

Antagonist Test of *Trichoderma* sp and *Gliocladium* sp Againts Fungal Pathogens That Cause Diseases on Tomato Plant

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ABSTRACT

Tomatoes (*Solanum lycopersicum*) are one of the widely cultivated crops consumed by people worldwide, including Indonesia. Tomato farming often faces various challenges that result in reduced tomato productivity. One of the challenges is the presence of diseases that affect tomatoes, leading to crop failure for farmers. Some important diseases that commonly affect tomato plants include Fusarium wilt and anthracnose. Most farmer use chemical fungicides to control the diseases. These methods have greater negative impacts on both plants and the surrounding environment. One alternative control technique that can be used is the use of biological agents with antagonistic properties, such as *Trichoderma* sp. and *Gliocladium* sp. This research aims to assess antagonistic abilities of endophytic fungi *Trichoderma* sp. and *Gliocladium* sp. againts the pathogens responsible for suppressing the growth of these disease-causing phatogens in tomato plants. in the Laboratory of Plant Pest and Disease Science, OECF Building, Faculty of Agriculture, Universitas Mulawarman. The experiment was designed using a completely randomized single-factor design with four treatments and five replications. The data obtained were analyzed using Analysis of Variance and the least significant difference test at a 5% significance level. The research results indicate that the antagonistic tests of *Trichoderma* sp. and *Gliocladium* sp. in vitro significantly differ againts the pathogenic fungi *Fusarium* sp. and *Colletotrichum* sp. Furthermore, itwas found that the antagonistic fungus *Gliocladium* sp. is the best fungus capable of suppressing the growth of important pathogenic fungi in tomato plants.

Keyword: *Gliocladium* sp., *Trichoderma* sp., *Fusarium* sp., *Colletotrichum* sp. and Tomato (*Solanum lycopersicum*)

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the cultivated plants widely consumed by people worldwide, including Indonesia. In Indonesia itself, tomatoes are one of the staple crops with high production figures. However, tomato farming often encounters various obstacles that result in a decrease in tomato productivity (Sopialena et al, 2022). One of the challenges is the presence of diseases that affect tomatoes, leading to crop failures for farmers. The presence of plant pests (OPT) is one of the factors that hinder tomato growth. These plant pests can cause various disease symptoms in plants. Some important diseases that frequently affect tomatoes include anthracnose and Fusarium wilt caused by *Fusarium* sp. and *Colletotrichum* sp. One control technique that can be used is the use of biological agents with antagonistic properties such as *Trichoderma* sp. and *Gliocladium* sp.

MATERIALS AND METHODS

This research was conducted from July to October 2022. The research was carried out at the Laboratory of Pest and Disease Plant, Faculty of Agriculture, Mulawarman University.

Materials and Equipment

The materials used in this study include *Potato Dextrose Agar* (PDA), tomato plants affected by Fusarium wilt and anthracnose, 70% alcohol, plastic, distilled water (aquades), chloramphenicol, spiritus (denatured alcohol), cotton, aluminum foil, and tissue.

The equipment used in this study includes a microscope, Petri dishes, Erlenmeyer flasks, cover glasses, object glasses, needle holders, measuring cups, Bunsen burner, autoclave, spatula, plastic cling wrap, Optilab, hemocytometer, writing tools, ruler, and camera.

Experimental Design

This research utilized a Completely Randomized Design (CRD) with one factor, consisting of 4 treatments, each with 5 replications. The treatments include:

FK = Control

FT = *Fusarium* sp. vs *Trichoderma* sp

FG = *Fusarium* sp. vs *Gliocladium* sp.

CT = *Colletotrichum* sp. vs *Trichoderma* sp.

CG = *Colletotrichum* sp. vs *Gliocladium* sp.

Research Procedure

The research activities consist of:

a. Propagation of antagonistic fungi

The antagonistic fungi used in this study are *Trichoderma* sp. and *Gliocladium* sp. Both isolates of *Trichoderma* sp. and *Gliocladium* sp. were obtained from the collection of the Laboratory of Pest and Disease Plant, Faculty of Agriculture, Mulawarman University. The fungal isolates were cultured on *Potato Dextrose Agar* (PDA) medium in 9 cm petri-dishes and incubated until they covered the entire petri-dish.

b. Propagation of pathogenic fungi

The isolates of pathogenic fungi used in this study are *Fusarium* sp. and *Colletotrichum* sp. These fungal isolates were obtained from tomato plants showing symptoms of Fusarium wilt and anthracnose. Both pathogen isolates were cultured on PDA medium in 9 cm diameter petri dishes and incubated until the fungi covered the entire petri dish.

c. Fungal Identification

The isolated fungi will be identified using a binocular microscope. Fungal samples will be collected using a needle holder and placed on an object glass, followed by adding a drop of methylene blue and covering it with a cover glass. Fungal isolates will be identified based on their microscopic and macroscopic characteristics such as color, hyphae, colony counter and others.

d. Purification

Fungi that have been successfully identified will then be purified using a 9 cm diameter petri dish filled with PDA. The fungi will be streaked using a needle holder and placed in the center of the petri dish. The petri dish will be labeled and incubated until the fungi cover the dish.

e. In vitro Antagonistic Test

Pathogenic fungi *Colletotrichum* sp. and *Fusarium* sp. will be isolated in petri dishes containing PDA. Then, the antagonistic fungi *Trichoderma* sp. and *Gliocladium* sp. will be grown on the opposite side at a distance of 5 cm from the colonies of pathogenic fungi. In the control group, isolates of *Colletotrichum* sp. and *Fusarium* sp. will be isolated without antagonistic fungi. Observations will be made every 24 hours regarding the growth of both antagonistic and pathogenic fungi.

Data Analysis

The data obtained will be analyzed using analysis of variance (ANOVA) at a 5% significance level. If the ANOVA results indicate a significant difference, then a Least Significant Difference (LSD) test will be conducted at a 5% significance level.

RESULTS AND DISCUSSION

Results

The fungal morphology based on observations

The morphology of antagonistic fungi *Trichoderma* sp. and *Gliocladium* sp. and the pathogenic fungi *Colletotrichum* sp. and *Fusarium* sp. were obtained. Fungal morphology was examined both microscopically and macroscopically. The observed morphological characteristics include color, hyphae, colony shape, and others.

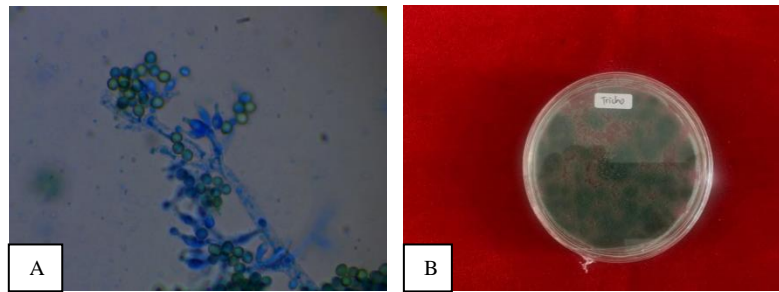


Figure 1. (A) *Trichoderma sp.* colony (400x magnification) (B) *Trichoderma sp.* colony

In the observation of *Trichoderma sp.* macroscopically, at the early stage of colony formation, it appeared white and then changed to light green and eventually turned dark green as the fungus aged. This is consistent with the findings of Sopialena (2020), who noted that *Trichoderma sp.*

The color of *Trichoderma* colonies green, or olive-green with the The texture of colonies are velvety. When young, the colonies may appear fluffy, and as they mature, they often become more compact. *Trichoderma sp.* are fast-growing fungi, and colonies can spread rapidly on growth media. The surface of *Trichoderma* colonies may be smooth or slightly wrinkled. As the colony matures, concentric rings or sectors may develop, giving the colony a zoned appearance. When grown on solid media, the underside of *Trichoderma* colonies may appear similar to the surface but may be slightly lighter in color. *Trichoderma* colonies typically produce abundant conidia (asexual spores) on the surface, which can contribute to the colony's appearance. These conidia are typically produced in slimy masses or brush-like structures called conidiophores.

Under a microscope, *Trichoderma sp.* can be identified by their characteristic conidiophores, conidia, and branching pattern. Conidiophores are usually long, branched, and bear phialides (structures that produce conidia) in a brush-like arrangement.

Microscopically, it can be observed that *Trichoderma sp.* has distinctive characteristics, such as trident-shaped colony tips. The fungal hyphae are septate, with conidiophores branching extensively and elongated conidia, in agreement with the description provided by Sopialena (2020). The observed characteristics of *Trichoderma sp.*'s morphology have been matched with fungal identification books by Barnett (1998) and Watanabe (2002).

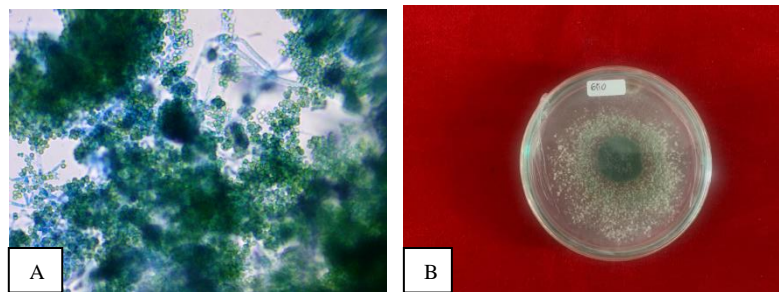


Figure 2. (A) *Gliocladium sp.* colony (400x magnification) (B) *Gliocladium sp.* colony

Macroscopically, the colony of *Gliocladium sp.* appears dark green and gradually fades to white as it approaches the edge of the petri dish. When compared, *Gliocladium sp.* does not grow as extensively as *Trichoderma sp.* *Gliocladium sp.* tends to grow with more spacing between colonies.

Microscopically, *Gliocladium sp.* has septate conidiophores that branch upwards with a similar brush-like shape (pencil-like). Each branching forms a spiral groove with 4-5 groups of conidia. The conidia of this fungus are round to flattened and hyaline in appearance (Rahma,2021).

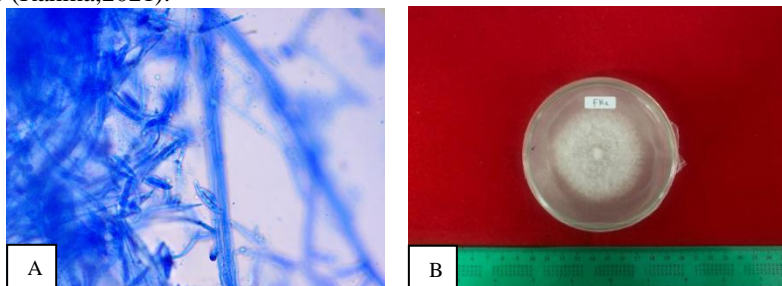


Figure 3. (A) *Fusarium* sp. colony (400x magnification) (B) *Fusarium* sp. Colony

Macroscopically, it can be observed that *Fusarium* sp. has colonies that appear white, resembling thick cotton, and grow to nearly fill the petri dish. Microscopically, the hyphae of *Fusarium* sp. are long and septate. The macroconidia of the fungus are round to oval in shape. These hyphae also produce asexual spores in the form of microconidia and macroconidia (Sholihah, 2019).

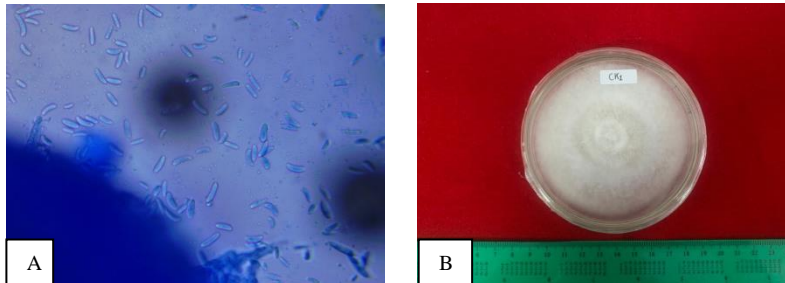


Figure 4. (A) *Colletotrichum* sp. colony (400x magnification) (B) *Colletotrichum* sp. Colony

Based on the observations, the macroscopic characteristics of *Colletotrichum* fungus include colonies that appear white like cotton, with a yellowish-white center and bright white hyphae. Microscopically, this fungus exhibits morphological characteristics such as oval-shaped conidia, septate hyphae, and arched conidia (Wiyana, 2022). In general, this fungal species has transparent, elongated cylindrical and septate hyphae with lengths ranging from 10-16 µm and widths of 5-7 µm, with conidia colonies that are black in color.

Growth rate

Table 1. Growth rate of antagonistic and pathogenic fungi

Fungus Name	Day							Avarage
	1	2	3	4	5	6	7	
<i>Trichoderma</i> sp.	0,7	1,62	2,06	2,77	2,98	3,24	3,38	2,39
<i>Gliocladium</i> sp.	0,73	2,24	3,61	3,96	4	4,17	4,41	3,30
<i>Colletotrichum</i> sp.	0,37	0,66	0,94	1,24	1,36	1,31	1,28	1,02
<i>Fusarium</i> sp.	0,32	0,78	1,27	1,47	1,84	1,85	1,88	1,34

Spore density

Table 2. Conidia density of antagonistic and pathogenic fungi

Fungus Name	Spore Density
<i>Trichoderma</i> sp	9,3 x 10 ⁶
<i>Gliocladium</i> sp	3,4 x 10 ⁶
<i>Fusarium</i> sp	1,4 x 10 ⁶
<i>Colletotrichum</i> sp	2,7 x 10 ⁶

Percentage of inhibition

Table 3. Results of the analysis of variance for the inhibitory effect of antagonistic fungi on pathogenic fungi

Treatment	Avarage
FG	0,82a
FT	0,84ab
CT	1,02bc
CG	1,05c

Note: Numbers followed by lowercase letters are significantly different at a 5% alpha level according to the Least Significant Difference (LSD) test (LSD = 3.04), while numbers with the same lowercase letter are not significantly different at a 5% alpha level.

Antagonistic mechanisms

Table 4. Antagonistic mechanisms of antagonistic fungi against pathogenic fungi

Treatment	Type of Mechanism		
	Competition	Parasitism	Antibiosis
FT (<i>Fusarium</i> sp. vs <i>Trichoderma</i> sp.)	+	+	-
FG (<i>Fusarium</i> sp. vs <i>Gliocladium</i> sp.)	+	+	-
CT (<i>Colletotrichum</i> sp. vs <i>Trichoderma</i> sp.)	-	-	-
CG (<i>Colletotrichum</i> sp. vs <i>Gliocladium</i> sp.)	-	-	-

Discussion

Fungal Morphology

a. *Trichoderma* sp.

The color of *Trichoderma* colonies ranges from green to olive-green, while their texture is described as velvety. When in their early stages, the colonies may exhibit a fluffy appearance, which tends to become more compact as they mature. *Trichoderma* sp. are known for their rapid growth, spreading swiftly on growth media. The surface of these colonies can either be smooth or slightly wrinkled, with the mature colonies often developing concentric rings or sectors, giving them a zoned appearance. On solid media, the underside of *Trichoderma* colonies may resemble the surface but with a slightly lighter hue. Abundant conidia (asexual spores) are typically produced on the surface of *Trichoderma* colonies, often in slimy masses or brush-like structures known as conidiophores (Sopialena and Wati, 2018).

Under microscopic observation, *Trichoderma* sp. can be distinguished by their characteristic conidiophores, conidia, and branching pattern. Conidiophores are typically elongated and branched, bearing phialides (structures responsible for conidia production) arranged in a brush-like manner (Chaveri and Samuel, 2013).

b. *Gliocladium* sp.

Macroscopically, *Gliocladium* sp. colonies appear dark green and gradually fade to white as they approach the petri dish's edge. Compared to *Trichoderma* sp., *Gliocladium* sp. doesn't grow as extensively and tends to have more spaced colonies (Sopialena et al. 2020). Microscopically, *Gliocladium* sp. has septate conidiophores that branch upwards with a similar brush-like shape (pencilate). Each branch forms a spiral groove with 4-5 groups of conidia. The conidia of this fungus are round to flattened and hyaline (Rahma, 2021). *Gliocladium* sp. thrives at temperatures around 26-28°C with a pH level of 5-6.

c. *Fusarium* sp.

Macroscopically, *Fusarium* sp. colonies appear white, resembling thick cotton, and grow to nearly fill the petri dish. Microscopically, *Fusarium* sp. hyphae are elongated and septate. The macroconidia of the fungus are round to oval. These hyphae also produce asexual spores in the form of microconidia and macroconidia. *Fusarium* sp. undergoes both pathogenic and saprophytic phases in its life cycle, commonly known as a saprophyte but can become a plant pathogen. It can persist in the soil as chlamydospores are abundant in diseased roots. This fungus enters through root wounds and grows inside the host plant. In advanced infections, *Fusarium* sp. mycelium can spread through parenchyma vessels, producing numerous spores within plant tissues (Sholihah, 2019).

d. *Colletotrichum* sp.

This fungus can grow and thrive with environmental support that includes an optimum temperature range of 24-30°C. Other factors, such as pH, humidity, planting distance, and cleanliness around the planting area, also play a role. When all these aspects are favorable, the spread and infection process becomes faster, and the plants are more likely to die (Inaya, 2022). The fungus's spores can spread through wind, rain splashes, and adhere to suitable host plants, where they reproduce rapidly. Moist soil conditions can influence fungal growth and accelerate the infection process.

The initial step in calculating fungal growth rate began by measuring the diameter of each antagonistic and pathogenic fungus colony for seven days, starting one day after incubation. From the observations, it was found that the diameter of the *Trichoderma* sp. antagonistic fungus colony was 3.38 cm, and that of *Gliocladium* sp. was 4.41 cm. In contrast, the pathogenic fungi, *Fusarium* sp. and *Colletotrichum* sp., had colony diameters of 1.88 cm and 1.28 cm, respectively. The diameter of the antagonistic fungi was larger than that of the pathogenic fungi, demonstrating that the antagonistic fungi were capable of inhibiting the growth of the pathogenic fungi. The results of the analysis of variance for the inhibitory effect of antagonistic

fungi on pathogenic fungi showed significant differences. The inhibition percentage of fungi was determined by calculating the growth of pathogenic fungi in proximity to endophytic fungi in confrontation plates, measured every 24 hours for seven days after inoculation (HSI). The highest inhibition percentage in the testing of *Fusarium* sp. vs. *Gliocladium* sp. was on average 0.63%. Based on the LSD test at a 5% significance level, it can be seen that the treatments of *Gliocladium* sp. and *Trichoderma* sp. had a significant difference in inhibiting important plant diseases caused by *Fusarium* sp. and *Colletotrichum* sp. in tomato plants.

The results of spore density calculations indicated that the highest spore density was observed in the *Trichoderma* sp. antagonistic fungus, with a value of 9.3×10^6 , followed by *Gliocladium* sp. with a value of 3.4×10^6 . Among the pathogenic fungi, the highest spore density was found in *Colletotrichum* sp., with a spore density value of 2.7×10^6 , while *Fusarium* sp. had the lowest spore density at 1.4×10^6 . Spore density was calculated using a hemocytometer and observed under a microscope.

The results of the analysis of variance for the inhibitory effect of antagonistic fungi on pathogenic fungi showed significant differences (Table 3.) The inhibition percentage of fungi was determined by calculating the growth of pathogenic fungi in proximity to endophytic fungi in confrontation plates, measured every 24 hours for seven days after inoculation (HSI). The highest inhibition percentage in the testing of *Fusarium* sp. vs. *Gliocladium* sp. was on average 0.63%. Based on the LSD test at a 5% significance level, it can be seen that the treatments of *Gliocladium* sp. and *Trichoderma* sp. had a significant difference in inhibiting important plant diseases caused by *Fusarium* sp. and *Colletotrichum* sp. in tomato plants.

Observations of antagonistic mechanisms of antagonist fungi against pathogenic fungi causing important diseases in tomato plants were conducted for seven days after incubation. Antagonistic mechanisms can be observed when both tested fungi suppress each other's growth. In this observation, three types of mechanisms were: competition, antibiosis, and parasitism (Table 4.). Based on observations made over 7 days, it can be concluded that the mechanism of interaction falls into the category of competition, where the faster growth of antagonistic fungi leads to competition for space and nutrients with the pathogenic fungi.

CONCLUSION

Based on the research results, it can be concluded that *Trichoderma* sp. and *Gliocladium* sp. antagonistic fungi are capable of suppressing and inhibiting the growth of pathogenic fungi *Fusarium* sp. and *Colletotrichum* sp., which are important plant diseases in tomato plants. Among these two antagonistic fungi, *Gliocladium* sp. proves to be the most effective antagonist capable of inhibiting the growth of important pathogenic fungi in tomato plants.

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