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Utilization of Neem Leaves as a Biological Pesticide for the Control of Anthrachnose Diseases in Dragons (*Hylocereus* sp.)

Nolla Dwi Elvira[1], Yenny Wuryandari[1,2]*

[1]Agrotechnology Study Program, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya 60294, Indonesia
[2]Innovation Center of Appropriate Food Technology for Lowland and Coastal Area, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya 60294, Indonesia

Corresponding author:
E-mail:
yennywuryandari@upnjatim.ac.id

ABSTRACT

Dragon fruit (*Hylocereus* sp.) has problems in its cultivation related to Plant Pest Organisms (OPT). One of the pathogens that attack dragon fruit plants is Colletotrichum sp. which can cause anthracnose. dragon fruit pest control method can be done by using biological pesticides from neem leaf extract. Pesticides from plant extracts are sources of various compounds that are rich in various types of active ingredients such as secondary metabolites that play an important role in the process of interacting or competing and protecting themselves from competitors. The purpose of this study was to determine the effectiveness of neem leaf pesticide for controlling anthracnose on dragon fruit plants. The method used is testing the inhibitory power of neem leaf biological pesticides against anthracnose on dragon fruit plants in vitro. The concentration treatments of biological pesticides included 0%, 20%, 40%, and 60%. The results obtained showed that the biological pesticide neem leaf with a concentration of 20% already had an inhibitory power against the pathogen Colletotrichum sp. which is quite good because at that concentration it already has a fairly high antifungal compound. The magnitude of the inhibitory power of neem leaf biological pesticides at a concentration of 20% was 53.94%.

Keywords: Dragon fruit plant, Anthracnose, Biological pesticides, Neem Leaves

Introduction

Dragon fruit (*Hylocereus* sp.) is a horticultural commodity that has many benefits and has been cultivated in many countries, including Australia, China, Israel, Malaysia, Nicaragua, Taiwan, and Vietnam (Luders, 2004). Dragon fruit cultivation is usually done with a monoculture system. Planting with a monoculture system can trigger the occurrence of attacks by types of pathogens that have never existed before and have the potential to be destroyed it can reduce productivity yields.

Anthracnose is a disease that often attacks dragon fruit plants. The fungus that can cause anthracnose disease is *Colletotrichum* sp. According to Syafnidrati et al. (2013), Anthracnose is one of the important diseases in dragon fruit plants because it can cause losses of up to 99.5%. This fungus can attack stems, flowers, and fruit. The damage it causes failure of fruit formation and damage to fruit quality from planting to post-harvest (Phoulivong, 2011).

The method of controlling dragon fruit plant pests, especially anthracnose, needs to be shifted to methods that do not leave residues and prevent environmental damage, namely more environmentally friendly control using biological pesticides. According to Tjahjani and Rahayu (2003), neem (*Azadirachta indica*) is a plant that can be used as a botanical pesticide and is known to have antifungal properties that have the potential to be used as a botanical pesticide. Neem leaf extract is reported to contain the active ingredients azadirachtin, solanine, melantriol and nimbin which function as pesticides. According to Ruskin (1993) Neem leaf extract is known to produce compounds that inhibit the production of mycotoxins by pathogenic fungi, these compounds include Azadirachtin, nimbin and nimbindin which are able to inhibit the growth of pathogenic fungi. nimbin and nimbidine compounds

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contained in neem leaf extract have a high fungicidal effect, causing the growth of pathogenic mycelium to be inhibited.

Material and Methods

This research was carried out from January 18 to February 18 2021 at the Plant Protection Laboratory of the Ketindan Agricultural Training Center, Lawang, East Java. The research methods were a sampling of dragon fruit plants that were attacked by anthracnose, isolation, and purification of plant pathogens, microscopic and macroscopic identification, manufacture of vegetable pesticides from neem leaves, and in vitro testing of the inhibitory power of neem leaf vegetable pesticides against anthracnose disease on dragon fruit plants.

A sampling of dragon fruit plant parts affected by anthracnose

Sampling is done by observing the symptoms and signs of a visible pathogen attack. Plant samples taken by plants with symptoms of stem parts such as dry rot are brownish yellow. The rotten part is concave and dark brown in the middle. According to Faidah et al. (2017), Anthracnose disease shows early symptoms of spots with wide, round brown spots surrounded by brown and yellow halos. Further symptoms in the tendrils are straw brown spots, starting from the edges of the tendrils, you can see blackish brown spots that are lined up regularly and some are also found with spots.

Isolation and purification of plant pathogens

Isolation of the pathogen is cutting the infected plant samples that have been obtained, namely ½ sick parts and ½ healthy plant parts. Next, dip the plant parts in chlorox for 2 minutes, then 70% alcohol and sterile distilled water to minimize the growth of other microorganisms or to minimize contamination. Planting the pieces of the plant on PDA media that has been prepared or that has been Plated. Store the isolated results in a storage rack or culture for approximately 3 to 5 days. The results of the isolation are then carried out by purification of the pathogen isolates obtained.

Microscopic and macroscopic identification

Identification of pathogenic fungi that infect dragon fruit plants was carried out based on macroscopic and microscopic characteristics. Macroscopic identification was carried out with the naked eye, while microscopic identification was carried out using an Olympus microscope. The macroscopic observation of the fungus was by observing the shape of the colony, color of the colony, and structure of the colony. While the microscopic observation of fungi is by observing the hyphae structure and conidia shape.

Making biological pesticides from neem leaves

The work of making neem leaf pesticide is to collect 1 kg of fresh neem leaves. Separate the stems and leaves. Wash the collected neem leaves using water. The clean neem leaves are chopped in order to get a more concentrated extract of biological pesticide content. Put 1 kg of neem leaves into the distillator and add 4 liters of water. Close the distillator and light the fire to heat the distillation. Wait until the first drop of biological pesticide residue comes out, then reduce the heat so that the dripping that comes out remains stable. Wait up to ± 4 hours or until the last drop. Next, separate the essential oils and biological pesticide residues remaining in the connecting pipe (adapter). The essential oil is located above the residue or the oil usually floats on the surface of the water (residue) during the distillation process because the oil will not be mixed with the residue. There is a difference between the mass of oil and the residue whose mass is water. Biological pesticide residues and essential oils are then stored for use when testing biological pesticides.

In Vitro testing of pesticide inhibitory power of neem leaves against Anthracnose on dragon fruit plants

The concentrations of neem leaf pesticides used were 0%, 20%, 40%, and 60%. Concentration was made by mixing the residue from the distillation of neem leaves with sterile distilled water. The

concentration of 0% or control is derived from 100 ml of sterile distilled water. The concentration of 20% is by mixing 20 ml of neem leaf distillation residue versus 100 ml of aquadest. The concentration of 40% is by mixing 40 ml of neem leaf distillation residue compared to 100 ml of aquadest. The 60% concentration is by mixing 60ml of neem leaf distillation residue versus 100ml of distilled water.

The next way of working is that for each treatment of biological pesticide concentration, 8 ml of liquid PDA medium is poured and mixed with 1 ml of a solution of biological pesticide concentration, homogenized until evenly mixed. Wait for the medium to solidify. Pure cultures of mushrooms are drilled with a cork drill. The culture is placed right in the middle of the petri dish which already contains the medium mixed with the concentration of biological pesticides. Cultures were incubated at room temperature. The parameters observed were the diameter of the fungal colonies in the control and compared with the diameter of the fungus at the concentration treatment. The inhibitory power of neem leaf biological pesticides on the diameter of fungal colony growth in each treatment was determined using the formula:

$$DH = \frac{(a-b)}{a} \times 100\%$$

Information:
DH : Inhibitory power of vegetable pesticides on fungal colony diameter
a : Colony diameter in control treatment
b : Colony diameter in the treatment of botanical pesticide concentration formulation

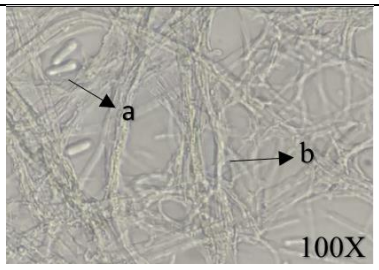
Results and Discussion

The results of macroscopic and microscopic observations of purified fungal isolates have characteristics such as the fungus *Colletotrichum* sp. the cause of anthracnose on dragon fruit (*Hylocereus* sp.). The visible macroscopic characteristics were the color of the colonies were grayish white, the shape of the colonies was circular and spread on the petri dish, and the colony structure was smooth like cotton (Figure 1). According to Sudirga (2016) the fungus *Colletotrichum* spp. isolates in PDA media produced a lot of mycelium, the colonies were white and gray, on the other hand, the colonies were blackish brown, slow growth (3-6 mm in 24 hours), and in old cultures (more than 15 days) black spots appeared. on the colony surface.

The results of microscopic observations showed that the hyphae were not insulated and hyaline. In addition to hyphae, another visible morphological feature is the shape of the fungal conidia. Conidia are oval, slightly elongated, have blunt ends, and are slightly rounded, sometimes with slightly protruding ends (Figure 2). According to Faidah et al. (2017) *Colletrotichum* sp. microscopically the conidia are cylindrical with blunt ends, sometimes slightly oblong with slightly rounded ends, not insulated and single-nucleated and hyaline or slightly brownish in color. This fungus has hyphae that are not insulated, single-nucleated (Semangun, 2007).

Inhibition test was carried out using the poisoned food technique. Testing the inhibitory power of neem leaf biological pesticides against anthracnose on dragon fruit (*Hylocereus* sp.) was carried out using 4 treatments with 6 replications. The concentration treatments of biological pesticides were control, 20%, 40%, and 60%. Mixing each concentration in the media by adding 1 ml of the concentration mixed into the PDA media until homogeneous in each petri dish.

Table 1. Macroscopic and microscopic identification results

Identification Results	Macroscopic Observation	Microscopic Observation
Anthracnose (*Colletotrichum* sp.)		

To be continued…

Figure 1. Macroscopic identification of the fungus *Colleto-trichum* sp.

Figure 2. Microscopic identification of the fungus *Colletotrichum* sp. : (a) Conidia, (b) Hyphae

Table 2. Observation of Inhibitory Power of Biological Pesticides from Neem Leaves on Control of Anthracnose (*Colletotrichum* sp.) on Dragon Fruit Plants (*Hylocereus* sp.).

Treatment	Replication						Total	Average
	I	II	III	IV	V	VI		
0%	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
20%	30,92	58,94	58,82	63,87	54,77	56,33	323,66	53,94
40%	23,19	29,67	56,21	50,32	29,33	34,93	223,66	37,28
60%	0,00	31,71	34,97	41,29	36,40	29,69	174,06	29,01
Total							721,37	

The results of the observation of the percentage of inhibitory power showed that the treatment with the smallest concentration had a large enough inhibitory power compared to the largest concentration. Concentration of 20% has an average percentage of inhibition of 53.94%; a concentration of 40% has an average percentage of inhibition of 37.28%; and a concentration of 60% had an average percentage of inhibition of 29.01%. Each concentration has a different inhibitory power because the active substances contained in each concentration are different (Table 2.).

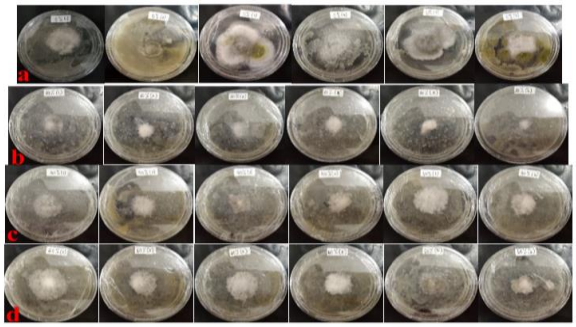


Figure 3. Results of Testing of Biological Pesticides on Neem Leaves for the Control of Anthracnose (*Colleto-trichum* sp.) on Dragon Fruit Plants (*Hylocereus* sp.): (a) control treatment, (b) 20% concentration treatment, (c) 40% concentration treatment, (d) treatment with 60% concentration.

Observations showed that a concentration of 20% already had a fairly good inhibitory power (Figure 3.). This is in accordance with Barnet's (1995) statement that the difference in the size of the inhibition area for each concentration can be caused by differences in the amount of the active substance content. According to Mulyati (2009), the difference in inhibitory power produced in the antifungal activity test can also be influenced by the type of test isolate used. Because each isolate has a different sensitivity to the given extract. So that the antifungal compounds will form resistance which is done naturally in maintaining its life. The effect of the concentration of the given sample can also produce differences in inhibiting the growth of fungi and bacteria.

Conclusion

Based on the description above, it can be concluded that the test of the inhibitory power of the neem leaf biological pesticide against anthracnose (*Colletotrichum* sp.) shows that at a concentration of 20% it has a fairly good inhibitory power because at that concentration it already has a fairly high antifungal

compound. This can be used as an alternative in an effort to minimize the use of chemicals to control anthracnose on dragon fruit plants.

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