Journal of Biotechnology and Natural Science
Vol. 3, No. 2, December 2023, pp. 41-46
ISSN 2808-2508

Antagonistic Activities of Endophytic Fungi from Chili Pepper (*Capsicum frutescens* L.) Stems and Leaves against *Colletotrichum sp*.

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ARTICLE INFO

Article history
Received: December 1th 2023
Revised: December 29th 2023
Accepted: December 31th 2023

Keywords
Chilli
Endophytic fungi
Growth area
Percentage of inhibition

ABSTRACT

Colletotrichum sp. is one of the type pathogenic fumgi that can cause antachnose diseases. One of the plants that can be attacked by antrachnose is cayenne pepper (*Capsicum frutescens* L.). This study attempts to determine the ability of endophytic fungi from the stems and leaves cayenne pepper (*Capsicum frutescens* L.). **Methods:** This study used dual culture method to determine the percentage of resistence to patogen *Colletotrichum sp*. and the area of growth endophytic fungi planted on PDA (Potato Dextrose Agar). **Results:**. There are 3 isolates from the stems and leaves of cayenne pepper that is JE-B4-C, JE-D5-C, dan JE-B1-C. Among all the isolates, the largest percentage of inhibition and growth area endophytic fungi were found in isolate JE-B4-C with each of 29,41% dan 58,04 cm^2. **Conclusions:** Expected isolates JE-B4-C derived from the genus eupenicillium, while isolates JE-D5-C and JE-B1-C respectively derived from the genus *Aspergillus* and *Neoscytalidium*.

How to Cite: Puspitarini, S. & Aji, O.R. (2023). Antagonistic Activities of Endophytic Fungi from Chili Pepper (*Capsicum frutescens* L.) Stems and Leaves against *Colletotrichum sp*. *Journal of Biotechnology and Natural Science*, 3(2):41-46.

1. Introduction

Colletotrichum sp. is one type of pathogenic fungus that can cause diseases and inhibit the growth of various types of plants, such as vegetables, fruits, and others (Ainy et al., 2015). The disease caused by *Colletotrichum sp*. is known as anthracnose. Anthracnose can affect plants on leaves, stems, and fruits. One of the plants susceptible to anthracnose is chili pepper (*Capsicum frutescens* L.).

Farmers have traditionally employed synthetic fungicides to control anthracnose due to their economic advantages (Arif, 2015). However, the use of multiple types of synthetic fungicides at high doses and relatively short spraying intervals, typically every 1-3 days, can have negative impacts such as killing beneficial microorganisms, pathogens developing resistance, and environmental pollution (Sumartini, 2012). This is supported by Oktavia et al (2015), who stated that natural enemies exposed to these synthetic fungicides would die, affecting the ecosystem. Additionally, exposure to synthetic fungicides can lead to contamination of water and soil. Due to the adverse effects of pesticide use, one alternative approach is to use bio-agents that are environmentally friendly and more effective than synthetic fungicides in inhibiting the growth of *Colletotrichum sp*. One such bio-agent is endophytic fungi.

Endophytic fungi can produce mycotoxins, enzymes, and antibiotics (Sinaga, 2009). Therefore, it is expected that research on endophytic fungi from chili pepper plants (*Capsicum frutescens* L.), which are antagonistic and conducted in vitro, can suppress the growth of *Colletotrichum sp*. This could be a recommendation for environmentally friendly management of anthracnose. In conclusion, exploring alternative strategies such as bio-agents like endophytic fungi presents promising avenues for sustainable and eco-friendly control of anthracnose in agricultural settings.

2. Methods

2.1. Research Design

The type of research employed is experimental, aiming to determine the inhibition percentage and growth area of endophytic fungi against the pathogen. This study utilizes the dual-culture method to observe the interaction between endophytic fungi from chili peppers and the pathogenic fungus *Colletotrichum sp.*, grown in the same petri dish.

2.2. Tools and Materials

Erlenmeyer flask 250 mL, tray, petri dish, analytical balance, weighing spoon, watch glass, bunsen burner, tweezers, scissors, graduated cylinder 100 mL, stirrer, needle holder, oven, incubator, autoclave, micropipette, PDA (Potato Dextrose Agar), chloramphenicol, 70% Alcohol, aluminum foil, cotton, HVS paper, distilled water (aquadest), toothpick, plastic straw, clear plastic, rubber, tissue, permanent marker, black asturo paper, label, plastic wrap, and Endophytic fungi isolates from the UAD laboratory collection.

2.3. Dual Culture

Testing using the dual-culture method involves pure isolates of endophytic fungi and the pathogen with punctures made on their edges using a plastic straw. The obtained samples are then transferred with a toothpick to the PDA medium, maintaining a distance of 3 cm. Subsequently, the cultures are incubated at 30°C for 7 days. Observation and measurement of the diameter of endophytic fungi and pathogen colonies are conducted daily. To determine the percentage of the inhibition zone, the following equation (Seema & Devaki, 2012) can be utilized:

$$P\ (\%) = \ R1 - R2/R1\ \times 100\%$$

Where:
P = Antagonist Percentage (%)
R1 = Radius of the *Colletotrichum sp*. colony growing opposite to the antagonistic microorganism
R2 = Radius of the *Colletotrichum sp*. colony growing towards the antagonistic microorganism

2.4. Growth Area

Calculating the growth area of pathogen and antagonist colonies involves drawing the growth patterns on transparent plastic. The plastic is then cut and transferred to HVS paper, which is further cut according to the growth pattern and measured using the formula (Hutabalian et al., 2015):

$$\frac{A}{B} = \frac{A'}{B'}$$

Where:
A = Weight of the plastic (size of a 9 cm petri dish) (g)
B = Area of the 9 cm petri dish (cm^2)
A′ = Weight of the plastic after the fungus has grown each day (g)
B′ = Area of the petri dish after the fungus has grown each day (cm^2)

3. Results and Discussion

The antagonistic activity of endophytic fungi from bird's eye chili (*Capsicum frutescens* L.) against the pathogenic fungus causing anthracnose, *Colletotrichum sp*., was assessed using the dual-culture method. The determination of antagonistic mechanism interactions was conducted through direct macroscopic observations in the dual-culture setup. Three endophytic fungal isolates were employed, originating from both the stems and leaves of bird's eye chili plants (*Capsicum frutescens* L.). The

colony diameters resulting from the interaction between endophytic fungi and the pathogenic fungus, assessed using the dual-culture method, are presented in Figures 1 and 2.

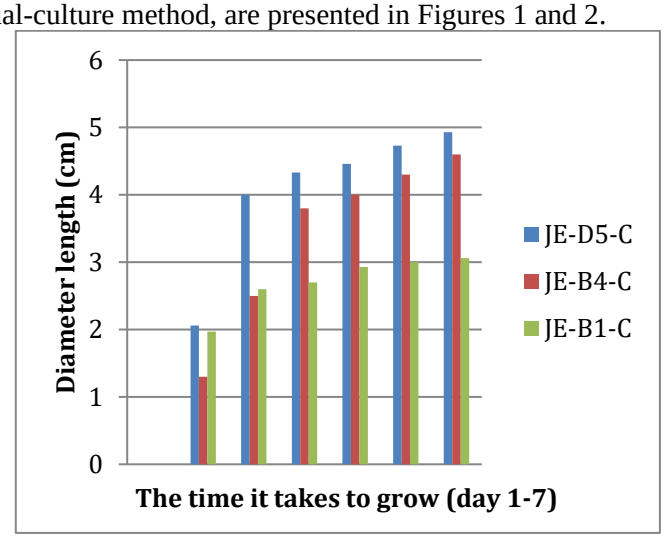

Figure 1. Colony diameter length of the *Colletotrichum sp*. using the dual-culture method for 7 days

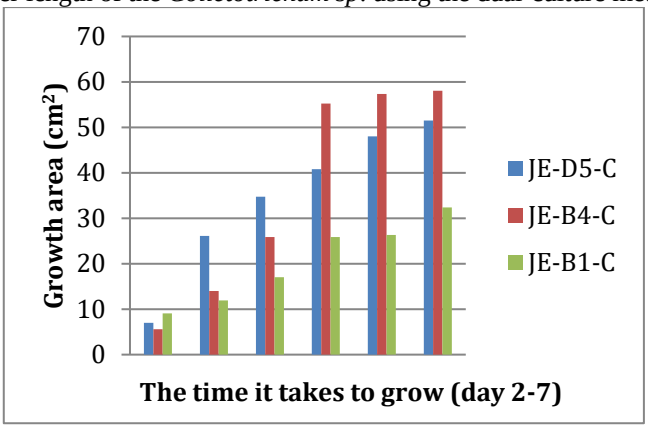

Figure 2. Colony diameter length of the endophytic fungi using the dual-culture method for 7 days

The results obtained from Figure 1 indicate that each isolate of the *Colletotrichum sp.* pathogen, when grown together with endophytic fungi in a petri dish, experienced an increase in colony diameter each day. One factor contributing to the increase in pathogen colony diameter is the nutrition obtained from the Potato Dextrose Agar (PDA) medium. This is supported by Octavia & Wantini (2017), stating that PDA consists of three components, namely potato as a carbon source, vitamins, and energy; dextrose as an energy and sugar source; and agar to solidify the medium. Additionally, Chang & Miles (2004) mentioned that the carbon source plays a role in forming mold cells, and the mold produces enzymes to break down complex compounds into simple ones. The mycelium can then absorb these simple compounds as energy for growth. The measurement of the pathogen *Colletotrichum sp*.'s colony diameter was conducted by measuring the length of the colony diameter in the petri dish using a ruler. Based on Figure 1, it can be observed that the shortest colony diameter of the *Colletotrichum sp*. pathogen is found when grown with the endophytic isolate JE-B1-C, while the longest growth diameter is observed when grown with the isolate JE-B4-C. According to the results obtained from Figure 2, the diameter of endophytic fungi cultivated with the pathogenic fungus in a petri dish increased each day. The endophytic fungi's colony diameter that exhibited the longest growth, based on Figure 2, was found in the JE-D5-C isolate, while the shortest endophytic fungi diameter was observed in JE-B1-C.

A comparison between Figures 1 and 2 shows that the growth of bird's eye chili (*Capsicum frutescens* L.) endophytic fungi colonies has a faster growth rate compared to the *Colletotrichum sp*. pathogenic fungus. On the 7th day of observation, the JE-D5-C endophytic fungi isolate had an average diameter growth rate of 4.93 cm, while the *Colletotrichum sp*. pathogenic fungus had a diameter growth rate of 3.93 cm. The JE-B4-C endophytic fungi isolate had an average diameter of 4.60 cm, compared to the pathogenic fungus with a diameter of 4 cm, and the JE-B1-C endophytic fungi isolate had an average diameter of 3.06 cm, while the pathogenic fungus had a diameter of 2.60 cm.

Other than the diameter of endophytic fungi and the pathogen, another observed parameter in this study is the percentage inhibition of endophytic fungi against the growth of *Colletotrichum sp*. According to Nahdah et al (2020) the inhibition percentage is measured to demonstrate that the presence of endophytic fungal isolates has antagonistic capabilities in inhibiting pathogen growth. The inhibition percentage of endophytic fungi from the stems and leaves of bird's eye chili (*Capsicum frutescens* L.) against the pathogenic fungus *Colletotrichum sp*. on the 7th day is illustrated in Figure 3. Notably, this parameter provides crucial insights into the biocontrol potential of the endophytic fungi against *Colletotrichum sp*.

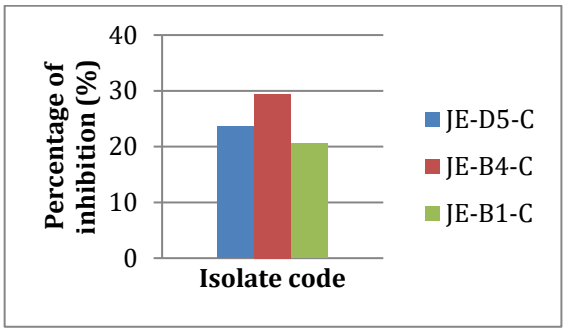

Figure 3. Average percentage inhibition of endophytic fungi against the pathogenic fungus *Colletotrichum sp*. on day 7 (%)

In addition to measuring the diameter between endophytic fungi and the pathogenic fungus, observations were made on the colony conditions of *Colletotrichum sp*. The macroscopic observation of the pathogenic colony was conducted. The visual representation of the interactions occurring among the three isolates of endophytic fungi inhibiting *Colletotrichum sp*. is presented in Figure 4. Another parameter observed for both the pathogenic and endophytic fungi, aside from the inhibition percentage, is determining the growth area of endophytic fungi against the pathogen. The growth areas obtained by endophytic fungi and the pathogen are illustrated in Figure 5 as follows.

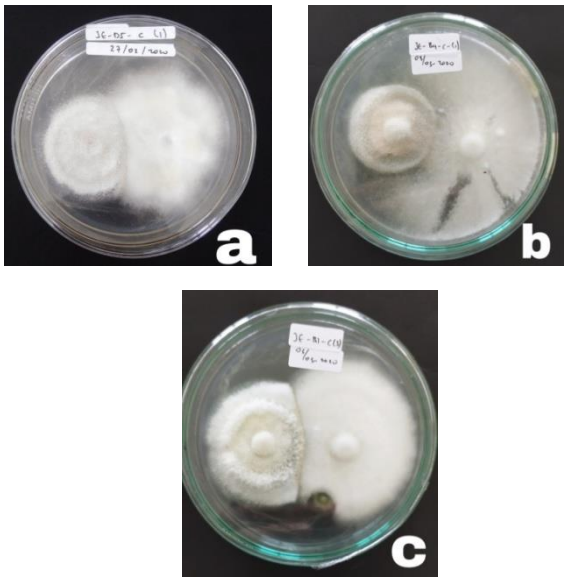

Figure 4. Antagonistic activity using the dual-culture method (a) dual culture of isolate je-d5-c, (b) dual culture of isolate je-b4-c, (c) dual culture of isolate je-b1-c (personal documentation)

The inhibition percentage in Figure 3 was calculated on the 7th day after inoculation between the pathogenic colony radius moving away from the antagonistic mold colony with the pathogenic colony radius approaching the antagonistic mold colony. The inhibition percentage results for each isolate were JE-D5-C at 23.63%, JE-B4-C at 29.41%, and JE-B1-C at 20.52%. The difference in inhibition percentage results is due to each isolate having different types of endophytic fungi. This is supported by Kim et al (2013), stating that isolates taken from different parts of the plant have varying physical characteristics, leading to microbial diversity in a habitat or on plant parts. Furthermore, the highest inhibition percentage of endophytic fungi from bird's eye chili (*Capsicum*

frutescens L.) stems and leaves against the *Colletotrichum sp*. pathogenic fungus is found in the JE-B4-C isolate at 29.41%. This test indicates that a high inhibition percentage has the potential to be a biological agent in inhibiting pathogenic growth (Amaria et al., 2015).

Based on the observations in Figure 4, it is evident that the JE-D5-C, JE-B4-C, and JE-B1-C isolates exhibit antagonistic mechanisms through spatial competition. The competition mechanism occurs because the endophytic fungi from *Capsicum frutescens* L. outgrow the pathogen present in the PDA medium, preventing the *Colletotrichum sp*. pathogenic fungus from having space to grow. This is supported by Kusumawardani et al (2015), stating that the competition mechanism can be shown by slower pathogen radius growth. Additionally, this statement is supported by Octariana (2011), indicating that the competition mechanism of endophytic fungi against pathogens inhibits pathogenic growth as they lack space to live.

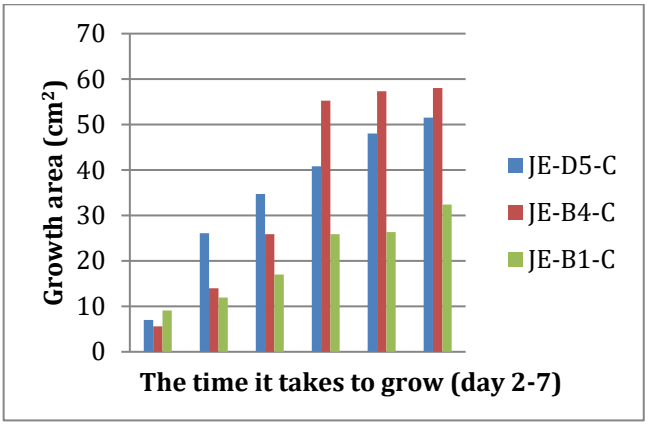

Figure 5. Growth area of endophytic fungi colonies with the pathogen cultivated in a single PDA medium for 7 days

Figure 5 shows that the growth area of endophytic fungi colonies for each isolate differs. This is in line with the statement by Sunarwati & Yoza (2010), who mention that the difference in the surface area of mold on media is an indication of competition mechanisms for space and food. Another statement by Soesanto (2008) suggests that each biological agent has different inhibitory abilities and mechanisms, leading to differences in growth area. The growth area of endophytic fungi against the pathogen in Figure 5 indicates that the JE-B4-C isolate has the highest growth area on the 7th day at 58.04 cm2, while the lowest growth area is found in the JE-B1-C isolate at 32.40 cm2. These results suggest that the JE-B4-C isolate has a higher growth area for endophytic fungi colonies. This is in line with Tan & Zou (2011), who state that endophytic fungi generally have rapid growth and are commonly used as biological agents or to inhibit pathogenic growth. Moreover, the size of the growth area of endophytic fungi colonies indicates their ability to compete with pathogenic fungi, meaning a larger growth area of endophytic fungi or biological agents signifies a greater ability to compete with pathogens.

4. Conclusion

Based on the research findings, it can be concluded that there are three isolates obtained from chili pepper plants (*Capsicum frutescens* L.), namely JE-B4-C, JE-B1-C, and JE-D5-C. The antagonistic activities of these three isolates exhibit a spatial competition mechanism. JE-B4-C, in particular, shows higher inhibition percentages and surface areas compared to the other isolates. It is suspected that JE-B4-C may belong to the genus *Eupenicillium*, while JE-D5-C and JE-B1-C are presumed to belong to the genera Aspergillus and *Neoscytalidium*, respectively.

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