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The Influence of Papaya Leaf Extract to Anthracnose Disease in Papaya Fruit

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Authors' contributions

This work was carried out in collaboration among all authors. Authors Sudiono and EY designed the study and wrote protocol. Author MAA performed statistical analysis and made some literature searches. Author Sudiono wrote the first draft of manuscript and managed the analyses of the study. All authors read, contributed and approved the final version of the manuscript.

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ABSTRACT

Aims: The objective of this research was to find out the influence of papaya leaf extract to the *in-vitro* growth of *Colletotrichum gloeosporioides* and *in-vivo* anthracnose disease occurrence at papaya fruit.

Methodology: This research used completely randomized design with seven treatments and four replications. Obtained data were analyzed by using analysis of variance and mean scores were compared and tested with least significance difference (LSD) at 5% and polynomial test at 5%.

Results: The research result showed the significant influence of papaya leaf extract and synthetic fungicide in inhibiting the growth of *Colletotrichum gloeosporioides* colony and the disease occurrence at papaya fruit. The polynomial test result of papaya leaf extract showed linier pattern of *C. Gloeosporioides* colony diameter and disease course progression.

Conclusion: The papaya leaf extract inhibited *C. Gloeosporioides* colony growth at 2 up to 7 days after inoculation, but it did not inhibit spore germination and its density. The papaya leaf extract also inhibit the disease occurrence at 5 and 6 days after application.

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Keywords: *Colletotrichum gloeosporioides*; vegetative fungicide; disease occurrence; papaya.

1. INTRODUCTION

Papaya (*Carica papaya* L.) is a common fruit plant in Indonesia and its fruit has rich nutrition. Papaya fruit contains of enzymes, vitamin A, B, C, E, minerals, chymopapain and protease III enzymes [1,2]. Papaya fruit domestic and export demands are increasing annually, but in fact, the papaya fruit production in Indonesia decreases. Papaya production in Indonesia was 904,284 tons in 2016 [3], and it decreased into 875,112 tons in 2017 [4]. This production decrease was caused by fruit damage from improper post-harvesting handling or because of pest organism attack such as pathogenic fungi [5].

One of pathogenic fungi is *Colletotrichum gloeosporioides* that causes anthracnose disease at papaya fruit. Anthracnose disease causes low papaya fruit quality and it is considered as post-harvesting fruit disease, but the pathogen can also infect the plant at pre-harvesting stage. This pathogenic fungi causes red-brownish, wet, small sized, and rounded spots at fruits near to ripening period. At ripen fruit, these spots grow wider quickly to form rounded, red-brownish, and a little bit depositing spots. Then, this pathogenic fungi will continue to grow and rotten inside part of the fruit so that fruit tissues go rotten, softening, and dark coloring [6,7].

Farmers commonly control anthracnose disease by using synthetic fungicide. Continuous fungicide use will increase cost production, farmers' health risks, and deteriorate environment. Considering the bad effects of synthetic fungicide, a more secure alternative for health and environment needs to make. Using vegetative fungicide from vegetative extracts shall be an alternative to overcome anthracnose disease more safely and environmentally friendly [8].

Papaya leaf extract potential can be used as vegetative fungicide to replace synthetic fungicide. The objective of this research was to find out the influence of papaya leaf extract to the *in-vitro* growth of *Colletotrichum gloeosporioides* and *in-vivo* anthracnose disease occurrence at papaya fruit (*Carica papaya* L.).

2. MATERIALS AND METHODS

This research used completely randomized design with seven treatments and four

replications. They were Antracol 70 WP with pronipep 70% active material as synthetic fungicide (P1), control without fungicide (P2), 10% concentrated papaya leaf extract (P3), 20% concentrated papaya leaf extract (P4), 30% concentrated papaya leaf extract (P5), 40% concentrated papaya leaf extract (P6), and 50% concentrated papaya leaf extract (P7). Each treatment was done in *duplo* (2 sets). This research was conducted in two stages; testing inhibition ability of papaya extract leaf to *C. gloeosporioides* growth *in-vitro* and testing papaya leaf extract *in-vivo* to intensity of anthracnose disease in papaya fruit. Obtained data were analyzed with analysis of variance and then mean scores were compared and tested with least significant difference (LSD) test at 5% level and polynomial test at 5% level. *In-vitro* observation parameters were diameter of *C. gloeosporioides* colony, *C. gloeosporioides* spore density, *C. gloeosporioides* spore germination.

The colony diameter estimation was done with the following formula:

$$D = \frac{D1 + D2 + D3 + D4}{4} \times 100\%$$

Description:

D = *C. Gloeosporioides* colony diameter (cm)
D1, D2, D3, D4 = *C. Gloeosporioides* colony length (cm) as results of four different directions.

C. Gloeosporioides spore density can be estimated by using the following formula [9]:

$$C = \frac{t}{n} \times 0.25 \times 10^6$$

Description

C = Spore density /ml of *C. gloeosporioides* suspension
t = spore total amount in observed sample box
n = sample box numbers (5 middle sized boxes)
0.25 = Correction factor of middle scaled sample box use at *haemocytometer*

Spore germination can be estimated by using the following formula:

$$\text{Spore germination} = \frac{\text{Numbers of germinated spore}}{\text{Numbers of not germinated spore}} \times 100\%$$

In-vivo observation parameter was the anthracnose disease occurrence at papaya fruit.

The disease occurrence can be estimated by using the following formula [10]:

$$\text{Occurrence} = \frac{\text{Numbers of spots showing symptoms}}{\text{Numbers of observed spots (sample)}} \times 100\%$$

3. RESULTS AND DISCUSSION

3.1 The Influence of Papaya Leaf Extract to *C. Gloeosporioides* Growth

The analysis of variance of papaya leaf extract and synthetic fungicide to *C. gloeosporioides* colony growth shows a significant influence in inhibiting the colony growth (Table 1). Papaya leaf extract and synthetic fungicide inhibit the *C. gloeosporioides* colony at 2 up to 7 days after inoculation (dai). At 8 up to 10 days after inoculation, the papaya leaf extract does not influence significantly in inhibiting *C. gloeosporioides* colony growth.

The least significant difference (LSD) test of papaya leaf extract treatment at 10% concentration shows significant difference in inhibiting *C. gloeosporioides* colony growth compared to control without fungicide at 2 up to 4 days after inoculation. 20%, 30%, 40%, and 50% concentrations of papaya leaf treatments show different significances in inhibiting *C. gloeosporioides* colony growth in 2 up to 7 days after inoculation compared to control without fungicide. In addition, the synthetic fungicide with propineb 70% active material shows significant difference in inhibiting the *C. gloeosporioides* colony growth compared to control without fungicide in 2 up to 7 days after inoculation. Based on this finding, the abilities of papaya leaf

extract at 20%, 30%, 40%, and 40% concentrations in inhibiting *C. gloeosporioides* colony growth are the same with the control ability of synthetic fungicide with propineb 70% active material.

The papaya leaf extract and synthetic fungicide have inhibiting ability to *C. gloeosporioides* colony growth compared to control without fungicide (Fig. 1). 50% concentration of papaya leaf extract (P7), that has been the best concentration, has strongest inhibiting ability to the *C. gloeosporioides* colony growth compared to other papaya leaf extract concentrations.

The polynomial test result of papaya leaf extract concentrations to *C. gloeosporioides* colony growth shows a linier pattern, and it is presented with equation of ($Y = -0,1857 x + 23,476$). This result shows that the higher is the papaya leaf extract concentration, then the stronger is the inhibiting ability to *C. gloeosporioides* colony growth (Fig. 2).

The observation results of the influence of papaya leaf extract and synthetic fungicide to spore density are presented in Table 2, and the spore germination results after 14 hours are presented in Table 3. The analysis of variance to the influence of papaya leaf extract and synthetic fungicide to spore density and germination show no significant influence in inhibiting the progress of spore density and germination. This indicates that papaya leaf extract and synthetic fungicide with propineb 70% active material is unable to inhibit *C. gloeosporioides* spore density and germination.

Table 1. Effect of papaya leaf extract and synthetic fungicide to *C. gloeosporioides* colony growth

Treatment	Diameter average (cm)									
	2 dai	3 dai	4 dai	5 dai	6 dai	7 dai	8 dai	9 dai	10 dai	
P1	0,500 a	0,738 a	1,200 a	1,819 a	2,394 a	3,094 a	3,731	4,238	4,600	
P2	1,038 b	1,988 b	3,138 b	4,250 b	5,369 c	6,656 c	7,794	8,531	8,900	
P3	0,638 a	1,225 a	2,063 a	3,100 ab	3,981 bc	5,094 bc	6,013	7,013	7,919	
P4	0,500 a	0,806 a	1,788 a	2,669 a	3,575 ab	4,663 ab	5,694	6,700	7,700	
P5	0,544 a	0,944 a	1,731 a	2,663 a	3,375 ab	4,319 ab	5,269	6,181	7,219	
P6	0,531 a	0,800 a	1,475 a	2,281 a	3,319 ab	4,431 ab	5,519	6,650	7,625	
P7	0,500 a	0,681 a	1,394 a	2,225 a	2,969 ab	3,888 ab	4,744	5,650	6,531	
F _{count}	6,586	4,614	3,354	3,093	3,123	2,781	2,486	2,192	2,041	
F _{table}	2,573	2,573	2,573	2,573	2,573	2,573	2,573	2,573	2,573	
Conclusion	*	*	*	*	*	*	NS	NS	NS	
LSD 5%	0,225	0,630	1,034	1,320	1,557	1,953	-	-	-	

Description: P1 = Synthetic fungicide; NS = Not Significant; P2 = Control without fungicide; * = Significant
P3 = 10% papaya leaf extract; dai: days after inoculation; P4 = 20% papaya leaf extract; P5 = 30% papaya leaf extract; P6 = 40% papaya leaf extract; P7 = 50% papaya leaf extract

Table 2. The influence of papaya leaf extract and synthetic fungicide treatments to spore density

Treatment	Spore density/ml
Control with synthetic fungicide	87.500
Control without fungicide	900.000
10% papaya leaf extract	275.000
20% papaya leaf extract	212.500
30% papaya leaf extract	162.500
40% papaya leaf extract	300.000
50% papaya leaf extract	925.000
F _{count}	2.437 NS
F _{table}	2.573

Description: * = Significant NS = Not Significant

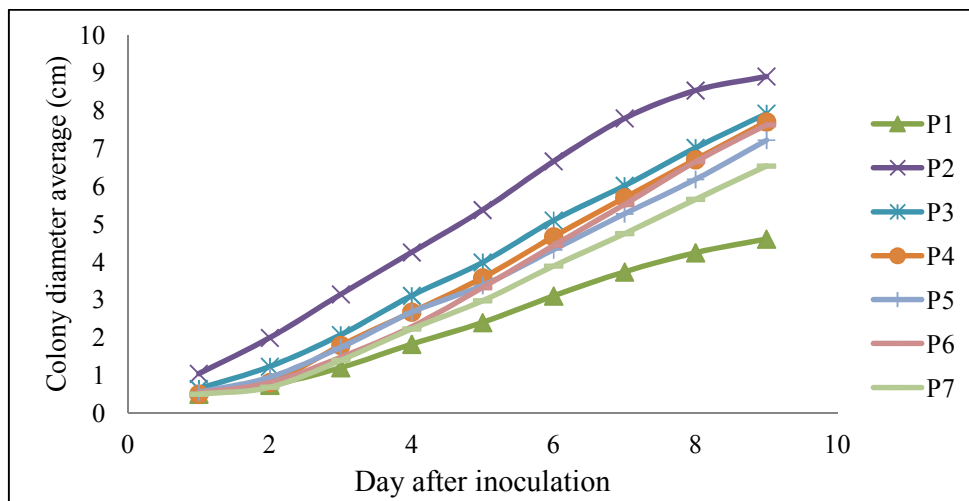


Fig. 1. Effect of papaya leaf extract and synthetic fungicide influences to *C. gloeosporioides* colony growth at 2 up to 10 days after inoculation

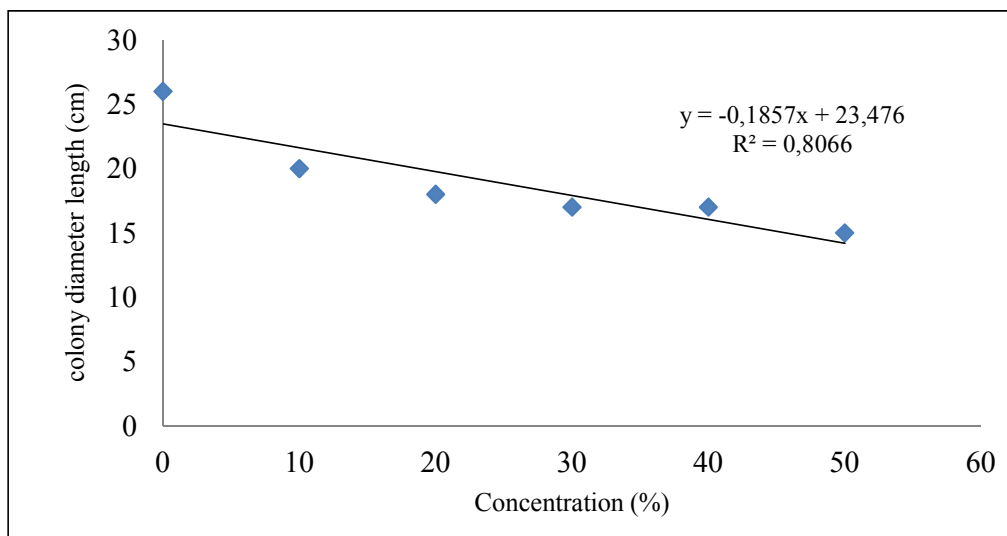


Fig. 2. Growth *C. gloeosporioides* on certain concentrations of papaya leaf extract after 7 days of inoculation

Table 3. The influence of papaya leaf extract and synthetic fungicide to spore germination after 14 hours

Treatment	Spore germination (%)
Control with synthetic fungicide	91.50
Control without fungicide	79.00
10% papaya leaf extract	83.75
20% papaya leaf extract	93.75
30% papaya leaf extract	95.00
40% papaya leaf extract	44.50
50% papaya leaf extract	90.00
F _{count}	2.474 NS
F _{table}	2.573

Description: * = Significant NS = Not Significant

The *in-vitro* testing result shows that the papaya leaf extract inhibits *C. gloeosporioides* colony growth at 2 to 7 days after inoculation. This is assumed because there is an active compound in young papaya leaf such as papain and alkaloid. Papain is a protease enzyme that decomposes proteins [11]. Decomposed proteins will cause protein denaturation so that the fungal protein is damaged and the fungal growth is inhibited. Alkaloid is antifungal compound that is able to inhibit nucleic acid biosynthesis [12]. Inhibited nucleic acid biosynthesis will disturb protein composition process so that fungal growth will be disturbed. However, papaya leaf extract does not inhibit spore density and germination. It is assumed that the active compound in the papaya leaf extract is unable to inhibit *C. gloeosporioides* at generative phase. This finding shows that the papaya leaf extract has fungi-static characteristic (inhibiting fungal growth).

3.2 The Influence of Papaya Leaf Extract to Anthracnose Disease Occurrence in Papaya Fruit

Testing papaya leaf extract influence to anthracnose disease occurrence in papaya fruit was done by using the best papaya leaf extract treatment from *in-vitro* testing results. The *in-vitro* test result obtained four treatments with 20%, 30%, 40% and 50% concentrations. Based this finding, the anthracnose disease occurrence at papaya fruit was done by using six treatments. They were P1 (control with synthetic fungicide), P2 (control without fungicide), P4 (10% papaya leaf extract), P5 (30% papaya leaf extract), P6 (40% papaya leaf extract), and P7 (50% papaya leaf extract).

The analysis of variance result of the influence of papaya leaf extract and synthetic fungicide to anthracnose disease occurrence at papaya fruit showed that there was a significant influence in inhibiting anthracnose disease at 5 and 6 days after application (Table 4). Meanwhile, at 4, 7, and 8 days after application the papaya leaf extract and synthetic fungicide did not influence significantly in inhibiting the anthracnose disease occurrence in papaya fruit.

The least significant difference (LSD) test showed that the 30% and 50% papaya leaf extract concentrations and synthetic showed significant difference in inhibiting anthracnose disease occurrence at papaya fruit compared to control without fungicide at 7 days after application. At 6 days after application, only 30% papaya leaf extract concentration that showed significant difference in inhibiting the anthracnose disease compared to control without fungicide. These findings showed that the abilities of 30% and 50% papaya leaf extract concentrations in inhibiting anthracnose disease at papaya fruit was the same with control with synthetic fungicide with propineb 70% at 5 days after application. At 6 days after application, only 30% papaya leaf extract concentration that was able to inhibit the anthracnose disease at papaya fruit.

In-vivo testing result showed that papaya extract leaf inhibited anthracnose disease intensity, occurrence, and development at papaya fruit, and even it had the same effectiveness with synthetic fungicide with propineb 70% active material. However, the papaya leaf extract and synthetic fungicide did not show significant influence to papaya fruit disease severity. The research result showed that 5% papaya leaf extract concentration and female sex were the best extract to suppress anthracnose disease in red chili fruit compared to other papaya leaf extract concentrations and other papaya leaf extract types. In addition, used papaya leaf extract as vegetative fungicide to control *Colletotrichum* sp. fungi that caused anthracnose disease at chili (*Capsicum annuum* L.), and the research result showed that papaya leaf extract was able to slow down symptoms in chili fruit [13].

The similar research result was obtained that used papaya leaf extract to slow down the *Alternaria solani* fungal mycelium at sweet potato. The research result showed that the higher was the papaya leaf extract concentration, then the strongest will be the

Table 4. The influence of papaya leaf extract and synthetic fungicide to anthracnose disease in papaya fruit

Treatment	Disease occurrence average (%)				
	4 daa	5 daa	6 daa	7 daa	8 daa
P1	0	12.5 a	65 ab	85	92.5
P2	25	62.5 b	82.5 b	95	95
P4	22.5	42.5 ab	65 ab	75	82.5
P5	0	12.5 a	22.5 a	55	85
P6	22.5	60 ab	90 ab	95	95
P7	10	22.5 a	57.5 ab	80	90
F _{count}	1.171	3.241	2.819	1.251	0.350
F _{table}	2.773	2.773	2.773	2.773	2.773
Conclusion	NS	*	*	NS	NS
LSD 0.05	-	3.767	4.176	-	-

Description: P1 = Synthetic fungicide; NS = Not Significant; P2 = Control without fungicide; * = Significant; P4 = 20% papaya leaf extract; daa = days after application; P5 = 30% papaya leaf extract; P6 = 40% papaya leaf extract; P7 = 50% papaya leaf extract

inhibiting ability to *Alternaria solani* fungal mycelium growth. This research showed that the papaya leaf extract has the same effectiveness with synthetic fungicide. Papaya leaf extract and synthetic fungicide are effective in inhibiting *C. gloeosporioides* fungal colony growth, disease occurrence, and anthracnose disease progress at papaya fruit. These findings show that papaya leaf extract can be used as an alternative to control anthracnose disease at papaya fruit compared to synthetic fungicide with propineb 70% active material.

4. CONCLUSION

The papaya leaf extract inhibits *C. gloeosporioides* fungal colony growth at 2 up to 7 days after inoculation, but it does not inhibit spore density and germination. The papaya leaf extract also inhibits anthracnose disease occurrence at papaya fruit in 5 and 6 days after application.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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