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Antagonistic test of *Penicillium* sp. against the pathogen *Colletotrichum* sp. causing anthracnose disease *in-vitro*

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A B S T R A C T

Anthracnose is a type of plant diseases caused by the pathogen *Colletotrichum* sp. Anthracnose disease can infect all plant organs, such as leaves, branches, twigs, and fruits. The initial symptoms of anthracnose disease are dry and dark brown spots. The process of anthracnose disease spread is so fast that it can reduce agricultural productivity. One alternative to environmentally friendly plant control is the use of biological control agent. *Penicillium* sp. is one of the fungi used as a biological control agent. This study aims to determine the antagonistic ability of *Penicillium* sp. against the pathogen *Colletotrichum* sp. causing anthracnose disease *in-vitro*. This research is classified as experimental research using the *dual culture* method. This research consists of two treatments and two replications. The control treatment was conducted using only the pathogen *Colletotrichum* sp. This research was conducted for 7 days at a temperature of 25°C using PDA medium. The observation process was carried out by measuring the diameter of the control and treatment pathogens for 7 days. Data analysis in this study was conducted using descriptive statistics to determine the mean and standard deviation of a treatment. The results show that *Penicillium* sp. can inhibit the pathogen *Colletotrichum* sp. with the highest inhibition percentage on the 7th day after inoculation of 49.14 ± SD 2.39%, which falls within the medium criteria. The inhibition mechanism of *Penicillium* sp. against the pathogen *Colletotrichum* sp. occurs through competition and antibiosis.

Keywords: Antagonistic; Biological agents; *Colletotrichum* sp; *Penicillium* sp.

1. INTRODUCTION

Plant diseases have become one of the main problems in cultivation engineering activities. Plant diseases can occur due to attacks by pathogens such as pests, fungi, bacteria, and viruses. One type of plant disease is anthracnose. Anthracnose disease is caused by the infection of the fungus *Colletotrichum* sp. throughout all parts of the plant, such as leaves, branches, twigs, and fruit (Ramdan et al., 2019). According to Nurjasmi & Suryani (2020), the early symptoms of anthracnose disease appear as dry, dark brown spots on the surface of the fruit. The dark brown spots are caused by the presence of setae on the fungal body of *Colletotrichum* sp. Setae are part of the fungus that forms on the acervulus (Wardoyo et al., 2020). The pathogen that causes anthracnose, *Colletotrichum* sp., is known to reduce agricultural productivity by up to 45-60% (Palupi et al., 2015).

Decreased productivity due to anthracnose disease will impact the income losses of farmers and producers. This can be prevented with efforts to control plant diseases. The disease control technique for anthracnose used by most farmers is the application of synthetic fungicides. The choice of synthetic fungicides is due to their practicality, speed, and efficiency. However, on the other hand, excessive use of synthetic fungicides can lead to negative effects on the environment, such as resistance in pathogenic fungi, killing other microorganisms around the plants, and health issues for humans. This is caused by the residue of chemical substances in the composition of fungicides (Saleh *et al*., 2021). The use of synthetic fungicides can also damage the sustainability of ecosystems; therefore, there is a need for plant disease control techniques that are easily accessible and environmentally friendly.

Biological agents have become one of the environmentally friendly biocontrol alternatives that can boost productivity levels. Biological agents consist of various types, including fungi, bacteria, viruses, nematodes, insects, and others. Control of anthracnose disease can be carried out with biological agents in the form of fungi. Biological fungal agents are included in the group of antagonistic microorganisms that can directly inhibit the growth of pathogens by producing antibiotics (Alifia *et al*., 2023). The types of biological fungal agents include *Trichoderma* sp., *Aspergillus* sp., *Gliocladium* sp., *Penicillium* sp., and others (Ayaz *et al*., 2023).

Penicillium sp. is one of the biological agents used to control anthracnose disease. According to Astuti and Respati (2022), *Penicillium* sp. has antibiosis capabilities that can produce secondary metabolites, thereby

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inhibiting pathogen growth. These secondary metabolites include penicillin (Correia *et al*., 2023), griseofulvin (Panda *et al*., 2005), agroclavin and ergometrine (Arvina, 2023). According to research by Ramdan *et al*. (2017), *Penicillium* strain MAG1 and *Penicillium* strain PAB2 can inhibit the growth of Phytophthora capsici in vitro by demonstrating colonisation ability and antibiosis mechanisms, thereby controlling the potential for stem base rot disease by 25.5-35.5%. Additionally, according to research by Alifia *et al*. (2023), *Penicillium* sp. as a biological agent can act as a parasite that is suspected to suppress the growth of *Colletotrichum gloeosporioides* fungus in orchids through the process of destroying pathogenic hyphae until they undergo lysis. Research by Noviyanti *et al*. (2022) also shows that endophytic fungal isolates *Penicillium* sp. (E3) and (E5) can inhibit the growth of the pathogenic fungus *Colletotrichum* sp., which causes anthracnose disease, and has the potential to suppress the occurrence of anthracnose disease in chilli plants with an inhibition test percentage of 49.73% and 53.09%.

Based on this description, it can be seen that research on the antagonism test of *Penicillium* sp. against *Colletotrichum* sp. causing anthracnose disease needs to be done because it can be used as an alternative to integrated pest and disease control that is environmentally friendly. In addition, research on the antagonism test of *Penicillium* sp. against *Colletotrichum* sp. is very limited so further research needs to be done to get significant results and ensure its effectiveness. Therefore, researchers conducted a study that aims to determine the antagonistic ability of *Penicillium* sp. against the pathogen *Colletotrichum* sp. causing anthracnose disease *in-vitro*.

2. MATERIAL AND METHODS

2.1 Time and place of research

This research was conducted in from 18 May to 3 June 2024 at the Microbiology Laboratory, Faculty of Science and Technology, Sunan Ampel State Islamic University Surabaya at a temperature of 21℃ and an oxygen level of 57.9.

2.2 Preparation of *Potato Dextrose Agar* (PDA) media and rejuvenation of fungal culture

PDA media was weighed as much as 39 grams, put into an erlenmeyer and then dissolved with 1000 ml of distilled water. Erlenmeyer containing PDA media was placed on a hotplate to boil and homogenized with a stirring rod. After boiling and homogeneous, the hotplate was turned off and the solution was left until it was lukewarm. Erlenmeyer was covered with cotton and alumunium foil. Furthermore, it was sterilized by autoclaving for 15 minutes at 121°C and 1 atm pressure (Jamilatun *et al*., 2020). Fungal rejuvenation is carried out by taking 1 plate of culture using a sterile needle and inoculating it into a Petri dish containing PDA medium, then incubating it at 21°C for 4-7 days (Pratama *et al*., 2018).

2.3 Antagonistic test of *Penicillium* sp. against the pthogen *Colletotrichum* sp.

The antagonistic test was conducted using the *dual culture* method, which consisted of two treatments and two replicates. Pure cultures of *Penicillium* sp. and *Colletotrichum* sp. fungi were prepared on one plate and then inoculated into a Petri dish containing PDA medium. The two cultures were placed opposite each other at a distance of 3 cm. They were then incubated for 7 days in dark conditions at a temperature of 21℃ and an oxygen level of 57.9. The diameter of the pathogen mycelium was observed 2-7 days after inoculation. The control treatment was carried out by only inoculating the *Colletotrichum* sp. pathogen into a petri dish containing the medium. The diameter of the pathogen mycelium was measured twice using a ruler.

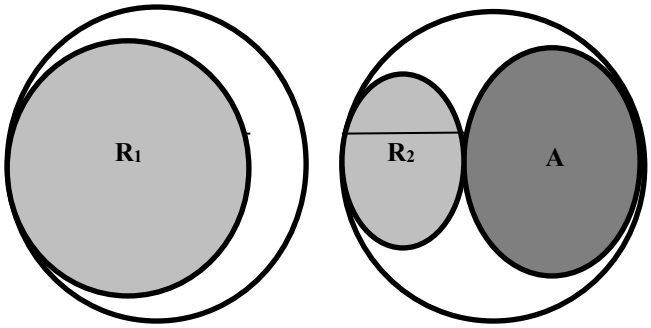

Figure 1. Antagonistic test with *dual culture* method. (R_1): Mycelial diameter of *Colletotrichum* sp. in control petri dish; (R_2) Mycelial diameter of *Colletotrichum* sp. in treatment petri dish; (A) fungi *Penicillium* sp.

Observation of the percentage of inhibition can be done by calculating the formula of Izzatinnisa (2020) as follows:

$$P1=\frac{R1-R2}{R1} \times 100\% \quad (1)$$

Description:
P1 = Percentage of mycelial growth inhibition (%)
R1 = Diameter of pathogenic mycelium in control petri dish (cm)
R2 = Diameter of pathogenic mycelium in treatment petri dish (cm)

The results of the inhibition percentage measurements were then analysed based on the inhibition criteria. Inhibition percentage criteria according to Zivkovic *et al*. (2010); Cendrawati *et al*. (2020) are 1-25% low, 26-50% medium, 51-75% high, 72-100% very high.

2.4 Data analysis

Data from the calculation of the percentage of inhibition was analysed by descriptive statistics. In descriptive statistics, the average (mean), standard deviation, variance, maximum, and minimum are used to provide an overview or description of the data. Data from descriptive statistics were then tabulated in microsoft excel.

3. RESULT AND DISCUSSION

The antagonist test aims to determine the ability of *Penicillium* sp. in inhibiting the pathogen *Colletotrichum* sp. The antagonist test was carried out using the *dual culture* method, namely inoculating antagonistic fungi and pathogens in one petri dish containing agar medium. Antagonistic test of *Penicillium* sp. against *Colletotrichum* sp. can be seen in figure 2 as follows:

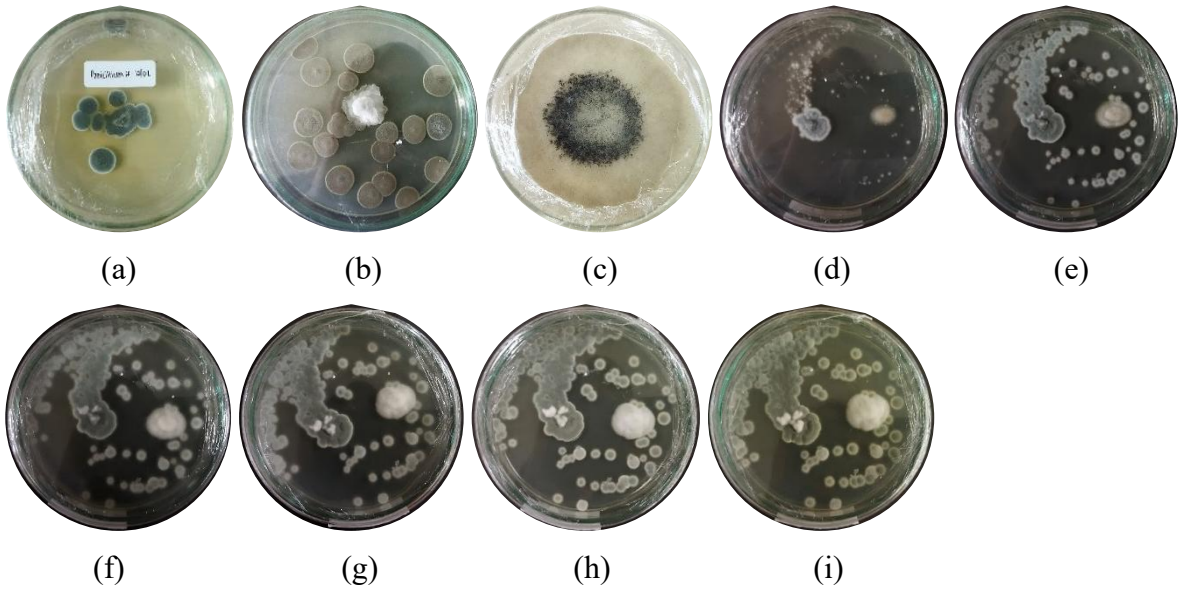

Figure 2. Results of antagonistic test of *Penicillium* sp. against *Colletotrichum* sp. (a) *Penicillium* sp. control at 7 days old; (b) *Penicillium* sp. control at 14 days old; (c) *Colletotrichum* sp. control (d) Incubation period of 2 days; (e) Incubation period of 3 days; (f) Incubation period of 4 days; (g) Incubation period of 5 days; (h) Incubation period of 6 days; (i) Incubation period of 7 days

The parameters used in the antagonist test are by calculating the diameter of the pathogen in the control treatment and the diameter of the pathogen in the antagonist treatment. The inhibitory potential of *Penicillium* sp. in the antagonist test was seen from the percentage of inhibition on days 2 to 7 after inoculation. The existence of an inhibitory mechanism proves that there is inhibitory potential of *Penicillium* sp. The test results of the percentage of inhibition of *Penicillium* sp. against *Colletotrichum* sp. can be seen in figure 3 as follows:

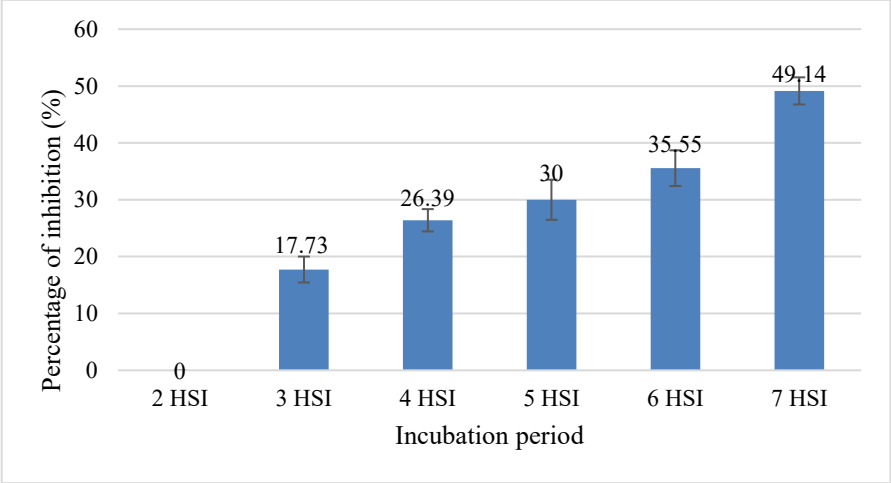

Figure 3. Diagram of antagonistic test of *Penicillium* sp. against *Colletotrichum* sp.

Based on figure 3 shows that the percentage of inhibition of *Penicillium* sp. against the pathogen *Colletotrichum* sp. began to appear on the 3rd day after inoculation. Data on the percentage of inhibition of *Penicillium* sp. against *Colletotrichum* sp. consecutively on the 2nd day after inoculation was 0,00 ± SD 0,00%, the 3rd day was 17,73 ± SD 2,28%, the 4th day was 26,39 ± SD 1,96%, the 5th day was 30,00 ± 3,53%, the 6th day was 35,55 ± 3,14%, and the 7th day was 49,14 ± SD 2,39%. The inhibition of *Penicillium* sp. against *Colletotrichum* sp. was included in the medium criteria with the highest inhibition percentage of 49,14 ± SD 2,39% on day 7 after inoculation. This is supported by Zivkovic *et al*. (2010); Cendrawati *et al*. (2020) which stated that the percentage of inhibition consists of four criteria, namely low with a percentage of 1-25%, medium 26-50%, high 51-75%, and very high 76-100%.

Research by Muhibbudin *et al*. (2024) stated that the inhibition ability of *Penicillium* spp. against *Colletotrichum* sp. was included in the medium category with the highest percentage of inhibition of 31,58% on the 5th day after inoculation. In addition, there is research by Noviyanti *et al*. (2022) stated that the average percentage of inhibition of *Penicillium* sp. (E3) and (E5) against *Colletotrichum* sp. was 49,73% and 53,09%. However, the inhibition percentage results of this study were lower than those reported by Sanothan *et al*. (2023), which stated that the inhibition percentage of *Trichoderma* sp. against *Colletotrichum* sp. on the 7th day after inoculation could reach 100%. Differences in inhibition percentages can be influenced by several factors, including isolate type, inoculation time, age, spore density, antagonist growth rate, and inhibition mechanism. According to Yulianto (2014), low growth of antagonistic fungi will affect the low ability to suppress pathogens. The activity of suppressing target pathogens is determined by the high growth rate of antagonistic fungi (Halwiyah *et al*., 2019).

Penicillium sp. has potential as a biological agent against the pathogen *Colletotrichum* sp. because it has a percentage of inhibition of more than 40%. Although the inhibition reached 49,14% which falls within the medium category, further testing under *in vivo* or field conditions is necessary to confirm its efficacy. This is supported by the statement Otten *et al*. (2004); Marlina and Hakim (2023) that fungal isolates can become biological agents if they have a minimum percentage of inhibition of 30% and a maximum of more than 60%. The percentage inhibition of *Penicillium* sp. against *Colletotrichum* sp. was produced through the mechanisms of competition inhibition and antibiosis. The competition mechanism is shown by the growth of *Penicillium* sp. which spreads faster and fills the space around the petri dish (Figure 2). This is in accordance with the research of Muhibuddin *et al*. (2024) that the competition mechanism occurs between the antagonistic fungus *Penicillium* sp. against pathogen *Colletotrichum* sp. which is characteristed by the growth of colonies competing for more growth space and nutrients. In addition, there is research by Bartholomew *et al*. (2022) stated that the control of microorganisms by *Penicillium* sp. can be through the mechanism of competition and obtaining nutrients from infected areas.

Research by Aji *et al*. (2022) stated that isolates of *Penicillium* sp. can inhibit *Fusarium oxysporum*. According to Sudewi *et al*. (2023) stated that the nature of antagonism can develop because of the mastery of the same life needs.

The mechanism of antibiosis inhibition is indicated by the presence of a clear zone between the colonies of *Penicillium* sp. and *Colletotrichum* sp. (Figure 2). This is in accordance with the research of Asniah *et al*. (2024) stated that isolate EB02 (*Penicillium* sp.) has an antibiotic mechanism characteristed by the pathogenic fungus *Colletotrichum* sp. cannot pass through endophytic fungi and there is a distance between pathogenic fungi and endophytes. Research by Safitri *et al*. (2022) also stated that the antibiosis mechanism of *Penicillium* sp. JRTK2 with *Fusarium* sp. JPTK1 formed a clear zone in the middle of the colonies of the two fungi and caused the lower surface of the *Penicillium* sp. JRTK2 colony to change colour pigment. In addition, Aji *et al*. (2022) also stated that isolate A2 *Penicillium* sp. showed an antibiotic mechanism against *Fusarium oxysporum* which was characteristed by the formation of a clear zone between the two colonies. Research by Rotasouw *et al*. (2020) also stated that *Penicillium* sp. has antibiotic ability in inhibiting the growth of *Rhyzoctonia solani* penyebab pathogen that causes frond rot disease.

The mechanism of antibiosis inhibition between organisms can produce a substance. The substance is a secondary metabolite compound that can cause the pathogen cell wall to be damaged. Secondary metabolite compounds function as a means of self defence or competition. *Penicillium* fungi can produce several secondary metabolite compounds one which is penicilin. According to Putra and Purwantisari (2018), penicillin compounds can prevent the formation of intact peptidoglycan so that the cell wall weakens and causes lysis. In addition, *Penicillium* sp. can produce citrinin and griseofulvin compounds that act as protectors against infection by several pathogenic microbes (Nugrahani *et al*., 2012). According to Angkur *et al*. (2024) *Penicillium* sp. also has alkaloid group compounds in the form of agroclavine and ergometrin which act as antifungal against several pathogens such as *Botrytis cinerea*, *Fusarium solani*, dan *Alternaria tenuissima*.

Based on the results of the antagonistic test, it shows that *Penicillium* sp. has antagonistic ability against the pathogen *Colletotrichum* sp. causing anthracnose disease so that it has potential as a biological agent. The inhibitory mechanism occurs through competition and antibiosis. Biological agents can be applied directly with microorganisms or secondary metabolite compounds. The application of biological agents must consider the toxicity that can affect the environment and humans. The toxicity of *Penicillium* sp. as a biological agent is known to have no fixed standard because toxicity depends on the species, secondary metabolites, dosage, and mode of use. However, there is research by Subowo (2015) which states that the use of *Penicillium* sp. as a biofertilizer can increase the growth of rice plants because the ability to decompose lignin and phosphate is quite high and can produce IAA. In addition, there is research by Skora *et al*. (2016) which stated that *Penicillium* sp. is able to produce mycotoxins such as rugulovasines, quinocitrinines, chanoclavines which can affect human health. Therefore, the use of appropriate dosage, isolate sorting, and thorough toxicity evaluation are key to ensuring its safety as a biocontrol agent. Secondary metabolite compounds contained in *Penicillium* sp. including the class of compounds can be used as antimicrobial substances. Testing secondary metabolite compounds of *Penicillium* sp. is necessary because it can be used an environmentally friendly alternative to pathogen control.

4. CONCLUSION

Based on the results of the research that has been done, it can be concluded that *Penicillium* sp. has antagonistic ability against the pathogen *Colletotrichum* sp. causing anthracnose disease with the percentage of inhibition included in the medium criteria. The mechanism of inhibition is done through competition and antibiosis.

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