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# Antagonist Activity of *Gliocladium* sp. Against *Fusarium* Wilt Fungi in Chili Plant (*Capsicum frutescens* L.)

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## ABSTRACT

*Fusarium* sp. is a pathogen that causes wilt disease in chili plant (*Capsicum frutescens* L.), which has a significant impact on crop production. One of the environmentally friendly control strategy is the use of biological agents, such as *Gliocladium* sp. This study aimed to evaluate the antagonistic ability of *Gliocladium* sp. to inhibit the growth of *Fusarium* sp. in vitro. The experiment was conducted using the dual culture method on Potato Dextrose Agar (PDA) medium. *Fusarium* sp. isolates were obtained from the culture collection of BPPMTPH Kalasey. Observations were carried out of seven days to assess colony growth and calculate the percentage of inhibition. *Gliocladium* sp. demonstrated the ability to inhibit *Fusarium* sp. growth, with a maximum inhibition percentage of 51.52% on the seventh day. *Gliocladium* sp. exhibited rapid growth and dominated the medium, covering the pathogen colony entirely, although no clear inhibition zone was formed. The antagonistic mechanism is presumed to involve competition for space and nutrients, and parasitism. The mechanism of competition was demonstrated by the ability of *Gliocladium* sp. to grow more rapidly and dominate the PDA medium compared to *Fusarium* sp. in the dual culture treatment. Parasitism was identified through the coiling of *Fusarium* sp. hyphae by *Gliocladium* sp. hyphae, accompanied by morphological abnormalities observed in *Fusarium* sp. These findings indicate that *Gliocladium* sp. has potential to be developed as a biological control agent against *Fusarium* wilt desease in chili plants.

***Key words:*** *Antagonism; Dual Culture; Fusarium sp.; Gliocladium sp.; In vitro*

## INTRODUCTION

Chili (*Capsicum frutescens* L.) is a strategic horticultural commodity in Indonesia, valued for its high economic potential and steadily increasing market demand. In addition to its central role as a culinary staple, chili also holds significant export potential (Ahdiat, 2024). Despite its importance, chili production in Indonesia faces major constraints, among which plant diseases are a dominant limiting factor. Wilt disease caused by *Fusarium* sp. is among the most destructive, capable of reducing yields by 50% (Abdila and Madurat, 2021). In North Sulawesi—a key chili-producing region with 2,847 hectares of cultivated area—disease outbreaks have led to production instability and price inflation, affecting both farmers and market supply chains (Badan Pusat Statistik Sulawesi Utara, 2022).

*Fusarium* wilt, caused by *Fusarium oxy*sp*orum* f.sp. *capsici*, is a soilborne disease that infects the plant's vascular system through the roots and spreads via the xylem. This leads to progressive wilting symptoms, beginning with chlorosis in young leaves and often ending in total plant collapse (Ngittu et al., 2014). The pathogen is particularly challenging to control due to its adaptability to various environmental conditions and its ability to persist in soil through chlamydospores, even in the absence of host plants. It can infect chili plants from seedling stage to maturity (Nensi, 2021; Imah et al., 2022), and the tropical climate of Indonesia—characterized by high humidity—further supports its endemic presence.

Conventional control methods relying on synthetic fungicides have shown diminishing effectiveness and raise multiple concerns (Fikri, 2022). Overuse of active ingredients such as carbendazim and thiophanate-methyl has led to the

emergence of resistant *Fusarium* strains, reducing the long-term efficacy of chemical treatments (Liu et al., 2019; Li et al., 2022). Moreover, continuous fungicide application can cause chemical residue buildup in agricultural soils and produce, posing potential risks to human health and non-target organisms, as well as damaging the surrounding ecosystem (Singkoh and Katili, 2019; Muslim and Suwandi, 2023; Romdhani et al., 2024). These challenges highlight the urgent need for alternative disease management approaches that are both environmentally sustainable and economically viable for smallholder farmers.

Biological control using antagonistic microorganisms has emerged as a promising alternative. Such organisms compete with pathogens for space and nutrients and can produce bioactive compounds that suppress pathogen growth (Rusli et al., 2021). One such potential biocontrol agent is *Gliocladium* sp., a saprophytic and endophytic fungus known for its ability to produce secondary metabolites with antifungal properties (Herlina, 2013). While various studies have highlighted the effectiveness of *Gliocladium* sp. against *Fusarium* spp. in horticultural crops, specific research on its use against *Fusarium* wilt in chili plants remains limited, especially under the unique agroecological conditions of Indonesia.

In North Sulawesi, where chili farming is a major agricultural activity, the application of *Gliocladium* sp. as a biological control agent has not yet been explored. To date, there has been no recorded application of the antagonistic fungus *Gliocladium* sp. as a biological control agent in plant disease management programs in North Sulawesi. This highlights a critical knowledge gap and an opportunity for localized innovation. Therefore, this study aims to evaluate the antagonistic potential of *Gliocladium* sp. against *Fusarium* sp. under *in vitro* conditions.

## METHODS

This study employed exploratory and experimental methods. The research was conducted from August to December 2024 at the Laboratory of the Center for Plant Protection and Quality Testing of Food Crops and Horticulture (BPPMTPH), Kalasey, North Sulawesi. Samples of wilted chili plants (*Capsicum frutescens* L.) were collected from the BPPMTPH experimental field. The *Gliocladium* sp. isolate used in this study was part of the culture collection at BPPMTPH, originally obtained from the Center for Forecasting of Plant Pest Organisms, Jatisari, Karawang, West Java.

The preparation stage for *Fusarium* sp. isolates began with the exploration of chili plants (*Capsicum frutescens* L.) exhibiting wilt symptoms. Diseased plant tissues were isolated on Rose Bengal Chloramphenicol (RBC) medium and incubated at room temperature for 3–5 days. Fungal colonies showing morphological characteristics typical of *Fusarium* sp. were then subcultured onto Potato Dextrose Agar (PDA) medium to obtain pure isolates. Macroscopic observations of *Fusarium* sp. and *Gliocladium* sp. included colony color, pigmentation, and colony shape. Microscopic characterization was conducted by observing conidiophores, phialides, and conidia morphology using the slide culture method (Tjampakasari et al., 2024).

Viability testing of *Gliocladium* sp. was carried out to determine conidial germination capacity on PDA medium (Khaqim, 2007). The method followed the

Indonesian National Standard (SNI) for Biological Control Agents of *Trichoderma* spp. (Badan Standarisasi Nasional, 2014). PDA medium was cut using a cork borer (0.5 cm diameter) into three replicate discs and placed in a row on an object glass. The conidia of *Gliocladium* sp. were suspended in sterile distilled water (aquadest). One drop of the conidial suspension (1 mL pipette) was placed on each agar disc and covered with a cover glass. The object glass was then placed in a petri dish containing moistened cotton or tissue paper to maintain humidity and incubated at room temperature for 24 hours. After incubation, each disc was observed under a microscope, and the number of germinated and non-germinated conidia was counted. The conidial germination percentage was calculated using the following formula:

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

According to the Indonesian National Standard (SNI), the minimum viability quality requirement for antagonistic fungal conidia is ≥ 60%.

Koch's postulates test was conducted to confirm whether the isolated *Fusarium* sp. was the causal agent of wilt disease in chili plants (Asharo et al., 2022). Four chili seedlings were used, and a fungal suspension of *Fusarium* sp. was prepared by dissolving the isolate in 50 mL of sterile distilled water and placing it in a beaker. The roots of the chili plants were wounded using a sterile scalpel and immersed in the fungal suspension (Sudarma et al., 2014). The plants were observed until they exhibited symptoms similar to those of *Fusarium* wilt. If the same symptoms appeared, the isolate could be confirmed as the causal pathogen of *Fusarium* wilt disease (Alifia et al., 2023).

The antagonistic assay was conducted in vitro using the dual culture method on PDA medium. Inocula of the pathogenic and antagonistic fungi were cut into circular plugs using a cork borer with a diameter of 0.5 cm. Both inocula were placed on a 9 cm petri dish containing PDA medium, positioned 2 cm from the edge of the petri dish and facing each other. This study employed dual culture treatment, antagonist control, pathogen control, and a negative control consisting of uninoculated PDA medium to ensure that colony growth in other treatments originated solely from the introduced inoculum. The inoculum placement followed the guidelines of the Indonesian National Standard (Badan Standarisasi Nasional, 2014), as illustrated in **Figure 1**.

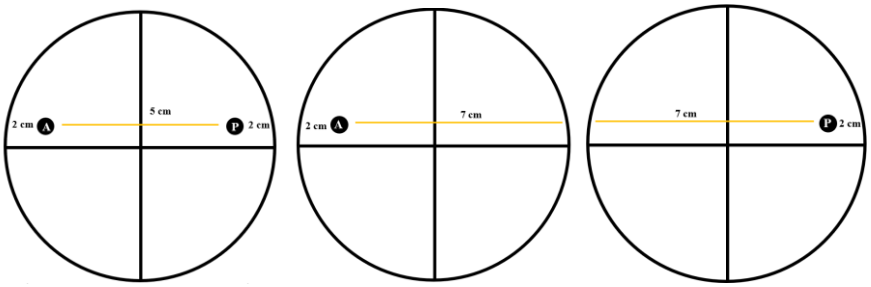


**Figure 1.** Inoculum placement of antagonistic assay
Description: A= Antagonist fungi *Gliocladium* sp., P= Pathogen fungi *Fusarium* sp.

The observed variables included (1) the morphological characteristic of each fungal isolate under different treatments, (2) colony growth, (3) the antagonistic

mechanisms involved, including competition, antibiosis, and parasitism, and (4) the percentage of inhibition, calculated based on the formula from the SNI (Badan Standarisasi Nasional, 2014; Alifia et al., 2023).

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

Description:
R1= Radius of the pathogen colony in the control treatment (cm),
R2= Radius of the pathogen colony in the dual culture treatment (cm)

## RESULTS

*Exploration and isolation of Fusarium sp.*

*Fusarium* sp. was found on chili (*Capsicum frutescens* L.) plants showing typical wilt symptoms in the experimental field of the Plant Protection and Quality Testing Center for Food and Horticultural Crops (BPPMTPH). Observed symptoms included yellowish-green leaves, wilting plants, and white patches resembling fungal mycelium at the base of the stem (**Figure 2**). Samples were taken from the basal root area for fungal isolation.

**Figure 2.** Chili plants exhibiting symptoms of *Fusarium* wilt

*Viability test of Gliocladium sp.*

The viability test of *Gliocladium* sp. conidia on PDA medium showed a germination rate of 66.51%. This indicates that most of the observed conidia were still capable of germinating and forming hyphae. According to the Indonesian National Standard (SNI) for biological agents, a viability rate of at least 60% is considered good (Badan Standarisasi Nasional, 2014), suggesting that the *Gliocladium* sp. isolate used was suitable for antagonism testing.

*Koch's postulate test*

The Koch's postulate test showed that healthy chili plants developed symptoms similar to naturally infected plants after their roots were immersed in a *Fusarium* sp. suspension. As seen in **Figure 3**, the control plants grew optimally, marked by an increase in leaf number, while the treated plants showed stunted growth from the first day of observation. Early symptoms appeared in the first week, with young leaves turning yellow and gradually spreading to older leaves in the second week.

By the third week, wilting and white patches at the root base were observed—symptoms that closely resembled those found in infected field samples.

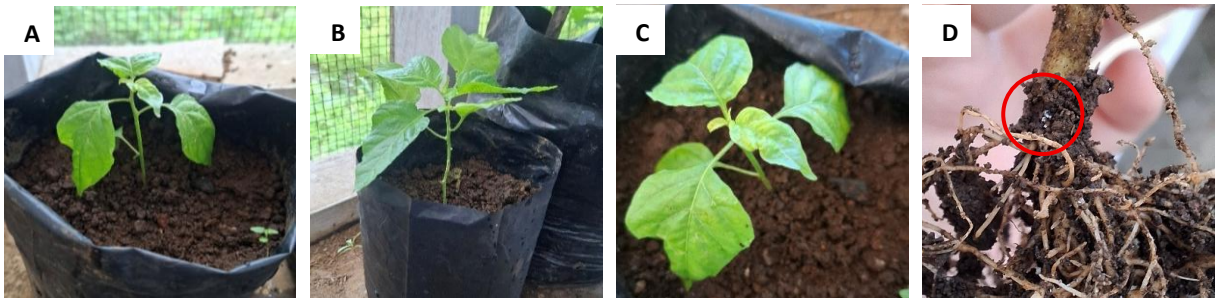


**Figure 3.** Result of Koch's postulate test on chili plants. (A) plant treated with *Fusarium* sp.; (B) healthy control plant; (C) chlorosis on young leaves as initial symptom; (D) white patches at the basal root area

*Morphological characterization of Gliocladium sp. and Fusarium sp.*

Macroscopic observations were conducted to examine the color, pigmentation, texture, and growth pattern of the fungal colonies. Microscopic observations focused on the morphology of conidia, conidiophores, hyphae, and phialides, using a light microscope at 40x magnification. The results of the macroscopic and microscopic characterization of both fungal isolates are presented in **Table 1**.

**Table 1.** Morphological characteristic of *Fusarium* sp. and *Gliocladium* sp.

| **Morphological Characteristic** | **Isolates** | |
|---|---|---|
| | ***Fusarium* sp.** | ***Gliocladium* sp.** |
| Macroscopic characteristics | | |
| Colony color[a] | White | Intilially white, turning light green to dark green |
| Pigmentation[b] | Orange | Pale yellowish brown |
| Texture | Cotton-like | Smooth and velvety |
| Colony growth pattern | Radial, aerial mycelium | Radial, aerial mycelium |
| Microscopic characteristics | | |
| Microconidia shape | Round to oval | Round |
| Macroconidia shape | Crescent-shaped, curved | - |
| Conidia color | Hyaline | Green |
| Conidia septation | Multiseptate, commonly 3 | Aseptate |
| Hyphae | Hyaline, branched | Hyaline, branched, smooth |

Notes: [a]Colony color was determined by observing the top surface of the colony. [b]Pigmentation was determined by observing the underside of the colony.

The *Fusarium* sp. isolate obtained from symptomatic chili plants showed distinctive macroscopic features: the colony surface was white with a pale yellowish-brown reverse, aerial mycelium with a cottony texture, and a radial growth pattern on PDA medium extending from the inoculation point across the

plate surface. Microscopic examination revealed that *Fusarium* sp. produced two types of conidia: microconidia and macroconidia. The conidiophores were upright and unbranched. Microconidia were initially spherical and matured into oval shapes, while macroconidia were falcate (crescent-shaped), hyaline, typically with three septa (**Figure 4**). No chlamydospores were observed in this isolate.

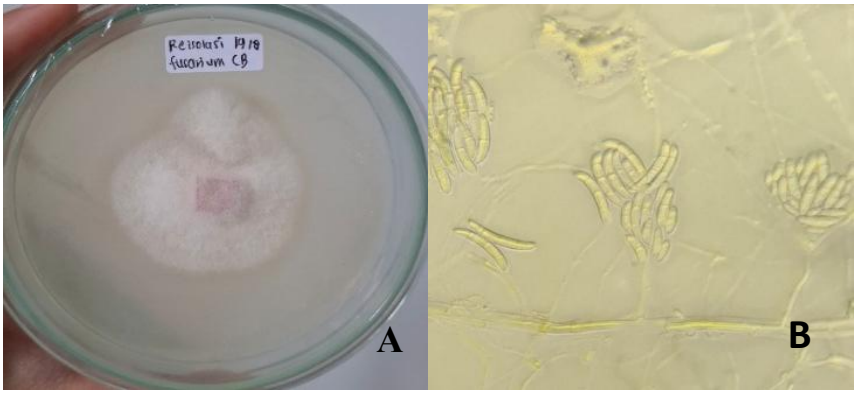


**Figure 4.** Colony of *Fusarium* sp. on PDA 4 days after isolation (A); macroconidia, hyphae, and conidiophores (B)

The *Gliocladium* sp. isolate exhibited initial white colonies that gradually turned light to dark green with a fine, velvety surface texture, aerial mycelium, and circular colony form. The *Gliocladium* sp. isolate displayed hyaline hyphae, upright conidiophores with branched phialides, and bright green, round conidia that clustered into dense brush-like (penicillate) structures at the tip of the conidiophores (**Figure 5**).

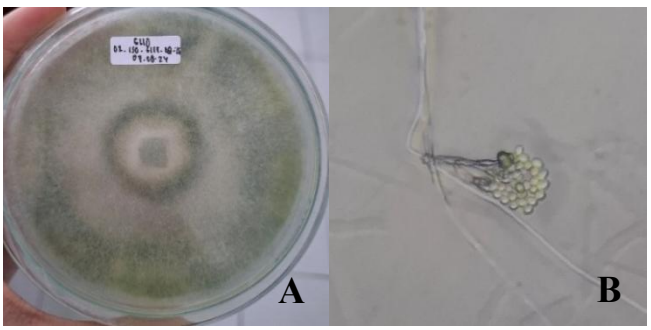


**Figure 5.** Colony of *Gliocladium* sp. 7 days after isolation (A); conidia, conidiophores, hyphae (B)

*Antagonism test*

The antagonistic assay was conducted to assess the ability of *Gliocladium* sp. to inhibit the growth of *Fusarium* sp. The colony growth of both fungi was measured daily, and the percentage of inhibition was calculated by comparing the radial growth of *Fusarium* sp. in the control and dual culture treatments. The results show that *Gliocladium* sp. significantly reduced the radial growth of *Fusarium* sp (**Figure 6**). In the control, *Gliocladium* sp. exhibited rapid growth, reaching an average colony radius of 7 cm by day seven. In contrast, *Fusarium* sp. only reached about 3.96 cm. In dual culture, *Fusarium* sp. growth was substantially suppressed, with average colony radii between 1.84–1.92 cm from days four to seven.

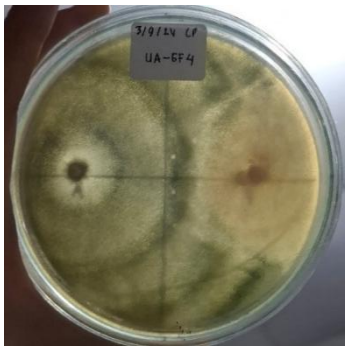

**Figure 6.** Results of antagonistic test using dual culture method

The percentage of inhibition by *Gliocladium* sp. increased daily, reaching 51.52% on day seven (**Figure 7**), which is categorized as moderate inhibition according to PIRG classification (Picardal et al., 2019).

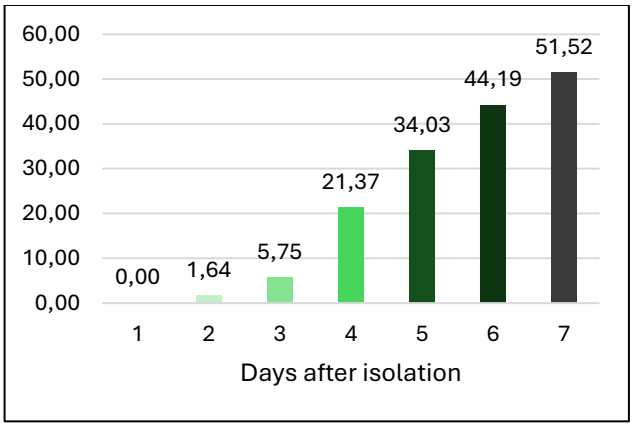


**Figure 7.** Percentage of inhibition of *Gliocladium* sp. against *Fusarium* sp.

The antagonistic interaction between *Gliocladium* sp. and *Fusarium* sp. was observed through dual culture assays. The results indicated that *Gliocladium* sp. inhibited *Fusarium* sp. growth via competition and parasitism mechanisms (**Table 2**).

**Table 2.** Antagonistic mechanisms of *Gliocladium* sp. against *Fusarium* sp.

| Antagonist Mechanism | Description |
|---|---|
| Competition | + |
| Parasitism | + |
| Antibiosis | - |

Note: (+) antagonistic mechanism occurs; (-) antagonistic mechanism does not occur

In the dual culture plates, *Gliocladium* sp. grew rapidly, dominating the surface of the PDA medium and suppressing the radial growth of *Fusarium* sp. Microscopic examination at the contact zone between hyphae revealed morphological changes in Fusarium sp. hyphae. These included coiling by *Gliocladium* sp. hyphae,

swelling of Fusarium hyphae, and the presence of *Gliocladium* sp. conidia clusters on the Fusarium sp. growth area (**Figure 8**). No inhibition zones or discoloration were observed at the interface between the colonies, indicating that antibiosis was not clearly detected in this study.

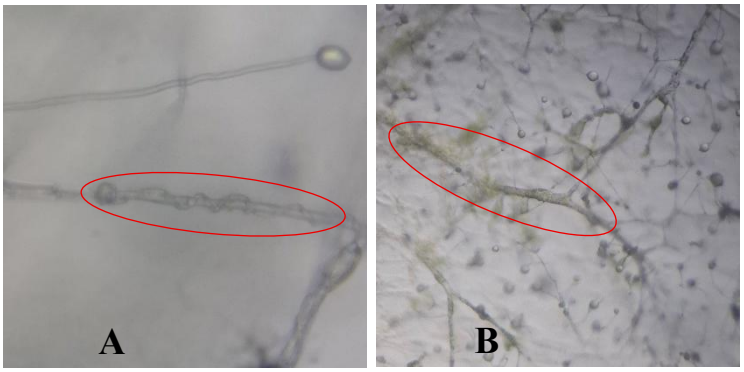


**Figure 8.** Hyphal coiling by *Gliocladium* sp. (A); swelling of *Fusarium* sp. hyphae

## DISCUSSION

The wilt symptoms observed in chili plants, such as yellowing of young leaves and white fungal patches on the basal root, are consistent with typical *Fusarium* wilt infection. According to Syaifudin (2020), *Fusarium* infections in chili plants are characterized by leaf yellowing, wilting, and often the presence of mycelial growth at the root collar, which aligns with the field observations in this study. *Fusarium* sp. is known as a soil-borne pathogen capable of surviving in soil without a host and infecting plants through wounds on the root surface (Ulya et al., 2020; Bahadur, 2020). The successful isolation of *Fusarium* sp. from symptomatic plant roots confirms its association with the wilt symptoms and supports the hypothesis that this pathogen is responsible for the observed disease incidence. These findings provide the foundation for further pathogenicity testing and antagonistic evaluation.

The viability of *Gliocladium* sp. conidia at 66.51% was close to the minimum standard for high-quality biological agents but still considered acceptable for use. This result is lower than findings by Andari et al. (2020), who reported 100% germination in *Trichoderma* sp. stored for 3–15 days. Viability tends to decline with storage time, and factors such as storage temperature, culture media enrichment, subculture generations, and preservation methods influence conidial quality (Pramesti et al., 2014; Andari et al., 2020). Arias-Chavarria et al. (2025) demonstrated that encapsulated *Trichoderma longibrachiatum* conidia retained 100% viability for two months while maintaining high antagonistic activity. Similarly, Løvschall et al. (2024) reported that encapsulation enhances spore stability during storage. These findings highlight the importance of maintaining high viability, as it directly affects hyphal growth and the antagonistic capability of fungi in suppressing pathogens (Rachmawati et al., 2016).

The emergence of identical wilt symptoms in previously healthy plants following *Fusarium* sp. inoculation confirms the pathogenicity of the isolate, fulfilling Koch's postulates. The development of yellowing in leaves and overall growth inhibition indicates disruption in vascular function, as *Fusarium* colonizes xylem tissues and impedes water and nutrient transport (Bahadur, 2022). These

symptoms are consistent with those reported by Syaifudin (2020), who described *F. oxy*sp*orum* infection in chili plants as causing yellowing, wilting, and suppressed vegetative development. This confirms that the *Fusarium* sp. isolate is the causal agent of wilt disease in chili plants used in this study.

The observed colony morphology of *Fusarium* sp. aligns with previous studies reporting white to pink, orange, yellow, or purple colonies with aerial mycelium resembling cotton (Riyanti & Liani, 2023; Syaifudin, 2020). The radial spreading pattern and cottony texture are also characteristic traits of *Fusarium* species grown on PDA. The *Gliocladium* sp. isolate displayed colony characteristics consistent with earlier findings, where mature colonies appeared greenish with fine hairs and spherical shape (Sopialena et al., 2024; Rizal, 2017; Mattola et al., 2025). The color change from light to dark green indicates active sporulation, a key taxonomic trait for identifying *Gliocladium* (Ahmadi, 2023). Microscopically, *Fusarium* sp. showed typical features with distinct micro- and macroconidia and erect conidiophores (Sutejo, 2008; Syaifudin, 2020). The absence of chlamydospores is likely due to the nutrient-rich PDA medium used, which may not induce their formation. Studies have shown that chlamydospores are more commonly produced on media such as CLA, SNA, or Soil Agar (Leslie & Summerell, 2006; Crous et al., 2021; Bahadur, 2022). The *Gliocladium* sp. isolate showed penicillate conidiophore structures, hyaline phialides, and bright green round conidia forming clustered masses, in agreement with descriptions by Mattola et al. (2025), Alifia et al. (2023), and Sopialena et al. (2024). These features confirm the identity of the isolate and its taxonomic placement in the genus *Gliocladium*.

The findings confirm that *Gliocladium* sp. is capable of inhibiting the growth of *Fusarium* sp. through rapid colony expansion and spatial competition. The antagonist's faster growth rate allows it to colonize space more efficiently, as also described by Hartal et al. (2010). The inhibition began on day four and gradually increased, indicating effective antagonistic interaction. This is consistent with Herlina (2013) and Fardhani et al. (2024), who stated that *Gliocladium* sp. suppresses pathogens by overgrowing them and producing antifungal enzymes. Although contamination was observed in all treatments (including controls), it did not interfere with the main growth zone and did not affect data validity. Negative controls confirmed that the culture media was sterile. The 51.52% inhibition level, while moderate, may have been influenced by the viability of *Gliocladium* sp. conidia, which was slightly below optimal. According to Rachmawati et al. (2016), low conidial viability may reduce antagonistic performance due to delayed germination and hyphal development. Maintaining high viability is crucial for effective biocontrol. Future efforts should include improving conidia viability and exploring new isolates of *Gliocladium* sp. with stronger antagonistic properties.

The rapid surface colonization by *Gliocladium* sp. suggests that competition for space and nutrients played a role in suppressing *Fusarium* sp. growth. This is common in dual culture settings where multiple fungi compete for limited resources such as carbohydrates and vitamins provided by PDA medium (Asdinar et al., 2024). Microscopic changes observed in *Fusarium* sp. hyphae—including hyphal coiling, swelling, and green discoloration from *Gliocladium* sp. conidia—support the occurrence of parasitism. These observations are in line with Muslim & Suwandi (2023), who described fungal parasitism as involving physical contact,

penetration, and internal proliferation. Previous studies have also shown that *Gliocladium* sp. produces hydrolytic enzymes such as cellulases that degrade pathogen cell walls (Herlina, 2013; Rusli et al., 2021; Fardhani et al., 2024). Widyawati (2008), as cited in Ainy et al. (2015), stated that these enzymes can cause mycolysis, or abnormal hyphal growth due to internal cell wall degradation.

No evidence of antibiosis was detected, as no clear inhibition zones or media color changes were found. While *Gliocladium* sp. is known to produce antibiotics such as gliotoxin, gliovirin, and viridin (Risthayeni et al., 2018; Sopialena et al., 2024), this study suggests that antibiosis was not a dominant mechanism under the conditions tested. Consistent with Howell (2000) in Juariyah et al. (2018), a biocontrol agent may not employ all antagonistic mechanisms simultaneously. Previous research supports that *Gliocladium* sp. can act through different mechanisms depending on the pathogen and conditions—ranging from competition (Dailah et al., 2020), parasitism (Sopialena et al., 2024), to antibiosis (Sofian et al., 2025), or a combination of all three (Kalimutu et al., 2020).

## CONCLUSION

The results of this study indicate that *Gliocladium sp.* has potential as a biocontrol agent against *Fusarium sp.*, the causal agent of wilt disease in cayenne pepper. In vitro antagonism tests using the dual culture method showed an inhibition percentage of 51.52%, which falls within the moderate category. This inhibitory effect is supported by the faster growth rate of *Gliocladium sp.* compared to *Fusarium sp.*, as well as observed antagonistic mechanisms including space and nutrient competition and parasitism. Parasitism was confirmed through microscopic observations showing hyphal coiling and structural damage to the pathogen's hyphae. These findings reinforce the potential of *Gliocladium sp.* as an effective biological control agent against *Fusarium*-induced wilt in cayenne pepper.

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