JURNAL
BIOS LOGOS
Accredited by Ministry of Research, Technology and Higher Education No. 105/E/KPT/2022

Vol. 15 (No.2), Juli 2025, 219–225
DOI: https://doi.org/10.35799/jbl.v15i2.62836
E-ISSN: 2656-3282
P-ISSN: 2088-9569

Antagonist Test of *Gliocladium* sp. against *Fusarium* sp. Cause of Wilt Disease in Shallot Plants In Vitro

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(Article History: Received-July 14, 2025; Revised-August 18, 2025; Accepted-August 30, 2025)

ABSTRACT

This study aims to analyze the morphological characteristics of the fungus *Gliocladium* sp., measure its inhibition against the growth of the fungus *Fusarium* sp. causing wilt disease in shallot plants (*Allium ascalonicum* L), and examine its antagonistic mechanism in vitro. This research method includes exploration and sampling, sterilization of tools and materials, making PDA media, isolation, purification, and identification of morphological characteristics and measurement of antagonistic fungal inhibition. The antagonist test uses the dual culture method, and data analysis with a completely randomized design (CRD) and ANOVA test followed by the Honestly Significant Difference (HSD) Test at the 5% significance level. The results showed that *Gliocladium* sp. was able to inhibit the growth of *Fusarium* sp. with a percentage inhibition of 25.63% and spore viability of 66.52%. Antagonistic mechanisms observed include competition for space and nutrients, antibiosis, lysis, and mycoparasitism.

Keywords: *antagonist; Fusarium sp.; Gliocladium sp.; dual culture*

INTRODUCTION

Shallot (*Allium ascalonicum* L.) is one type of vegetable that has high economic value, so its development potential is very promising because the demand for shallots continues to increase (Prakoso et al., 2016). According to BPS (Central Bureau of Statistics) data, shallot production in Indonesia, especially in North Sulawesi in 2018-2022, has increased. In 2022 shallot production reached 5,020 tons, while in 2023 shallots experienced a decrease in production to 3,153 tons. The difficulty faced is the increasing demand but inconsistent productivity. This is caused by crop failure or reduced shallot yield due to pest and disease attacks (Rosyidah, 2019).

Diseases caused by the pathogenic fungus Fusarium sp. pose a significant threat to shallot farmers, given their extensive impact on crop productivity and quality. According to Maqbool et al. (2017) this pathogen causes a serious wilt disease that reduces yield and quality of shallots making them unfit for sale or consumption. Infection damages plant roots and tissues, reduces water and nutrient uptake, and causes damage to bulbs.

Fusarium wilt disease control generally uses chemical fungicides. The use of chemical fungicides risks polluting soil and water, triggering pathogen resistance, and leaving harmful residues on agricultural products that endanger human health. (Abo-Elyousr et al., 2014; Singh et al., 2015). To reduce the negative impact of chemical fungicides, the use of biological agents such as *Gliocladium* sp. is a more environmentally friendly solution. These fungi effectively control pathogens through antagonistic mechanisms, while being safer for the environment and human health.

Gliocladium sp. fungi control plant pathogens through three main mechanisms: competition for space and nutrients, where *Gliocladium* sp. fungi grow faster and dominate the environment. Antibiosis, where the *Gliocladium* sp. fungus produces

pathogen-inhibiting compounds. Mycoparasitism, where the *Gliocladium* sp. fungus attacks the pathogen directly (Sharma et al., 2019).

Several recent studies have shown the effectiveness of the fungus *Gliocladium* sp. in controlling various plant diseases. The results of research by Gupta et al. (2018), showed that the application of *Gliocladium* sp. fungi in rice plants can reduce Fusarium infection and increase yield significantly. Similar research on corn plants by Singh et al. (2019), indicated that the fungus *Gliocladium* sp. was effective in controlling fungal diseases and improving plant health.

Based on the background description of the problem above, it is necessary to conduct research to analyze the morphological characteristics of *Gliocladium* sp. and *Fusarium* sp., measure the inhibition of *Gliocladium* sp. against the growth of Fusarium sp. and examine the antagonistic mechanism of *Gliocladium* sp. against *Fusarium* sp. causing Fusarium wilt disease in shallot plants.

METHODS

The testing phase includes morphological characteristics of *Gliocladium* sp. and *Fusarium* sp. fungi, measurement of the inhibition of antagonistic fungi (growth rate, percentage of inhibition, mechanism of antagonism, and viability test).

Characterization of Fungal Morphology

Characteristics of antagonistic fungal isolates *Gliocladium* sp. and pathogenic fungus *Fusarium* sp. were observed macroscopically and microscopically for fungal growth and development.

Measurement of Antagonistic Fungal Inhibition

Growth Rate

Measuring the growth rate of antagonistic fungi and pathogenic fungi used four treatments, namely KA = Antagonist Control, KP = Pathogen Control, AP = Antagonist Treatment, PP = Pathogen Treatment. Each treatment with 3 replications and incubated at room temperature for 7 days. Colony diameter was measured using a ruler through the intersection of vertical and horizontal lines at the center of the colony. Growth rate using the formula:

$$D = \frac{d1 + d2}{2}$$

Description:
D = diameter of fungal colonies
d1= vertical diameter of fungal colonies
d2= horizontal diameter of fungal colonies

Percentage of Inhibition

Calculated using the Fokkema and Meuleun formula (Alifia et al., 2023):

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

Description:
P = Percentage of inhibition
R1 = Radius of pathogenic colonies in control treatment
R2 = Radius of pathogenic colonies in dual culture treatment

According to Izzatinnisa et al. (2020), the criteria for the percentage of growth inhibition (%) are as follows:
1. High percentage inhibition: 70-100%
2. Medium percentage inhibition: 40-69%
3. Low percentage inhibition: 0-39%

Mechanisms of Antagonism

Identified in the mechanism of antagonism, namely competition for space and nutrients, antibiosis, lysis and parasitism.

Viability Test

Spore viability is calculated using the Indonesian National Standard formula, as follows:

$$VK = \frac{\sum KB}{\sum KB + \sum KTB} \times 100\%$$

Description:
VK = Conidium viability
KB = Germinated conidium
KTB = Non-germinated conidium

The design used in this study was a completely randomized design (CRD) with 3 replicates. The data obtained were analyzed using the F test in ANOVA followed by the Honestly Significant Difference (HSD) Test at the 5% error level.

RESULTS AND DISCUSSION

Characteristics of antagonist fungi *Gliocladium* sp. and pathogen *Fusarium* sp. were shown in **Table 1**.

Table 1. Characteristics of antagonist fungi on PDA media after 7 days incubation period

No	Types of Fungi	Macroscopis Characteristics	Microscopic Characteristics
1	Antagonistic *Gliocladium* sp.	Colony color: 3rd day to 7th day half white half greenish white color. Front colony section: greenish white colony edge with dense texture. Back colony section: white with coscentric lines.	Hyphae: quite densely arranged, transparent hyaline. Conidiophores: slender and branched. Conidia: transparent ovoid.
2	Patthogenic *Fusarium* sp.	Colony color: white. Front colony: white with circular and spreading colonies. Back colony: white with yellowish center with smooth and transparent colony surface.	Hyphae: Septate branched Conidia: ovoid microconidia and sickle-like macroconidia. Chlamydospores in the form of Central spores.

Based on the results of the analysis conducted, it shows that the average colony diameter of the antagonist fungus *Gliocladium* sp. grows faster when compared to the average colony diameter of the pathogenic fungus *Fusarium* sp. both from the test results and from the control treatment (**Figure 1**).

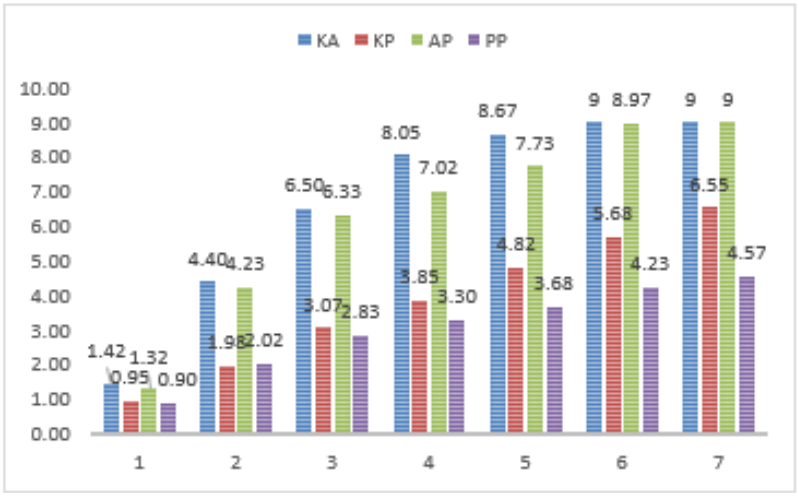

Figure 1. Colony Diameter Growth Rate Diagram of Antagonist Fungi *Gliocladium* sp. and Pathogenic *Fusarium* sp.
(blue color: KA, red color: KP, green color: AP, purple color: PP).

During the 7 days of observation, the Antagonist Control (KA) and Antagonist Treatment (AP) treatments showed the fastest colony growth, reaching a maximum diameter of 9 cm on day 7. Pathogen Control (KP) and Pathogen Treatment (PP) grew more slowly, with KP reaching 6.55 cm and PP only 4.57 cm on day 7. These results prove that *Gliocladium* sp. has the ability to grow and master the media faster than *Fusarium* sp.

Table 2. Growth diameter of antagonistic fungus *Gliocladium* sp. and pathogenic fungus *Fusarium* sp.

Treatment	Average Growth Diameter
KA	6.72 ± 2.87 a
AP	6.37 ± 2.77 a
KP	3.84 ± 2.00 b
PP	3.07 ± 1.28 b

Description: KA= Antagonist Control, KP= Pathogen Control, AP= Pathogen Antagonist, PP= Pathogen Treatment. Data followed by different lowercase signs in the same column indicate significant differences ($P < 0.05$).

Based on **Table 2**, the results of measuring the colony growth diameter from 3 replicates show that the KA treatment has the largest average, which is 6.72 with a standard deviation of ±2.87. The AP treatment has an average of 6.37 with a standard deviation of ±2.77. These two treatments show a relatively similar growth pattern with a difference that is not too large. KP and PP treatments have values of

3.84 ± 2.00 and 3.07 ± 1.28. These two treatments showed lower results than the KA and AP treatments.

The KA and AP treatments are significantly different from the KP and PP treatments because they have a larger average colony diameter and the difference exceeds the minimum HSD value limit. The KA treatment is not significantly different from AP because these two treatments have the notation 'a' which means they are not statistically significantly different. KP and PP treatments are not significantly different because the difference is not large enough to exceed the minimum HSD value or not statistically significant.

The amount of inhibition of the tested antagonistic fungi against pathogenic fungi tested by double culture with an incubation period of 7 days, characterized by the magnitude of the inhibition area. In this study, the inhibition zone was observed to obtain a clear picture of the interaction of the two fungi in vitro. During the observation period, the inhibition of the fungus *Gliocladium* sp. against the pathogen *Fusarium* sp. was observed against the zone of inhibition that occurred periodically to see the level of inhibition that occurred. The results of the antagonistic test in (**Figure 2**) show the inhibitory activity by the fungus *Gliocladium* sp. against the growth of the pathogenic fungus *Fusarium* sp.

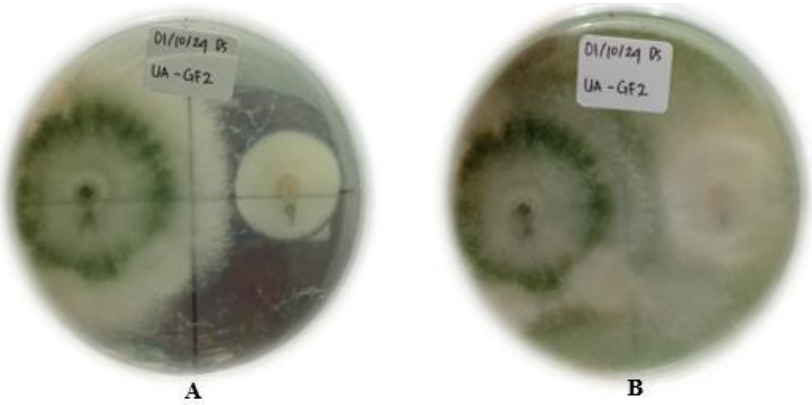

Figure 2. Antagonist test. (A) 3rd day after inoculation (B) 7th day after inoculation

Table 3. Percentage of Inhibition of *Gliocladium* sp. against Fusarium sp.

Treatment	Percentage of Obstacles	Category
Inhibition	25.63%	Low

From Table 3, it can be seen that the percentage of inhibition quantitatively falls into the low inhibition category. Although *Gliocladium* sp. showed an antagonistic effect against the pathogen *Fusarium* sp., the inhibition ability shown has not reached the optimal level expected for application as an effective biological agent. The low percentage of inhibition is thought to be influenced by various environmental factors that affect the activity and growth of these antagonistic fungi.

The antagonistic mechanism of the fungus *Gliocladium* sp. against other organisms is the mechanism of competition, antibiosis, lysis and parasitism (Rizal,

2017). Based on observations, the antagonistic fungus *Gliocladium* sp. grew and developed well, while the pathogen experienced a decrease in cell tugidity due to cytoplasmic leakage. In the antibiosis test, the colony meeting area of the two fungi did not form a clear zone which is usually an indicator of the presence of antibiotic compounds that inhibit the growth of pathogens directly (**Figure 2**). The nature of the growth of the fungus *Gliocladium* sp. which tends to be aggressive and dominantly spreads in the test media. This antagonistic fungus only grows side by side, but also physically wraps or coats the pathogen colonies. This statement is in accordance with research by Herlina (2013), that the fungus *Gliocladium* sp. parasitizes the host pathogen *Fusarium* sp. by covering or wrapping the pathogen. Another mechanism is mycoparasitism which occurs through the entanglement of *Gliocladium* sp. hyphae on *Fusarium* sp., followed by penetration of host hyphae to absorb nutrients.

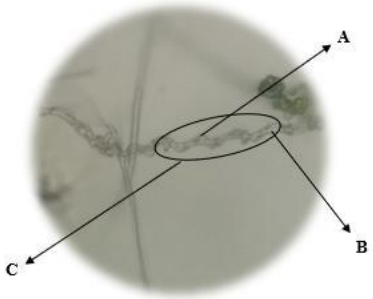

Figure 3. Mycoparasitic Antagonistic Fungus *Gliocladium* sp. against Pathogenic *Fusarium* sp.

The mechanism of mycoparasitic antagonism can be seen in (**Figure 3**). The results of mycoparasitic observations on day 6 can be seen that the hyphae of the pathogen began to swell due to the activity of the compounds produced by the antagonist. In **Figure 3**, the antagonistic fungus *Gliocladium* sp. will attach to the pathogenic fungus *Fusarium* sp. then will penetrate into the cell wall of the pathogenic fungus so that the hyphae of *Fusarium* sp. are damaged. This process is carried out by hydrolytic enzymes produced by *Gliocladium* sp. to facilitate penetration. After penetrating the cell wall of *Fusarium* sp., *Gliocladium* sp. develops hyphae inside the cells of *Fusarium* sp. which results in damage to the pathogen cells which ultimately inhibits growth and causes the death of the pathogen.

This is in line with Soesanto's research (2008), which states that the hyphal wall infected by mycoparasitics is seen to penetrate due to a combination of hydrolytic enzymes and mechanical pressure. The infecting hyphae will penetrate the host cell wall, causing disruption to the cytoplasm and leading to necrosis.

Viability was conducted to determine whether *Gliocladium* sp. could be used as a sample in antagonistic testing with the pathogen *Fusarium* sp. The test results showed an average spore viability of 66.52%, which means that more than half of the spores were able to germinate on the media provided. This level of viability above 60% is considered adequate because it shows good growth ability, allowing *Gliocladium* sp. to compete effectively with the pathogen. High viability is a key

factor in the success of *Gliocladium* sp. as a biological control agent in inhibiting the growth of *Fusarium* sp.

CONCLUSION

The macroscopic and microscopic morphological characteristics of *Gliocladium* sp. Fungi are white to greenish surface and back colonies with fast growth, hyphae are concentrated, conidia are ovoid with concentrated and branched conidiophores. While *Fusarium* sp. has white colonies with slower growth, branched septate hyphae, conidia in the form of ovoid microconidia and crescent-like macroconidia with calyxdospores in the form of central spores.

The results of measuring the inhibitory power of the antagonistic fungus *Gliocladium* sp. against the pathogenic fungus *Fusarium* sp. showed that the antagonistic fungus *Gliocladium* sp. was able to inhibit the growth of *Fusarium* sp. characterized by a larger colony diameter in the KA treatment of 9 cm and a clear zone diameter of AP with an inhibitory power of 25.63%, this is classified as a low category but the *Gliocladium* sp. fungus still shows potential as an antagonistic agent that can suppress the growth of pathogens that cause wilt disease in shallot plants, and viability of 66.52%. *Gliocladium* sp. fungus was able to inhibit the growth of *Fusarium* sp. by the mechanism of competition, antibiosis, lysis, and parasitism.

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