Potential of ethyl acetate and methanol extract from *Eupatorium odoratum* and *Morinda citrifolia* plants as *Fusarium Oxysporum* fungicide

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Abstract. *Fusarium oxysporum* is a significat plant pathogen that affects various crops. Fusarium causing diseases and substantial agricultural losses. This research aims to determine the effect of methanol extract and ethyl acetate of *Eupatorium odoratum* (roots, leaves and stem) and *Morinda citrifolia* (fruits, leaves and twig) plants on the growth of fusarium fungi. To determine the optimal extract concentration to inhibit fungal growth. This research is a factorial study with independent variables in the form of extract type, ingredients of plant (root, leaves, stem and twig) and differences in concentration. The activity of the extract as a biofungicide was analyzed based on the fungal growth inhibition zone. The results were analyzed using Anava and continued with the Duncan test. The results of the research show that methanol extract and ethyl acetate extract of *Morinda citrifolia* have potential as biofungicides for the fungus *Fusarium oxysporum*. The root of *Eupatorium odoratum* and the fruit of *Morinda citrifolia* ethyl acetate extract at a concentration of 80% is optimum inhibiting the growth of *Fusarium oxysporum*.

1 Introduction

Fusarium oxysporum is a significant plant pathogen that affects various crops, causing diseases such as vascular wilts, rots, and damping-off [1]. Fusarium oxysporum is a soil-borne pathogen. This pathogen has a very wide host plant range and is distributed in all subtropical and tropical climate zones [2]. This fungus is known to infect a wide range of economically important crops like cotton, banana, tomato, and potato, leading to Fusarium wilt disease and substantial agricultural losses [3,4]. Fusarium oxysporum is a cosmopolitan plant pathogen causing Fusarium wilt and root rot in many economically important crops [5]. It is crucial to note that Fusarium oxysporum is a devastating plant-pathogenic fungus that causes vascular wilt disease in many economically important crops worldwide, including watermelon [4]. The pathogen initiates a necrotrophic growth phase towards the later stages of its lifecycle, contributing to the development of Fusarium wilt disease on numerous legume crops globally [6].

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In general, the Fusarium oxysporum fungus has a characteristic 24 mycelium morphology which has a smooth, white, cotton-like texture, sometimes pink to purplish in color [7]. Fusarium oxysporum is a soil pathogen that can survive for long periods by forming chlamydospores [8]. Fusarium oxysporum can grow at temperatures of 9-38°C in vitro conditions, but the optimum temperature is 22-30°C, where the average plantation temperature is 29°C [9]. Fusarium fungus colonies are white with a pink center of the colony.

Fusarium wilt disease can cause plant death and crop failure. Fusarium pathogens are transmitted through water flow, so land on slopes spreads more quickly. Plants affected by Fusarium disease have symptoms of the lower leaf blades curling and folding, the leaves changing color from initially green to yellow, and over time they dry out, then the stem buds rot, and finally, the plant dies [10]. Naturally, Fusarium attacks plant tissue that experiences necrosis [11]. This fungus gets nutrients from healthy plants and can reproduce in the tissue [9].

To combat Fusarium disease, researchers have explored different strategies, including the use of fungicides, plant extracts, nanoparticles, peptides, and biocontrol agents. The efficacy of six fungicides against Fusarium oxysporum f. sp. Lycopersici was evaluated, highlighting the potential of benomyl, carbendazim, and other fungicides in managing the disease [12]. Similarly, carbendazim found to be effective against Fusarium oxysporum wilt in tomatoes [13].

These studies underscore the importance of fungicides in controlling Fusarium wilt. In addition to chemical fungicides, natural compounds have shown promise in combating Fusarium oxysporum. For instance, Wee et al. demonstrated the antifungal activity of magnetic magnesium oxide nanoparticles against Fusarium oxysporum [14]. Furthermore, the fungitoxic properties of plant extracts on Fusarium oxysporum, suggesting the potential of botanical agents as alternatives to synthetic fungicides [15]. Moreover, biocontrol agents have been investigated for their efficacy against Fusarium wilt. The antifungal activity of plant growth-promoting fungi against Fusarium oxysporum, indicating the potential of biological control in managing the disease [16]. A novel Streptomyces strain identified with bio fungicidal properties against Fusarium oxysporum in soil [17]. Overall, the research indicates a multifaceted approach to combat Fusarium oxysporum, encompassing chemical fungicides, natural compounds, biocontrol agents, and novel peptides. The use of biofungicides would be better used to protect, facilitate and encourage sustainable use of terrestrial ecosystems in accordance with SDG's goal number 15.

Plants that have the potential to become biofungicides are plants that contain large amounts of chemical compounds. Morinda citrifolia, commonly known as noni, has been extensively studied for its various pharmacological activities, including anticancer properties [18], antimicrobial efficacy against various pathogens, including Candida albican [19], and antimicrobial activity extends to other bacteria as well, as evidenced by studies on Salmonella typhi and Enterococcus faecalis [20].

In the agricultural context, Morinda citrifolia has been explored for its potential in managing plant diseases caused by pathogens like Fusarium oxysporum. Investigated the antibacterial activity of endophytic fungi from Morinda citrifolia against bacterial spot in passion fruit trees [21]. Furthermore, studied the antioxidant activity of Morinda citrifolia polysaccharides, highlighting its potential in enhancing immunity and balancing physiological functions [22]. Noni fruit (Morinda citrifolia) contains scopolectin as an analgesic, anti-inflammatory, and antibacterial [23]. Apart from scopoletin, noni fruit also contains anthraquinone compounds, alkaloids, tannins, flavonoids, steroids, triterpenoids, saponins, xeronin, proxeronin, ascorbic acid, linoleic acid, caprylic acid and beta carotene [24]. Meanwhile, the leaves contain active compounds such as saponins, flavonoids, polyphenols, tannins, and triterpenes [25]. In conclusion, Morinda citrifolia exhibits a wide range of pharmacological activities, including anticancer, antimicrobial, and antioxidant

properties. While research on Morinda citrifolia's direct fungicidal effects on Fusarium oxysporum is limited, its overall antimicrobial properties suggest potential for managing fungal pathogens. Further studies specifically targeting Fusarium oxysporum with Morinda citrifolia extracts or compounds could provide valuable insights into its fungicidal potential.

Apart from noni, a plant that has potential as an antifungal is Eupatorium odoratum. Eupatorium odoratum, also known as "Krinyuh or Wedhusan" has been studied for its antimicrobial properties [26]. The plant has shown significant pharmacological activities, including anti-inflammatory and antibacterial effects. Additionally, the ethanolic extract of Eupatorium odoratum has demonstrated anthelmintic activity [27]. Krinyuh contains bioactive compounds such as saponins, tannins, alkaloids, steroids, and flavonoids which can be used as fungicides [28]. These findings suggest that Eupatorium odoratum possesses bioactive compounds that could be beneficial in combating various pathogens include Fusarium oxysporum.

This research aims to determine the effect of methanol and ethyl acetate extracts of Eupatorium odoratum and Morinda citrifolia as fungicides, to determine the effect of Eupatorium odoratum (root, leaves and stem) and Morinda citrifolia (fruits, leaves, and twig) which are more effective as fungicides, to determine the optimal concentration that is effective as a biofungicide for the Fusarium oxysporum fungus, and to determine the presence of the interaction is the influence of a combination of extracts, plant material and concentration as a biofungicide.

2 Material and Methods

2.1 Sample Preparation

The Krinyuh and Noni plants are obtained around UAD campus 4. The part of Krinyuh (root, stem and leaves) and Noni (fruit, leves and twig) are cleaned with water. The clean ingredients are air-dried in the laboratory. The Krinyuh and Noni are air dried indoors exposed to sunlight until the sample is completely dry, easy to break, light, turns brownish with weight constant dry [29]. Once the ingredients are dry, they are then thinly sliced and blended until smooth.

2.2 Extract Preparation

The Krinyuh and Noni plants extraction processed by maceration. According to [30] maceration is carried out using put the simplicia into a jar and add solvent until simplicia completely submerged. In this research, a solution was used 80% concentration methanol was made with a dilution of 4166.7 mL of methanol was mixed with 833.3 mL of distilled water. Put 150 grams of noni simplicia powder into a jar, add 1000 mL methanol solvent, stir and then cover. After 24 hours, stir thoroughly and then filter using a sieve filter paper to obtain filtrate and dregs. Next the dregs the mixture was reused by adding 1000 mL of methanol and stirred once every 24 hours after which it was filtered with a sieve and filter paper. The evaporation process uses a simple distillation tool.

The distillation equipment is assembled until everything is installed properly. The filtrate solution was added to a 250 mL distillation flask. Hose water is poured into the distillation and the electric stove is turned on until it is hot and boiling water until the solution evaporates. The distillation process is carried out 8-9 hours until the methanol no longer drips so that an extract is obtained thick from the distillation flask [30]. Fractionation is carried out by adding a thick extract 100 mL of 80% methanol in a separating funnel then added with 40 mL ethyl acetate solvent. Then shake gently until mixed, open the lid of the separating funnel once to

let the gas into the funnel. Separate it out, then let it sit until two layers are visible. The two layers are separated by opening the separating funnel tap. The lower solution and upper solution are collected in separate containers. Then the bottom solution becomes methanol extract and the top solution as ethyl acetate extract. The methanol extract was added back into the separating funnel then fractionated again using ethyl acetate two more repetitions. The resulting ethyl acetate extract solution was left open so that it can evaporate [31]. The concentration of noni methanol and ethyl acetate extract consists of 6 types: 10%, 20%, 40%, 60%, 80%, 100%.

Making PDA media is done by heating 500 mL distilled water in a beaker on a hot plate. After boiling add 15.8 grams of PDA powder then stir until dissolved and boils. Then the media solution is poured into the Erlenmeyer. Put the Erlenmeyer in an autoclave for 15 minutes at 1210C. After 15 minutes, the PDA solution was removed. PDA solution slant media taken using a pipette and placed in a test tube as much as ¾ of the test tube. Next, the slanted media is arranged on tube rack. In the petridish medium, the solution is poured ¾ of the petridish. Wait for the PDA media until it solidifies and then put it in the refrigerator

SDB media is made with 1.5g of SDB powder added to it tube containing 100 mL of distilled water. Then one round of mushrooms is taken then added to 1 mL SDB liquid media. Then incubate for 24 hours at room temperature 37ºC. The fungal suspension was taken 0.1 mL was then given 0.9% physiological NaCl solution until turbidity is the same as the Mc Farland standard (on a scale of 0.5 and fungal concentration 1.5 x 108 CFU/mL). After obtaining the desired turbidity, 0.1 mL is then taken and then added into SDB media to 10 mL and shake until homogeneous (concentration 106 CFU/mL).

2.3 Antifungal activity test

The antifungal activity test was carried out using the disc diffusion method (Kirby-Baeur). Cotton swab dipped in fungal inoculum tube. Then it is scratched on the entire surface so that the PDA is smooth equally. Leave for 5 minutes, then each paper discs with 3 repetitions were soaked in methanol extract concentration 10%, 20%, 40%, 60%, 80%, 100% ethyl acetate extract concentration of 10%, 20%, 40%, 60%, 80%, 100% distilled water solution as negative control and one of the more active positive control solutions for 15 minutes then removed. Media should be labeled so that it is not confused when attaching the disc paper. After the disc paper is dry, each paper disc is placed on the surface of the agar media use tweezers to make it stick perfectly. Next in incubate for 24 hours at 37ºC. After completion of incubation the diameter of the inhibition zone was measured.

2.4 Inhibition zone measurement

The clear zone formed around the paper disc was measured by unit mm. Measurements are made by measuring the horizontal diameter and vertical diameter.

$$\frac{(Dv-Dc)+(Dh-Dc)}{2} \quad (1)$$

Information:
Dv: vertical diameter (mm)
Dh: horizontal diameter (mm)
Dc: disc diameter (mm)

There is classification for determine the power of extracts on inhibit the Fusarium growth. Determining the inhibitory power of extracts on Fusarium growth based on the Table 1.

Table 1. Inhibitory power classification

Inhibition zone (mm)	Inhibitory power
>30 mm	Very active
21-30 mm	Active
11-20 mm	Medium
1-10 mm	Weak
0 mm	Not active

3 Results and Discussion

Based on the research results, it can be seen that the effect of the extract, differences in plant materials, and differences in concentration inhibit the growth of Fusarium. The effect of extract use on the fungal growth inhibition zone presented in Table 2.

Table 2. Effect of extract to the fungal growth inhibition

Treatment	Average inhibition zone (mm)	Inhibition power
Negative control (aquades)	0.00	Not active
Methanol extract	4.67^{b}	Weak
Ethyl acetate extract	7.72^{c}	Weak
Positive control (dithane)	12.83^{d}	Medium

Based on Table 2, it can be seen that the two extracts have the same inhibitory power category weak. However, ethyl acetate extract has a wider average area of inhibition than methanol extract. Therefore, ethyl acetate would be better if used as a solvent in this research. Ethyl acetate is a widely used solvent in the extraction of bioactive compounds from various plant materials due to its effectiveness in isolating phytochemicals with antioxidant, anticancer, and antimicrobial properties. Several studies have demonstrated successful extraction of compounds such as andrographolide, flavonoids, and terpenoids using ethyl acetate as a solvent [32–34]. Ethyl acetate, a moderately polar solvent with a high content of COO groups, was more effective than methanol at elevated temperatures [32]. The effect of plant materials on the fungal growth inhibition zone is presented in Figure 1.

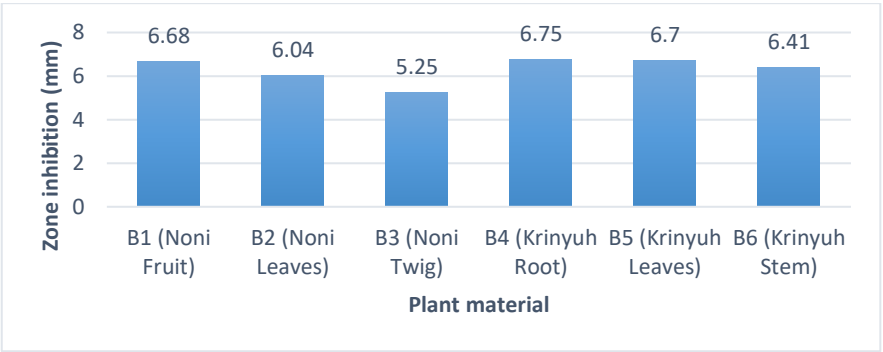


Fig. 1. The effect of plant materials on the fungal growth inhibition

Based on Figure 1, it can be seen that each part of Krinyuh and Noni has a different average area of inhibition. However, the resistance is still in the weak category because it is still below 10 mm. In Krinyuh plants, the highest average area of inhibition is produced from the roots. In Noni plants, the highest average area of inhibition is in the fruit. The effect combination of extract and plant materials to the fungal growth inhibition zone presented in Table 3.

Table 3. The effect combination of extract and plant materials to the fungal growth inhibition

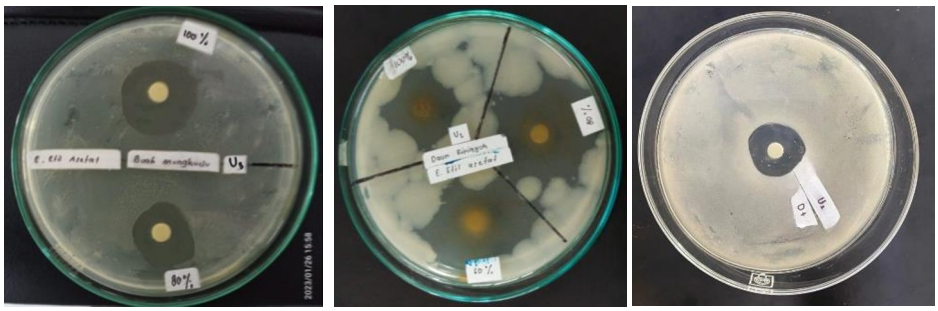
Treatment	Average inhibition zone (mm)	Inhibition power
Extract Methanol of Noni fruit	4.28[b]	Weak
Extract Methanol of Noni Leaves	4.83[cd]	Weak
Extract Methanol of Noni Twig	2.94[b]	Weak
Extract Methanol of Krinyuh Root	5.03[cd]	Weak
Extract Methanol of Krinyuh Leaves	5.56[de]	Weak
Extract Methanol of Krinyuh Stem	5.39[d]	Weak
Extract Ethyl acetate of Noni fruit	9.61[g]	Weak
Extract Ethyl acetate of Noni Leaves	6.50[ef]	Weak
Extract Ethyl acetate of Noni Twig	5.23[cd]	Weak
Extract Ethyl acetate of Krinyuh Root	8.94[g]	Weak
Extract Ethyl acetate of Krinyuh Leaves	8.611[g]	Weak
Extract Ethyl acetate of Krinyuh Stem	7.42[f]	Weak
Positive control	12.83[h]	Medium
Negative control	0[a]	Weak

Based on the data in Table 3, it can be seen that the combination of noni fruit ethyl acetate extract has the highest average zone of inhibition compared to extracts from other plant parts. However, based on Duncan's test with α=5%, the results were not significantly different from krinyuh root ethyl acetate extract and krinyuh leaf ethyl acetate extract. This shows that the ethyl acetate extract from noni fruit, krinyuh roots and krinyuh leaves has potential as a biofungicide. The effect combination of concentration, extract and plant materials to the fungal growth inhibition zone (mm) presented in Table 4.

Table 4. The effect combination of concentration, extract and plant materials to the fungal growth inhibition

Treatment	Concentration					
	10%	**20%**	**40%**	**60%**	**80%**	**100%**
Extract Methanol of Noni fruit	3.50	3.83	4.17	4.33	4.83	5.00
Extract Methanol of Noni Leaves	3.67	3.83	4.33	4.67	6.00	6.50
Extract Methanol of Noni Twig	0.83ab	1.50abc	2.17abcd	3.67	4.67	4.83
Extract Methanol of Krinyuh Root	2.67	3.67	4.83	5.33	6.17	7.50
Extract Methanol of Krinyuh Leaves	4.50	5.00	5.33	5.67	6.33	6.50
Extract Methanol of Krinyuh Stem	3.50	5.00	5.17	5.67	6.33	6.67
Extract Ethyl acetate of Noni fruit	7.50	8.67	8.83	8.83	**11.33**	**12.50**
Extract Ethyl acetate of Noni Leaves	3.83	4.50	5.83	6.67	8.33	9.83
Extract Ethyl acetate of Noni Twig	1.57abcd	3.83	4.50	5.50	8.00	8.00
Extract Ethyl acetate of Krinyuh Root	4.67	7.67	8.50	9.33	**11.17**	**12.33**
Extract Ethyl acetate of Krinyuh Leaves	6.33	7.17	8.33	8.33	**10.10**	**11.50**
Extract Ethyl acetate of Krinyuh Stem	4.83	6.00	6.33	8.67	9.00	9.67
Positive control	12.83					
Negative control	0.00					

Table 4 shows that the combination of noni fruit ethyl acetate extract at concentrations of 80% and 100% has an inhibition zone of more than 10mm. This shows that the strength of the inhibition zone is in the medium category. Likewise, the ethyl acetate extract of krinyuh roots and krinyuh leaves at concentrations of 80% and 100% showed an inhibition zone of more than 10 mm. Therefore, the ethyl acetate extract of noni fruit, krinyuh roots and krinyuh leaves have potential as a bio fungicide. The optimal concentration that can be used as a biofungicide is a concentration of 80% ethyl acetate extract of noni fruit, krinyuh roots and krinyuh leaves. The optimum concentration is the minimum concentration of the ethyl acetate extract of the plant material used which is capable of having an inhibition zone of >10 mm. The inhibition zone >10mm shows the medium's inhibitory power as is the inhibition zone of Dhitane solution (positive control) which is a chemical fungicide. The fungal inhibition zone yield by 80% noni fruit ethyl acetate extract reached 11.33 mm, while for krinyuh root ethyl acetate extract it reached 11.17 mm. The strength of the inhibition zone for both extracts is still in the medium category Based on Table 4, it can also be seen that the combination of ethyl acetate extract has better inhibitory power compared to methanol extract. In the methanol extract, the widest inhibition zone is in the krinyuh root methanol extract with a concentration of 100% (7.00mm), then the krinyuh stem methanol extract with a concentration of 100% (6.67mm). The strength of the inhibition zone for both extracts is still in the low category. Images of the inhibition zones of noni and krinyuh fruit extracts are presented in Figure 2.



A **B** **C**

Fig. 2. *Fusarium oxysporum* inhibition zone by extract etyl acetat: A) Noni's Fruit at 80% and 100%; B) Krinyuh's Leaves at 80% and 100%; C) Dhitane (positive control)

4 Conclusion

Methanol and ethyl acetate extracts of the Krinyuh and Noni plant have potential as biofungicides. There is a difference in inhibitory activity between methanol extract and ethyl acetate extract. Ethyl acetate extract was more active in inhibiting the growth of Fusarium compared to methanol extract. There are differences in the activity of Krinyuh and Noni plant extracts as biofungicides. The root and leaves of Krinyuh and Noni fruits is more active than the Noni's leaves and twig. There is an effect of extract concentration on inhibiting the growth of Fusarium. Extract with a concentration of 80% is optimal in inhibiting fungal growth. There is an interaction between the combination of extract types, ingredients and concentration on the inhibition of fungal growth. The extract ethyl acetate of krinyuh roots and noni fruits at a concentration of 80% is the optimal extract in inhibiting the growth of the *Fusarium oxysporum*.

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