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Potential of *Trichoderma asperellum* in Suppressing the Growth of Several Fungal Pathogens in Rice In Vitro

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ABSTRACT

Rice (*Oryza sativa L.*) is an important food crop with *Helminthosporium oryzae* B. de Haan, *Curvularia* sp., and *Rhizoctonia solani* as three major associated pathogenic fungi. In controlling these pathogens, the use of antagonistic fungus, such as *Trichoderma asperellum*, is still considered the most effective and environmentally friendly. The study aimed to determine the potential of T. asperellum in suppressing several pathogenic fungi that cause disease in rice plants in vitro. The study used a completely randomized design (CRD) with 4 treatments and 10 replicates. The treatments were several pathogens that cause disease in rice plants with antagonistic fungi *T. asperellum* to inhibit several pathogens in rice plants, namely *R. solani*, *Curvularia* sp., *H. oryzae*., and untreated control. Testing the antagonistic power of T. asperellum against several pathogenic fungi was carried out by double culture method and culture vapor method. Observations made included the percentage of inhibition, invasion rate, colony area, changes in morphological characters, the number of conidia and the number of germinated conidia. The results showed that T. asperellum has the potential to suppress the growth of several pathogenic fungi studied in vitro. In the double culture method, T. asperellum suppressed the growth of R. solani fungus with the highest inhibition percentage of 68.40%, whereas in the vapor culture method, T. asperellum suppressed the growth of Curvularia sp. fungus with the highest effectiveness of 62.04%.

INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial cultivated crop for humans, as more than half of the world's population relies on it as a staple food source (Ningrat et al., 2021). National rice productivity fluctuated between 2020 and 2024, with recorded yields at 5.12, 5.22, 5.23, 5.28, and 5.24 tons.ha^{-1}, respectively. Meanwhile, rice productivity in West Sumatra from 2020 to 2022 was 4.69, 4.83, and 5.05 tons.ha^{-1}, respectively (BPS, 2024). However, these productivity levels remain below the potential yield, which ranges from 10 to 11 tons.ha^{-1} (Karim and Aliyah, 2019).

Several factors hinder the achievement of optimal rice productivity, one of which is the attack of various plant pathogens, particularly fungi. Major fungal diseases affecting rice include brown spot disease caused by *Helminthosporium oryzae* B. de Haan, narrow brown leaf spot by *Cercospora oryzae*, stackburn disease by *Alternaria padwickii*, blast disease by *Pyricularia grisea* (Cke) Sacc.,

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sheath blight by *Rhizoctonia solani* Kuhn, leaf spot disease by *Curvularia* sp., and Fusarium wilt by *Fusarium* sp. (IRRI, 2018).

Several dominant rice diseases in West Sumatra include brown spot disease caused by *H. oryzae*, leaf spot disease by *Curvularia* sp., and sheath blight by *R. solani*. In general, disease control in the field is carried out using synthetic fungicides. However, continuous use of synthetic fungicides can lead to pathogen resistance and environmental pollution. Therefore, an environmentally friendly alternative, such as biological control, is needed. Biological control is a technique that utilizes naturally occurring microbes to control pest and diseases, one of which is the biological agent *T. asperellum* (Febriza et al., 2024).

T. asperellum is a species of *Trichoderma* that has been widely reported as antagonist agent to be successful in biological control of various plant pathogens. The mechanisms by which *Trichoderma* spp. inhibit the growth of fungal pathogens include direct competition, antibiosis, and lysis. The antibiosis mechanism of *Trichoderma* spp. against fungal pathogens involves the production of toxic enzymes such as β-1,3-glucanase, cellulase, and chitinase, which can inhibit pathogen growth and even kill fungal pathogens (Ruswandari et al., 2020). Muhibuddin et al. (2021) reported that *T. harzianum* is capable of suppressing *R. solani* on rice plants. Additionally, *T. asperellum* has been shown to inhibit the growth of *Colletotrichum* sp. in large red chili peppers by 40–70% *in vitro* (Sutarman et al., 2022).

The evaluation of *T. asperellum* in suppressing the growth of *H. oryzae*, *R. solani* and *Curvularia* sp., serves as a foundation for further research on the biological control of fungal pathogens using *T. asperellum*. The successful application of *T. asperellum* in controlling rice diseases is expected to reduce the use of synthetic fungicides in the field. Therefore, this study was conducted to investigate the potential of *T. asperellum* in inhibiting the growth of several fungal pathogens that cause diseases in rice *in vitro*. This antagonistic assay aims to determine the ability of *T. asperellum* to suppress various fungal pathogens affecting rice plants *in vitro*.

MATERIALS AND METHODS

The research was conducted from October to December 2024 at the Phytopathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Andalas University. The isolates used in the study were pathogenic *R. solani*, *Curvularia* sp., and *H. oryzae* from Laboratory of Phytopathology's culture collection, and *T. asperellum* from Laboratory of Biological Control's culture collection.

This study used a completely randomized design (CRD) consisting of 4 treatments and 10 replicates. The treatments were pathogenic fungi as follows: *R. solani*, *Curvularia sp.*, *H. oryzae*, and Control.

Rejuvenation of Pathogenic Fungi

Rejuvenation was carried out by taking pathogenic fungal cultures (*Curvularia* sp., *R. solani*, and *H. oryzae*) that had been propagated on PDA medium in petri dishes using a cork borer with a diameter of 5 mm and grown on PDA medium, incubated for 7 days at room temperature. The macroscopic and microscopic forms were observed.

Virulence test of Pathogenic Fungi

The virulence test aimed to determine the level of ability of the fungal isolate to cause disease in its host plant. For the pathogens *Curvularia* sp. and *H. oryzae*, the test was carried out by wounding the leaves of rice plants aged 30 days after planting (DAP) using a sterile needle. The 10 ml fungal suspension, with a density of 10^6 conidia.ml^{-1}, was sprayed on the rice leaves. The rice plants were subsequently covered with a clear plastic to maintain humidity. They were observed until symptoms appear. As for the pathogen *R. solani*, it is done by inoculating one sclerotia into each leaf midrib of rice plants aged 30 DAP. The development of the disease was observed for 7-14 days until symptoms appear.

Propagation of *T. asperellum*

Propagation of *T. asperellum* was conducted by cutting the culture of *T. asperellum* with a cork borer (5 mm) and transferred to a Petri dish containing PDA medium and incubated at

room temperature until the Petri dish was full. The fungal culture was observed macroscopically and microscopically.

Testing the Antagonistic Power of *T. asperellum* Against Several Pathogenic Fungi

Dual Culture Method

Dual culture testing between pathogenic fungi and *T. asperellum* was carried out as follows. Pathogenic fungi and *T. asperellum* fungi were grown in the same Petri dish. The pathogenic fungi were placed 3 cm from the left edge of the Petri dish while *T. asperellum* was placed 3 cm from the right opposite edge of the petri dish (Figure 1). After that, it was incubated for 7 days while measuring the radius of the pathogen.

The percentage of inhibition of fungal growth are observed and calculated based on the formula as follows:

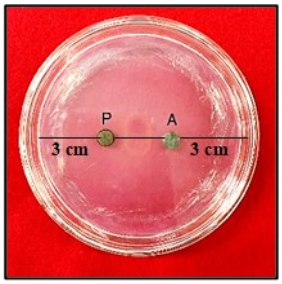

Figure 1. Dual culture method of pathogenic fungi (P) and *T. asperellum* (A)

$$PI = \frac{r1-r2}{r1} \times 100\%$$

Note:
PI = percentage of inhibition (%)
r1 = radius of the pathogen away from the antagonistic fungus.
r2 = radius of the pathogen approaching the antagonistic fungus

Vapor Culture Method

The vapor culture test between pathogenic fungi and *T. asperellum* (Figure 2) was carried out by means of a culture snip of pathogenic fungi was taken with a 5 mm corn borer from a 7-day-old culture. The cutting placed in the center of a Petri dish containing PDA media. At the bottom of the Petri dish, a 7-day-old *T. asperellum* culture was placed facing each other (Sudantha and Suwardji, 2021). For the control, the center of the upper petri dish was placed with the pathogenic fungal colony and the lower petri dish contained only PDA media. Then incubated at room temperature for 7 DAP.

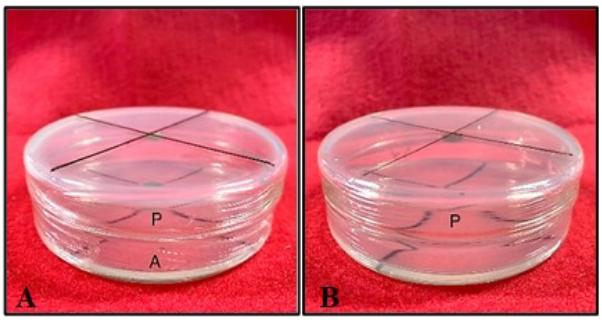

Figure 2. Vapor culture method on several pathogenic fungi and T. asperellum; (A) Treatment; (B) Control)

The effectiveness calculation was performed using the following formula:

$$E = (Acc-Act)/Lk \times 100\%$$

Note:
E = effectiveness.
Acc = area of pathogen colonies in the control.
Act = area of pathogen colonies in the treatment.

Observations included the percentage of inhibition, invasion rate, colony area, changes in morphological characters, number of conidia and number of germinated conidia.

RESULTS AND DISCUSSION

Antagonistic Mechanism of *T. asperellum*

Antagonistic mechanism of *T. asperellum* against plant pathogens such as inhibition, antibiosis and invasion level are shown in Table 1. The inhibition of *T. asperellum* against pathogenic fungi showed significant differences between treatments.

The results show that, based on dual culture method, *T. asperellum* can suppress the growth of *R. Solani* with the highest percentage of inhibition reaching 68.40%,. *T. asperellum* can suppress the growth of several pathogenic fungi with antagonistic mechanisms such as competition, mycoparasitism and antibiosis (Win *et al*., 2021). The competition mechanism is characterized by dominant growth of *T. asperellum* as compared to several pathogenic fungi (*Curvularia* sp., *R. solani*, and *H. oryzae*)

Table 1. Antagonism power of *T. asperellum* against pathogenic fungi including inhibition, antibiosis and invasion rate

Treatment	Antagonistic mechansms		
	Inhibition (%) ± SD	Anti-biosis	Invasion level
R. solani	68.40 ± 5.48 a	-	4
Curvularia sp.	67.27 ± 6.69 ab	+	4
H. oryzae	62.21 ± 4.64 b	+	4
	CV = 8.59		

on day 7 (DAP) to obtain space and nutrients (Figure 3). According to Kumar *et al*. (2023), antagonistic effects are due to competition between two fungal species grown side by side. This is due to that each fungus needs space and nutrients from the media for its growth.

The mechanism of mycoparasitism is the ability to inhibit the growth of pathogenic fungi through the release of various metabolites. *T. asperellum* can recognise and attach to the targeted pathogen, penetrate deeper into the host and cause the pathogen to die. In addition, *T. asperellum* produces N-acetyl-glucosaminidase, chitinase and glucanase to degrade the cell wall of the pathogen (Asad, 2022).

The antibiosis mechanism was indicated by the formation of a clear zone on the 4th day of observation (Table 1 and Figure 4). This may be caused by the presence of antagonistic activities against pathogenic fungi such as mycoparasitism and the activity of metabolite compounds. Metabolites play an important role in the antagonistic ability of *Trichoderma.* Volatile compounds like aldehyde, ester and terpene produced by *Trichoderma* sp. fungi are known to be important in antagonistic activity (Siahaan *et al*., 2024).

Table 1 shows that in the treatment of *H. oryzae* and *Curvularia* sp, the antibacterial mechanism characterized by the formation of a

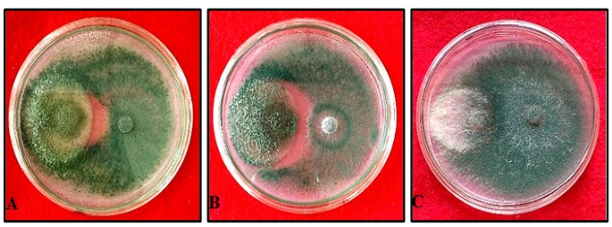

Figure 3. Colony area of some pathogenic fungi in the double culture method (7 DAP). (A) *H. oryzae*; (B) *Curvularia* sp.; (C) *R. solani.*

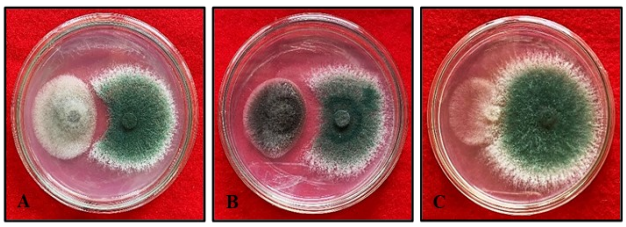

Figure 4. Clear zone of *T. asperellum* fungus treated with several pathogenic fungi (4 DAP). (A) *H. oryzae*; (B) *Curvularia* sp.; (C) *R. solani.*

clear zone. Meanwhile, the treatment of *R. solani* showed no clear zone between the pathogen and fungus *T. asperellum*. According to Mifthahul *et al*. (2023), the clear zone formed between the two fungal colonies is resulted by the presence of secondary metabolites produced by the antagonistic fungi, so that the pathogenic fungi are unable to grow in the surroundings of the antagonistic fungi. The mechanism of antibiosis is due to the presence of secondary metabolites produced by microbes in the form of antibiotics and mycotoxins.

The invasion rate of *T. asperellum* against several pathogenic fungi showed that *T. asperellum* could invade all pathogenic fungi with a very high level of invasion on the 6th day of observation (Table 1). *T. asperellum* grew faster than pathogenic fungi because it has a high competition mechanism for space and nutrients (Figure 5). In this case, the need for the same food substances can cause competition between the two fungi. The fungus that can adjust the fastest will experience more fertile growth.

According to Lila *et al*. (2023), Trichoderma's competition mechanism involves a mycoparasite mechanism, which is the entanglement of Trichoderma hyphae with pathogenic hyphae so that pathogenic hyphae become flattened and then lysed. The mycoparasite interaction is followed by the involvement of antibiosis mechanisms. The antibiosis mechanism of *Trichoderma* sp. is capable of producing different types of antibiotics that actively induce cell death in the pathogenic fungi.

Mycoparasitic mechanism

The process of parasitism exhibited by *T. asperellum* against various pathogenic fungi (*Curvularia* sp., *R. solani*, and *H. oryzae*) can

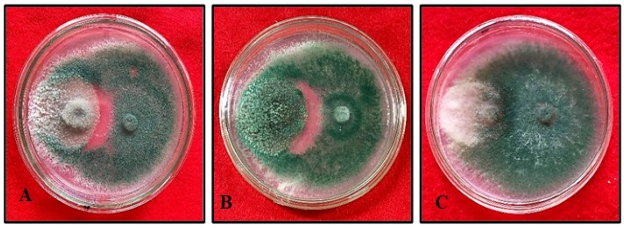

Figure 5. Invasion rate of *T. asperellum* against pathogenic fungi (6 DAP) (A) *H. oryzae*; (B) *Curvularia sp.*; (C) *R. solani*.

be observed through the occurrence of attachment, penetration, and entanglement, resulting in the lysis of the fungal hyphae. The observation of parasitism is facilitated by a dual culture test, whereas the mechanism of parasitism is conducted through the slide culture method. The mechanism of parasitism can be observed in Figure 6.

As illustrated in Figure 6, the mechanism of parasitism exhibited by *T. asperellum* involves the lysis of pathogenic hyphae through the action of various enzymes, including chitinase, glucanase, and protease. These enzymes are capable of degrading the cell walls of pathogenic fungi, leading to their demise (Mirsam *et al*., 2023). The mechanism of mycoparasites involves the rolling of the hyphae of pathogenic fungi by the hyphae of antagonistic fungi, which involves cell wall-degrading enzymes consisting of chitinase, glucanase and protease enzymes that can degrade the cell walls of pathogenic fungi, thereby lysing the hyphae of pathogenic fungi (Febriza *et al*., 2024).

As stated by Mifthahul *et al*. (2023), *Trichoderma* has the capacity to produce enzymes that function as antifungals, including trichodermin, gliotoxin, and gliovirin. These enzymes have the ability to damage the pathogen cell wall, leading to the death of the organism and the inhibition of the development of pathogenic fungi. Furthermore, Trichoderma produces the enzyme chitinase, which is capable of breaking down chitin into N -acetylglucosamine polymers. This process leads to the lysis of the pathogen cell wall due to the breakage of the polymer bond.

The results of the observation of the colony area of several pathogenic fungi (*Curvularia* sp., *R. solani*, and *H. oryzae*) in the culture vapor method demonstrated that *T. asperellum* produced a significantly different effect on the colony area of these organisms (Table 2).

In Table 2, it is evident that *Curvularia* sp. treated with *T. asperellum* demonstrates the most significant inhibitory effectiveness, reaching 62.04% compared to other pathogenic fungi treated with *T. asperellum*. It is evident from Figure 7 that the inhibitory effectiveness of *T. asperellum* is directly proportional to the colony area of the pathogenic fungi treated, with a corresponding increase or decrease. Observations of the morphological changes in the pathogenic fungi (*Curvularia* sp., *R. solani*, and *H. oryzae*) after being treated with *T. asperellum* can be seen in Figure 7.

As shown in Figure 7, different pathogenic fungus showed different morphological changes in the presence of *T. asperellum* as compared to the control without the antagonist. In the absence of *T. asperellum*, morphological growth of pathogenic fungus showed features like thick, circular edges and spread across the plate. However, in the presence of *T. asperellum,* the growth of pathogenic fungi of *Curvularia* sp., *R. solani* and *H. oryzae* changed a lot, becoming thin, white, thick in the middle and not growing well. According to Chilosi *et al*. (2020), *Trichoderma* is capable of producing simple and non-volatile toxic metabolites,

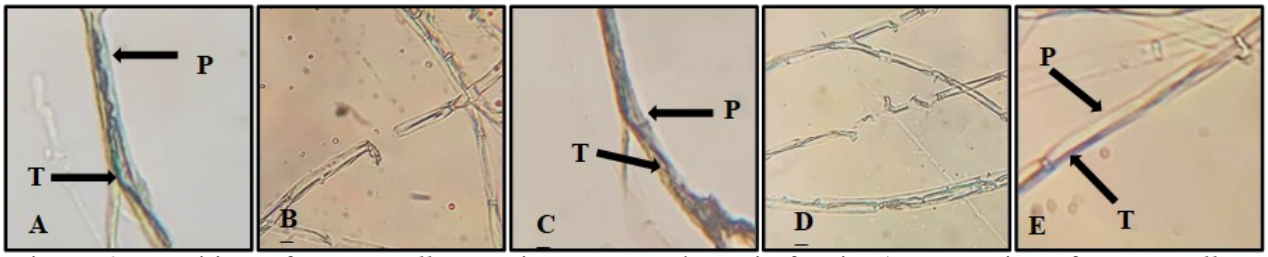

Figure 6. Parasitism of *T. asperellum* against some pathogenic fungi. A) Penetration of *T. asperellum* against *H. oryzae*, B) Lysis of *T. asperellum* against *H. oryzae*, C) Penetration of *T. asperellum* against *Curvularia* sp., D) Lysis of *T. asperellum* against *Curvularia* sp., E) Penetration of *T. asperellum* against *R. solani*. Notes: P= Pathogen; T= *T. asperellum*

Table 2. Colony area of some pathogenic fungi in the culture vapor method at 8 DAP

Treatment	Colony area (cm^2) ± SD	Effective-ness (%)
Control *Curvularia* sp.	8.54 ± 0.37 a	0.00
Control *R. solani*	8.53 ± 0.79 a	0.00
Control *H. oryzae*	8.29 ± 0.63 a	0.00
H. oryzae	6.27 ± 0.68 b	41.80
R. solani	5.25 ± 0.67 c	61.01
Curvularia sp.	5.22 ± 0.40 c	62.04
	CV = 8.77%	

Note: numbers in the same column and followed by the same letter are not significantly different (according to DNMRT at the 5% level).

such as the production of harzianic acid, alamethicin, tricholine, peptaibol, and antibiotics, that can inhibit the growth of pathogens, thereby inducing changes in their morphological characteristics.

The study revealed that *T. asperellum* was capable of suppressing the formation of conidia in multiple pathogenic fungi, as indicated by the absence of conidia in the observed samples (Table 3). According to Dendang (2015), *Trichoderma* spp. possess a high degree of antagonistic power and are capable of releasing toxins (mycotoxins), defined as compounds that can inhibit and even kill other fungi. The compounds produced by *Trichoderma* spp. are both volatile and non-volatile in nature, and have the capacity to inhibit the growth and production of conidia of the test pathogen.

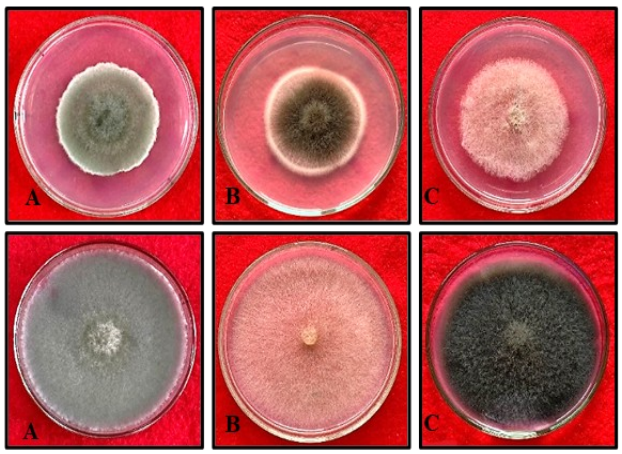

Figure 7. Morphological changes of pathogenic fungi after being treated with *T. asperellum* (above)as compared to normal Pathogenic fungus in control fungus (below) at 8 DAP. A) *H. oryzae*, B) *Curvularia* sp., C) *R. solani*.

Table 3. Observation of the number of conidia and the number of germinated conidia of pathogenic fungi after being treated with *T. asperellum*

Treatment	Conidia count/ml	Number of germi-nating conidia
H. oryzae	0	0
R. solani	0	0
Curvularia sp.	0	0

CONCLUSION

The antagonistic fungus *Trichoderma asperellum* has been observed to inhibit the growth of *Curvularia* sp., *Rhizoctonia solani*, and *Helminthosporium oryzae* from rice with an inhibition rate of up to 62%-68% in *in vitro* conditions. The mechanisms responsible for this inhibition include antibiosis, competition for resources, hyperparasitism, and the production of volatile organic compounds.

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