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Biological control of stem canker and black scurf of potato (*Solanum tuberosum* L.) using antagonistic microorganisms and commercial biofungicides

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Abstract

Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide, valued for its nutritional quality, accessibility, and economic importance. It is a rich source of proteins, vitamins, minerals, essential amino acids, and carbohydrates, while being relatively low in energy content. However, potato production is significantly constrained by fungal diseases, among which *Rhizoctonia solani*—the causal agent of stem canker and black scurf—is of major concern due to its wide host range and global distribution. This study evaluated the management of *R. solani* both in vitro and in vivo using fungal bio-agents, bacterial isolates, and commercial biofungicides. The fungal antagonists tested included *Trichoderma harzianum* (7 and 10) and *T. asperellum*. The bacterial antagonists included *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus megaterium*, and *Paenibacillus polymyxa*. Commercial biofungicides tested were T34 (Biocontrol), Bio-Arc, and Bio-Zeid. All treatments demonstrated significant inhibitory effects against *R. solani*. *In vitro*, *P. fluorescens*, *P. polymyxa*, *T. asperellum*, and T34 (Biocontrol) produced the highest inhibition of mycelial growth. *In vivo* experiments during the 2021/2022 season confirmed that these bio-agents and biofungicides significantly reduced the severity of stem canker and black scurf compared with untreated controls. Overall, biological agents and commercial biofungicides proved to be highly effective, safe, and eco-friendly alternatives for managing *R. solani* in potato production.

Keywords: Potato, *Rhizoctonia solani*, stem canker, black scurf, *Trichoderma* spp., biological control.

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1. Introduction

Potato (*Solanum tuberosum* L.) is an important tuberous crop of the family Solanaceae. It is the world's fourth largest food crop after rice, wheat, and maize. Next to cereals, potato is the only crop capable of supplementing global food demand (Das *et al*., 2000). It is often referred to as the "King of Vegetables" because of its wide cultivation, high productivity, and large-scale consumption. In Egypt, potato cultivation contributes significantly to the national economy, producing approximately 6.87 million tons annually (FAOSTAT, 2023). However, the crop is increasingly affected by fungal diseases, particularly stem canker and black scurf. Potato is considered a highly economical and accessible food source, even for low-income populations. Although relatively low in caloric energy, it is nutritionally rich, containing high-quality protein, essential vitamins, minerals, and amino acids such as leucine, tryptophan, and isoleucine (Mehdi *et al*., 2008). In addition to direct consumption, potato has broad industrial uses including starch and alcohol production, and processing into chips, French fries, granules, and canned products. The green vegetative parts may also serve as fodder, while tubers are consumed as a staple vegetable. Potato production is constrained by multiple factors, including insects, abiotic stresses, and pathogens. Among these, diseases caused by *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris*) are particularly destructive. Stem canker and black scurf are serious soil-borne diseases with global distribution and a wide host range. *R. solani* infects potato stems and stolons below the soil surface, resulting in stem canker, while sclerotia produced on tubers manifest as black scurf. These symptoms may appear on all underground parts of the plant at different growth stages, and disease severity is pronounced when abundant black sclerotia cover the tuber surface. The pathogen survives between cropping seasons primarily as sclerotia in soil or on seed tubers, and also as mycelium in plant debris. Sclerotia can persist in soil for up to six years under favorable conditions, serving as primary inoculum for subsequent infections when potatoes are continuously cultivated (Banville *et al*., 1996). Biological control of soil-borne pathogens has been successfully demonstrated in laboratory studies using both bacterial and fungal antagonists. Such organisms are not pathogenic to plants but may parasitize pathogens (mycoparasitism), lyse their mycelia (mycolysis), compete for resources, or produce inhibitory metabolites (Agrios, 1997; Hoitink *et al*., 2001). *Trichoderma* species are widely reported to control soil-borne pathogens, including *R. solani*, through mechanisms such as microbial competition, antibiosis, hyperparasitism, and induction of systemic resistance (Brimner and Boland, 2003; Grosch *et al*., 2007; Schubert *et al*., 2008; Verma *et al*., 2007). Given their safety for humans and the environment, biocontrol agents represent a promising alternative to

synthetic pesticides. The objective of this study was to (i) determine the pathogenicity of *R. solani* isolates obtained from Menofia, Kaliobeia, Minia, and Assiut governorates, Egypt; (ii) assess the susceptibility of different potato cultivars; (iii) evaluate the efficacy of biological control agents in suppressing stem canker and black scurf; and (iv) compare the effectiveness of biofungicides with chemical inducers in minimizing disease severity.

2. Materials and methods

2.1 In vitro studies

2.1.1 Evaluation of antagonistic bacteria against R. solani isolates

The bacterial antagonists used in this study were *Bacillus subtilis*, *B. megaterium*, *Paenibacillus polymyxa*, and *Pseudomonas fluorescens*. These cultures were obtained from the Faculty of Agriculture, Ain Shams University, Egypt. The antagonistic activity of these bacteria was tested against four pathogenic *R. solani* isolates (Nos. 3, 7, 9, and 11). Bacteria were cultured on nutrient sucrose agar (NSA) medium consisting of peptone (5 g), beef extract (3 g), sucrose (5 g), yeast extract (2 g), and agar (20 g) dissolved in 1 L of distilled water. Cultures were incubated at 25 °C for 3 days. Mycelial discs (6 mm diameter) were excised from 7-day-old *R. solani* colonies and placed in the center of 9-cm Petri dishes containing sterilized medium. Each plate was streaked with an antagonistic bacterial culture at a distance of 2 cm from the edge in a semicircular pattern. Plates inoculated only with *R. solani* served as controls. Each treatment was replicated five times and incubated at 25 ± 1 °C for 5 days. Mycelial growth inhibition was calculated when the pathogen reached the plate margins in control dishes, using the formula:

$$Inhibition\ (\%) = \frac{A - B}{A} \times 100$$

where A = mycelial diameter in the control, and B = mycelial diameter in treated plates.

2.1.2 Evaluation of Trichoderma spp. against R. solani isolates

The tested antagonistic fungi included *Trichoderma asperellum* (T7, T10, and T34 Biocontrol), *T. harzianum*, and *T. album*. Fungal isolates were obtained from the Biological Control Unit, ARC, Giza, Egypt. The T34 (Biocontrol) strain of *T. asperellum* was provided by Shoos Conical Company. Dual culture assays were performed on potato dextrose agar (PDA) plates (9 cm diameter). One 6 mm disc of the pathogenic *R. solani* isolate was placed on one side of the plate, while a disc of the *Trichoderma* isolate was placed on the opposite side. Plates containing only *R. solani* served as controls. Each treatment was replicated five times and incubated at 25 ± 1 °C until the pathogen covered the control plates. The percentage of mycelial growth inhibition was calculated as:

$$Inhibition\ (\%) = \frac{A - B}{A} \times 100$$

where A = growth diameter in control

plates, and B = growth diameter in dual culture plates.

2.1.3 Effect of culture filtrates of Trichoderma spp. on R. solani

Trichoderma isolates were grown separately in 100 mL Erlenmeyer flasks containing 50 mL of Gliotoxin Fermentation Medium (GFM) as described by Brain and Hemming (1945). Cultures were incubated at 27 °C under dark conditions for 1, 2, and 3 weeks. Mycelial mats were removed by filtration, and culture filtrates were sterilized through filter paper. Sterile filtrates were incorporated into autoclaved PDA medium at concentrations of 5, 10, and 15% (v/v). Plates (9 cm diameter) containing treated and control PDA media were inoculated in the center with a 6 mm disc of *R. solani* (isolates 3, 7, 9, and 11). Each treatment was replicated five times and incubated at 25 °C until controls were fully colonized. Growth inhibition was calculated as described previously.

2.2 In vivo studies

2.2.1 Antagonistic effect of Trichoderma spp. under greenhouse conditions

Pot experiments were conducted during the 2021/2022 and 2022/2023 winter seasons using the potato cultivar Spunta. The antagonists tested included *T. harzianum*, *T. asperellum*, *T. album*, and T34 (Biocontrol). Inocula of antagonistic fungi were prepared on barley grain medium following procedures used for pathogenicity tests. *Trichoderma* isolates were incorporated into infested soil at rates of 1, 2, and 3% (w/w). Infestation with *R. solani* was performed one week before sowing, using 1% inoculum (w/w). Five potato tubers were planted per pot (50 cm diameter), with five replicate pots per treatment. Pots infested only with *R. solani* served as controls. Plants were maintained under standard irrigation and fertilization. Disease severity was assessed 90 days after sowing.

2.2.2 Evaluation of commercial biofungicides under field conditions

Field trials were carried out during the 2023/2024 winter season on the potato cultivar Caruso. The biofungicides tested were T34 (Biocontrol, *T. asperellum*), Bio-Zeid (*T. album*), and Bio-Arc (*Bacillus megaterium*). Each was applied as a seed tuber dressing at three concentrations (1, 2, and 3 g/kg soil). The experiment was arranged in a randomized complete block design (RCBD) with 12 rows per treatment, each 5 m long and 80 cm wide. Each treatment was replicated four times, with untreated rows as controls. A total of 25 seed tubers were planted per row at 20 cm spacing. Disease severity of stem canker and black scurf was recorded 90 days after planting.

2.3 Statistical analysis

Data were analyzed using analysis of variance (ANOVA) according to Gomez and Gomez (1984). Treatment means were compared using the least significant

difference (LSD) test at the 5% probability level.

3. Results

3.1 Effect of bacterial bio-agents on mycelial growth of R. solani (in vitro)

The antagonistic effects of four bacterial bio-agents were evaluated against four isolates of *R. solani* (Table 1). All bacterial treatments significantly inhibited mycelial growth compared with the control. *Pseudomonas fluorescens* exhibited the highest inhibition rates (68.79–81.10%), followed by *Paenibacillus polymyxa* (61.11–79.19%). *Bacillus megaterium* provided moderate inhibition (54.66–73.39%), while *B. subtilis* showed the lowest inhibition (34.22–68.66%). These results are consistent with earlier reports demonstrating the strong antagonistic potential of *P. fluorescens* and *P. polymyxa* against *R. solani* and *Fusarium oxysporum* (Abou-Aly, 2008; Montealegre *et al*., 2003; Rini and Sulochana, 2007).

Table (1): Effect of antagonistic bacteria on the mycelial growth inhibition (%) of *R. solani* isolates.

Bacterial bio-agents	Mycelial growth inhibition (%)			
	R. solani (3)	*R. solani* (7)	*R. solani* (9)	*R. solani* (11)
B. subtilis	34.22	37.55	63.55	68.66
B. megaterium	57.55	54.66	71.33	73.39
Paenibacillu polymyxa	66.78	61.11	72.50	79.19
P. fluorscens	68.79	71.40	77.33	81.10
Control	0.0	0.0	0.0	0.0
L.S.D at 5%	0.83	0.92	0.54	0.72

3.2 Effect of Trichoderma spp. on mycelial growth of R. solani (in vitro)

All tested *Trichoderma* isolates significantly inhibited the growth of *R. solani* (Table 2). The greatest inhibition was recorded with *T. asperellum* T34 Biocontrol (82.44–86.30%), followed by *T. asperellum* (70.66–84.0%). *T. harzianum* isolates (7 and 10) showed moderate inhibition (46.11–68.33%), while *T. album* exhibited the lowest inhibitory effect (54.55–59.65%). The observed inhibition zones in dual cultures suggest that *Trichoderma* spp. may secrete diffusible non-volatile inhibitory substances, in addition to direct hyphal interactions such as coiling around pathogen hyphae (Adekunle *et al*., 2006; Chu and Wu, 1981; Ha, 2010). These findings agree with previous studies demonstrating the antagonistic activity of *Trichoderma* against *R. solani* and other soil-borne pathogens (Eshetu *et al*., 2013; Ezzat *et al*., 2015; Mishra *et al*., 2013).

3.3 Effect of Trichoderma culture filtrates on R. solani (in vitro)

Culture filtrates of *T. asperellum*, *T. harzianum* (7 and 10), and *T. album* significantly reduced pathogen growth,

with inhibition increasing at higher filtrate concentrations (Table 3). *T. asperellum* consistently showed the highest inhibition across concentrations, reaching 39.10% against isolate R11 at 15%. *T. album* was most effective at 15% concentration against R11 (39.50%), though it showed weaker inhibition at lower concentrations. *T. harzianum* isolates exhibited moderate inhibition across all concentrations. These results support previous findings that *Trichoderma* spp. produce secondary metabolites with antifungal activity against various pathogens (Sivasakthi *et al*., 2014; Vinale *et al*., 2008).

Table (2): Effect of *Trichoderma* spp. on mycelial growth inhibition (%) of *R. solani* isolates.

Fungal bio-agents	Mycelial growth inhibition (%)			
	R. solani (3)	*R. solani* (7)	*R. solani* (9)	*R. solani* (11)
T. harzianum (7)	54.33	57.66	61.15	64.33
T. harzianum (10)	58.45	46.11	66.66	68.33
T. asperellum	74.33	70.66	81.15	84.0
T. album	56.45	54.55	57.22	59.65
T34 (biocontrol)	68.33	66.66	82.44	86.3
Control	0.0	0.0	0.0	0.0
L.S.D at 5%	2.15	1.46	2.29	3.58

Table (3): Effect of culture filtrates of *Trichoderma* spp. on mycelial growth inhibition (%) of *R. solani* isolates.

Trichoderma isolates	Concentration (%)	Mycelial growth inhibition (%)											
		One week				Two weeks				Three weeks			
		R 3	R7	R9	R11	R 3	R7	R9	R11	R 3	R7	R9	R11
T. harzianum (7)	5 %	12.65	18.24	14.33	27.04	28.25	22.14	19.25	30.10	46.10	51.10	32.33	33.33
	10 %	19.41	22.10	17.19	29.89	33.33	26.35	30.20	34.84	48.75	54.20	38.66	37.75
	15 %	23.33	27.33	21.65	32.59	36.10	3.65	32.75	38.40	51.33	61.27	40.18	39.15
T. harzianum (10)	5 %	16.66	20.33	18.65	24.81	23.33	25.75	21.35	26.80	31.33	33.35	26.80	29.35
	10 %	19.10	24.15	24.40	27.66	29.10	29.10	31.0	28.33	35.66	38.43	33.66	30.40
	15 %	23.33	23.40	29.33	30.31	37.15	34.15	39.66	30.66	40.40	41.56	39.75	33.66
T. asperellum	5 %	24.10	26.66	25.10	29.63	37.55	40.33	29.0	31.75	39.66	48.33	31.67	33.66
	10 %	32.15	28.10	32.15	33.65	41.40	42.66	34.40	35.30	44.45	52.66	37.22	36.25
	15 %	36.33	33.65	36.90	39.10	43.65	47.15	38.85	41.33	51.10	57.75	39.70	43.15
T. album	5 %	11.65	14.65	11.85	22.10	19.33	27.35	16.75	24.50	23.15	32.16	35.81	38.11
	10 %	16.10	17.40	15.19	33.36	23.40	28.41	20.15	38.75	29.45	38.27	38.59	39.67
	15 %	21.33	21.22	22.59	39.50	29.10	29.66	23.33	41.40	33.75	40.41	40.88	41.48
Control	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D at 5%	T	1.13	1.10	0.71	4.33	0.94	0.84	1.36	0.82	1.58	0.82	1.16	0.70
	C	1.10	0.96	0.57	1.17	0.82	0.79	0.76	0.79	1.35	0.79	1.10	0.63
	T × C	2	1.92	1.25	2.36	1.84	1.61	1.62	2.60	2.69	1.32	1.82	1.28

3.4 Effect of Trichoderma spp. under greenhouse conditions

Greenhouse experiments confirmed the antagonistic effect of *Trichoderma* spp. against stem canker and black scurf (Table 4). Disease severity decreased with increasing application rates (1–3%). *T. asperellum* was the most effective, reducing disease severity to as low as 0–2.33% in some isolates at 3% application rate. *T. harzianum* (10) and *T. harzianum*

(7) also significantly reduced disease severity, though less than *T. asperellum*. These findings align with previous studies reporting that *Trichoderma* spp. suppress *R. solani* through antibiosis, mycoparasitism, and induction of systemic resistance (Usamah *et al*., 2024; Verma *et al*., 2007).

Table (4): Effect of *Trichoderma* spp. on disease severity (%) of stem canker and black scurf under greenhouse conditions.

Trichoderma isolates	Rate of application	Severity of stem canker and black scurf diseases (%)															
		Season 2022								Season 2023							
		Stem canker				Black scurf				Stem canker				Black scurf			
		R3	R7	R9	R11	R3	R7	R9	R11	R3	R7	R9	R11	R3	R7	R9	R11
T. harzianum (7)	1 %	18.33	24.44	13.88	14.20	14.44	15.35	13.33	11.11	14.13	15.83	10.44	9.66	9.66	13.32	11.32	10.88
	2 %	16.66	17.77	11.11	11.33	13.32	11.11	8.88	8.33	11.26	13.33	9.10	7.33	7.63	10.15	7.75	7.75
	3 %	10.15	10.55	8.33	8.67	8.33	9.55	6.66	5.55	8.06	4.53	2.66	1.70	2.71	9.30	5.55	1.45
T. harzianum (10)	1 %	31.33	22.21	15.55	17.77	16.33	17.77	11.32	9.87	12.71	13.0	9.15	7.88	4.70	19.85	10.44	11.33
	2 %	20.0	15.55	8.33	14.33	11.33	8.88	6.67	8.80	7.77	11.66	4.33	4.33	3.67	9.44	4.55	7.88
	3 %	9.60	8.33	5.55	10.22	6.67	4.67	2.47	2.77	2.14	4.08	2.71	0.80	0.0	5.55	2.10	1.65
T. asperellum	1 %	33.33	18.88	19.77	22.21	10.55	12.21	4.33	3.88	12.71	12.33	8.88	6.65	10.85	18.65	11.13	2.62
	2 %	25.0	10.22	8.33	7.55	6.32	7.77	2.45	1.88	7.63	4.26	4.20	4.88	4.33	10.69	9.03	1.67
	3 %	7.67	4.88	2.66	2.33	3.44	3.33	1.67	0.0	7.0	1.66	1.38	0.90	8.10	9.66	4.88	0.0
Control	-	92.66	88.33	86.66	80	55.55	66.33	45.10	48.67	90.45	86.66	87.33	82.0	56.66	68.20	48.0	40.0
L.S.D at 5%	T	0.96	0.72	1.58	1.61	0.83	2.79	0.80	1.39	0.91	1.59	0.82	2.6	0.70	1.38	0.86	1.31
	R	0.82	0.68	1.40	1.41	0.73	2.43	0.70	1.19	0.81	1.42	0.72	1.11	0.60	1.17	0.73	1.11
	T × R	1.69	1.22	2.79	2.71	1.36	4.88	1.31	2.49	1.35	2.59	1.14	2.06	1.31	2.55	1.44	2.4

3.5 Effect of commercial biofungicides under field conditions

Field trials showed that all commercial biofungicides significantly reduced disease severity compared with untreated controls (Table 5). The degree of control was concentration-dependent. T34 Biocontrol was the most effective, completely suppressing disease at 3 g/kg soil. Bio-Arc also reduced disease effectively at higher concentrations, while Bio-Zeid provided moderate control. These results corroborate previous findings that T34 Biocontrol is highly effective against soil-borne diseases of potato and other crops (Hussein *et al*., 2021; Sarhan, 2020).

Table (5): Effect of commercial biofungicides on disease severity (%) of stem canker and black scurf under field conditions.

Bio-fungicides	Rate of application (g/kg)	Disease severity (%)							
		Stem canker				Black scurf			
		R3	R7	R9	R11	R3	R7	R9	R11
T34 Bio-control	1	7.88	8.62	7.65	6.88	5.41	5.88	4.33	4.46
	2	5.33	5.43	4.36	2.47	3.46	4.36	3.25	3.33
	3	1.36	2.67	0.0	0.0	1.50	2.17	0.0	0.0
Bio-Arc	1	10,83	10.87	7.70	6.63	6.46	8.35	5.77	4.46
	2	6.45	6.33	4.29	3.25	4.35	4.88	3.46	2.75
	3	2.57	1.65	1.16	0.0	2.36	3.14	1.07	0.0
Bio-Zeid	1	12.50	15.63	14.42	14.13	8.65	10.19	12.0	11.40
	2	9.62	9.65	12.65	11.24	4.25	6.48	9.62	5.36
	3	3.57	4.46	8.77	5.66	1.57	3.70	5.15	4.47
control	-	46	49.33	39.33	9.85	30.94	31.65	29.75	28.73
L.S.D at 5%	B	0.81	0.44	1.46	1.23	1.16	1.26	1.18	1.14
	R	0.66	0.36	1.11	1.17	1.12	1.23	1.14	1.10
	B × R	1.47	0.75	2.58	2.38	2.29	2.39	2.33	2.24

4. Discussion

The present study demonstrated that both bacterial and fungal bio-agents, as well as commercial biofungicides, were effective in suppressing stem canker and black scurf of potato caused by *Rhizoctonia solani*. By integrating *in vitro*, greenhouse, and field experiments, this research provides evidence supporting the use of eco-friendly biological strategies as viable alternatives to chemical fungicides. Among the bacterial isolates tested, *Pseudomonas fluorescens* and *Paenibacillus polymyxa* consistently exhibited the highest inhibition of *R. solani* mycelial growth in vitro. These findings align with previous studies where *P. fluorescens* showed strong antagonism against *R. solani* and *Fusarium oxysporum* (Montealegre *et al*., 2003; Rini and Sulochana, 2007). Similarly, Abou-Aly (2008) demonstrated the effectiveness of *P. polymyxa* and *B. megaterium* as biocontrol agents. The mechanisms underlying their activity likely involve production of antibiotics, competition for nutrients, and induction of plant resistance, as reported in other pathosystems (Abdelrehem *et al*., 2021; Hassan *et al*., 2013). The in vitro results confirmed the strong inhibitory activity of *Trichoderma* isolates, particularly *T. asperellum* and the commercial T34 Biocontrol strain. This observation agrees with previous reports demonstrating the antagonistic potential of *Trichoderma* against *R. solani* and other soil-borne pathogens (Eshetu *et al*., 2013; Ezzat *et al*., 2015; Mishra *et al*., 2013). The inhibition observed in dual culture assays, often without direct hyphal contact, suggests that diffusible metabolites play a central role (Adekunle *et al*., 2006; Chu and Wu, 1981). In addition, mechanisms such as mycoparasitism, antibiosis, and induced systemic resistance likely contribute (Hoitink *et al*., 2001; Verma *et al*., 2007). Culture filtrates of *Trichoderma* spp. further confirmed that secondary metabolites are important determinants of antagonism. In line with earlier findings, these filtrates suppressed pathogen growth in a dose-dependent manner (Sivasakthi *et al*., 2014; Vinale *et al*., 2008). Such metabolites may also enhance systemic resistance in plants, suggesting their dual role as antifungal compounds and elicitors. Greenhouse trials validated the efficacy of *Trichoderma* spp., with *T. asperellum* showing superior disease suppression compared to *T. harzianum* and *T. album*. The reduction in disease severity at higher inoculum rates (3% w/w) highlights the importance of application dosage. Similar results have been reported where *Trichoderma* spp. reduced *R. solani* incidence and promoted potato growth (Usamah *et al*., 2024). Field trials provided further confirmation, showing that commercial biofungicides—particularly T34 Biocontrol—were highly effective under natural conditions. Complete suppression of disease was achieved at 3 g/kg soil, consistent with earlier findings in potato (Hussein *et al*., 2021) and soybean (Sarhan, 2020). The slightly lower efficacy of Bio-Zeid compared to Bio-Arc and T.34 may reflect strain-specific differences in antagonistic potential. The results emphasize that biological control agents, particularly *Trichoderma* spp. and *Pseudomonas fluorescens*, represent promising tools for integrated management of stem canker

and black scurf. Their efficacy, environmental safety, and compatibility with sustainable agriculture make them attractive alternatives to synthetic fungicides, whose overuse can cause environmental pollution and pathogen resistance (Brimner and Boland, 2003). Nevertheless, certain limitations should be acknowledged. The performance of bio-agents can be influenced by environmental conditions, soil microbiome interactions, and application methods. Long-term field trials across diverse agro-ecological zones are therefore needed to validate their consistency. Furthermore, integration of biocontrol agents with cultural practices and resistant cultivars may provide more durable management strategies.

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