Research Article

Secondary Metabolite *Ceiba pentandra* Gaertn. as Biological Control to Canker Disease on Dragon Fruit

Rachmi Masnilah ^{1*}, Ival Oktavian Nurtia Budi ², Ankardiansyah Pandu Pradana ¹, Fariz Kustiawan Alfariy ³

¹ Department of Plant Protection, University of Jember, Jember 68121, Indonesia

² Department of Agrotechnology, University of Jember, Jember 68121, Indonesia

³ Department of Agriculture Science, University of Jember, Jember 68121, Indonesia

Article history:

Submission February 2021

Revised April 2021

Accepted July 2021

*Corresponding author:

E-mail: rachmimasnilah@gmail.com

ABSTRACT

Canker diseases caused by *Neoscytalidium dimidiatum* are a serious threat for dragon fruit production. Comprehensive and environmentally friendly control efforts are needed to reduce yield losses due to this disease. Cottonwood (*Ceiba pentandra*) is one of the plants that contain complex phytochemicals that can control phyto-pathogens. This study aimed to examine the potential of secondary metabolites produced from the leaves and bark of the *C. pentandra* as botanical fungicides. Parts of dragon fruit that are attacked by canker are isolated on Potato Dextrose Agar media. Observation of conidia and fungal spores using a binocular microscope with a magnification of 100×. The hyphal form of the *N. dimidiatum* is rectangular and clear. As botanical fungicides, the leaves and the bark of the *C. pentandra* are dried for 14 days. The extract solution then analyzed for its secondary metabolites. The results of the qualitative analysis showed that each leaf extract and bark (+) compound of flavonoids, alkaloids, tannins, and saponins. The quantitative results of secondary metabolites detected 304.3 mg.kg⁻¹, Flavonoids and Tannins of 1.6 mg.kg⁻¹. Secondary metabolites obtained were tested on the fungus *N. dimidiatum* with inhibitory parameters *in vitro* and *in vivo*. This study followed a factorial completely randomized design consisting of concentration factors and types of extract parts. The data obtained were then analyzed by ANOVA and significant treatments were further tested by Duncan Multiple Range Test (DMRT) at $\alpha = 5\%$. The combination treatment with a concentration of 40 mg ml⁻¹ is more effective in controlling canker in indicators of inhibitory, the extent of the attack, and intensity of disease severity.

Keywords: Canker, Inhibition, *Neoscytalidium dimidiatum*, Severity

Introduction

Canker is one of the primary diseases of dragon fruit and is potentially endemic in almost all regions [1, 2]. A pathogenic fungus *Neoscytalidium dimidiatum* causes canker is dermatomycosis and attacks the stem and fruit that cause serious yield loss in high intensity infection [3]. The symptoms caused by their infection are white patches that spread on the bark and the surface of dragon fruit. Pathogenic fungus *N. dimidiatum* not only attacks dragon fruit but can also infect Royal, Almond, Ficus, and Randu plants [4 - 7].

In Indonesia, control of this disease is still limited to the use of synthetic pesticides. Whereas, the impact of using synthetic pesticides unwisely will cause resistance to pathogens. In addition, pesticide residues will be stored in the soil and fruit surface. It is well known that the active ingredients of pesticides are difficult to decompose and potentially cause land degradation [8]. Previous studies reported that synthetic pesticides can suppress the growth of *N. dimidiatum* mycelium [9]. Even when there was an endemic canker in West Sumatra, it still used a mixture of synthetic pesticides

How to cite:

Masnilah R, Budi ION, Pradana AP, Alfariy FK (2021) Secondary Metabolite *Ceiba pentandra* Gaertn. as Biological Control to Canker Disease on Dragon Fruit. Journal of Tropical Life Science 11 (3): 331 – 337. doi: 10.11594/jtls.11.03.10.

and botanical pesticides, although the results of this combination treatment study can reduce the intensity of the disease was 58%.

An environmental factor also influences high intensity of disease attacks. The high temperature and humidity can significantly increase the pathogen virulence. The increase of CO₂ levels goes up an infected rate of fungus. The mycelium is carried from the host to the healthy plant by the wind [10]. The ecosystem must be considered to maintain the humidity around the plant cultivation. The mitigation can be made to prevent canker attacks by mapping epidemiological diseases based on regional topography. The Canopy treatment affects disease incidence and severity for management control of dragon fruit [11, 12].

Control of canker diseases in dragon fruit is still done conventionally. Even in some dragon fruit-producing countries, control of this disease is still carried out using synthetic pesticides unwisely [13, 14]. Seeing this phenomenon, alternative efforts to control cankers that are effective and environmentally friendly are needed. An effort that can be made is to use extracts from cottonwood (*Ceiba pentandra*). Currently, *C. pentandra* extract has not been widely used as a botanical pesticide. On the other side, the extract of these plants is usually used in the pharmaceutical industry [15, 16, 17]. The cottonwood contains phytochemicals and ethanol that are potential as antimicrobials [18]. From the above backgrounds, this study aimed to determine the potential of cottonwood extracts' secondary metabolites to control canker diseases on dragon fruit.

Material and Methods

Study area

The *N. dimidiatum* infected dragon fruit was taken from Banyuwangi Regency, East Java, Indonesia (8013'17.98"S114021'21,29"E). In vitro and in vivo test conducted at the Plant Disease Laboratory, University of Jember.

Isolation of pathogens *Neoscytalidium dimidiatum*

Samples of infected plant stems were cut using a sterile knife (1 × 1) cm. The sample was soaked in 70% ethanol for 3 minutes then rinsed with sterile distilled water. The sample was planted on Potatoes Dextrose Agar (PDA) media. All steps were conducted in aseptic conditions. Furthermore, the isolates were incubated for 3-5 days [2].

Extraction of stems and leaves of the cottonwood

Leaves and bark of 2 kg cottonwood were washed and dried for 14 days at a controlled temperature of 20 – 32°C. Then the sample is mashed until it turns into flour. A total of 250 g of sample was then put into the macerator. The methanol was added with a ratio of 1 : 5 (w/v). The mixture was then incubated for 72 hours and filtered using filter paper. The solution formed was then put into a rotary evaporator to separate the solvent from the crude extract.

Analysis of secondary metabolites

The crude extract was analyzed for its compounds qualitatively and quantitatively. The qualitative test aimed to detect and determine the presence of secondary metabolite compounds, while the quantitative test aimed to determine the total concentration in the extracted sample.

Anti-microbial test in vitro and in vivo

The concentrations used in the experiment were 0 mg.ml⁻¹, 10 mg.ml⁻¹, 20 mg.ml⁻¹, 30 mg.ml⁻¹, and 40 mg.ml⁻¹. The concentration calculated using the following formula:

$$N1 \times V1 = N2 \times V2$$

Note: (N1) concentration of stock solution; (N2) concentration of solution to be made; (V1) volume of solution 1; and (V2) volume solution 2.

The test was carried out by pouring 9 ml of media mixed with 1 ml of extract into a petri dish. Furthermore, pathogens with a diameter of 50 mm were grown on the media. After the process, the fungus was then incubated.

In vivo testing was carried out using concentrations that were effective in suppressing the growth of *N. dimidiatum*. Observation of the symptoms of attack was observed by the detached leaf assay method. Stems of plants are injured with a sterile needle of 3 points. The stem was sprayed with the cottonwood raw extract and put in a container for 24 hours. The stem of the treated plant was inoculated by dropping the suspension of inoculum *N. dimidiatum* at a density of 2.7×10^7 conidia per ml, and it was incubated for 14 days.

Experimental design

This study was designed with Factorial Completely Randomized Design, which consisted of

Table 1. Treatments used in this study

Concentration (K)	Extraction from part	
	Leaf (D)	Bark (B)
0 mg.ml ⁻¹	K0D	K0B
10 mg.ml ⁻¹	K1D	K1B
20 mg.ml ⁻¹	K2D	K2B
30 mg.ml ⁻¹	K3D	K3B
40 mg.ml ⁻¹	K4D	K4B

Table 2. Result of the qualitative secondary metabolite from leaves and bark

Secondary metabolite	Stand-ard color	Sample			
		Leaf		Bark	
		Color	Result	Color	Result
Flavonoid	Yel-low	Yel-low	+	Yel-low	+
Alkaloid	Or-ange	No color	-	No color	-
Tanin	Black	Black	+	Black	+
Saponin	Purple	Pur-ple	+	Pur-ple	+

Note: (-) undetected, (+) detected

Table 3. Result of the quantitative secondary metabolite from leaves and bark extraction

Secondary Quantitative	Sample (mg/kg)	
	Leaves	Bark
Flavonoid	304.30	215.90
Tanin	1.60	1.40

two factors: factor 1 was concentration and factor 2 was extracted from the cottonwood. The details of the treatment used were: factor 1: consisting of variations in concentration (0 mg.ml⁻¹, 10 mg.ml⁻¹, 20 mg.ml⁻¹, 30 mg.ml⁻¹, and 40 mg.ml⁻¹), factor 2: consisting of extracts of parts. Details of treatment combinations are presented in Table 1.

Data analysis

The infection data was observed based on the symptoms that appeared and calculated using the following formula:

$$l = (d1 + d2)^2 \times \pi \frac{1}{4}$$

Note: (I) area infected, (d1) diameter *N.dimidiatum* infection - transverse, (d2) diameter *N. dimidiatum* infection - longitudinal, (π) pi value 3.14

The disease severity was observed and calculated using the following formula:

$$\frac{ni \times vi}{N \times V} \times 100\%$$

Note: (D) diseases severity, (ni) number of plant parts infected, (vi) score of infection, (N) number of all plants observed, (V) highest score

Data were analyzed using ANOVA, if the data were significantly different, then it was followed by the DMRT (Duncan Multiple Range Test) at α = 5%.

Results and Discussions

Secondary metabolite of *Ceiba pentandra* raw extract

The analysis showed that the raw extract contains flavonoids, tannins, and saponins (+) (Table 2). In contrast, the alkaloids from the leaves and bark of the cottonwood were not detected (-). The quantitative analysis showed that the concentration of flavonoid compounds is greater than the tannin. Flavonoid compounds from cottonwood leaves were higher by 304.3 mg.kg⁻¹ when compared to extracts from stem bark (215.9 mg.kg⁻¹). At the same time, tannin compounds from leaves and stem bark of kapok plants were detected with minimal concentrations of 1.6 mg.kg⁻¹ and 1.4 mg.kg⁻¹ (Table 3). Secondary metabolites from several plants are reported to be able to affect microbial metabolism. Previous studies said that the application of raw extracts from medicinal plants could suppress the growth of *Pseudomonas aeruginosa*. The suppression of growth was due to the presence of antimicrobial compounds contained in the extract. In addition, the antioxidant content in the extract can inhibit microbial growth [19].

Inhibition tests in vitro

The study examines the potential of secondary metabolites to inhibit the development of the *N. dimidiatum*. The inhibition process varies depending on the concentration used (Figure 1). Figure 1a-e showed the development of isolates from the treatment of the extract from the leaves of the cottonwood. Figure 1f-j showed the development of the isolates from the extract of the bark. The difference in growth was influenced by the concentration used in the test. Treatment with 0 mg.ml⁻¹ has faster fungal development than other treatment used in this study. The result was significantly different from the 40 mg.ml⁻¹ treatment.

Figure 1. Inhibition of metabolites secondary to *N. dimidiatum* on PDA media on day 5 after inoculation with different treatment concentrations

Figure 2. Symptoms of canker diseases tendrils, (A) symptoms on the day 8 after inoculation, (B) symptoms on the day 14 after inoculation, (C) initial symptoms of *N. dimidiatum* at day 8 after inoculation, and (D) advanced symptoms of *N. dimidiatum* at day 16 after inoculation

The morphological characteristics of *N. dimidiatum* colony are blackish gray and grow feathers on the colony. In the previous study [2], the macroscopic character of *N. dimidiatum* is hairy colonies with black pigmentation. Color changes occur with the growth of fungi, ranging from gray to black. Based on observations in this study within 3 days, the fungus can spread to 3 cm per day on the media.

The diameter of the colonies of *N. dimidiatum* treated with extracts from cottonwood leaves showed a smaller size (Figure 1A-E) than the diameters of the colonies treated with bark extract (1F-J). This phenomenon is thought to be due to differences in the metabolite content of the two extracts. The number of flavonoids and tannins contained in the leaf extract was higher than that of the bark extract. These results are consistent with previous studies, which stated that the higher the

concentration of plant secondary metabolites, the higher the potential to inhibit the growth of pathogens [19, 20]. The higher the concentration given, the smaller the number of spores formed. Inhibition of the formation of the spores will result in a reduction in the number of inoculums during the infection process.

Inhibition test in vivo

N. dimidiatum infected dragon fruit was characterized by the occurrence of spots on the stem. After macroscopic identification, the microscopy identification was then tested with the Postulate Koch protocol. The Postulate Koch test was carried out by taking pure cultures of *N. dimidiatum* to the stems of healthy plants. The *N. dimidiatum* showed a symptom of patches with small brownish-white spots (Figure 2A). The initial symptoms in dragon fruit stem from concave brownish to yellow lesions [2]. After seven days, the infected

Figure 3. Effect of concentration on the extensive attack disease *N. dimidiatum*. (Note: K: Concentration, 0: 0 mg.mL⁻¹, 1: 10 mg.mL⁻¹, 2: 20 mg.mL⁻¹, 3: 30 mg.mL⁻¹, 4: 40 mg.mL⁻¹, D: leaf, and B: bark)

Figure 4. Effect of serial concentration on the diseases severity

stem turned dark brown. Then the infected part formed black conidia on the surface. The formation of conidia is relatively slow, requiring ± 14 days (Figure 2B). It is shown that the color changes to brownish-yellow spots and there is a large patch accompanied by decay [13].

The incubation period was also tested in this study. Figure 2C-D showed the experiment to determine the incubation period of the disease. The incubation period is the time needed by pathogens to infect the host plants. Figure 2C shows symptoms eight days after inoculation, while Figure 2D shows symptoms 16 days after infection. The symptoms were increasingly widespread with a

wider size of spots, brownish discoloration, and decay. This study's symptoms are similar to the symptoms reported in the previous research [21].

Infectious diseases

Based on the development of the observed symptoms, it is known that the raw extract of cottonwood is fungistatic. This means it cannot kill the fungal pathogens but can inhibit the growth of fungal pathogens. The mode of action is different from toxic fungi, which can kill pathogens. The fungus will continue to develop when the secondary metabolite compounds are no longer available (Figure 3). On day 0 to day 16, symptoms on petri

dish media are increasing. In the treatment of concentration with 0 mg ml⁻¹ has the most extensive symptoms compared to other treatments. In opposite, treatment with a concentration of 40 mg.ml⁻¹ has the lowest area of symptoms. It is proven that the role of secondary metabolite compounds significantly influences the extent of pathogen infection.

Diseases severity

Figure 4 shows the effect of concentration treatment on disease severity. The highest disease severity was found in the treatment with concentrations 0 mg.ml⁻¹ (K0) and 10 mg.ml⁻¹ (K1) with average disease severity of 40%. The K2 treatment showed a total of 38% diseases severity, while the K3 treatment showed a total of 34% diseases severity. The best treatment was K4 (40 mg.mg⁻¹ extract) with severity of 20.67%. The data above shows that the severity of the disease is inversely proportional to the concentration of the extract used. The higher the concentration used, the lower the severity of the infection. The Duncan Multiple Range Test with $\alpha = 5\%$ results showed that the treatments (K0) and (K1) were not significantly different but it was significantly different from the treatment (K3) and (K4) was the highest concentration value.

Conclusion

The raw extracts of leaves and bark of the cottonwood potentially suppress canker disease on dragon fruit. The extract contains flavonoids, tannins, and saponins. The secondary metabolite content was detected higher in the leaves than in the bark. The study results show that the interaction treatment of cotton leaf extract as much as 40 mg.ml⁻¹ can inhibit the growth of colony diameter and the formation of spores of the pathogen *N. dimidiatum*.

Acknowledgment

The authors thank the Plant Disease Laboratory and work with the Plant Clinic Program to support facilities and infrastructures for the study.

References

1. Jumjunidang, Yanda RP, Rizka, Emilda D (2019) Identification and characterization of stem and fruit canker diseases on dragon fruit (*Hylocereus spp.*) in Indonesia. *Journal of Horticulture* 29(1): 103–110.
2. Mohd MH, Salleh B, Zakaria L (2013) Identification and

- molecular characterizations of *Neoscytalidium dimidiatum* causing stem canker of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. *Journal of Phytopathology* 161: 841–849. doi: 10.1111/jph.12146.
3. Florez-Munoz SV, Gómez-Velásquez JC, Loaiza-Díaz N et al. (2019) ITS rDNA gene analysis versus MALDI-TOF MS for identification of *Neoscytalidium dimidiatum* isolated from Onychomycosis and Dermatormycosis cases in Medellin (Colombia). *Microorganism* 7 (306): 1-10. doi:10.3390/microorganisms7090306.
4. Nouri MT, Laurence DP, Yaghmour MA et al. (2018) *Neoscytalidium dimidiatum* causing canker, shoot blight and fruit rot of almond in California. *Plant Disease X(X)*: 1–10. doi: 10.1094/PDIS-12-17-1967-RE.
5. Al Raish SM, Saeed EE, Sham A et al. (2020) Molecular characterization and disease control of stem canker on royal poinciana (*Delonix regia*) caused by *Neoscytalidium dimidiatum* in the United Arab Emirates. *International Journal of Molecular Science* 21 (1033): 1–17. doi:10.3990/ijms21031033.
6. Afrifa AB, Boateng AY (2012) Controlling dieback disease and its effect on the growth performance of a six-year *Ceiba pentandra* Stand. *Journal of Agriculture & Social Sciences* 8 (3): 106–108.
7. Ray JD, Burgess T, Lanoiselet V M (2010) First record of *Neoscytalidium dimidiatum* and *N. novaehollandiae* on *Mangifera indica* and *N. dimidiatum* on *Ficus carica* in Australia. *Australasian Plant Disease Notes* 5: 48–50. doi:10.1071/DN10018.
8. Jacobsen RE, Fantke P, Stefan T (2015) Analyzing half-lives for pesticide dissipation in plants. *SAR and QSAR in Environmental Research* 26 (4): 325–342. doi: 10.1080/1062936X.2015.1034772.
9. Widiastuti A, Agustina W, Wibowo A, Sumardiyono C (2011). In vitro test pesticides effectiveness against some pathogens of important diseases in dragon fruit (*Hylocereus spp.*). *Jurnal Perlindungan Tanaman Indonesia* 17(2): 73–76.
10. Pathak R, Singh AK, Alkes T, Gehlot P (2018) Impact of climate change on host, pathogen, and plant diseases adaptation regime: a review. *Bioscience Biotechnology Research Asia* 15(3): 529–540. doi:10.13005/bbra/2658.
11. Yáñez-López R, Torres-Pacheco I, Guevara-González RG et al. (2012) The effect of climate change on plant diseases. *African Journal of Biotechnology* 11 (10): 2417–2428. doi: 10.5897/AJB10.2442.
12. Hieu NT, Thu NTA, Linh DT et al. (2018) Effect of various degree of canopy pruning on plant growth, yield, and control of canker disease of dragon fruit crop: 2018; International Conference on Tropical Fruit Pests and Diseases.
13. Fullerton RA, Sutherland PA, Rebstock RS, Giang T (2015) The life cycle of dragon fruit canker caused by *Neoscytalidium dimidiatum* and implications for control. *Dragon Fruit Regional Network Initiation Workshop*. 71–80.
14. Hidayat NA, Sofian, Akhsan N (2018) Intensity of stem rot disease in dragon fruit (*Hylocereus polyrhizus*) at Samboja Regency. *Agroteknologi Lembab* 1(1): 53–60.
15. Peraza-ku SA, Cervantes-Uc JM, Morales BE, Calderon JAU (2020) Modification of *ceiba pentandra* cellulose for drug release applications. *e-Polymare* 20: 194–202. doi: 10.1515/epoly-2020-0025.
16. Kar S, Nehru J, Vishwavidyalaya et al. (2020) Cotton

- wood tree [*Ceiba pentandra* (L.) Gaertn]-an overview: 22 June 2020; Tikamgarh, College of Agriculture.
17. Syihabudin V, Sari BL, Utami NF, Apriliani NA (2018). Inhibitory activity of α -glucosidase of bark of *Ceiba pentandra*. Indonesian Journal of Pharmacology 29 (4): 206–213. doi:10.14499/indonesianjpharm29iss4pp206.
 18. Pratiwi RH (2014) Potential cottonwood (*Ceiba pentandra*) GAERTN. on traditional medicine service. E-Journal Widya Kesehatan dan Lingkungan 1 (1): 53-60.
 19. Raji P, Samrot AV, Rohan DB et al. (2019) Extraction, characterization and invitro bioactivity evaluation of alkaloids, flavonoids, saponins and tannins of *Cassia Alata*, *Thespesia Populnea*, *Euphorbia Hirta* and *Wrightia Tinctoria*. Rayasan Journal Chemical 12 (1): 123–137. doi: 10.31788/RJC.2019.1214054.
 20. Ajayi OE, Awala SI, Olalekan OT, Alabi OA (2012) Evaluation antimicrobial potency and phytochemical screening *Persea americana* leaf extract against selected bacterial and fungal isolates of clinical important. Microbiology Research Journal International 20 (1): 1-11. doi: 10.9734/MRJI/2017/24508.
 21. Athipunyakom P, Seemadua S, Doungsa-ard C (2012) Dragon fruit (Pitaya) diseases in Thailand : 2012: Improving Pitaya Production and Marketing, Thailand.

This page is intentionally left blank.