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Original Research Paper

# Inhibition of *Gliocladium sp* against plant pathogenic fungus and their exoenzyme activity

**Dinar Mindrati Fardhani[1*], Dzakiya Alda Fuadiyah[1], Wisnu Adhi Susila[2], Ika Afifah Nugraheni[1]**
[1] Department of Biotechnology, Universitas Aisyiyah Yogyakarta, Indonesia
[2] PT Biotek Cipta Kreasi, Yogyakarta, Indonesia
dinar@unisayogya.ac.id

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## Abstract
*Gliocladium sp*. It is known to have an antagonistic mechanism against other organisms by means of hyper parasitism, antibiosis and lysis, as well as competition. This study aims to determine the effectiveness of *Gliocladium sp*. in inhibiting several types of plant pathogenic fungi and their exoenzyme activity. Inhibition testing was carried out by the Dual Culture Assay method on several plant pathogenic fungi including *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Fusarium sp*., and *Phytophthora sp.* The exoenzyme activity tested includes cellulolytic tests, amylolytic tests, and chitinolytic tests. Antagonist testing showed that *Gliocladium sp*. able to inhibit the growth of all pathogenic fungi tested with varying percentage of inhibition. High percentage of inhibition was shown in the pathogens *Colletotrichum gloeosporioides* and *Rhizoctonia solani* with percentages of 78.75% and 75%, respectively. Then, the antagonist activity against Fusarium sp. has a moderate resistance percentage of 56%. Meanwhile, a low percentage of inhibition is shown in *Gliocladium sp.* against *Phytophthora sp*. with a figure of 25%. From the characterization of exoenzyme activity in *Gliocladium sp*., negative results were obtained in liquid and solid *Carboxymethyl Cellulose*, amylum, and chitin media. Meanwhile, in the amylolytic test, positive growth was marked by the appearance of mycelia on the surface of the amylum liquid medium, but in the solid media negative results were obtained against the amylase enzyme. *Gliocladium sp* fungus has the potential to be a biocontrol agent against various plant pathogenic fungi

**Keywords:** antagonist test; exoenzyme; *Gliocladium sp*.; plant pathogenic fungus

## 1. Introduction

*Gliocladium sp*. is a soil fungus that is commonly found in various types of soil and the rhizosphere of plants. These fungi can live as sporophytes or as parasites on other fungi. In addition, these fungi can compete for food sources, produce inhibitory substances, and are hyperparasitic (Rahma & Karimah, 2021). *Gliocladium sp*. is essential for the soil as it helps control pathogenic fungi. These fungi colonize plant roots to increase nutrient availability, accelerate plant growth, and protect plants through the production of phytohormone compounds, antimicrobials, toxins, and enzymes (Herlina, 2013). One of the superior products of PT Biotek Cipta Kreasi (PT BCK) is "GRO-MAXX Golden Tricho", one of which is *Gliocladium sp*. As the basic ingredient of GRO-MAXX Golden Tricho bio fungicide, *Gliocladium sp*. provides important benefits in improving soil fertility and health. The content of microbes in the soil plays a role in maintaining soil fertility. By using GRO-MAXX Golden Tricho, the soil can have a population of *Gliocladium sp.* which helps in controlling pathogenic fungi as well as providing benefits for plant growth and protection.

In its control, *Gliocladium sp*. has the ability to produce different types of antibiotics and diverse inhibition mechanisms. These fungi form fine hyphae that grow rapidly, and there are fine hairs that

can come into contact with pathogenic fungi. With this structure, *Gliocladium sp*. can interact with its host through enzymatic activity without physical penetration. This is seen when the hyphae *Gliocladium sp*. is circular on its host (Castillo et al., 2016).

*Gliocladium sp*. has antagonistic mechanisms against other organisms which include hyperparasitism, antibiotics, lysis, and competition. The mechanism of hyperparasitism suggests that the antagonist agent directly promotes and takes food sources from the pathogen tested (Rahma & Karimah, 2021). Antibiotics are the process of inhibiting other organisms through secondary metabolites produced by the organism. Antibiotic activity generally inhibits growth and can lead to the death of other organisms, while lysis usually causes damage, decomposition, or decomposition of biological substances (Dailah et al., 2020). Meanwhile, the competition mechanism involves competition between antagonistic fungi and pathogens tested to obtain nutrients and limited space (Rahma & Karimah, 2021).

*Gliocladium sp* can inhibit various plant pathogens with varying degrees of inhibition such as *Fusarium oxysporum* capsici, *Fusarium udum*, *Botryodiplodia theobromae* and *Colletotrichum* sp (Mulyani, et al., 2024; Sopialena et al., 2024; Kalimutu et al., 2020; Agustina et al., 2020; Singh & Singh, 2019). *Gliocladium sp* showed inhibition activity against *Fusarium* sp of 35.20% on the sixth day, while inhibition activity against *Phytophthora* sp was 54.89% on the fifth day (Hidayat et al., 2020; Malik et al., 2022). The application *of Gliocladium sp* in vivo on wheat plants also showed an inhibition against *Fusarium* sp by 76-92% (Er, 2020). Nonetheless, *Gliocladium sp* has varying inhibitory activity against various pathogens.

This study aims to determine the effectiveness of *Gliocladium sp*. in inhibiting several types of plant pathogenic fungi including *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Fusarium sp.*, and *Phytophthora sp*. In addition, *Gliocladium sp* is tested for its exoenzyme activity through cellulolytic assay, amylolytic assay, and chitinolytic assay.

## 2. Research Methods

### 2.1.Time and Place

This research was conducted for one month, from February 1 to March 4, 2023, at the PT BCK Laboratory located at Jl. Kyai Samiyoredji, Gondang Lutung village, Donoharjo, Ngaglik District, Sleman Regency, Special Region of Yogyakarta.

### 2.2.Tools and Materials

The tools used in this study were petri cups, glass beakers, erlenmeyers, analytical scales, hot plates, stirring rods, refrigerators, bunsen, scissors, cutters, autoclaves, ovens, incubators, *hand sprayers*, ose needles, tweezers, *object glass*, *laminar air flow* (LAF), cameras and stationery. Meanwhile, the materials used include *Gliocladium sp., Colletotrichum gloeosporioides, Rhizoctonia solani, Fusarium sp., and Phytophthora sp*. The media used includes *Potato Dextrose Agar* (PDA) media. Meanwhile, in the medium *Carboxymethyl Cellulose* (CMC); amylum and chitin are used liquid and solid media, as well as iodine and lugol.

### 2.3.Characterization of Exoenzyme Activity in *Gliocladium sp.*

In this study, tests were carried out to determine the presence of exoenzyme activity in *Glocladium sp.* The tests carried out include cellulolytic tests, amylolytic tests, and chitinolytic tests. The first test was carried out by inoculating *Gliocladium sp*. to solid media CMC (cellulolytic test); amylum (amylolytic test); chitin (chitinolytic test), then incubated for 7 days. After 7 days, CMC media is given 1.5 ml of iodine, and amylum media is given three drops of lugol to the entire surface

of the media. Positive results will later have clear zones around the isolate grown. In the second test, CMC, amylum and chitin liquid media were used. *Gliocladium sp.* Then it is inoculated into the liquid medium. Furthermore, observations were made on the 3rd, 7th, 10th, and 14th days after inoculation (hsi). A positive result will indicate the growth of fungal mycelia on the surface of the liquid medium.

### 2.4.Test of the antagonist *Gliocladium sp.* against some plant pathogens

The antagonist fungus used is *Gliocladium sp.* Meanwhile, the pathogenic fungi used include *Colletotrichum gleosporides, Rhizoctonia solani, Fusarium sp*., and *Phytophthora sp.* The antagonist test was carried out by the dual culture assay method using PDA media on a petri dish (Figure 1). *Gliocladium sp.* and pathogenic isolates are cultured in a petri dish measuring 8 cm. The control treatment is a medium that is only inoculated with pathogen isolates. Inoculation is carried out in the LAF to avoid contamination. The antagonist's ability can be determined based on the percentage of inhibition by assessing the presence or absence of an inhibition zone. The development of the area of the control mushroom colony was calculated using the average length of the farthest finger and the middle finger. Observations were made on the colony area on the 3rd, 7th, 10th and 14th days. The growth inhibition percentage is calculated by the formula:

$$\text{Percentage of Inhibition} = \frac{\text{R1} - \text{R2}}{\text{R2}} \text{x100\%}$$

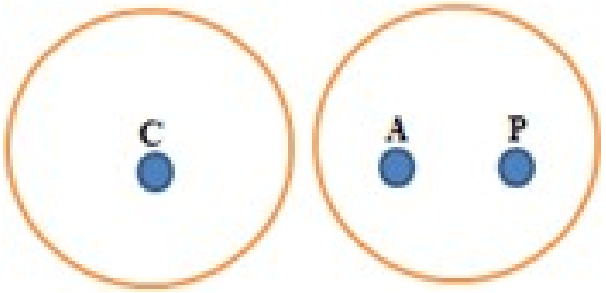



**Figure 1.** Test of the antagonist *Gliocladium sp.*

Information:
C = Pathogen control
A = Antagonist fungus (*Gliocladium sp*.)
P = Pathogenic fungi (*Colletotrichum gleosporides, Rhizoctonia solani, Fusarium sp*., and *Phytophthora sp.)*

## 3. Results and Discussion

### 3.1.Test of the antagonist *Gliocladium sp*. against some plant pathogens

*Gliocladium sp.* Marasis the host by covering or enveloping the pathogen, producing enzymes and destroying the pathogen's cell wall until the pathogen dies. In addition, *Gliocladium sp* can live both as saprophytes and parasites on other fungi, can compete for food, can produce inhibitors and are hyper parasitic (Agustina et al., 2013). In this study, an antagonist test was carried out *Gliocladium sp.* against some plant pathogens such as *Colletotrichum gloeosporioides* (chili), *Rhizoctonia solani* (chili), *Fusarium sp.* (chili), and *Phytophthora sp.* (shallots) as follows.

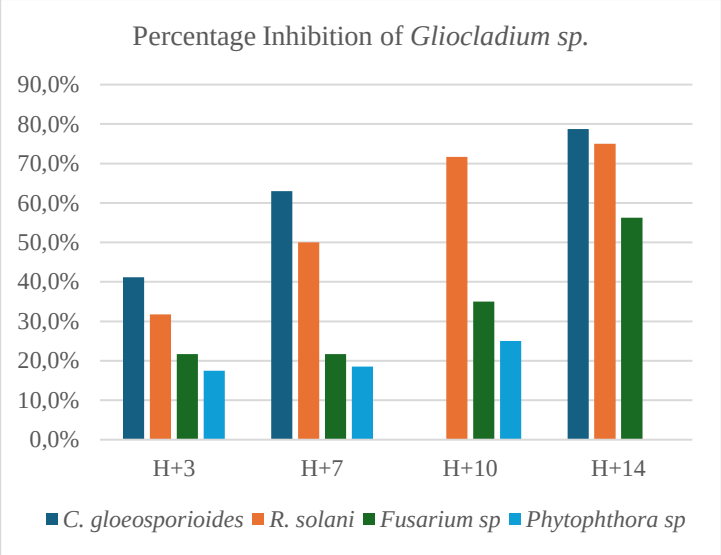



**Figure 2.** Results of Percentage Inhibition of *Gliocladium sp.* Against Some Plant Pathogens

Figure 2 shows the growth of pathogenic fungi which include *C. gloeosporioides*, *R. solani*, *Fusarium sp., and Phytophthora sp*. and the percentage of their resistance to antagonist fungi. In addition, pathogenic fungal control tests were also carried out without the administration of the antagonist fungus *Gliocladium sp*. In the isolates *Colletotrichum gloeosporioides* and *Rhizoctonia solani*, maximum growth occurred on the 14th day observation with a radius of 4 cm, which means that this isolate grows to fill a petri dish with a diameter of 8 cm. Then in the control treatment of *Fusarium sp.* The growth was faster than the previous growth of the pathogen, namely on the 10th day of observation this isolate had covered the Petri dish. Meanwhile, in the control of *Phytophthora sp.* has the fastest growth characterized by the fact that the entire petri dish has been covered by this fungus only on the 3rd day of observation.

From this observation, it was shown that the fastest growth of pathogenic fungi was in the isolate of *Phytophthora sp.* that has managed to grow covering the Petri dish on the 3rd day. Then the second position was occupied by *Fusarium sp.* which on the 10th day had completely covered the Petri cup. Meanwhile, in the isolate *C.gloeosporioides* and *Rhizoctonia sp.* The growth is quite slow with the maximum growth shown on the 14th day. According to Herlina (2013), the optimal growth of *Gliocladium sp.* occurred at a temperature of 25-32° C. Inhibition of *Gliocladium sp* extract was able to show percentages of 11-59% and 14-85% by chloroform and ethyl acetate extraction techniques respectively (Hassine et al., 2022).

After the observation of pathogenic fungus control, then the observation of the antagonist test shown in table 1 was continued. According to Izzatinnisa et al. (2020), a high percentage of inhibition has a range between 70-100%, while 40-69% indicates a moderate percentage of inhibition, while a low percentage of inhibition is shown between 0-39%. From these parameters, it can be seen in table 1 that *Gliocladium sp.* had a high percentage of inhibition against pathogens *C. gloeosporioides* and *R. solani* with a percentage of 78.75% and 75%, respectively. Then in the isolate *Fusarium sp.* has a moderate resistance percentage of 56%. While the percentage of inhibition of *Gliocladium sp.* against *Phytophthora sp.* low with a figure of 25%. The following observations on day-14 show the inhibition of *Gliocladium sp.* against several plant pathogens tested by showing (Figure 2).


**Figure 2.** *Gliocladium sp* Antagonist Test Against Several Plant Pathogens on the 14th Day of Observation. (a) *Colletotrichum gloeosporioides*, (b) *Rhizoctonia solani*, (c) *Fusarium sp.*, (d) *Phytophthora sp*.

*Gliocladium sp.* It is known to produce a wide variety of antibiotics and various inhibition mechanisms. *Gliocladium sp.* produces fine hyphae that grow quickly and there are fine hairs that can come into contact with pathogenic fungi. With this structure, *Gliocladium sp.* It can act with its enzymatic activity without penetration or by physical action so that it can be seen circling the hyphae around the host. Some examples of pathogenic fungi that can be controlled by *Gliocladium sp.* such as *Colletotrichum sp., Rhizoctonia, Fusarium,* and so on (Castillo et al., 2016).

*Gliocladium sp.* are known to have antagonistic mechanisms against other organisms by means of hyper parasitism, lysis and antibiotics, as well as competition (Rahma and Karimah, 2021). The hyperparasite mechanism shows that antagonistic agents directly parasitized and take food from the test pathogen (Rahma and Karimah, 2021). Antibiosis is the process of inhibition of an organism by secondary metabolites produced by other organisms, antibiotic activity generally inhibits growth and possibly kills other organisms, while lysis usually causes damage, decomposition or decomposition of biological substances (Dailah et al. 2020).

*Gliocladium sp.* It is known to have an antagonistic mechanism against other organisms by means of hyper parasitism, lysis and antibiotics, as well as competition. The hyperparasite mechanism shows that antagonistic agents directly parasitized and take food from the test pathogen (Rahma and Karimah, 2021). Antibiosis is the process of inhibition of an organism by secondary metabolites produced by other organisms, antibiotic activity generally inhibits growth and possibly kills other organisms, while lysis usually causes damage, decomposition or decomposition of biological substances (Dailah et al. 2020). The inhibition activity of *Gliocladium sp* is influenced by various factors including pH, temperature, intensity and duration of exposure (Hidayat et al., 2020; Triasih & Widyaningsih, 2023)

## 3.2.Characterization of Exoenzyme Activity in *Gliocladium sp.*

*Gliocladium sp.* It has an inhibition mechanism in the form of a mycoparasitism mechanism, this mechanism allows the hyphae wall infected with parasitic microbes to be seen penetrated by a combination of hydrolysis enzymes and mechanical stress. The infected hyphae will penetrate the cell wall, causing the host's cytoplasm to undergo necrosis (Rahma and Karimah. 2021). *Gliocladium sp.* also has the ability to produce extracellular products in the form of toxins that can suppress the growth of pathogens (Soenartiningsih, 2014).

*Gliocladium sp.* produces fine hyphae that grow quickly and there are fine hairs that can come into contact with pathogenic fungi. With this structure, *Gliocladium sp*. can act with its enzymatic activity without penetration or by physical action so that it can be seen to circle the hyphae around the host (Castillo et al., 2016). Toxins and enzymes released *by Gliocladium sp.* can cause the cell wall to collapse so that the pathogen's cell wall becomes destroyed (Herlina 2013).

In this study, a characterization test was carried out for *Gliocladium sp.* on several exoenzyme

activities produced by *Gliocladium sp.* Positive control is produced when *Gliocladium sp.* It is proven that the production of cellulose, amylum, and chitin enzymes is by the formation of a clear zone around the isolate that is grown. The formation of clear zones is caused by the inhibition of antimicrobial compounds against microbial cells. The mechanism of action of an antimicrobial compound can be carried out by disrupting or damaging the constituents of the cell wall, reacting with the cell membrane causing increased cellular permeability, inactivation of essential enzymes and destruction or inactivation of genetic functions and material (Sari et al., 2013). The positive control shown by *Gliocladium sp.* to the cellulolytic test, amylolytic test, and chitinolytic test (Figure 3).

Then in the research activity, cellulolytic, amylolytic, and chitinolytic testing was carried out on *Gliocladium sp.* The results after 7 days of incubation were obtained that no clear zones were formed in the cellulolytic, amylolytic, and chitinolytic tests (Figure 3).

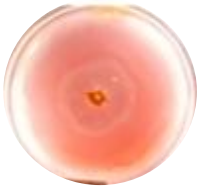
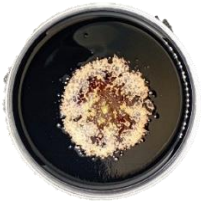
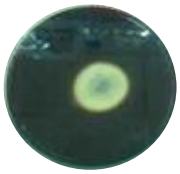
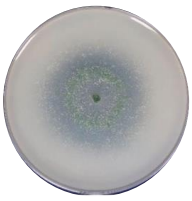
| | Result | Refference |
|---|---|---|
| Cellulolytic test |  |  |
| | | (Putri *et al*., 2019) |
| Amylolytic test |  |  |
| | | (Tangapo & Mambu, 2021) |
| Chitinolytic test |  |  |
| | | (Chung et al., 2022) |

**Figure 3.** Characterization of *Gliocladium sp.* On solid media (a) cellulolytic test, (b) amylolytic test, (c) Chitinolytic Test

Because all the results were negative, a second test was carried out by replacing the solid media with liquid media CMC, amylium, and chitin. In this study, *Gliocladium sp*. incubated into liquid medium in a test tube for 14 days. In this test, the growth of *Gliocladium sp*. fungi was only found on the amylum medium, while the CMC (cellulolytic test) and chitin (chitinolytic test) media did not find the growth of fungal mycelia (Figure 4).

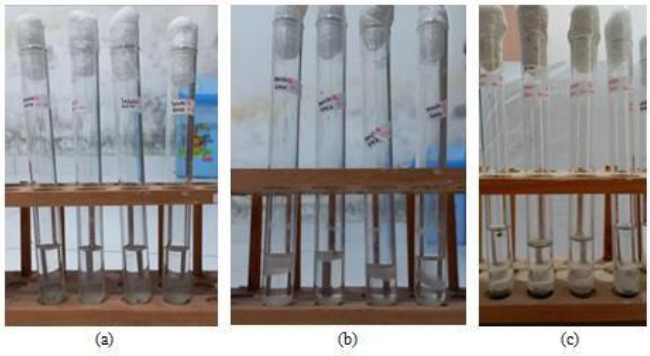



**Figure 4.** Characterisation of *Gliocladium sp*. in Liquid Media. (a) Cellulolytic test, (b) Amylolytic Test, (c) Chitinolytic Test

The results of the above study are not in accordance with the results of Adila (2022) research, that *Gliocladium sp*. is effective in degrading amyl, cellulose, and chitin substrates. According to Herlina (2013), *Gliocladium sp.* can produce cellulase enzymes that are able to break down cell walls composed of a mixture of polysaccharides and proteins, and chitin (β-1,4-N Acetyl glucosamine) and β-1,3-glucose or β-1,6 glucose. Cellulase enzyme is an enzyme that is able to degrade cellulose by breaking the bonds of β-1,4 glycosides which produce cellulose-derived oligosaccharides and glucose. In the growth medium of cellulolytic fungi contains a substrate of CMC (*Carboxy Methyl Cellulase*) which can be degraded by the enzyme cellulase. If the fungus can grow in the medium, it can be indicated that the fungus is a cellulolytic fungus (Murtiyaningsih & Hazmi, 2017).

In addition, *Gliocladium sp.* produce extracellular enzymes such as amylase, which produce mycotoxins namely aflatoxin and ochratoxin which act as antibiotics to inhibit the growth of pathogens (Rusli et al. 2021). The formation of the clear zone is the result of the activity of the amylase enzyme in the area. The clear zone formed around the isolate after being dripped by a solution of lugol iodine indicates that the amylase enzyme is produced by the isolate, so that in that area the starch has been hydrolyzed into simpler compounds. Meanwhile, the formation of blackish-blue color is caused by a reaction between iodine solution and unhydrolyzed starch. Amylolytic fungi are able to hydrolyze amylum with the help of amylase enzymes (Silitonga et al., 2020).

According to Corneliyawati et al, (2018), the chitinase enzyme can be found in *Gliocladium sp.* Chitin in fungi is the main component that constitutes the cell wall of the fungi class Ascomycetes, Basidiomycetes and Deuteromycetes. Chitinolytic fungi synthesize chitinase to degrade chitin as a nutrient source for cell division. Colloidal chitin can be hydrolysed by chitin deacetylase which produces chitosan and chitosanase which produces chitobiosa (Akhsani and Suprihadi, 2019). The chemical structures of chitin and chitosan are shown in Figure 5.

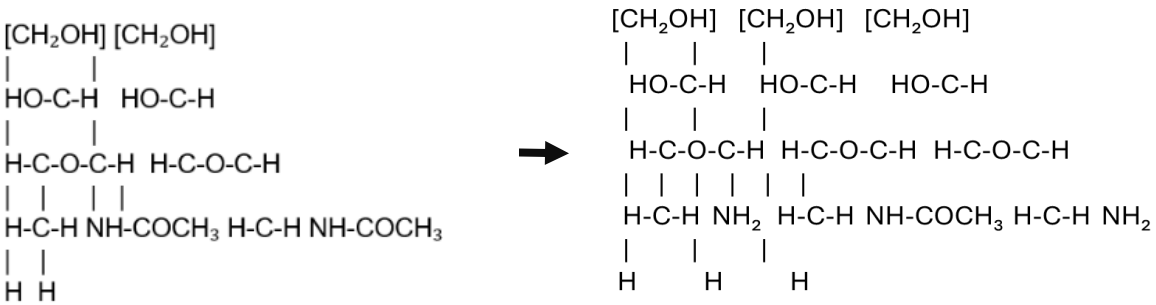



**Figure 5.** The Structure of Chitin and Hydrolyzed Chitin Into Chitosan

No growth of *Gliocladium sp*. In the media, the media can be affected by temperature. Enzymes have a certain temperature that can cause the activity of the enzyme to work optimally. The higher the temperature, the higher the enzyme activity will be. In a study by Fitrianti (2014), the highest enzyme activity data was obtained at a temperature of 50°C. The addition of temperature that exceeds the optimum limit can cause the enzyme to be denatured and shut down the activity of the enzyme catalyst.

In addition to the influence of temperature, enzyme activity can be affected by pH. Enzymes cannot work in enzymes that are too low (acidic) or at pH that is too high (alkaline). This is because, at the pH, enzymes can be denatured so that the active side of the enzyme can be disturbed. Each of the enzymes produced also has a different optimum pH. Cellulase enzymes with one of their components, namely CMC, tend to have an acidic optimum pH with a pH range of 4-6.5 (Fitrianti, 2014). Carvalho et al. (2008 in Istia'nah et al. 2020), stated that the optimum pH activity of amylase enzyme ranged from 4.0-8.0. Meanwhile, the chitinase enzyme has an optimal pH of 6 (Purkan et al., 2016).

## 4. Conclusion

The antagonist test using the Dual Culture Assay method showed that *Gliocladium sp*. can inhibit the growth of all pathogenic fungi with varying percentage of inhibition. A high percentage of inhibition was shown in the pathogens *Colletotrichum gloeosporioides* and *Rhizoctonia solani* with percentages of 78.75% and 75%, respectively. Then against *Fusarium sp.* has a moderate resistance percentage of 56%. While the percentage of inhibition of *Gliocladium sp.* against *Phytophthora sp.* low with a figure of 25%. From the research that has been carried out on the characterization of exoenzyme activity in *Gliocladium sp.*, several sources state that *Gliocladium sp.* can produce cellulase, amylase, and chitin enzymes. However, in this study, the results of cellulolytic and chitinolytic testing *of Gliocladium sp*. showed negative results for liquid and solid media. Meanwhile, in the amylolytic test, positive growth was marked by the appearance of mycelia on the surface of amylase liquid media, but in solid media negative results were obtained against amylase enzymes. The non-appearance of this mold growth can be influenced by temperature and pH factors.

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