DEVOTION
Journal of Research and Community Service
Volume 7, Number 1, January 2025
e-ISSN: 2797-6068 and p-ISSN: 2777-0915

Indigenous Fungi as Controllers of Fruit Rot Disease in Organic and an Organic Cocoa Plants

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KEYWORDS
Endophytic fungi; fruit rot disease; *P. palmivora*; organic and non-organic cocoa

ABSTRACT
Exploration of endophytic fungi from the rhizosphere of organic and inorganic cocoa plants shows potential as a biocontrol for fruit rot caused by *Phytophthora palmivora*. These fungi can mitigate the adverse effects of chemical pesticides on yields impacted by plant pathogens. This study aimed to identify endophytic fungi from the rhizosphere of organic cocoa plantations in *Palolo Village* and inorganic plantations in *Sidondo Village*, and to test the inhibitory ability of the obtained fungi against cocoa fruit rot disease. The research comprised two stages: Stage I, exploration of endophytic fungi; and Stage II, selection of rhizosphere endophytic fungi (*in vitro* testing). Results identified several endophytic fungi from the inorganic cocoa rhizosphere, namely isolates *SAO1* (*Gliocladium* sp.), *SAO2* (*Aspergillus niger* mf-1), *SAO3* (*Aspergillus niger* mf-2), and *SAO4* (*Trichoderma* sp.). From organic cocoa fields, the isolates were *NO1* (*Gliocladium* sp. mf-1), *NO2* (*Aspergillus niger* mf-1), *NO3* (*Gliocladium* sp. mf-2), *NO4* (*Aspergillus terreus*), *NO5* (*Trichoderma* sp.), and *NO6* (*Aspergillus niger* mf-2). Isolates from organic fields (*NO5*) and inorganic fields (*SAO4*) inhibited *P. palmivora* growth by up to 70% via mechanisms such as space competition and volatile exudates.

INTRODUCTION

Endophytic fungi are fungi that live inside plant organs, do not cause disease, and form mutualistic symbioses by exchanging nutrients and releasing chemical compounds such as nitrogen and carbohydrates, which the fungi then reuse to help increase plant lifespan (Hakim, 2015; Sukapiring & Nurliana, 2022). Additionally, endophytic fungi are known to have antioxidant activity from the terpenoids they produce, as well as antibacterial activity against infectious diseases (Silva et al., 2022; Ratnawati et al., 2024).

In recent years, various studies have demonstrated the ability of endophytic fungi to suppress the growth of plant pathogens. For example, the endophytic fungus *Aspergillus* sp. has been proven effective in inhibiting the development of *Phytophthora palmivora* in cocoa seedlings, while *Trichoderma virens* and *Aspergillus fumigatus* are capable of suppressing *Rhizoctonia solani* in rice plants (Simamora et al., 2021; Rizali et al., 2021; Motlagh et al., 2022).

Central Sulawesi Province, as one of Indonesia's largest cocoa production centers, is located in the Wallacea region, which is known for its high level of biodiversity, including microorganisms such as endophytic fungi. The presence of endophytic fungi associated with cocoa plants has the potential to be utilized as biological agents for controlling plant disease pathogens, particularly fruit rot, which is one of the main problems in cocoa cultivation.

This research is important because there is still limited data and understanding of local endophytic fungal species that live in cocoa plants, especially in Central Sulawesi. Revealing the diversity and functional potential of endophytic fungi not only provides useful basic information for the development of agricultural biotechnology but also opens opportunities for their use as an environmentally friendly and sustainable alternative for biological control.

The results of the study show that cocoa rhizosphere endophytic fungi, namely *Gliocladium* sp., *Aspergillus niger*, and *Trichoderma* sp., can inhibit the growth of *Phytophthora palmivora* by up to 70% *in vitro* through mechanisms of space competition and the release of volatile exudates. These findings reinforce the potential of endophytic fungi as effective biological control agents. In addition, the main motivation for this study is to support sustainable agricultural systems and reduce dependence on chemical pesticides, which often have negative impacts on the environment and human health.

Thus, the exploration of endophytic fungi from cocoa plants has the potential to generate innovations in ecological plant disease management and contribute to increasing the productivity and quality of national cocoa. This study aims to further examine the role of endophytic fungi in controlling fruit rot disease in cocoa plants, thereby providing an environmentally friendly, sustainable biological control alternative that supports national cocoa productivity.

RESEARCH METHOD

This research was conducted in two stages. The first stage involved collecting rhizosphere samples from organic cocoa plants in Palolo Village and samples from non-organic cocoa plants in Sidondo Village, Sigi Biromaru District, Sigi Regency. The second stage, laboratory research, was conducted at the Faculty of Agriculture Laboratory, Alkhairaat University, from May to September 2024.

This research consists of two stages.

Stage I: exploration of endophytic fungi

The first stage of the research began with a survey to identify organic and non-organic cocoa farmer groups. Interviews with cocoa farmers revealed that the category of organic farmers consisted of farmers who did not spray pesticides (prima 3 certified), while the category of inorganic farmers consisted of farmers who sprayed pesticides once or twice a week, either in combination or individually. The data obtained was used to determine the sampling of the rhizosphere in cocoa plants, both organic and inorganic (Ratnawati, 2019).

Based on information from agricultural extension workers and farmers, the sampling locations consisted of organic cocoa plantations and non-organic cocoa plantations. The rhizosphere samples of organic cocoa plants were taken in Palolo Village, while the rhizosphere samples of non-organic cocoa plants were taken in Sidondo Village, Sigi Biromaru Subdistrict, Sigi Regency.

Rhizosphere sampling

Rhizosphere samples were taken from organic cocoa plants belonging to farmers in Palolo Village and non-organic cocoa plants in Sidondo Village, Sigi Biromaru District. Soil samples were taken using the authority sampling method, with the stipulation that rhizosphere samples were taken from cocoa plants that were growing dominantly and were free from cocoa pod rot disease. At each sampling point, 300 g of soil was collected from the root surface to a

depth of 10-20 cm, then placed in a plastic bag labeled with the location and date of sampling, which was then taken to the laboratory for identification and testing.

Isolation and Identification of Phytopthora palmivora

P. palmivora was isolated from infected cocoa pods, which were then sterilized using 70% alcohol, and the outer skin of the cocoa pods was removed using a sterile knife. A 0.5×0.5 cm^2 piece of fruit flesh was taken from the border between diseased and healthy tissue and planted in V8 juice medium. The culture was then incubated for 3 days until it grew on the surface of the tissue. The growing fungus was purified in potato dextrose agar (PDA) medium, incubated at room temperature, and then identified.

Isolation and Purification of Endophytic Fungi from the Cocoa Rhizosphere

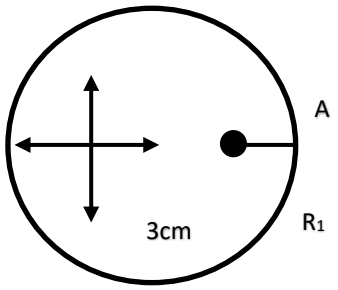
This method can be carried out through the following series of steps:

1. Dry the rhizosphere sample at room temperature for 3 days.
2. Grind the soil sample with a porcelain mortar.
3. Weigh and place 1 g of the sample into an Erlenmeyer flask containing 99 ml of sterile distilled water (10-2). Then vortex until homogeneous (preparation I).
4. Add 1 ml of preparation I to 9 ml of sterile distilled water (10-3), vortex until homogeneous (preparation II).
5. Continue the dilution process until a dilution of 10-4 (preparation III) and 10-5 (preparation IV) is obtained.
6. Pour or spread 0.1 ml of preparations II, III, and IV onto PDA medium.

Stage 2 Selection of Endophytic Fungi from the Cocoa Rhizosphere (In Vitro Test)

Dual Culture Test

The dual culture test used rhizosphere fungal colonies and pathogens with a diameter of 1 cm placed on a Petri dish containing PDA medium, with a distance of 5 cm between the two colonies. Observations were made by calculating the diameter of the fungal colony and the presence or absence of a clear zone between the two colonies. Observations of the percentage of inhibition were measured at 3 HSI, using the formula P = ((R1-R2))/R1 X 100% (Ratnawati, et al., 2023).



Description:
C = Pathogenic fungal colony
A = Antagonistic fungal colony
R1 = Average diameter of pathogenic fungal colonies cultured with antagonistic fungi.

Where:
P = Percentage of growth inhibition (%).
R1 = Radius of the pathogenic fungus colony away from the antagonistic fungus colony.
R2 = Radius of the pathogenic fungus colony approaching the antagonistic fungus colony.

Next, all Petri dishes were incubated at room temperature in a laminar air flow. The variables observed were as follows:

a. Percentage of inhibition measured from 3 HSI to 7 HSI.
b. Antagonistic mechanism

Volatility Test

The volatility test was conducted to observe the effect of the compounds produced on pathogenic fungi. The test was conducted using the vapor method reported by Dennis and Webstar (1971) in Amaria et al. (2016). Observations were made by measuring the diameter of the *P. palmivora* fungal colony every 24 hours until the pathogenic fungal culture was five days old or had filled the Petri dish. The volatile test results were calculated using the formula (Liswarni et al., 2018), namely:

$$V = \frac{V0 - V1}{Vo} x100\%$$

Description
V: Inhibition rate (%)
Vo: Control colony diameter
V1: Average colony diameter

Morphological Identification of Endophytic Fungi

Pure endophytic fungal isolates were observed for their macroscopic morphological characteristics (color, colony surface, and hyphae color) and microscopically for their conidia shape, hyphae structure, and reproductive structure using a microscope. Identification was performed by matching the fungal characteristics obtained from the observations with reference books (Barnett & Hunter, 1998; Alexopoulos & Mims, 1996; Ratnawati & Jaya K, 2021).

Percentage of Cocoa Fruit Infected by P. palmivora (In Vivo)

Spore density data were analyzed using the following formula by Gabriel and Riyanton (1989):

$$C = \frac{t\ x\ d}{n\ x\ 0{,}25}\ 10^6$$

Description:

C	=	Spore density per ml of solution
T	=	Total number of spores in the sample box observed
n	=	Number of sample boxes (5 large boxes x 16 small boxes)
0.25	=	Correction factor for the use of small sample boxes on a haemacytometer.
d	=	Dilution factor if dilution is required (d = 1 means no dilution; d = 10 means 1:10 dilution)
10^6	=	Good spore density standards were reported by the Directorate of Plantation Protection, Ministry of Agriculture, in 2014 (F. Rozy Iftaqul, 2017).

The percentage of cocoa fruits infected with *P. palmivora* was tested using a completely randomized design (CRD) with 4 treatments repeated 5 times, resulting in 20 treatment units. The treatments tested were as follows:

K0(+) = Control (without fungus)
K0(-) = Phytophthora palmivora
K1 = Organic endophytic fungus + *P. palmivora*

K2 = Inorganic endophytic fungus + *P. palmivora*

The data were analyzed using Analysis of Variance (ANOVA), and treatments that were significantly different were followed up with the Least Significant Difference (LSD) test at a 95% confidence level.

RESULTS AND DISCUSSION

Isolation and Identification of *P. palmivora* Fungi

Based on the results of macroscopic observation of *P. palmivora* fungi incubated for 3 days, the growth of *P. palmivora* fungi is smooth and white like cotton, with characteristic fungal hyphae and sporangia, although this mycelium is hyaline (transparent) in form.

Figure 1. (a) Front view of pure culture of Phytopthora palmivora fungus (b) Back view of pure culture of Phytopthora palmivora fungus

Meanwhile, the yellowish appearance observed on the back is caused by pigments or metabolites produced by the fungus during its growth. These pigments can be compounds such as pectinase enzymes or other decomposing enzymes that are released when the fungus breaks down components of the medium. Microscopic observation of *P. palmivora* fungi at 40x magnification shows that *P. palmivora* fungi have irregular hyphae with no boundaries between segments on their cell walls. The hyphae look like long tubes that function as the main dispersal tool of the fungus. In this observation, the spores were located at the ends of the hyphae and looked like round clusters.

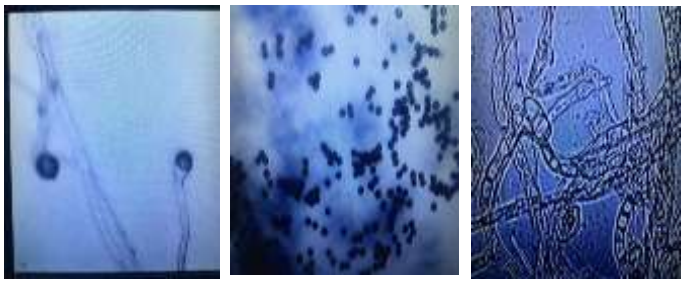


Figure 2. Microscopic view of Phytophthora palmivora fungus a. Hyphae (40x magnification) b. Spores (40x magnification) c. Sporangium (40x magnification)

Based on the isolation results, four isolates with different morphological characteristics were found in the rhizosphere of organic cocoa plants. The identified isolates included Gliocladium sp, characterized by a greenish-gray color and irregular, flour-like colony shape. This morphology indicates a growth pattern typical of the Gliocladium species, which is known

for its role in biological control and as a saprophytic fungus. The Aspergillus niger isolates, namely mf 1 (SAO 2) and mf 2 (SAO 3), were black in color with irregular colony shapes and a rough texture. Black A. niger colonies with white edges were also reported by Sudipa et al. (2023). The morphological characteristics observed, consistent with the typical description of A. Niger, were also reported by Erdiansyah & Zaini (2023), whose initial isolates were white and round in shape, gradually turning black as the colonies aged. Trichoderma sp (SAO4) is characterized by a green color in its isolates, with the colony surface forming a perfect circle and floating with white mycelium at the edges. The Trichoderma species is known for its biological control properties against various plant pathogens and is often used in agricultural practices to improve soil health and crop yields (Table 1).

Table 1. Macroscopic Characteristics of Endophytic Fungi Originating from the Rhizosphere of Inorganic Cocoa Plants

No	Isolate Code	Macroscopic Appearance		Colony Form	Genus
		Top View	Bottom View		
1	S.A.O 1	Grayish green	White	The surface of the colony is floury, irregular in shape.	*Gliocladium sp.*
2	S.A.O 2	Black	White	The surface of the colony is irregular, with a rough texture.	*Aspergilus niger* mf 1
3	S.A.O 3	Black	White	The surface of the colony is irregular, with a rough texture.	*Aspergilus niger* mf 2
4	S.A.O 4	Green	White	The surface of the colony forms a perfect circle and floats with white mycelium around the edges.	*Trichoderma sp*

Microscopic observation (conidia and phialides) on PDA culture medium. Gliocladium sp isolates are characterized by erect conidiophores that appear on the substrate or from clear, colorless septate hyphae, with branching at the ends, peniculate in shape, and heads that produce phialid spores and are sometimes bottle-shaped (Barnett & Hunter, 1998). Aspergillus niger isolates are characterized by long conidiophores, spherical conidia, slightly rounded vesicles, and septate hyphae. Trichoderma sp. isolates are characterized by green colonies and numerous conidiophore branches and conidia formation in clusters on the surface of the conidiophore cells.

Based on the results of isolation in the rhizosphere of organically grown cocoa plants, six endophytic fungal isolates were found. The macroscopic characteristics of the endophytic fungi observed included colony color (top and bottom) and colony shape (Table 2). Microscopic morphological observations can be seen through the color and shape of the colonies on PDA culture media. Based on macroscopic characteristics, the identified fungi were isolate NO1 (Gliocladium sp mf 1.), which was grayish green and round in shape. Isolate NO2 (Aspergillus niger mf1) had a black surface with a round shape, isolate NO3 (Gliocladium sp mf2.) had a gray surface, isolate NO4 (Aspergillus tereus) was pink, isolate NO5 (Trichoderma sp.) was green, isolate NO6 (Aspergillus niger mf 2) was green, and all six isolates isolated from organic cocoa plants were spherical in shape (Table 2).

Table 2. Macroscopic Characteristics of Endophytic Fungi from the Rhizosphere of Organic Cocoa Plants

No.	Code Isolate	Colony Color		Shape Colony	Genus
		Top View (A)	Bottom View (B)		
1.	N.O.1	Grayish green	Yellow	Smooth, irregular	Gliocladium sp mf 1
2.	N.O.2	Black	White	Round	Aspergilus niger mf 1
3.	N.O.3	Gray	Yellow	Round, irregular	Gliocladium sp mf 2
4.	N.O.4	Pink	White	Smooth, irregular edges	Aspergilus terreus
5.	N.O.5	Green	Green	Circular, irregular	Trichoderma sp
6.	N.O.6	Black	Yellow	Round, irregular	Aspergilus niger mf 2

Antagonistic Test (Dual Culture) Inorganic

Antagonism activity testing using the dual culture method is carried out by growing endophytic fungi and pathogenic fungi in culture media simultaneously. This is done to determine the mechanism of antagonism of endophytic fungi against pathogens. Antagonistic properties will appear in the two types of fungi grown side by side, encouraging competition for space and nutrients for fungal growth (Dwiastuti et al., 2015). Fungi with antagonistic properties can control pathogens through nutrient competition and antibiosis (Fontana et al., 2021).

The results of the antagonistic test of four endophytic fungal isolates using the double culture method showed that all four isolates had the ability to inhibit the growth of the pathogenic fungus Phytophthora Palmivora with an inhibition rate of ≥ 75%. Isolate SAO 4 had the best inhibition percentage of 70.85%, followed by SAO 1 at 64.24%, SAO 3 at 63.00%, and SAO 2 at 60.91%. The analysis of variance results showed that the percentage of inhibition at 3.5 days after planting had no significant effect and only had a significant effect at 7 days after planting, as shown in Table 3.

Table 3. Average Percentage of Inorganic Endophytic Fungus Inhibition

Isolate Code	Age (HSI)		
	3	5	7
S.A.O 1	49.23	59.22	69.60a
S.A.O 2	46.48	52.62	60.78b
S.A.O 3	54.67	49.54	63.09b
S.A.O 4	66.89	66.06	73.13a
BNT α = 0,05	tn	tn	4.33

Note: Numbers followed by the same letter in the same column are not significantly different at the BNT α = 0.05 test level.

The results of the study indicate that isolate S.A.O.4. produced the best results with a percentage. Although not significantly different from S.A.O.1, it was significantly different

from treatments S.A.O 2 and S.A.O 3. (Table 4). This indicates that the four endophytic fungal isolates from organic cocoa have the potential as biological agents to control Phytophthora palmivora pathogen attacks in efforts to control fruit rot disease in cocoa plants.

Antagonistic Test (Dual Culture) Organic

The results of observations of antagonistic tests between isolates of endophytic fungi originating from the rhizosphere of organic cocoa plants showed the ability to inhibit the growth of the pathogenic fungus Phytophthora palmivora.

Table 4. Average Percentage of Organic Endophytic Fungus Inhibition

Isolate Code	Age (HSI)		
	3	5	7
NO1	53.64c	62.00c	70.67b
NO2	60.00b	62.67bc	68.89d
NO3	59.09b	62.96bc	70.00bc
NO4	60.00b	63.81b	70.67b
NO5	65.00a	69.89a	72.17a
NO6	59.00b	63.07bc	69.39cd
BNT α = 0,05	1.39	1.42	1.06

Note: Numbers followed by the same letter in the same column are not significantly different at the BNT α = 0.05 test level.

The results of the BNT α = 0.05 follow-up test show that the percentage of inhibition of organic endophytic fungi against the pathogen *P. palmivora* indicates that isolate NO5 provides a higher inhibitory effect and is significantly different from other treatments, and the lowest was found in isolate NO1 at the beginning of observation (ages 3, 5, and 7 HIS).

Inorganic Volatile Compound Test

The results of the test on the effect of volatile compounds on the growth of *P. palmivora* fungi showed inhibition of colonies with inhibition percentages as shown in the following table.

Table 5. Percentage Inhibition of Test Results for Volatile Compounds Isolated from Endophytic Fungi Originating from the Rhizosphere of Inorganic Cocoa Plants Against the Pathogen *P. palmivora*

Isolate Code	Colony Diameter (cm)	Percentage Retention (%)
P. palmivora (Kontrol)	9.00	0.00
SAO1	7.67	14.81
SAO2	7.87	12.59
SAO3	7.70	14.44
SAO4	6.90	23.33

Observation of colony diameter and percentage inhibition of endophytic fungal isolates and organic compounds in volatile compound tests showed insignificant results, but the organic isolate SAO4 was able to inhibit the growth of *P. palmivora* by up to 23.33% compared to other isolates which gave lower results (12-14%) (Table 5). The inhibition of the growth of the pathogenic fungus *P. palmivora* colony proves that there are several endophytic fungi that

release volatile antibiotic or alkaloid compounds. The diameter of the pathogenic fungus colony grew rapidly six times after 2 HSI (Table 6).

Table 6. Colony Diameter of the Pathogenic Fungus Phytophthora palmivora

Isolate Code	Colony Diameter (cm) On Day	
	1 HSI	2 HSI
SAO1	1.33	7.67
SAO2	1.00	7.87
SAO3	1.00	7.70
SAO4	2.33	6.90
BNT α = 0,05	0.38	0.49

Note: Numbers followed by the same letter in the same column are not significantly different at the BNT α = 0.05 test level.

Testing Volatile Organic Compounds

The results of testing the effect of volatile compounds on the growth of *P. palmivora* fungi showed inhibition of colonies, with the percentage of inhibition shown in Table 7. The results of the colony diameter of six organic endophytic fungal isolates showed no significant difference, with the ability to inhibit the growth of *P. palmivora* ranging from 12 to 17%.

Table 7. Percentage Inhibition of Test Results for Volatile Compounds Isolated from Endophytic Fungi Originating from the Rhizosphere of Organic Cocoa Plants Against the Pathogen *P. palmivora*

Isolate Code	Colony Diameter (cm)	Percentage Retention (%)
P. palmivora (Control)	9.00	0.00
NO1	7.90	12.22
NO2	7.73	14.07
NO3	7.60	15.56
NO4	7.50	16.67
NO5	7.47	17.07
NO6	7.73	17.04

The results of the BNT α = 0.05 follow-up test (Table 10) showed that treatment NO5 produced the smallest endophytic fungus colony diameter at 2 HSI, which was significantly different from treatments NO6, NO2, and NO1 but not significantly different from treatments NO3 and NO4.

Table 8. Diameter of organic *P. palmivora* pathogenic fungus colonies

Isolate Code	Colony Diameter (cm) On Day	
	1 HSI	2 HSI
NO1	3.90	7.90c
NO2	3.80	7.73bc
NO3	3.77	7.60ab
NO4	3.73	7.50ab
NO5	3.67	7.47a
NO6	3.80	7.73bc
BNT α = 0,05	tn	0.22

Note: Numbers followed by the same letter in the same column are not significantly different at the BNT α = 0.05 test level.

Molecular Identification

Based on the results of macroscopic selection of Trichoderma sp. isolates, molecular identification was performed on two different isolates, namely isolates NO5 and RAN16. Identification of isolate NO5 used the ITS1 primer pair 5' – TCCGTAGGTGAACCTGCGG – 3' with a target band of 575 bp, while isolate RAN16 used the ITS4 primer pair 5' – TCCTCCGCTTATTGATATGC – 3' with a target band of 576 bp. The results of the two test isolates were visualized by electrophoresis to visualize the DNA amplification results. The sequencing analysis results of T. asperellum strain NG125 and T. asperellum strain NECC30406 showed 100% similarity, based on data from the gene bank as shown in the following table:

Table 11. Sequencing analysis of Trichoderma sp. isolates.

Isolate	Organism	Similarity	Accession No.
Trichoderma sp	*Trichoderma asperellum* strain NG125	100%	MW287256.1
	Trichoderma asperellum strain NECC30406		
Trichoderma sp		100%	MH153622.1

Although both were identified as T. asperellum, the T. asperellum isolate from the rhizosphere of organic cocoa had different morphological characteristics from the T. asperellum isolate from the rhizosphere of non-organic cocoa. This can be proven from the total score of the sequencing analysis, namely the total score for T. asperellum from the rhizosphere of organic cocoa was 1062, while the total score for T. asperellum from the rhizosphere of conventional cocoa is 1064, which is slightly higher by 2 points.

Percentage of Cocoa Fruits Infected with *P. palmivora* (In vivo)

The data on the percentage of infected cocoa fruits in the first observation is presented in Table 9 below:

Table 9. Percentage of Fruit Infected by *P. palmivora*

Treatment	Percentage of fruit attacked by *P. palmivora*		
	Observation 1	Observation 2	Observation 3
K0 (+)	0.6 ab	1.0 b	1.0
K0 (-)	0.9 b	1.0 b	1.0
K1 (NO5)	0.5 ab	0.4 a	0.8
K2 (RAN16)	0.2 a	0.3 a	0.8
BNT α 0,5	0.46	0.43	tn

The results of the BNT test (α 0.05) showed that the percentage of cocoa pods infected with *P. palmivora* in vivo at the first observation was lower in the endophytic fungus and organic treatment (K2) compared to the other treatments and was significantly different from the treatment with the *P. palmivora* pathogen (K0-). However, there was no significant difference between the organic endophytic fungus treatment (K1) and the treatment without fungus (K0+). This study also showed that the treatment with the pathogen *P. palmivora* (K0-) had a higher percentage of cocoa pods infected with *P. palmivora* compared to the other treatments.

The results of the study show that morphologically, several species of endophytic fungi were found, including Gliocladium sp, Aspergillus niger, and Trichoderma sp. Antagonistic tests showed that fungi isolated from inorganic cocoa plantations have been proven to exhibit significant antagonistic activity against the pathogen *P. palmivora*. These isolates, derived from Gliocladium sp., Aspergillus niger, and Trichoderma sp., were obtained through isolation (Figure 5 and Table 1) and were able to inhibit the growth of fruit rot-causing fungi in cocoa plants by forming resistance zones in Petri dishes, demonstrating their potential as biological control agents. This research is still limited to identification and characterization, and it is hoped that it can be continued with an in-depth analysis of the inhibition mechanism of the three types of microbes that produce disease-antagonistic compounds. However, several previous researchers have reported that the genus Trichoderma sp. is known as a highly active saprophytic fungus in controlling plant pathogens, including *P. palmivora*.

All four isolates, whether isolated from organic or inorganic cocoa, have the ability to inhibit the growth of the pathogenic fungus Phytophthora palmivora through a series of tests, including dual culture antagonism tests (Figures 7 and 8) and volatile compound tests (Tables 7 and 9). The inhibitory potential of the isolates against the pathogen *P. palmivora* was at a level of 60 to 70% (Tables 3 and 5). The isolate identified as Trichoderma sp., from inorganic cocoa land, had an inhibition level of 73.13%. Trichoderma sp. is known for its role in biological control due to its ability to competitively inhibit the growth of pathogenic fungi. One of the main strategies used by Trichoderma sp. is nutrient competition, whereby Trichoderma sp. rapidly colonizes plant roots and other surfaces to secure essential nutrients and space (Ahlawat et al., 2010; Oszust et al., 2020; Sanothan et al., 2023).

Gliocladium sp. isolates inhibited growth by 72.17%. Previous research conducted by Malik et al. (2022) reported that Gliocladium sp. effectively inhibited the growth of Phytophthora capsici, an important pathogen that causes disease in chili plants, with the highest percentage of 54.89% after five days of inoculation. The mechanisms used by Gliocladium sp. to provide an inhibitory effect include nutrient competition, pathogen cell lysis, and mycoparasitism. This strategy allows it to effectively suppress the growth of competing pathogenic fungi (Suryanto & Nugraha., 2018; Malik et al., 2022;). The effectiveness of Gliocladium sp. is influenced by environmental conditions, particularly pH levels. Research shows that at pH 5.5, the inhibition percentage reaches 35.2%, while at pH 7, it drops to 14%. These findings underscore the importance of optimizing growth conditions to maximize the antifungal activity of Gliocladium sp. (Halim & Setiawan., 2020).

Isolates SAO2 and 3 showed inhibition percentages against *P. palmivora* of 60.78% and 63.89% after 7 days of incubation (Table 4). These results were lower than those reported by Sudarma et al. (2017), who showed a significant inhibition percentage of 90% against the growth of *P. palmivora*. The antifungal activity of A. niger is associated with the production of bioactive metabolites that inhibit the growth of pathogenic fungi. Specific metabolite content has been reported to contain compounds such as Scopularide A and B, which have been identified as having strong antifungal activity against Aspergillus niger by (Niazi et al., 2023; Singab et al., 2023), with minimum inhibitory concentration (MIC) values ranging from 3.9 to 31.25 µg/mL, indicating its potential as an effective antifungal agent (Singab et al., 2023). This demonstrates a strong capacity to suppress the growth of this pathogen.

Another study reported an inhibition percentage of 5 ± 0.1% for A. niger when applied to infected fruit, demonstrating its effectiveness in reducing infection rates compared to the control group (Sudarma et al., 2017). The results of this study indicate that fungi morphologically confirmed to belong to the genus Trichoderma have the highest inhibitory capacity against the development of *P. palmivora* disease (Tables 3 and 5). Woo et al. (2014) mention that Trichoderma sp. is the most widely used biological control agent in controlling pathogens that attack roots, shoots, and post-harvest. Trichoderma produces various metabolites, including peptibols that function as fungicides. These toxic compounds can extrude the pathogen's cell membrane, thereby disrupting the pathogen's physiological processes (Sudarma et al., 2020).

Trichoderma also produces lytic enzymes, glucanase, cellulase, and chitinase. These enzymes can break down the structure of pathogen polysaccharides, making it difficult for pathogens to reproduce and spread (Khumar et al., 2012). The composition of plant pathogen cell walls is an important factor in inducing the production of these enzymes.

In addition, there are other mechanisms, such as the production of volatile compounds that are toxic to pathogens, enabling Trichoderma sp to directly inhibit pathogen growth. The results of volatile compound testing showed that isolates identified as Trichoderma had the highest inhibition rate of 23% compared to other isolates (Table 7). Qualhato et al. (2013) found in their study that three of the four types of Trichoderma tested showed the production of volatile and toxic metabolites, which had a significant effect on the growth and development of plant pathogens. Compounds such as n-alkanes, cyclohexane, cyclopentane, fatty acids, alcohols, esters, sulfur-containing compounds, simple pyranes, and benzene derivatives have been identified in T. harzianum and T. atroviride cultures (Siddiquee et al., 2012).

The antagonistic ability of Trichoderma sp. is a combination of several mechanisms, including direct mycoparasitism (Lorito et al., 2010), Qualhato et al. (2013) found four Trichoderma species growing in liquid culture with cell walls from S. sclerotiorum, F. solani, and R. solani, each producing different enzymes, such as b-1,3-glucanase, NAGAse, chitinase, phosphatase, protease, and alginate-lyase, all of which depend on the cell wall as an inducer. Efficient hydrolysis of cell wall components is likely one of the main factors in the ability of these fungi to biologically control a wide spectrum of plant pathogens. Previously, Lewis and Papavizas (1984) were able to produce a number of extracellular enzymes, ß (1,3) gluconase and chitinase, which can dissolve the cell walls of pathogens.

Macroscopic and microscopic characteristics, isolate NO5, previously suspected to be Trichoderma sp., based on sequencing test results, has a genetic similarity of 100% with Trichoderma asperellum strain NG125. The strain was confirmed through access number MW287256.1 in the genetic database. The high level of similarity (100%) indicates that the tested isolate is very closely related to Trichoderma asperellum, which is known to have potential as a biocontrol agent, especially in controlling plant pathogens. The presence of these two strains in the identification indicates that the isolate obtained may also have superior properties in biological control.

In line with previous research documenting the potential of Trichoderma asperellum as a biocontrol agent, Zhang et al. (2020) stated that T. asperellum is effective in inhibiting the growth of various plant pathogens, including Fusarium spp. and Rhizoctonia spp., through mechanisms of space competition, secretion of hydrolytic enzymes, and production of

antimicrobial compounds. Similar research by Harman et al. (2018) also shows that T. asperellum can increase plant resistance to abiotic stress, such as drought, due to Trichoderma's ability to improve nutrient absorption and root growth. The genetic similarity of isolate NO5 to strains of T. asperellum that have been tested for effectiveness in biological control supports the assumption that isolate NO5 may have a similar function.

CONCLUSION

This study concludes that endophytic fungi isolated from the rhizosphere of organic cocoa trees include SAO1 (*Gliocladium* sp.), SAO2 (*Aspergillus niger* mf-1), SAO3 (*Aspergillus niger* mf-2), and SAO4 (*Trichoderma* sp.), while those from non-organic cocoa rhizospheres comprise NO1 (*Gliocladium* sp. mf-1), NO2 (*Aspergillus niger* mf-1), NO3 (*Gliocladium* sp. mf-2), NO4 (*Aspergillus terreus*), NO5 (*Trichoderma* sp.), and NO6 (*Aspergillus niger* mf-2); notably, isolates NO5 (*Trichoderma* sp.) from organic cocoa and SAO4 (*Trichoderma* sp.) from non-organic cocoa demonstrated the highest inhibitory effect, suppressing *Phytophthora palmivora* growth by up to 70%. For future research, field trials should evaluate the efficacy of these promising *Trichoderma* sp. isolates under in vivo conditions across diverse cocoa cultivation systems in Central Sulawesi, including assessments of their persistence, environmental impacts, and integration with organic farming practices to optimize sustainable fruit rot control.

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