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Antagonistic activity of phyllosphere fungi isolated from medicinal plants against *Colletotrichum sp.* causing anthracnose in chilli (*Capsicum annuum* L.)

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Abstract

Anthracnose disease caused by the fungus Colletotrichum sp. is one of the main problems in chili cultivation (*Capsicum annuum* L.) and can cause significant yield losses. The intensive use of chemical pesticides in controlling this disease has a negative impact on the environment and health. This study aimed to evaluate the antagonistic potential of phyllosphere fungi isolated from five medicinal plants, namely *Phaleria macrocarpa, Curcuma domestica, Kleinhovia hospita, Hibiscus rosa-sinensis*, and *Morus macroura*, against the pathogen Colletotrichum sp. Isolation was performed from leaf surfaces, and a total of 7 phyllosphere fungal isolates were tested using a double culture method with three replicates. The results showed that all isolates were able to inhibit the growth of *Colletotrichum sp.* through mechanisms of competition for space and nutrients as well as antibiosis, with inhibition levels ranging from high to very high. Among the tested isolates, Trichoderma sp. exhibited the highest inhibitory activity with an inhibition percentage of 92.78%, categorized as very high. Macroscopic and microscopic characterization supported the identification of several isolates belonging to the genera Trichoderma. This study concluded that phyllosphere ungi from medicinal plants have the potential as environmentally friendly biological control agents in combating anthracnose disease in chili plants.

Introduction

Indonesia is one of the countries with the highest utilization of medicinal plants, comparable to other Asian countries such as China and India. This is supported by its tropical climate, which provides rich biodiversity and abundant natural resources for traditional and herbal medicine (Widjaja *et al*., 2014; Hafid, 2019). Medicinal plants generally contain secondary metabolites such as essential oils, alkaloids, flavonoids, and tannins, which play important roles as bioactive compounds, including antimicrobial and antifungal agents (Chatri *et al*., 2022).

Several medicinal plants, including *Phaleria macrocarpa*, *Curcuma domestica*, *Kleinhovia hospita*, *Hibiscus rosa-sinensis*, and *Morus macroura*, are known to contain active compounds such as flavonoids, tannins, polyphenols, curcumin, stilbenoids, and essential oils with antifungal potential (Mustapha *et al*., 2017; Febriyossa & Rahayuningsih, 2021; Budiarti & Jokopriyambodo, 2020; Zulkurnain *et al*., 2023; Aseny *et al*., 2021). Previous studies have reported that various phyllosphere fungi have been isolated and identified from medicinal plants, including *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*, and *Chaetomium* (Oladeji *et al*., 2025).

The phyllosphere, defined as the aerial surface of plant leaves, serves as a habitat for diverse microorganisms, including fungi. High microbial diversity in the phyllosphere is beneficial for plant health through mechanisms such as competition for space and nutrients and suppression of plant pathogens (Bashir *et al*., 2022; De Mandal & Jeon, 2023). Although phyllosphere research has predominantly focused on bacteria, recent studies indicate that phyllosphere fungi actively colonize leaf surfaces and may possess ecological functions similar to those of soil fungi, including potential roles as biological control agents (Vorholt, 2012; Kembel & Mueller, 2014).

Fungal antagonists have been widely reported to inhibit phytopathogens through integrated mechanisms such as nutrient competition, antibiosis, and mycoparasitism, which collectively contribute to the suppression of pathogen development and disease incidence in crops (Nassary, 2025). Several studies have demonstrated the antagonistic potential of phyllosphere fungi against plant pathogens. Sulaiman *et al.* (2021) reported that phyllosphere fungi isolated from chili plants inhibited the growth of *Colletotrichum acutatum* by up to 52.89%, while Ibrahim *et al.* (2021) showed that phyllosphere fungi isolated from maize suppressed *Aspergillus flavus* growth by up to 75%.

Colletotrichum spp. are among the most important plant pathogenic fungi worldwide, with more than 200 species reported to infect various crops (Udayanga *et al*., 2013; Marin-Felix *et al*., 2017). These fungi cause anthracnose disease in a wide range of host plants, including chili (*Capsicum annuum* L.), resulting in significant yield losses ranging from 30% to 80% in the field and during postharvest handling (Chung *et al*., 2020). However, studies investigating the antagonistic potential of phyllosphere fungi derived from medicinal plants against indigenous *Colletotrichum* sp. infecting chili plants remain limited. Therefore, this study aimed to evaluate the antagonistic activity of phyllosphere fungi isolated from several medicinal plants against *Colletotrichum* sp., the causal agent of anthracnose disease in chili, in order to explore their potential as environmentally friendly biological control agents for sustainable disease management in chili cultivation.

Materials and methods

This study was conducted at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang. The research was carried out from April 2025 to Agustus 2025.

1. Materials

The tools use in this study included a Petri dishes, a laminar air flow cabinet, an autoclave, and an incubator. The materials consisted of phyllosphere fungal isolates, *Colletotrichum* sp., and Potato Dextrose Agar (PDA) medium. Antagonistic activity was evaluated based on the inhibition of pathogen growth by the antagonistic fungi compared with the control treatment.

2. Method and Research Design

This study was conducted as a laboratory-based experimental research to evaluate the antagonistic activity of phyllosphere fungi isolated from several medicinal plants against *Colletotrichum* sp., the causal agent of anthracnose disease in chili plants, using the dual culture method. The experiment was arranged in a completely randomized design (CRD) with three replicates.

3. Procedures

a. Media Preparation

Potato Dextrose Agar (PDA) medium was prepared by dissolving 39 g of ready-to-use PDA powder (Merck, Darmstadt, Germany) in 1 L of distilled water, followed by heating and homogenization. The medium was sterilized in an autoclave at 121 °C under a pressure of 2 atm for 20 minutes.

b. Fungi Reculture

Reculture of phyllosphere fungal isolates obtained from the Laboratorium Biota Sumatera culture collection, Universitas Andalas, and Colletotrichum isolates was carried out in test tubes by transferring a portion of fungal hyphae previously grown on Potato Dextrose Agar (PDA) plates into sterile PDA medium in test tubes. The recultured fungi were incubated at room temperature (25–27 °C) for 7 days until abundant mycelial growth was obtained.

c. Morphological Characterization of *Colletotrichum* sp.

The characterization of *Colletotrichum* sp. was carried out through macroscopic and microscopic observations. Microscopic characteristics were observed using a photomicrograph (microscope). A small portion of hyphae was carefully taken using a sterile inoculating needle and placed on a glass slide previously dropped with sterile distilled water. The observation was then performed using a photomicrograph (microscope). The microscopic characteristics observed included hyphal structure, conidia, and conidiophores. Meanwhile, macroscopic characteristics observed included colony shape, color, and growth pattern (Halwiyah, 2019).

d. Antagonistic Assay

The antagonistic activity of phyllosphere fungal isolates from several medicinal plants against the pathogenic fungus *Colletotrichum* sp. was evaluated using the dual culture method with three replications. Mycelial plugs (5 mm) of the antagonist and pathogen were taken from 7 day old cultures using a sterile cork borer and placed opposite each other (3 cm apart) on the same Petri dish. Plates were incubated at 25–28°C for 5–7 days, and inhibition was assessed based on radial growth reduction of the pathogen compared to the control (R1 = control; R2 = treatment), following Dennis and Webster (1971).

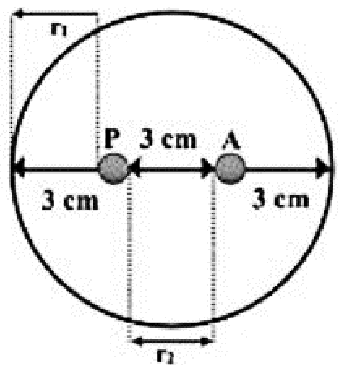

Figure 1. Schematic arrangement of antagonistic fungi and pathogenic fungi using the dual culture method.
Description: A = antagonistic fungi; P = pathogenic fungi (*Colletotrichum*)

e. Measurement of Percentage Inhibition

The percentage of inhibition (PI) was determined by measuring the radius of the pathogenic fungal colony growing toward and away from the antagonistic fungal colony. According to Ningsih *et al.* (2016), the percentage of inhibition (PI) was calculated using the following formula:

$$R = \frac{R1-R2}{R1} x\ 100\ \%$$

R = percentage of growth inhibition (%);
R_1 = diameter of pathogen growth in the control treatment (mm);
R_2 = diameter of pathogen growth in each treatment (mm).

f. Data Analysis

The data were analyzed for inhibition and fungi growth diameter using ANOVA. If a significant difference was observed, it was followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level.

Results and discussion

1. Morphological Characterization of *Colletotrichum* sp.

The morphological characteristics of the pathogenic fungus *Colletotrichum* sp. are shown in Figure 2.

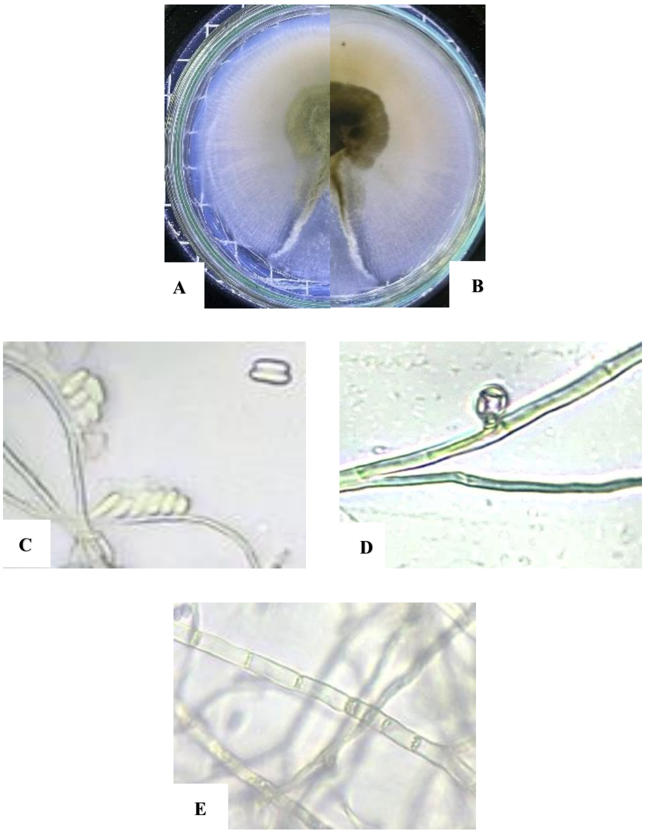

Figure 2. Macroscopic and Microscopic Characteristics of *Colletotrichum* spp. (40× magnification). Notes: (a) Front view, (b) reverse view, (c) conidia, (d) conidiophores, (e) septate hyphae on a slide.

The results of the study showed in Figure 2. the *Colletotrichum* isolate exhibited distinctive morphological characteristics, with colonies appearing whitish-gray and a darker central area. The colony texture was smooth, with radial growth characterized by even margins and a denser central zone. During the early stage of growth, the conidia appeared orange in color. Microscopically, the isolate showed septate hyphae with smooth and branched structures. The conidiophores were observed in clusters, with terminal structures producing chains of conidia. The conidia were oval to fusiform in shape, small in size, and evenly distributed along the conidiophores.

The colony texture of *Colletotrichum* varies depending on the species and the growth medium used. Generally, on Potato Dextrose Agar (PDA), Colletotrichum colonies exhibit cottony to velvety textures, with surfaces that are often smooth or slightly fluffy. In some species, such as Colletotrichum gloeosporioides, colonies tend to be grayish to dark in the central area with white margins. In contrast, *Colletotrichum acutatum* typically forms colonies that are orange to pinkish in color, with a more compact and smoother texture (Wakhidah & Sari, 2021). As the colonies mature, the central region may become darker due to spore formation. Colony growth patterns may also display concentric rings formed as a result of variations in hyphal and conidial production (Marizka *et al*., 2025).

2. Antagonistic Activity Assay Between Phyllosphere Fungi and the Pathogenic Fungi *Colletotrichum* sp.

The percentage of inhibition of each phyllosphere fungi isolate against *Colletotrichum* sp. can be seen in Table 1.

Table 1. Percentage and Categories of Inhibition of Phyllosphere Fungi Against the Pathogen *Colletotrichum* sp.

No	Isolate	The percentage of inhibition (%)	Inhibition Categories
1	*Trichoderma* sp. (1)	89.67 cd	Very High
2	*Trichoderma* sp. (2)	92.78 d	Very High
3	*Trichoderma* sp. (3)	85.77 cd	Very High
4	*Trichoderma* sp. (4)	86.89 cd	Very High
5	*Trichoderma* sp. (5)	89.00 cd	Very High
6	*Trichoderma* sp. (6)	85.55 cd	Very High
7	*Trichoderma* sp. (7)	65.44 b	High

Numbers followed by different letters indicate significant differences in the DNMRT 5% test.
Note: The inhibition percentage was classified into four categories:
Low : (1–25%),
Moderate : (26–50%),
High : (51–75%),
Very high : (76–100%) (Živković et al., 2010).

Based on the data in Table 1, all isolates of phyllosphere fungi from several medicinal plants showed antagonistic activity against the pathogen Colletotrichum sp. The highest inhibition percentage was produced by the *Trichoderma* sp. (2) isolate (92.78%), and it was not significantly different from *Trichoderma* sp. (1) (89.67%), *Trichoderma* sp. (5) (89.00%), *Trichoderma* sp. (4) (86.89%), *Trichoderma* sp. (3) (85.77%) and *Trichoderma* sp. (6) (85.55%) isolates. However, it was significantly different from the *Trichoderma* sp. (7) (65.44%) isolates. All isolates of phyllosphere fungi have the potential to inhibit the growth of the pathogen *Colletotrichum* sp.

Competition occurs when two microorganisms require nutrients and living space in limited quantities. In this mechanism, antagonistic fungi are able to gain more control over resources than pathogenic fungi, thereby inhibiting their growth. The suppression of pathogens by antagonistic fungi can occur through competition for nutrients and space, the production of antibiotic compounds (antibiosis), and parasitic mechanisms (Ainy, 2015). According to a study by Kamel *et al.* (2020), microscopic observations of mycoparasitic interactions show various forms of different interactions. Examples include the abundant growth of antagonistic hyphae around the pathogenic hyphae, hyphae coiling around pathogenic hyphae, and lysis of the hyphae.

The process of antagonistic mechanisms through competition occurs because two or more microorganisms require nutrients and space with limited availability (Karim *et al.*, 2020). The antibiosis process involves the production of chemical compounds by antagonistic fungi that are toxic to the pathogen. These compounds can be antibiotics that directly inhibit or kill the pathogen. In parasitism, antagonistic fungi directly attack the pathogen by attaching to, coiling around, or penetrating the pathogen's hyphae, which ultimately causes damage or death to the pathogen (Muhibuddin *et al.*, 2021).

The rapid growth of antagonistic fungi plays a crucial role in suppressing target pathogens through competition for space and nutrients. Factors that influence the growth rate of a pathogen are its viability and conidia density. Antagonistic fungi with slow growth generally have a lower inhibition potential against faster-growing pathogens (Yulianto, 2014).

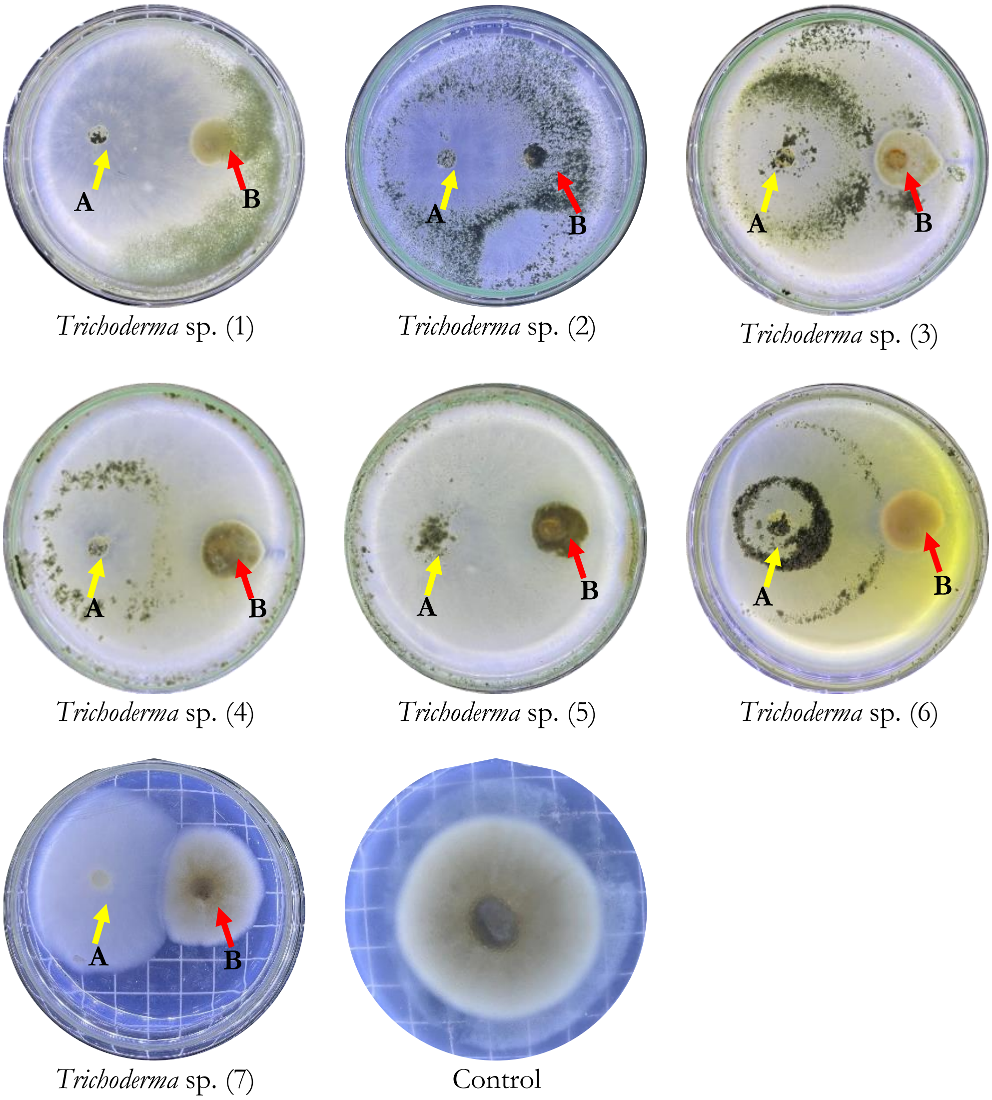

Figure 3. Antagonistic Test of Phyllosphere Fungi against the Pathogen *Colletotrichum* sp. after 7 days of incubation (A = Phyllosphere fungi, B = *Colletotrichum* sp.).

Figure 3 shows the results of the antagonistic test between the phyllosphere fungi (yellow arrow, A) and the pathogen *Colletotrichum* sp. (red arrow, B). All isolates of the phyllosphere fungi showed inhibitory activity against the growth of *Colletotrichum* spp., which was indicated by the presence of an inhibition zone or suppressed growth around the pathogen. In the isolates of *Trichoderma* sp. (1), *Trichoderma* sp. (2), *Trichoderma* sp. (5), and *Trichoderma* sp. (7). The phyllosphere fungi were observed to occupy the entire Petri dish, indicating that the fungi can inhibit the growth of pathogenic fungi through competition for space and nutrients.

According to Cendrawati *et al.* (2020), the grouping of antagonistic mechanisms can be based on the growth rate of the fungi in occupying the media surface in a Petri dish. If the antagonistic fungi grow faster until they occupy the entire Petri dish, this indicates a competition mechanism. This is a factor that triggers antagonism through competition. This is due to the presence of several microorganisms that compete with each other to obtain nutrients and space with limited availability.

In *Trichoderma* sp. (3), *Trichoderma* sp. (4), and *Trichoderma* sp. (7), the inhibition zone (empty space) separating the mycelium of the antagonistic and pathogenic fungi was not visible, but a change in the color of the media indicated antibiosis activity in the dual culture test. According to Jayalakshmi *et al.* (2021), Previous studies reported that *Trichoderma* spp. produce antifungal secondary metabolites such as gliotoxin that contribute to the inhibition of plant pathogenic fungi. The production of these metabolites during fungal interaction may also influence changes in the culture medium. The antibiosis mechanism of antagonism is suspected to occur as a result of compounds produced by a microorganism, such as antibiotics, alkaloids, metabolites, or toxins, which can inhibit the growth of other microorganisms (Berlian *et al.*, 2013).

An organism can be called a biological control agent if it is able to multiply in conditions that are not favorable for other organisms and can inhibit their growth and development (Muslim, 2019). Antagonistic fungi groups are known for their ability to control or suppress the growth of plant pathogens (Widiyanti *et al.*, 2022). Antagonistic isolates with high inhibition are those whose colonies grow faster than the pathogen's colonies, thus inhibiting pathogen growth. This rapid growth allows antagonistic fungi to dominate in utilizing nutrients and space, ultimately suppressing the pathogen's development (Sari & Setiawanto, 2015).

Overall, these results show that the 7 isolates of phyllosphere fungi have the potential to be used as biological control agents against *Colletotrichum* sp., although their effectiveness can vary depending on the type of isolate used. These findings affirm the importance of selecting isolates with the highest antagonistic activity, as each isolate may have a different mechanism of action, such as nutrient competition, production of antifungi compounds, or parasitism, which need to be studied further before being applied in the field. Thus, the utilization of phyllosphere fungi can be a promising and environmentally friendly alternative for biological control.

Conclusion

All phyllosphere fungal isolates obtained from the medicinal plants *Phaleria macrocarpa*, *Curcuma domestica*, *Kleinhovia hospita*, *Hibiscus rosa-sinensis*, and *Morus macroura* exhibited antagonistic activity against the pathogenic fungus *Colletotrichum* sp. Morphological characterization revealed that several isolates were identified as *Trichoderma* sp. Among the tested isolates, *Trichoderma* sp. demonstrated the strongest inhibitory effect, with a growth inhibition percentage of 92.78%, categorized as very high. These findings highlight the potential of phyllosphere fungi associated with medicinal plants as environmentally friendly biological control agents for the sustainable management of anthracnose disease in chili cultivation.

Author Statements

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Competing of Interest: The authors declare no competing interests.

Author's contributions: Ayumi Rizci Puspita contributed to conceptualization, methodology, investigation, data analysis, and writing of the original draft. Feskaharny Alamsjah contributed to methodology, validation, and supervision. Mildawati contributed to data analysis and manuscript review. Anthoni Agustien contributed to supervision, project administration, and manuscript editing. All authors have read and approved the final version of the manuscript.

Generative AI: Generative artificial intelligence tools were not used in the preparation of this manuscript.

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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