity against *P. infestans* and other phytopathogens [9]. Recent studies have further highlighted the multifaceted mechanisms by which *Trichoderma* and *Streptomyces* contribute to pathogen suppression and host defense modulation [10–12].

Despite increasing interest in biological control of potato late blight, significant knowledge gaps remain regarding the efficacy of indigenous endophytic microorganisms adapted to local agroecological conditions, their underlying mechanisms of pathogen suppression, and the stability of their performance under field conditions across multiple growing seasons. Most previous studies have focused primarily on in vitro antagonism, greenhouse experiments, short-term evaluations, or individual strains examined in isolation, limiting understanding of their practical applicability and long-term effectiveness. Furthermore, comprehensive investigations integrating antagonistic activity, molecular characterization, microscopic evidence of mycoparasitism, host biochemical defense responses, multivariate analyses, and multi-season field validation remain scarce in potato late blight research. In particular, the combined contribution of direct antagonistic mechanisms, such as mycoparasitism and structural disruption of pathogen propagules, together with modulation of host antioxidant defense responses, remains insufficiently characterized in endophytic *Trichoderma* and *Streptomyces* associated with potato.

Therefore, the present study investigated indigenous endophytic *Trichoderma* and *Streptomyces* isolates recovered from healthy potato plants in Egypt using an integrated framework encompassing molecular identification, in vitro antagonistic assays, microscopic characterization, oxidative stress and antioxidant profiling, multivariate statistical analyses, and two-season field evaluation. Specifically, this study aimed to isolate and characterize effective endophytic bioagents, elucidate their mechanisms of pathogen suppression and host defense modulation, and assess their consistency and efficacy under field conditions. The findings provide mechanistic and applied insights into the development of sustainable, locally adapted biocontrol strategies that may reduce dependence on synthetic fungicides while supporting integrated disease management and sustainable potato production.

2. Materials and Methods

2.1. Isolation and Identification of Phytophthora infestans

In November 2022, potato leaves and stems showing typical late blight symptoms were collected from naturally infected plants grown in Beni-Suef Governorate, Egypt. Small tissue segments, approximately 0.5 cm, were excised from the advancing margins of lesions to include both symptomatic and apparently healthy tissue. The segments were surface-sterilized in 2% sodium hypochlorite for 2 min, rinsed three times with sterile distilled water, dried on sterile filter paper, and plated on potato dextrose agar (PDA). Plates were incubated at 25 °C for 7 days and examined regularly for pathogen growth. Emerging colonies showing morphological features consistent with *Phytophthora* spp. were purified by hyphal-tip or single-spore isolation. Preliminary identification was based on colony morphology, mycelial characteristics, and sporangial features according to standard descriptions and [13]. Pathogenicity was confirmed by Koch's postulates, and the pathogen was re-isolated from artificially infected potato tissues [14].

2.2. Isolation of Endophytic Bioagents from Potato Tissues

Healthy, asymptomatic 'Lady Rosetta' potato plants were collected from the same agroecological region. Leaves, stems, and roots were washed thoroughly under running tap water, cut into small segments, and surface-sterilized sequentially using 95% ethanol,

2% sodium hypochlorite, and 95% ethanol. The tissues were then rinsed three times with sterile distilled water and dried under aseptic conditions. To verify surface sterilization efficiency, aliquots from the final rinse water were plated on PDA and nutrient agar.

Sterilized tissue segments were macerated aseptically and plated on PDA for fungal endophytes and nutrient agar or starch nitrate agar for actinomycetes. Plates were incubated at 25–27 °C for 5–7 days. Emerging colonies were purified and grouped according to their morphological and cultural characteristics [14]. A total of 39 endophytic isolates were recovered, comprising 37 putative *Trichoderma* isolates and two putative *Streptomyces* isolates. Based on preliminary screening, eight representative *Trichoderma* isolates and two *Streptomyces* isolates were selected for further evaluation.

The isolates selected for field experiments and subsequent mechanistic analyses were those demonstrating the strongest in vitro antagonistic activity against *P. infestans*.

Specifically, the following isolates were selected: *Streptomyces parvus* (Act 28), *Streptomyces globisporus* (Act 29) and *Trichoderma* isolates T1–T8. These same isolates were subsequently used for microscopy studies, antioxidant enzyme analyses, lipid peroxidation measurements, phenolic content determination, proline assays, and field evaluation.

2.3. In Vitro Antagonistic Activity Against P. infestans

The antagonistic activity of 37 putative *Trichoderma* isolates and two putative *Streptomyces* isolates was evaluated against *P. infestans* using a dual-culture assay. A 5 mm mycelial disc of actively growing *P. infestans* was placed near one edge of a PDA plate. For *Trichoderma* treatments, a 5 mm disc from a young culture was placed on the opposite side of the same plate. For *Streptomyces* treatments, the bacterial isolate was streaked on the opposite side using a sterile loop [14]. Control plates contained only *P. infestans.* Each treatment was replicated three times and incubated at 22 ± 2 °C for 7 days.

Pathogen radial growth was measured and compared with the untreated control. The percentage inhibition of mycelial growth was calculated as follows:

$$\text{Inhibition (\%)} = [(C - T)/C] \times 100$$

where C is the radial growth of *P. infestans* in the control and T is the radial growth of *P. infestans* in the presence of the bioagent.

2.4. Molecular Identification of Selected Bioagents

Selected high-performing *Streptomyces* isolates were grown in broth culture, and genomic DNA was extracted using a standard bacterial DNA extraction protocol. The 16S rRNA gene was amplified using universal bacterial primers 27F and 1492R. PCR products were purified and sequenced commercially. The resulting sequences were compared with reference sequences available in the NCBI GenBank database using BLAST (v2.14.1).

For selected *Trichoderma* isolates (three isolates), genomic DNA was extracted from seven-day-old cultures grown on PDA. The internal transcribed spacer region of rDNA was amplified using universal ITS primers. Purified PCR products were sequenced bidirectionally, and the resulting sequences were compared with reference sequences in GenBank. Multiple sequence alignment was performed using ClustalW (v2.1) implemented in MEGA X software (v10.2.6). Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method with the Kimura two-parameter model, and branch support was estimated using 1000 bootstrap replicates. Closely related reference sequences retrieved from NCBI GenBank were included in the analysis, and outgroup taxa were selected to root the phylogenetic trees. Accession numbers were deposited or retrieved for all identified isolates.

2.5. Preparation of Bioagent Inocula

For *Trichoderma atrobrunneum*, 5 mm mycelial discs from four-day-old cultures were transferred into glucose fermentation medium and incubated at 25 °C for 12 days on a rotary shaker at 170 rpm. The resulting suspension was filtered and adjusted to approximately 1.5×10^8 CFU mL^{-1} before application.

For *Streptomyces* isolates, cultures were grown in starch nitrate broth at 28 °C for 7 days on a rotary shaker at 180 rpm. The cell suspension was adjusted to approximately 1.5×10^8 CFU mL^{-1}. All bioagent suspensions were prepared immediately before field application.

2.6. Field Evaluation of Bioagent Efficacy

Field experiments were conducted during the 2022 and 2023 growing seasons at the Experimental Farm of Sids Agricultural Research Station, Beni-Suef Governorate, Egypt. The experiment was designed to evaluate the efficacy of selected endophytic bioagents against potato late blight and their effects on plant growth and tuber yield. The labels T1–T8 represent treatment codes corresponding to the eight selected fungal isolates.

Twelve treatments were evaluated:

1. Act 28: *Streptomyces parvus*;
2. Act 29: *Streptomyces globisporus*;
3. T1: *Trichoderma* isolate T1;
4. T2: *Trichoderma* isolate T12;
5. T3: *Trichoderma* isolate T15;
6. T4: *Trichoderma* isolate T21;
7. T5: *Trichoderma* isolate T22;
8. T6: *Trichoderma* isolate T27;
9. T7: *Trichoderma* isolate T28;
10. T8: *Trichoderma* isolate T30;
11. Mancozeb fungicide treatment;
12. Untreated control.

The experiment was arranged in a randomized complete block design with three replicates per treatment. Each plot measured 3 × 3.5 m. Bioagent suspensions were applied as foliar sprays. Mancozeb was applied according to the manufacturer's recommended dose. Disease incidence, disease severity, growth traits, and yield parameters were recorded during both seasons.

2.7. Disease Incidence and Severity Assessment

Disease incidence was calculated as the percentage of infected plants relative to the total number of plants assessed in each plot.

Disease severity was estimated using a 0–4 scale, where 0 = no visible symptoms, 1 = less than 25% leaf area affected, 2 = 26–50% affected, 3 = 51–75% affected, and 4 = 76–100% affected. Disease severity percentage was calculated as follows:

$$\text{Disease severity (\%)} = [\Sigma\ (n \times \text{r})/(N \times \text{R})] \times 100$$

where n is the number of leaves in each rating class, r is the disease rating, N is the total number of assessed leaves, and R is the maximum rating value.

2.8. Light and Scanning Electron Microscopy Analysis of Bioagent–Pathogen Interactions

Light microscopy and scanning electron microscopy (SEM) were employed to investigate the antagonistic interactions between selected endophytic bioagents and *Phytophthora*

infestans. Samples were collected from the interaction zones of dual-culture assays at different incubation periods. For light microscopy, excess agar was carefully removed, and specimens were stained with 1% safranin solution for 5 min before mounting on glass slides. Samples were examined under a light microscope at different magnifications to observe morphological changes associated with antagonistic activity, including hyphal coiling, attachment, penetration, sporangial deformation, and pathogen cell disruption.

For SEM analysis, samples from the interaction zones were fixed in 2.5% glutaraldehyde for 18 h at 4 °C, washed repeatedly with 0.1 M phosphate buffer (pH 7.2), and post-fixed in 1% osmium tetroxide. Samples were subsequently dehydrated through a graded ethanol series, dried, mounted on aluminum stubs, and sputter-coated with gold prior to examination using a scanning electron microscope. SEM observations were performed to characterize ultrastructural alterations induced by the antagonistic isolates, including hyphal deformation, collapse, mycoparasitic penetration, and structural disruption of *P. infestans*. Combined microscopic analyses provided detailed insights into the mechanisms underlying antagonistic interactions between the selected bioagents and the pathogen [15].

2.9. Antioxidant Enzyme Assays

Fresh leaf tissue (1 g) collected from each treatment was homogenized in 3 mL of potassium phosphate buffer (10 mM, pH 7.0) containing 4% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000× *g* for 20 min at 4 °C, and the resulting supernatant was used as the crude enzyme extract. Total soluble protein concentration was determined according to the Bradford method [16].

Catalase (CAT) activity was assayed by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. One unit (U) of CAT activity was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per minute under assay conditions and was expressed as U mg^{-1} protein min^{-1}.

Peroxidase (POD) activity was determined according to Dias and Costa (1983) [17]. by measuring guaiacol oxidation at 470 nm. One unit (U) of POD activity was defined as the amount of enzyme catalyzing the formation of 1 µmol of tetraguaiacol per minute and was expressed as U mg^{-1} protein min^{-1}.

2.10. Lipid Peroxidation Assay

Lipid peroxidation was estimated by measuring malondialdehyde content using the thiobarbituric acid method. Fresh leaf tissue, 500 mg, was homogenized in 0.1% trichloroacetic acid and centrifuged at 10,000× *g* for 20 min [18]. An aliquot of the supernatant was mixed with 0.5% thiobarbituric acid prepared in 20% trichloroacetic acid. The mixture was heated in a boiling water bath for 30 min, rapidly cooled, and centrifuged again at 10,000× *g* for 5 min. Absorbance was measured at 532 nm, and nonspecific absorbance was corrected when required.

2.11. Biochemical Analyses

Total phenolic content: Total phenolic content was determined in ethanolic leaf extracts using the Folin–Ciocalteu reagent (MilliporeSigma, Saint Louis, MO, USA). Absorbance was measured at 765 nm, and results were expressed as milligrams of gallic acid equivalents per gram of fresh weight [19].

Proline content: Proline content was determined using the acid ninhydrin method. Fresh leaf tissue, 0.5 g, was homogenized in 3% sulfosalicylic acid and filtered. The filtrate was reacted with acid ninhydrin and glacial acetic acid, heated at 100 °C for 1 h, and then cooled. The reaction mixture was extracted with toluene, and absorbance was measured at 520 nm. Proline concentration was calculated using a standard curve [19].

2.12. Statistical Analysis

Data were collected from two independent growing seasons using three biological replicates per treatment. Disease incidence and severity were assessed following successive spray applications, whereas growth and yield parameters were recorded at the appropriate developmental stage or at harvest. Data were analyzed using analysis of variance (ANOVA) to evaluate treatment effects. For field experiments conducted across two growing seasons, treatment, season, and their interaction were included as fixed factors in a two-way ANOVA model. Prior to analysis, normality and homogeneity of variance assumptions were evaluated using the Shapiro–Wilk and Levene tests, respectively. When significant effects were detected, means were separated using Fisher's least significant difference (LSD) test at $p \leq 0.05$. Results are presented as mean $\pm$ standard error (SE). Statistical analyses were performed using SPSS software (Version 27.0; IBM Corp., Armonk, NY, USA).

To facilitate integrative comparison among treatments, mean values were standardized within each season using Z-score transformation. Disease incidence and severity variables were sign-inverted prior to standardization so that higher standardized values consistently represented improved treatment performance (i.e., lower disease levels and enhanced agronomic responses). Principal component analysis (PCA) was performed on centered and scaled trait means using standardized variables, and eigenvalues > 1 were used to identify major components contributing to total variation. Heatmaps based on standardized values were generated to summarize treatment performance across disease, physiological, growth, and yield traits.

3. Results

3.1. Isolation and Identification of Fungal Pathogen

A number of *Phytophthora* species, such as *P. infestans*, cause leaf blight in plants like potato. As shown in Figure 1A–C, symptoms begin as small flecks that expand into large lesions within 3–5 days. The initially water-soaked tissue turns necrotic (brown or black), often surrounded by a light green halo. The chosen leaf and stem tissue should ideally comprise both diseased and healthy parts. After surface sterilization, the tissue must be placed on an appropriate selective medium, with regular examination of the plates for the slow emergence of non-septate hyphae as shown in Figure 1D,E.

3.2. Identification of Fungal Endophytes Using Morphological and Molecular Markers

The colony appearance and sporulation patterns were recorded from cultures grown on PDA or cornmeal at 25 °C in darkness at the Mycological Center of Assiut University (AUMC), Faculty of Science, located in Assiut, Egypt. Molecular identification and phylogenetic analysis of selected endophytic bioagents confirmed the taxonomic affiliation of the most effective antagonistic isolates (Figure 2). Phylogenetic analysis based on 16S rRNA gene sequences identified the bacterial isolates as *Streptomyces globisporus* (strain AUMC B-569) and *Streptomyces parvus* (Act 28; accession no. PX414630), showing 99.93% and 99.13% sequence identity, respectively, with their corresponding type strains retrieved from GenBank, each with 100% query coverage (Figure 2A). Similarly, ITS rDNA-based phylogenetic analysis classified the fungal isolates FMF1 (PX417884), FMF2 (PX417885), and FMF3 (PX417889) as *Trichoderma atrobrunneum*, exhibiting 100% sequence identity and 97% query coverage with the reference strain *T. atrobrunneum* CBS124620 (accession no. NR_137304) (Figure 2B). The generated phylogenetic trees showed clustering of the recovered isolates with closely related reference sequences, supporting their molecular identification (Table S1). Arrowheads indicate isolates obtained in the present study.

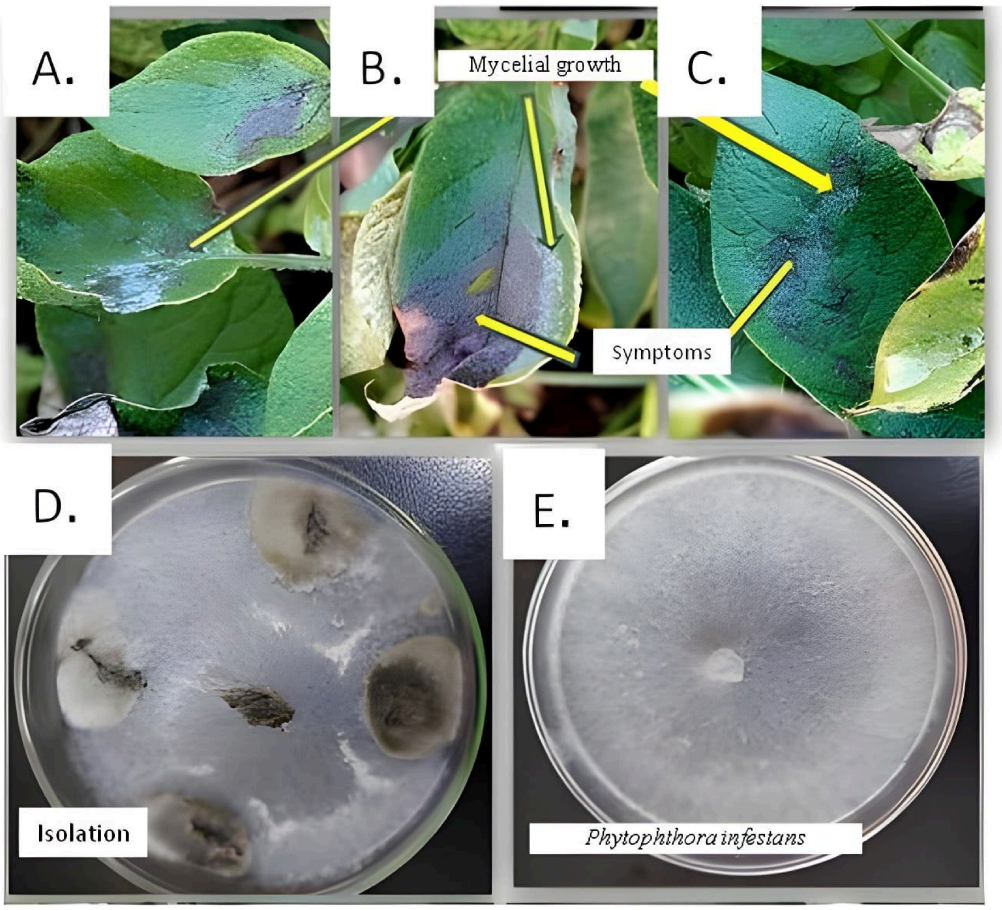


Figure 1. Isolation and symptoms of *Phytophthora infestans* associated with potato late blight. (**A**–**C**) Symptoms of late blight on potato leaves showing necrosis, mycelial growth, and sporangial development (**D**) Pure culture of *P. infestans* on PDA; (**E**) Isolation from infected potato tissue.

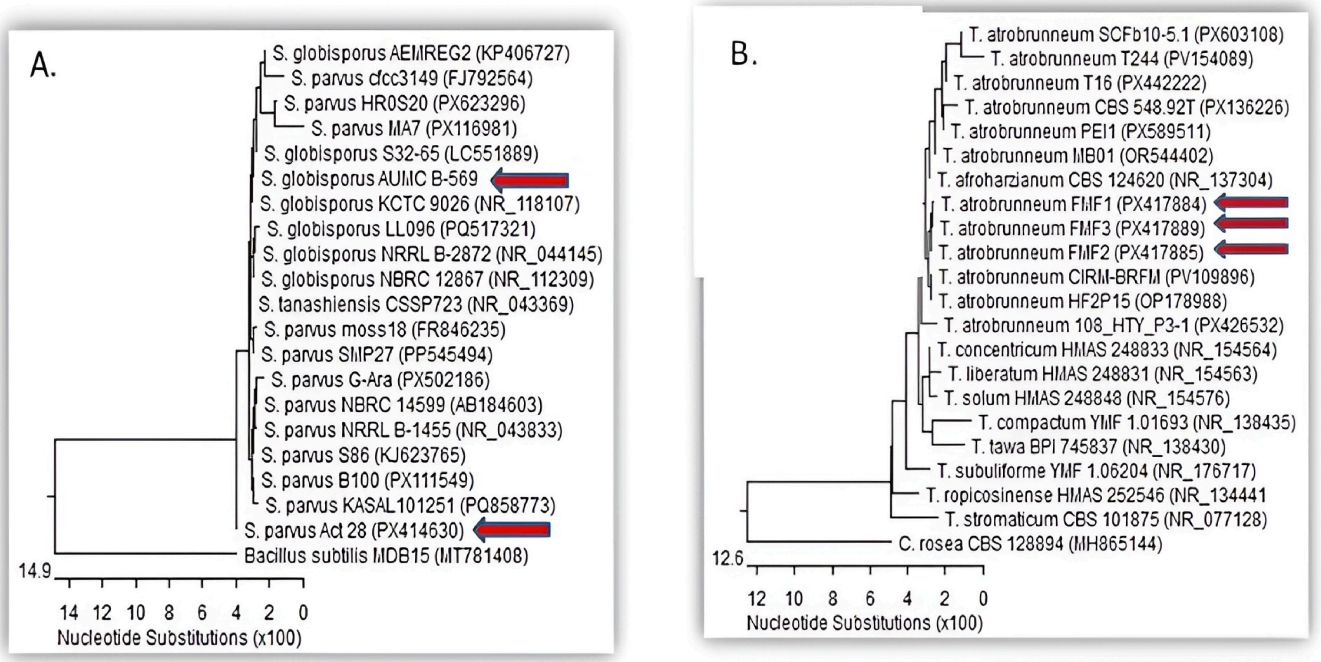


Figure 2. Molecular identification of selected endophytic bioagents based on phylogenetic analysis. (**A**) Phylogenetic relationships of the bacterial isolates *Streptomyces globisporus* (strain AUMC B-569)

and *Streptomyces parvus* (Act 28; accession no. PX414630) inferred from 16S rRNA gene sequences and aligned with closely related reference strains retrieved from GenBank. *Streptomyces globisporus* showed 99.93% sequence identity and 100% query coverage with the type strain NBRC 12867 (accession no. NR_112309), whereas *S. parvus* exhibited 99.13% identity and 100% coverage with the type strain NRRL B-1455 (accession no. NR_043833). *Bacillus subtilis* was used as the outgroup. (**B**) Phylogenetic relationships of the fungal isolates *Trichoderma atrobrunneum* strains FMF1 (accession no. PX417884), FMF2 (PX417885), and FMF3 (PX417889), inferred from ITS rDNA sequences and aligned with closely related reference strains from GenBank. The isolates exhibited 100% sequence identity and 97% query coverage with the type strain *Trichoderma atrobrunneum* CBS124620 (accession no. NR_137304). *Clonostachys rosea* was included as the outgroup. Arrowheads indicate isolates recovered in the present study.

3.3. In Vitro Antagonistic Activity of Endophytic Bioagents

Thirty-seven *Trichoderma* sp. isolates and two *Streptomyces* sp. isolates, obtained from healthy potato tissues in Beni Suef, Egypt, were evaluated for antagonistic activity against *Phytophthora infestans*. All tested isolates significantly suppressed pathogen growth compared with the untreated control (9.0 cm).

The highest antifungal activity was observed in Act 29 and T4, which reduced mycelial growth by 82.56%, with corresponding diameters of 1.57 ± 0.07 cm. Similarly, Act 28 and T1 achieved an inhibition rate of 81.44%, with growth reduced to 1.67 ± 0.20 cm. Additional isolates, including T8, T3, T6, T7, T2, and T5, also demonstrated substantial suppression, with inhibition percentages ranging from 77.78% to 79.67% (Tables 1 and S2). Based on Fisher's LSD test ($p \leq 0.05$), isolates could be grouped into performance classes: highly effective (Act 29, T4, Act 28, T1), moderately high (T3, T6–T8), moderate (T2, T5, T19, T23, T26, T33, T35), low–moderate (T9–T10, T12–T18, T20–T21, T24–T32, T34, T36–T37), and least effective (T11, T22) (Table S2). This grouping highlights a small subset of bioagents with the strongest potential for in vivo and field-level evaluation.

Table 1. Antagonistic effect of selected *Trichoderma* and *Streptomyces* isolates against *Phytophthora infestans*.

Category	Isolates (Examples)	Linear Growth (cm)	Growth Reduction (%)
Highly effective	Act 29, T4	1.57 ± 0.07	82.56
	Act 28, T1	1.67 ± 0.20	81.44
Moderately high	T3, T6–T8	1.83–1.90	78.89–79.67
Moderate	T2, T5, T19, T23, T26, T33, T35	2.00 ± 0.11–0.29	77.78
Low–moderate	T9–T10, T12–T18, T20–T21, T24–T32, T34, T36–T37	2.13–2.50	71.11–76.33
Least effective	T11, T22	3.00 ± 0.12	66.67

Control (untreated): 9.0 cm (0% reduction) Values are mean ± SE (n = 3). Isolates sharing similar letters in the original analysis were not significantly different according to Fisher's LSD test ($p \leq 0.05$).

3.4. Mechanisms of Antagonism and Field Suppression of Phytophthora infestans by Selected Endophytic Bioagents

Selected endophytic *Trichoderma* and *Streptomyces* isolates exhibited pronounced antagonistic activity against *Phytophthora infestans*, as demonstrated by dual culture assays and microscopic analyses (Figure 3). Dual culture assays revealed marked inhibition of *P. infestans* mycelial growth by *Trichoderma* isolates (T1–T8) and *Streptomyces* isolates (Act. 28 and Act. 29) compared with untreated controls. Microscopic observations showed sequential antagonistic interactions, including hyphal attachment, coiling around pathogen hyphae, and direct penetration of *P. infestans* structures by *Trichoderma atrobrunneum*. Morphological alterations in pathogen propagules included sporangial deformation, swelling, collapse, lysis, and zoospore release. Light microscopy further demonstrated coating and colonization of *P. infestans* hyphae and sporangia by antagonist structures, accompanied

by progressive structural degradation. These observations were confirmed by scanning electron microscopy (SEM), which revealed extensive deformation, collapse, and disruption of *P. infestans* hyphae in treated samples compared with the intact morphology observed in untreated controls (Figure 3). Collectively, microscopic analyses indicated substantial structural damage to pathogen hyphae and sporangia following interaction with the selected antagonistic isolates.

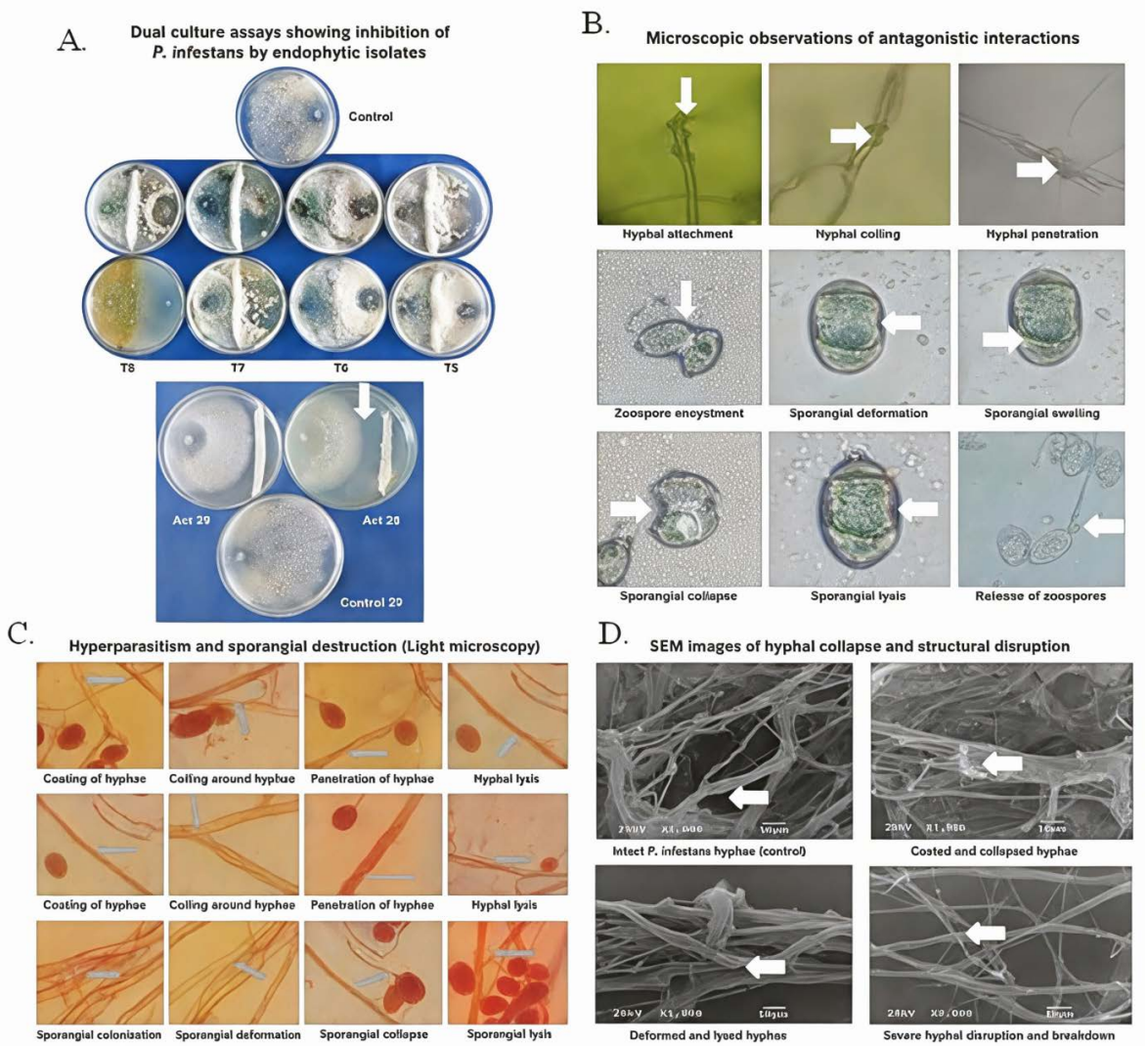


Figure 3. Mechanisms of antagonism exhibited by endophytic *Trichoderma* spp. and *Streptomyces* spp. against *Phytophthora infestans*. Dual culture assays (top left: (**A**)) demonstrate the inhibitory effects of selected *Trichoderma* isolates (T1–T8) and *Streptomyces* isolates (Act 28 and Act 29) on the mycelial growth of *P. infestans* compared with untreated controls. Microscopic observations (top right, (**B**)) illustrate sequential antagonistic interactions, including hyphal attachment, coiling, penetration, zoospore encystment, sporangial deformation, swelling, collapse, lysis, and release of zoospores. Light microscopy images (bottom left; (**C**)) reveal hyperparasitism of *P. infestans* hyphae and sporangia by *Trichoderma atrobrunneum*, showing coating and coiling around pathogen hyphae, direct penetration, hyphal lysis, sporangial colonization, deformation, collapse, and structural disruption. Scanning electron microscopy (SEM) images (bottom right; (**D**)) further confirm antagonistic activity through visualization of intact control hyphae compared with coated, collapsed, deformed, and severely disrupted *P. infestans* hyphae following interaction with antagonistic bioagents. These observations collectively indicate that the selected endophytic isolates suppress *P. infestans* through multiple mechanisms, including mycoparasitism, direct penetration, and structural degradation of pathogen propagules.

Based on their superior in vitro antagonistic activity, ten endophytic isolates were selected for biochemical assays and multi-season field evaluation against potato late blight.

Under field conditions, treatments with endophytic bioagents generally reduced disease incidence and severity relative to untreated controls, although treatment efficacy varied between seasons (Table 2; Figure 4). During the 2022 season, *Trichoderma* isolate T4 exhibited the strongest performance, recording the lowest disease incidence (3.00 ± 0.00%), followed by T8 (4.67 ± 1.68%), T5 (4.89 ± 1.60%), and T3 (5.11 ± 1.24%). The chemical fungicide mancozeb showed comparable efficacy, with a disease incidence of 6.33 ± 1.20%. Similarly, T4 demonstrated the greatest reduction in disease severity (2.18 ± 1.31%), followed by mancozeb (2.70 ± 0.79%) and T1 (2.93 ± 0.18%). Other isolates, including the evaluated *Streptomyces* strains, exhibited moderate disease suppression with no significant differences among them. During the 2023 season, T1, T4, T8, and mancozeb remained among the most effective treatments for reducing disease incidence and severity, although treatment rankings differed from those observed in 2022. Across both growing seasons, several treatments, including Act. 28, Act. 29, T1, and T4, consistently maintained comparatively low disease levels relative to untreated controls. Overall, selected *Trichoderma* isolates exhibited sustained disease suppression comparable to chemical control, indicating stable efficacy under field conditions (Table 2; Figure 4).

Table 2. Representative effects of selected endophytic treatments on key growth and yield traits of potato across the 2022–2023 growing seasons (mean ± SE).

Treatment	Plant Height (cm)	Tuber Weight/Plant (g)	Yield/Plot (kg)	Plant Height (cm)	Tuber Weight/Plant (g)	Yield/Plot (kg)
	2022			2023		
Act. 29	53.11 ± 4.71	1136.27 ± 181.29	13.00 ± 3.61	62.60 ± 1.24	884.02 ± 52.14	21.37 ± 1.96
Trich. 1	50.94 ± 5.07	1451.28 ± 557.99	13.83 ± 2.17	63.60 ± 1.04	515.34 ± 42.17	7.67 ± 1.85
Trich. 4	55.78 ± 2.89	1519.49 ± 216.46	17.73 ± 6.60	66.01 ± 1.46	1360.55 ± 73.35	8.43 ± 2.31
Mancozeb	53.33 ± 2.14	1156.69 ± 270.14	13.33 ± 2.91	55.06 ± 1.95	1400.38 ± 83.78	9.74 ± 2.97
Control	48.34 ± 7.12	701.55 ± 46.23	10.00 ± 2.52	46.70 ± 1.82	1505.31 ± 171.69	15.98 ± 0.88

Values are mean ± SE. Complete treatment responses and statistical comparisons are provided in Supplementary Tables S3 and S4. Surface are of the ploy is 10.5 m^2.

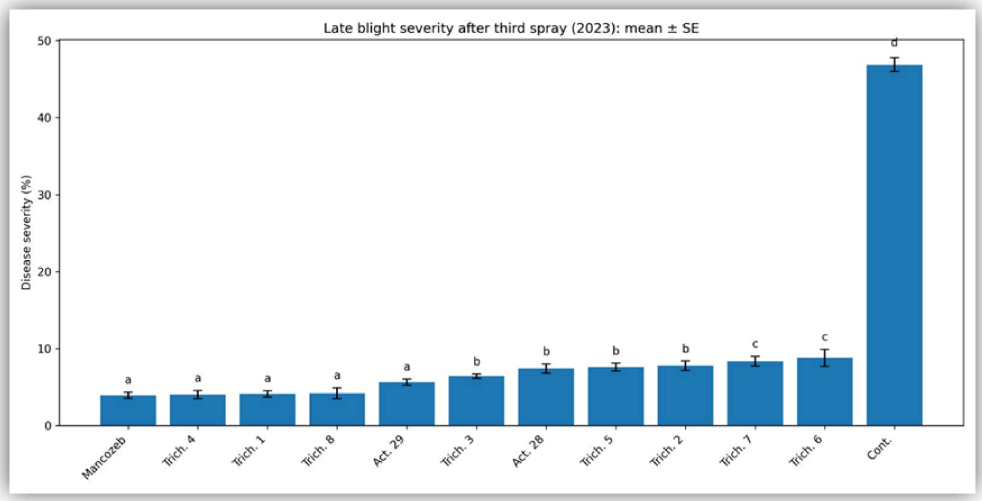


Figure 4. Effect of endophytic bioagents on late blight severity (%) after the third spray during the 2023 season. Values represent mean ± SE; different letters indicate significant differences (LSD, $p \leq 0.05$).

3.5. Field Efficacy of Selected Endophytic Bioagents Against Potato Late Blight Under Two Growing Seasons

The effects of endophytic bioagents on potato late blight incidence and severity exhibited generally consistent patterns across the 2022 and 2023 growing seasons, although treatment efficacy varied among isolates (Table 2). Untreated plants consistently recorded the highest disease incidence and severity, with disease severity increasing from 23.29% in 2022 to 31.64% in 2023, indicating greater disease pressure during the second season. In contrast, several treatments, particularly Act. 28, Act. 29, and Trich. 4, maintained comparatively low disease incidence and severity across both seasons, suggesting stable disease suppression under field conditions.

Field evaluations demonstrated that all tested endophytic bioagents significantly reduced late blight incidence and severity compared with the untreated control (Table 2). In 2022, the lowest disease incidence was observed in Trich. 5 (12.63%), followed by Act. 29 (14.25%), Act. 28 (14.39%), and Trich. 4 (14.41%), whereas untreated plants exhibited the highest incidence (20.55%). Disease severity was markedly reduced by Trich. 4 (8.14%), *Trich.* 3 (8.78%), and Act. 29 (8.87%), compared with 23.29% in the control treatment. Similarly, during the 2023 season, Act. 28, Act. 29, and Mancozeb exhibited the lowest disease incidence (13.34–14.14%) and disease severity (9.15–10.28%), while untreated plants recorded the highest incidence (22.60%) and severity (31.64%) (Table 3).

Table 3. Effect of endophytic bioagent treatments on late blight incidence and severity in potato across the 2022 and 2023 growing seasons. Values are mean ± SE (n = 3). Different letters within each column indicate significant differences according to Fisher's LSD test at $p \leq 0.05$.

Treatment	Disease Incidence (%)	Disease Severity (%)	Disease Incidence (%)	Disease Severity (%)
	2022		2023	
Act. 28	14.39 ± 1.15 a	10.49 ± 1.74 a	13.34 ± 0.73 a	10.28 ± 1.04 a
Act. 29	14.25 ± 0.61 a	8.87 ± 0.12 a	14.14 ± 1.48 a	9.15 ± 0.52 a
Trich. 1	16.41 ± 0.55 b	9.10 ± 0.76 a	15.27 ± 0.73 a	12.04 ± 0.28 b
Trich. 2	17.59 ± 1.01 b	10.97 ± 0.73 a	17.19 ± 1.07 b	8.61 ± 0.55 a
Trich. 3	16.33 ± 0.68 b	8.78 ± 1.19 a	17.20 ± 0.47 b	13.31 ± 0.58 b
Trich. 4	14.41 ± 0.81 a	8.14 ± 1.51 a	17.77 ± 0.64 b	8.96 ± 0.98 a
Trich. 5	12.63 ± 2.58 a	11.17 ± 1.71 a	20.56 ± 0.92 c	15.07 ± 0.74 c
Trich. 6	14.98 ± 0.87 a	9.82 ± 2.25 a	20.44 ± 1.00 c	15.41 ± 0.71 c
Trich. 7	16.54 ± 1.21 b	9.74 ± 0.70 a	20.38 ± 0.79 c	13.63 ± 0.82 b
Trich. 8	18.95 ± 0.76 b	9.89 ± 0.72 a	17.37 ± 0.47 b	10.10 ± 1.14 a
Mancozeb	17.73 ± 1.24 b	10.91 ± 1.23 a	13.34 ± 0.73 a	10.28 ± 1.04 a
Control	20.55 ± 1.18 c	23.29 ± 1.59 b	22.60 ± 0.89 c	31.64 ± 1.97 d

Although some *Trichoderma* isolates displayed variable performance between seasons, selected endophytic treatments, particularly Act. 28 and Act. 29, consistently maintained low disease incidence and severity across both years, demonstrating stable field efficacy comparable to, or in some cases exceeding, the chemical fungicide treatment.

3.6. Effect of Endophytic Bioagents on Late Blight Severity After the Third Spray During the 2023 Season

The effects of selected endophytic bioagents on late blight severity following the third spray application during the 2023 season are presented in Figure 4. Unlike the season-wide disease assessments shown in Table 3, Figure 4 represents disease severity evaluated immediately after the third application, reflecting short-term treatment efficacy. All treatments significantly reduced disease severity compared with the untreated control ($p \leq 0.05$). The lowest disease severity values were recorded for Mancozeb (3.91%), followed by Trich.

4 (4.03%), Trich. 1 (4.10%), and Trich. 8 (4.18%), with no significant differences among these treatments (group a). Intermediate disease severity was observed for Act. 29, Trich. 3, Act. 28, Trich. 5, and Trich. 2 (5.58–7.79%), whereas Trich. 7 and Trich. 6 showed relatively higher severity values (8.37–8.84%) but remained significantly lower than the untreated control. The control treatment exhibited the highest disease severity (46.67%), indicating severe disease development in the absence of bioagent or fungicide application. The tested endophytic bioagents effectively suppressed late blight severity under field conditions, with several treatments demonstrating disease control comparable to the chemical fungicide treatment (Figure 4).

3.7. Effect of Endophytic Treatments on Growth and Yield Parameters

Selected endophytic treatments enhanced growth and yield parameters relative to untreated plants across both growing seasons (Table 4). Growth and yield responses varied among treatments and between seasons, with *Trichoderma* isolate 4 (Trich. 4) consistently promoting plant height and exhibiting superior performance for selected growth traits, particularly during the 2022 season. Act. 29 also demonstrated favorable agronomic performance, especially for yield-related traits in 2023. In contrast, untreated controls generally showed lower vegetative growth compared with selected bioagent treatments. Complete treatment responses are provided in Supplementary Tables S3 and S4, and Figure S1.

Table 4. Biochemical responses of potato leaves to endophytic bioagent treatments under late blight infection, showing changes in oxidative stress markers, antioxidant enzyme activities, and total phenolic content.

N.	Bioagents	Total Phenols (mg/g Dry Weight)	MDA	Proline	Enzymes U/mg Protein	
					CAT	POD
1	Act 28	3.28 ± 0.06	128.96 ± 6.67	0.0748 ± 0.0004	0.004321 ± 0.00001	0.91 ± 0.02
2	Act29	5.46 ± 0.09	68.2 ± 3.35	0.15 ± 0.006	0.0123 ± 0.00034	0.94 ± 0.02
3	T1	5.64 ± 0.09	95.48 ± 4.56	0.14 ± 0.005	0.00249 ± 0.00007	0.97 ± 0.02
4	T4	13.10 ± 0.18	62.00 ± 3.11576	0.09 ± 0.004	0.0123 ± 0.00035	0.95 ± 0.02
5	T8	5.82 ± 0.09	100.44 ± 5.34	0.07 ± 0.003	0.00238 ± 0.00007	0.95 ± 0.02
6	Control	11.11 ± 0.16	146.32 ± 7.98	0.17 ± 0.006	0.0236 ± 0.00007	0.91 ± 0.02

Values are mean ± SE.

3.8. Principal Component Analysis (PCA) of Growth and Yield Traits Across the 2022–2023 Growing Seasons

Principal component analysis (PCA) was performed to evaluate the relationships among growth and yield traits and their association with endophytic bioagent treatments averaged across the 2022–2023 growing seasons (Figure 5). The first two principal components explained 85.2% of the total variation, with PC1 and PC2 accounting for 52.2% and 33.0%, respectively. Yield and plant weight were positively associated and clustered in the upper-left quadrant, indicating a strong correlation between these traits. Similarly, plant height showed a positive association with plant weight, whereas tuber weight per plant and branch number clustered together in the lower-left quadrant.

Among treatments, Act. 29 was closely associated with increased yield and plant biomass traits, while Act. 28 and Trich. 6 were positioned in the positive PC1 region, indicating distinct influences on growth-related parameters. In contrast, untreated plants (Control) were separated from most treatments along PC1, reflecting a different growth and yield response profile. Several *Trichoderma* isolates exhibited variable distributions across quadrants, suggesting differential effects on growth and productivity traits.

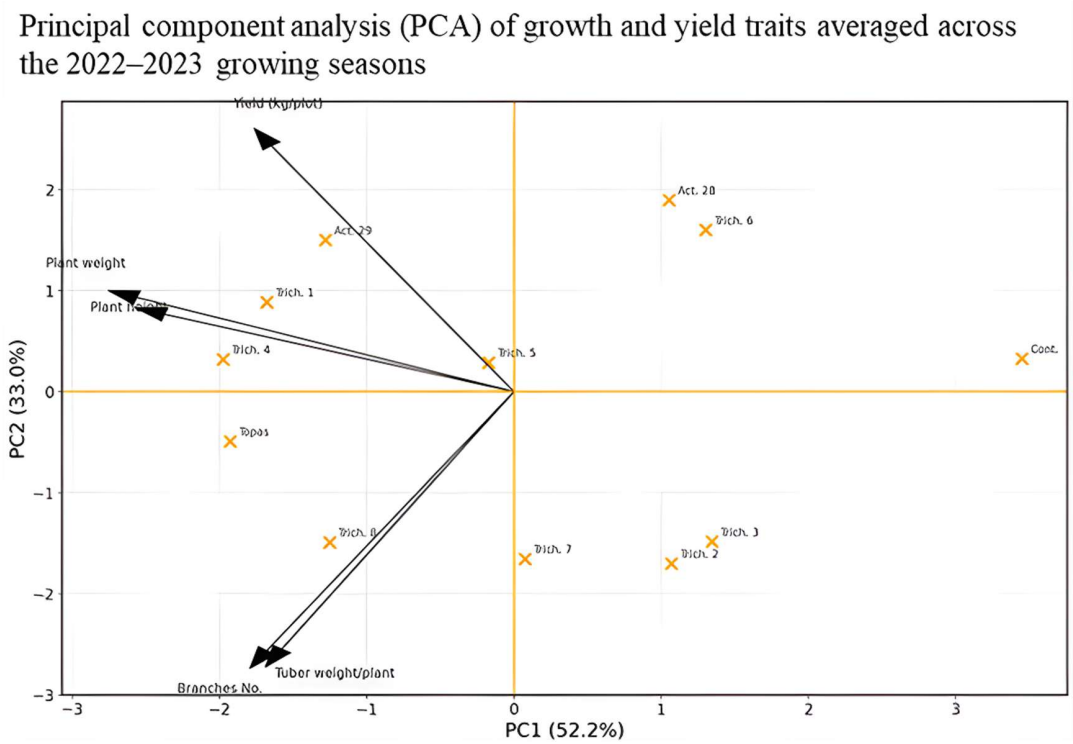


Figure 5. Principal component analysis (PCA) of growth and yield traits averaged across the 2022–2023 growing seasons. PC1 and PC2 explained 52.2% and 33.0% of the total variance, respectively. Arrows represent trait loadings, and treatment positions indicate associations with specific growth and yield characteristics.

3.9. Integrated Treatment Performance Across Seasons

Integrated treatment profiles based on standardized Z-scores revealed clear differences among bioagents in disease suppression, plant growth, and yield performance across the 2022 and 2023 growing seasons (Figure 6). Top-performing treatments, particularly Topas, *Trichoderma* isolate 4 (Trich. 4), and *Trichoderma* isolate 1 (Trich. 1), consistently exhibited favorable profiles characterized by reduced disease incidence and severity together with improved growth and yield traits. In contrast, untreated controls showed uniformly low Z-scores across most variables, indicating inferior overall performance. Although the magnitude of treatment responses varied between seasons, the relative ranking of high-performing treatments remained largely stable. The separation between seasonal profiles further suggests that environmental conditions influenced the contribution of disease suppression and agronomic traits to overall treatment efficacy. Nevertheless, selected endophytic bioagents maintained superior performance over untreated plants across both seasons, highlighting their sustained effectiveness under varying field conditions (Figure 6).

Tuber weight per plant in 2023 differed significantly among treatments based on LSD groupings, indicating a strong treatment effect on yield response under prevailing disease pressure. Several biocontrol treatments and the fungicide standard produced significantly greater tuber weights than the untreated control, which recorded the lowest mean value. These findings suggest that effective disease management contributed to enhanced tuber development and biomass accumulation.

3.10. Effect of Endophytic Bioagents on Oxidative Stress Markers and Antioxidant Responses in Potato Plants Under Late Blight Infection

Late blight infection induced pronounced oxidative stress in potato plants, as evidenced by elevated malondialdehyde (MDA), total phenolic compounds, and proline levels relative to healthy controls (Table 4). Application of endophytic bioagents, including Act. 28,

Act. 29, T1, T4, and T8, markedly mitigated oxidative damage. Among the evaluated treatments, T4 at 1000 mg/L produced the greatest reduction in MDA content (62.00 nmol/g), followed by Act. 29 and T1, indicating enhanced protection against lipid peroxidation and membrane damage. Similarly, proline accumulation, a key indicator of stress response, was significantly reduced in T4-treated plants (0.0886 µmol/g) compared with infected controls (0.1748 µmol/g), suggesting alleviation of pathogen-induced stress. Bioagent treatments modulated antioxidant enzyme activities in a treatment-dependent manner rather than inducing uniform increases or decreases. Variations in catalase (CAT) and peroxidase (POD) activities reflected differential regulation of antioxidant defense mechanisms, with T1 exhibiting the highest POD (0.965 U/mg protein) and CAT (0.00249 U/mg protein) activities among the tested treatments. In addition, total phenolic content decreased across all treated groups, with Act. 28 showing the lowest concentration (3.276 mg GAE/g) compared with infected controls (11.10 mg GAE/g). The reduction in MDA, proline, and phenolic content, together with treatment-specific modulation of antioxidant enzymes, indicates that endophytic bioagents alleviated oxidative stress and contributed to improved physiological stability in potato plants challenged with late blight infection (Table 4).

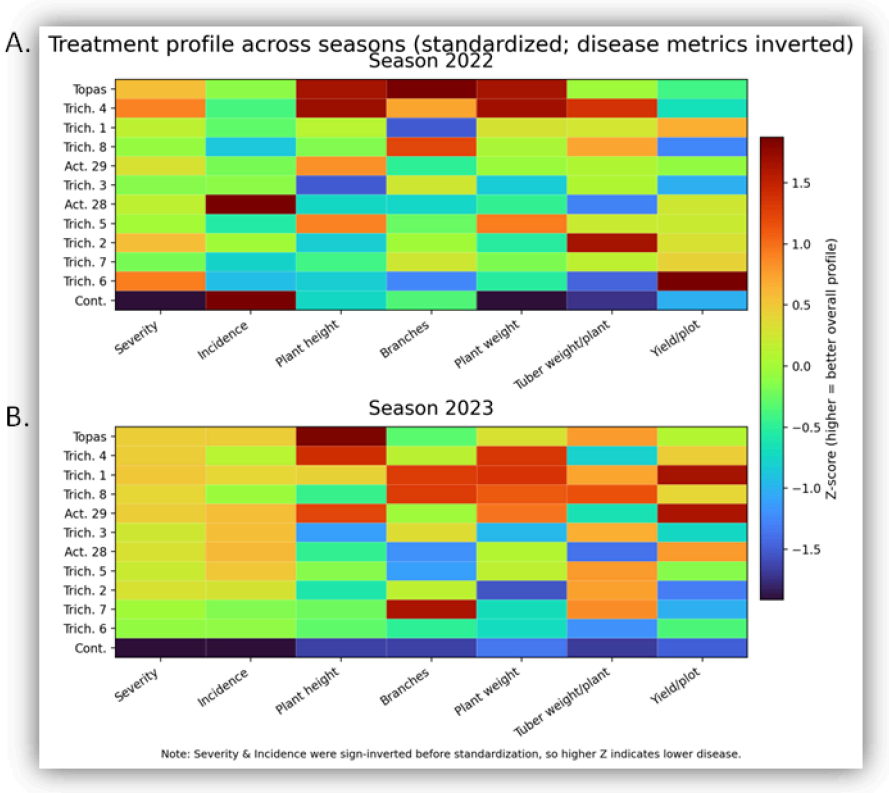


Figure 6. Integrated treatment performance of endophytic bioagents across two growing seasons (2022–2023) based on standardized Z-scores of disease, growth, and yield traits. Panel (**A**) represents treatment profiles during the 2022 season, while panel (**B**) represents treatment profiles during the 2023 season. Disease incidence and severity values were sign-inverted before standardization; therefore, higher Z-scores indicate lower disease pressure and improved overall treatment performance. Color gradients indicate relative performance across disease severity, disease incidence, plant height, branch number, plant weight, tuber weight per plant, and yield per plot.

4. Discussion

Effective management of potato late blight requires an integrated disease management (IDM) approach that combines complementary strategies, including cultural practices, host resistance, biological control, and judicious fungicide use. Biological control agents,

particularly *Trichoderma* spp., have attracted considerable attention due to their capacity to suppress *Phytophthora infestans* through multiple mechanisms, including direct antagonism via antibiotics and lytic enzymes, as well as induction of host defense responses that enhance systemic resistance. These multifaceted actions support the incorporation of *Trichoderma* into sustainable disease management programs.

In the present study, thirty-nine endophytic isolates belonging to *Trichoderma* and *Streptomyces* were recovered from healthy potato tissues and evaluated for antagonistic activity against *P. infestans*. White velvety growths, appearing at the lesion edges and primarily on the underside of leaves, indicate the presence of *Phytophthora*. This growth allows for the production of numerous sporangia spores, which may become airborne in dry conditions or transform into zoospores in wet conditions. Zoospores can form new lesions on the same plant or spread to neighboring plants through contact. Isolation of *P. infestans* is required special techniques because rather slowly in vitro compared with saprophytic fungi and bacteria which suppress the growth of *Phytophthora* by direct competition. Successful isolation of *P. infestans* from diseased plant tissue requires careful selection of freshly infected material, specifically from the edge of an actively growing lesion. Among these, selected *Trichoderma* isolates and two actinomycete strains, *Streptomyces globisporus* and *S. parvus*, exhibited the strongest inhibitory effects in both in vitro and field evaluations. Molecular identification of effective isolates was confirmed using ITS and 16S rDNA sequencing, following approaches described by Alhaithloul et al. [19].

Molecular identification and phylogenetic analysis confirmed the identity of the selected endophytic bioagents as bacterial isolates *Streptomyces parvus*, *Streptomyces globisporus* and *S. parvus*. Similarly, phylogenetic relationships identified the fungal isolates as *Trichoderma atrobrunneum* strains FMF1, FMF2 and FMF3 confirming the taxonomic placement of the selected endophytic bioagents. However, additional loci such as tef1-α, rpb2, and calmodulin may improve taxonomic resolution. Similarly, closely related species often require multilocus sequence analysis (MLSA) or whole-genome sequencing for definitive identification.

The observed antagonistic activity agrees with previous studies demonstrating that *Trichoderma* spp. effectively inhibit *P. infestans* growth through production of antifungal metabolites, antibiotics, and cell wall–degrading enzymes [20,21]. Earlier investigations also confirmed the ability of *Trichoderma* spp. to parasitize and lyse *Phytophthora* species) [22–24]. Similarly, Loliam et al. [25] reported strong antagonistic activity of *Streptomyces* rubrolavendulae against *P. infestans*, with inhibition rates reaching 83.33%. Beyond potato pathosystems, *Trichoderma* spp. have demonstrated broad-spectrum biocontrol efficacy against other phytopathogens, including *Alternaria alternata*, through mechanisms involving mycoparasitism, nutrient competition, and production of antifungal compounds such as gliotoxin and trichodermin [26,27].

Microscopic analyses in the present study identified two distinct antagonistic mechanisms employed by *T. atrobrunneum* against *P. infestans*. Initially, *T. atrobrunneum* formed dense coils around pathogen hyphae, followed by penetration and structural disruption leading to hyphal collapse. SEM observations further revealed wrinkling, deformation, and destruction of *P. infestans* hyphae. These findings are consistent with reports that *Trichoderma* species suppress *P. infestans* through mycoparasitism and antibiosis [11,28]. It has been reported that *Trichoderma* spp. were used as biological control agent for potato late blight. In another study, antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao* [28]. The sensitivity of *P. infestans* to cell wall–degrading enzymes, including cellulases, pectinases, and proteases produced by *Trichoderma* species, may further explain the structural degradation observed in this study [29–32].

In addition to direct antagonism, selected bioagents modulated antioxidant responses and reduced oxidative stress markers associated with late blight infection. Lower concentrations of MDA and proline in treated plants suggest mitigation of pathogen-induced oxidative damage, whereas treatment-dependent variations in CAT and POD activities indicate differential regulation of antioxidant defense mechanisms. Similar responses have been reported following *Streptomyces*-mediated activation of induced systemic resistance, which enhances activities of defense-related enzymes and promotes accumulation of phenolic compounds involved in pathogen restriction [10]. Likewise, *Trichoderma* species induce production of defense enzymes such as peroxidase, chitinase, and glucanase, thereby strengthening host resistance [33]. The *T. harzianum* fungus has been found to enhance the protective defenses of host plants by stimulating the production of various protective enzymes, such as peroxidase, chitinase, and glucanase, which serve against pathogens [33]. This indicates that the protective mechanisms enacted by *T. harzianum* are a result of its interaction with the host plants. Additionally, similar to other species within the *Trichoderma* genus, *T. harzianum* is capable of producing antibiotics and enzymes that contribute to the breakdown of cell walls, specifically glucanase, chitinase, and cellulase [30].

Across two growing seasons (2022–2023), all tested treatments suppressed late blight development relative to untreated controls, although treatment efficacy varied among years and disease pressure levels. Selected isolates, particularly T1, T4, and T8, maintained disease suppression comparable to the chemical fungicide mancozeb under field conditions. The consistency of disease suppression across seasons is notable given the strong influence of environmental conditions on late blight epidemics and supports the robustness of these bioagents under variable disease pressure [34,35]. It has been discussed how *Streptomyces* spp. activate both induced and systemic resistance in plants, leading to heightened defense through increased production of defense-related enzymes like peroxidase, polyphenoloxidase, and chitinase [10]. This process also boosts the accumulation of total free phenolic compounds and antioxidant enzymes in leaves, fortifying the cell wall against phytopathogenic fungi and facilitating the oxidation of phenolic compounds to mycotoxic quinones.

The observed reduction in disease severity was accompanied by improvements in selected growth and yield traits. *Trichoderma* species are known to enhance nutrient acquisition, root colonization, and micronutrient availability, thereby promoting plant growth and productivity [11]. Compounds such as harzianic acid and gibberellins produced by *Trichoderma* contribute to improved root development, mineral uptake, and biomass accumulation [36]. Previous studies have also linked *Trichoderma* inoculation with enhanced physiological performance and improved crop quality traits [37,38]. In the present study, however, responses of specific vegetative traits varied among treatments and seasons, indicating that growth promotion effects may depend on environmental conditions and baseline soil fertility.

The multifunctional nature of *Trichoderma* and *Streptomyces* likely explains their simultaneous effects on disease suppression and plant performance. These microorganisms operate through complementary mechanisms including competition, antibiosis, mycoparasitism, enhancement of nutrient availability, and induction of systemic resistance [39,40]. Such multifunctionality supports their integration into sustainable disease management frameworks aimed at reducing reliance on repeated fungicide applications. Resistance-management principles emphasizing diversification of control strategies and reduced chemical selection pressure further reinforce the value of biological control within integrated pest management programs [41]. This need is increasingly important given emerging fungicide resistance in *P. infestans*, including carboxylic acid amide resistance alleles detected through droplet digit PCR (ddPCR)-based approaches [41].

Despite the promising antagonistic activity and field performance observed, several limitations should be addressed. This study was conducted within a single geographical region in Beni-Suef governorate, over two growing seasons, which may limit extrapolation of the findings to broader agroecological conditions. In addition, species-level identification of some *Streptomyces* isolates based solely on 16S rRNA sequences should be interpreted cautiously because closely related species may require multilocus sequence analysis or whole-genome comparisons for definitive taxonomic resolution. Across the two seasons (2022/2023) and the three sequential spray assessments, the figures collectively indicate that the tested treatments suppressed late blight development relative to the untreated control, with clearer contrasts under the higher disease pressure observed in 2023. This pattern is epidemiologically important because late blight epidemics are strongly driven by seasonal variability in infection-conducive conditions, and disease progress often accelerates over time when inoculum and weather align. Such season-dependent shifts in epidemic behavior have been emphasized in late blight forecasting and multi-country syntheses, where robustness and temporal consistency of disease risk are central considerations [34,35]. From a mechanistic plant-pathology perspective, the combined pattern of disease reduction together with improved agronomic traits is consistent with the multifunctional nature of prominent biocontrol fungi and actinomycetes. It has been reported that *Trichoderma* as a biocontrol genus acting through multiple, often complementary mechanisms competition, antibiosis, mycoparasitism, and induction of systemic resistance features that plausibly translate into both lower disease expression and better plant performance under field conditions [39]. In parallel, Khan et al. (2023) highlighted *Streptomyces* as a rich source of antimicrobial metabolites with broad biocontrol potential, supporting a biological explanation for consistent suppression across treatments and seasons when rhizosphere establishment is successful [40]. These outcomes also support an IPM framing where effective biological options can help reduce reliance on repeated fungicide inputs and thereby contribute to fungicide stewardship. Core resistance-management principles such as limiting repeated selection pressure and integrating non-chemical tactics—have been synthesized in fungicide resistance management reviews [41]. In late blight specifically, the need for monitoring and resistance-aware programs is reinforced by molecular work demonstrating ddPCR-based detection of CAA resistance alleles in *Phytophthora infestans*, enabling more responsive management decisions [42].

Although the selected bioagents exhibited sustained disease suppression and modulation of host antioxidant responses, persistence and colonization efficiency within potato tissues and the rhizosphere were not evaluated, limiting understanding of their long-term establishment and stability under field conditions. Furthermore, metabolomic analyses were not performed to identify specific antifungal metabolites, antibiotics, or secondary compounds associated with antagonistic activity. Likewise, molecular validation of induced resistance pathways, including expression profiling of defense-related genes, was beyond the scope of the present study. Future investigations should therefore validate these isolates across broader agroecological regions and integrate colonization studies, metabolomic profiling, transcriptomic or gene expression analyses, formulation optimization, and assessment of long-term field persistence to further elucidate mechanisms underlying disease suppression and improve their potential for large-scale agricultural application.

5. Conclusions

This study demonstrates that selected indigenous endophytic *Trichoderma* and *Streptomyces* isolates effectively suppress potato late blight caused by *Phytophthora infestans* under both in vitro and field conditions, through direct antagonism and modulation of host defense responses. The most effective isolates reduced disease incidence and severity while

modulating oxidative stress markers and antioxidant enzyme activities, suggesting complementary roles in direct antagonism and regulation of host defense responses. Although growth and yield responses varied between seasons, the consistent reduction in late blight severity across two growing seasons highlights the potential of these endophytic bioagents as sustainable components of integrated disease management strategies. Nevertheless, validation through multilocation trials, formulation optimization, colonization studies, and molecular characterization of induced resistance mechanisms will be essential before large-scale field application.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae12070899/s1, Table S1: Molecular phylogenetic characterization of selected endophytic biocontrol agents based on 16S rDNA and ITS rDNA sequences Table S2: Effect of thirty-seven *Trichoderma* spp. isolates and two *Streptomyces* spp. isolates on *Phytophthora infestans* linear growth;; Table S3: Influence of various endophytes on growth parameters of potato plants during the 2022 growing season; Table S4: Influence of various endophytes on growth parameters of potato plants during the 2023 growing season; Figure S1: Effect of selected endophytic biocontrol agents and fungicide treatment on tuber weight per plant (g) in potato during the 2023 season. Data are presented as mean ± SE (n = 3), and different letters indicate significant differences among treatments according to Fisher's LSD test ($p \leq 0.05$).

Author Contributions: Conceptualization, A.K. and M.F.A.; methodology, M.F.A., F.M.F. and S.M.H.; software, F.M.F.; validation, M.F.A., Z.A.M. and A.K.; formal analysis, F.M.F., M.F.A. and A.K.; investigation, M.F.A., S.M.H. and N.A.; resources, Z.A.M., N.A. and A.K.; data curation, M.F.A. and F.M.F.; writing—original draft preparation, M.F.A. and A.K.; writing—review and editing, A.K., Z.A.M. and F.M.F.; visualization, F.M.F. and M.F.A.; supervision, A.K. and Z.A.M. project administration, A.K.; funding acquisition, Z.A.M. and N.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available within the article and Supplementary Materials. Additional datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Full term
Act.	Actinomycete isolate
AUDPC	Area under the disease progress curve
CAT	Catalase
CAPMAS	Central for Public Mobilization and Statistics
DI	Disease incidence
DS	Disease severity

GAE	Gallic acid equivalent
IAA	Indole-3-acetic acid
LB	Late blight
LSD	Least significant difference
MDA	Malondialdehyde
PCA	Principal component analysis
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
POD	Peroxidase
PGP	Plant growth-promoting
PGPR	Plant growth-promoting rhizobacteria
ROS	Reactive oxygen species
SD	Standard deviation
SE	Standard error
TPC	Total phenolic content
Trich.	*Trichoderma* isolate
VOCs	Volatile organic compounds

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