Evaluation of Antagonistic Activity of *Trichoderma Spp.* For Sustainable Management of Plant Diseases

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ABSTRACT

Plant diseases caused by soil-borne phytopathogenic fungi are a major constraint to global agricultural productivity, leading to significant yield losses and increased dependence on chemical fungicides. Sustainable management of plant diseases has therefore gained considerable attention, with biological control emerging as an eco-friendly alternative. The present study was undertaken to evaluate the antagonistic activity of different *Trichoderma spp*. against selected soil-borne plant pathogenic fungi under *in vitro* conditions. Antagonistic potential was assessed using the dual culture technique on potato dextrose agar, and percent inhibition of mycelial growth was calculated. The results revealed significant variation among *Trichoderma* isolates in suppressing pathogen growth. Maximum mycelial growth inhibition ranging from 68.4% to 82.7% was recorded with *Trichoderma harzianum*, followed by *Trichoderma viride* (61.2%–74.5%), while comparatively lower inhibition (45.3%–56.8%) was observed with other isolates. The strong antagonistic activity exhibited by effective isolates was characterized by rapid overgrowth, hyphal coiling, and clear inhibition zones, indicating mechanisms such as competition, mycoparasitism, and antibiosis. Statistical analysis confirmed that the inhibition effects were significant ($p \leq 0.05$). The study demonstrates that selected *Trichoderma spp*. possess substantial biocontrol potential against soil-borne pathogens and can be effectively utilized as sustainable alternatives to chemical fungicides. These findings highlight the role of *Trichoderma*-based biocontrol agents in environmentally safe plant disease management and support their further evaluation under field conditions.

Keywords: *Trichoderma spp*.; Antagonism; Biocontrol; Soil-borne pathogens; Sustainable agriculture

1. INTRODUCTION

Plant diseases remain one of the most serious constraints to agricultural productivity worldwide, causing substantial qualitative and quantitative losses across a wide range of crops. It is estimated that plant pathogens are responsible for annual yield losses of nearly 20–30% globally, posing a major threat to food security, farmer livelihoods, and agricultural sustainability (Oerke, 2006; Savary *et al*., 2019). Soil-borne fungal pathogens such as *Fusarium*, *Rhizoctonia*, *Sclerotium*, and *Pythium* are particularly destructive due to their long-term persistence in soil, wide host range, and difficulty in effective control using conventional methods. These pathogens not only reduce crop yield but also impair crop quality, resulting in significant economic losses.

Chemical fungicides have traditionally been employed as the primary strategy for managing plant diseases. Although they provide rapid and effective disease suppression, their continuous and indiscriminate use has led to several adverse consequences, including the development of fungicide-resistant pathogen populations, accumulation of toxic residues in soil and water, and negative impacts on non-target organisms and human health (Brent & Hollomon, 2007; Pimentel, 2009). Moreover, increasing regulatory restrictions and consumer demand for residue-free agricultural produce have necessitated the search for alternative disease management strategies that are environmentally benign and economically viable.

In this context, sustainable plant disease management has emerged as a key component of modern agriculture, emphasizing reduced chemical inputs, conservation of ecological balance, and long-term disease suppression (Cook, 2010). Sustainable approaches integrate cultural practices, host resistance, and biological control methods to minimize pathogen pressure while maintaining soil health and biodiversity. Among these approaches, biological control using antagonistic microorganisms has gained significant attention due to its eco-friendly nature and compatibility with integrated disease management systems.

Biological control agents (BCAs) suppress plant pathogens through diverse mechanisms and offer several advantages over chemical fungicides, including target specificity, minimal environmental impact, and reduced risk of resistance development (Pal & McSpadden Gardener, 2006). Fungal BCAs, particularly species belonging to the genus *Trichoderma*, are among the most extensively studied and commercially exploited microbial antagonists for plant disease management. These fungi are naturally abundant in soil and rhizosphere ecosystems and exhibit strong adaptability to diverse environmental conditions.

Species of *Trichoderma* have been widely recognized for their ability to control a broad spectrum of plant pathogenic fungi and for their additional plant growth-promoting effects. Several studies have reported the successful use of *Trichoderma* spp. against soil-borne pathogens through direct antagonism as well as indirect enhancement of plant defense responses (Harman *et al*., 2004; Vinale *et al*., 2008). Their rapid growth rate, ease of mass multiplication, and rhizosphere competence make them ideal candidates for sustainable disease management programs.

The antagonistic activity of *Trichoderma* spp. is attributed to multiple mechanisms, including competition for nutrients and space, secretion of antifungal metabolites (antibiosis), and direct mycoparasitism involving hyphal coiling and enzymatic degradation of pathogen cell walls (Benítez *et al*., 2004; Howell, 2003). The production of hydrolytic enzymes such as chitinases, glucanases, and proteases plays a crucial role in the lysis of pathogenic fungal hyphae. Additionally, volatile and non-volatile secondary metabolites produced by *Trichoderma* spp. contribute significantly to pathogen suppression.

Despite extensive research on *Trichoderma*-based biocontrol, the antagonistic potential varies considerably among species and isolates due to genetic diversity, ecological adaptability, and interaction with specific pathogens. Many indigenous isolates remain unexplored, and comparative evaluations under standardized conditions are limited. Furthermore, the effectiveness of *Trichoderma* spp. against locally prevalent plant pathogens requires systematic investigation to ensure reliable field application.

Therefore, the present study was undertaken to evaluate the antagonistic activity of *Trichoderma* spp. against selected plant pathogenic fungi for sustainable disease management.

2. MATERIALS AND METHODS

2.1 Collection and Isolation of *Trichoderma* spp.

Soil and rhizosphere samples were collected from healthy crop fields showing no visible symptoms of disease. Rhizosphere soil was obtained by gently uprooting plants and collecting soil tightly adhering to the roots. Samples were placed in sterile polyethylene bags, labeled properly, and transported to the laboratory for further processing. Isolation of *Trichoderma* spp. was carried out using the serial dilution technique. One gram of soil sample was suspended in 9 mL of sterile distilled water and serially diluted up to 10^{-5}. Aliquots (0.1 mL) from appropriate dilutions were spread on Trichoderma Selective Medium (TSM) or potato dextrose agar (PDA) amended with streptomycin to suppress bacterial growth (Elad *et al*., 1981). Plates were incubated at 25 ± 2^0C for 5–7 days. Distinct greenish colonies resembling *Trichoderma* were sub-cultured repeatedly to obtain pure cultures. The purified isolates were maintained on PDA slants at 4^0C for short-term storage and periodically sub-cultured to maintain viability.

2.2 Identification of *Trichoderma* Isolates

Morphological identification of *Trichoderma* isolates was performed based on colony characteristics, growth pattern, pigmentation, and microscopic features such as conidiophore branching, phialide arrangement, and conidial shape. Microscopic observations were made using lactophenol cotton blue staining under a light

microscope following standard mycological keys (Barnett & Hunter, 1998; Rifai, 1969). Where molecular identification was performed, genomic DNA was extracted from fresh mycelium using a standard fungal DNA extraction protocol. The internal transcribed spacer (ITS) region of rDNA was amplified using universal primers ITS1 and ITS4. PCR products were purified and sequenced, and the obtained sequences were compared with available sequences in the NCBI GenBank database using BLAST to confirm species identity (White *et al*., 1990).

2.3 Plant Pathogenic Fungi Used

Plant pathogenic fungi used in the study included soil-borne pathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotium rolfsii*. These pathogens were obtained from infected plant tissues or maintained cultures available in the departmental culture collection. Isolation of pathogens was carried out using standard tissue isolation techniques on PDA. Pure cultures were obtained through hyphal tip or single-spore isolation and maintained on PDA slants at 4^0C for further experimentation.

2.4 *In vitro* Antagonistic Activity (Dual Culture Assay)

The antagonistic activity of *Trichoderma* spp. against plant pathogenic fungi was evaluated using the dual culture technique described by Dennis and Webster (1971). PDA medium was poured into sterile Petri plates and allowed to solidify. A 5-mm diameter mycelial disc from the actively growing margin of a *Trichoderma* isolate was placed on one side of the Petri plate, while a similar disc of the test pathogen was placed at an equal distance on the opposite side. Control plates were inoculated with the pathogen alone. All plates were incubated at 25 ± 2^0C for 7 days. Radial growth of the pathogen was measured when the control plate was fully covered. Percent inhibition of mycelial growth was calculated using the formula:

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

where C is the radial growth of the pathogen in control and T is the radial growth in the presence of *Trichoderma*.

2.5 Experimental Design and Statistical Analysis

All experiments were conducted following a Completely Randomized Design (CRD) with three replications for each treatment. Data on percent inhibition of mycelial growth were subjected to statistical analysis using appropriate software such as SPSS or OPSTAT. Analysis of variance (ANOVA) was performed, and treatment means were compared at a 5% level of significance ($p \leq 0.05$).

3. RESULTS

3.1 Antagonistic Activity of *Trichoderma* spp.

The in vitro antagonistic activity of different *Trichoderma* isolates against selected soil-borne plant pathogenic fungi was evaluated using the dual culture technique. All *Trichoderma* isolates exhibited varying degrees of inhibition against the test pathogens compared to the untreated control. The percent inhibition of mycelial growth ranged from 45.3% to 82.7%, indicating significant variability among the isolates.

Among the tested isolates, *Trichoderma harzianum* recorded the highest antagonistic activity, showing maximum mycelial growth inhibition of 82.7% against *Fusarium oxysporum*, followed by 78.9% inhibition against *Rhizoctonia solani*. *Trichoderma viride* also showed strong antagonistic potential, with inhibition values ranging from 61.2% to 74.5% against different pathogens. Other *Trichoderma* isolates exhibited moderate inhibition, with values ranging between 45.3% and 58.6%. In contrast, control plates inoculated with pathogens alone showed complete radial growth without any inhibition.

3.2 Comparative Performance of Isolates

Statistical analysis revealed significant differences ($p \leq 0.05$) among *Trichoderma* isolates with respect to their antagonistic activity against the tested pathogens. The variation in percent inhibition clearly indicated differential antagonistic efficiency among the isolates. *Trichoderma harzianum* consistently performed better

across all pathogens tested, followed by *Trichoderma viride*, while the remaining isolates showed comparatively lower inhibitory effects. The interaction between *Trichoderma* isolates and pathogens resulted in distinct growth suppression patterns, as evident from reduced pathogen colony diameter in treated plates compared to control.

Table 1. *In vitro* antagonistic activity of *Trichoderma* spp. against soil-borne plant pathogenic fungi

***Trichoderma* isolate**	***Fusarium oxysporum* (%)**	***Rhizoctonia solani* (%)**	***Sclerotium rolfsii* (%)**
T. harzianum	82.7 ± 1.9	78.9 ± 2.1	75.4 ± 1.8
T. viride	74.5 ± 2.3	68.6 ± 1.7	61.2 ± 2.0
T. asperellum	58.6 ± 1.5	54.2 ± 1.9	49.8 ± 1.6
T. hamatum	52.4 ± 1.8	48.7 ± 2.2	45.3 ± 1.4
Control	0.0	0.0	0.0

Values are mean ± SE of three replications.

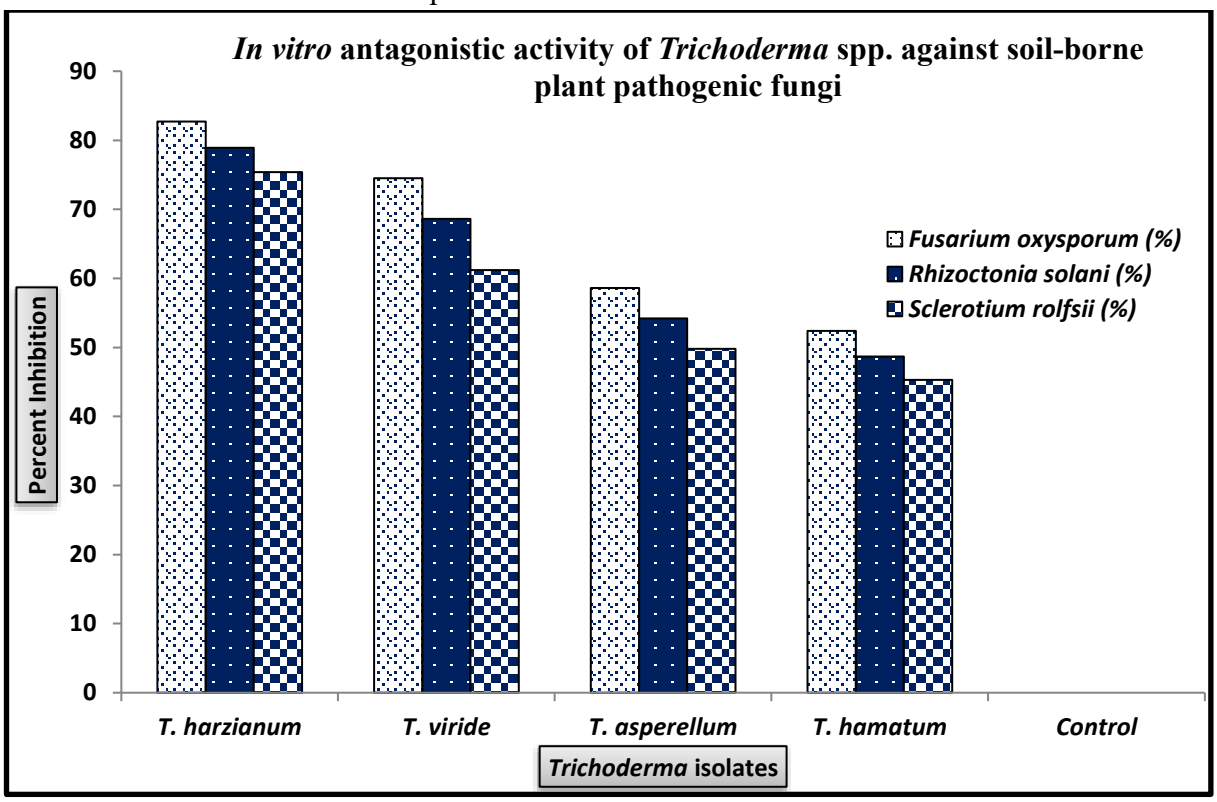

Figure 1: *In vitro* antagonistic activity of *Trichoderma* spp. against soil-borne plant pathogenic fungi

4. DISCUSSION

The present investigation demonstrated that *Trichoderma* spp. exhibited pronounced antagonistic activity against selected soil-borne plant pathogenic fungi under in vitro conditions, confirming their potential as effective biological control agents. The differential inhibition observed among *Trichoderma* isolates indicates that antagonistic efficacy is isolate- and species-specific, which is consistent with earlier reports emphasizing the genetic and physiological diversity within the genus (Benítez *et al*., 2004; Harman *et al*., 2004). Such variability highlights the importance of screening and selecting efficient local isolates rather than relying on generalized species-level assumptions.

The superior performance of certain *Trichoderma* isolates, particularly *T. harzianum*, may be attributed to their rapid growth rate, strong competitive ability for nutrients and space, and aggressive colonization of the growth medium. Faster colonization allows *Trichoderma* to deprive pathogens of essential resources, resulting in reduced pathogen growth. Similar observations have been reported by Howell (2003) and Vinale et al.

(2008), who emphasized competition as a primary mechanism contributing to pathogen suppression in dual culture assays.

In addition to competition, multiple antagonistic mechanisms likely contributed to the inhibitory effects observed in this study. Direct mycoparasitism, characterized by hyphal coiling and penetration of pathogen hyphae, is a well-documented feature of *Trichoderma*–pathogen interactions (Chet & Inbar, 1994). The secretion of cell wall–degrading enzymes such as chitinases, β-1,3-glucanases, and proteases enables *Trichoderma* to degrade the structural components of pathogenic fungi, leading to growth inhibition and lysis (Benítez *et al*., 2004; Lorito *et al*., 2010). Furthermore, antibiosis mediated by volatile and non-volatile secondary metabolites, including peptaibols and polyketides, has been reported to play a significant role in suppressing pathogen development (Vinale *et al*., 2009).

The findings of the present study are in agreement with earlier studies reporting strong antagonistic activity of *Trichoderma* spp. against soil-borne pathogens such as *Fusarium*, *Rhizoctonia*, and *Sclerotium* (Dennis & Webster, 1971; Harman *et al*., 2004; Mukherjee *et al*., 2012). Several researchers have reported that *T. harzianum* and *T. viride* consistently outperform other species due to their metabolic versatility and adaptability to diverse environmental conditions. The consistency of these findings across different studies strengthens the reliability of *Trichoderma*-based biocontrol strategies.

From an applied perspective, the observed antagonistic potential of *Trichoderma* spp. underscores their relevance in sustainable agriculture. The use of biological control agents offers an environmentally safe alternative to chemical fungicides, reducing chemical residues, minimizing ecological disruption, and lowering the risk of resistance development in pathogens (Pal & McSpadden Gardener, 2006; Cook, 2010). Incorporation of *Trichoderma*-based formulations into integrated disease management programs can contribute to long-term disease suppression while promoting soil health and microbial diversity.

Overall, the results of this study reinforce the role of *Trichoderma* spp. as promising biocontrol agents for sustainable plant disease management and support their further evaluation under greenhouse and field conditions to validate their efficacy in real agro-ecosystems.

5. CONCLUSION

The present study clearly demonstrated the strong antagonistic potential of *Trichoderma* spp. against selected soil-borne plant pathogenic fungi under in vitro conditions. All tested isolates exhibited varying degrees of mycelial growth inhibition, indicating their ability to suppress pathogen development. Among the evaluated isolates, *Trichoderma harzianum* emerged as the most effective antagonist, consistently exhibiting superior inhibitory activity against multiple pathogens. This highlights the importance of isolate selection in maximizing the efficacy of biological control strategies. The antagonistic performance of *Trichoderma* spp. can be attributed to their rapid growth, competitive colonization, and multifaceted mechanisms such as competition for nutrients and space, production of antifungal metabolites, and direct mycoparasitism. These attributes collectively contribute to effective pathogen suppression without the adverse environmental impacts associated with chemical fungicides. The findings reinforce the role of *Trichoderma*-based biocontrol agents as eco-friendly and sustainable alternatives for plant disease management. Furthermore, the consistent *in vitro* efficacy of *Trichoderma* spp. suggests their potential applicability under greenhouse and field conditions. However, large-scale field evaluations are essential to validate their performance under diverse agro-climatic environments and cropping systems. Integration of effective *Trichoderma* isolates into integrated disease management programs could significantly reduce chemical fungicide dependency while promoting soil health and sustainable agricultural productivity. Overall, the study supports the development and application of *Trichoderma*-based formulations as a promising strategy for environmentally safe plant disease management.

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