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Various Applications of Active Materials *Trichoderma* sp. RC 3 Isolate for Antracnosa Disease Control Caused by *Colletotrichum gloeosporioides* in Chilli

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A B S T R A C T

Anthracnose, caused by the fungal pathogen *Colletotrichum gloeosporioides*, poses a significant threat to chili plant cultivation, resulting in yield losses and environmental concerns. This study aimed to obtain the best active ingredients from *Trichoderma* sp. RC3 isolates to control anthracnose diseases caused by *C. gloesporoides* in chilies. The research used a Randomized Block Design (RBD) with four treatments and three replications, each repetition consisting of 9 chilies. The treatments used were Control (sterile aquadest/ without isolates), administration of liquid culture, conidia suspension, and filtrate from *Trichoderma* sp. RC3 isolate. The variables observed were the incubation period, the percentage of fruit attacked, and the intensity of anthracnose disease in chilies. The results showed that the active ingredient was a filtrate containing secondary metabolites from Trichoderma sp. RC3 isolate was the best treatment with an effective disease intensity of 58.45%, the percentage of fruit attacked by 44.45%, and slowed the appearance of the first symptoms (incubation period) for 13 days. These findings underscore the potential of *Trichoderma* sp. RC3 isolates are an eco-friendly solution for anthracnose management in chili plants, offering promise for sustainable disease control in agriculture.

INTRODUCTION

The productivity of chili plants in West Sumatra is still relatively low; in 2019, it was only 11.006 tons/ha (BPS Provinsi Sumatera Barat, 2019). This condition is still far from the potential productivity of chili, which can reach 20-30 tons/ha (Syukur et al., 2010). One of the obstacles to increasing chili production is *the infection of Colletotrichum gloeosporioides,* which causes anthracnose disease and can cause high yield losses (Rohmawati, 2002). In Indonesia, three species of Colletotrichum are associated with anthracnose on chili pepper: *C. acutatum*, *C. capsici*, and *C. gloeosporioides* (Widodo and Hidayat, 2018).

Some standard methods of controlling *C. gloeosporioides* are technical culture and using chemicals. The continuous use of chemicals can harm the environment. An environmentally friendly alternative control is needed to avoid these negative impacts, namely using biological control by utilizing fungi antagonistic to pathogens.

One of the antagonistic fungi reported to inhibit various causes of plant pathogenic fungi successfully is *Trichoderma* spp. in strawberries, and *Phytophthora infestans* causes fruit rot in potato plants. Nurbailis and Martinius (2015) reported that out of 9 antagonistic fungi isolates from the chili rhizosphere. Three isolates could potentially inhibit the growth of *C. gloeosporioides* in chili: *Trichoderma* RC1 isolate, *Trichoderma* RC3 isolate, and *Paecilomyces* RC1 isolate.

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The active ingredient used influences the effectiveness of antagonistic fungal applications against pathogens. The active ingredient will determine whether or not a biological agent effectively suppresses the growth of pathogens. Active ingredients that can be used include conidia suspension, liquid culture, and filtrate. Conidia suspension contains conidia produced by the fungus during its growing period. Liquid culture in the form of mushroom cultures propagated on liquid media. Liquid culture contains fungal cultures and metabolites produced during the incubation period. The filtrate results from the propagation of fungi in the liquid medium during the incubation period and is then separated between the fungal cells and the supernatant (filtrate). The filtrate contains metabolites produced by fungi during the propagation process (Nurbailis, 2008).

Some researchers have reported that *Trichoderma* produces various antibiotics that can inhibit the growth of pathogenic fungi. Dermadine is a product of this antagonist, an active unsaturated acid against many fungi and gram-positive and negative bacteria. Suzukacillin and Alamethicine, which have been registered, are peptides produced by *Trichoderma* with antifungal and bacterial properties (Meyer and Reusser, 1967). Trichoderma species are known for their ability to produce lytic enzymes, such as exoglucanases, endoglucanases, chitinases, and proteases, which play important roles in cell wall degradation of phytopathogens (Ribeiro et al., 2019). Chitinase enzymes degrade pathogenic fungal cell walls and inhibit spore germination from fungi (Daguerre et al., 2014; Lorito et al., 1993). The purpose of this study was to obtain the active ingredient *Trichoderma* sp. RC3 isolates are the best for controlling anthracnose diseases caused by *C. gloeosporioides* in chili.

METHODS

This research used a randomized block design (RBD) consisting of 4 treatments with three replications, and each repetition consisted of 9 chili plants. The treatment consists of:

A = Control (sterile aquadest)
B = Liquid culture of *Trichoderma* sp. RC3 isolate
C = Konidia Suspension *Trichoderma* sp. RC3 isolate
D = Filtrate *Trichoderma* sp. RC3 isolate

Data were analyzed using variance and, if significantly different, continued with Tukey's test at a 5% level.

Fungal propagation

Fungal isolate *Trichoderma* sp. and *C. gloeosporioides* used came from the collection of Phytopathology Laboratories in the Department of Pests and Plant Diseases. *Trichoderma* and *C. gloeosporioides* fungi were propagated in PDA media.

Preparation of various active ingredients Trichoderma sp. RC3 isolates

Conidia suspension

Trichoderma sp. RC3 isolates were reproduced in PDA media using Petri dishes and then incubated for 14 days. The conidia formed were removed by adding 10 ml of sterile distilled water to the petri and then brushed using a soft brush. The suspension was transferred into a test tube and homogeneous using a vortex. After that, 1 ml of the suspension was taken using a dropper pipette to calculate the number of conidia at a density of 106 / ml using the Improved Neubauer Nesco Haemocytometer.

Liquid culture

Trichoderma sp. RC3 isolates incubated for 14 days were reproduced in liquid media (PDB). A total of 150 ml of Potato Dextrose Broth (PDB) media was put into a 250 ml Erlenmeyer, then added with pieces of the fungal culture of *Trichoderma* sp. RC3 isolates have a diameter of 5 mm. Incubation was conducted on a rotary shaker at 180 rpm for three days (Nurbailis, 2008). The liquid culture of *Trichoderma* sp. RC3 isolates can be seen in Figure 1.

Figure 1. Liquid culture of *Trichoderma* sp. RC3 isolate after incubation for three days in a Rotary shaker with a speed of 180 rpm.

Fungal filtrate of Trichoderma sp. RC3 isolate

Trichoderma sp. RC3 isolates incubated for 14 days were reproduced in liquid media (PDB). 150 ml of PDB media

was put into a 250 ml Erlenmeyer, then added with pieces of the fungal culture of *Trichoderma* sp. RC3 isolate with a diameter of 5 mm. Incubation was conducted on a rotary shaker at 180 rpm for three days (Nurbailis, 2008). The results of fermentation in the form of liquid culture were obtained by separating the liquid culture between cells and filtrate using a centrifuge of 2000 rpm for 30 minutes, separating between fungal cells and filtrate using filtrate filter paper into centrifuge tubes. The filtered filtrate was centrifuged at a speed of 2000 rpm for 30 minutes and then filtered. Filtering was conducted using a millipore membrane filter measuring 0.2 µm to completely separate the cell from the filtrate. *Trichoderma* sp. RC3 isolates can be seen in Figure 3.

Preparation of chili plants

Soil preparation

Chili growing media is a mixture of soil and manure with a ratio of 2:1. The soil was put into a 10 kg plastic bag and sterilized using a pot for 1 hour.

Chili seed preparation

Chili seeds or seeds are taken from a healthy chili fruit, and the chili fruit is cut into three parts, each of which is the same length. Seeds for seeds are taken from the middle cut; then the cut is split, the seeds are removed, then air-dried until dry. After the chili seeds are obtained, seed selection is carried out to get good chili seeds. Selection is made by putting chili seeds in a bucket and stirring it. In this way, it will be seen that there are seeds that float and those that sink. Floating seeds are seeds that are not good for seed. On the other hand, sunken seeds are filled seeds suitable for seed use.

Seeding and planting

The seeding of chili seeds is carried out on a seed tray containing soil that has been sterilized. Seedling is done by planting the seeds as deep as 0.5 cm. Seeds that have been planted must be protected from direct sunlight and rain. The time for sowing chili is three weeks.

Planting chili seeds is done by transferring chili seeds into 10 kg polybags containing sterile soil. Two chili seedlings were planted in each polybag; the best ones were selected, and one stem was left.

Chili fruit treatment

The chilies that were treated were still on the plant's stem. Each active ingredient of *Trichoderma* sp. isolate RC3 was treated on chili peppers one month after flowering. The application technique is wounding the chili fruit's surface by stabbing it in two parts using a needle. Each of the active ingredients of the fungus *Trichoderma* sp. RC3 isolate was applied as much as 0.5 ml using a soft brush to the surface of the chili fruit. The treatment of chili fruit using the active ingredient of the fungus *Trichoderma* sp. RC3 isolate can be seen in Figure 4.

Figure 4. Treatment of chili fruit using the active ingredient *Trichoderma* sp. Isolate RC3 (A. Chili fruit is pierced using a needle. B. The active ingredient *Trichoderma* sp. isolate RC3 is smeared on the surface of the fruit)

Inoculation of pathogenic fungi C. gloeosporioides

Fungal isolates of *C. gloeosporioides* were rejuvenated and tested for virulence using healthy chilies. Two parts were pierced on the surface of the chili, and then the suspension of *C. gloeosporioides* was applied using a soft brush on the surface of the chili.

After the virulence test, the isolates of *C. gloeosporioides* were multiplied and incubated for 14 days. Pathogen inoculation was carried out after seven days of application of the active ingredient *Trichoderma* sp. Isolate RC3 by making conidia suspension of *C. gloeosporioides*. The conidia formed by the fungus *C. gloeosporioides* were removed by adding 10 ml of sterile distilled water to the petri dish, which was brushed using a soft brush. The suspension was transferred to the test tube and homogenized using a vortex. The suspension was taken at 1 ml using a dropper, and the number of conidia was calculated at a density of 106/ml using an Improved Neubauer Nesco Haemocytometer. The application technique is to apply 0.5 ml of the conidia suspension of *C. gloeosporioides* evenly using a soft brush on the surface of the chili.

Observation

The level of anthracnose disease in chili was observed by observing several parameters, including:

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Incubation period

The incubation period was observed every day after inoculation of the pathogen until day 18. Symptoms of anthracnose on chili can be seen in Figure 5

Figure 5. Symptoms of anthracnose on chili peppers. (A. Early symptoms. B. Late symptoms).

Percentage of fruit attacked

The percentage of infected fruit was observed by counting the number of fruit infected with anthracnose in each treatment. Observations were made from the onset of the first symptoms until 18 days after inoculation, with an interval of 3 days. The percentage of infected fruit can be calculated using the formula:

$$P = \frac{A}{B}\ x\ 100\%$$

P = Level/intensity of anthracnose disease on chili fruit
A = Number of fruits with anthracnose symptoms
B = Total number of fruits, including symptomatic fruit

The effectiveness of each treatment on the percentage of infected fruit can be calculated using the formula:

$$\mathrm{E} = \frac{IK - IP}{IK}$$

E = Suppression effectiveness
PK = Percentage of fruit attacked in control
PP = Percentage of fruit attacked in treatment

Disease intensity

The intensity of the disease was determined by observing the fruit with anthracnose symptoms. Observations started three days after inoculation and continued until 18 days after inoculation, with an interval of 3 days. The intensity of anthracnose disease was determined based on the percentage of affected fruit using the formula for disease intensity (Zadoks and Schein, 1979):

$$\mathrm{I} = \frac{\sum(n\ x\ v)}{N\ X\ V}\ x\ 100\%$$

I = Disease intensity
n = Number of pieces for each attack category
v = Value of each attack category
N = Number of fruits observed
V = The highest scale value

The effectiveness of each treatment on disease intensity can be calculated using the formula:

$$\mathrm{E} = \frac{IK - IP}{IK}$$

E = Suppression effectiveness
IK = intensity of disease in control
IP = intensity of disease in treatment

The attack category (scale) value for anthracnose disease is based on the scale of damage to plants affected by the disease (Herwidyarti, 2011) (modified). The attack category value (scale) is as follows:
Scale 0 = No damage
Scale 1 = Symptoms occur in 1 – 20%
Scale 2 = Symptoms occur in 21 – 40%
Scale 3 = Symptoms occur in 41 – 60%
Scale 4 = Symptoms occur in > 60%

The scale of damage to fruit affected by anthracnose can be seen in Figure 6

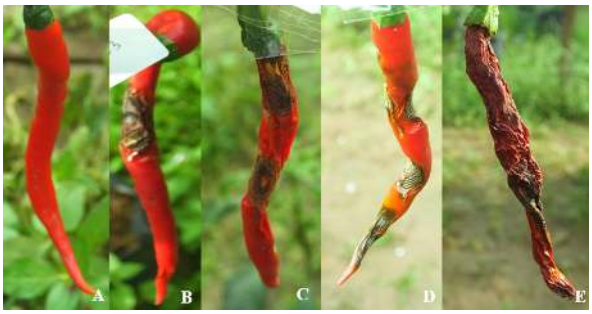

Figure 6. The scale of damage to fruit affected by anthracnose. (A. Scale 0. B. Scale 1. C. Scale 2. D. Scale 3. E. Scale 4)

RESULTS AND DISCUSSION

Incubation period

The incubation period of *C. gloeosporoides* with the treatment of various active ingredients of *Trichoderma* sp. RC3 isolate showed significantly different results. Tukey's test results at a 5% significance level can be seen in Table 1.

Table 1. The incubation period of *C. gloeosporoides* on chili peppers treated with various active ingredients *Trichoderma* sp. RC3 isolate

Treatment	Average incubation period ± sd	Asymptomatic until day 18
Filtrate	13,85 ± 1,22 a	12
Liquid culture	12,00 ± 0,40 ab	5
Conidia suspension	11,66 ± 1,01 ab	1
Control (aquadest)	10,10 ± 0,90 b	0

Table 1 shows that the incubation period of *C. gloeosporioides* on chili peppers was treated with filtrate, liquid culture, and conidia suspension of Trichoderma sp. RC3 isolate was not significantly different, but the filtrate treatment was significantly different from the control and not significantly different from the conidia suspension and liquid culture. In general, the application of the active ingredient Trichoderma sp. isolate RC3 was able to delay the incubation period of *C. gloeosporoides* compared to controls.

Percentage of fruit attacked

Observation of the percentage of fruit attacked by treating several active ingredients of *Trichoderma* sp. RC3 isolate showed significantly different results. Tukey's test results at a 5% significance level can be seen in Table 2.

Table 2. Percentage of chilies that were attacked by *C. gloeosporoides* causing anthracnose disease with various active ingredients of *Trichoderma* sp. RC3 Isolate

Treatment	Percentage of infected fruit ± sd (%)	Suppression effectiveness (%)
Control (aquadest)	100,00 ± 0,00 a	0,00
Conidia suspension	96,29 ± 6,42 a	3,71
Liquid culture	81,47 ± 6,41 ab	18,53
Filtrate	55,55 ± 22,00 b	44,45

The percentage of infected fruit (Table 2) shows the filtrate treatment of *Trichoderma* sp. RC3 isolate was significantly different from the control and conidia suspension, while the filtrate treatment was not significantly different from the liquid culture. The highest fungal attack of *C. gloeosporioides* was in the control treatment, with a percentage of fruit attacked by 100%, while the lowest percentage was in the filtrate treatment, which was 55.55%.

Applying various active ingredients of *Trichoderma* sp. RC3 isolate, in general, can reduce the attack rate, including the incubation period, percentage of attack, and intensity of attack of *C. gloeosporoides,* which causes anthracnose in chili. The filtrate application suppressed more than the liquid culture and conidia suspension. It is due to the filtrate of *Trichoderma* sp. isolate RC3 containing secondary metabolites from various compounds, including enzymes and antibiotics. Enzymes produced by Trichoderma can destroy the cell walls of the hyphae of pathogenic *C. gloeosporioides*, which contain chitin and cellulose. Several researchers have reported that Trichoderma can produce lytic enzymes such as B. 1.3 glucanase and chitinase, which play a role in degrading the cell wall of pathogenic fungi. This enzyme can also inhibit spore germination (Daguerre et al., 2014; Lorito et al., 1993).

Other secondary metabolites produced by Trichoderma are antibiotics that inhibit the growth of various pathogenic fungi. From the in vitro test results, it can be detected that *Trichoderma* sp. RC3 isolate showed an antibiosis mechanism against *C. gloesporoides* with an inhibition zone between the two fungal colonies meeting in the double culture method test. It indicates that *Trichoderma* sp. RC3 isolate produces a compound that can inhibit the growth of the fungus *C. goeosporoides*. Several researchers have reported that Trichoderma produces various antibiotics that can inhibit the growth of pathogenic fungi. Meyer and Rousser (1967) stated that Dermadin is a product of the fungal antagonist Trichoderma, an unsaturated acid active against a wide range of gram-positive and gram-negative fungi and bacteria.

Disease intensity

The intensity of anthracnose on chili peppers with the treatment of various active ingredients of the fungus *Trichoderma* sp. RC3 isolates showed significantly different results. Tukey's test results at the 5% significance level can be seen in Table 3.

Table 3. The intensity of anthracnose on chili peppers with treatment of various active ingredients of *Trichoderma* sp. RC3 isolate.

Treatment	Disease intensity ± sd (%)	Suppression effectiveness (%)
Control (aquadest)	46,75 ± 6,07 a	0,00
Conidia suspension	34,25 ± 3,21 b	26,73
Liquid culture	32,40 ± 3,21 b	30,69
Filtrate	19,44 ± 4,81 c	58,45

The intensity of anthracnose on chili peppers with filtrate treatment significantly differed from the control, conidia suspension, and liquid culture. The highest fruit suppression effectiveness in the treatment filtrate was

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58.45%, while the effectiveness of the liquid culture treatment and conidia suspension was lower at below 50%.

Treatment with filtrate of *Trichoderma* sp. RC3 isolate showed higher suppression effectiveness than conidia suspension and liquid culture. The low effectiveness of suppression in treating liquid culture and conidia suspension is thought to be due to the conidia of *Trichoderma* sp. RC3 isolate could not grow and develop appropriately on chilies due to unsuitable environmental conditions and nutritional sources. According to Kredics et al. (2004), the growth and development of Trichoderma on its substrate were significantly influenced by the availability of nutrients..

The rate of anthracnose development caused by *C. gloeosporioides* increased every day of observation. The rate of disease development in each treatment generally increased after the sixth day. The liquid culture treatment experienced a drastic increase after the 12th day. The anthracnose development rate on chili peppers can be seen in Figure 7.

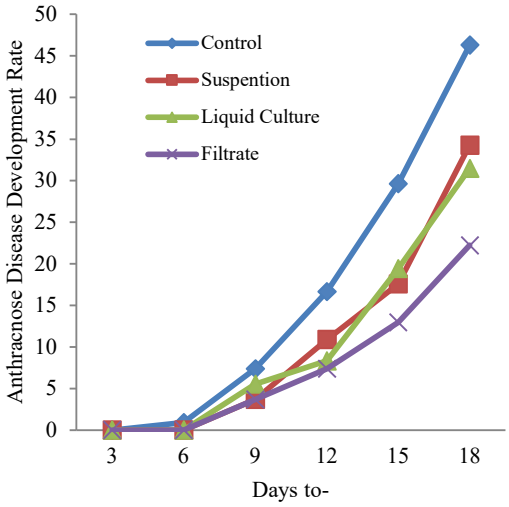

Figure 7. The rate of development of anthracnose on chili peppers with various active ingredients treatment of *Trichoderma* sp. RC3 isolate

In general, the rate of development of anthracnose in chili peppers was inhibited by the treatment of various active ingredients of *Trichoderma* sp. RC3 isolates were compared with controls. It is because all the active ingredients of Trichoderma can inhibit the growth of *C. gloesporoides* by various mechanisms such as competition, hyperparalysis, and antibiosis.

CONCLUSIONS

The results showed that the active ingredient was a filtrate containing secondary metabolites from *Trichoderma* sp. RC3 isolate was the best treatment with an effective disease intensity of 58.45%, the percentage of fruit attacked by 44.45%, and slowed the appearance of the first symptoms (incubation period) for 13 days. From the results of this study, it can be concluded that the active ingredient of *Trichoderma* sp. RC3 isolate is in the form of a filtrate containing various secondary metabolites that are effective for controlling anthracnose caused by *C. gloeosporioides* in chili*es*.

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