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BIOLOGICAL CONTROL OF WHEAT ROOT ROT CAUSED BY *FUSARIUM* SPP USING MICROBIAL BIOCONTROL AGENTS

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Abstract
This study aimed to evaluate the ability of the fungus *Trichoderma harzianum* and the bacteria *Bacillus subtilis* to resistant disease Wheat root rot caused by the fungus *Fusarium culmorum*. The pathogenic fungus was isolated from the roots of diseased wheat plants, and the effect of two biological agents on it was tested in vitro and under greenhouse conditions. The laboratory results showed that *Trichoderma* was superior in inhibiting the growth of the pathogenic fungus 77.29% followed by *Bacillus* with a rate of 69.64%, while the use of both biocontrol agents showed 82.94% and the average diameter of *F.culmorum* was 19.3 with *T.harzianum*, followed by B. *subtilis*, it was 25.8, while in treatment of both biocontrol agents was 14.5 compared to control treatment which was 85. In greenhouse experiments, the disease severity was 21.4% in plants treated with *T.harzianum* and the reduction percentage damage was 74.2%, while it was 28.6% in the treatment with *Bacillus*, and the reduction percentage damage was 65.6%. The treatment with both biocontrol agents was 17.6% and the reduction percentage of damage was 78.8% compared to control (C2) the disease severity was 83.2%. These results suggest that *Trichoderma* and *Bacillus* can be used to control wheat root rot instead of chemical pesticides. Field trials are recommended to confirm these findings and to develop biocontrol agents.

1. Introduction

Wheat (*Triticum aestivum* L.) is the world's leading strategic crop, providing sustenance for more than a third of the world's population. However, its productivity faces significant challenges, foremost among them root rot caused by various species of the genus *Fusarium*. This fungus attacks the plant's roots and stem base, leading to rotting, stunted growth, and yellowing of the leaves. Crop losses can range from 8% to 36%, and are particularly severe in semi-arid conditions (Duveiller et al. 2007). Even more concerning is that some *Fusarium* species produce mycotoxins that accumulate in the grain and pose a health risk to both humans and animals (Nelson et al . 1993). For decades farmers have relied on chemical pesticides to control this disease. However, these pesticides have proven to be of limited effectiveness, in addition to polluting the soil and groundwater, harming beneficial organisms, and leading to the emergence of resistant fungal strains. Furthermore, commercially available genetically resistant varieties of this disease remain unavailable.

Faced with these challenges, biological control has emerged as a safe and sustainable alternative, relying on the use of beneficial organisms to suppress plant pathogens (Petrucci et al. 2023). Among the most important organisms used in this field is the fungus *Trichoderma* spp., which has proven highly effective in combating *Fusarium* wilt through multiple mechanisms, including fungal parasitism, production of fungal cell wall-degrading enzymes (such as catetinase and protease), competition for nutrients and space, and stimulation of the plant's systemic immunity. *Bacillus* spp., particularly *Bacillus subtilis*, also stand out as promising biological agents. They are characterized by their ability to form resistant spores that withstand harsh environmental conditions, produce antifungal compounds, and reshape the microbial communities in the root zone in favor of beneficial organisms (Oulkhir et al. 2025).

Since the effectiveness of these biological agents depends largely on the isolates used and the prevailing environmental conditions in each region, this study aimed to evaluate the efficiency of local isolates of *Trichoderma harzianum* fungus and *Bacillus subtilis* in resisting *Fusarium* (Hjelijord et al. 2001; Krause et al . 2001)., the causative agent of wheat root rot disease, under laboratory and greenhouse conditions, in preparation for their use within integrated disease management programs as a safe and effective alternative to chemical pesticides. (Mahmoud. 2016).

2. Materials and methods

2.1 Collection and isolation of the pathogen

The isolates of pathogen (*Fusarium*) were isolated from wheat plants which exhibiting symptoms of root rot (yellowing of lower leaves, stunted growth, and reddening of the crown and roots) from agricultural fields in Wasit, Iraq (Al-Dujaili, Numaniyah, Badra(during Agricultural season 2026. Infected tissue was cut into small pieces (5–10 mm) and cultured on potato dextrose agar (PDA) (Mehrotra and Aggarwal .2003). Supplemented with streptomycin (100 mg/L). The plates were then incubated at 25 ± 2°C for 5–7 days. Fungal colonies growing on the PDA were purified, and the purified isolates were stored in a refrigerator at 4°C (Al-Askar and Rashad. 2021).

2.2 Isolation of the *Trichoderma harzianum*

Soil samples were collected from the rhizosphere of healthy wheat plants. Five grams of soil were suspended in 100 mL of sterile distilled water, and serial dilutions were made up 10^{-4}to10-6 , the dilutions were spread onto PDA plates and incubated at 28°C for 3–5 days. *T. harzianum* colonies were identified based on growth rate, the green color of the colonies, and the presence of branched conidia. They were purified and stored on a PDA slanted plate at 4°C (Rahman and Begum, 2018).

2.3 Isolation of *Bacillus subtilis*

One gram of rhizosphere soil was suspended in 9 mL of sterile saline and then serial dilutions were made up to 10^{-5}. One hundred microliters of the appropriate dilutions were spread onto nutrient agar (NA) plates and incubated at 30 ± 2°C for 24–48 hours. Irregular, creamy-white colonies (Hashem and Abd_Allah, 2019).

2.4 The antagonistic efficacy of biocontrol agents against the fungus in vitro

The antagonistic activity of the fungus *T. harzianum* and the *B.subtilis* against the pathogenic *Fusarium* spp. was evaluated using a dual culture technique on PDA medium. A plug of the pathogenic fungus (5 mm diameter) was placed in the center of a Petri dish, two plugs of *T. harzianum* (5 mm diameter) were placed 2.5 cm from the center, and B. *subtilis* was placed 3 cm from the center. The dishes were incubated at 25 ± 2°C for 7 days. The inhibition ratio was calculated using the equation: (R1-R2)/R1 × 100,

where:

R1 is the diameter of the pathogen in the control, R2 is the diameter of the pathogen in the treatment (Awad et al. 2018).

2.5 The effectiveness of biocontrol agents under greenhouse conditions

The efficacy of the fungus *T.harzianum* and the B. *subtilis* in reducing the severity of wheat root rot disease caused by the *Fusarium culmorum* was evaluated under greenhouse conditions. The soil (sandy loam) was autoclaved at 121°C for 20 minutes. The soil was inoculated with a 5% (w/w) fungal pathogen inoculant Wheat seeds (*Triticum aestivum* L). The seeds were then treated with either a bacterial suspension (1×105 CFU/ml) or a fungal spore suspension (1 × 106 spores/ml) by soaking them for 30 minutes. The experiment included five treatments: a negative control (C1) (uncontaminated soil, untreated seeds), a positive control (C2) (contaminated soil, untreated seeds), contaminated soil + seeds treated with *T. harzianum* (T1), contaminated soil + seeds treated with B. *subtilis* (B1), and contaminated soil + seeds treated with both agents (T+B). The pots were arranged in a completely randomized block design with five replicates for each treatment under greenhouse condition. The plants were grown at 22 ± 3°C, 65 ± 5% humidity. Twenty-eight days after planting, the disease severity index was calculated on a scale of (0–4) (Abdel-Kader et al. 2021).

This experiment was carried out according to the equation:

E = (C - T) / C × 100

Where:

E = Efficacy (%), C = Control disease severity, and T = Treated disease severity

2.6 Statistical analysis

The research employed a completely randomized design (CRD) with three replicates per treatment. After collecting infection severity data (%), the analysis showed highly significant differences between the biological control treatment and the infected control at a significance level of ($P \geq 0.05$) (Gomez and Gomez,1984).

3. Result and Discussion

3.1 Effect of the biocontrol agents against the pathogenic fungus in vitro

The inhibitory efficacy of the two biological control agents (*Trichoderma harzianum* and *Bacillus subtilis*) against the pathogenic fungus *Fusarium culmorum* was isolated from wheat roots infected with root rot, and then evaluated using subculture on PDA medium. The results showed that both agents were able to inhibit the diametric growth of the pathogenic fungus, with an inhibition rate of 19.3 (77.2 %) for *T. harzianum* and 25.8(69.6%) for B. *subtilis*, the inhibition rate of both biocontrol agents after 7 days of incubation at 28°C was 14.5 (82.9%) (see Table 1). Under the microscope, it was observed that *T. harzianum* caused coiling and penetration of the *Fusarium* filaments with their decomposition, while B. *subtilis* showed a clear inhibition zone around the discs without direct contact. The result showed significant differences between the treatments *T. harzianum* and B. *subtilis* against *Fusarium*. The current results indicate that both agents are effective against *Fusarium*, with *Trichoderma* having a clear advantage.

As shown in Table 1, *Trichoderma harzianum* achieved the highest inhibition rate against *Fusarium culmorum* compared with *Bacillus subtilis*, reaching 77.2%, while the combined treatment increased the inhibition percentage to 82.9%, indicating a synergistic interaction between both biocontrol agents. These findings are consistent with the results reported by El-Mohamedy (2020) regarding the efficiency of antagonistic microorganisms against *Fusarium* species.

Table 1. Percentage inhibition of biocontrol agents against the fungus in vitro

Biocontrol agent	Average diameter of *Fusarium* growth (mm)	Percentage inhibition %
Control	85	0.0
T. *harzianum* (T1)	19.3	77.2
B. *subtilis* (B1)	25.8	69.6
T. *harzianum* + *B.subtilis*	14.5	82.9

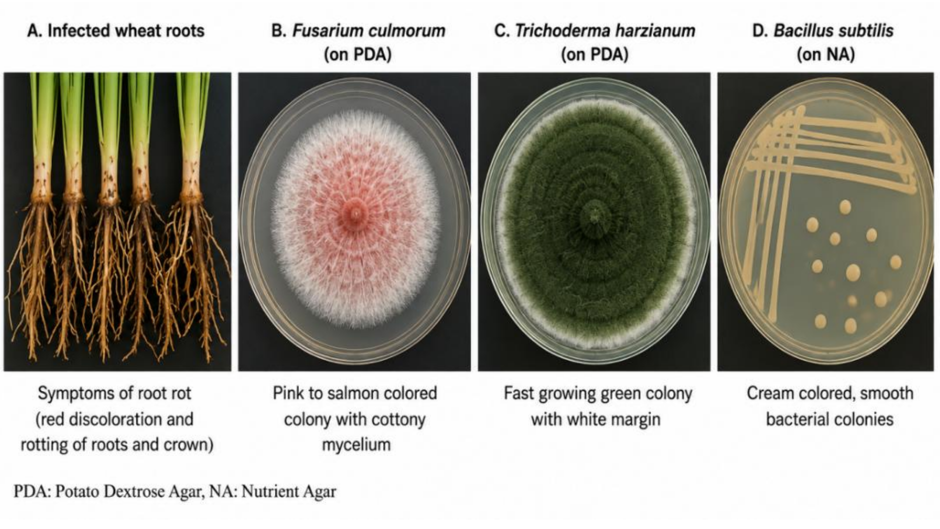

Figure 1. Morphological Characteristics of *Fusarium culmorum*, *Trichoderma harzianum*, and *Bacillus subtilis* Isolated from Wheat Rhizosphere

Figure 1 illustrates the morphological characteristics of the pathogenic fungus and the biocontrol agents isolated from the wheat rhizosphere. *Trichoderma harzianum* showed fast-growing green colonies, whereas *Bacillus subtilis* produced creamy-white bacterial colonies, consistent with the characteristics described by Hashem et al. (2019).

3.2 Effect of the biocontrol agents against the fungus under greenhouse conditions

The efficacy of the biological agents *T. harzianum* and *Bacillus subtilis* in controlling wheat root rot caused by *Fusarium culmorum* was evaluated under greenhouse conditions. The results showed a significant decrease in disease severity in all biological treatments compared to the control , the root rot infection rates reaching 21.4% in the *T. harzianum* and 28.6% in the B. *subtilis* and 17.6% in the treatment content both biocontrol agents , while the control (C2) recorded 83.2%. The efficacy of *T. harzianum* in reduction percentage damage reached 74.2%, while the efficacy of B. *subtilis* reached 65.6% and 78.8% in the treatment that includes both biocontrol agents (see Table 2).

Table 2 demonstrates a significant reduction in disease severity under all biological treatments compared with the infected control treatment. The lowest disease severity (17.6%) was observed in the combined treatment of *T. harzianum* and B. *subtilis*, which agrees with the findings of Abdel-Kader et al. (2021) concerning the effectiveness of biocontrol agents under greenhouse conditions.

Table 2: Effectiveness of biological agents in reducing the severity of wheat root rot under greenhouse conditions

Treatment	Disease severity (%)	(%) reduction percentage Damage
Control (C1)	00.0	-
Control(C2)	83.2	00.0
T. harzianum(T1)	21.4	74.2
B. subtilis (B1)	28.6	65.6
T. harzianum+B.subtilis (T+B)	17.6	78.8

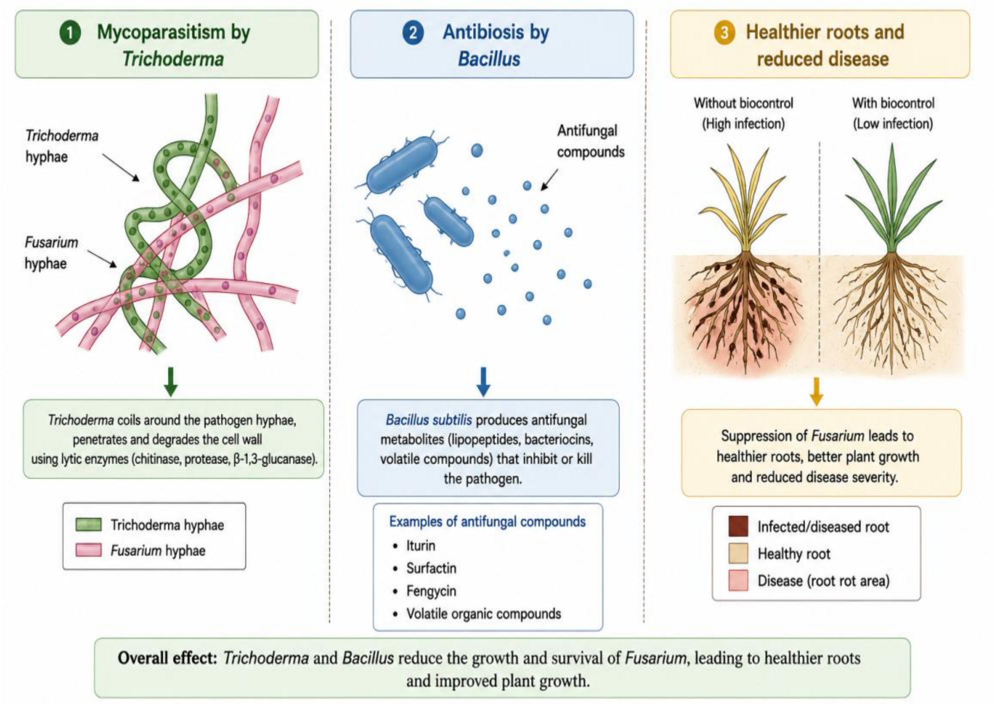

Figure 2. Schematic Representation of Antagonistic Mechanisms Employed by *Trichoderma harzianum* and *Bacillus subtilis* Against *Fusarium culmorum*

As demonstrated in Figure 2, the antagonistic mechanisms of the biocontrol agents include mycoparasitism by *Trichoderma harzianum* and antibiotic production by *Bacillus subtilis*. Similar mechanisms were previously reported by Petrucci et al. (2023) and Oulkhir et al. (2025). As illustrated in Table 3, clear morphological differences were observed among the pathogenic fungus and the biocontrol agents. *Trichoderma harzianum* was characterized by rapid green colony growth and branched conidia, which is in agreement with the description reported by Rahman and Begum (2018).

Table 3. Morphological characteristics of *Fusarium culmorum* and biocontrol agents

Isolate	Colony color	Texture	Growth rate	Microscopic characteristics
Fusarium culmorum	Pink-salmon	Cottony	Moderate	Septate hyphae
Trichoderma harzianum	Green	Compact	Fast	Branched conidia
Bacillus subtilis	Creamy white	Smooth	Rapid	Rod-shaped cells

Figure 3 clearly shows the inhibitory effects of the microbial biocontrol agents on the mycelial growth of *Fusarium culmorum*. The combined treatment exhibited the lowest fungal growth and the highest inhibition percentage, confirming the enhanced antagonistic effect of using both agents together. The statistical analysis presented in Table 4 revealed significant differences among treatments. The combined treatment (T+B) recorded the highest inhibition percentage (82.9%) and was classified within statistical group (A), indicating superior antagonistic activity against *Fusarium culmorum*.

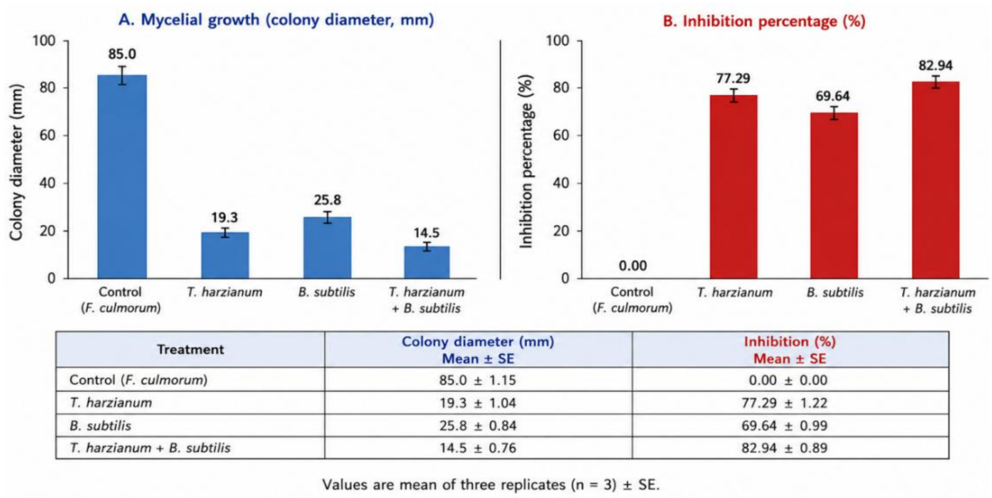

Figure 3. Comparative Inhibitory Effects of Microbial Biocontrol Agents on the Mycelial Growth of *Fusarium culmorum* Under Laboratory Conditions

Table 4. Statistical analysis of treatments against *Fusarium culmorum*

Treatment	Mean inhibition (%)	Statistical group
Control	0.0	C
T. *harzianum*	77.2	A
B. *subtilis*	69.6	B
T+B	82.9	A

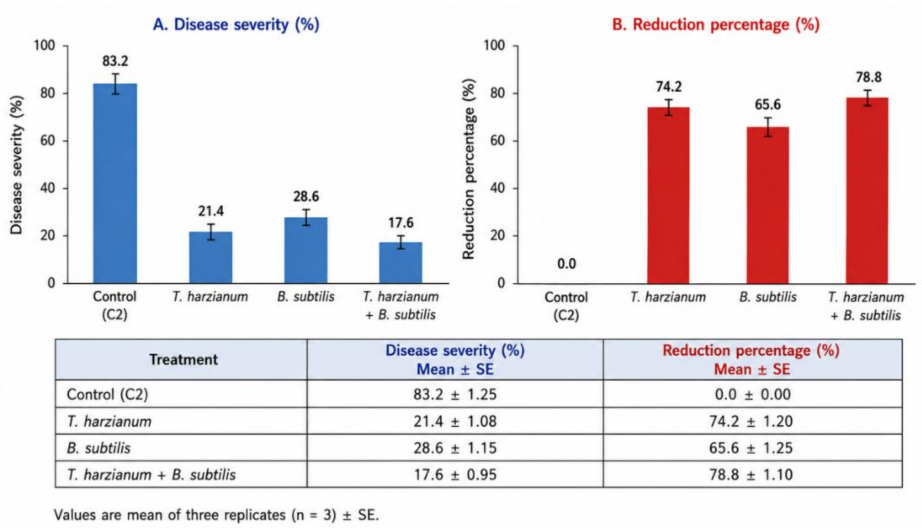

Figure 4. Efficacy of *Trichoderma harzianum* and *Bacillus subtilis* in Suppressing Wheat Root Rot Caused by *Fusarium culmorum* Under Greenhouse Conditions

As shown in Figure 4, the application of *Trichoderma harzianum* and *Bacillus subtilis* significantly reduced wheat root rot severity under greenhouse conditions. The combined treatment achieved the greatest disease suppression and the highest reduction percentage, indicating the strong effectiveness of integrated biological control. Table 5 compares the present findings with previous studies and indicates that the inhibition percentage obtained in this study was comparable to those reported by Saadaoui et al. (2024) and El-Mohamedy (2020) in biological control studies against *Fusarium* species. As shown in Table (6), biocontrol agents possess several advantages over chemical fungicides, including higher environmental safety, lower risk of resistance development, and improved soil health, which supports the observations reported by Woo et al. (2014).

Table 5. Comparison between previous studies and current findings

Study	Pathogen	Biocontrol agent	Inhibition (%)
El-Mohamedy (2020)	*Fusarium* oxysporum	*Trichoderma*	>80
Saadaoui et al. (2024)	F. *culmorum*	*Bacillus subtilis*	40–70
Present study	F. *culmorum*	T+B	82.9

Table 6. Advantages of biological control compared with chemical fungicides

Parameter	Biocontrol agents	Chemical fungicides
Environmental safety	High	Low
Resistance development	Rare	Common
Soil health	Improved	Reduced
Sustainability	High	Moderate

In dual culture assays of *T.harzianum* significantly inhibited the growth of the pathogenic *F.culmorum* responsible for root rot. Clear inhibition zones were observed between the colonies of the pathogen and the antagonistic *T.harzianum* . These inhibition zones are likely attributed to the production of diffusible inhibitory secondary metabolites by the *T.harzianum* which effectively restrict the growth of the pathogen. The presence and diameter of such inhibition zones have long been considered reliable indicators of antibiotic production by *T.harzianum* as part of their biocontrol mechanisms (Sempere and Santamarina, 2010). This is consistent with what was found in the study (El-Mohamedy, 2020), which confirmed the efficiency of *T. harzianum* in inhibiting *Fusarium* oxysporum by more than 80% in vitro. and The results agreed with (El-Sayed, and Shalaby, 2025) In the use of biological control agents against the fungus causing root rot disease.

The Competition between *Bacillus* spp. and root rot inducing fungi consistently resulted in the formation of clear inhibition zones were observed between the bacterial colony and the fungal mycelium (Dawar et al. 2010). This result is largely in agreement with the study of (Petrucci et al. 2023) which confirmed that the *T.harzianum* fungus secretes enzymes that break down the fungal wall, as well as with the study of (Saadaoui et al. 2024) who found that B *subtilis* bacteria isolated from wheat roots achieved 40-70% inhibition against *Fusarium culmorum*. This present results consistent with the study of (Winter et al. 2019) indicated regarding the ability of biological control agents to induce resistance in wheat which evaluated under controlled greenhouse conditions. The study of *Trichoderma* inoculations showed a decrease in both the frequency and intensity of foot and root rot disease in wheat accompanied by substantial increases in grain weight and total productivity (Woo et al.2014).

These results indicate that the superiority of *T. harzianum* in reducing root rot severity under greenhouse conditions, which is agreement with previous laboratory results and with the findings of (Abdel-Kader et al. 2020), who recorded control efficacy ranging from 43.7% for *T. harzianum* to 93.6% for some bacterial agents against *Fusarium*. graminearum on wheat. The high efficacy of *Trichoderma* is also attributed to its ability to produce cell wall-dissolving enzymes such as chitinase and glucanase, in addition to its mechanisms of parasitism and direct antagonism (El-Sharkawy, H. H., & El-Komy, M. H. 2024).

4. Conclusion

This study demonstrated the effectiveness of *Trichoderma harzianum* and *Bacillus subtilis* as biological control agents against wheat root rot caused by *Fusarium culmorum*. Both agents significantly inhibited the growth of the pathogenic fungus under laboratory conditions, with *T. harzianum* showing greater antagonistic activity than B. *subtilis*. The combined treatment of both agents achieved the highest inhibition percentage, indicating a synergistic effect between the fungal and bacterial biocontrol agents.

Under greenhouse conditions, all biological treatments significantly reduced disease severity compared with the infected control. The combined application of *T. harzianum* and B. *subtilis* resulted in the lowest disease severity and the highest reduction percentage, confirming their potential role in sustainable disease management.

The results suggest that biological control agents can serve as environmentally safe and effective alternatives to chemical fungicides. Their use may reduce environmental pollution, decrease the risk of fungicide resistance, and improve soil health. Further field studies are recommended to evaluate their effectiveness under natural agricultural conditions and to support the development of commercial biocontrol formulations.

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