Jurnal Biologi Tropis

Original Research Paper

Effect of Miana Leaf Extract (*Plectranthus scutellarioides* (L.) R.Br.) as a Natural Fungicide against *Colletotrichum capsici* on Chili Plants

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Article History

Received : February 25th, 2025

Revised : March 10th, 2025

Accepted : March 13th, 2025

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Abstract: Anthracnose disease caused by the fungus *Colletotrichum capsici* causes rotting of ripe chili fruits and causes a decrease in chili production up to 80%. Excessive use of synthetic pesticides can cause problems for humans and the environment. Therefore, as an alternative, natural/vegetable pesticides that are more environmentally friendly can be used. Miana leaves contain flavonoids, tannins, saponins and steroid compounds that are known to be responsible for pesticide effects on plants. The purpose of this study was to determine the effectiveness of miana leaf extract as a natural fungicide and the best concentration in inhibiting the growth and development of *Colletotrichum capsici* fungus that causes anthracnose disease of chili plants (*Capsicum annuum* L.). The research method used a completely randomized design (CRD). The treatment used in the study was the concentration of miana leaf extract. The treatment used 5 concentrations of miana leaf extract (*Plectranthus scutellarioides* L.), namely: 0%, 5%, 10%, and 15% and 20%. Each treatment was repeated 5 times. The parameters observed were the intensity of *C. capsici* fungus attack, plant height, plant wet weight, and plant dry weight. The results showed that the application of miana leaf extract (*Plectranthus scutellarioides* L.) had no effect on each parameter, namely the intensity of *C.capsici* fungal attack, plant height, plant wet weight, and plant dry weight.

Keywords: Anthracnose, *Colletotrichum capsici*, Chili plants (*Capsicum annuum* L.), miana leaf extract (*Plectranthus scutellarioides* L.).

Introduction

Red chili (*Capsicum annuum* L.) is one of the important horticultural crops because it has great development potential (Lubis *et al*., 2021). Chili peppers contain various nutrients and vitamins, such as vitamins A and C, as well as minerals including iron, potassium, calcium, phosphorus, and niacin. In addition, chilies also have nutritional content in the form of 15 grams of protein, 11 grams of fat, 35 grams of carbohydrates, 150 mg of calcium, and 9 mg of iron (Suharman *et al*., 2022). Apart from being used in household needs, chili peppers are also used in various industries, including the seasoning industry, food, and medicine or herbal medicine. Chili (*Capsicum annum* L.) is one of the vegetable commodities that is widely cultivated by farmers in Indonesia because it has a high selling value and a variety of health benefits. One of the important ingredients in chili is capsaicin (Pratama, 2017).

Capsaicin is a bioactive compound that gives a spicy sensation. Capsaicin is often used as an anti-inflammatory to relieve pain (Renate *et al*., 2014). Capsaicin belongs to the capsasinoid group, which are spicy compounds found in plants, are fat-soluble (non-polar), and easily oxidized during storage (Dyastuti *et al*., 2013). Chili has a high economic value, both for domestic and international markets, and provides substantial income from local and export markets (Ziaulhaq & Amalia, 2022). The Central Bureau of Statistics (2021) reported that national chili

production in 2021 reached 1.36 million tons, an increase compared to 2020 of 1.26 million tons. This increase shows that the demand for chili continues to grow along with population growth and the development of the food industry that uses chili as a raw material. However, the increase in demand is not accompanied by improvements in chili quality. Phoulivong, (2012) stated that diseases caused by microorganisms such as bacteria, viruses, and fungi contribute to the decline in chili quality, especially in the post-harvest period. One of the diseases caused by fungi is anthracnose.

Anthracnose caused by the fungus disease *Colletotrichum capsici*. This disease can significantly reduce yield, with symptoms of black spots on various parts of the plant. Anthracnose attack can cause rotting of ripe fruit, resulting in large production losses. This problem has urgency because the impact of anthracnose disease can cause substantial economic losses for large red chili farmers, with potential losses of up to 80% in a year (Ziaulhaq & Amalia, 2022).

Typically, farmers use synthetic insecticides to manage pests that attack plants. However, because the toxin can kill pests' natural adversaries, the ongoing use of synthetic pesticides might upset the agricultural land ecosystem (Kumar *et al*., 2012). In addition, pesticide residues can cause pests to become resistant to the pesticides used (Permatasari & Asri, 2021) and pollute the environment, especially soil and water, due to the accumulation of residues from synthetic pesticides (Chen *et al*., 2011). Given the various negative impacts caused, it is necessary to develop pesticides that are more environmentally friendly in order to support sustainable development (Suhartini *et al*., 2017). Therefore, as an alternative, natural/vegetable pesticides can be used that are more environmentally friendly (Manullang *et al*., 2020).

The use of plant-based pesticides is a solution for farmers in reducing dependence on harmful synthetic pesticides. In addition, its use can improve the quality and yield of agricultural production while being more environmentally friendly (Sutriadi *et al*., 2020). In addition to providing benefits to farmers, the ingredients for making vegetable pesticides are also easy to obtain (Windriyati *et al*., 2020). Vegetable pesticides contain active compounds derived from plants, such as eugenol, alkaloids, polyphenols, tannins, and saponins (Kusumawati & Istiqomah, 2022).

Miana leaf with the scientific name *Plectranthus scutellarioides* L. is included in herbaceous plants or shrubs. This plant can be propagated through roots, leaves, and stems, making the planting process easier. Miana is also used as an ornamental and medicinal plant. Miana leaves vary in color, ranging from brownish purple to blackish purple, with some varieties having green or yellow tones on the edges. Miana leaves that are solid purple on the entire surface indicate a high anthocyanin content (Ta'dung *et al.*, 2023). Miana (*Plectranthus scutellarioides* L.) is a plant rich in metabolite compounds. leaves miana contain flavonoids, tannins, saponins and steroids. The four compounds are known to be responsible for pesticidal effects on plants (Artantyo *et al*,. 2022).

Various studies on leaves miana by Syahira in 2024 leaves miana contain alcohol as an antiseptic. In the following year research on miana by Makatempuge in 2023, namely leaves miana as anti-microbial. From the research that has been done, it is suspected that plants miana can be used to control anthracnose disease caused by the fungus *Colletotrichum capsici.* The concentration of leaves miana that has been carried out is one of Ardillah Wasiah research in 2014 with concentrations of 0%, 20%, 40%, 60%, and 100%. Therefore, this study used leaf extract miana to be 0%, 5%, 10%, 15% and 20%. So far, there has been no research on leaf extract miana to control anthracnose disease caused by the fungus *Colletotrichum capsici.* Based on the description above, it is necessary to conduct research that aims to determine the effectiveness of miana leaf extract as a natural fungicide and the best concentration in inhibiting the growth and development of *Colletotrichum capsici* fungi that cause anthracnose disease of chili plants (*Capsicum annuum* L.).

Material and Methods

Time and Place of Research

This research was conducted at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung from October to December 2024.

Tools and Materials

The tools used in this research are petri dishes, test tubes, ose needles, glass objects, glass covers, erlenmeyer flasks, beaker glass, measuring cups, funnels, drop pipettes, tweezers, bunsen, stirrers, microscopes, analytical balances, vortex mixers, electric stoves, autoclaves, incubators, ovens, refrigerators, blenders, aluminum foil, filter paper, polybags, sprays, rulers, matches, scissors, and stationery. While the materials used are miana leaves (*Plectranthus scutellarioides* L.), red chili plant seeds (*Capsicum annuum* L.) local cultivars, isolates of *Colletotrichum capsici* fungus obtained from IPB, 70% alcohol, distilled water, ready-made PDA media, soil, and manure.

Research Design

This study used a completely randomized design (CRD). With treatments, namely 0%, 5%, 10%, 15% and 20% concentrations with 5 replications. Concentration of miana leaf extract with distilled water solvent 0% (0 mg miana leaf flour + distilled water 100 ml), concentration of miana leaf extract with distilled water solvent 5% (5 mg miana leaf flour + distilled water 100 ml), concentration of miana leaf extract with distilled water solvent 10% (10 mg miana leaf flour + distilled water 100 ml), concentration of miana leaf extract with distilled water solvent 15% (15 mg miana leaf flour + distilled water 100 ml), concentration of miana leaf extract with distilled water solvent 20% (20 mg miana leaf flour + distilled water 100 ml).

Data Analysis

Data obtained from each variable taken were tested for homogeneity by Bartlett's test then continued with ANARA test. If there is a difference in treatment, further test with the Least Significant Difference (BNT) test α 5% to see the difference between treatments.

Rejuvenation of Pure Isolates of *Colletotrichum capsici* Fungus

Fungal colonies were taken from isolates from the Bogor Agricultural University (IPB). Then the fungus was inoculated into petri dishes containing PDA media and incubated for± 5 days at.

Planting Media Preparation and Soil Sterilization

The planting of large red chili seeds is a mixture of soil and manure, with a ratio of 2 kg soil : 1 manure put into polybags with a diameter of 25 cm and a height of 30 cm with a weight of 3 kg each.

Sowing and Planting Red Chili Seeds

Red chili sprouts are planted and watered every morning and evening. After the chili seedlings are 15 days old, they are ready to be inoculated with fungi.

Preparation of Miana Leaf Extract (*Plectranthus scutellarioides* L.)

Making miana leaf extract (*Plectranthus scutellarioides* L.) is made using distilled water. Miana leaves that have been washed clean, thinly sliced and then dried in a shaded place. After drying, miana leaves are blended until miana leaf flour is obtained.

Inoculation

A 7-day-old culture of *Colletotrichum capsici* was scraped and sterile water was added. The *C. capsici* suspension was sprayed to chili plants as much as 15 ml for each plant in polybags a week before spraying miana leaf extract, then covered for two days.

Application of Miana Leaf Extract

After two days of hooding, the miana leaf extract was sprayed when the plants were two weeks old. The concentrations used were 0%, 5%, 10%, 15%, and 20% for each extract.

Observation

One week after the application of miana leaf extract, several parameters were observed, including the intensity of *C. capsici* fungal attack, plant height, wet weight and dry weight. The intensity of *C.capsici* fungal attack is the level of plant damage due to pathogen attack or the presence of disease, so the variables observed:

$$IS = \frac{\sum(n \times v)}{N \times V} \times 100\% \qquad \textbf{(1)}$$

IS = Attack intensity (%)
n = Number of plants in each attack category
N = Number of plants observed
v = Numerical value for each attack category
V = Highest score value

The disease scores used are :
0 = No attack
1 = Affected plant parts reach 0 - 20% of the plant
2 = Affected parts of the plant reach 20 - 40% of the plant
3 = Affected plant parts reach 40 - 60% of the plant
4 = The affected part of the plant reaches 60-80% of the plant
5 = Affected plant parts reach 80 -100 % of the plant

Result dan Disscusion

Result

Infestation Intensity of Anthracnose Fungus *C.capsici* on Red Chili Plants (*Capsicum annuum* L.)

The highest mean intensity of attack was found in the 0% concentration treatment or without the application of miana leaf extract, while the lowest intensity of attack was found in the 15% concentration treatment (Table 1). The analysis of variance results demonstrated that the fungus *Colletotrichum capsici* level of attack on red chili plants was not significantly impacted by the concentration of miana leaf extract.

Table 1. Mean intensity of *C. capsici* fungal attack on red chili plants treated with with miana leaf extract (*Plectranthus scutellarioides* L.)

Miana leaf extract concentration	μ	SD
A (0%)	1,598±	0,2236
B (5%)	1,517±	0,2766
C (10%)	1,529±	0,2609
D (15%)	1,324±	0,1323
E (20%)	1,474±	0,2016

Notes: μ indicates Mean Attack Intensity of *C.capsici* Fungus.

To find out the effect of miana leaf extract on the average intensity of attack can be seen in the figure below:

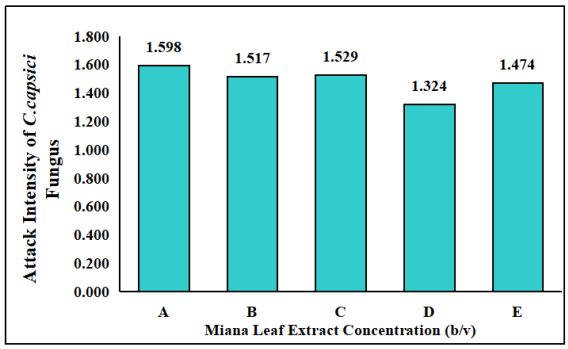

Figure 1. Graph of *C.capsici* Fungal Attack Intensity
Description:
A: Miana Leaf Extract Concentration 0%
B: Miana Leaf Extract Concentration 5%
C: Miana Leaf Extract Concentration 10%
D: Miana Leaf Extract Concentration 15%
E: Miana Leaf Extract Concentration 20%

According to the graph above, applying miana leaf extract can lessen the severity of the fungal assault caused by C. capsici when compared to applying no miana leaf extract. The application of miana leaf extract with a concentration of 15% showed the lowest intensity of *C. capsici* fungal attack.

Plant Height of Red Chili (*Capsicum annuum* L.)

The 5% concentration treatment had the lowest mean plant height, while the 15% concentration treatment had the highest mean plant height (Table 2). The results of the analysis of variance showed that the treatment of miana leaf extract concentration had no significant effect on plant height in red chili plants.

Table 2. Average height of red chili plants after inoculation *C. capsici* fungus and treated with miana leaf extract (*Plectranthus scutellarioides* L.)

Miana leaf extract concentration	μ	SD
A (0%)	25,540±	4,8397
B (5%)	22,980±	2,4261
C (10%)	24,690±	4,8473
D (15%)	27,610±	2,0879
E (20%)	26,630±	5,9468

Notes : μ shows the average height of red chili plants.

Figure 2 illustrates how the average plant height is affected by miana leaf extract. The graph above shows that giving 15% concentration of miana leaf extract will increase plant height compared to other concentrations.

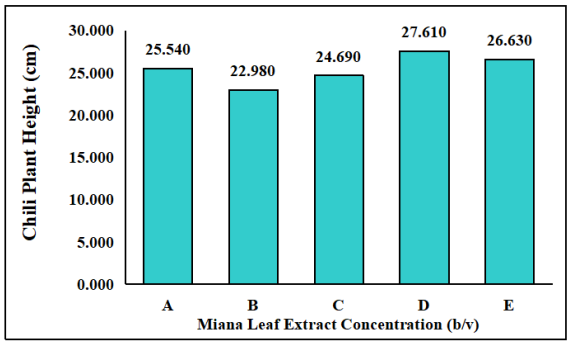

Figure 2. Chart of Red Chili Plant Height
Description:
A: Miana Leaf Extract Concentration 0%
B: Miana Leaf Extract Concentration 5%
C: Miana Leaf Extract Concentration 10%
D: Miana Leaf Extract Concentration 15%
E: Miana Leaf Extract Concentration 20%

Wet Weight of Red Chili Plants (*Capsicum annuum* L.)

The lowest average wet weight of plants was found in the 5% concentration treatment and the highest average wet weight of plants was found in the 15% concentration treatment (Table 3). The analysis of variance's findings demonstrated that the concentration of miana leaf extract had no discernible impact on the red chili plants' wet weight.

Table 3. Average wet weight of red chili plants after inoculated with miana leaf extract (*Plectranthus scutellarioides* L.)

Miana leaf extract concentration	μ	SD
A (0%)	6,711±	2,3523
B (5%)	5,229±	1,5915
C (10%)	6,399±	2,6383
D (15%)	7,563±	1,0527
E (20%)	7,273±	3,1146

Notes : μ indicates the Average Wet Weight of Chili Plants Red

Figure 3 illustrates the impact of miana leaf extract on the average wet weight of plants. The graph above shows that giving 15% concentration of miana leaf extract will increase the wet weight of plants compared to other concentrations.

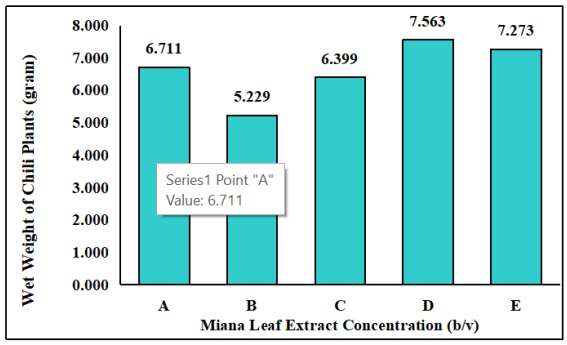

Figure 3. Graph of Wet Weight of Red Chili Plants
Description:
A: Miana Leaf Extract Concentration 0%
B: Miana Leaf Extract Concentration 5%
C: Miana Leaf Extract Concentration 10%
D: Miana Leaf Extract Concentration 15%
E: Miana Leaf Extract Concentration 20%

Dry Weight of Red Chili Plants (*Capsicum annuum* L.)

The 5% concentration treatment had the lowest average dry weight of plants, while the 20% treatment had the highest average dry weight of plants (Table 4). The analysis of variance results indicated that the dry weight of red chili plants was not substantially impacted by the treatment of miana leaf extract concentration.

Table 4. Average dry weight of red chili plants after inoculated with the fungus *C. capsici* and treated with miana leaf extract (*Plectranthus scutellarioides* L.)

Miana leaf extract concentration	μ	SD
A (0%)	0,939±	0,0683
B (5%)	0,867±	0,0875
C (10%)	0,923±	0,0157
D (15%)	0,967±	0,0536
E (20%)	0,968±	0,0948

Notes: μ indicates Average Dry Weight of Red Chili Plants

Figure 4 illustrates the impact of miana leaf extract on the average wet weight of plants. The graph above shows that giving 20% concentration of miana leaf extract will increase the dry weight of plants compared to other concentrations.

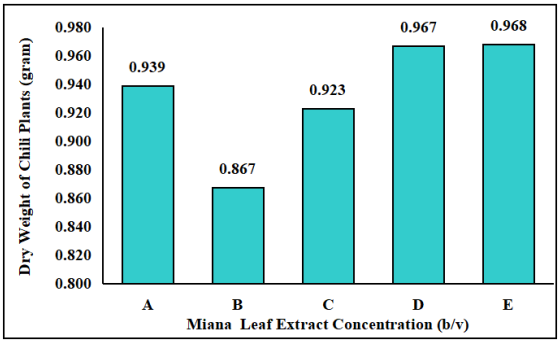

Figure 4. Dry Weight Chart of Red Chili Plants
Description:
A: Miana Leaf Extract Concentration 0%
B: Miana Leaf Extract Concentration 5%
C: Miana Leaf Extract Concentration 10%
D: Miana Leaf Extract Concentration 15%
E: Miana Leaf Extract Concentration 20%

Disscusion

Attack of *C. capsici* Fungus on Chili Plants (*Capsicum annuum* L.)

The application of miana leaf extract at a concentration of 0% showed the highest intensity of *C. capsici* fungal attack and the lowest was at a concentration of 15%. The results of the analysis of variance showed that the application of miana leaf extract did not affect the intensity of the attack of the *C. capsici* fungus, but judging from the average value there was a tendency that the application of miana leaf extract was able to reduce the intensity of the attack of the *C. capsici* fungus. This is different in the treatment without the application of miana leaf extract which shows the highest value. In chili plants that are not given miana leaf extract, plants will generally produce compounds that are toxins to resist the attack of the *C.capsici* fungus. Plants infected with fungi will respond by producing phytoalexin compounds as a defense mechanism against pathogens. Phytoalexins are antimicrobial compounds produced due to infection (Triadiati *et al*., 2016).

The concentration of 15% miana leaf extract was able to reduce the intensity of *C.capsici* fungal attack, because the miana leaf simplisia sample contained flavonoid compounds. The reduction in the severity of anthracnose disease proves the content of flavonoid compounds that can fight anthracnose disease caused by the attack of the fungus *C.capsici*. Like the research of Artantyo (2022), that the content of miana leaf extract contains flavonoid compounds, saponins, steroids and tannins. The four compounds are known to be responsible for the pesticidal effect on plants.

Plant Height of Red Chili (*Capsicum annuum* L.)

The application of miana leaf extract had no influence on plant height, according to the analysis of variance data; nevertheless, based on the average height of the chili plant, the greatest concentration was 15%. This proves the negative correlation in the intensity of disease attack. The lower the intensity of disease attack, the higher the chili plants. Chili plants still experience growth even though they have been attacked by disease. This is because the treatment with miana leaf extract gets additional macro elements in the form of N where this element is very important for plant growth. This is in accordance with Satria *et al*., (2015) which states that the provision of additional nitrogen to plants will accelerate the division of apical and lateral meristem cells in plant height growth.

Wet Weight of Red Chili Plants (*Capsicum annuum* L.)

The analysis of variance results show that the fresh weight of the plants is unaffected by the different treatments with miana leaf extract. Although the greatest average concentration is 15%, this suggests that miana leaf extract has not been able to satisfy the nutrient requirements of chili plants. This is because the sample material from the leaves used contains Besi (Fe) and Magnesium (Mg) compound elements. Iron and magnesium elements have an important role in various processes that affect plant growth. According to (Adelia *et al*., 2013), the function of Fe is to play a role in the formation of chlorophyll. Therefore, optimal Fe availability is needed by plants. If Fe in the nutrient solution is not sufficient, chlorophyll formation will not be perfect. Meanwhile, according to Neneng & Saraswati, (2019), Mg is an important element in the green color of leaves that cannot be replaced by other elements. The element Mg has a crucial role in the formation of chlorophyll. This is going to improve it by increasing cell division, and the water content of the plant also affects fresh weight.

Dry Weight of Red Chili Plants (*Capsicum annuum* L.)

The analysis of variance data show that the dry weight of the plants is unaffected by the different treatments with miana leaf extract. According to the average dry weight of the chili plants, the highest concentration of miana leaf extract is 20%, which suggests that it has not been able to satisfy the nutrient requirements of the plants. Wet weight and dry weight did not positively correlate, according to the results. The highest wet weight was at 15% concentration while the highest dry weight was at 20% concentration. This is thought to be the effect of differences in water availability between concentrations of 15% and 20%. In accordance with the statement of (Solichatun *et al*., 2005), the availability of sufficient water will affect cell turgor in addition to affecting cell expansion so that it will determine the growth rate (biomass accumulation-dry weight).

Conclusion

Spraying of miana leaf extract (*Plectranthus scutellarioides L.*) did not give any effect to each parameters, namely the intensity of fungal attack of *C.capsici*, plant height red chili, wet weight of red chili plants, and dry weight of red chili plants.

Acknowledgments

Thank you to all those who have helped in carrying out research and making this article.

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