



ACTIVITY OF NONI EXTRACT (*Morinda citrifolia* L.) AS A BIOFUNGICIDE AGAINST THE GROWTH OF THE FUNGUS *Fusarium oxysporum* IN VITRO

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ARTICLE INFO		ABSTRACT
Article history		<i>Fusarium oxysporum</i> is a fungus that attacks various plants and causes wilting. Several plants, including bananas, have experienced a decrease in productivity due to the <i>Fusarium</i> fungus. This research aims to determine the effect of the whole plant of noni plants (fruits, leaves, and twigs), methanol extract, and ethyl acetate on the growth of <i>Fusarium</i> 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, ingredients (fruit, leaves, and twigs), and differences in concentration. The activity of the extract as a fungicide was analyzed based on the fungal growth inhibition zone. The results were analyzed using Anava, a statistical method for comparing means, and continued with the Duncan test, a post hoc test that allows for multiple comparisons. The research results showed that methanol extract and ethyl acetate extract of noni could be bio fungicides for the fungus <i>Fusarium oxysporum</i> . Noni fruit ethyl acetate extract at a concentration of 80% was optimum for inhibiting the growth of <i>Fusarium oxysporum</i>
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INTRODUCTION

Fusarium wilt is a disease caused by the fungus *Fusarium oxysporum*. *Fusarium* fungi can form chlamydospores to survive in the soil for quite a long time, even without a host (Aprilia et al., 2020). *Fusarium oxysporum* is a pathogen whose habitat is in the soil. Fungal activity in the roots makes it easy for this fungus to infect other plants through

soil. The spread of fungal pathogens through soil is carried out using soil adhering to agricultural equipment, transfer of infected seeds, and water flow by infecting plant roots. The distance between the roots of the diseased plant and other plants and the soil condition influences the plant infection process. Plants infected with *Fusarium oxysporum* are characterized by wilting of the leaves, causing death within a few weeks (Asrul et al., 2021).

Pathogens (including *Fusarium*) can survive long without riding on host plants or weeds, making it challenging to control pathogenic fungi in the soil. Farmers usually eradicate the fungus using chemical fungicides. However, chemical fungicides have many disadvantages, including environmental pollution and cost. They can also make it challenging to develop resistant plant varieties (Pinaria & Assa, 2017). Efforts to maintain plants using chemical fungicides have many negative impacts, so another alternative is bio-fungicides made from plants.

One of the plants that can be used as a fungicide is the noni plant (*Morinda citrifolia* L). This noni plant is easy to find and abundant, but only some people use it as a fungicide. The noni plant is a plant that is used by the community as a natural medicinal ingredient. Almost all parts of the noni plant can be used as medicine, including stems, leaves, fruit, and seeds. This is because noni plants contain abundant secondary metabolites. This secondary metabolite compound has many pharmacological and polyvalent activities that can treat various diseases (Pamungkas et al., 2019).

The noni plant is rich in various active substances, including scopoletin, alkaloids, xeronine, vitamin C, anthraquinone, proxeronine, linoleic acid, ascorbic acid, β -carotene, caproic acid, caprylic acid, and flavonoids. Of particular interest are the anthraquinones, which have demonstrated potential antiseptic, antifungal, antibacterial, and anti-inflammatory effects. These anthraquinones include aloin, barbaloin, tannin, emodin, saponin, and sterol.

Methanol is a solvent with universal properties, namely being able to bind chemical components in plants, both polar and non-polar. Examples of compounds that can dissolve in methanol are flavonoids, phenolics, and tannins (Muaja et al., 2017). Therefore, methanol has the potential to dissolve these compounds contained in the noni plant. Meanwhile, according to (Gazali et al., 2019), ethyl acetate solvent has semi-polar properties so that it can dissolve compounds with polar, semi-polar, or non-polar

properties. Compounds that can be dissolved in ethyl acetate are phenolics, tannins, and steroids. It is estimated that the amounts of flavonoid and phenolic compounds differ between methanol and ethyl acetate extracts (Situmeang et al., 2024). Therefore, these two solvents were used in this study.

The current state of research on plant-based bio fungicides, particularly those derived from *Morinda citrifolia* (noni), demonstrates promising potential. Recent studies have emphasized the antifungal activity of noni-derived compounds, including anthraquinones, alkaloids, and flavonoids, in combating various plant pathogens, such as *Fusarium oxysporum*. Despite these advancements, ongoing research is crucial to optimize extraction methods and identify the most effective solvents to enhance the bioactive potential of noni plants as antifungal agents. This study aims to evaluate the fungicidal properties of methanol and ethyl acetate extracts from noni plants, assess the efficacy of different noni plant materials (fruit, leaves, and twigs), and determine the optimal concentration for biofungicide activity against *Fusarium oxysporum*. Your continued engagement and commitment to this field are essential for its progress.

MATERIALS AND METHODS

Sample Preparation

Noni fruit and leaves were obtained around the Universitas Ahmad Dahlan. The fruit and leaves were cleaned with water. The clean ingredients were air-dried in the laboratory. The fruit and leaves were air-dried indoors and exposed to sunlight until the samples were dehydrated, easy to break, light, and turned brownish with a constant dry weight (Savitri et al., 2017). Once the ingredients were dry, they were thinly sliced and blended until smooth

Extract Preparation

The extraction process of the noni plant, a pivotal step in our research, was performed using maceration. This method, as described by Haryanti et al. (2020), involved submerging the simplicia into a jar filled with solvent. In our study, we prepared a solution with 80% methanol concentration by diluting 4166.7 mL of methanol with 833.3 mL of distilled water. One hundred fifty grams of noni simplicia powder was placed

into a jar, 1000 mL of methanol solvent was added, and the mixture was stirred before being covered. After 24 hours, the mixture was stirred thoroughly and filtered using sieve filter paper to obtain the filtrate and the dregs. The dregs were then reused by adding 1000 mL of methanol and stirring every 24 hours before filtering with a sieve and filter paper.

The evaporation process was carried out with precision using a simple distillation apparatus. All the distillation equipment was assembled and checked to ensure proper installation. The filtrate solution was transferred to a 250 mL distillation flask. Water was added to the distillation apparatus, the electric stove was turned on, and the solution was heated until it began to boil, causing the solvent to evaporate. The distillation continued for 8-9 hours until the methanol no longer dripped, leaving a thick extract in the distillation flask (Savitri et al., 2017).

Fractionation was performed by adding 100 mL of the thick extract and 80% methanol solution into a separating funnel, followed by 40 mL of ethyl acetate solvent. The mixture was shaken gently until well mixed, and the funnel was briefly opened to release any gas buildup. The mixture was then allowed to settle until two layers formed. The layers were separated by opening the tap of the separating funnel, and the lower and upper solutions were collected in separate containers. The lower solution, the methanol extract, and the upper solution, the ethyl acetate extract, were identified. The methanol extract was returned to the separating funnel and fractionated with ethyl acetate for two additional repetitions. The resulting ethyl acetate extract solution was left open to evaporate (Runtuwene et al., 2021). The concentrations of the noni methanol and ethyl acetate extracts were prepared in six different concentrations: 10%, 20%, 40%, 60%, 80%, and 100%.

Potato Dextrose Agar Preparation

Potato Dextrose Agar (PDA) was prepared by dissolving 15.8 grams of PDA powder in 500 mL of sterile distilled water. The solution was divided into six petri dishes. The PDA solution was heated using a hot plate and stirred until fully dissolved. The media was then sterilized in an autoclave at a temperature of 121°C for 15 minutes at 2 atm pressure. After sterilization, the media was allowed to cool until lukewarm. One milliliter of fungal suspension was added to each petri dish. The lukewarm PDA media was poured into the petry dishes containing the fungal suspension and left to solidify (Azzahra et al., 2020).

Mc Farland standard preparation

The McFarland 0.5 standard, a critical component of our experiment, was prepared with utmost precision. This involved mixing 0.05 mL of 1% BaCl₂ with 9.95 mL of 1% H₂SO₄ in a test tube. The solution was vortexed to ensure homogeneity, then sealed with aluminum foil and stored in the refrigerator (Avyani & Piyanto, 2020). This McFarland standard served as the comparative basis for preparing the *Fusarium oxysporum* suspension.

Suspension of Fusarium oxysporum

One streak of *Fusarium oxysporum* culture was added to 1 mL of liquid Potato Dextrose Broth (PDB) in a test tube. The solution was incubated for 24 hours at room temperature (25°C). After incubation, 0.1 mL of the fungal culture was taken, and 0.9% physiological NaCl solution was added to adjust the turbidity to match the McFarland standard (scale 0.5, corresponding to a fungal concentration of 1.5×10^8 CFU/mL). Once the turbidity of the fungal suspension reached the equivalent of the McFarland standard, 0.1 mL of the suspension was transferred to a test tube containing 9 mL of PDB media, and the mixture was homogenized by shaking and vortexing.

Antifungal activity test

The antifungal activity was tested with thoroughness using the disc diffusion method (Kirby-Bauer) with three repetitions. A cotton swab was dipped into the fungal inoculum and then streaked evenly across the entire surface of the Potato Dextrose Agar (PDA) plate. After allowing the plate to sit for 5 minutes, paper discs were soaked in the following solutions for 15 minutes: methanol extract concentrations (10%, 20%, 40%, 60%, 80%, 100%), ethyl acetate extract concentrations (10%, 20%, 40%, 60%, 80%, 100%), distilled water (negative control), and Dhitane, a known active positive control solution. The discs were then removed and placed onto the surface of the PDA plate, ensuring they adhered properly using tweezers. The plates were incubated for 24 hours at 37°C. After incubation, the diameter of the inhibition zones around the discs was measured (Putri et al., 2019).

Inhibition zone measurement

The clear zones formed around the paper discs were measured millimeters (mm). The measurements were taken by determining the inhibition zone's horizontal and vertical diameters.

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

Keterangan :

Dv = diameter vertical (mm)

Dh = diameter horizontal (mm)

Dc = diameter cakram (mm)

Furthermore, the data were meticulously analyzed using ANOVA to determine whether there were significant differences, and a subsequent DMRT test was conducted to identify the influencing factors. This thorough research process instills confidence in the validity of the findings.

RESULTS AND DISCUSSION

Building on the ANOVA results, the effect of the extract, ingredients, and concentration used showed a significant difference, indicating a profound impact of the treatments on the inhibitory power of the *Fusarium oxysporum* fungus. The Duncan 5% test was then conducted to pinpoint the treatment that had the most significant effect, adding to the intrigue of the research.

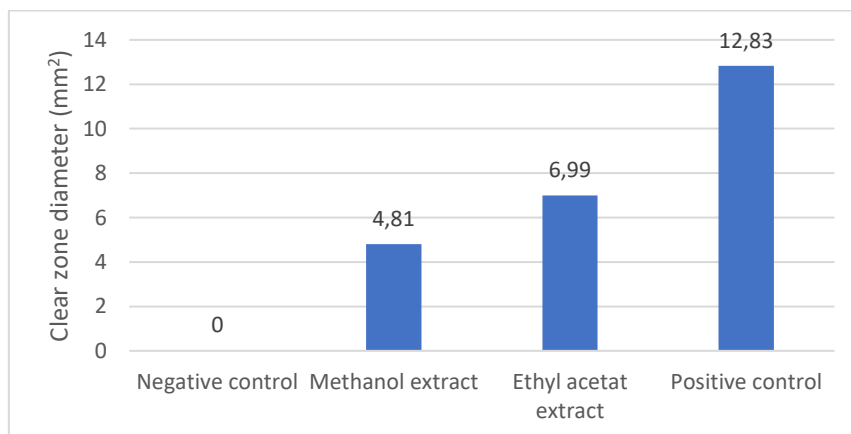


Figure 1. The inhibition of fungal growth of *Fusarium oxysporum* due to the effect of extract treatment of *Morinda citrifolia* L. was significantly different in the Duncan test ($\alpha < 0.05$).

Inhibition of the *Fusarium oxysporum* growth was indicated by a clear zone around the paper discs that had been soaked in the extract treatment. The clear zone suggests the inability of the fungus to grow around the extract. The wider the clear zone, the higher the inhibitory power against fungal growth. The study showed that ethyl acetate extract from the Noni plant produced an average clear zone of 6.99 mm². The clear zone treated with ethyl acetate extract was more comprehensive than methanol extract (4.81mm²). The extraction process using ethyl acetate is particularly effective due to its ability to dissolve semi-polar phytochemicals, which are often responsible for biological activity (Toh et al., 2023). This solvent has been shown to extract a range of bioactive compounds, including flavonoids, terpenoids, alkaloids, and saponins, which contribute to the antifungal activity of the noni plant (Aji & Romawati, 2020). As a universal solvent, methanol can bind plant chemical compounds that are polar, non-polar, and semi-polar (Suryani et al., 2016); (Muaja et al., 2017). The methanol extract of noni fruit has demonstrated potent antifungal activity, with studies reporting inhibition rates of up to 79.3% against *Trichophyton mentagrophytes* and significant activity against other fungal species, including *Penicillium* and *Fusarium* (approximately 50%) (Ali et al., 2016). The presence of phenolic compounds, particularly flavonoid glycosides and phenolic acids, has been linked to the antifungal properties of noni, as these compounds can interfere with the metabolic processes of fungi (Meilawati et al., 2021).

Furthermore, methanol's high polarity allows for the effective extraction of these bioactive compounds, enhancing their bioavailability and potential efficacy (Zhang et al., 2016). This study's results show that the treatment of noni plants with ethyl acetate extract has a wider apparent zone diameter than that of methanol extract. This indicates that in vitro, the ethyl acetate extract of the noni plant can inhibit the growth of the *Fusarium oxysporum* fungus more strongly than the methanol extract. The results of this study differ from those of a study by Jayaraman et al. (2008), which demonstrated that the methanol extract of noni fruit was more effective (48.5%) in inhibiting *Fusarium* sp. compared to the ethyl acetate extract. The discrepancy in results may be attributed to differences in the ripeness of the noni fruit used in the studies and the growing location of the noni plant, which can influence the composition of compounds in the fruit.

The research on the noni plant, focusing on its fruit, leaves, and twigs, is of significant importance. The ANOVA analysis reveals that the different parts of the Noni

plant have significantly different effects on the growth inhibition zone of the *Fusarium oxysporum*. This finding, presented in Figure 2, underscores the integral role of this research in advancing our understanding of the antifungal properties of noni.

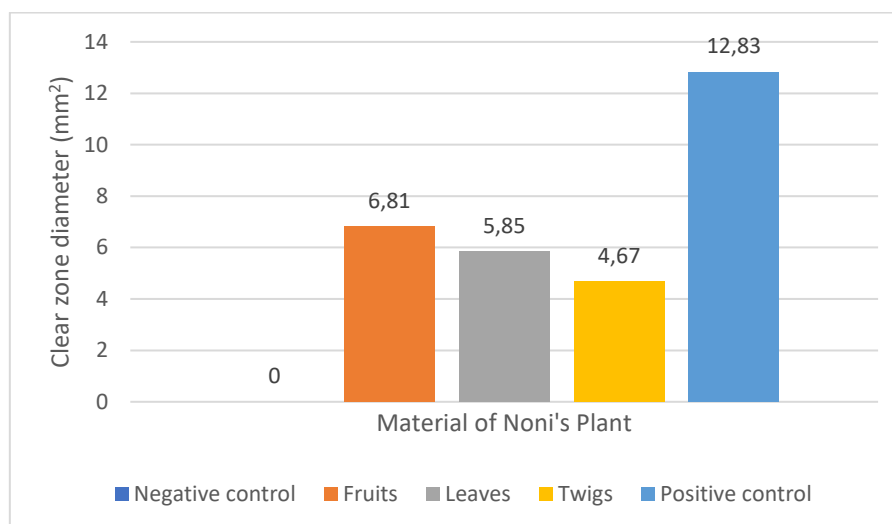


Figure 2. The inhibition of *Fusarium oxysporum* growth due to the effect of different plant organ of *Morinda citrifolia* L. (negative control is aquades, positive control is dhitane) was significantly different in the Duncan test ($\alpha < 0.05$).

The results show that noni fruit extract is a potent antifungal agent, with a more transparent, expansive zone than other leaves and twigs. The extract can inhibit the growth of several fungal species, making it a potential candidate for natural antifungal agents. The key to its antifungal activity lies in its rich array of bioactive compounds, including flavonoids, terpenoids, and iridoids. Specific compounds such as scopoletin and various flavonoids have been linked to the antifungal properties of noni. Scopoletin, a coumarin derivative found in noni, has been shown to inhibit the production of inflammatory cytokines, potentially enhancing the overall antifungal efficacy (Torres et al., 2017). Similarly, flavonoids like quercetin and rutin, prevalent in noni fruit, possess antioxidant and anti-inflammatory properties that may further boost their antifungal activity (Kim et al., 2024). Research has also indicated that noni fruit extracts can effectively combat various fungal pathogens, including *Trichophyton mentagrophytes*, *Penicillium*, *Fusarium*, and *Rhizopus* species, with varying degrees of inhibition reported (Ali et al., 2016).

Table 1. Diameter (mm²) of the inhibition zone of the *Fusarium oxysporum* due to the effect of concentration of noni fruit extract

Concentration	Inhibition zone by Methanol extract	Inhibition Zone by Ethyl Acetate Extract
10%	3.50 ^b	7.50 ^{cd}
20%	3.83 ^b	8.67 ^{de}
40%	4.17 ^b	8.83 ^{de}
60%	4.83 ^{bc}	8.83 ^{de}
80%	4.83 ^{bc}	11.33^{ef}
100%	5.00 ^{cd}	12.50 ^f
Negative control		0 ^a
Positive control		12.83 ^f

Note: Superscripts with different letters in columns and rows indicate significant differences in the Duncan Test ($\alpha < 0.05$).

Table 2 reveals that the 80% concentration of noni fruit ethyl acetate extract exhibits an inhibition zone of > 10mm², indicating a moderate inhibitory power. This is comparable to the 100% concentration (11.50mm²) and the positive control/distance (12.83mm²), highlighting the similar effectiveness of these two concentrations.

Table 2. Diameter (mm²) of the inhibition zone of the *Fusarium oxysporum* due to the effect of concentration of noni leaves extract

Concentration	Inhibition Zone by Methanol extract	Inhibition Zone by Ethyl Acetate Extract
10%	3.67 ^b	3.83 ^b
20%	3.83 ^b	4.50 ^{bc}
40%	4.33 ^{bc}	5.83 ^{bc}
60%	4.67 ^{bc}	6.67 ^{cd}
80%	6.00 ^{bc}	8.33^{de}
100%	6.50 ^{cd}	9.83 ^e
Negative control		0 ^a
Positive control		12.83 ^f

Note: Superscripts with different letters in columns and rows indicate significant differences in the Duncan Test ($\alpha < 0.05$).

The potential of noni leaves as natural antifungal agents is a significant area of research, given their rich composition of bioactive compounds. Studies have shown that various secondary metabolites in noni leaves, including terpenoids, alkaloids, flavonoids, and anthraquinones, exhibit antifungal activity. These compounds disrupt the structural integrity of fungal cell membranes, thereby inhibiting fungal growth and spore development (Aji & Roosyidah, 2020); (Zhang et al., 2016). Specifically, terpenoids and steroids found in noni leaves have been shown to possess potent antifungal effects. For instance, it highlighted that terpenoid compounds can compromise the cytoplasmic membrane of fungi, leading to their inhibition (Aji & Roosyidah, 2020).

Moreover, the identified anthraquinones, another class of compounds present in noni leaves, have demonstrated antifungal activity against various pathogens (Zhang et al., 2016). The presence of these compounds suggests that noni leaves could be a valuable resource for developing natural antifungal agents, offering a hopeful prospect for the future of antifungal therapies. In addition to the compounds above, flavonoids in noni leaves also contribute to their antifungal properties. These compounds not only exhibit direct antifungal activity but also enhance the overall efficacy of other bioactive compounds present in the leaves (Wang et al., 2021). The synergistic effects of these phytochemicals can lead to a more pronounced antifungal action, making noni leaves a promising candidate for natural antifungal therapies. Moreover, studies have shown that extracts from noni leaves can inhibit the growth of specific fungal pathogens, such as *Malassezia globosa*, associated with dandruff and other scalp conditions (Hamilton & Jaikishun, 2023). This demonstrates the potential application of noni leaf extracts in dermatological formulations to treat fungal infections.

The antifungal properties of noni leaves can be attributed to their diverse bioactive compounds, including terpenoids, flavonoids, and anthraquinones. Flavonoids can interact with microbial DNA and cause damage to the permeability of microbial cell walls, microsomes, and lysosomes (Sari et al., 2022). These compounds work synergistically to inhibit fungal growth and could be a basis for developing effective natural antifungal treatments. Based on the results in Table 2, it can be seen that the most expansive inhibition zone is in the 100% concentration ethyl acetate extract, which was 9.83mm². An inhibition zone of less than 10mm² is classified as weak. The result showed that the ethyl acetate extract of noni fruit has more potent antifungal activity than that of noni leaves. The fruit and leaves of noni may contain different concentrations of bioactive compounds. The fruit might have higher concentrations of compounds with more substantial antifungal properties, such as flavonoids, alkaloids, or terpenes, compared to the leaves.

Table 3. Diameter (mm²) of the inhibition zone of the *Fusarium oxysporum* due to the effect of concentration of noni twigs extract

Concentration	Inhibition zone by Methanol extract	Inhibition zone by Ethyl acetate Extract
10%	0.83 ^a	1.57 ^{ab}
20%	1.50 ^{ab}	3.83 ^{cd}
40%	2.17 ^{abc}	4.50 ^d
60%	3.67 ^{cde}	5.50 ^d

Concentration	Inhibition zone by Methanol extract	Inhibition zone by Ethyl acetate Extract
80%	4.67 ^d	8.00 ^e
100%	4.83 ^d	8.00 ^e
Negative control		0 ^a
Positive control		12.83 ^f

Note: Superscripts with different letters in columns and rows indicate significant differences in the Duncan Test ($\alpha < 0.05$).

The study results showed that noni twig extract had the lowest inhibition zone of *Fusarium oxysporum* compared to noni leaf and fruit extract, indicating that noni leaf and fruit extract were more effective than noni twig. The results in Table 3 show that the average inhibition zone for noni twig extract is 4.83mm² (methanol extract) and 8.00mm² (ethyl acetate extract). This showed that the content of anti-fungal chemical compounds in the twigs is lower than in noni leaves and fruit. The twigs contain some of the same compounds found in the leaves and fruits, such as flavonoids and terpenoids, but typically in lower amounts (Deng et al., 2010; Chan-Blanco et al., 2006). The specific chemical constituents of the twigs have not been as thoroughly documented as those of the leaves and fruits, indicating a need for further research to fully understand their potential benefits. These findings are significant in the context of antifungal research, as they provide valuable insights into the comparative effectiveness of different noni extracts.

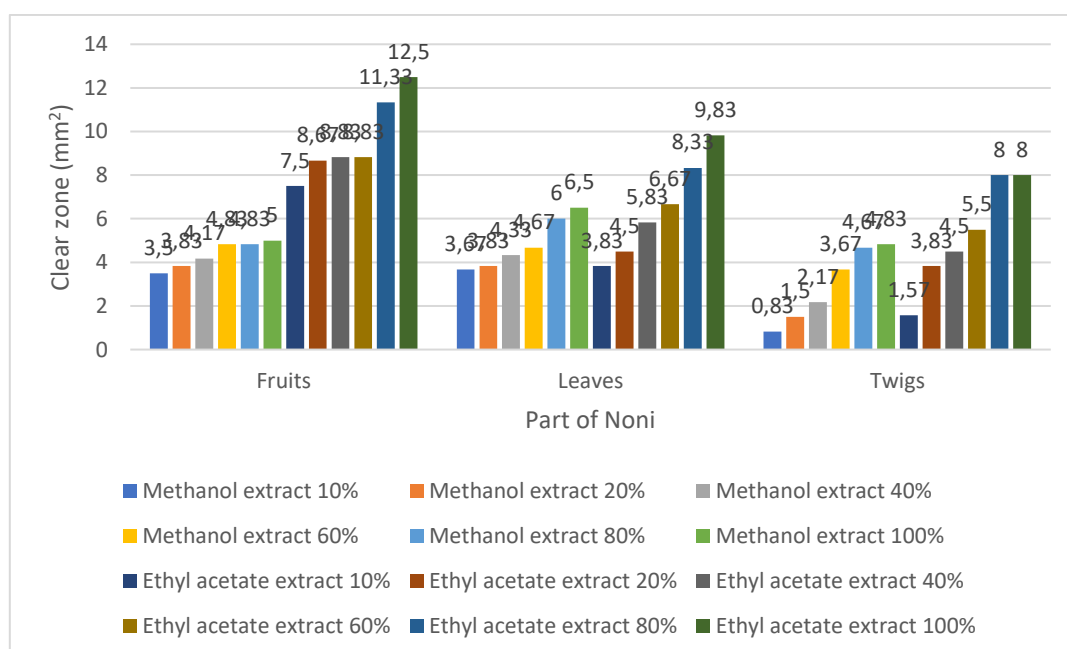


Figure 3. The inhibition of *Fusarium oxysporum* growth due to the effect of different plant organ of *Morinda citrifolia* L. (negative control is aquades, positive control is dhitane) and concentration was significantly different in the Duncan test ($\alpha < 0.05$).

Comparing the overall effect of noni plant parts (fruit, leaves, and twigs) on different extracts and concentrations, it can be seen that the most effective treatment is noni fruit ethyl acetate extract at a concentration of 100%. However, the ethyl acetate extract of noni fruit at a concentration of 100% (12,5f mm²) was not statistically significantly different from the ethyl acetate extract of noni fruit at a concentration of 80% (11,33ef mm²). This similarity in the effects of the 80% and 100% concentrations provides reassurance about the reliability of the *research*. The optimum concentration is a lower extract concentration, significantly inhibiting fungal growth. Based on the results of this research, it is known that noni fruit ethyl acetate extract at a concentration of 80% has moderate inhibitory power similar to noni fruit ethyl acetate extract at a concentration of 100% and positive control (white). The effect of methanol extract on all parts of the noni plant was considered weak. The inhibition zone for the growth of the fungus *Fusarium oxysporum* by methanol and ethyl acetate extracts of noni plants in vitro is presented in Figure 4.

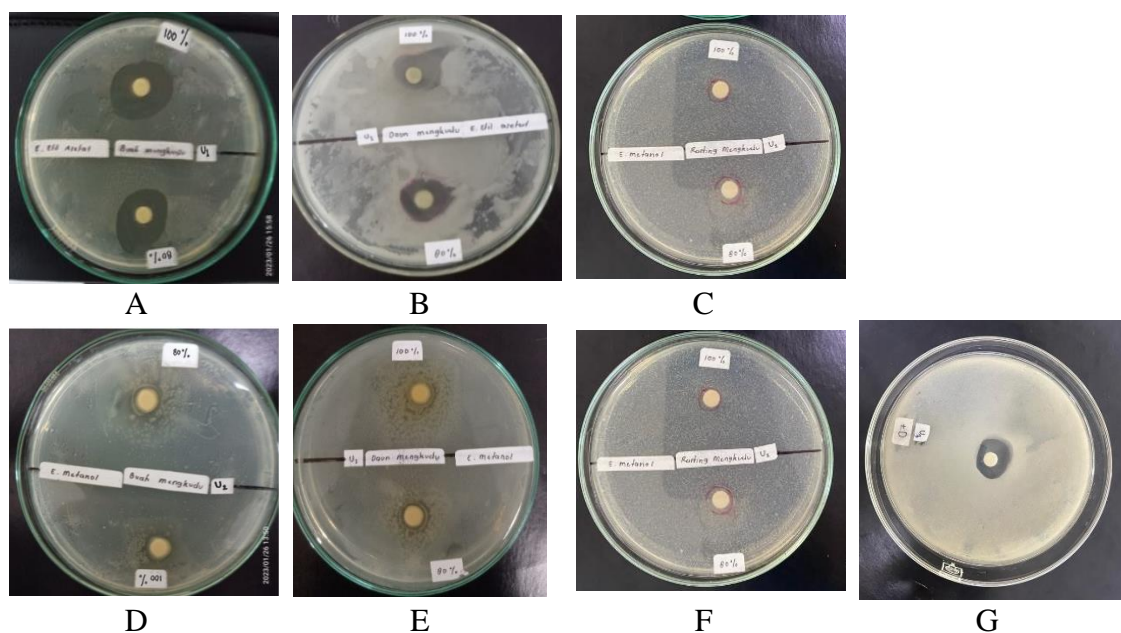


Figure 4. The inhibition zone of ethyl acetate extract: A) fruits, B) leaves and C) twigs of Noni, the inhibition zone of methanol extract: D)) fruits, E) leaves and F) twigs of Noni and positive control/Dhitane (G)

CONCLUSION

The research results concluded that ethyl acetate extract and methanol extract of the Noni plant affect the growth of the *Fusarium oxysporum*. The ethyl acetate extract of

the noni plant was more effective in inhibiting the growth of the *Fusarium oxysporum* in vitro compared to the methanol extract. The part of the plant with the most potent inhibitory power against the development of the *Fusarium oxysporum* fungus was noni fruit. These findings could potentially be applied in the development of new antifungal agents. The optimum concentration that could inhibit the development of the *Fusarium oxysporum* fungus was ethyl acetate extract for noni at a concentration of 80%.

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