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Differential Efficacy of Fungal and Bacterial Antagonists against *Fusarium verticillioides*, *Macrophomina phaseolina*, and *Dickeya zeae*: Stalk Rot Pathogens of Maize

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Authors' contributions

This work was carried out in collaboration among all authors. Author MRR designed, wrote the protocol and performed the study, Author BDP wrote the first draft of the manuscript, assisted in performing study and statistical analysis. Authors VS and ISK managed the literature searches and assisted in laboratory studies. All authors read and approved the final manuscript.

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Abstract

Aim: This study evaluated the *in vitro* antagonistic efficacy of selected fungal and bacterial bioagents against the major maize stalk rot pathogens *Fusarium verticillioides*, *Macrophomina phaseolina*, and *Dickeya zeae*.
Study Design: The experiment was arranged in a completely randomised design.
Place and Duration of Study: The study was conducted at the Agricultural Research Station, Peddapuram, ANGRAU, Andhra Pradesh, India, during 2025–26.

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Methodology: Six bioagents, namely *Trichoderma harzianum*, *T. asperellum*, *T. koningiopsis*, *Pseudomonas fluorescens*, *P. chlororaphis*, and *Bacillus subtilis*, were tested. Antagonism against the fungal pathogens was assessed by the dual-culture technique, whereas activity against *D. zeae* was evaluated through an inhibition-zone assay. Mycelial growth inhibition, inhibition-zone diameter, and reduction in sclerotial production were recorded and statistically analysed.
Results: The bioagents differed in their activity against the tested pathogens. *Trichoderma koningiopsis* and *T. asperellum* were the most effective against *F. verticillioides* and *M. phaseolina*, producing 56.67–71.25% inhibition over the control. Fungal bioagents also reduced sclerotial production of *M. phaseolina* more effectively than the bacterial bioagents. *Bacillus subtilis* and *P. chlororaphis* showed moderate, broader antagonistic activity against the fungal and bacterial pathogens, with inhibition generally ranging from 36.67% to 46.67%.
Conclusion: The findings indicate that *T. koningiopsis*, *T. asperellum*, *B. subtilis*, and *P. chlororaphis* merit further validation as components of integrated maize stalk rot management under greenhouse and field conditions.

Keywords: Biological control; Charcoal rot; Dickeya zeae; Fusarium verticillioides; Macrophomina phaseolina; Maize; Post-flowering stalk rot; Pseudomonas chlororaphis; Trichoderma asperellum; Trichoderma koningiopsis.

1. Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide and contributes substantially to food, feed and industrial sectors. Global maize production is estimated at nearly 1.2 billion tonnes annually, making it the most widely produced cereal crop (FAO, 2025). In India, maize production increased from 31.65 million tonnes in 2021-22 to an estimated 43.41 million tonnes in 2024-25 (APEDA, 2025). The crop is cultivated over approximately 10-11 million hectares, mainly in Madhya Pradesh, Karnataka, Maharashtra, Bihar, Telangana, and Andhra Pradesh. India contributes nearly 3% of global maize production, with more than 60% utilised as animal feed. Growing demand from the ethanol and starch industries has further increased its economic importance.

Maize productivity is severely affected by post-flowering stalk rot (PFSR), which cause significant yield losses during both kharif and rabi seasons. In India, stalk rots of maize have been reported to cause annual yield losses of approximately 10-42% (ICAR-IIMR, 2015). PFSR in maize is caused by a complex of fungal and bacterial pathogens. *Fusarium verticillioides* is the major pathogen under irrigated conditions, whereas *Macrophomina phaseolina* predominates in rainfed regions during the kharif season (Kaur & Mohan, 2012). Bacterial stalk rot caused by *Dickeya zeae* [formerly *Erwinia chrysanthemi pv. zeae*] is an emerging disease of maize that causes severe yield losses under warm and humid conditions (Kumar et al., 2015). Among the component pathogens, charcoal rot caused by *M. phaseolina* has been reported to cause grain yield losses of 25-32.2%, reaching 39.5% in susceptible genotypes and as high as 63.5% under severe natural disease conditions. Under artificial inoculation, an 85.5% yield reduction has been recorded in susceptible varieties (Khokhar et al., 2014).

Previous studies have demonstrated the high efficacy of carbendazim, carbendazim + mancozeb, hexaconazole, propiconazole, thiophanate methyl, and azoxystrobin + tebuconazole against *Macrophomina phaseolina*, with several fungicides producing complete inhibition of mycelial growth under *in vitro* conditions (Khamari & Patra, 2018; Khaire et al., 2018; Patil et al., 2024). Similarly, azole fungicides such as prochloraz, tebuconazole, and difenoconazole have shown strong antifungal activity against *Fusarium spp.*, whereas azoxystrobin was comparatively less effective (Amini & Dzhalilov, 2010; Rekanovic et al., 2010). In the case of bacterial stalk rot, stable bleaching powder, streptocycline, and copper-based antibiotic combinations effectively suppressed *Dickeya zeae* and reduced disease severity under field conditions (Kumar et al., 2016). Reliance on chemical fungicides may increase the risk of fungicide resistance development in plant pathogenic fungi and has raised concerns regarding environmental contamination and pesticide residues (Islam et al., 2024). In addition, the development of resistant cultivars is complicated by the involvement of multiple pathogens in the maize stalk rot complex. Owing to the soil-borne nature of these pathogens, disease management through a single control measure is often inadequate. Therefore, biological control using microbial antagonists has emerged as an eco-friendly, sustainable, and promising strategy for the management of maize stalk rot diseases. The effectiveness of *Trichoderma spp.* against several soil-borne pathogens can be attributed to different mechanisms of

antagonism, including mycoparasitism, antibiosis, and enzyme production (Harman et al., 2004). *Pseudomonas spp.* have been reported to suppress pathogens through the production of siderophores and antimicrobial metabolites (Pieterse et al., 2014), whereas *Bacillus subtilis* produces lipopeptide antibiotics that inhibit pathogen growth (Shoda, 2000). Earlier studies have focused on evaluating the efficacy of bioagents against individual stalk rot pathogens (Kaur & Mohan, 2013; Meena & Gangopadhyay, 2016; Jambhulkar et al., 2022). In this context, safe and eco-friendly strategies such as resistance management and biocontrol of stalk rots in maize are becoming practical options for farmers. In some instances, mixed infection by stalk rot pathogens has been reported to cause significant yield losses in maize (Dodd, 1980; Khokhar et al., 2014; Nair & Rashid, 2024), thereby prompting researchers to identify bioagents or chemicals with combined efficacy against major stalk rot pathogens. Recent screening of maize inbreds against post-flowering stalk rot also indicated variable resistance responses across *Fusarium* isolates, reinforcing the need for complementary management options (Harish et al., 2024). Recent reviews on plant-microbe interactions suggest that beneficial microbes can support plant defence through induced systemic resistance and antimicrobial compound production, although their effectiveness may be influenced by environmental conditions (Sahoo et al., 2024). Recent maize-based microbiome work also indicates that *Bacillus-associated* microbiota may contribute to defence against corn stalk rot (Xia et al., 2024). Other recent studies reported antifungal activity of *Bacillus velezensis* and *Paenibacillus ottowii* metabolites against *Fusarium verticillioides* and documented disease reduction from seed treatment with *Bacillus velezensis* CNPMS-22 under greenhouse and field conditions (Diniz et al., 2024; Figueiredo et al., 2025). However, comparative evidence on the efficacy of the same set of fungal and bacterial bioagents against the major fungal and bacterial components of the maize stalk rot complex under uniform *in vitro* conditions remains limited. Therefore, the present study was undertaken to evaluate six bioagents under *in vitro* conditions against three major stalk rot pathogens of maize, namely *Fusarium* stalk rot, charcoal rot, and bacterial stalk rot, using dual-culture and inhibition-zone assays.

2. Materials and Methods

The experiment was conducted at the Agricultural Research Station, Peddapuram (ANGRAU), Kakinada district, Andhra Pradesh. Six ANGRAU bioagents, namely *Trichoderma asperellum*, *T. harzianum*, *T. koningiopsis*, *Pseudomonas chlororaphis*, *Pseudomonas fluorescens*, and *Bacillus subtilis*, were evaluated against the major stalk rot pathogens of maize, i.e., *Fusarium* stalk rot caused by *Fusarium verticillioides*, charcoal rot caused by *Macrophomina phaseolina*, and bacterial stalk rot caused by *Dickeya zeae*. The pathogens used in the study were isolated from diseased specimens collected from maize-growing areas of the Godavari zone of Andhra Pradesh during 2024-25. Standard laboratory procedures were followed for the isolation, purification, maintenance, preservation of cultures, and assay of bioagents against the stalk rot pathogens under study (Dhingra & Sinclair, 1995).

2.1 Collection and Isolation of Pathogens

Diseased maize plants exhibiting characteristic stalk rot symptoms were collected from farmers' fields in the major maize-growing regions of the Godavari zone of Andhra Pradesh. Freshly collected samples showing visible lesions were brought to the laboratory, washed under running tap water to remove dirt, air-dried, and then small portions from the diseased and healthy tissues were sliced with a surgical knife and used for pathogen isolation.

2.1.1 Isolation of Fungal Stalk Rot Pathogens

Surface-sterilised (1% sodium hypochlorite) diseased plant tissue pieces (approximately 5 mm x 5 mm) were plated on potato dextrose agar (PDA) and incubated at 28 +/- 2 deg C for 5-7 days. Fungal colonies with morphological characteristics consistent with *Fusarium verticillioides* and *Macrophomina phaseolina* were identified based on cultural and morphological features. Preliminary identification of the fungal isolates was carried out based on colony characteristics, pigmentation, growth pattern, and microscopic features using standard taxonomic descriptions (colony color, septation, condiophore and conidia production etc.). The isolates were identified as *Fusarium verticillioides* and *Macrophomina phaseolina*. The identity of *Fusarium verticillioides* and *Macrophomina phaseolina* was further confirmed through the pathogen identification service of the Indian Type Culture Collection (ITCC), ICAR–Indian Agricultural Research Institute (ICAR-IARI), New Delhi.

2.1.2 Isolation of Bacterial Stalk Rot Pathogen

Fresh samples of diseased tissue, along with adjacent healthy plant tissue, were collected and macerated in sterile distilled water, and the bacterial suspensions were streaked under aseptic conditions on nutrient agar plates, followed by incubation for 48 hours. Bacterial colonies with morphological characteristics of *Dickeya zeae* were selected based on colony morphology, and pathogen identity was confirmed based on pathogenicity on susceptible maize. The isolate was further characterized using standard biochemical tests, including the KOH solubility test (Gram reaction), catalase test, oxidase test, and pectolytic activity on carrot and potato tissues. Based on the morphological, pathogenicity and biochemical characteristics, the isolate was identified as *Dickeya zeae* and used for subsequent *in vitro* evaluation of bioagents. Pure bacterial cultures were maintained on nutrient agar slants for further study.

2.2 Bioagents for Evaluation

The bioagents evaluated in the present study included *Trichoderma asperellum* (Amaravati isolate; NCBI GenBank accession no. ON909211), *Trichoderma harzianum* (Guntur isolate; GenBank accession no. PQ045539), *Trichoderma koningiopsis* (Peddapuram isolate; identified through the Indian Type Culture Collection (ITCC), ICAR–Indian Agricultural Research Institute, New Delhi), *Pseudomonas fluorescens* (Guntur isolate; GenBank accession no. ON964472), *Pseudomonas chlororaphis* (Anakapalli isolate; GenBank accession no. ON116377), and *Bacillus subtilis* (Anakapalli isolate). The fungal bioagents were maintained on potato dextrose agar (PDA), whereas the bacterial bioagents were maintained on nutrient agar (NA) medium under laboratory conditions for subsequent experiments.

2.3 *In vitro* Evaluation of Bioagents Against Fungal Stalk Rot Pathogens

The antagonistic activity of *Trichoderma* bioagents against the fungal stalk rot pathogens was evaluated using the dual-culture technique on potato dextrose agar (PDA) plates (Dennis & Webster, 1971). A 5 mm diameter mycelial disc of the stalk rot fungus (*Fusarium verticillioides*/*Macrophomina phaseolina*) was placed 2 cm away from the margin of a sterile Petri dish containing solidified PDA. Simultaneously, a 5 mm diameter mycelial disc of the bioagent under evaluation was placed on the opposite side of the plate at a distance of 5 cm from the pathogenic fungal disc. Plates containing only the pathogenic fungus without any antagonist served as the control. Three replications were maintained for each treatment. All plates were incubated at 28 +/- 2 deg C, and pathogen growth (colony diameter) was recorded in each replication for each evaluated bioagent when complete pathogen growth was observed in the control Petri plate. The mean inhibition-zone diameter, i.e., the clear zone of inhibition between the two antagonistic organisms, was also measured.

The percentage inhibition of mycelial growth was calculated using the formula % Inhibition = [(R1 – R2) / R1] × 100, where R1 is the radius of the pathogenic fungal colony in the control plate and R2 is the radius of the pathogenic fungal colony in the presence of the antagonist.

2.4 *In vitro* Evaluation of Bioagents against Bacterial Stalk Rot Pathogen

The antagonistic potential of bioagents against the bacterial pathogen *Dickeya zeae* was assessed using the inhibition-zone assay on nutrient agar medium (Bauer et al., 1966). A bacterial culture of *D. zeae* (approximately 10^8 CFU/mL) was seeded in lukewarm nutrient agar and poured into plates. After solidification, wells of 5 mm diameter were aseptically cut into the agar at the centre of each plate using a sterile cork borer. For bacterial antagonists (*P. fluorescens*, *P. chlororaphis*, and *B. subtilis*), a 5 mm disc from a 24 h old culture of the bacterial bioagent on a nutrient agar plate was cut with a sterilised cork borer and placed at the centre of Petri plates seeded with the bacterial pathogen. For fungal bioagents, a 5 mm disc from a 5-day-old actively growing culture was transferred to the centre of the seeded agar medium under aseptic conditions. The positive control plate was maintained by placing a Whatman No.4 filter paper disc (5 mm) soaked in plantomycin solution (100 ppm) at the centre of the seeded plates, which were incubated in a refrigerator overnight to allow chemical diffusion. Three replications were maintained for each treatment. Plates were incubated at 28 +/- 2 deg C for 48 hours, and the diameter of the clear inhibition zones formed around the discs was measured (cm) and expressed as inhibition annulus (cm^2) and relative efficacy (%) over positive control.

2.5 Statistical Analysis

Data on colony diameter, inhibition zone diameter, sclerotia count and percent inhibition of mycelial growth were subjected to analysis of variance (ANOVA) to determine the significance of treatment effects at the 5% level of significance. Percentage inhibition data were subjected to angular (arc sine) transformation prior to analysis, wherever applicable, to stabilize the variance. Treatment means for percent inhibition, inhibition zone diameter, and relative efficacy were compared using the Critical Difference (CD) test at the 5% level of significance ($P \leq .05$). Sclerotial count data were further subjected to Duncan's Multiple Range Test (DMRT) at $P \leq .05$ for mean separation, and the resulting significance groupings are presented in Figure 2. The original (untransformed) means are presented in heatmap for ease of interpretation. Heat map, and bar graph were prepared using R STUDIO Version (2024.12.1+563).

3. Results and Discussion

The antagonistic potential of different bioagents against the major stalk rot pathogens of maize, viz., *Fusarium verticillioides*, *Macrophomina phaseolina*, and *Dickeya zeae*, was studied under laboratory conditions. Considerable variation in bioagent efficacy against the stalk rot pathogens was observed.

3.1 Efficacy against *Fusarium verticillioides* (*Fusarium* Stalk Rot Pathogen)

Among the bioagents evaluated against the *Fusarium* stalk rot pathogen, *Trichoderma koningiopsis* (local isolate) and *Trichoderma asperellum* (Amaravati isolate) were the most effective and statistically comparable in suppressing the growth of *Fusarium verticillioides*, resulting in 2.30 cm and 2.43 cm colony growth, respectively, compared with 8.00 cm recorded in the control (Table 1 and Plate 1). Fungal bioagents demonstrated superior efficacy against FSR, exhibiting 66.25-71.25% inhibition relative to bacterial bioagents, which exhibited 6.25-46.67% inhibition of pathogen growth under *in vitro* conditions (Figure 1). Among the bacterial bioagents, *Bacillus subtilis* and *Pseudomonas chlororaphis* were moderately effective against FSR, with 46.67% and 41.67% inhibition, respectively, compared with the untreated control (pathogen alone), whereas *Pseudomonas fluorescens* showed minimal suppression (6.25%) of the FSR pathogen.

3.2 Efficacy against *Macrophomina phaseolina* (Charcoal Rot)

The local *Trichoderma* isolate was superior, reducing pathogen growth to 3.0 cm and recording 62.50% inhibition compared with the control (Table 1). *Trichoderma harzianum* was the next most effective treatment, with a mean colony diameter of 3.47 cm and 56.67% inhibition, followed by *Trichoderma asperellum* with a mean colony diameter of 4.33 cm and 45.83% inhibition. Among the bacterial bioagents, *Bacillus subtilis* and *Pseudomonas chlororaphis* showed moderate inhibition of 40.42% and 36.67%, respectively, whereas *Pseudomonas fluorescens* showed minimal suppression (6.25%) of the *Macrophomina* pathogen (Plate 2). All bioagents significantly reduced sclerotial production of *Macrophomina phaseolina* compared with the untreated control (Figure 2). *T. asperellum* was the most effective and statistically superior in inhibiting sclerotial production in *M. phaseolina* (8.67 sclerotia/cm2), followed by *T. koningiopsis* (12.67 sclerotia/cm2) and *T. harzianum* (14.00 sclerotia/cm2), compared with 40.33 sclerotia/cm2 in the control (pathogen alone). Among the bacterial antagonists, *B. subtilis* and *P. chlororaphis* showed moderate suppression (19.0-29.0 sclerotia/cm2), whereas *P. fluorescens* was the least effective.

3.3 Efficacy against *Dickeya zeae* (bacterial Stalk Rot)

In vitro screening revealed that bacterial antagonists significantly outperformed fungal antagonists in restricting the BSR pathogen *Dickeya zeae* through antibiosis. *Bacillus subtilis* was identified as the most potent bioagent, yielding an inhibition zone of 0.50 cm (42.38% relative efficacy), followed by *Pseudomonas chlororaphis* at 0.43 cm (38.86% relative efficacy). The efficacy of both strains was statistically lower than that of the antibiotic plantomycin at 100 ppm (1.1 cm zone). In contrast, *Trichoderma species* lacked zone-forming capability but showed aggressive competitiveness, as indicated by vigorous vegetative growth and dense green sporulation against *D. zeae* in the pathogen-seeded agar medium (Plate 3).

The heatmap (Figure 1) illustrates the differential efficacy profiles of the bioagents against the tested pathogens. *Trichoderma* isolates exhibited superior antagonistic activity against fungal pathogens, whereas bacterial antagonists showed greater effectiveness against *D. zeae*. Notably, *Trichoderma* isolates achieved the highest inhibition of *Fusarium verticillioides* and *Macrophomina phaseolina*, while *Bacillus subtilis* and *Pseudomonas*

chlororaphis were more effective against *Dickeya zeae*. These findings align with previous reports demonstrating the strong antagonistic potential of *Trichoderma spp.* against *M. phaseolina* (Gajera et al., 2012; Srinivas et al., 2022; Fatoh et al., 2024). The substantial reduction in sclerotial production documented in the present investigation corroborates earlier findings by Shekhar and Kumar (2010), who demonstrated effective suppression of sclerotial formation by *Trichoderma-based* bioagents. Similarly, the marked inhibition of *Fusarium spp.* by *Trichoderma spp.* reported herein is consistent with observations from Sobowale et al. (2007), Saravanakumar et al. (2017), and Cuervo-Parra et al. (2022).

Table 1. *In vitro* Efficacy of Bioagents against Major Stalk Rot Pathogens of Maize

S. No.	Treatments	*Fusarium verticillioides*		*Macrophomina phaseolina*		*Dickeya zeae* Mean zone of inhibition (cm)
		Mean colony diameter (cm)	Mean inhibition zone (cm)	Mean colony diameter (cm)	Mean inhibition zone (cm)	
1	*Trichoderma harzianum*	2.70	0.33	3.47	0.67	0.01
2	*Trichoderma asperellum*	2.43	0.37	4.33	1.03	0.01
3	*Trichoderma koningiopsis*	2.30	0.50	3.00	1.13	0.01
4	*Pseudomonas fluorescens*	7.50	0.83	7.50	0.10	0.01
5	*Pseudomonas chlororaphis*	4.67	1.03	5.07	0.60	0.43
6	*Bacillus subtilis*	4.27	1.17	4.77	0.80	0.59
7	Control	8.00	-	8.00	-	1.10
	C.D. (P = .05)	0.25	0.06	0.24	0.05	0.04
	SE(m)	0.08	0.02	0.08	0.02	0.01
	C.V. (%)	3.11	4.86	2.57	4.00	7.37

**values presented in table are mean of three replications; *Plantomycin (100ppm) was used as positive control for Dickeya zeae*

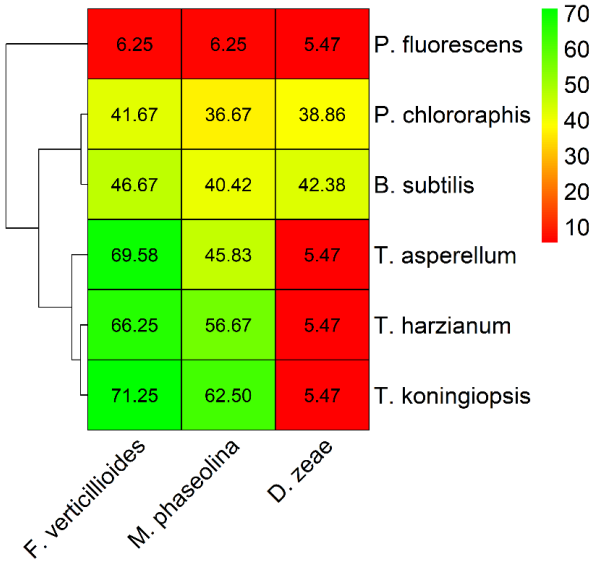

Fig. 1. Heat map showing efficacy of bioagents on major stalk rot pathogens of maize

Bioagents: significant from untreated control at P < 0.05

Values represent mean inhibition of growth (%) for fungal pathogens and mean relative efficacy over plantomycin (%) for bacterial pathogen of three replications. Color intensity corresponds to antagonistic activity, with green indicating higher inhibition and red indicating lower inhibition

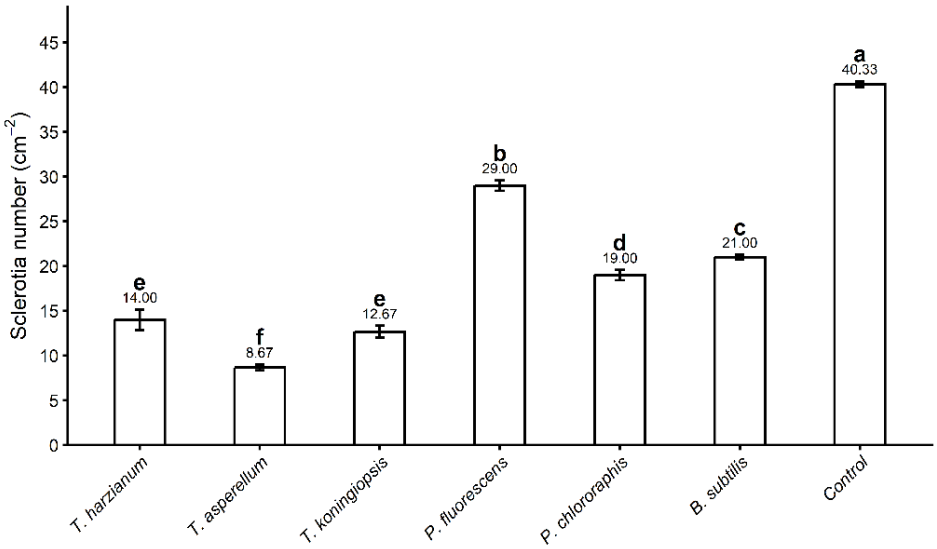

Fig. 2. Efficacy of bioagents on inhibtion of sclerotia production in *Macrophomina phaseolina* causing charcoal rot in maize under *in vitro* conditions

*Bioagents: significant from normal control, * P < 0.05*

Mean ± S.E.M = Mean values ± Standard error of means of average number of sclerotia per cm^2 from three replications. Different lowercase letters indicate significant differences among treatments according to Duncan's Multiple Range Test (DMRT) at P ≤ .05

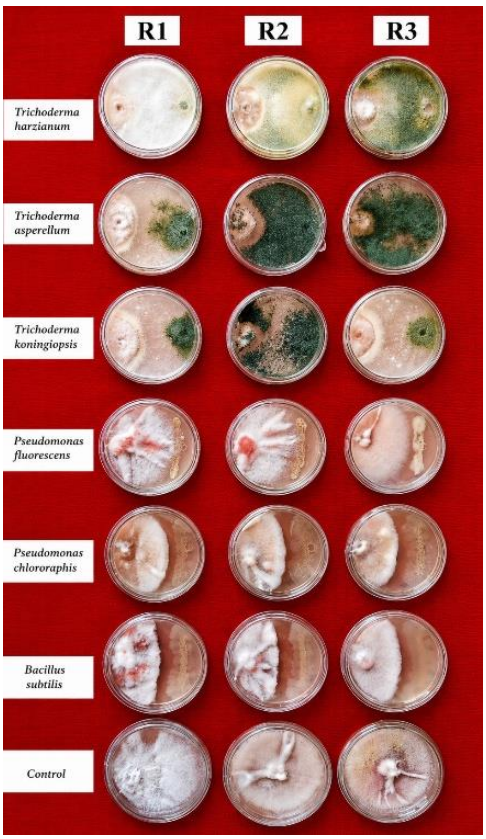

Plate 1. *In vitro* antagonistic efficacy of fungal and bacterial bioagents on growth of *Fusarium verticillioides* (dual culture assay)

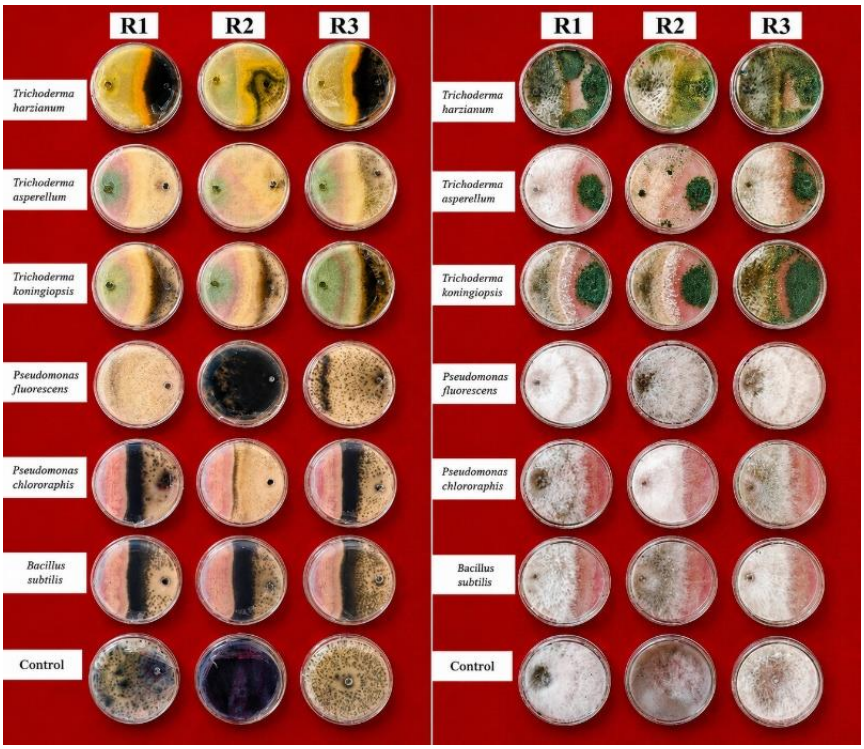

Plate 2. *In vitro* antagonistic efficacy of fungal and bacterial bioagents on sclerotial production and growth of *Macrophomina phaseolina* (dual culture assay)

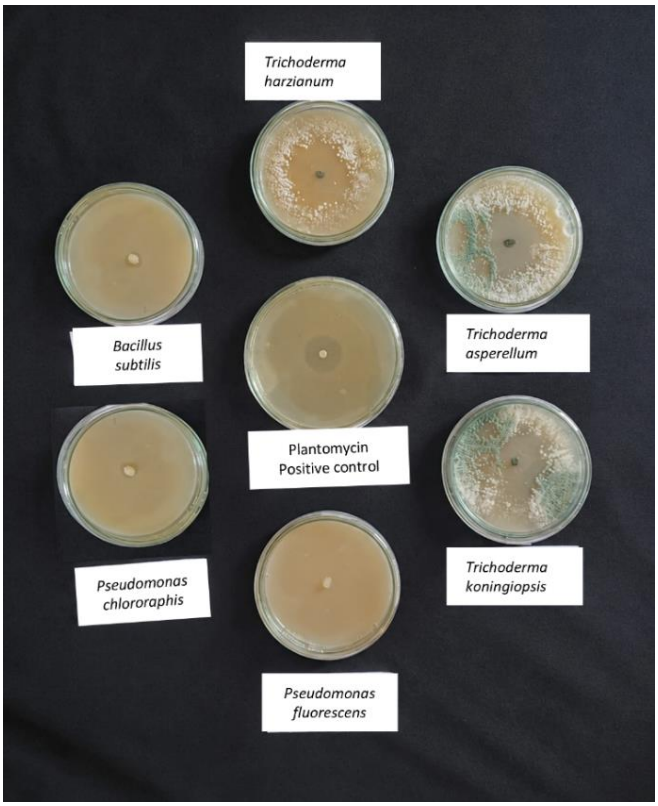

Plate 3. *In vitro* antagonistic efficacy of fungal and bacterial bioagents against *Dickeya zeae* (Inhibition zone assay)

The superior antagonistic activity of *Bacillus subtilis* and *Pseudomonas chlororaphis* against *D. zeae* is consistent with reports by Baez-Astorga et al. (2022) and Feng et al. (2025), who attributed pathogen suppression to the production of antimicrobial metabolites and other antagonistic compounds. The reduced efficacy of *Pseudomonas fluorescens* may reflect strain-specific variation in antagonistic potential. Furthermore, Ajijah et al. (2024) demonstrated enhanced suppression of *Fusarium spp.* through co-culturing of *Pseudomonas* and *Trichoderma species*, suggesting the potential of microbial consortia for improved disease management. Collectively, these findings suggest that *Trichoderma koningiopsis* and *T. asperellum* were comparatively superior in inhibiting the growth of *Fusarium verticillioides* and *Macrophomina phaseolina* (56.67-71.25% inhibition of growth over the control) compared with 5.47% inhibition of the bacterial stalk rot pathogen. In contrast, among the bacterial antagonists under study, *B. subtilis* and *P. chlororaphis* were moderately effective against both fungal and bacterial stalk rots, recording growth inhibition in the range of 36.67-46.67%. These results underscore the potential of deploying *Trichoderma koningiopsis*, *T. asperellum*, or *Bacillus subtilis* as constituent components of microbial consortia within integrated management strategies for the maize stalk rot complex.

4. Conclusion

The present *in vitro* study demonstrated differential antagonistic responses of fungal and bacterial bioagents against the principal stalk rot pathogens of maize. Among the tested antagonists, *Trichoderma koningiopsis* and *T. asperellum* were comparatively more effective against *Fusarium verticillioides* and *Macrophomina phaseolina*. *T. koningiopsis* recorded the highest inhibition of mycelial growth of both fungal pathogens, whereas *T. asperellum* showed the greatest reduction in sclerotial production by *M. phaseolina*. The bacterial antagonists *Bacillus subtilis* and *Pseudomonas chlororaphis* provided moderate suppression of the fungal pathogens and produced measurable inhibition zones against *Dickeya zeae*. *Pseudomonas fluorescens* showed comparatively low antagonistic activity under the tested conditions. These results suggest that selected *Trichoderma*, *Bacillus*, and *Pseudomonas* isolates may be useful candidates for further evaluation in integrated management of the maize stalk rot complex. However, the findings are based on laboratory assays only, and their practical relevance should be confirmed through greenhouse, pot-culture, and multi-location field studies before recommendation for disease management.

5. Limitations

This study was restricted to *in vitro* assays involving six bioagents and three maize stalk rot pathogens under controlled laboratory conditions. Field performance may vary with soil type, temperature, moisture, host genotype, pathogen pressure, resident microbial communities.

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Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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