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The effect of cogongrass extract in inhibiting the *Colletotrichum* sp. that causes anthracnose disease in bird's eye chili

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Abstract

Anthracnose can be caused by fungi consisting of two types, *Gloeosporium piperatum* and several species of *Colletotrichum* sp. Avoiding the negative effects of chemical fungicides to control anthracnose, biofungicides are more ecologically safe. Cogongrass (Imperata cylindrica) extract contains secondary metabolites in the form of saponins, alkaloids, terpenoids, flavonoids, and tannins which can play a role as biofungicides. Aimed to determine the effect of cogongrass extract in inhibiting the growth of *Colletotrichum* sp. on Bird's eye chili and to know the best concentration of Cogongrass extract to inhibit the growth of the *Colletotrichum* sp. on bird's eye chili. This research was conducted from April to August 2022 at the Agroecotechnology Production Laboratory, Faculty of Agriculture, University of Lambung Mangkurat. This study used a research design in the form of a completely randomized design (CRD) with 5 treatments and 5 replications, so that 25 experimental units were obtained. The levels of treatment given were as follows: A0(-) = negative control (distilled water), A0(+) = positive control (Tandem 325 SC), A1 = 10% cogongrass extract, A2 = 20% cogongrass extract, and A3 = 30% cogongrass extract. The results showed that cogongrass extract had a positive effect on inhibiting the growth of *Colletotrichum* sp. which isolated from bird's eye chili on PDA media. The results of the analysis of variance and the DMRT test showed that A1, A2, and A3 were significantly different from A0(-). Treatment with the best concentration to inhibit the growth of *Colletotrichum* sp. is A3 (30% cogongrass extract).

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Introduction

Bird's eye chilies are horticultural commodity that are preferred and widely consumed by the public. Bird's eye chilies are often used as a flavor enhancer in every dish that is served every day. Therefore, the demand for and consumption of bird's eye chilies is always high every year. Based on data from BPS (2021), bird's eye chilies production in Indonesia in 2020 reached 1.51 million tons, while the production in South Kalimantan only reached 15,616 tons in the same year (South Kalimantan Food Crops and Horticulture Service, 2022).

This low productivity is caused by several problems in bird's eye chili cultivation. Problems that commonly occur in the planting and maintenance of bird's eye chili are pests and diseases. Farmers often experience difficulties in cultivating bird's eye chili due to diseases that attack it. One of the important diseases in bird's eye chili is anthracnose, which is an air-borne and seed-borne disease that spreads quickly, especially during the rainy season and can reduce production and even cause crop failure. If the part of the fruit that is affected by this disease, the market price drops or even does not sell at all (Wardana, 2014).

Yield losses in the field caused by anthracnose disease reach 80% in the rainy season and 20–35% in the dry season (Widodo, 2007). Anthracnose disease can cause losses reaching 5–65% (Ratulangi *et al*., 2012). Anthracnose is recorded as a disease in chili with the highest attack in Indonesia, which is 26% (Directorate of Horticultural Protection, 2019). Anthracnose disease can be caused by fungi consisting of two types, *Gloeosporium piperatum* and several species of *Colletotrichum* sp. such as *C. acutatum*, *C. gloeosporioides*, and *C. capsici* (AVRDC, 2003).

Farmers often use chemical fungicides to control anthracnose disease in bird's eye chili. The use of chemical fungicides provides effective and fast results, but if used without paying attention to recommended dosages for a long time, it can cause pathogens to become resistant and have a negative impact on the environment and the health of the human body. According to Sumartini (2010), disease control is recommended to be executed by combining several environmentally friendly control components. This is intended to support sustainable agriculture. Therefore, an alternative control to avoid the negative impacts that have been mentioned is the use of fungicides derived from natural ingredients.

Biofungicides is an ecologically safe method and has been developed to control disease. Biofungicides uses plants that can produce secondary metabolites, such as steroids, alkaloids, flavonoids, tannins, saponins, triterpenoids and others (Nurmansyah, 1997). One of the plants that can be used as a biofungicides is cogongrass.

Cogongrass (*Imperata cylindrica*) is a grass weed that grows wild and widespread in forests, rice fields, gardens, or yards, and other open environments (Fujiyanto *et al*., 2015). Cogongrass produce secondary metabolites which can be used as biofungicides. According to Aryani *et al*. (2020), cogongrass extract contains saponins, alkaloids, terpenoids, flavonoids, and tannins which act as biofungicides.

Several studies have stated that cogongrass extract can control anthracnose disease. One of them is research from Gusmarini *et al*. (2014), cogongrass had an effect on reducing the severity of anthracnose disease in chili peppers. In the research by Arie *et al*. (2015), it was also stated that cogongrass extract had a significant effect on suppressing the growth and sporulation of the *Colletotrichum* sp. on Cavendish bananas. In the same study, it was also stated that the concentration of 10% was still not optimal in inhibiting the growth of the *Colletotrichum* sp.

Based on some of the problems and research results above, it is necessary to conduct research to determine the optimal concentration of cogongrass extract in inhibiting the growth of the *Colletotrichum* sp. which is the cause of anthracnose disease in bird's eye chilies.

Materials and methods

Materials and tools

The materials and tools used were ORI 212 variety bird's eye chili, cogongrass, Tandem 325 SC fungicide, 70% ethanol, distilled water, PDA media, 96% ethanol, oven, cling wrap, scales, hotplate, Erlenmeyer, autoclave, petri dish, blender, LAF, ent needle, bunsen burner, rotary evaporator, and microscope slide cover glass.

Research design

This study used a research design in the form of a completely randomized design (CRD) with 5 treatments and 5 replications, so that 25 experimental units were obtained. The levels of treatment given were as follows: A0(-) = negative control (distilled water), A0(+) = positive control (Tandem 325 SC fungicide), A1 = 10% cogongrass extract, A2 = 20% cogongrass extract, and A3 = 30% cogongrass extract.

Study site

This research was conducted from April to August 2022 at the Agroecotechnology Production Laboratory, Faculty of Agriculture, University of Lambung Mangkurat, Banjarbaru, South Kalimantan.

Tool sterilization

All tools made of glass were washed and dried. Tools that have mouths were plugged using cotton, then the tools were wrapped using newspaper. Dry sterilized the tools using an oven for 1 hour at 170°C.

Making potato dextrose agar (PDA) media

In making PDA media, it took 1 liter of distilled water, 20g of agar, 20g of sugar, and 200g of potatoes. The potatoes were cut into small pieces and boiled in 1 liter of water while stirring, then the boiled potatoes were filtered into an Erlenmeyer tube. Put the filtered potato juice into a solution of sugar and agar, stirred until homogeneous and boiled for 30 minutes, then put it in an Erlenmeyer tube. The Erlenmeyer tube containing PDA was sterilized using an autoclave at 121°C and 1 atm pressure for ±15 minutes.

Isolation of the Colletotrichum sp.

Bird's eye chili fruits that have symptoms of anthracnose disease are taken and isolated. Furthermore, the fungi that grow were identified macroscopically and microscopically to ensure that the isolated fungi were *Colletotrichum* sp. The fungi identified as *Colletotrichum* sp. purified on fresh PDA media.

Making Cogongrass Extract

The extract of cogongrass rhizome was prepared by washing the rhizomes and then drying them in the open air until they were completely dry. Then the rhizome powder of the cogongrass was macerated for 3 days in 96% ethanol solution. This was done in a closed state so that the powder of the rhizome of the cogongrass and the ethanol solution really blend together. Filtered and took the juice then evaporated it at a temperature of 40°C–70°C using a rotary evaporator to obtain the final result in the form of cogongrass rhizome extract with a concentration of 100% (Sari, 2015). Extracts with a concentration of 100% was stored in the refrigerator until needed. The extract was diluted first using distilled water to obtain 20 mL of 10%, 20%, and 30% extract.

Testing of Cogongrass Extract against Colletotrichum sp. on PDA media

PDA media was sterilized in an autoclave at 121°C, with a pressure of 1 atm for 15 minutes. Then the PDA media was put into a measuring cup as much as 9 ml and 1 ml of test extract for each cup, the two were mixed until evenly distributed.

After that, it was put into the petri dish. This activity was carried out for each treatment (Yendi *et al*., 2015). As a negative control (A0(-)) PDA media was used with the addition of sterile distilled water and positive control (A0(+)) with the addition of Tandem 325 SC fungicide. Fungi colonies in pure cultures were taken using an ent needle and placed in the middle of a petri dish. The petri dishes were wrapped in cling wrap and incubated at room temperature.

Inhibition test

The parameters observed in this study were the inhibition of cogongrass extract on the growth of the *Colletotrichum* sp. Inhibition was calculated on day 3, day 5, and day 7 after incubation. Fungi growth inhibition can be calculated using the formula (Astuti *et al*., 2014).

Inhibition = $\frac{C-T}{C}$ x 100%

Note: C = Diameter of the mycelia in the control

T = Diameter of the mycelia in the treatment

The data obtained from this study were tested for homogeneity with the Bartlett test. After that, the data were analyzed using ANOVA (Analysis of Variant). If the analysis of variance showed that administration of cogongrass extract had an effect ($P<0.05$) on the observed variables, then continued with the Duncan's Multiple Range Test at α = 5%.

Results and discussion

Isolation of anthracnose disease in bird's eye chili

Fig. 1. Anthracnose disease symptomatic bird's eye chili.

Bird's eye chili fruit infected with anthracnose was received from farmers' fields in Landasan Ulin area. The fruit collected had symptoms of anthracnose in the form of small round spots that grew larger and merged with other spots, dark yellow to brown in color, and had irregular edges. According to Martoredjo (2009), symptoms of anthracnose disease on the fruit which are quite severe often cause spots which are brown on the edges and could cause the whole fruit to dry out and shrink (wrinkled). The spots are usually slightly concave or curved inward and have a layered circle in the middle. Symptoms will be more severe when the air is humid, causing the fruit to become soft rot and fall off (Soesanto, 2019).

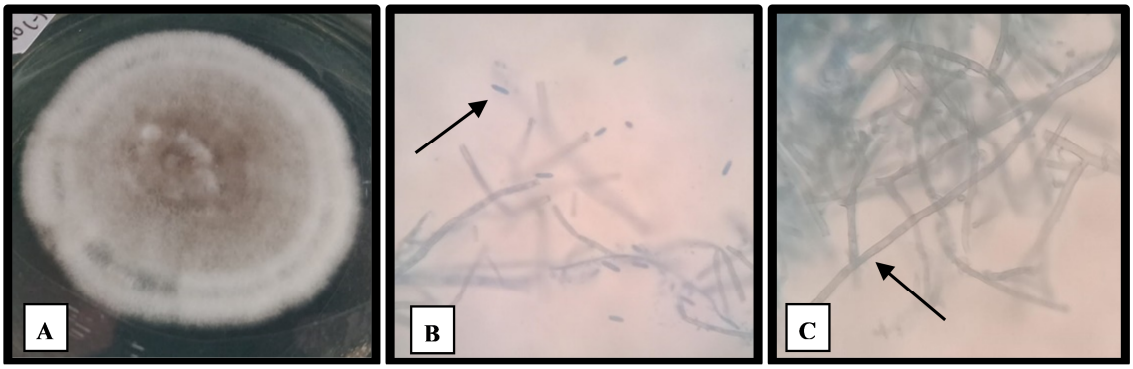

Fig. 2. *Colletotrichum* sp. in the laboratory (A) colonies, (B) conidia, (C) hyphae.

The results of the isolation of the fruit parts showed that there were colonies that grew white to gray in color with mycelium arising on a smooth, cotton-shaped surface with flat colony edges. According to Suwardani (2014), *Colletotrichum* sp. has colonies with a smooth

texture like cotton, colonies have a white color. Microscopically, conidia are cylindrical in shape with one end slightly curved, hyphae are insulated, elongated, branched, and hyaline in color. According to Barnett & Hunter (1972), *Colletotrichum* sp. have hyaline conidia with one cell, ovoid to sickle-shaped. *Colletotrichum* sp. has many variations in the shape of the conidia such as *C. gloesosporioides* has oval conidia with blunt ends, *C. acutatum* has cylindrical conidia with tapered or curved ends (Hyde *et al*., 2009). On microscopic observation, no setae, sclerotia, and aservulin were found. Therefore, the observed fungi were identified as *C. acutatum*. This is in accordance with Zivkovic *et al*. (2010) who stated that *C. acutatum* has microscopic characteristics in the form of septate hyphae, hyaline, cylindrical conidia with tapered ends, and does not have sclerotia, seta, or aservulin. PDA colonies are pale gray in color, white aerial mycelia, thick and cottony.

Inhibition Percentage

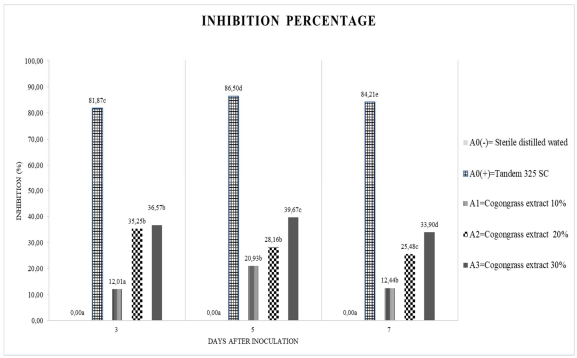

Fig. 3. Inhibition percentage of each treatment against *Colletotrichum* sp.

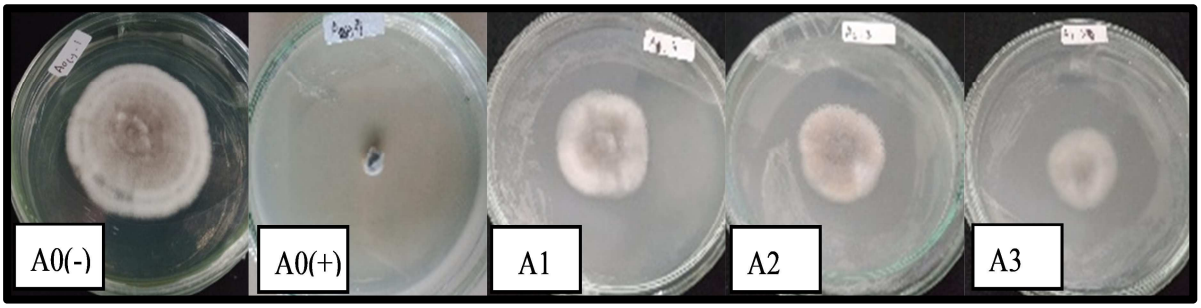

Fig. 4. The results of the inhibition test for each treatment on *Colletotrichum* sp.

Based on the results of Bartlett's test, the inhibition percentage of Cogongrass extract on the *Colletotrichum* sp. showed homogeneous data. The results of the analysis of variance showed that the extract had a significant effect on inhibiting the growth of the *Colletotrichum* sp. The percentage of inhibition for each treatment can be seen in Fig. 3.

Treatment on each PDA media was given cogongrass extract with different concentrations. The results showed that the cogongrass extract on PDA media inhibited the growth of *Colletotrichum* sp. On 3 days after inoculation, the media that was given cogongrass extract with a concentration of 10% (A1) had an inhibition percentage of only 12.01%, but the inhibition percentage of 20% (A2) was 35.25%, and the percentage of inhibition that is the highest occurred at 30% extract (A3) that is equal to 36.57%. On 5 days after inoculation, A1 and A2 were not significantly different with 20.93% and 28.16%, while treatment A3 showed the highest inhibition of 39.67%. Likewise on the 7th day, the A3 treatment was the extract treatment with the highest inhibition of 33.90%. Treatment A1 could inhibit the growth of *Colletotrichum* sp. to 12.44% while the A2 treatment was 25.48%. From these results it could be seen that the higher the concentration of the extract given to PDA media, the higher the percentage of inhibition.

The growth of the *Colletotrichum* sp. on PDA media with control treatment that was not mixed with cogongrass extract had good growth and the growth of the colonies was not inhibited.

This was due to the absence of compounds that could inhibit the growth of fungi in the culture media. Whereas on PDA media treated with cogongrass extract, growth was inhibited where the higher the concentration of the extract given to PDA media, the higher the ability to inhibit colony growth, so that the diameter of the colonies growing on the culture media was getting smaller. This was in accordance with Cahyani *et al*. (2015) who stated that concentration is closely related to the amount of active ingredients in a formulation, where the higher the concentration of a formulation, the higher the active ingredients it contains so that the ability to suppress pathogen growth will be more optimal. Vice versa, the lower the concentration of a formulation, the less active ingredients it contains, and the lower the ability to suppress the growth of pathogens.

Table 1. Phytochemical test results of reed extract.

Parameter	Result
Alcaloid	+
Flavonoid	+
Saponin	+
Steroid	-
Triterpenoid	+
Tanin	+

The process of inhibiting the growth of fungal colonies could occur because the extracts of cogongrasss contain secondary metabolites. Based on the Phytochemical Test conducted, the extract of cogongrasss contains alkaloids, flavonoids, saponins, triterpenoids, and tannins which act as biofungicides and could suppress the growth of *Colletotrichum* sp. Alkaloids can damage the cell components of the fungus by forming a kind of hole which causes the cell membrane to leak, thereby damaging the fungal cells and causing the death of the fungi (Bhaskara, 2012). This is in line with Berlian *et al*. (2016) who stated that alkaloids can cause damage to cell membranes, by binding to ergosterone and form a pore which results in leakage of the cell membrane causing permanent cell damage and resulting the death of the fungi. Flavonoids can interfere with the process of diffusion of food into the fungal cells so the growth of the fungi stop (Supriati *et al*., 2016). Flavonoids are also able to inhibit cell division, bind to proteins in fungal cells, and interfere with mitotic function, and inhibiting the growth of fungi (Siswandono & Soekatjo, 2000).

Saponins are surfactants (can unite liquid oil and water) in a polar form which have the ability to act as an antifungal by working to reduce the surface tension of the sterol membrane of the cell wall and can cause the more concentrated intracellular fluid to be pulled out of the cell so that the fungi die (Pulungun, 2017). Saponins have surfactant properties in a polar form which will break down the fatty layer on the cell membrane and disrupt the permeability of the cell membrane, this results in the process of diffusion of materials or substances needed by the fungi being disrupted, eventually the cell swells and bursts (Sugianitri, 2011).

Triterpenoid compounds act as antifungals by inhibiting the growth and development of the cytoplasmic membrane of fungal spores (Lutfiyanti *et al*., 2012). Triterpenoids can also affect the integrity of the fungal cell membrane by inhibiting enzymes which are the main components of the fungal cell wall (Ghannoum *et al*., 2020). The tannin compounds contained in cogongrass extract can damage the permeability of the fungal cell wall so that the cells cannot develop and cause the growth of fungal hyphae to be inhibited (Idris & Nurmansyah, 2015). The mechanism of these different secondary metabolic compounds interacts with each other and results in the inhibition or death of the cell of the *Colletotrichum* sp. on PDA media.

Positive control treatment (A0(+)) using a synthetic fungicide (Tandem 325 SC) with the active ingredients of azoxystrobin and diphenoconazole was used as a comparison of the inhibition of Cogongrass extract on the growth of

the *Colletotrichum* sp. The inhibition of the treatment (A0(+)) has the highest inhibition due to the presence of fungicide active ingredients (azoxystrobin and difenoconazole) which can cause hyphae to swell, causing the hyphae to not develop and expand and reduce the function of the haustoria as a tool for food absorption. Azoxystrobin compounds are antisporulant, which can inhibit the formation and germination of conidia and the growth of mycelia (Istifadah *et al*., 2017). Therefore, the fungi *Colletotrichum* sp. cannot produce spores and the hyphae do not develop or expand.

Conclusions

1. Cogongrass extract could inhibit the growth of the *Colletotrichum* sp. isolated from bird's eye chili on PDA media in vitro as seen from the inhibition percentage of the cogongrass extract treatment.
2. Treatment A3 (30% cogongrass extract) was the treatment with the best concentration in inhibiting the growth of the *Colletotrichum* sp. isolated from bird's eye chili on PDA media in vitro with the highest percentage of inhibition at 36.57% on the 3rd day, 39.67% on the 5th day, and 33.90% on the 7th day.

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