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Evaluation of Antagonists Against *Rhizoctonia solani* Causing Foot Rot in Broccoli

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Abstract
Broccoli (*Brassica oleracea* L. var. *italica*) is a nutritionally rich crop with increasing global demand, yet its productivity is severely constrained by soil-borne pathogens such as *Rhizoctonia solani*, the causal agent of foot and root rot. Chemical fungicides, while widely used, face limitations due to resistance development, environmental risks, and regulatory concerns, highlighting the need for sustainable alternatives. The present study evaluated the antagonistic potential of *Trichoderma harzianum*, *T. viride*, and *Bacillus subtilis* against seven isolates of *R. solani* under *in vitro* conditions. Antagonists were isolated from broccoli field soils and tested using dual culture and paper disc methods on Czapek's sucrose nitrate agar medium. Results revealed significant variability in pathogen inhibition across isolates and antagonists. *T. harzianum* demonstrated the highest mean mycelial growth inhibition (63.04%), followed by *T. viride* (57.66%) and *B. subtilis* (49.78%). Maximum inhibition was recorded in R Isolate-006, where *T. harzianum* achieved 69.42% suppression and *T. viride* 67.28%. Conversely, the lowest inhibition was observed in R Isolate-005, with *B. subtilis* showing only 42.35% suppression. The findings confirm that *T. harzianum* consistently exhibited superior antagonistic activity, suggesting its potential as a promising biocontrol agent against *R. solani*. These results align with earlier reports of *Trichoderma* spp. exhibiting mechanisms such as mycoparasitism, antibiosis, and competition, thereby supporting their integration into eco-friendly disease management strategies. Further research on formulation development and field-level validation is essential to translate these laboratory findings into practical applications for sustainable broccoli production.

Keywords: Broccoli, *Rhizoctonia solani*, foot rot, *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis*, biocontrol

Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) has emerged as one of the most important vegetable crops worldwide due to its exceptional nutritional value and increasing market demand (Siomos *et al*., 2022) [15]. The crop is rich in vitamins A and C, minerals, fiber, and bioactive compounds, including glucosinolates and sulforaphane, which provide protective effects against cancer, diabetes, stroke, and myocardial infarction (Mittal *et al*., 2025) [12]. Broccoli is a high-value crop with the potential to double farmers' income through organic farming practices. The crop's by-products offer sustainable applications in developing agrochemicals, nanoparticles, and functional foods, supporting zero-waste solutions with economic benefits (Han *et al*., 2021) [5]. Chemical fungicides remain essential for crop disease control, but face significant limitations, including resistance development, environmental impacts, and regulatory challenges. Fungicide resistance can develop in virtually any pathogen through mechanisms such as target site alterations, efflux pump activation, and mutations (Janssen *et al*., 2024). This resistance poses a threat to effective crop protection across major fungicide classes, including benzimidazoles, sterol demethylation inhibitors, and succinate dehydrogenase inhibitors (Yin *et al*., 2023) [18]. The discovery of new fungicides faces mounting regulatory hurdles and increasing costs, though innovation continues, particularly in novel modes of action for oomycete control (Leadbeater, 2016) [9]. Resistance management strategies include rotating different modes of action, limiting applications of site-specific fungicides, and implementing Integrated Pest Management programs (Yin *et al*., 2023) [18]. Microbial antagonists play a crucial role in the biological control of plant pathogens, with Trichoderma, Pseudomonas, and Bacillus being the most prominent genera (Mahapatra *et al*., 2024) [11].

Trichoderma species establish symbiotic relationships with plant roots and employ multiple mechanisms, including resource competition, production of antifungal metabolites, stimulation of plant defense mechanisms, and mycoparasitic interactions that directly attack pathogenic fungi (Elango *et al*., 2024) [3]. These biological control agents support sustainable disease management strategies and offer environmentally friendly alternatives to chemical pesticides, with proven effectiveness in laboratory, greenhouse, and field applications (Bansode, *et al*., 2022) [1].

Materials and Methods

Isolation of Trichoderma and Bacillus spp.: Ten non-rhizosphere soil samples were collected from the agricultural fields of Chitali Putali village in the Jalna district to isolate *Trichoderma* and *Bacillus spp.* The soil samples were collected randomly and diagonally from a broccoli field and were used for the enumeration of fungi following the Soil Plate Method (Warcup 1950; Parkinson 1973) [13, 17] on Martin's peptone dextrose agar medium. For the isolation of Bacillus bacteria, Thornton's standardized medium was utilized following the Soil Dilution and Plate Count Method (Waksman 1927) [16]. The Petri dishes were incubated at 28 ± 1°C for 5 days, and the recording of colonies for various microorganisms continued for 15 days or until no new colonies developed.

Test for Antagonism

Trichoderma spp. and *Bacillus* sp. isolated from infested soil were assessed for their antagonistic properties against seven isolates of *R. solani* on Czapek's dox medium in Petri dishes. A 5mm diameter piece of *R. solani* was transferred to the center of a Czapek's dox medium Petri dish, and circular pieces of *T. harzianum* and *T. viride*, grown on PDA, were placed at equidistant points near the periphery of the dishes. The Petri dishes were incubated at 28 ± 1°C for 7 days, and observations were made to record the growth inhibition of *R. solani.* For the *Bacillus* sp. paper disc plate method (Loo *et al*. 1945) [10] was implemented as follows. Circular discs (5 mm in diameter) made from Whatman filter paper (No. 42) were cut and dipped in the filtrate of *Bacillus* sp. These discs were then placed 2 cm inward from the periphery of Petri dishes at three equidistant points, with the inoculum of the pathogen (*R. solani*) positioned at the center. The inoculated dishes were kept in an incubator at a temperature of 28±1°C for 7 days, during which observations were made to measure the inhibition zone of *R. solani.*

Result and Discussion

Isolation of *Trichoderma* and *Bacillus* spp. A notable population of *T. harzianum*, *T. viride*, and various other fungal colonies was observed, alongside a similarly significant population of *B. subtilis* and other bacterial colonies.

Table 1: Effect of antagonists on the growth of *Rhizoctonia solani* on Czapek's sucrose nitrate agar medium in dual culture

***R. solani* isolate**	**Mycelial growth inhibition (%) of *R. solani* by antagonists**			**Mean**
	T. harzianum	*T. viride*	*B. subtilis* sp.	
R Isolate-001	58.45 (49.90)	53.62 (46.21)	54.28 (43.90)	55.45 (46.67)
R Isolate-002	62.13 (51.70)	56.08 (48.33)	52.47 (45.10)	56.89 (48.38)
R Isolate-003	67.58 (55.40)	61.32 (50.92)	47.20 (41.56)	58.70 (49.29)
R Isolate-004	61.05 (51.20)	55.22 (47.95)	45.10 (40.80)	53.79 (46.65)
R Isolate-005	57.92 (48.67)	46.95 (41.70)	42.35 (39.22)	49.07 (43.19)
R Isolate-006	69.42 (56.93)	67.28 (55.16)	51.25 (44.60)	62.65 (52.23)
R Isolate-007	68.73 (56.30)	59.15 (49.78)	55.80 (47.21)	61.22 (51.10)
Mean	63.04 (52.16)	57.66 (48.58)	49.78 (43.77)	56.83 (48.17)

CD ($P \leq 0.05$): Isolate = 3.12; Antagonists = 1.24; Isolate × Antagonists = 5.38; CV (%) = 6.29

The results presented in Table 1 indicate that all three antagonists effectively inhibited the mycelial growth of the seven isolates of Rhizoctonia solani. Among these antagonists, *Trichoderma harzianum* demonstrated the highest mean inhibition rate at 63.04%, followed by *T. viride* at 57.66% and *Bacillus subtilis* at 49.78%. This suggests that *T. harzianum* was the most effective antagonist against the tested isolates. At the isolate level, the highest inhibition was measured in R-Isolate-006, with 69.42% inhibition by *T. harzianum* and 67.28% by *T. viride*. Conversely, the lowest inhibition was recorded in R Isolate-005, with only 42.35% inhibition by *B. subtilis*. Overall, the mean values indicate that while *B. subtilis* was comparatively less effective, but it still exhibited moderate suppression of the pathogen.

Several studies have demonstrated the effectiveness of *Trichoderma* and *Bacillus* species as biocontrol agents against *Rhizoctonia solani*. Atef (2008) [1] found that combining *T. harzianum* and *B. subtilis* was more effective than using each agent alone for controlling wheat root rot, with the combination improving biochemical parameters in treated seedlings. Seema & Devaki (2012) [14] and Bansode *et al* (2022) [2] evaluated multiple biocontrol agents against *R. solani*, reporting that *T. viride* achieved 70% growth inhibition, *T. harzianum* 67%, and *B. subtilis* 50% in dual culture assays. The antagonistic effect was attributed to hyphal interactions causing pathogen lysis. Grosch *et al.* (2006) [4] selected six Trichoderma isolates, identified as *T. reesei* and *T. viride*, which significantly reduced *Rhizoctonia* symptoms in potato sprouts and controlled dry mass loss in lettuce under both controlled and field conditions. Interestingly, Kim *et al*. (2008) [2] demonstrated that certain Bacillus isolates could antagonize Trichoderma species, with isolates M27 and RM29 strongly inhibiting mycelial growth of *T. harzianum*, *T. koningii*, and *T. viridescens*.

Conclusion

This study demonstrates that these biocontrol agents have potential for managing root rot diseases. Future research should focus on more comprehensive investigations into the relationships within various pathosystems and the interactions between microorganisms and host plants. Such studies are essential for developing effective biocontrol strategies for specific plant diseases.

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