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Assessment of *Trichoderma asperellum* as a Beneficial Soil Fungus against Soil-Borne Pathogen of Chilli

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ABSTRACT

Soil-borne diseases represent a major constraint to chilli (Capsicum spp.) production due to the persistence of pathogenic fungi in soil and their strong association with soil health conditions. Conventional management practices relies on chemical fungicides often provide limited control of soil-resident inoculum and may negatively affect soil biological activity. Biological control agents with the ability to suppress soil-borne pathogens are increasingly explored as sustainable alternatives. Among these, Trichoderma are widely recognized for their antagonistic activity against a range of soil-borne fungal pathogens. The present study aimed to evaluate the antagonistic potential of T. asperellum against selected soil-borne fungal pathogens of chilli. Antagonistic activity was assessed under laboratory conditions using dual culture assays to determine the inhibitory effects of T. asperellum isolates on pathogen growth. By using soil dilution plating method, T. asperellum was isolated from the healthy chilli rhizosphere cultivated in MARDI Organic Farm and fungal pathogens were obtained from the infected chilli plants. The results demonstrated strong antagonistic activity of T. asperellum with inhibition of pathogen growth ranging from 65.5% to 81.1%. These findings confirm the role of T. asperellum as a beneficial soil fungus and highlight its potential as a biocontrol for managing chilli diseases.

Key words: *Trichoderma, Soil-borne pathogens, chilli, dual culture assay*

INTRODUCTION

Soil-borne fungal pathogens are major constraint in agriculture due to their ability to survive in soil for extended periods and cause persistent infections. These pathogens persist in soil as chlamydospores, sclerotia or oospores which allow them to remain viable even in the absence of a host. Pathogenic soil-borne and soil-inhabiting fungi may disrupt plant health by weakening root systems, making plants more susceptible to environmental stress and nutritional deficiencies (Yang *et al.*, 2024). Their persistence in soil and plant debris provides a continuous inoculum reservoir that facilitates recurrent infection cycles. Once the environment becomes favorable, it may infect the plant and cause severe damage including root rot, wilting and stunting to the plant. Soil health influences the severity and prevalence of soil-borne diseases which encompasses the biology, chemical and physical soil properties. Healthy soils rich in organic matter and microbial diversity and can naturally suppress pathogen proliferation through competition, antagonism and other biological interactions (Trivedi *et al*., 2022). Conversely, degraded soils with low microbial activity, poor structure and nutrient imbalance are more conducive to the establishment and spread of soil-borne pathogens (Larkin *et al*., 2020).

Chilli (*Capsicum spp.*) is a high-value vegetable crop widely cultivated in tropical and subtropical regions including Southeast Asia. It is susceptible to many soil-borne pathogen that cause stunted growth, root necrosis and wilting. Soil-borne fungi are common almost in all farm soils where it survives through chlamydospores that germinate after contacting with

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susceptible plant roots and directly influence water and nutrient uptake capacity (Sharma *et al*., 2020).

Conventional management relies on chemical fungicides that can lead to the development of resistance in pathogens, frequently fail to eliminate soil-resident inoculum and may disrupt beneficial microorganisms (Zhao *et al*., 2024). This development has prompted researchers to explore alternate options for controlling pathogens. Biological control strategies offer a sustainable alternative, targeting pathogens directly in the soil while supporting soil ecosystem functioning.

Among potential agents, a beneficial soil fungus; *Trichoderma* have been studied for their antagonistic activity against soil-borne pathogens. *Trichoderma* can inhibit pathogen growth through mechanisms such as competition for nutrients, mycoparasitism, production of antifungal metabolites and cell wall-degrading enzymes (Zeilinger *et al*., 2016). They are saprophytic fungi with fast mycelial growth and strong adaptability to the environment. The growth rate of *Trichoderma* is much faster than that of plant-pathogenic fungi; therefore, it can effectively inhibit the growth of plant-pathogenic fungi (*Mohiddin et al*., 2021). Through its rapid growth and reproduction, it can seize nutrients and space near the plant rhizosphere, consume oxygen in the air, and weaken the growth of plant pathogenic fungi (Panchalingam *et al*., 2022). In vitro, studies indicated that *Trichoderma* can inhibit *Fusarium*, *Rhizoctonia*, and *Pythium* in chilli suggesting their promising as biocontrol agents (Kumar *et al*., 2023). *Trichoderma* based biocontrol agents have better ability to promote plant defense response, promote plant growth and soil remediation (Shirke *et al.,* 2025). Therefore, the objective of this study was to evaluate the antagonistic potential of *Trichoderma* against various soil-borne pathogens associated with chilli. The results obtained can be used as baseline information for future field evaluation in support of sustainable disease management and improved soil health.

MATERIALS AND METHODS

Soil chemical analysis

Soil samples were air-dried, ground using an agate mortar, and passed through a 2-mm sieve prior to chemical analyses. The soil parameters analysed included pH, organic carbon content and available phosphorus (P).

Soil pH (H_2O)

One gram of air-dried soil was mixed with 25 mL of distilled water at a soil-to-water ratio of 1:2.5 (w/v). The suspension was stirred and left overnight before pH measurement using a calibrated pH meter (Mettler Toledo Delta 320).

Organic Carbon

Soil organic carbon was determined by the Walkley–Black dichromate oxidation method (Walkley & Black, 1934)**.**

Available Phosphorus (P)

Available phosphorus was determined using the Bray and Kurtz No. 1 extraction method. Two grams of air-dried soil (<2 mm) were extracted with 20 mL of a solution containing 0.03 N NH_4F and 0.1 N HCl. The mixture was shaken for 45 s and filtered through Whatman No. 42 filter paper. Phosphorus concentration in the extract was determined using a UV–Vis spectrophotometer (Shimadzu UV-1700 Double Beam Scanning UV–Vis Spectrophotometer).

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Isolation and Identification of Biocontrol Agent (*Trichoderma asperellum*) and Soil-Borne Pathogens

Soil sample collection

Soil samples were collected from the root zone of healthy chilli (*Capsicum spp.*) plants grown at the MARDI Organic Farm, Serdang. The soil samples (200 g) were collected in triplicate by using a sterile trowel at depth 10 cm within a radius of 0.5 m around the trunk or stem of chilli plants (Sharifah *et al*., 2021). The samples were placed in sterile plastic bags, transported to the laboratory in cool conditions and and stored at 4 °C until being used.

Trichoderma isolation from the soil

Ten grams (10 g) of soil was suspended in 100 mL of sterile distilled water prior agitation on a shaker at 100 rpm for 10 minutes. Serial dilutions were prepared up to 10^{-5}. One milliliter (ml) of each dilution was spread onto Trichoderma Selective Medium (TSM) plates (Kumar *et al*., 2023) using a sterile spreader and incubated at 28 ± 1°C for 72 hours. *Trichoderma* like colonies formed as green conidia and fast-growing hyphae were sub-cultured onto fresh potato dextrose agar (PDA) plates to obtain pure cultures.

Pathogen isolation from infected chilli plants

Infected chilli plants were collected based on characteristic disease symptoms as described by Kumari *et al*. (2025). Root and plant tissues were first rinsed under running tap water to remove soil and debris. Small pieces (~0.5 cm^2) were excised from the margin of diseased lesions and surface-sterilized in 10% sodium hypochlorite for 5 minutes. Sterilized tissues were rinsed twice with sterile distilled water and air-dried on sterile filter paper. The dried tissue pieces were then plated onto PDA and incubated at 25 ± 1°C for 5–7 days until fungal growth was observed. Emerging fungal colonies were sub-cultured to obtain pure isolates. *Trichoderma* and the pathogenic fungi were initially characterized based on their morphological characteristics before subjected to ITS region-based molecular identification.

Dual Culture Assay for antagonistic activity

A single isolate of identified *Trichoderma* was selected for antagonistic screening against the isolated chilli pathogens. The dual culture method was performed as follows; a 0.5 cm agar disc of *Trichoderma* was placed 2 cm from the edge of a PDA plate. On the opposite side, a 0.5 cm agar disc of the pathogen was placed 2 cm from the plate edge. For control plates, only the pathogen disc was placed in the same position on fresh PDA plates. Each treatment, including control, was replicated four times. All plates were incubated at 28 ± 1°C for 7 days under dark conditions to allow interaction between the antagonist and pathogen.

Measurement of antagonistic activity

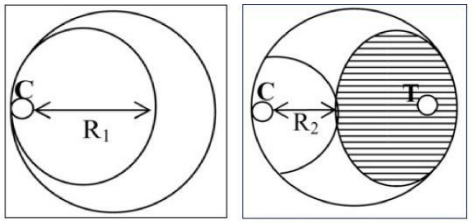
Observations included colony morphology, presence of mycelial inhibition zones and overgrowth of *Trichoderma* on the pathogen. The mycelial growth of the pathogen toward the antagonist (R_2) and in control plates (R_1) was measured in centimeters. The percentage inhibition of radial growth (PIRG) was calculated according to Awad *et al*. (2018) using the formula:

$$PIRG\ \% = \frac{(R_1 - R_2)}{R1} \times 100$$

where:

PIRG = Percent inhibition of radial growth
R_1 = mycelial growth of the pathogen in the control plate (cm)
R_2 = mycelia growth of the pathogen toward the *Trichoderma* colony (cm)

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Control plate (R1) Dual culture plate (R2)

Descriptive assessment of the antagonistic activity was scale following Soytong (1988):

++++ = very high antagonistic activity (> 75% PIRG)
+++ = high antagonistic activity (61-75% PIRG)
++ = moderate antagonistic activity (51-60% PIRG)
+ = low antagonistic activity (< 50% PIRG)

The ability of *T. asperellum* to overgrow and inhibit the growth of each pathogen was then scored according to Bell's scale (Suresh *et al*., 2017) where:

R1 = 100% overgrowth;
R2 = 75% overgrowth;
R3 = 50% overgrowth;
R4 = locked at the point of contact.

Statistical analysis

Data was analyzed using SAS version 9.4 (Statistical Analysis Software) following the procedures of Gomez and Gomez (1984). The differences in means were separated by Duncan multiple range test (DMRT).

RESULTS AND DISCUSSION

In the present study, a total of 27 *Trichoderma* isolates were recovered from the soil sample. Based on morphological characteristics and ITS-based molecular analysis, five representative isolates were confirmed as *Trichoderma asperellum*. The morphological characteristic and ITS molecular analysis also revealed the pathogens as *Colletotrichum gloeosporioides*, *C. capsici*, *Fusarium oxysporum*, *F. solani*, *Curvularia* sp. and *Alternaria* sp.

Soil chemical properties such as soil pH and nutrient concentrations are key factors influencing microbial communities. **Table 1** showed the soil chemical properties of chilli cuktivated soil in Integrated Organic Farming, MARDI Serdang. The occurrence of *Trichoderma* spp. in soil is likely influenced by favourable physicochemical conditions, particularly the moderately acidic pH which supports the growth of acid-tolerant fungi such as *Trichoderma*. According to Kolli *et al*. (2012), *Trichoderma* can live well on acid soils and even some of them are able to show optimal activity at a pH of less than 5.5. High organic carbon content enhances fungal growth, sporulation and secondary metabolite production (Yang *et al*., 2025). The available phosphorus content of the chilli cultivation soil was relatively high indicating favorable soil fertility. Phosphorus adequacy increased the disease-suppressive potential of rhizosphere microbiome (Cao *et al*., 2024). A conducive environment may promotes the abundance and ecological success of *Trichoderma* spp. in soil ecosystems.

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Table 1: Soil chemical analysis of chilli cultivated soil

No	Soil properties	Mean+ SD
1	pH	5.22 ± 0.08
2	Organic Carbon (%)	11.39 ± 0.67
3	Available P (mg kg^{-1} or ppm)	211.08 ± 35.53

Dual culture assays demonstrated varying degrees of growth inhibition indicating the potential of the biocontrol agent to suppress the pathogenic fungi. The complete course of interaction between *T. asperellum* and the pathogen was observed up to 10 days. Rapid growth with heavy and quick sporulation activity of *T. asperellum* is the advantage to its ability to inhibit the growth of the pathogen. In the present study, *T. asperellum* exhibited mycelial growth on all pathogens as measured in percentage inhibiton growth (PIRG) in **Figure 1**. The results demonstrated that *T. asperellum* exhibited strong inhibitory effects against all tested pathogens, with maximum growth inhibition percentage inhibition of radial growth (PIRG) ranging from 65.5% to 81.1%. The maximum growth inhibition was observed in *C. gloeosporioides* (81.1%) followed by *Curvularia* sp. (80.6%), *C. capsici* (70.5%), *Alternaria* sp. (69.9%), *F. solani* (67.4%) and *F. oxysporum* (65.5%).

Fusarium wilt is a destructive disease caused by fungal soil-borne pathogens such as *F. oxysporum* or *F. solani* that causes significant losses for many important vegetable and crop plants (Ajilogba and Babalola, 2013). In the present study, *T. asperellum* exhibited 65.5% inhibition against *F. oxysporum* and scored 100% overgrowth (R1) for Bell's Scale on *F. oxysporum* (**Table 2**). This result is comparable to Sharma (2011), who reported 69% inhibition of *F. oxysporum* using a *Trichoderma* isolate (T15) which suggests consistent antagonistic potential across studies. Petra e*t al*. (2019) however reported *T. asprellum* inhibited 88.3% of mycelia growth of *F. oxysporum* isolated from chilli. Similarly, Singh *et al*. (2019) reported *T. asperellum* BHU-P1 have potential for management of fusarium wilt of tomato and enhancement of growth and productivity. Reddy *et al*. (2018) observed antagonistic potential of *Trichoderma* isolates against *F. oxysporum* ranged from 10.12% to 70.70%. The inhibition recorded in the present study falls within this reported range and is closer to the higher end, indicating a comparatively strong antagonistic activity of *T. asperellum*. Hastuti *et al*. (2016) indicated that antagonistic efficiency varies depending on the *Trichoderma* species on *F. solani*. The inhibition of *F. solani* by *T. citrinoviride* (66.82%) and *T. koningii* (68.90%) were found comparable to *T. asperellum* in the present study.

The inhibition of *Curvularia* sp. by *T. asperellum* observed in the present study showed lower PIRG (80.6%) compared to a study by Ramirez-Olier *et al.* (2019) who reported 100% growth inhibition on *C. lunata* by *T. asperellum*, however scored R1 for Bell's Scale indicating similar strong antagonistic activity. In Malaysia, chilli anthracnose caused by *Colletotrichum* spp. has been reported as one of the major production constraints and significantly reduce fruit quality and yield under field condition (Noor and Zakaria, 2024). *T. asperellum* exhibited the highest level of inhibition against the anthracnose causal agent, *C. gloeosporioides*. Ramírez-Olier *et al*. (2019) reported R1 for Bell's classification (100% overgrowth) on *C. gloeosporioides* by *T. asperellum* which is found similar to the present study. Reynaldo *et al*. (2018) however observed lower inhibition by *T. asperellum* T2-10 on *C. gloeosporioides* with PIRG only at 22.5%. *T. asperellum* also exhibited a high antagonistic effect against *C. capsici.* This inhibition was substantially higher than that reported by Yan and Tu Anh (2018), who recorded a maximum inhibition of 30.87% on the third day of incubation. In the case of Alternaria blight disease, *T. asperellum* suppressed *Alternaria* sp. growth by up to 69.9% in the present study. However, *T. asperellum* TH inhibited the growth of *Alternaria* spp. only at 56% (Muhamad Usman *et al.,* 2019).

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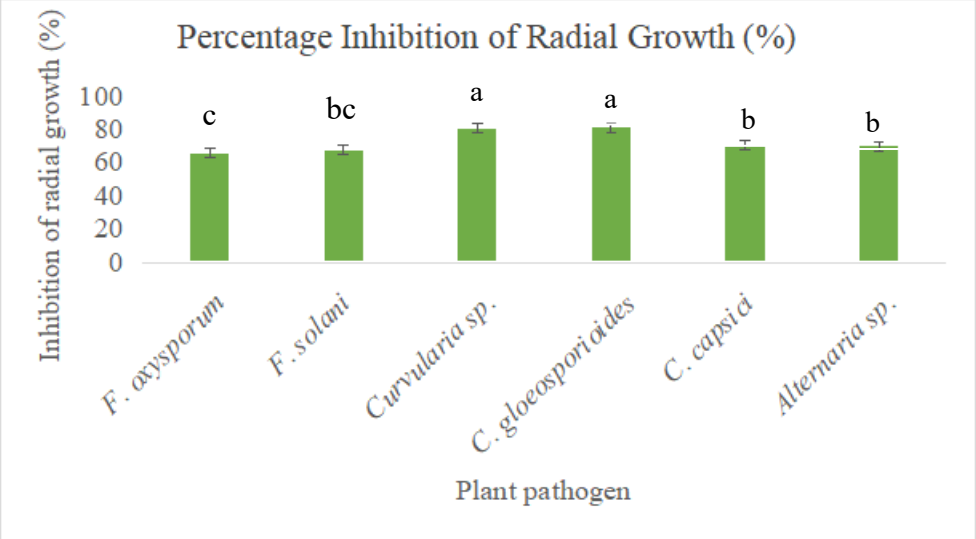


Figure 1: Antagonistic potential of *T. asperellum* on plant pathogen in dual culture assay. Means followed by the same letter are not significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

Table 2: Antagonistic activity and Bell's scale classification of *T. asperellum* against selected soil-borne fungi

No.	Pathogen	Antagonistic activity	Bell's Scale
1	*oxysporum*	+++	R1
2	*F.solani*	+++	R2
3	*Curvularia* sp.	++++	R1
4	*gloeosporioides*	++++	R1
5	*capsici*	+++	R1
6	*Alternaria* sp.	+++	R2

The direct interaction between *Trichoderma* and pathogen in dual culture assay is called mycoparasitism as observed in the dual culture plates. (**Figure 2**). The results showed that *T. asperellum* is an excellent competitor which successfully competes for space and nutrients due to its ability to grow faster. With increasing incubation time, the mycelia of *T. asperellum* were overgrown on pathogens colony and sporulated on the colony for space to live. The strong antagonistic activity observed in this study may be attributed to the rapid growth rate and aggressive colonization ability of *Trichoderma*, which enables it to compete for nutrients and space. SEM observations by Sehim *et al*. (2023) demonstrated that *T. asperellum* grew over, coiled around and lysed the hyphae of *F. oxysporum*, highlighting the role of mycoparasitism as an important antagonistic mechanism against soil-borne pathogens. The production of hydrolytic enzymes such as β-1,3-glucanase, chitinase, protease, and cellulase contributes to the degradation of pathogen cell walls during mycoparasitism, leading to suppression of fungal growth. Widely recognized as a beneficial soil fungus, *Trichoderma* plays a crucial role in suppressing plant pathogens and maintaining microbial balance in the rhizosphere. As a native rhizosphere microorganism, *Trichoderma* possesses strong ecological adaptability, allowing it to colonize the root zone effectively and outcompete pathogenic fungi. The isolation of *T. asperellum* from rhizosphere of healthy plant further highlights its role as a beneficial soil fungus capable of naturally suppressing soil-borne pathogens and enhancing plant health under sustainable agricultural systems.

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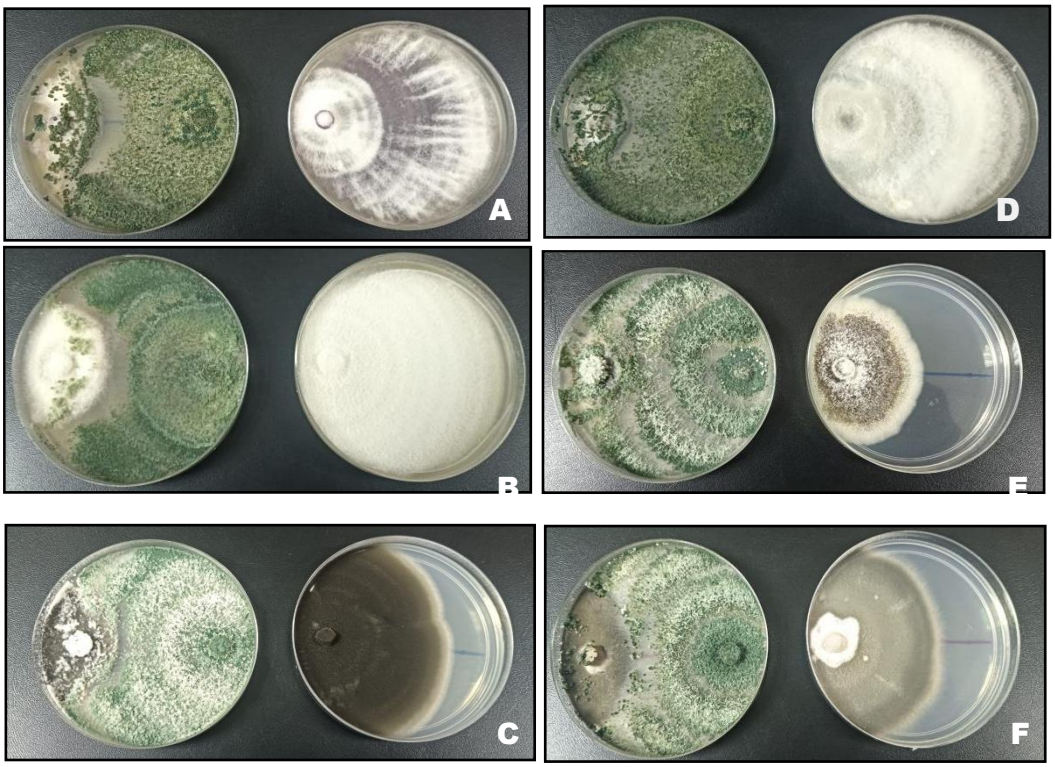


Figure 2. Dual culture assays of *T.asperellum* against (A) *F. oxysporum*, (B) *F. solani*, (C) *Curvularia* sp. (D) *C. gloeosporioides*, (E) *C. capsici*, and (F) *Alternaria* sp. at day 10; left, dual culture (pathogen + *T. asperellum*); right, pathogen alone (control).

CONCLUSION

The study concluded indigenous *T. asperellum* isolates from MARDI Organic Farm demonstrated significant potential to control different agriculturally important plant pathogenic fungi. These findings provide baseline information on the biocontrol potential of *Trichoderma* and support further soil-based and field investigations aimed at managing soil-borne diseases and improving soil health in sustainable chilli production systems. The potential use of *T. asperellum* can further be enhanced through advancement in the formulation and application especially in field conditions.

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