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THE ANTAGONISM MECHANISM OF ENDOPHYTIC FUNGI FROM MUNG BEAN TO *Cercospora canescens*

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ABSTRACT

Cercospora leaf spot disease is severely damaging to mung bean plants and causes yield loss of 60% more. Pathogenic infections can give rise to leaf spots, widen rapidly, and cause premature defoliation of the leaves so they are often considered a sign of harvest. Biological control using endophytic fungi derived from mung bean plants themselves is very necessary. The purpose of the study was to evaluate the antagonism mechanism of endophytic fungi to *Cercospora canescens*. The endophytic fungus was isolated from the mung bean plant tissue and double-cultured with *C. canescens*. The percentage of inhibition and the mechanism of antagonism of each endophytic fungus was observed. From the results of the study, 15 species of fungi were obtained, namely *Aspergillus flavus* 1, *Fusarium* sp. 1, CE5, CE10, *Rhizoctonia* sp., *Aspergillus* sp.1, *Fusarium* sp. 2, *Cladophialophora* sp., CE6, *Aspergillus* sp. 2, *Phytium* sp., CE4, CE13, *Aspergillus flavus* 2, and *Aspergillus* sp. 3.The percentage of inhibition of 15 species of endophytic fungi against *C. canescens* ranged from 5.13 to 50.0% with antagonistic mechanisms in the form of competition for space, nutrients, and oxygen; some species of endophytic fungi have antibiotics and lysis-parasitism. Endophytic fungi are able to compete in the absorption of carbohydrates, proteins, essential amino acids, minerals, and microelements such as phosphates, magnesium, potassium, C vitamins, and B vitamins. Antibiotics through the production of antimicrobial compounds in the form of enzymes, toxins, or antibiotics are produced by endophytic fungi. The coil around of pathogenic hyphae until pathogenic hyphae lysis and die is the last mechanism carried out by endophytic fungi. The role of endophytic fungi as an inducer of plant resistance to pathogens in the field needs to be evaluated.

Keyword: *Cercospra leaf spot, endophytic fungi, mung bean*

ABSTRAK

[MEKANISME ANTAGONISME CENDAWAN ENDOFIT DARI KACANG HIJAU TERHADAP *Cercospora canescens*]. Penyakit bercak daun *Cercospora* sangat merusak tanaman kacang hijau dan menyebabkan kehilangan hasil 60% lebih. Infeksi patogen dapat menimbulkan bercak daun, melebar dengan cepat, dan menyebabkan daun defoliasi prematur sehingga sering dianggap sebagai pertanda panen. Pengendalian hayati dengan menggunakan cendawan endofit yang berasal dari tanaman kacang hijau sendiri sangat diperlukan. Tujuan dari penelitian adalah mengevaluasi mekanisme antagonisme cendawan endofit terhadap *C. canescens*. Cendawan endofit diisolasi dari jaringan tanaman kacang tanah dan diuji biakan ganda dengan *C. canescens*. Persentase penghambatan dan mekanisme antagonisme setiap cendawan endofit diamati. Hasil penelitian memperoleh 15 species cendawan, yaitu *Aspergillus flavus* 1, *Fusarium* sp. 1, CE5, CE10, *Rhizoctonia* sp., *Aspergillus* sp.1, *Fusarium* sp. 2, *Cladophialophora* sp., CE6, *Aspergillus* sp. 2, *Phytium* sp., CE4, CE13, *Aspergillus flavus* 2, dan *Aspergillus* sp. 3. Persentase penghambatan dari 15 species cendawan endofit terhadap *C. canescens* berkisar antara 5,13 – 50,0 % dengan mekanisme antagonism berupa persaingan ruang, nutrisi, dan oksigen; sebagian species cendawan endofit dengan antibiosis dan lisis-parasitisme. Cendawan endofit mampu bersaing dalam penyerapan karbohidrat, protein, asam amino esensila, mineral, dan mikro elemen seperti fosfat, magnesium, kalium, vitamin C, dan vitamin B. Antibiosis melalui produksi senyawa anti mikroba dalam bentuk enzim, toksin, atau antibiotik diproduksi candawan endofit. Pelilitan hifa patogen sehingga hifa patogen lisis dan mati merupakan mekanisme terakhir yang dilakukan cendawan endofit. Peranan cendawan endofit sebagai penginduksi ketahanan tanaman terhadap patogen di lapangan perlu dievaluasi.

Kata kunci: *bercak daun Cercospra, cendawan endofit, kacang hijau*

INTRODUCTION

Leaf spot disease caused by the pathogen *Cercospora canescens* can infect almost all parts of the mung bean plant (Iqbal *et al*., 1995 in Sumartini, 2017). Cercospora leaf spots on mung bean leaves are easy to recognize, the initial symptoms are small brown spots and subsequently develop into larger spots (Semangun, 2004). This symptom can appear about 30-40 days after planting, depending on temperature and humidity. *C. canescens* spreads rapidly in susceptible varieties, causing premature defoliation and swelling of the size of the grains (Grewal *et al*., 1980 in Sumartini, 2017). Therefore, this disease can cause yield loss of up to 61% (Semangun, 2004))

Adriani (2006) stated that the use of sinthetic pesticides to control leaf spot disease can cause air pollution, water and soil pollution, damage ecosystem balance, resurgence, resistance, and can have an impact on health. Therefore, environmentally friendly disease control with biological control is very necessary. Endophytic fungi are currently widely developed as biological control (Durham, 2004). Endophytic fungi have great potential as biological control because they live in plant tissue and can stop the growth of pathogens directly (Niere, 2002).

Endophytic fungi are fungi that occupy living plant tissue throughout their life cycle but do not show symptoms of disease infection. Endophytic fungi are known to be good for plant through several mechanisms, namely increasing resistance to several pathogens and pests, enhancing growth, and increasing plant resistance to unfavorable environmental conditions (Setyaningrum *et al*., 2016).

Kurnia *et al*. (2014) reported that endophytic fungi can inhibit the growth of pathogens *Alternaria solani* and *Fusarium oxysporum* with inhibition percenttages of 54.10% and 56.89% while *Aspergillus* sp. able to suppress the growth of the pathogen *F. oxysporum* by 67.83%. Sumartini (2017) has isolated nine endophytic fungi from mung bean plants in Malang, East Java, which are identified as *Fusarium* sp., *Curvularia* sp., *A. flavus*. The three fungi have the potential as growth inducing agents and biological agents that can control leaf spot disease in mung bean plants.

It is necessary to isolate endophytic fungi from mung bean that are planted in Bengkulu to control leaf spot disease. The purpose of the study was to measure the percentage of inhibition and to evaluate the mechanism of antagonism of endophytic fungi of mung bean against the pathogen *C. canescens*.

MATERIALS AND METHODS

Study site and materials

The research was carried out from November 2023 to February 2024 at the Plant Protection Laboratory, Faculty of Agriculture, University of Bengkulu. The research stages include isolation of endophytic fungi from mung bean plants and *Cercopora canescens* from mung bean plants infected with leaf spot disease by tissue planting method and a double culture test between endophytic fungi and *C. canescens*. Endophytic fungi were isolated from root, stem, and leaf of Kutilang mung bean variety using tissue planting method.

Treatments

Double culture testing was carried out, by taking pure cultures of endophytic fungi and pathogens *C. canescens* with a diameter of 7 mm, then inoculated into petri dishes containing PDA media at a distance of 30 mm (Dharmaputra *et al*., 1999). This test was repeated 3 times. The placement scheme is shown in the following figure:

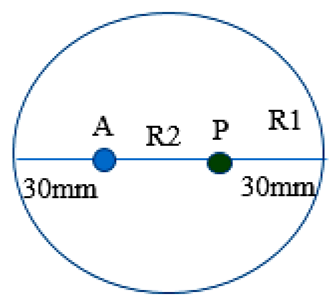

Figure 1. Dual culture test between endophytic fungi and patogen
Note : A = Endophytic fungi colony, Pathogenic fungal colony, R1 = Radius of pathogen colony that stay away from endophytic fungal colony; R2 = Radius of pathogens approaching endophytic fungal colony.

Next, all petri dishes are incubated at room temperature (about 29 ^oC) with RH 85% and 12 hours light - 12 hours dark.

Variable observed

Percentage of inhibition (%)

Radius of pathogen colony is measured by ruler on the 7th day after incubation. The percentage of inhibition is calculated with the formula:

Inhibition = (R1-R2)/R1 x 100%

Note : R1 = Radius of pathogen colony that stays away from endophytic fungal colony; R2 = Radius of pathogen colony approaching endophytic fungal colonies

The mechanism of antagonism

The mechanism of antagonismis identified based on Farida (1992) which includes: space, nutrient, and oxygen competition; antibiosis and lysis and parasitism.

Space, nutrient, and oxygen competition between endophytic fungi and pathogenic fungi is observed by looking at the types **o**f fungi that fill the petri faster. Antibiosis observation is carried out by measuring the width of the empty zone (barrier) and seeing whether or not there is a color change in the medium due to antibiotic compounds produced by endophytic fungi. Observation of the mechanism of lysis and parasitism is carried out by observing the hyphae of test endophytic fungi that grow on pathogenic fungi microscophycaly.

Data analysis

The data obtained is analyzed descriptively and presented in the form of tables and images.

RESULTS AND DISCUSSIONS

The percentage of endophytic fungus inhibition against the pathogen C. canescens varies from 5.13% - 50%. *Rhizoctonia* sp. has the highest percentage of inhibition of 50% (Table 1). This difference in inhibitory ability indicates the diversity of endophytic fungi that are tested as antagonistic agents (Putri *et al*., 2022).

The high and low percentage of inhibiton can be influenced by environmental conditions and other factors that can affect the endophyti fungus. Acording to Putri *et al*. (2022), the inhibition of endophytic fungi against the pathogen *Pyricuaria oryzae* is influenced by environmental conditions that vary between all isolates. In addition, the difference in the environment of origin of endophytic fungi in the field can affect the mechanism of action of endophytic fungi against pathogenic fungi. Based on the diameter growth data, *Rhizoctonia* sp. has a fast growth ability compared to others. It also shows that the isolate of *Rhizoctonia* sp. has the ability to grow quickly and adapts to live better in an artificial environment (outside the plant tissue).

Tabel 1. Percentage of inhibition of 15 endophytic fungi to *C. canescens* on 7 days after incubation

Endophytic fungi isolate	Percentage of inhibition
Aspergillus flavus 1	5.13%
Fusarium sp. 1	24.29%
CE5	40.44%
CE10	23.75%
Rhizoctonia sp.	50.00%
Aspergillus sp. 1	32.38%
Fusarium sp 2	30.57%
Cladophialophora sp.	8.87%
CE6	22.43%
Aspergillus sp. 2	31.25%
Pythium sp.	47.15%
CE4	5.67%
CE13	32.12%
Aspergillus flavus 2	26.55%
Aspergillus sp. 3	27.67%

The difference in the inhibition of the fifteen isolates tested is also due to the difference in the growth rate of each isolate and its ability to compete in obtaining nutrients from the growing medium, as the results of research by Liswarni *et al*. (2018). *Rhizoctonia* sp. has a fast-growing diameter, on the 6th day it is almost full of petri dishes. This ability to grow quickly affects the ability to compete that endophytic fungi have.

In addition, the difference in the ability of endophytic fungi to suppress the growth of pathogens is due to the difference in biological compounds produced. Fungi that are able to suppress the growth of pathogens produce more biological compounds compared to other endophytic fungi so that they can suppress the growth of pathogens (Manurung, 2014). However, in the double culture test that has been carried out, there are endophytic fungi that have a lower growth than *C. canescens*. This is related to the fact that antibiotics produced from endophytic fungi are less effective in suppressing pathogen growth so that the diameter of the pathogen colony becomes higher than that of endophytic fungi and the percentage of inhibition of the endophytic fungus becomes low.

The mechanisms of antagonism that occur in fifteen endophytic fungi isolates vary, ranging from competition between nutrient, spaces, and oxygen, antibiotics, and parasitism.

Observation of the antagonism mechanism was carried out on the 7th day after incubation and the results were obtained as shown in Figure 2 and Table 2.

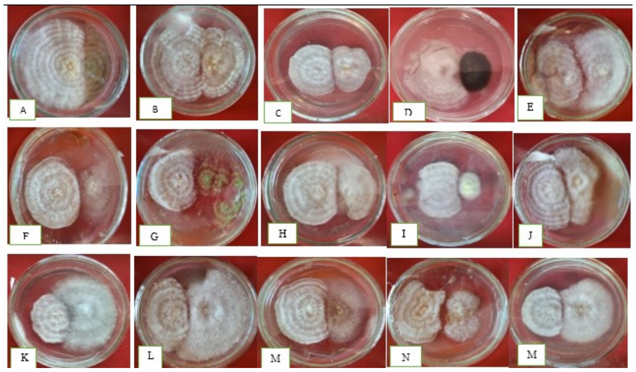

Figure 2. Antagonist mechanism of endophytic fungi to *C. canescen*

Note : A-M= *C. canescens* vs *Aspergillus flavus 1*, *C. canescens* vs *Fusarium* sp. 1, *C. canescens* vs CE5, *C. canescens* vs CE10, *C.canescens vs Rhizoctonia* sp., *C.canescens* vs Aspergillus sp. 1, *C.canecens* vs *Fusarium* sp. 2, *C. canescens* vs *Cladophialophora* sp., *C. canescens* vs CE6 , *C. canescens* vs *Aspergillus* sp. 2, *C. canescens* vs *Phytium* sp., *C. canescens* vs CE4, *C.canescens* vs CE13 , *C. canescens* vs *Aspergillus flavus* 2, *C.canescens* vs *Aspergillus* sp.3

The type of competition mechanism of spaces, nutrients and oxygen occurs in all endophytic fungi against the pathogen *C. canescens*. This competition occurs due to the same needs of each fungi, namely the need for a place to grow and nutrients from the medium used to grow (Liswarni *et al*., 2018). Each isolate shows different competitive abilities. This is influenced by the ability of each endophytic fungi to grow. Endophytic fungi are able to push *C. canescens*, causing *C. canescens* to lose more and more space to grow. The competition also occurs because each fungi tested requires the same nutrients. According to Mukarlina (2010), the need for nutrients contained in the antagonist test medium for its survival is in the form of carbohydrates, proteins, essential amino acids, minerals and microelements such as phosphorus (P), magnesium (Mg) and potassium (K), C vitamin (ascorbic acid), and some B vitamins (thiamine, niacin, and B6 vitamin).

Another antagonistic mechanism that occurs is antibiosis. There are eight endophytic fungi with an antibiotic mechanism, namely,CE5,*Aspergillus flavus 2*, *Aspergillus* sp. 2, *Aspergillus* sp. 3, and *Cladophialophora* sp. Antibiotics are growth inhibition characterized by the presence of an inhibition zone (Mejia *et al.*, 2008). This is in accordance with the antibiosis that occurs as shown by the presence of a clear zone in the medium between endophytic fungi and *C. canescens*. The clear zone that occurs in the media is caused by endophytic fungi that are able to produce metabolite compounds that can be antifungal for pathogens. This is in accordance with Kusumawardani (2015), which states that endophytic fungi that secrete antibiotic substances can also be proven by the absence of pathogenic fungi on pieces of media with clear zones. Antimicrobial compounds produced by endophytic fungi can be in the form of enzymes, toxins, or antibiotics and these compounds are able to inhibit and kill organisms (Manurung, 2014).

Table 2. Antagonist mechanism of endophytic fungi to *C. canescen*

	Competation of nutrient, spaces, and oxygen	Antibiosis	Lysis and Parasitism
Aspergillus flavus 1	+	-	+
Fusarium sp. 1	+	-	+
CE 5	+	+	-
CE 10	+	+	-
Rhizoctonia sp.	+	-	+
Aspergillus sp. 1	+	-	+
Fusarium sp. 2	+	+	-
Cladophialophora sp.	+	+	-
CE6	+	-	+
Aspergillus sp. 2	+	-	+
Pythium sp.	+	-	+
CE 4	+	-	+
CE 13	+	-	+
Aspergillus flavus 2	+	+	-
Aspergillus sp. 3	+	+	-

The third mechanism is the mechanism of hyper-parasitism that causes lysis (Figure 3). There are 3 fungi that carry out the mechanism of hyperparasitism, namely *Aspergillus flavus*, *Fusarium* sp.1 and *Fusarium* sp. 2, *Rhizoctonia* sp., *Pythium* sp., *Aspergillus* sp., CE10, CE4, and CE13.

Figure 3. Lysis and parasitism between endophytic fungi and *C. canescens*

Note: Hypha *Rhizoctonia* sp. coil around *C. canescns*

Arnold *et al*. (2003) stated that the mechanism of action of endophytic fungi consists of a direct mechanism, namely the interaction of endophytic fungi with pathogens and an indirect mechanism, namely an increase in plant defense. The mechanism directly produces antibiotics that help suppress pathogens. Hyphae endophytic fungi can form hooks around them before penetration or direct entry. Antimicrobial mechanisms combat pathogenic microorganisms by destroying cell walls, disrupting microbial cell metabolism, preventing microbial cell synthesis, reducing microbial cell membrane permeability, and preventing microbial cell protein and nucleic acid synthesis (Dolakatabadi *et al*., 2012).

Shittu *et al*. (2011) stated that the potential benefits of endophytic fungi can be used to treat infectious diseases, especially those caused by pathogens. Baron and Rigobelo (2022) and Shah *et al*. (2024) also stated that endophytic fungi have the potential to support plant growth because they have various positive benefits for their host plants, including increasing the absorption of plant nutrients, increasing plant resistance to disease and being able to produce secondary metabolites.

CONCLUSIONS

A total of 15 endophytic fungi isolates were found in mung bean plants, consisting of 2 isolates of *Fusarium* sp., 3 isolates of *Aspergillus* sp., 2 isolates of *Aspergillus flavus*, 1 isolates of *Rhizoctonia* sp., 1 isolates of *Cladophialophora* sp., and 1 isolates of *Pythium* sp. In addition, there are 5 endophytic fungi isolates that have not been identified, namely CE4, CE5, CE6, CE10, and CE13. *Rhizoctonia* sp. had the highest percentage of inhibition against *Cercospora canescens* (50%). The mechanism of endophytic fungal antagonism against *C. canescens* includes competition of spaces, nutrients, and oxygen, antibiotics,and hyperparasitism.

It is recommended to be able to study the potential of endophytic fungi for induction testing of mung bean plant resistance to the pathogen *C. canescens* that causes leaf spot disease in vivo.

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