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Research article

Use of Ficus Septica Leaf Extract for Biological Control of Anthracnose Disease in Carica Papaya Caused by Colletotrichum spp

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Abstract.
The fig (*Ficus septica*) is a widespread wild plant whose leaf extract contains bioactive compounds with antifungal properties, including alkaloid and phenolic compounds, both of which are antioxidants. *Colletothricum* spp. is a pathogenic fungus found in cultivated plants that can cause anthracnose, a plant disease. Papaya (*Carica papaya*) is one of the cultivated plants that frequently displays symptoms of anthracnose disease caused by *Colletothricum* spp. The focus of this research was to determine whether *F. septica* leaf extract could inhibit the growth of *Colletothricum* spp. on *C. papaya*. The antifungal activity of *F. septica* leaf extract against *Colletothricum* spp. was measured using the well diffusion method. The extract concentrations, which were determined by measuring the extract's MIC value, were 0.5%, 1%, 2%, 3%, 4% and 5% (b/v), with methanol and synthetic fungicides serving as controls. According to the findings, the MIC value of the *F. septica* leaf extract on *Colletothricum* spp. growth was 0.4%, with an inhibition zone of 2.37 mm. Meanwhile, the inhibition zones formed by the *F. septica* leaf extract treatment were 2.5 mm, 3.4 mm, 7.6 mm, 9.7 mm, 12.7 mm, and 13.5 mm, respectively, and 0 mm for the negative control and 30 mm for the positive control. According to the analysis of variance results, all of the treatments produced results that were significantly different from those of the negative control, and the 4% extract was the most effective in vitro. We conclude that *F. septica* crude leaf extract contains bioactive compounds with antifungal properties and it can be used as a safe alternative to control *Carica papaya* anthracnose disease.

Keywords: anthracnose, *Carica papaya*, *Colletothricum* spp., *Ficus septica.*

1. Introduction

Anthracnose is the main postharvest disease caused by the fungus *Colletotrichum* spp. which attacks fruits, including papaya. Anthracnose disease in addition to causing a decrease in yield can also damage the aesthetic value of fruits. This pathogen attack can occur both before and after harvest. Yield reduction due to anthracnose disease in papaya (Carica papaya L.) can reach 50% or more [1]. The control of anthracnose still relies on the use of synthetic fungicides. The continuous use of synthetic fungicides can lead to the emergence of pathogen resistance, pollute the environment and be harmful to consumers. Pollution by pesticides is not only in the agricultural environment but can also endanger human and animal life where pesticide residues accumulate in agricultural products and in waters [2]. Based on this, it is necessary to find an alternative to control anthracnose in fruits including papaya by utilizing plants that have the potential as botanical fungicides that are not harmful to consumers and the environment.

According to Sudirga et al. [3] found 5 plant species from 20 species tested for their antifungal activity against the fungus *Colletotrichum* spp which causes anthracnose on large chili peppers. The five plants, namely *Ficus septica*, *Piper rectopertum*, *Piper cubeba*, Among the 5 types of plants *Ficus septica* gave the highest inhibition zone of 30 mm.

Based on the problems and the results of the research above, it is necessary to conduct further research on the test of botanical fungicides of *Ficus septica* leaf extract against other types of pathogenic fungi that can cause symptoms of anthracnose in papaya fruit. So that the biofungicide effect of *Ficus septica* leaf extract can be used effectively to control the fungus that causes anthracnose on papaya fruit.

2. Methodology

2.1. Methods of leaf extraction

For extraction of leaf extract, the *Ficus septica* leaves were chopped, then dried at room temperature, and after that the dry material was made into powder by means of a blender. *Ficus septica* leaf powder (100 grams) was then macerated with 1000 ml of methanol PA (Pro-Analysis) for 72 hours at room temperature and dark place. The filtrate was obtained by filtering and the residue obtained was then macerated again with 1000 ml of methanol as much as two times. The filtrate obtained are combined and then evaporated using a vacuum rotary evaporator (Iwaki, Japan) at 40°C, to obtain a crude extract that was used for further testing.

2.2. Isolation and Identification of Pathogen

Papaya fruit showing symptoms of anthracnose was cleaned with running water followed by sterile water and then cut to a size of 1 cm x 1 cm on the part of the fruit with symptoms of the disease and the healthy part then placed on PDA media in a Petri dish. This culture is then placed in a dark place at room temperature until mold grows from the edge of the papaya fruit. The growing fungi were isolated and purified and identified morphologically by observing the shape of their spores and hyphae. To ensure that the isolated and purified fungus causes anthracnose disease in papaya fruit, the Koch Postulate test was carried out by inoculating pure isolates of fungi obtained from papaya fruit that showed symptoms of anthracnose disease followed by reisolation of the fungus, then identified morphologically, and if the same as morphological characteristics of the previous fungus, it can be said that the fungal isolate obtained is the fungus that causes anthracnose disease on papaya fruit. The fungal isolates were then maintained on inclined PDA media and were ready to be used for further testing. Pure cultures of pathogenic fungal isolates were identified macroscopically and microscopically based on morphological characteristics and pigmentation on PDA media using the identification key from Pit and Hocking (1997) [4].

2.3. Antifungal Activity Test.

The antifungal activity test of crude extract of *Ficua septica* against pathogenic fungi that cause anthracnose on papaya fruit was carried out by: Preparation of fungal suspensions by subcultured fungal culture in PDA media, filled with 10 ml of sterile distilled water and then scraped off with a hook needle so that the conidia were released, so that a mixture of mycelium and fungal conidia was obtained. Then filtered using gauze. The filtered filtrate is then centrifuged to obtain a concentrate. The fungal suspension was obtained by diluting the concentrate to reach a density of 2 x 105 conidia/ml. The petri dish containing 10 ml of PDA media and 200 l of the fungal suspension was allowed to solidify. After solidification, 2 diffusion wells were made in each petri dish using a cork borer. Each diffusion well was filled with 20 l of crude *Ficus septica* leaf extract using a micropipette. The diameter of the inhibition zone formed around the diffusion well is measured. According to Ardiansyah [5], if the inhibition zone is 20 mm (very strong inhibition), 10 – 20 mm (strong inhibition), 5-10 mm (moderate inhibition), and < 5 mm (low or weak inhibition).

3. Result and Discussion

3.1. Isolation of Fungus that Causes Anthracnose on Papaya Fruit

Papaya fruit (*Carica papaya* L.) that shows symptoms of anthracnose infection are picked from the tree, then taken to the laboratory to isolate the type of fungus that causes papaya fruit to show symptoms of anthracnose disease. The types of fungi that have been isolated from papaya fruit that show symptoms of anthracnose are presented in Figure 1.

Figure 1: Papaya fruit showing symptoms of anthracnose disease (A), pure isolate pathogen causing anthracnose disease in papaya (B).

The Koh Postulate test was carried out to ensure that the pure isolates obtained from the isolation of papaya fruit showing symptoms of anthracnose disease showed the same symptoms in the papaya fruit tested. The results of Koh's Postulate test are presented in Figure 2.

Figure 2: The results of Koch's Postulate test, papaya fruit shows the same symptoms as papaya fruit infected with anthracnose disease (A), pure isolate the results of re-isolation isolate fungus from papaya fruit showing symptoms of infection (B).

Based on the results of the study in Figure 2, it shows that the papaya fruit used as a test medium for Koch's Postulates showed the same symptoms as papaya fruit infected with anthracnose disease, so that the fungal isolate isolated from papaya fruit was indeed the cause of anthracnose disease in papaya fruit.

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3.2. Identification of Fungi that Cause Anthracnose on Papaya Fruit

Pure isolates of fungi that cause anthracnose obtained from Koh's Postulate test were identified macroscopically and microscopically. Macroscopic identification of fungi was carried out by observing the fungal isolates from the color, growth speed and morphology of the fungus. Macroscopic image of the fungus that causes anthracnose on papaya fruit as shown in Figure 3 A. Microscopic identification of fungi was carried out by observing fungal isolates from the mycelium form and fungal spores. Microscopic image of the fungus that causes anthracnose on papaya fruit as shown in Figure 3 B & C.

Figure 3: Macroscopic form (A), mycelium form (B) and spore form (C) isolates of fungi that cause anthracnose on papaya fruit.

Based on macroscopic observations (Figure 3 A) showed that the pathogenic fungus that causes anthracnose disease on papaya fruit has the characteristics of white color at a young age (1-2 weeks) and the color will change to gray and black after age (3-4 weeks). the edges are flat and the growth rate is 12.5 mm per day. While the microscopic characteristics of the pathogenic fungi that cause anthracnose disease on papaya fruit have the form of cylindrical spores, blunt spore tips, spore size 16.1 x 5.6 m, insulated and branched hypa shape (Figure 3 B & C). Based on the macroscopic and microscopic characteristics possessed, it can be seen that the fungus that causes anthracnose disease in papaya fruit is the fungus Colletothricum spp.

According to Dickman [6] the general characteristics of fungi from the Genus *Colletothricum* have insulated and branched hyphae, producing transparent and elongated conidia with rounded ends with a length of 10-16 m and a width of 5-7 m, conidia mass is black and hyphae are gray. ash. *Colletothricum* spp. is a facultative parasitic fungus with the characteristics of conidia (spores) arranged in acervules (asexual structures in parasitic fungi). Fungi of the genus *Colletothricum* are included in the Class Deuteromycetes which is an anamorphic form (asexual form) and when the fungus is in a telemorphic form (sexual form) it is included in the Class Ascomycetes known as the fungus Genus *Glomerella* [7].

According to Patel et al.[8] anthracnose disease in papaya fruit caused by the fungus *Colletothricum gleosporioide*s is indicated by early symptoms such as oval black spots, slightly watery, forming concave lesions on the fruit surface which will develop into necrosis, and tissue death. Based on the color of the white colony, the older the age of the colony, the color of the colony became gray, and the formation of acervulus with black conidia masses was identified as *C. gloesporioides.*

3.3. Inhibitory activity of Crude leaf Extract of Ficus septica.

The crude extract of the leaves of *Ficus septica* was tested for its inhibition on the growth of the fungus *Colletothricum* spp isolated from papaya fruit showing symptoms of anthracnose disease by the diffusion well method. The test results showed that the crude extract of the leaves of *F. septica* was able to inhibit the growth of the test fungus with the formation of an inhibition zone around the diffusion well of 23,54 mm as shown in Figure 4.

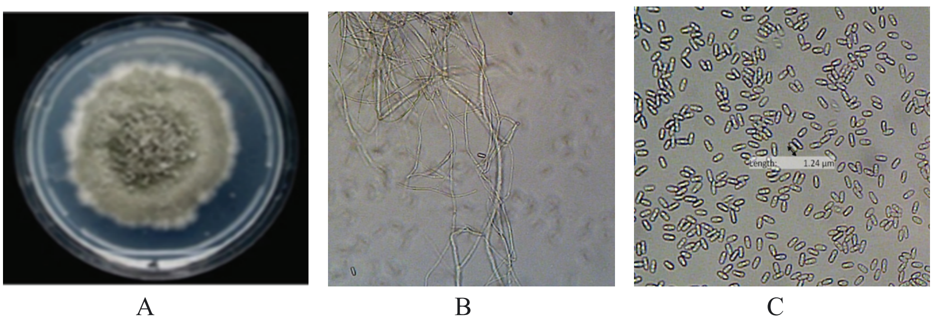

Figure 4: The results of the inhibition test of the crude extract of *Ficus septica* leaves against the fungus that causes anthracnose on papaya fruit using the diffusion well method, diffusion well (A), and the formed inhibition zone (B).

According to Sudirga [3] *Ficus septic*a leaf extract can inhibit the growth of the fungus *Colletothricum acutatum* which causes anthracnose disease in large chili (*Capsicum annuum* L.). The crude extract of *F. septica* leaves contains several active compounds that have antifungal properties including 2,3,5 trimethyl heptane, Sulfurous acid cyclohexyl methylhexadecyl ester, dodecanoic acid methyl ester, 3-Deoxy-d-mannonic acid, hexadecanoic acid methyl ester, octadecamethylcyclononasiloxane, 1 -Heptacosanol and 1,2-Benzenedicar boxylic acid mono (2-ethylhexyl) ester [9].

3.4. MIC of Leaf Crude Extract Ficus septica

Minimum inhibitory concentration (MIC) is the smallest extract concentration that is able to inhibit the growth of fungi that cause anthracnose on papaya fruit. MIC test results are presented in Table 1.

No.	Extract concentration (%)	Inhibition zone (mm)
1.	5	15,15
2.	4	14,78
3.	3	11,54
4.	2	9,33
5.	1	8,12
6.	0,9	7,25
7.	0,8	6,47
8.	0,7	5,25
9.	0,6	4,63
10.	0,5	3,33
11.	0,4*	2,34
12.	0,3	0
13.	0,2	0
14.	0,1	0

MIC = minimum inhibitory concentration

Figure 5: MIC test results of leaf extract of *Ficus septica* against *Colletotrichum* spp on PDA media.

Based on the results of the MIC test in Table 1, it can be seen that the MIC value of *Ficus septica* leaf extract on the growth of *C. gleosporioides* was 0.5% with an inhibition zone of 0.23 mm. Based on the MIC value, the extract concentration treatments to be used are: 0.5%, 1%, 2%, 3%, 4% and 5% while for negative control methanol is used and synthetic fungicide is used for positive control. The MIC value of an extract varies greatly, the smaller the MIC value of an extract, the higher its fungicidal activity [10]. According to Masangwa et al. [11] reported that differences in MIC in plant extracts can be caused by differences in compounds or solutions used when extracting these plants. Syzygium cordatum tested on *Colletotrichum dematium* had MIC values of 6.25 mg/ml when extracted with acetone, MIC of 3.13 mg/ml when extracted with water and MIC of 1.56 mg/ml when extracted with ethyl acetate. Meanwhile, *Allium sativum* extracted with water, ethyl acetate and acetone tested on *C. dematium* had MICs of 6.25 mg/ml, 3.13 mg/ml and 0.78 mg/ml, respectively.

3.5. Test the potential for inhibition of Ficus septica leaf extract

Based on the MIC value of the crude extract of *Ficus septica* leaves against the fungus *Colletotrichum* spp, it can be determined the treatment used in further research to determine the optimal concentration of the crude extract of *Ficus septica* leaves in inhibiting the growth of the fungus *Colletotrichum* spp in vitro, which is presented in Table 2

No.	Treatment of extract (%)	Inhibition zone (mm)
1.	Control negative	0^{a}
2.	Control positive	$30{,}23^{f}$
3.	0,5	$2{,}53^{b}$
4.	1	$3{,}42^{b}$
5.	2	$7{,}64^{c}$
6.	3	$9{,}63^{d}$
7.	4	$12{,}73^{e}$
8.	5	$13{,}51^{e}$

= values followed by different letters in the same column mean significantly different (P≤0,05) base on DMRT test at the level of 5%

Figure 6: The results of the inhibition test of the crude extract of *Ficus septica* leaves against the fungus *Colletotrichum* spp.

Based on the research data in table 2, shows that the extract treatment with a concentration of 4% is the most optimal when compared to the negative control. Gawade at al. [12], that leaf extract of *Aegle marmelos* (L). which was extracted with methanol and ether in a ratio of 1:1 inhibited the growth of the fungus *Colletotrichum acutatum* with a zone of inhibition diameter of 22 mm. Sayeed at al. [13] reported that *Moringa oleifera* Lam. inhibit the growth of the fungus *Colletotrichum* sp. with an inhibition zone diameter of about 11 mm at an extract concentration of 100 g/disc and 14 mm at a concentration of 200 g/disc. Another study showed that the methanol extract of the bark of *Leptadenia lancifolia* had antifungal activity against the fungi *Crytococcus neoformans* and *Candida albicans* with inhibition zone diameters of 30 mm and 28 mm, respectively [14]. Astiti and Suprapta [15] reported that teak leaf extract at a concentration of 1% was able to inhibit the growth of fungi Nigrospora sp., Penicillium citrinium, Aspergillus flavus, Arthrinium phaeospermum and Acremonium butyri with inhibitory powers of 95.76%, 63.12%, respectively. ., 90.59%., 92.0% and 77.43%.

4. Conclusion

Based on the research that has been done, it can be concluded that: The crude extract of *Ficus septica* leaves can inhibit the growth of the fungus *Colletotrichum* spp with an inhibition zone of 23.54 mm. The MIC value of the crude extract of *Ficus septica* leaves against the fungus *Colletotrichum* spp was 0.4% with an inhibition zone of 2.34 mm. The crude extract of *Ficus septica* leaves at a concentration of 4% optimally inhibits the growth of the fungus *Colletotrichum* spp in vitro

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