

## Antagonism *Trichoderma harzianum* Rifai in Suppressing the Intensity of Antraknosa (*Colletotrichum capsici* Sydow.) Disease

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### Abstract

Cayenne pepper (*Capsicum frutescens* L.) is a horticultural plant with considerable commercial worth and nutritional value. Cayenne pepper is one of the most important horticultural products at the national level, thus its production must be increased adequately. However, the obstacle of cayenne pepper production caused by anthracnose disease is a frequent stumbling block in the manufacturing of cayenne pepper. Anthracnose disease or fruit rot caused by the fungus *Colletotrichum capsici* Sydow can result in yield losses ranging from 20-90%. *Trichoderma harzianum* Rifai. is a soil saprophytic fungus that naturally can be used as a biological agent, because it has antagonism against pathogens in the form of competition for space and nutrients, mycoparasites and antibiosis. The experimental results in the laboratory showed that the biological agent *Trichoderma harzianum* Rifai was able to suppress the development of anthracnose disease.

**Keywords:** *Trichoderma harzianum*; biological agency; *Colletotrichum capsici*

### Introduction

Because Indonesian people are the most avid consumers of chili in the world, chili is one of the most essential components of traditional Indonesian cuisine (Paulus & Ellen, 2016). Cayenne pepper is widely used as raw material for the food industry such as sauces, chili powder, and the pharmaceutical industry (Sofiarani & Ambarwati, 2020). In addition, cayenne pepper is consumed in fresh or processed form which is generally used as an additional ingredient and flavoring in cooking to enhance the taste of food.

Because of its spicy taste and distinctive aroma, cayenne pepper is favoured with the substance capsaicin that

gives chili its spiciness. Capsaicin is useful for regulating blood circulation; strengthening the heart, pulse, and nerves; preventing flu and fever; raising enthusiasm in the body; and reducing rheumatism (Paulus & Ellen, 2016).

In Indonesia, the demand for cayenne pepper is rising in line with the country's population growth and the wide range of dishes and menu items that include this flavor (Irfandi, *et.al*, 2021). Various attempts to boost cayenne pepper production in Indonesia have been made in the past and continue to be made in the present to suit local consumption demands. Efforts to enhance cayenne pepper output often meet roadblocks, one of which is the interruption

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of cayenne pepper production, both in quality and quantity, caused by anthracnose disease.

Anthracnose or fruit rot is one of the important diseases in cayenne pepper plants caused by the fungus *Colletotrichum capsici* Sydow (Habibi & Wijayanto, 2019). Plants infected with *Colletotrichum capsici* can cause symptoms on the fruit in the form of concave circular spots that are brown in color in the center and light brown around the circle. The expansion of the spots during fruit growth causes the fruit to rot and dry up (Ministry of Agriculture, 2019) [i]. This disease can cause yield losses in cayenne pepper plants, which are estimated to range from 20-90%, especially during the rainy season (Agricultural Research and Development Agency. Anthracnose Disease Control in Chili Plants, 2016).

To prevent losses, efforts must be made to control anthracnose. The most common method of control used by farmers is the application of synthetic fungicides, often to combat anthracnose. Farmers use fungicides with active ingredients propineb, mankozeb, and benomyl. However, continual and irresponsible use of synthetic fungicides will leave residues on plants, harm the environment, and be hazardous to human health (Amelia, Marsuni, & Budi, 2020). Because using synthetic fungicides gives negative impact for the environment, one way to stop plant diseases without harming the environment is to use biological agents; for instance, by using fungi that are antagonistic to *Colletotrichum capsici*.

*Trichoderma harzianum* Rifai. is an antagonist fungus that naturally can be used as a biological agent, because it has antagonism against pathogens in the form of

competition for space and nutrients, mycoparasites and antibiosis. This mushroom is frequently used in the control of plant-disturbing organisms (Safitri, Martina, & Roza, 2019). In addition, this fungus also offers numerous benefits, including simple isolation, broad adaptability, easy detection in the planting area, the capacity to grow swiftly on varied substrates, a diverse spectrum of microparasitism, and the fact that it is not pathogenic to plants (Ministry of Agriculture, 2019).

### Research Methods

The research was carried out in Neglasari Village, Ciherang Village, Pacet District, Cianjur Regency. The region is at an elevation of 1,080 - 2,962 masl, with an average temperature ranging from 17°C to 26°C.

The following tools were utilized in the laboratory: autoclave, pan, spatula, measuring cup, erlenmeyer, petridish, testtube, scales, aluminum foil, newspaper, ose needle, bunsen, petri dish, lighter, microscope, cover glass, object glass, plastic clip, scissors, ruler, neubauer improve type haemacytometer, analytical weighing instrument, stove, laminar air flow, dropper, paper disc, cotton, newspaper, sampling spoon, newspaper, scissors, stationery and documentation tools.

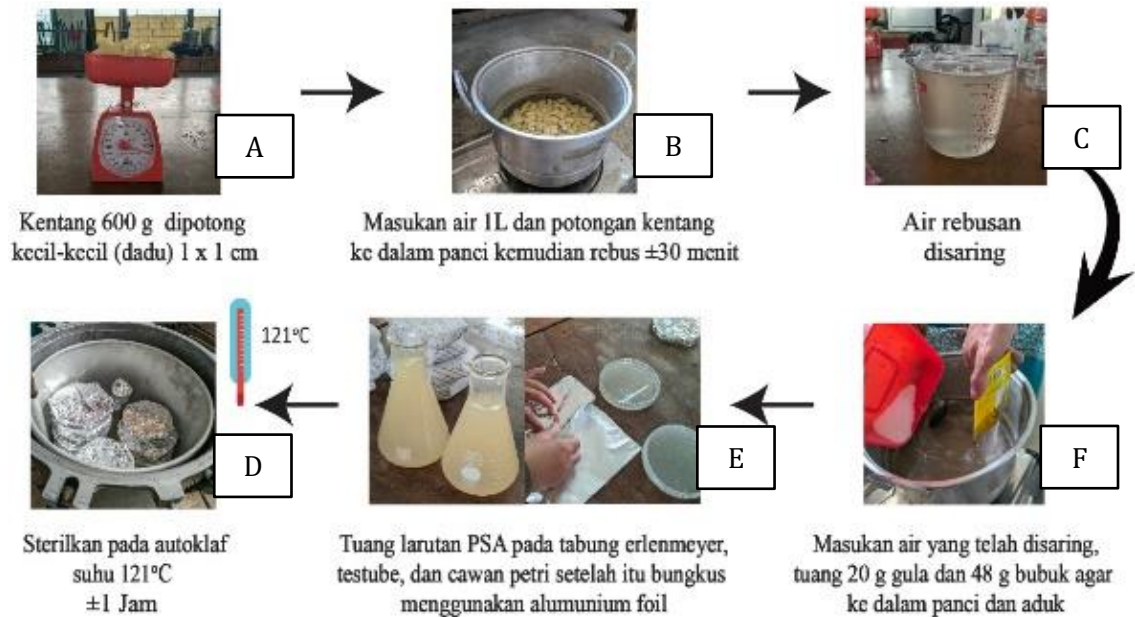
The ingredients were as follows: NaOCl, 70% alcohol, distilled water, PSA (Potato Sucrose Agar), *Trichoderma harzianum* Rifai isolate. rice. The materials utilized in the field are listed below: Manure, NPK fertilizer, compost, husk charcoal, Rawit Chilli Seeds of the Sihang Variety, *Trichoderma harzianum* Rifai culture.

## Search Methods

### Making PSA (Potato Sucrose Agar) Media

**Figure 1**

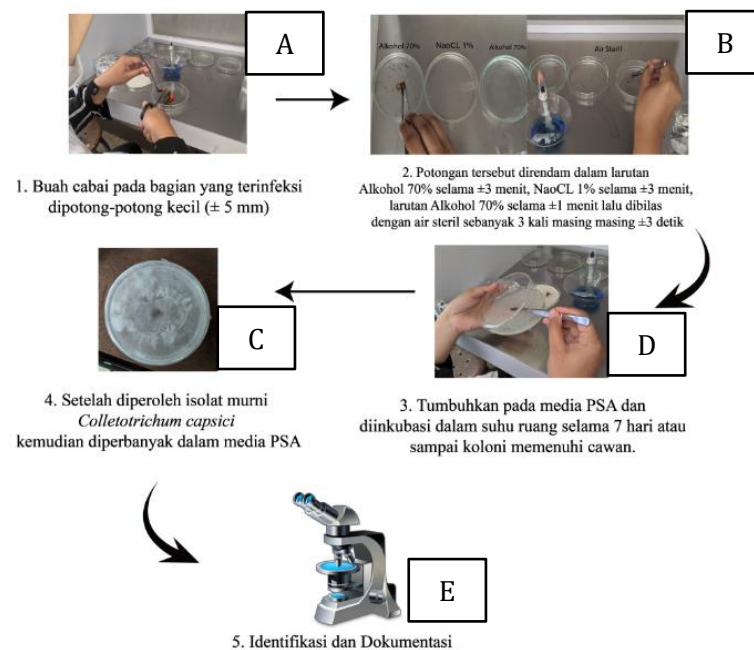
*Making PSA (Potato Sucrose Agar) Media*



### Isolation and Identification of Pathogens that Cause Disease

**Figure 2**

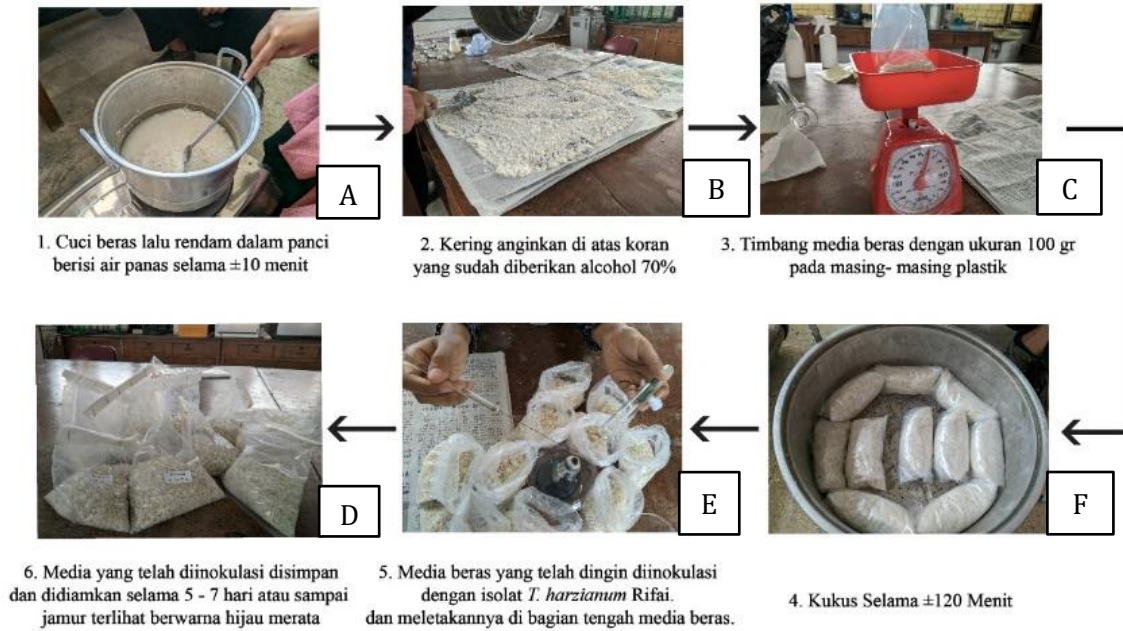
*Isolation and Identification of Pathogens that Cause Disease*



### Propagation of *Trichoderma harzianum* Rifai. on Rice Media

**Figure 3**

*Propagation of Trichoderma harzianum Rifai. on Rice Media*



### Calculating Spore Density

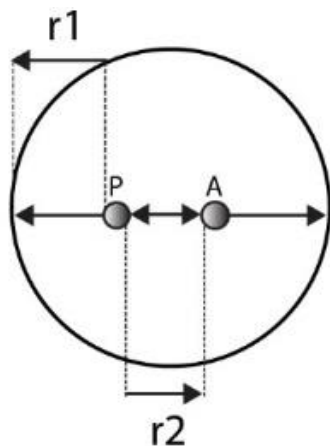
#### Trichoderma harzianum Rifai Antagonist Test

Antagonism test against *Colletotrichum capsici* was carried out in vitro by placing the two fungal colonies facing each other with a distance of 3 cm as shown in Figure 4 below.

Subsequently, it was incubated at room temperature and observed for its inhibitory power for  $\pm 7 \times 24$  hours.

**Figure 4**

*Multiple Culture Test on Antagonist Test of Trichoderma harzianum Rifai*



After inoculation, observations were taken daily by measuring the radius of the fungal colonies from the first day to the fifth day. Measurements were made using a millimeter ruler. The percentage growth inhibition data is calculated based on the following formula (Ningsih, Hastusi, & Listyorini, 2016):

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

Information:

R = Percentage of growth inhibition (%)

R1 = radius of the pathogenic colony away from the antagonist fungal colony

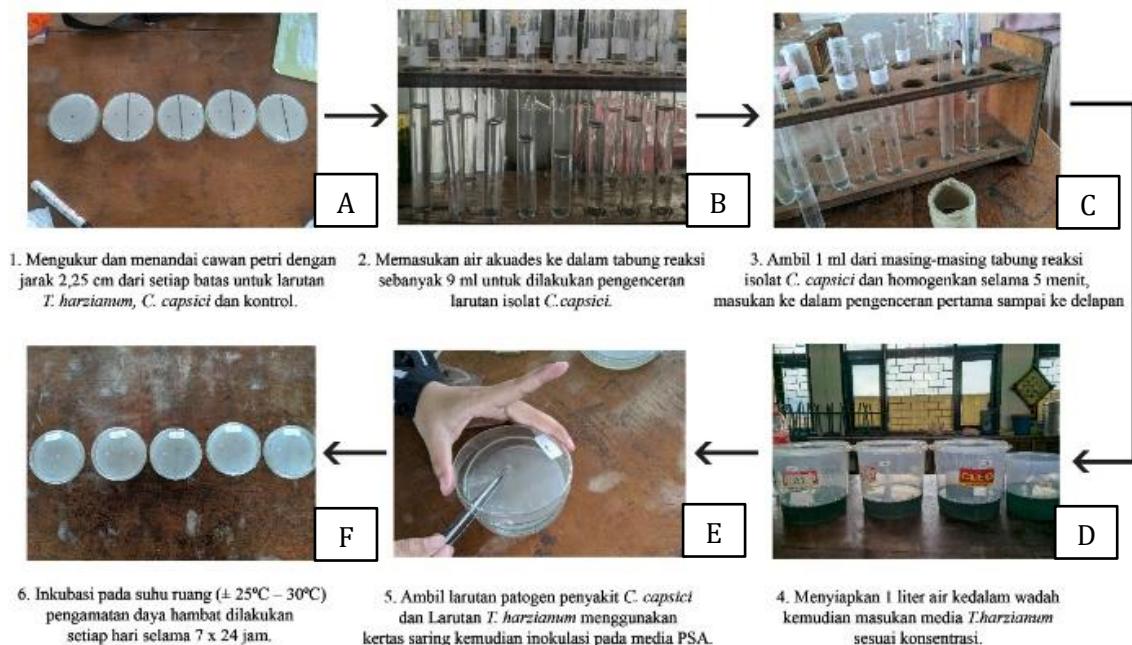
R2 = radius of the pathogenic colony approaching the antagonist fungal colony

**Concentration Test**

The concentration test was carried out to determine the range value of several concentrations of *Trichoderma harzianum* Rifai applications. against the intensity of the attack of the pathogen *Colletotrichum capsici* in vitro and will be used as a treatment for the application of *Trichoderma harzianum* Rifai on cayenne pepper plants. In vivo, the concentrations of *Trichoderma harzianum* Rifai tested were 10 g/l, 15 g/l, 20g/l and 25 g/l. The concentration test is carried out with the following working steps:

1. Measure each petri dish with a distance of 2.25 cm from each boundary for *Trichoderma harzianum* Rifai solution. and *Colletotrichum capsici* isolates and controls. Then mark the measured cup using a marker and ruler.

**Figure 5**  
*Trichoderma harzianum* Rifai . Concentration Test





2. Add 9 ml of distilled water into each test tube, except for the parent test tube, which is 10 ml for dilution of the *Colletotrichum capsici* isolate solution.
3. After that, take 1 ml from each test tube from *Colletotrichum capsici* isolate and homogenize for 5 minutes then put it into the first dilution, continue to do it until the eighth dilution
4. Prepare 1 liter of water in each measuring cup, then put the *Trichoderma harzianum* Rifai media. Into the cup according to concentration.
5. Take a solution of the disease pathogen *Colletotrichum capsici* and *Trichoderma harzianum* solution using filter paper and then inoculate it on PSA media.
6. Incubate at room temperature ( $\pm 25^{\circ}\text{C} - 30^{\circ}\text{C}$ ), the inhibition was observed every day for 7 x 24 hours. observe and calculate the effectiveness of the inhibition level by measuring the diameter of the mycelia growth.

## Results and Discussions

### Trichoderma harzianum Rifai Antagonist Test

The results of the Antagonist *Trichoderma harzianum* Rifai against *Colletotrichum capsici* Sydow disease using the dual culture method shows that the biological agent *Trichoderma harzianum* Rifai. effect in inhibiting the growth of *Colletotrichum capsici* Sydow up to 81% on day 5 after incubation. This result can be shown in the table 1

**Table 1**

*Antagonist Test Results (Average percentage inhibition of the antagonist fungus Trichoderma harzianum Rifai. against Colletotrichum capsici Sydow.)*

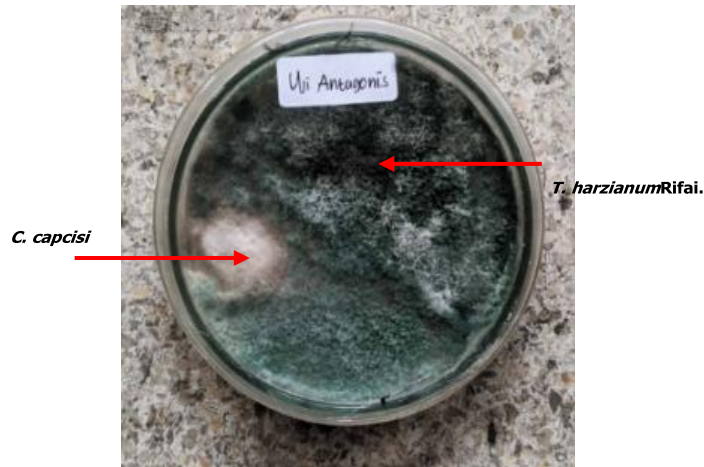
Day Observation -	Average Percentage of Inhibition (%)
Day - 1	-
Day 2	66%
Day - 3	75%
Day - 4	80%
Day 5	81%

The inhibition of the antagonist fungus *Trichoderma harzianum* Rifai against the fungus *Colletotrichum capsici* occurs by several mechanisms, including competition for space and nutrients between pathogens where *Trichoderma harzianum* Rifai can suppress the development of pathogens in their life cycle, therefore the nutrients needed by pathogens to thrive are not met. Furthermore, the mechanism of parasitism is able to wrap around the hyphae of

pathogenic fungi. Together with the coiling of the hyphae, enzymes are released that are able to remodel the cell walls of the hyphae of pathogenic fungi. The next mechanism is Antibiosis, in which it not only produces fungal cell wall enzymes, but also produces antibiotic compounds belonging to the furanon group which can inhibit the growth of spores and hyphae of pathogenic fungi (Ningsih, Hastusi, & Listyorini, 2016) (Suwahyono & Wahyudi, 2004).

**Figure 6**

*Test Results of Trichoderma harzianum Rifai Antagonists. against the fungus Colletotrichum capcisi Sydow*



**Concentration Test *Trichoderma harzianum* Rifai.**

The results of an in vitro concentration test with *Trichoderma harzianum* Rifai at 4

different concentrations are shown in table 2

**Table 2.**

*Concentration Test Results of Trichoderma harzianum Rifai.*

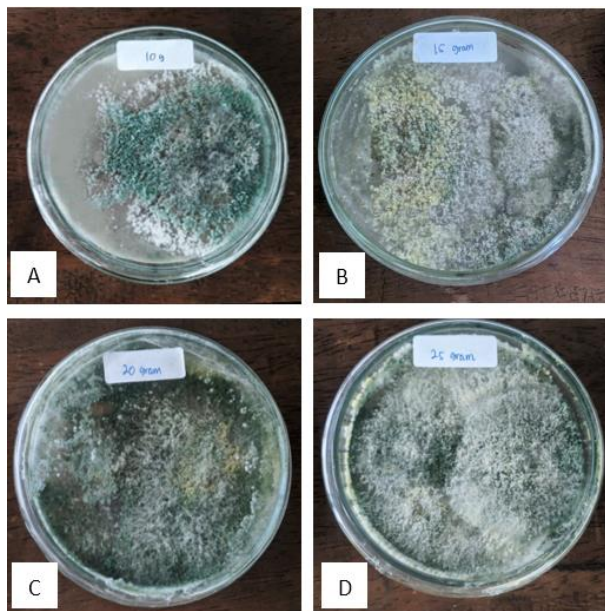
Concentration of <i>Trichoderma harzianum</i> Rifai.	Observation of Day to Concentration Test -					
	1 HSI	2 HSI	3 HSI	4 HSI	5 HSI	6 HSI
10 grams/liter of water	1.7 cm	2.3 cm	3.5 cm	4 cm	5 cm	6.5 cm
15 grams/ liter of water	1.7 cm	3 cm	4.5 cm	5 cm	6.5 cm	7.5 cm
20 grams/ liter of water	1.8 cm	3 cm	4.5 cm	5.5 cm	7 cm	7.8 cm
25 grams/ liter of water	1.8 cm	3 cm	5 cm	6.5 cm	7.8 cm	9 cm

The results of the determined concentration test showed that at 1 DAI to 6 DAI the inhibition of *Trichoderma harzianum* Rifai was observed. In the four tests, namely 10 grams; 15 grams; 20 grams;

25 grams, the inhibitory power is not much different, but 25 grams/liter of water is a high concentration of inhibition compared to a concentration of 10 grams; 15 grams; 20 grams; 25 grams with the growth of hyphae diameter reaching 9 cm.

### Figure 7

Concentration Test Results of *Trichoderma harzianum* Rifai. 10 grams (A). 15 grams (B). 20 grams (C). 25 grams (D).



### Conclusions

The biological agent *Trichoderma harzianum* Rifai is able to inhibit the growth of *Colletotrichum capsici* Sydow up to 81% on day 5 after incubation. The results of the concentration test showed that on the fourth day, the highest concentration was 25 grams with the growth of hyphae diameter reaching 9 cm.

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