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Short Communication: Effectiveness of cinnamon leaf extract to control anthracnose disease on large chilies in Bali, Indonesia

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Abstract. *Darmadi AAK, Suriani NL, Ginantra IK, Sudirga SK. 2022. Short Communication: Effectiveness of cinnamon leaf extract to control anthracnose disease on large chilies in Bali, Indonesia. Biodiversitas 23: 2859-2864.* The use of synthetic fungicides for plants is very dangerous because it has a negative impact on the environment. In the context of implementing eco-friendly, sustainable agriculture, efforts need to be made to reduce the negative impact caused by the use of synthetic fungicides. This study aimed to test the effectiveness of a crude extract of cinnamon (*Cinnamomum burmannii* Blume) leaves, observe the inhibition effect on fungi by scanning electron microscope, and test the formulation of cinnamon leaf extract against anthracnose disease on large chilies in the field. Cinnamon leaf crude extract showed inhibition zone of 35 mm diameter against *Colletotrichum acutatum.* The extract showed strong inhibition so further used as biofungicide in field experiments. Based on scanning electron microscopy, cinnamon leaf extract can damage to the fungal cell wall. Cinnamon leaf extract formula was found effective in reducing disease percentage and intensity of anthracnose disease in experimental fields. In the treatment, a concentration of 0.5% to 1.5% can inhibit the percentage of disease ranging from 29.4% to 100%. Concentrations of 0.5% to 1.5% can prevent disease severity from 35.8% to 100%.

Keywords: Antifungal, anthracnose, cinnamon leaf extract, *Colletotrichum acutatum*, *Capsicum annum*

INTRODUCTION

Anthracnose is one of the most common plant diseases that attacks large chili plants (*Capsicum annum* L.) in Bali. This disease can inhibit the growth and production of chili plants. Based on a survey conducted in March and April of 2018, the average percentage of anthracnose was 63% and the intensity of disease was 68%. *Colletotrichum acutatum* Simmonds is one of the fungi that cause anthracnose in chili plants in Bali. Other fungi that cause anthracnose disease are *Colletotrichum scovillei*, *Colletotrichum gloeosporioides, Colletotrichum nymphaeae*, *Colletotrichum fructicola* and *Colletotrichum truncatum* (Khalimi et al. 2019). The pathogen that causes fruit rot in chili plants, may attack many chili plants grown in lowland and highland areas. Symptoms of anthracnose disease include, at first a blackish brown spot is formed on the chili fruit, and over time it expands to soft rot. In the middle of the spot there are black spots consisting of seta and conidia. The affected fruit changes color from red to dry brown (Saxena et al. 2016; Ridzuan et al. 2018). According to Enyiukwu et al. (2021), anthracnose that attacks plant cells will cause these cells to lose important nutrients so that they become turbid, devoid of turgidity and vigor.

Synthetic fungicides used to control plant diseases caused by fungi are very harmful to the environment. Such fungicides become resistant to pathogen, which is not easy to decompose and can cause death of non-target organisms (Nicolopoulou-Stamati et al. 2016; Tudi et al. 2021). Synthetic fungicide residues are toxic to consumers, both animals and humans. Biofungicides are fungicides produced from natural ingredients, namely, roots, stems, branches, flowers, fruit, seeds, or leaves of plants. Extracts from these plant parts can be used to control plant diseases naturally. Plant extracts may contain phytochemical compounds that act as antifungal compounds. Plant extracts have several advantages, such as being economical, easy to decompose, and environmentally friendly (Shivanna and Garampalli 2014).

Several studies have been reported on the resistance of plant extracts to pathogenic fungi, such as resistance of *Ficus septica* leaf extract against anthracnose disease that attacks chili plants. This extract can control the growth of *C. acutatum in vitro*, a fungus that causes anthracnose disease in chili plants in Bali (Sudirga et al. 2014). The cinnamon plant (*Cinnamomum burmannii* Blume) has a tree habit and can even reach 50 meters in height. This plant can grow well at an altitude of 500 to 2500 m above sea level. Due to the aroma of cinnamon, its bark is widely used in the perfume industry, in cooking ingredients, and in medicine (Rao and Gan 2014; Błaszczyk et al. 2021). The use of *C. burmannii* for traditional medicinal ingredients is still limited to 4 ethnic groups, namely Sumba, Ternate, Flores and South Kalimantan (Hidayat et al. 2021).

Cinnamon contains phytochemical compounds such as cinnamaldehyde and trans-cinnamaldehyde, contributing to

its fragrance and various biological activities. Cinnamon bark oil, which is registered by European Phytomedicine, is used in teas for its anti-bacterial and fungi-killing properties. Darmadi et al. (2019) reported that cinnamon leaves can control Sigatoka disease attacking banana plants. Sigatoka disease is caused by the fungus *Pseudocercospora fijiensis*. This disease causes blackish brown spots on banana leaves. Cinnamon leaves can significantly inhibit the growth of spores and fungal colonies *in vitro*. At 0.1-0.3% concentration treatment, it could inhibit the growth of spores and fungal colonies by 52.6-89.5% and 65.3-86.9%, respectively. At 0.4-0.5% cinnamon leaves can inhibit the growth of fungal colonies and spores with each inhibition of 100%. At a concentration of 0.4% and 0,5%, the fungus died. Darmadi et al. (2017; 2021) have reported that the active compounds found in cinnamon leaf extract are azulene, phenol, and cinnamaldehyde compounds. These compounds have antifungal activity.

Therefore, the objectives of this study were: i) to make a formulation of cinnamon leaf extract, ii) to test the effectiveness of the extract against *C. acutatum in vitro*, iii) to observe the mechanism of inhibition of the extract against fungi by scanning electron microscope, iv) and application of cinnamon leaf extract on large chili plants affected by anthracnose disease.

MATERIALS AND METHODS

Extraction of cinnamon leaves

The cinnamon leaves (*C. burmannii* Blume) were collected from Belok village, Petang district, Badung-Bali. The cinnamon leaves used were the fourth to ninth leaves from the tip. The leaves were chopped into small pieces and then dried for 3 days at room temperature. Then, leaves were blended until they turned into fine powder. Leaf powder was macerated in methanol at a ratio of 1:10 (w/v) for 48 hours. The filtrate was obtained by filtration through four layers of gauze and Whatman filter paper no. 2. To obtain the crude extract, filtrate was evaporated using a vacuum rotary evaporator at 40°C temperature (Sudirga et al. 2014).

In vitro testing

A 200 µL spore suspension of *C. acutatum* was inoculate into a Petri dish and then poured 10 mL of Potato Dextrose Agar (PDA) medium at a temperature of around 40-45°C. The Petri dishes were shaken horizontally so that the fungus suspension was evenly mixed. After solidification, two diffusion wells with a diameter of 5 mm were made in each Petri dish using a cork borer. Each diffusion well was filled with 20 µL of cinnamon leaf crude extract with a concentration of 100%. The plates were incubated in the dark at room temperature and the formation of inhibition zones around the diffusion wells was observed for five days.

Observation of the inhibitory effect of cinnamon leaf extract against *Colletotrichum acutatum* by scanning electron microscopy (SEM).

For this, 1 mL of fungal spore suspension and 5 g of crude extract of cinnamon were transferred to erlenmeyer flasks and then 50 mL of potato dextrose broth (PDB) was added. The erlenmeyer flask was shaken horizontally to mix all the ingredients evenly. The erlenmeyer flask was placed into a shaker and incubated at 24°C for seven days. The fungal biomass was filtered using tissue paper and placed in an oven, then weighted until the fungi biomass was constant. The same approach was performed without using the extract (control). After that, the sample was prepared for scanning electron microscopy (SEM) (Darmadi et al. 2021).

Field experiment

The field research was carried out at the Experimental Garden of the Biology Study Program, FMIPA University of Udayana, located on the Bukit Jimbaran Campus, Badung-Bali, Indonesia. The experiment was performed in a Randomized Block Design (RBD) consisting of six treatments, namely: F0=control without extract, F1=cinnamon leaf extract 0.5%, F2=0.75% cinnamon leaf extract, F3=1% cinnamon leaf extract, F4=1.25% cinnamon leaf extract, F5=1.5% cinnamon leaf extract. Each treatment was repeated four times to form twenty-four experimental units, each consisting of three chili plants in a polybag. The research implementation included seeding, preparation of the planting media, planting seeds, fertilization, plant maintenance, inoculation of *C. acutatum*, application of vegetable fungicides, and harvesting. *Colletotrichum acutatum* was inoculated on plants by spraying fungal spores on chilies 122 days after planting (DAP). To obtain the spores of *C. acutatum*, pure culture of the fungus was filled with 10 ml sterile water, then scraped with a needle, vortexed for one minute and the suspension was filtered with Whatman No. 2 filter paper to separate fungal spores from their hyphae. The obtained fungal spores were then diluted in 1 L of sterile water.

Botanical fungicide formulation of cinnamon leaf extract was made by mixing 100 grams of crude cinnamon leaf extract in one liter of sterile water, then the stock solution was diluted to make various concentrations of 0.5%, 0.75%, 1%, 1.25%, 1.5%. Cinnamon extract was not added in the control treatment (0), but 1% agister and 5% Tween 80 were added. Cinnamon fungicide was applied to large chili plants in the morning or evening using hand sprayers. The first application was performed after twelve hours of inoculation of *C. acutatum* on the fruit of large chili plants. Subsequent applications were carried out every three days, such that the second application was 126 days after planting (DAP), the third application was 129 DAP, the fourth application was 132 DAP, and the fifth application was 135 DAP. The observed parameters included the percentage of disease and the intensity of anthracnose disease caused by *C. acutatum*. Observations were made after three days of treatment application by measuring the percentage and the intensity level of damage (intensity of disease) on chili plants. According to Cruz-

Rodríguez et al. (2020) the calculation of the percentage and intensity of damage level in chili may be represented by using the equation (1) to (4).

$$P = \frac{n}{N} \times 100\% \quad (1)$$

Where:
P : Percentage of disease (%)
n : Number of plants showing symptoms of illness
N : Total number of plants observed

$$RP = \frac{PC-PT}{PC} \times 100\% \quad (2)$$

RP : Inhibiting activity against disease percentage (%) versus control
PC : Percentage of anthracnose in control
PT : Percentage of anthracnose in treatment

$$IP = \frac{\Sigma(n_i.v_i)}{N.V} \times 100\% \quad (3)$$

IP : Disease intensity
ni : Number of chilies/plants in each category
vi : Score of each category
N : Highest score for each category
V : Number of chilies/plants observed

The score or scale graded for each category was rated on a scale from 0 to 5 where, 0 = no attack, 1 = very light attack (0-10% damaged fruit surface), 2 = light attack (10-30% fruit surface damaged), 3 = moderate attack (30-50% damaged fruit surface), 4 = heavy attack (50-75% fruit surface damaged), 5 = very heavy attack (75-100% damaged fruit surface)

$$RI = \frac{IC-IT}{PC} \times 100\% \quad (4)$$

RI : Inhibiting activity against disease intensity (%) versus control
IC : Intensity of anthracnose in control
IT : Intensity of anthracnose in treatment

Statistical analysis

This study was conducted in a randomized block design (RBD) with six treatments and fourteen replications. The obtained data were analyzed quantitatively using analysis of variance (ANOVA). If a significant difference was found in the obtained data, then data proceeded with the Duncans Multiple Range Test (DMRT). Statistical analysis was used with the help of SPSS software for Windows version 17.0 in 2009. In determining the number of treatments and replications, each research design was based on the minimum condition t (r-1) >15, where t = number of treatment and r = number of replication (Ihwah et al. 2018).

RESULTS AND DISCUSSION

In vitro* test against *Colletotrichum acutatum

The cinnamon leaf extract showed an inhibition zone of 35 mm against *C. acutatum* (Figure 1). The extract exhibited growth inhibition of the test fungus at a concentration of 100%. The diameter of inhibition zone was 35 mm, indicating a very strong inhibition category, so it was used as a bio-fungicide for field experiment.

Inhibitory effect of cinnamon leaf extract on the growth of *Colletotrichum acutatum*

Based on scanning electron microscopy (SEM), it can be seen that cinnamon leaf extract showed inhibitory effect on the fungus, resulting in damage to the cell wall structure. Scanning electron microscope with a magnification of 5000 times clearly demonstrated the cinnamon leaf extract has the ability to inhibit fungal growth. It was observed that the mycelium of the fungus becomes hollow (Figure 2).

Application of cinnamon leaf extract against anthracnose disease in field

The application of cinnamon leaf extract formulation in real terms ($P<0.05$) reduces the percentage of disease and intensity of anthracnose disease in large chili plants. The percentage of anthracnose disease on the 140th day after planting was highest at 59.7%, while the percentage of anthracnose disease on treatment varied between 0-42.1%. At a concentration of 0.5%, it can control the disease percentage with a bland force of 29.4%. The higher the concentration of the formula, the greater the bland power. At 1.5% concentration, cinnamon leaf extract formula can inhibit the percentage of disease with 100% bland power. In this condition, the cinnamon leaf extract formula serves as a fungicide, as it can kill test fungus (Table 1).

The disease intensity on 140th day after planting in control was 57.6%. Cinnamon leaf extract treatment at a concentration of 0.5% showed 35.8% inhibition on the test fungus. The higher the concentration, the greater the inhibition. Treatment with cinnamon leaf extract at a concentration of 1.5% can inhibit the growth of fungus by 100%. This indicates that the cinnamon leaf extract has the potential to as a fungicide (Table 2).

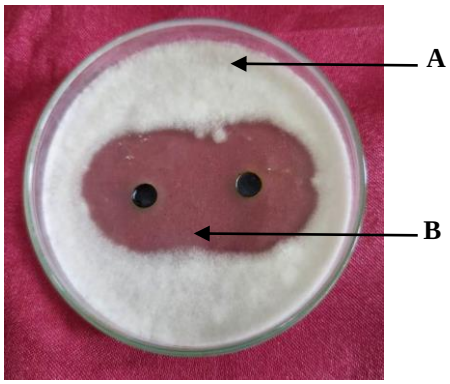

Figure 1. Inhibitory activity of cinnamon leaf extract on *Colletotrichum acutatum*: A. *Colletotrichum acutatum* fungus, B. inhibition zone

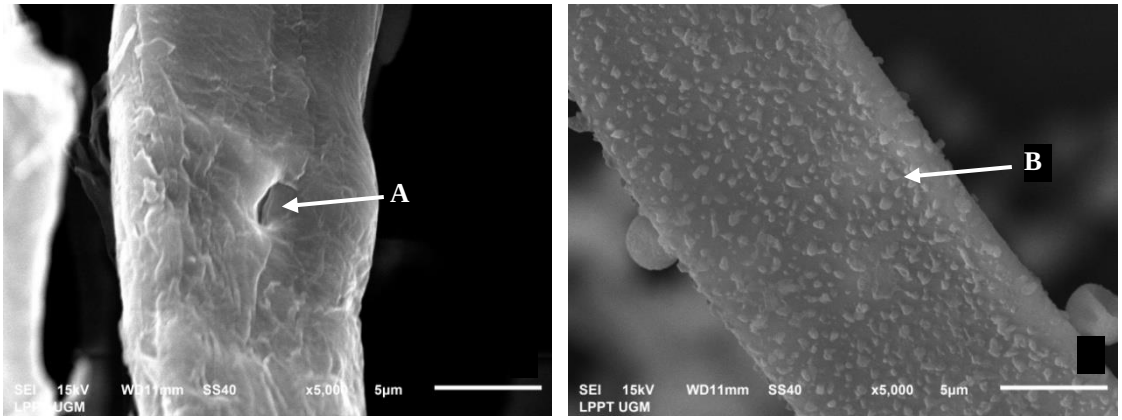

Figure 2. The inhibitory effect of cinnamon leaf extract on the growth of *Colletotrichum acutatum*: A. hollow hyphae of *Colletotrichum acutatum* (treated with extract). B. fine mycelium of *Colletotrichum acutatum* (control)

Discussion

Cinnamon leaf extract showed 35 mm inhibition zone, which was classified as very strong. This extract has the potential to be used as a bio-fungicide to control anthracnose that attacks large chili plants in Bali. There are several categories of diameters of plant inhibition zones against test fungi, such as if the diameter of the inhibition zone of plant extracts is less than 5 mm, it is categorized as weak, between 6 and 10 mm, it is categorized as moderate, for 11 to 20 mm it is categorized as strong, and for more than 20 mm it is categorized as very strong (Roza et al. 2019). Kudu et al. (2018) have reported that plant extract showed a very strong inhibition zone diameter on the test fungus. *Mitracarpus hirtus* plant extract can inhibit the growth of dermatophyte fungi, namely, *Epidermophyton floccosum*. A concentration of 1500 mg/mL can inhibit the growth of *E. floccosum* with a diameter of 22 mm. At the same concentration the plant extract of *M. hirtus* also inhibited the growth of *Trichophyton* sp. and *Microsporum* sp. with a diameter of 20 mm and 19 mm, respectively. Minimum inhibiting concentration (MIC) of *M. hirtus* plant extract on the three test fungi, namely *Microsporum* sp, *Trichophyton* sp. and *E. floccosum* at concentrations of 70µg/mL, 60µg/mL and 50µg/mL, respectively. Plant extracts can provide inhibition against plant pathogens because plant extracts contain active compounds that act as antifungals. Cinnamon leaf extract can inhibit the growth of colonies and spores of *Colletotrichum capsici*, which causes anthracnose disease. Based on the GC-MS test, cinnamon leaf extract contain 20 compounds but the 3 dominant compounds based on peak area are 3, namely 2.6-Dimethyl-6-nitro-2-hepten-4-one, 2H-I-Benzopyran-2-one (CAS) (Coumarin), and 1.2-Benzenedicarboxylic acid, mono 2-ethyl (Darmadi et al. 2019). *Avicennia marina* a mangrove plant in Saudi Arabia, can inhibit the growth of *Aspergillus fumigatus* and *Candida albicans*. The ethanolic extract of *A. marina* fruit could inhibit the growth of *A. fumigatus* and *C. albicans* with a minimum inhibiting concentration (MIC) of 0.26 ± 0.02 mg/mL and 0.25 ± 0.01 mg/mL, respectively. This MIC value is lower than the MIC value of the control (Fluconazole) 0.45 ± 0.02 mg/mL (Okla et al. 2021).

Table 1. Effect of cinnamon leaf extract on the percentage of anthracnose disease on large chili plants

Concentrations of cinnamon leaf extract formula (%)	Percentage of anthracnose disease in large chili plants after 140 days after planting (DAP)	Inhibiting activity against disease percentage (%) versus control
0	59.7[a*]	-
0.5	42.1[b]	29.4
0.75	26.3[c]	55.9
1	10.6[d]	82.3
1.25	1.4[e]	97.7
1.5	0[e]	100

Note: * Numbers followed by the same letter indicate an unreal difference based on Duncan's Multiple Range Test at 5%.

Table 2. Effect of cinnamon leaf extract on the intensity of anthracnose disease on large chili plants

Concentrations (%) of cinnamon leaf extract formula	Intensity of anthracnose on large chili plants after 140 days after planting (DAP)	Inhibition (%)
0	57.06[a*]	-
0.5	36.63[b]	35.8
0.75	23.01[c]	59.7
1	9.76[d]	82.9
1.25	1.45[e]	97.5
1.5	0[e]	100

Note: *Numbers followed by the same letter indicate an unreal difference based on Duncan's Multiple Range Test at 5%.

Based on scanning electron microscopy (SEM), it was clearly seen that the fungus treated with cinnamon leaf extract resulted in damage to the cell wall structure of the fungus The mycelia of the fungal cell wall became perforated. Perforation causes permeability in fungal cell membrane which causes imbalance. The contents of the fungal cells will spill out, fungal cells will undergo lysis, and over time the fungal cells die. This indicates that the cinnamon leaf extract contains compounds that act as antifungals. Ceruso et al. (2020) reported the impact of plant extracts on *Listeria monocytogenes*, a foodborne pathogen. In the pathogenic cells without treatment of plant extracts (control), the morphological structure of the cells is compact, with a smooth surface, and flagella are present. In the pathogenic cells treated with plant extracts, the cell structure is irregular, the cell wall is damaged, and lack of flagellum. Hashem et al. (2016) have reported that the hypha wall of *Fusarium solani* experienced impaired growth due to the treatment of *Forsskaolea tenacissima* extract at a concentration of 0.165 mg/mL. Cinnamon leaf formula can significantly inhibit the percentage and intensity of anthracnose disease in experimental gardens. This may be because the compounds found in cinnamon leaves are antifungal compounds. Some plant formulas have a very strong ability to inhibit fungal growth both *in vitro* and *in vivo*. *Cornus mas*, *Prunus laurocerasus*, *Morus nigra*, *Morus alba*, and *Rosa canina* had inhibitory effect on the growth of plant pathogenic fungi, namely, *Sclerotinia sclerotiorum*, *Monilinia fructigena*, and *Alternaria solani*, both *in vitro* and *in vivo*. At 100 mg/mL, 500 mg/mL, and 1000 mg/mL concentration of plant extract showed inhibition against the test fungus (*S. sclerotiorum*) from 7-90%, on *M. fructigena* from 4-89%, and on the fungus *A. solani* ranging from 7-47%. The methanol extract of *C. mas* leaves and a hexane extract of *C. mas* leaves and fruit at a concentration of 1000 mg/ml can inhibit the growth of *S. sclerotiorum* that attacks cucumbers (Onaran and Yanar 2016). At a concentration of 20 mg/mL, as much as 50 mL of *Crotalaria longirostrata* branch extract can reduce the incidence of root disease in maize caused by *Fusarium verticillioides* by 70.4-40.12% when compared to controls. The severity of leaf disease can also be reduced from 40.15% to 29.46% (Cruz-Rodriguez et al. 2020). *Vernonia amygdalina* extract formula can significantly control gray mold disease in tomato plants caused by *Botrytis cinerea in vivo*. At treatment concentrations of 400 mg/mL and 500 mg/mL, the disease was controlled with an inhibitory power of 50% and 53.12% when compared to the negative control, respectively. Tomato fruit treated with *V. amygdalina* at a concentration of 300-500 mg/mL was shown to have a lower disease severity than the positive control (Benomyl) (Yusoff et al. 2020). The cinnamon leaf extract formula could control Fusarium wilt disease in tomato plants. At a concentration of 1%, it can control Fusarium wilt disease by 47.62%. At a concentration of 2%, it was effective in reducing the incidence of Fusarium wilt disease and suppress yield loss in tomato plants (Darmadi et al. 2016).

Based on the results, it is concluded that the crude extract of cinnamon leaves can inhibit the growth of *C. acutatum in vitro*. Cinnamon leaf extract damages the cell wall of the *C. acutatum* causing the fungus to lyse, resulting in the death of fungus. Cinnamon leaf extract formula was significantly effective in controlling the percentage and intensity of anthracnose disease attacking large chili plants in Bali.

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