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RESEARCH

In-vitro Analysis of Different *Trichoderma* spp. Against *Fusarium* Rhizome Rot of Ginger (*Zingiber officinale* Roscoe)

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Abstract

This study evaluated the effectiveness of four isolates of *Trichoderma* spp. obtained from various plant protection divisions and commercial products for the biocontrol of *Fusarium* rhizome rot of ginger (*Zingiber officinale* Roscoe). Experiments were designed in a completely randomized design (CRD) with five treatments and ten replications. The National Agriculture Research Council (NARC) isolates of *Trichoderma* spp. were multiplied through direct inoculation on PDA media. Meanwhile, the commercial products of *T. harzianum* (TRICHO-HR) and *T. viride* (Biocide Trivi) were prepared as spore suspension. The dual culture technique was used to evaluate the interaction between *Trichoderma* spp. and *Fusarium* pathogen. The radial diameter of both *Trichoderma* and *Fusarium* was measured every 24 h for 7 days. The experiments in dual cultures demonstrated that various *Trichoderma* isolates and commercial products significantly inhibited *Fusarium* mycelial growth. Among the isolates tested, T1-NARC showed the highest efficacy at 57.91% growth inhibition, followed by T2-RPPL (52.50%) and T4-Biocide Trivi (40.98%). However, T3-TRICHO HR displayed lower performance with an inhibition rate of 24.75%. These findings offer valuable insights for the use of *Trichoderma* spp. as biocontrol agents in ginger cultivation, contributing to improved disease control and enhanced crop health. The observed differences in performance could be attributed to the genetic variations among the isolates.

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Statement of Sustainability: Using *Trichoderma* spp. as a biological control agent provides a sustainable and eco-friendly approach to mitigating diseases such as rhizome rot in ginger cultivation. *Trichoderma* contributes to a balanced agroecosystem by reducing reliance on chemical pesticides, enhancing soil health, naturally suppressing pathogens, and promoting resilience. This approach aligns with bio-rational strategies, ensuring long-term soil fertility, improved crop yield, and economic returns while minimizing environmental impact and supporting the continued viability of ginger production for both present and future generations.

1. Introduction

Ginger (*Zingiber officinale* Roscoe) is believed to have originated in the southern region of ancient China and was later distributed to India, the Maluku Islands, also known as the Spice Islands, and the rest of Asia and West Africa (Langner et al., 1998; Shahrajabian et al., 2019). According to MoAD (2020), Nepal produced 279,206 metric tons of ginger in 21,912 ha of land in Fiscal Year 2020/2021, with a productivity of 12.74 metric tons/ha. There is a promising trend in ginger production with an average annual growth rate of 6.67%. Although Nepal has a relatively small area, it has emerged as a significant contributor to global ginger production, accounting for approximately 9.2% of the total output (Joshi and Khanal, 2021). The Agriculture Development Strategy (ADS, 2014) is a long-term vision guiding Nepal's agriculture sector from 2015 to 2035, identifying ginger as a prioritized sub-sector for value chain development. The decision to prioritize ginger is based on its high export potential, which can significantly contribute to earning foreign currency, reducing poverty, improving the livelihoods of Nepalese farmers, and ultimately boosting the national economy (NTIS, 2016). Ginger is mainly used as a spice, condiment, food, medicine, and perfumery (Khatso and

Tiameren, 2013). Ginger consumption is known to ease stomachache, indigestion, diarrhea, and nausea, and is mostly used as a remedy for common cold, cough, and congestion (Wang, 2020).

Ginger is highly susceptible to various diseases and pests, which pose a significant challenge to its production. Of the many diseases affecting ginger, rhizome rot is the most prevalent and economically significant. This disease causes losses of up to 50-92% in yield (Meenu and Jebasingh, 2019). The primary pathogens responsible for rhizome rot in ginger are fungal, including *Pythium* and *Fusarium* spp., as well as bacterial (*Pseudomonas* [Ralstonia] *solaniserum*) (Rai, 2006). As noted by Prasath et al. (2023), worldwide, the impact of *Fusarium* yellows, caused by *F. oxysporum* f. sp. *zingiberi*, has been devastating. Their study confirms the pathogenicity of *Fusarium* spp. isolates on ginger by observing their ability to colonize and induce typical symptoms of *Fusarium* yellows. *F. oxysporum* f. sp. *Zinziberis* is primarily responsible for the dry rot type of rhizome rot in ginger, while *Pythium* spp. is primarily responsible for the soft rot of rhizome in ginger (Vivek et al., 2013; Rosangkima et al., 2018a).

Applying *T. harzianum* and *Bulkhoderia cepacian* resulted in a noteworthy decline in disease incidence by 80% and a corresponding increase in production efficiency by 84% according to Shanmugam et al. (2013). *T. harzianum* and *T. viride*, as biological control agents against *F. oxysporum* f.sp. *zingiberi* indicates maximum mycelial growth inhibition of 68.3% with *T. viride* and 66.7% growth inhibition with *T. harzianum*. Seed treatment with *T. viride* at a concentration of 4 g/10 mL of water per kg of seed under field conditions demonstrated a remarkable decrease in plant mortality along with disease control improving by 84.9% and plant stand improving by 32.8% (Khasto and Tiameren, 2013). Bandyopadhyay and Bhattacharya (2012) conducted a study on the management of ginger rhizome rot utilizing various methods. The combination of treating rhizomes with hot water at 51 °C for 10 min and applying *T. harzianum* inoculum mixed with 1 kg of neem cake in planting soil resulted in the lowest incidence of disease (27.14%) and highest yield of rhizomes. According to Meenu and Jebasingh (2019), treating seeds with 1% hypochlorous acid (HClO) followed by soaking in *Trichoderma* spp. and applying three rounds of talc-based formulation (3×10^6 CFU/g) in soil reduced disease occurrence. The incidence of disease was reduced by 80% and production efficiency was increased by 84% through the treatment with *T. harzianum* and *B. cepacian*, according to Shanmugam et al. (2013).

Trichoderma and *Pseudomonas* spp. suppress soil-borne pathogens through mechanisms such as faster nutrient uptake, production of plant-degrading enzymes, and toxic metabolites such as 2,4-diacetyl phloroglucinol and gliotoxin (Pal et al., 2006). The mycoparasitic character develops the plant resistance and production of antibiotics by the *Trichoderma* spp. Not only the production of potential antibiotics but also mycotoxins and more than 100 metabolites with antibiotics including polyketides, pyrones, terpenes, a metabolite derived from amino acids, and polypeptides can be obtained from the *Trichoderma* spp. can inhibit the growth of pathogenic fungi and enhance plant defense, rhizosphere colonization, and root growth (Odebode, 2006, Vinale et al., 2008, Bastakoti et al., 2017). The use of *Trichoderma* spp. as biological control agent represents a promising and environmentally friendly approach to managing soil-borne pathogenic fungal diseases by harnessing the antagonistic properties of *Trichoderma* spp. Farmers can potentially reduce disease incidence and enhance ginger production, resulting in improved crop yield and economic returns (Khasto and Tiameren, 2013).

This study hypothesizes that using *Trichoderma* spp. as biological control agent will significantly reduce the incidence of rhizome rot caused by *Fusarium* spp. in ginger cultivation. It is further expected that using *Trichoderma* spp. as biological control agent will enhance plant growth and resilience, resulting in improved ginger yield while minimizing the need for chemical interventions. The investigation aims to validate the efficacy of *Trichoderma*-based treatments as a sustainable solution for disease management in ginger production. This will contribute to the advancement of environmentally friendly and economically viable agricultural practices. The study aims to determine effective biological methods for controlling rhizome rot in ginger caused by *Fusarium* infection. The research evaluates the effectiveness of four different isolates of *Trichoderma* spp. against the pathogen causing rhizome rot in ginger. The findings of this research will contribute to a better understanding of potential strategies to manage and mitigate the impact of rhizome rot disease in ginger.

2. Materials and Methods

2.1. Sample Collection

The diseased sample was collected from the disease-prone areas. The samples of soil and diseased rhizome were collected from the ginger zone Sunsari, Prime Minister Agriculture Modernization Project (PMAMP), Nepal. The geographical coordinates of the sample collection site were latitude: 16°82′27.85″ N and longitude: 87°25′59.93″ E.

2.2. Preparation of Culture Media

Potato Dextrose Agar (PDA) was prepared by mixing 200 g of peeled and sliced potatoes with 20 g of dextrose (glucose) and 20 g of agar powder in 1000 mL of distilled water. The mixture was boiled until the potatoes became soft and their contents were released into the water. After boiling, the potato-water mixture was filtered to obtain a clear potato extract. To this clear extract, dextrose and agar powder were added while stirring to ensure complete dissolution. To prevent bacterial growth and contamination, 0.3 g of streptomycin, an antibiotic, was added to the mixture. The medium was poured into appropriate containers, such as flasks or bottles, and tightly sealed with nonabsorbent cotton and aluminum foil. The containers containing the PDA medium were then autoclaved at 121 °C and 15 psi for 15-20 min. This autoclaving process sterilizes the medium and eliminates any existing microorganisms. After autoclaving, the containers were allowed to cool to approximately 45–50 °C at room temperature. Once the PDA medium reached the desired temperature, it was carefully poured into sterilized Petri plates under a laminar airflow chamber to maintain sterility. The Petri plates containing the PDA medium were then allowed to solidify. The prepared PDA serves as a nutrient-rich substrate suitable for the growth of various microorganisms. It is commonly used in microbiological studies and research, including the evaluation of various *Trichoderma* spp. against the pathogen responsible for *Fusarium* rhizome rot of ginger. The addition of streptomycin to the PDA formulation prevents bacterial growth, allowing the interaction between *Trichoderma* spp. and the fungal pathogen to be studied in a controlled environment.

2.3. Isolation and Identification of Pathogen

The diseased sample was collected from the disease-prone areas (Figure 1). The samples of soil and diseased rhizome were collected from the ginger zone Sunsari, PMAMP Nepal. The isolation process was done at the Plant Protection Laboratory of GPCAR, Morang. Diseased rhizomes were specifically selected from the ginger zone in Sunsari, PMAMP. The collected samples were subjected to surface sterilization treatment using 2% sodium hypochlorite (NaOCl) solution for 5 min and rinsed twice with sterilized distilled water and the rhizome was dried using sterilized filter paper. Subsequently, inoculation was performed on PDA by transplantation method along with streptomycin to inhibit bacterial growth and incubated at 26±1 °C for 7 days. The resulting fungal colonies were sub-cultured by transferring 5 mm radial plugs from the colony edges. Pure culture was prepared by subculturing using the method of Siameto et al. (2010), (Mokhtar and Aid, 2013). According to Nelson et al. (1994), the identification of the pathogen was done by observing its morphological and cultural characteristics such as the production of macroconidia, microconidia, chlamydospores, and pigmentation (pink) of the colony.

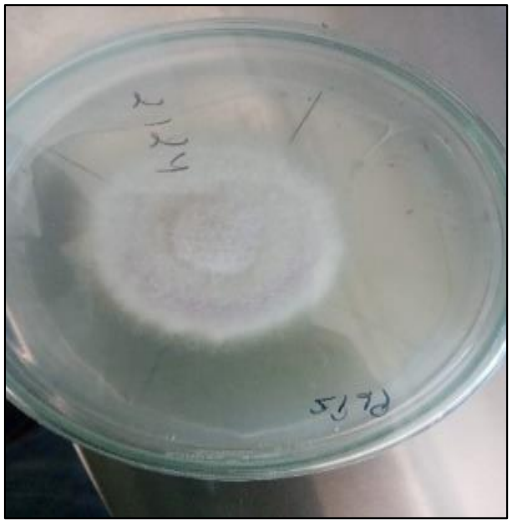

Figure 1. Isolated pathogen from the rotted rhizome of ginger.

2.4. Isolation and Identification of *Trichoderma* spp.

Four treatments of different isolates of *Trichoderma* spp. were obtained for the study i.e., T1 (Plant Protection Division at Nepal Agriculture Research Council, NARC), T2 (Regional Plant Protection Lab, RPPL, Koshi Province), T3 (*Trichoderma harzianum*, TRICHO HR, Excel Crop Care Limited, cell count: 2×10^6 cfu/g minimum), and T4 (*Trichoderma viride*, Biocide Trivi, Agricare Nepal Pvt Ltd., Bharatpur, Chitwan, cell count: 1×10^6 cfu/g, minimum). The *Trichoderma* isolated from NARC was kept in a Petri dish. Following the method of Siameto et al. (2010), a 5 mm radial disk NARC isolate of *Trichoderma* spp. was inoculated on PDA media. In addition, the *Trichoderma* products from RPPL (T2) and *T. harzianum* (T3) were in powder form. Therefore, 1 g of white powder *Trichoderma* from each treatment was separately mixed with 20 mL of distilled water. Subsequently, 1 mL of the resulting suspension was inoculated into PDA media to isolate the *Trichoderma* spp. from T2 and T3. As for the commercial product Biocide Trivi (T4), it was already in liquid suspension form and underwent the process for the isolation of *T. viride*. Then, incubated at 26±1 °C for 7 days and genus identification of the green fungus *Trichoderma* was carried out by using the method of (Hajieghrari et al., 2008; Domsch et al. 1980). The colonies were counted and transferred to PDA to prepare the pure culture of each treatment for further processing (Figure 2). The isolates were maintained on PDA and stored at 4 °C for further use.

Figure 2. Pure cultures of different isolates of *Trichoderma* spp.

2.5. Experimental Design and Treatments

The laboratory experiment was conducted using a completely randomized design (CRD) with five treatments and ten replications. The design was chosen to ensure the random assignment of treatments and to reduce bias in the experimental design. The CRD design allows for robust statistical analysis of the data and facilitates the assessment of treatment effects on the variables of interest. By replicating each treatment ten times, the experiment increases its precision and reliability, allowing more accurate conclusions to be drawn from the results. There was a total of five treatments in the experiment (Table 1). Among these treatments, four types of *Trichoderma* spp. were evaluated and there was a control treatment for comparison. The control treatment served as a natural control to evaluate the effect of different *Trichoderma* spp. on the pathogen causing *Fusarium* rhizome rot of ginger.

Table 1. List of different isolates of *Trichoderma* spp. used as a treatment in the experiment of the dual culture technique.

***Trichoderma* Isolates**	**Treatment Number**
NARC isolate of *Trichoderma* spp.	T1
RPPL isolate of *Trichoderma* spp.	T2
TRICHO HR- Commercial product of *T. harzianum*	T3
Biocide Trivi- Commercial product of *T. viride*	T4
Control	T5

These treatments were evaluated in the dual-culture experiment to determine the effectiveness of the different *Trichoderma* spp. in controlling the pathogen responsible for *Fusarium* rhizome rot in ginger. The CRD design with ten replications for each treatment was used to ensure the reliability and statistical significance of the study results.

2.6. Dual Culture Technique

On May 28, 2021, the solidified PDA medium was now ready for use, and the evaluation of *Trichoderma* and *Fusarium* spp. was carried out using the dual culture technique (Figure 3). For this evaluation, a dual culture technique according to Mokhtar and Aid. (2013) was followed. A 5 mm radial disc of 10 days old pure culture of different isolates of *Trichoderma* and *Fusarium* spp. were taken and placed on the edge of Petri plates, which were placed opposite to each other inoculum (90 mm diameter Petri plates containing PDA media). One disc of the pathogen was maintained as a control. Each treatment had ten replicates, and both dual culture and control plates were incubated at a controlled temperature of 28±1 °C for 7 days, and measurement of radial growth of dual culture and the pathogen was measured every 24 h.

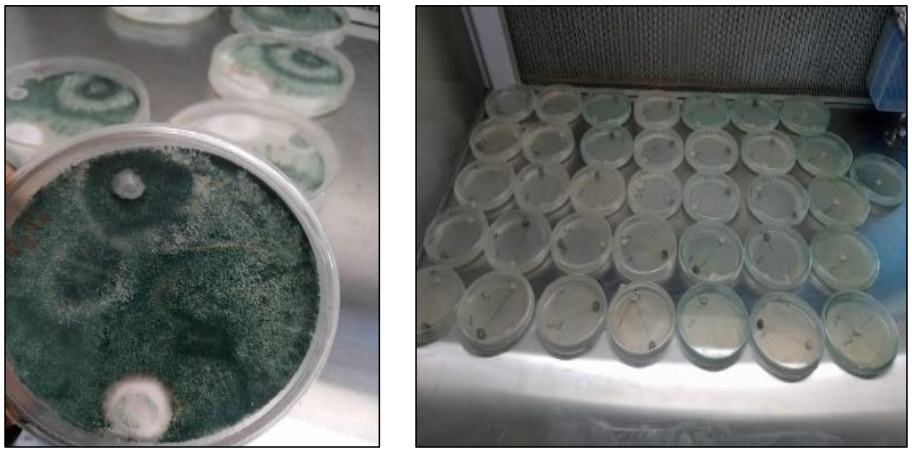

Figure 3. Pathogen and *Trichoderma* spp. in dual culture from 1st to 7th day of inoculation.

2.7. Growth Comparison and Data Collection

In this experiment, the growth comparison between different isolates of *Trichoderma* spp. was evaluated using 5 mm radial discs from 10 days old pure culture of each isolate. On May 28, 2021, the radial discs were inoculated in the center of sterile Petri plates containing an appropriate growth medium (PDA) and incubated at 28±1 °C. Throughout 24 h, the radial growth of *Trichoderma* spp. was measured from the center of the outer edge of the colony. This measurement was taken daily to follow the growth progress. After 24 h of inoculation, the radial diameter of both the pathogen (*Fusarium* spp.) and the *Trichoderma* spp. was measured from two directions on the Petri plates. These measurements were recorded daily for a total of 7 days, with readings taken at 24-h intervals. The purpose of this daily monitoring was to track the growth and interactions between the pathogen and *Trichoderma* spp. throughout the experiment and to observe any changes or effects of *Trichoderma* spp. on the growth of the pathogen. The mycelial growth of the pathogen was observed to be different in each treatment. To evaluate the effectiveness of the treatments in inhibiting the growth of the pathogen, the data from the different *Trichoderma* spp. treatments were compared with the control. To calculate the inhibition percentage of each treatment against the colony growth of the pathogen compared to the control, the formula given by Vincent (1947) was used:

$$\text{Inhibition Percentage (I: \%)} = (C - T)/C$$

Where "I" refers to the percent inhibition, "C" is the growth in the control, and "T" is the growth in the test treatment. By using this formula, the percentage of inhibition for each *Trichoderma* spp. treatment was determined based on the difference between the growth of the pathogen in the control and the respective treatment.

2.8. Statistical analysis

The data collected during the experiment, including the radial diameter measurements of *Trichoderma* spp. were organized in Microsoft Excel (Version 2007, Microsoft Corp., USA). To analyze and determine the statistical significance of the results, the data were subjected to analysis of variance (ANOVA) using R version 3.5.3 (RCT, 2023). After performing the ANOVA, mean comparisons were made between the significant variables. The Fisher's Least Significant Difference (LSD) test was used to compare means. This test helps to identify significant differences between the treatments and the control group at the 5% level of significance.

3. Results

3.1. Growth Comparison of Selected Isolates of *Trichoderma* spp.

During the experiment, the different isolates of *Trichoderma* spp. showed different growth patterns (Figure 4). After 48 h, the colony of T2 covered the largest area on the Petri plates with a diameter of 68 mm. This was followed by T1 and T4 with colony diameters of 56 and 54 mm, respectively. On the other hand, the growth rate of T3 was much slower, showing the smallest colony diameter of 36 mm at 48 h. At the 72-h mark, the colony diameters of T1, T2, and T4 treatments had increased to 72, 72, and 70 mm, respectively. Meanwhile, the colony diameter of the T3 treatment measured 54 mm, indicating slower growth compared to the other *Trichoderma* isolates. The different isolates of *Trichoderma* showed different growth patterns during the experiment. The colony of T2 covered 68 mm diameter of the Petri plates after 48 h. This was followed by T1 and T4 at 56 and 54 mm respectively. While the growth rate of T3 was very slow with the least colony diameter of 36 mm only at 48 h. At 72 h the colony diameter of treatments T1, T2, and T4 was measured 72, 72, and 70 mm, respectively whereas the colony diameter of treatment T3 was measured 54 mm. The experimental data highlight the different growth behaviors of the *Trichoderma* isolates studied. T2 showed the fastest growth, covering a substantial area within the Petri plates at 48 h. T1 and T4 also showed significant growth, while T3 lagged with the smallest colony diameter at 48 h. At 72 h, T2 continued to show robust growth, paralleling T1 and T4, while T3's growth remained comparatively limited. These results highlight the variability in growth patterns among the *Trichoderma* isolates tested and confirm the observations. Figure 4 shows the growth patterns of different *Trichoderma* spp. isolates during different incubation periods. After 96 h, T1, T2, and T4 completely covered the Petri plates with colony diameters of 89, 89, and 88 mm, respectively. In contrast, T3 had a smaller colony diameter of 72 mm at this time. However, by the 120-h mark, T3 showed improved growth, approaching the other three treatments with a colony diameter of 88 mm. Proceeding to the 144-h mark, complete coverage of 90-mm Petri plates was achieved by all treatments, including T3.

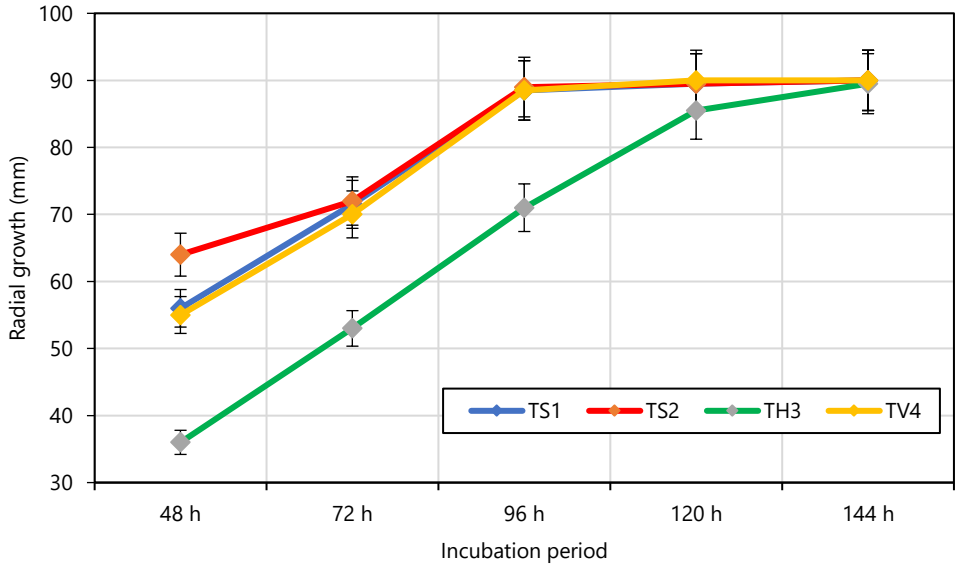

Figure 4. The growth pattern of different isolates of *Trichoderma* spp. at different incubation periods.

Despite T3's gradual start of growth within the first 24 h, it converged with the growth rates of the other treatments. This convergence resulted in T3 successfully covering the Petri dish by the 144-h mark. In contrast, T2, T1, and T4 had already achieved full coverage by the 96-h point. The results of the growth dynamics inherent to different *Trichoderma* isolates over time. It is evident that while T3 experienced a delay in growth initiation, it eventually reached growth comparability with other treatments and achieved complete Petri plate coverage within the same time frame. The graphical representation provides valuable insight into the growth trajectories of *Trichoderma* spp. This has implications for their potential efficacy in controlling *Fusarium* rhizome rot in ginger, highlighting T3's ability to effectively combat the pathogen (Table 2).

Table 2. Analysis of variance of growth of *Fusarium* spp. after 7th day of inoculation in dual culture.

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F-value	P (>F)
Treatment	4	1116.35	279.08	830.07	<0.001
Error	45	15.13	0.33		
Total	49	1131.48			
Coefficient of Variation: 3.97%					

3.2. Effect of Selected Isolates of *Trichoderma* spp. on *Fusarium* spp.

The results of this investigation have revealed remarkably significant variations (P≤0.001) among all treatment groups in terms of radial growth and *Fusarium* spp. inhibition by different *Trichoderma* spp. isolates in dual culture, as meticulously presented in Tables 3 and 4. Within the spectrum of *Trichoderma* isolates, T1 showed the least pathogen growth with a radial diameter of 9.50 mm, followed by T2 with a diameter of 10.70 mm and T4 with 13.30 mm. Notably, the presence of T3 facilitated the highest pathogen mycelium growth with a radial diameter of 16.95 mm. In terms of pathogen mycelial growth suppression, T1 stood out as the most potent isolate, achieving an impressive 57.91% inhibition percentage. T2 and T4 also exhibited remarkable inhibitory abilities, with percentages of 52.50% and 40.98%, respectively. In contrast, T3 showed the least inhibition of mycelial growth among the treatments, with an inhibition percentage of 24.75%.

Table 3. Effect of different isolates of *Trichoderma* spp. on inhibition of mycelial growth of *Fusarium* spp. after 7th day of inoculation.

Treatments	Radial Growth (mm) of *Fusarium*	Growth Inhibition (%)
TS1 (NARC isolate)	9.50 e	57.91 a
TS2 (RPPL isolate)	10.70 d	52.50 b
TV4 (Biocide Trivi)	13.30 c	40.98 c
TH3 (TRICHO-HR)	16.95 b	24.75 d
Control	22.55 a	0.00 e
LSD0.05	0.52	2.48
CV (%)	3.97	7.83
F-Test	***	***

CV: Coefficient of variation; ***: significant at 0.1% level of significance; LSD: least significant difference; values with the same letters in a column are not significantly different at 5% level of significance by Fisher-LSD test.

Table 4. Analysis of variance o growth inhibition of *Fusarium* spp. after 7th day of inoculation in dual culture.

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F-value	P (>F)
Treatment	4	21964.70	5491.20	721.71	<0.001
Error	45	342.40	7.60		
Total	49	22307.10			
Coefficient of Variation: 7.83%					

4. Discussion

The current study builds on a substantial body of research focused on the biocontrol potential of different *Trichoderma* spp. against various soil-borne pathogens. In particular, Bastakoti et al. (2017) laid the groundwork by isolating five different *Trichoderma* spp. from various soil samples and subjecting them to dual culture with *Sclerotium rolfsi* and *F. solani*, and their results highlighted the remarkable inhibitory effects by completely inhibiting the radial growth of *S. rolfsi* and 63.3%, respectively, highlighting the potential of *Trichoderma* spp. as effective biocontrol agents. Further highlighting the practical implications of *Trichoderma*-based biocontrol, Chao et al. (2019) evaluated the efficacy of different Trichoderma isolates against *Rhizoctonia solani*-induced root rot in *Vigna unguiculate*. Their study showed a significant reduction in disease severity, with significant reductions of up to 45% observed. In line with these findings, Hedge et al. (2017) reported the significant efficacy of *T. harzianum*, especially compared to other bioagents such as *P. fluorescens*, against *F. oxysporum* f.sp. *dianthus*-induced wilt of carnation. A remarkable reduction of 64.44% in the colony growth of the pathogen further substantiates the potential of *Trichoderma* spp. in biocontrol strategies. Contributing to the understanding of the antagonistic abilities of *Trichoderma*; Khasto and Tiameren (2013) investigated the inhibitory effects of *T. viride* and *T. harzianum* against *F. oxysporum* f.sp. *zingiberi*. Their work, which included both in vitro and in vivo evaluations, showed significant mycelial growth inhibition rates of 68.30 and 66.70%, respectively. The transition

from laboratory experiments to field applications was exemplified by a remarkable reduction in plant mortality (4.20%) and subsequent increase in disease control (84.90%) following seed treatment with *T. viride*. Kumar et al. (2021) further contributed to the understanding of the biocontrol efficacy of *Trichoderma* by evaluating five biocontrol agents, including *Trichoderma* spp. against *F. oxysporum* using dual culture techniques. Their results showed significant inhibition of mycelial growth for all bioagents tested, with *T. viride* and *T. harzianum* showing the maximum inhibition rates of 76.25 and 68.75% at 144 h, respectively.

Interestingly, Dar et al. (2013) highlighted the synergistic effect of combining *T. harzianum* and *T. viride*, resulting in a robust 87.33% growth inhibition of *Fusarium* mycelium. In addition, the individual application of this *Trichoderma* spp. resulted in significant mycelial growth inhibition of 73.33 and 60.00%, respectively. The antagonistic activity of *Trichoderma* against *Fusarium* pathogens has been demonstrated, and *T. harzianum* and *T. viride* exhibited potent inhibitory effects against *F. proliferatum* and *F. verticilliodes*, with mycelial growth inhibitions ranging from 68.38 to 80.17% (Yassin et al., 2021). Similarly, strong antagonistic activity of *T. viride* and *T. harzianum* against *Alternaria alternata* was observed, further highlighting the versatility of *Trichoderma* spp. in biocontrol strategies (Yassin et al., 2022). In the field of in vitro assays, *Aspergillus flavus* has demonstrated its ability as a potent bioagent against *Fusarium* spp. with an impressive inhibition rate of 50% (Boughalleb-M'Hamdi et al., 2018). Similarly, *A. niger* and *A. terreus* have demonstrated hyperparasitism against *F. oxysporum* f.sp. *melon*, further demonstrating the diverse antifungal capabilities within the *Aspergillus* genus. Furthermore, the inhibitory influence of *T. harzianum* ion on the mycelial growth of *Macrophomina phaseolina* was demonstrated, with a remarkable 44.42% reduction observed in the presence of this biocontrol agent (Boughalleb-M'Hamdi et al., 2018). The comprehensive work of Mustafa et al. (2009) has revealed the broad-spectrum inhibitory potential of different *Trichoderma* spp. against several seed-borne fungi. Their dual culture assay revealed substantial growth inhibition in six different fungal species, including *F. monoliforme*, *F. oxysporum*, *R. solani*, *F. solani*, *Botryoplodia theobromae*, and *A. alternate* (Mustafa et al., 2009).

In particular, chemical compounds extracted from *Trichoderma* spp., such as 6-pentyl-α-pyrone and cyclooctanol, have been identified as active ingredients contributing to their antagonistic properties (Yassin et al., 2021). Khatri et al. (2017) studied *T. hamatum* and *T. harzianum* and uncovered their potent enzymatic arsenal. They demonstrated chitinolytic activity and secretion of key antifungal enzymes. These *Trichoderma* strains were effective against fungal pathogens such as *A. niger*, *F. oxysporum*, and *S. rolfsi*. Key enzymes, including chitinase, protease, and β-glucanase, were secreted to degrade cell walls. *Trichoderma* spp. recognize hosts by wrapping themselves around hyphae and using these enzymes to break through the cell wall and absorb nutrients for their growth. Chitin, a mainstay of fungal cell walls with its β-1, 3-glucan content, is targeted by *Trichoderma*'s chitinases and β-1, 3-glucanases, reinforcing the mycoparasitic interaction and defining the competitive dynamic. In contrast to previous research indicating the strong efficacy of *T. harzianum* (T3-TRICHO HR), the current study has revealed a different result. In the present study, *T. harzianum* (T3-TRICHO HR) displayed a remarkably satisfactory growth inhibition rate of 24.75%, diverging from the more remarkable performances observed in other isolates such as T1-NARC (57.91%), T2-RPPL (52.50%), and *T. viride* (T3-Biocide Trivi with 40.98%). This variation in performance is likely due to genetic differences among these *Trichoderma* isolates. Genetic diversity is known to influence microbial functionality and interactions, suggesting that specific genetic traits of *T. harzianum* (T3-TRICHO HR) in this study differed from those in previous studies, contributing to the observed differences. This finding highlights the dynamic nature of biological interactions and underscores the importance of genetic diversity in assessing the efficacy of biocontrol agents. Further exploration through genetic analysis promises deeper insights into the mechanisms driving these performance differences, ultimately refining our approach to optimizing biocontrol strategies.

5. Conclusion

In this study, the efficacy of different *Trichoderma* spp. isolates, obtained from both plant protection departments and commercial products, were evaluated for biocontrol of *Fusarium* rhizome rot in ginger. The growth pattern of different Trichoderma spp. isolates varied during the experiment with T1-NARC covering the petri plate after 96 h, followed by T2-RPPL and T4-Biocide Trivi. T3-TRICHO HR showed a slower growth rate. The dual culture experiments showed significant inhibition of *Fusarium* mycelial growth by various *Trichoderma* isolates and commercial products. T1-NARC showed the highest efficacy with 57.91% growth inhibition, followed by T2-RPPL (52.50%) and T4-Biocide Trivi (40.98%). However, T3-TRICHO HR showed lower performance with 24.75% inhibition. Overall, these results provide

valuable insights into the use of *Trichoderma* spp. as a biocontrol agent in ginger cultivation to improve disease control and crop health. By integrating these biocontrol strategies, farmers can reduce the economic losses caused by rhizome rot diseases, leading to sustainable crop production and improved livelihoods. Further research to optimize application techniques and understand the genetic diversity of *Trichoderma* isolates could improve their biocontrol efficacy. In conclusion, *Trichoderma* spp. show promise as an effective biocontrol agent against *Fusarium* rhizome rot in ginger cultivation.

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