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Antifungal Activity of *Morinda citrifolia* Leaf Extracts Against *Colletotrichum acutatum*

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ABSTRACT

Anthraxnose is a plant disease that can infect a variety of plants worldwide. Fungal pathogen groups are the cause of anthracnose, of which is *Colletotrichum acutatum*. *Morinda citrifolia* is famous for having antimicrobial activity. This study aims to determine the antifungal activity of *M. citrifolia* leaves extract against the growth of the fungus *C. acutatum*. The extract solvent used was 96% ethanol. The experiment consisted of five treatments (0%, 20%, 40%, 60% and propineb 0.1% as positive control). The method used in this study was the poisoned food technique. In this technique, *C. acutatum* was grown on PDA media mixed with *M. citrifolia* leaves extract. Antifungal activity was observed based on reduced mycelium growth compared to control. Observations were made every day by measuring the diameter of the fungus mycelium for eleven days. The analysis showed that 60% *M. citrifolia* leaves extract effectively inhibited the growth of the mycelium *C. acutatum* on the eleventh day of observation.

Keywords: anthracnose; *Colletotrichum acutatum*; mycelium; noni, poisoned food technique**INTRODUCTION**

Anthraxnose is a common pathogen found in plants. This disease uses crop losses of up to 80-100% (Coelho *et al.*, 2013; Bill *et al.*, 2014). The symptoms are most easily observed on leaves and fruits. Initially, anthracnose appears as yellow, brown, dark brown, or black spot lesions and then spread widely. Anthracnose is transmitted through wind and water. Infection can occur directly on the fruit's skin cuticle, through wounds, or seeds. *Colletotrichum* species, including *Colletotrichum acutatum*, cause anthracnose.

Colletotrichum acutatum causes anthracnose in several important crops such as chili, citrus, mango, strawberry, and avocado (Wharton & Diéguez-Uribeondo, 2004; Ibrahim, 2017). This species is often mistakenly identified as *Colletotrichum gloeosporioides* hence it has a similar range of hosts and morphology. Molecular identification can be used to distinguish between these species. Colonies of *C. acutatum* are usually white-gray, pink, pale orange, or gray (Mari *et al.*, 2012; Gaffuri *et al.*, 2017; Ibrahim, 2017). The conidia shape of *C. acutatum* is elliptical and has a pointed end. Under suitable conditions, *C. acutatum* can grow quickly in

plants and cause severe symptoms. However, *C. acutatum* may also be quiescent in the host plant and then develop in the post-harvest stage. Preventive strategies must be employed to prevent these pathogens from causing anthracnose in crops.

Farmers usually use synthetic fungicides to control anthracnose. However, synthetic fungicides can be detrimental to the environment. Excessive and irrational use of synthetic fungicides can kill non-target organisms (Marcos *et al.*, 2012; Muñoz-Leoz *et al.*, 2012). Therefore, environmentally friendly disease control needs to be developed. This encourages the search for alternative products that can replace chemical components that are harmful to the environment. Some plants can produce metabolites that are beneficial to medicinal plants. These medical plant extracts have been shown to reduce the incidence of plant-pathogen infections in various crops.

Noni plants (*Morinda citrifolia*) is one of the well-known medical plants in Southeast Asia with antimicrobial activity. Noni extract has anthelmintic activity against *Pheretima posthuma* (Giri *et al.*, 2010), *Haemonchus contortus* (Cabardo Jr & Portugaliza, 2017), *Fasciola gigantica* (Hegazi *et al.*, 2018) worms,

antibacterial (Peixoto ⁷ *et al.*, 2011; Katata-Seru *et al.*, 2018), antiviral (Ratnoglik *et al.*, 2014; Wang *et al.*, 2016) and antifungal (Goss *et al.*, 2017) activity. Essential oils extracted from noni fruit can inhibit the pathogenic fungi *Exserohilum turcicum*, a pathogen that causes Northern Corn Leaf Blight (NCLB), and *Bipolaris maydis*, a pathogen that causes Southern Corn Leaf Blight (SCLB) (Silva *et al.*, 2017; Veloso *et al.*, 2020). Jayaraman *et al.* (2008) reported that methanol extract from noni fruit showed antifungal activity against *Candida albicans*, *Aspergillus niger*, *Trichophyton mentagrophytes*, *Penicillium* sp., *Fusarium* sp., *Mucor* sp., *Rhizopus* sp., *Aspergillus fumigatus*, and *Aspergillus flavus*. While several studies using plant extracts and evaluating their antifungal effects have been published, studies to investigate the potential of *M. citrifolia* extracts against *Colletotrichum* sp. are still scarce. Therefore, this study aims to analyze the antifungal activity of noni leaf extract against *C. acutatum* in vitro.

MATERIALS AND METHODS

Preparation of crude leaf extract. Noni (*Morinda citrifolia*) leaves are taken from the field in the Umbulharjo Yogyakarta. Noni leaves are sorted and selected with green characteristics, fresh, undamaged, and not rotten. The leaves are cleaned and rinsed with running tap water. Then, the leaves are dried using an oven at 60°C for 15 hours. Dried leaves were ground to obtain dry leaf powder.

Noni leaves (390 grams) were extracted using 96% ethanol (2.34 L) in a ratio of 1:6. The leaves were macerated for 24 hours. After maceration was completed, the mixture was filtered to obtain the filtrate. The filtrate was then evaporated using a vacuum rotary evaporator at 40°C for 10 hours. The extraction yield was calculated based on Wijaya *et al.* (2018).

$$\text{Yield \%} = \frac{\text{mass of extract}}{\text{mass of dry matter}} \times 100\%$$

Antifungal activity test against *Colletotrichum acutatum*. An antifungal activity test was carried out using the poisoned food technique (Anggreini *et al.*, 2016). Three kinds of crude leaf extract solutions were made

by dissolving the extract with distilled water so that the concentration of 20%, 40%, and 60% is obtained. Then, 1 mL of crude leaf extract solution for each concentration was added to the 5 mL PDA media. The mixture of media and crude leaf extract solution was poured into a sterile petri dish. Propineb 0.1% was used as a positive control, while distilled water was used negatively. One loop of *C. acutatum* culture was placed right in the middle of the media and then incubated at room temperature for 11 days. Fungal mycelium growth was observed every day by measuring the diameter of the fungus mycelium at each treatment. The percentage of antifungal activity was calculated using a formula, according to Iskarlia *et al.* (2014).

$$PA = \frac{D1 - D2}{D1} \times 100\%$$

PA = Percentage of antifungal activity (%)

D1 = Fungal mycelium diameter at negative control (cm)

D2 = Fungal mycelium diameter at various extract treatment (cm)

Data Analysis. All data analyses used ANOVA (Analysis of Variance) and Duncan's Multiple Range Test (DMRT) with a level of 5%.

RESULT AND DISCUSSION

Ethanol consists of polar -OH groups and non-polar CH₂CH₃ groups (Vrhovsek *et al.*, 2011; Inel *et al.*, 2016;), making it possible to dissolve polar and nonpolar secondary metabolites. The purpose of using ethanol solvents is to obtain potential secondary metabolite compounds as antifungal agents. Based on chemical analysis, *Morinda citrifolia* contains at least more than 200 bioactive compounds such as acids, alcohols, phenols, anthraquinones, carotenoids, esters, triterpenoids, flavonoids, glycosides, lactones, ketones, and aromatic compounds (Azeida *et al.*, 2019). Ethanol can dissolve secondary metabolites such as alkaloids, tannins, polyphenols, flavonoids, terpenoids, saponins, and polyphenolic compounds from noni leaves (Deng *et al.*, 2011; Zhang *et al.*, 2016). The yield in the noni leaf extraction process obtained in this study was 9.333%. Ethanol extract 96% of noni leaf has organoleptic

characteristics: the greenish-brown, distinctive odor of noni leaves, and a thick texture.

The antifungal activity test of 96% ethanol extract of *M. citrifolia* on the *C. acutatum* was carried out with the Poisoned Food Technique

(Anggreini *et al.*, 2016). In this technique, noni leaf extract was mixed into PDA media, used for fungal growth. Based on (Figure 1), each extract treatment can inhibit the growth of fungal mycelium.

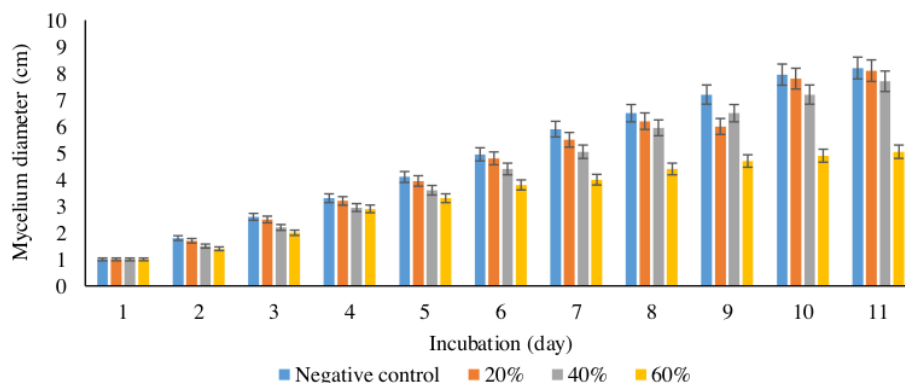


Figure 1. The effect of noni (*Morinda citrifolia*) leaf extract on mycelia growth diameter of *Colletotrichum acutatum*

Based on the fungal growth results, the size of fungal mycelium in all extract treatments was shorter than negative controls. The results showed that the fungal mycelium diameter at the 60% extract treatment was the shortest. The

results showed that 20% and 40% extract treatments had not shown any significant difference to negative controls, but 60% extract treatments had shown significant differences (Table 1).

Table 1. Fungal mycelium diameter on day 11

Concentration	Average diameter of fungus mycelium (cm)
Negative control (0%)	8.325 ^b
20%	8.125 ^b
40%	7.575 ^b
60%	5.075 ^a

The average diameter of fungal mycelium was smaller in extract treatment than the negative control. The percentage of antifungal activity was calculated based on the length of fungal mycelium in the treatment with fungal

mycelium in control. In this study, the highest percentage of antifungal activity (39%) was treated with the addition of 60% extract (Table 2).

Table 2. Percentage of antifungal activity of noni (*Morinda citrifolia*) leaf extract against *Colletotrichum acutatum*

Concentration	Percentage of Antifungal Activity
Negative control (0%)	0%
20%	2.4%
40%	9%
60%	39%
Positive control	87.98%

Colonies of *C. acutatum* used in this study were white and creams (Figure 2). *C. acutatum* colonies were initially white and then turned to

pink or orange. PDA media that has been mixed with noni leaf extract turns yellowish. If the addition of noni leaf extracts increases, the

yellow color was getting thicker. Fungal growth was observed for eleven days. After eleven days, fungal mycelium in the negative control covered all media areas in the petri dish (Figure

2b). However, fungal mycelium in media mixed with noni leaf extract had not yet covered the petri dish area, as well as a positive control.

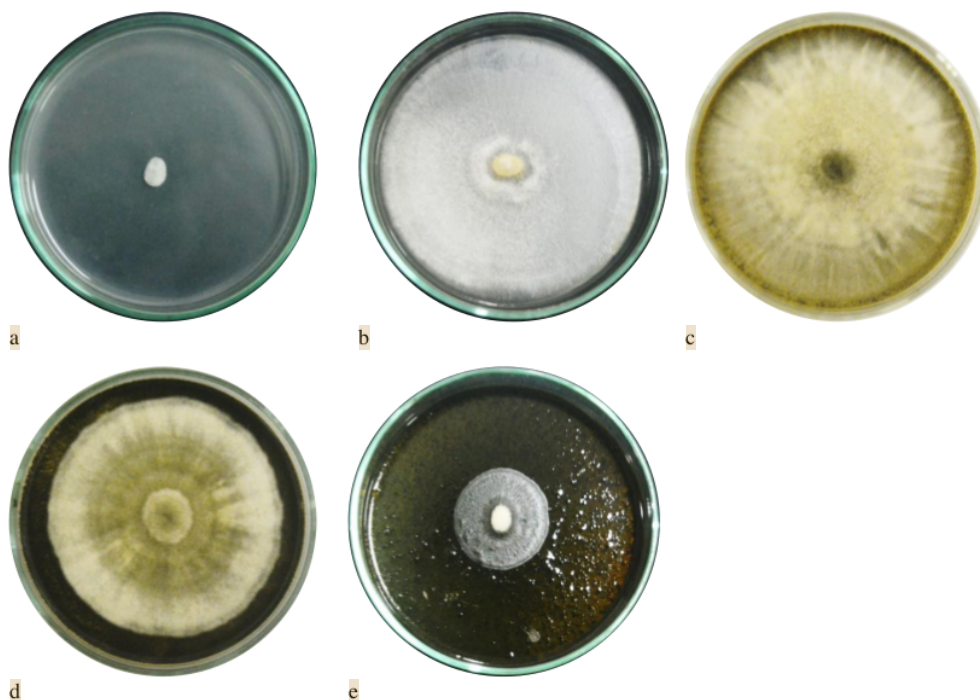


Figure 2. *Colletotrichum acutatum* mycelium: a. Positive control; b. Negative control (0%); c. 20%; d. 40%; e. 60%

C. acutatum growth is inhibited due to active compounds from secondary metabolites in noni leaves. Secondary metabolite compounds such as terpenoids, alkaloids, flavonoids, and saponins in noni leaves are thought to be antifungal. Terpenoid compounds, including triterpenoids and steroids, are natural bioactive compounds that can inhibit fungal growth by disrupting the structural and functional integrity of cytoplasmic membrane, and the development of fungal spores (Oros, 2010; Rao *et al.*, 2010; Tao *et al.*, 2014; Bilal *et al.*, 2018). Anggraini (2016) found that in *Colletotrichum capsici*, the higher the noni leaf extract concentration, the fewer spores produced by *Colletotrichum capsici*. Hydrophobic or lipophilic properties in terpenoid compounds are likely to cause cytoplasmic membrane damage, cell

coagulation, and proton disruption in fungal cells. Alkaloids are compounds that have antimicrobial activity by inhibiting the biosynthesis of fungal nucleic acids so that fungi cannot develop and eventually die (Jalianto, 2015).

Flavonoids are the largest compounds in nature that have antibacterial and antifungal effects because they contain phenol groups. Lipophilic properties in flavonoids can disrupt the microbial membrane (Jalianto, 2015). Saponin compounds in noni leaf extract contribute as an antifungal by reducing the surface tension of the sterol membrane from the fungus cell wall so that membrane permeability increases, which causes the movement of intracellular fluid out of the cell, provoking its death (Septiadi, 2013; Assi *et al.*, 2017). Based on this information, the noni leaf extract is

beneficial for a growth inhibitor of *C. acutatum*. Noni leaf extract can be developed into biofungicides to control *C. acutatum* that causes anthracnose. Therefore, further field-scale research needs to be done to obtain more complete data related to this application on crops.

CONCLUSION

The optimum concentration of noni leaf extract (*Morinda citrifolia*) in inhibiting the growth of *Colletotrichum acutatum* was 60%, with a percentage of antifungal activity of 39%.

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