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Research Article
Nano-Methanol Extract of Binahong (*Anredera cordifolia*) Leaves Inhibits *Fusarium oxysporum* Growth *in vitro* and Controls *Fusarium* Wilt in Shallot Plants

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Abstract

Background and Objective: *Fusarium oxysporum* f. sp. *cepae* (*Foc*) causes *Fusarium* wilt disease in shallots, with potential yield losses up to 50% if not properly managed. The widespread use of synthetic fungicides poses risks to human health and the environment. Binahong (*Anredera cordifolia*) leaves contain antifungal secondary metabolites and are a promising botanical alternative. Nano-sizing such plant-based extracts may enhance their efficacy. This study aimed to assess the effect of nano-sized methanol extract of Binahong leaves on the *in vitro* growth of *Foc* and the severity of *Fusarium* wilt in shallots and to compare it with the non-nano extract at higher concentrations. **Materials and Methods:** The study involved both *in vitro* and *in vivo* experiments. *In vitro* tests were arranged using a completely randomized design and *in vivo* experiments followed a randomized complete block design with four replications. Treatments included nano Binahong methanol extract at 0, 1,250, 2,500, 5,000 and 10,000 ppm; non-nano extract at 20,000 ppm and the synthetic fungicide mancozeb (1 g/L) as a standard. Key parameters measured included colony growth, conidial germination, disease incidence and disease intensity. Data were statistically analyzed using ANOVA at a significance level of 5% ($p<0.05$). **Results:** Nano Binahong extract significantly outperformed the non-nano extract in inhibiting *Foc* growth and suppressing disease development. At 1,250 ppm, it caused the highest inhibition of colony growth (37.77%) and conidial germination (90.52%), compared to 26.66 and 88.36% inhibition, respectively, by the non-nano extract at 20,000 ppm. The same nano concentration (1,250 ppm) reduced *Fusarium* wilt incidence and intensity in shallots by 92.36%, the highest among all treatments. **Conclusion:** Nano methanol extract of Binahong leaves is highly effective in controlling *Foc* and reducing *Fusarium* wilt in shallots, even at much lower concentrations than the non-nano extract. This indicates its potential as an efficient, eco-friendly alternative to chemical fungicides. Future studies should explore its field application and formulation stability.

Key words: Botanical pesticide, nano material, plant-based fungicide, *Fusarium oxysporum*, Binahong extract, nano formulation, *Fusarium* wilt, shallot disease control

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Shallot (*Allium cepa* L. *var. aggregatum*) is one of the major spice commodities cultivated in Indonesia. It is also a key commodity as it holds a high economic value and potential as an exported commodity[1]. One of the major constraints in shallot cultivation is *Fusarium* wilt, caused by the fungus *Fusarium oxysporum* f.sp. *cepae* (*Foc*). This soilborne pathogen causes wilt and leaf twisting and yellowing-greying on shallots and also bulb rot, so the plants will die eventually[2]. *Fusarium* wilt on shallots may cause up to 50% reduction in yield[3]. To overcome this problem, most farmers use synthetic fungicides. However, this may cause some negative impact on the environment.

The use of botanical fungicides to control fungal diseases on crops is considered as an environmentally safe and friendly control method[4]. Binahong *Anredera cordifolia* (Ten.) Steenis has the potential to be developed as a plant-based fungicide due to its secondary metabolites, such as flavonoids, alkaloids, polyphenols and saponins[5]. Binahong leaf extract was reported to be able to control sheath blight disease of rice paddy and *Alternaria solani* on tomato seedling at 2% concentration[6,7]. On the other hand, nanotechnology has been recently applied in plant pests and diseases management, since nano-sized materials have more advantages than the normal size[8].

Nano-size formulation of botanical fungicides improve water solubility, dissolution rate and uniform dispersion without any chemical changes on the fungicide compounds[9]. Moreover, nano-sized botanical fungicides can enhance their efficiency and effectiveness in controlling fungal plant diseases[10].

Although Binahong leaf extract was reported to control some fungal plant pathogens[6,7], there was no report on the use of nano-sized Binahong leaf fungicide. Expectedly, the use of nano Binahong leaf extract will be more efficient than the non-nano extract, as the need of the quantity of raw material will be less. Therefore, this study was conducted to evaluate the effect of nano-sized methanol extract of Binahong leaves on the *in vitro* growth of *Foc* and *Fusarium* wilt on shallot plants and also to determine the concentration level compared to the non-nano size.

MATERIALS AND METHODS

Study area and duration: The experiments were carried out at the Phytopathology Laboratory and Greenhouse of the Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran, from February to October, 2023. Contact angle observation was conducted at the Functional NanoPowder University Center of Excellence (Finder U-CoE), Universitas Padjadjaran. The Scanning Electron Microscope (SEM) was carried out at the National Research and Innovation Agency (BRIN) in Bandung, Indonesia.

The *in vitro* experiments were arranged in a completely randomized design, while the *in vivo* in a randomized complete block design, with 7 treatments and four replications. The treatments were application of nano extract of Binahong leaves at 0, 1250, 2500, 5000 and 10000 ppm, non-nano extract of Binahong leaves at 20000 ppm and mancozeb fungicide (0.1%).

Data analysis: The variables observed were colony diameter of *Foc* on PDA, number of germinated conidia, disease incidence, disease intensity and Area Under the Disease Progress Curve (AUDPC). Analysis of variants was performed on the data obtained by using SPSS version 26.0. If there were significant differences in the data between treatments, they were further analysed by Duncan's Multiple Range Test at a p-value of 0.05.

Culture and inoculum of *Fusarium oxysporum:* The *Foc* inoculum was the collection of the Phytopathology Laboratory, Department of Plant Pests and Diseases, Universitas Padjadjaran. The isolate was originally isolated from a shallot leaf with disease symptoms grown in Brebes, a shallot cultivation area in West Java, Indonesia. The *Foc* was cultured on the oxoid potato dextrose agar (PDA) at room temperature (24-30°C) for 5-7 days.

Rice medium was used to mass culture the *Foc* inoculum. The rice grains were soaked in water for 24 hrs and rinsed. Then 100 g of rice was placed in heat-resistant plastic bags and autoclaved at 121°C and 1 atm for 15 min. The rice medium was then inoculated with 0.5 cm plugs of *Foc* cultured on PDA, five plugs per bag and incubated at room temperature[11].

Ten grams of *Foc* mass culture in rice that had been homogenized was placed in 90 mL of sterile distilled water and vortexed for 5 min. The suspension was used to inoculate shallot seed bulbs. To count the conidial density, 1 mL of the suspension was dropped onto a hemocytometer plate. Then the conidial density was adjusted to 10^7/mL.

Extraction of Binahong leaves: The extraction method was a modification of the method of Yulia *et al.*[7]. Binahong leaf powder was macerated in 70% methanol (1:4) for 3×24 hrs, followed by two rinses. Then it was filtered using a glass funnel lined with filter paper. The filtrate was evaporated using a rotary evaporator at 50°C. The resulting powder of

Binahong extract was mixed well in Pro Analysis ethanol. This crude extract was then dissolved in sterile distilled water and sonicated using an Ultrasonic Processor UP200S HIELSCHER for 60 min at the Functional Nano Powder University Center of Excellence (Finder U-CoE) to obtain the nano-size Binahong leaf extract.

Colony growth of *Fusarium oxysporum*: The inhibition of *Foc*'s fungal colony growth was conducted using the poisoned food method[12]. Binahong leaf extract was poured into Schott bottles containing sterile liquid PDA and shaken thoroughly. Then it was poured into petri dishes, 10 mL each and left to solidify. A 0.5 mm plug of *Foc* culture (5 days) was taken and placed in the center of the PDA in the petri dish. The diameter of the fungal colonies was measured after 8 days/hrs.

Conidia germination of *Fusarium oxysporum*: The germination of *Foc* conidia was observed following the method by Yulia *et al.*[9] with slight modifications. Diluted *Foc* suspension was mixed with 3% water agar (1:1) at 45°C in an Erlenmeyer flask. The flask containing the mixture was shaken until thoroughly blended. The resulting mixture was poured into a petri dish and left to solidify. Then, the agar containing conidia was cut using a 0.5 cm cork borer and placed in the middle of an object glass.

The surface of the conidia agar was treated with five drops of the extract of nano methanol extract of Binahong leaves at different concentrations and covered with a cover glass. Object glasses with treated fungal agar were stored in a sterile plastic box lined with moist tissue and incubated for 5 hrs. The number of germinated conidia was counted. The inhibition of conidial germination was computed by comparing the number of germinated conidia in the control and treatments.

Disease incidence and intensity: The greenhouse experiment to observe the disease incidence was carried out[13]. The planting medium was soil+chicken manure+rice husk biochar (2:1:1)[14]. In each treatment, shallot seed bulbs were planted in a box, 10 cm deep, 10 seeds per box. The distance between holes was 15 cm. Before planting, the planting holes were inoculated with *Foc* inoculum, 10 mL/planting hole[15] and incubated for one day. One-third of the top of the shallot seed bulbs were cut to break the dormancy period and accelerate the growth of plant shoots. Before planting, shallot seeds were soaked in Binahong leaf extract according to the treatments for 10 min. Then they were planted in the moist and *Foc* inoculated soil and drenched with 10 mL of nano Binahong leaf extract/plant. Disease symptoms were observed 7 times weekly since planting.

Observations on the number of plants with disease symptoms such as wilt and leaf twisting and yellowing and the scoring of the disease symptoms were conducted every week. Disease incidence is the percentage of the number of diseased plants compared to the total number of observed plants[16]. The disease intensity was measured (Fig. 1) based on symptom scoring standards used[16] as follows:

0 = No symptoms
1 = 1-20% of the plant parts with symptoms
2 = 21-40% of the plant parts with symptoms
3 = 41-60% of the plant parts with symptoms
4 = 61-80% of the plant parts with symptoms
5 = 81-100% of the plant parts with symptoms

The disease intensity was calculated using the following formula[17]:

$$DI\ (\%) = \{\sum(n_i \times v_i) : (N \times V)\} \times 100$$

Where:
DI = Disease intensity
n_i = Number of diseased plants in each category
v_i = Score for each category
N = Total number of observed plants
V = Highest score

To compute the Area Under the Disease Progress Curve (AUDPC), the disease symptoms were observed and scored weekly, 7 times to the 8th week since inoculation. Based on the data of disease intensity, the AUDPCs were calculated with the formula[18]:

$$AUDPC = \sum_{i=1}^{n-1}\left(\frac{X_{i+1} \times X_i}{2}\right) \times (t_{i+1} - t_i)$$

Where:
X_i = Intensity of the disease at the first (previous) observation
X_{i+1} = Intensity of the disease at the next observation
t_i = Time of first (previous) observation
t_{i+1} = Time of next observation

The AUDPC represents the progress of the disease symptoms' development, hence, the disease progress became obvious.

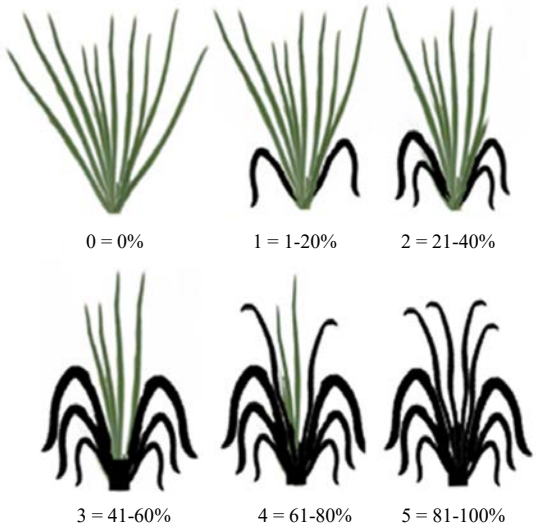

Fig. 1: Symptom scoring of disease intensity

Contact angle: The contact angle was observed using the tilting method[13] with slight modifications using a Canon G7X Mark II camera. The material used was shallot bulb peel, dropped with the Binahong extracts.

RESULTS AND DISCUSSION

***Fusarium oxysporum* colony growth:** The radial colony growth of *Foc* on the control had reached the edge of the petri dish at day 8 after inoculation. The colony growth of *Foc* treated with nano methanol extract of Binahong leaves was slower. At 8 days after inoculation, the average colony diameter was smaller than the control, ranging from 5.58 to 8.30 cm and significantly different from the control (Table 1). Overall, the shortest colony diameter was caused by the synthetic fungicide mancozeb. However, it was clear that the nano size of Binahong leaf extract at different concentrations, except 1,250 ppm, caused higher inhibition compared to the non-nano.

Macroscopic colony observations revealed differences in the thickness of the *Foc* colonies (Fig. 2). At the beginning of incubation, the mycelia formed white colonies and on the last day of observation (day 8), the colony color turned light purple, with a fine cotton-like texture. On the nano methanol extract of Binahong leaves at concentrations of 5,000 and 10,000 ppm, the mycelia were thick in the middle. Meanwhile, at 1,250 and 2,500 ppm, the colonies had a purple color with thinner mycelia compared to 5,000 and 10,000 ppm. The non-nano methanol extract of Binahong leaves at 20,000 ppm had white colonies and thinner mycelia compared to the other treatments.

Microscopic observations indicated the effect of nano methanol extract of Binahong leaves on the morphology of *Foc* hyphae. Damage to *Foc* hyphae was evident in the treatment with nano methanol extract from Binahong leaves. The hyphae were twisted and entwined, unlike the control, which exhibits a normal hyphal form (Fig. 3a-b). Compared to the non-nano p leaf methanol extract at 20,000 ppm, the *Foc* colony diameter affected by nano Binahong leaf methanol extract at 1,250 ppm was smaller. The smaller particle size of the nano extract (89.6 nm) is believed to enhance the activity of its secondary metabolites, leading to increased inhibition. Nanometer-sized particles increase the surface area, thereby enhancing the activity of the compounds in the material[19].

The treatments with nano Binahong leaf methanol extract at 2,500, 5,000 and 10,000 ppm were classified as weak inhibition, while Binahong leaf methanol extract at 1,250 ppm and non-nano extract 20,000 ppm were moderate. The fungicide mancozeb was classified as strongly inhibition[20]. The inhibition levels are categorized as fungistatic, indicating that they only inhibit fungal growth.

In this study, the Binahong leaf extract was prepared from a mixture of old and young leaves, which affected the quantity of its secondary metabolites. Lower concentration of the secondary metabolites may unexpectedly lead to weak inhibition. Older Binahong leaves reportedly contain higher levels of saponin compounds than younger ones[21].

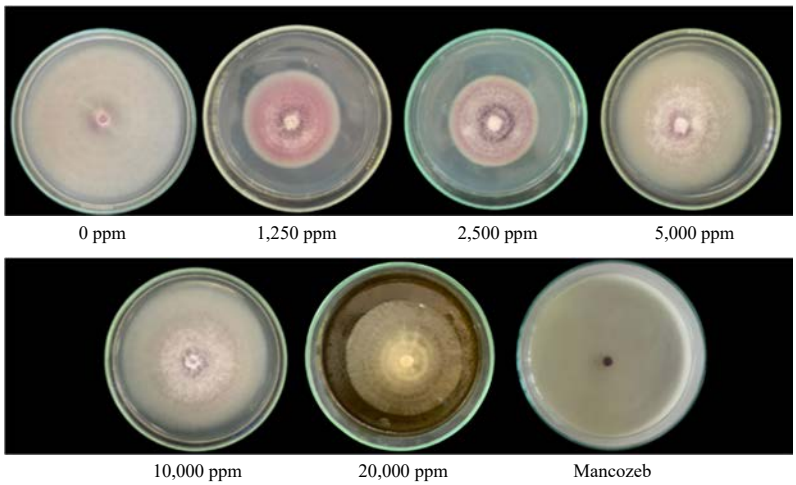

Fig. 2: Growth of *Fusarium oxysporum* colonies on PDA at various concentrations of nano methanol extract of Binahong leaves at day 8

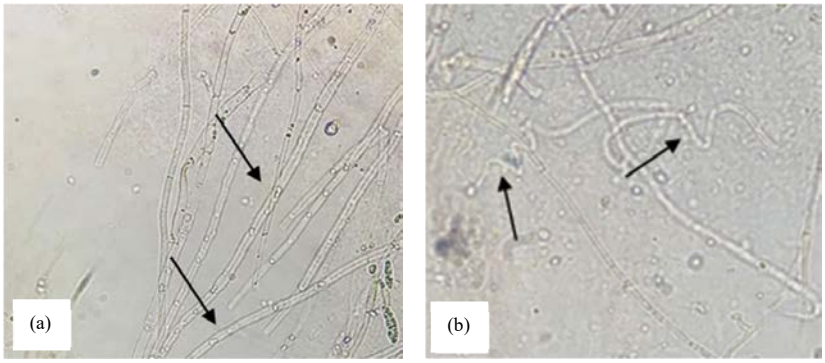

Fig. 3(a-b): *Foc* hyphae in the nano methanol extract of Binahong leaves (400x), (a) Normal and (b) Twisted and entwined

Table 1: Colony diameter and inhibition of *Fusarium oxysporum* radial growth at day 8 after inoculation

Code	Treatments	Colony diameter (cm)	Inhibition (%)	Antifungal activity
A	NBE 0 ppm	9.00^{f}	-	-
B	NBE 1,250 ppm	5.58^{b}	37.77	Medium
C	NBE 2,500 ppm	6.82^{c}	24.44	Weak
D	NBE 5,000 ppm	7.87^{d}	12.22	Weak
E	NBE 10,000 ppm	8.30^{e}	7.77	Weak
F	BE 20,000 ppm	6.60^{c}	26.66	Medium
G	Mancozeb 1%	0.50^{a}	94.44	Strong
CV		3.01		

Same letter in column 3 indicates no significant difference based on the Duncan's Multiple Range Test at 5%, NBE: Nano methanol extract of binahong leaves, BE: Non Nano methanol extract of binahong leaves and CV: Coefficient of variation

The study suggested decreased inhibition with increasing concentration, presumably due to excess sugar compounds in the higher concentrations, which subsequently stimulated fungal growth. Polysaccharides like L-arabinose, D-galactose, L-rhamnose and D-glucose in Binahong are prominent[22]. The sugar content in the extract may provide more nutrients to the fungus, thus accelerating the growth of *Foc*. Plant extract compounds, including sugars, can stimulate or promote fungal growth[23].

The inhibition of *Foc* colony growth was considered due to secondary metabolites produced by Binahong. Binahong contains several antifungal secondary metabolites, including flavonoids, alkaloids, tannins, saponins and terpenoids[23]. The disruption of fungal cell membrane permeability is caused by inhibition of mitochondrial electron transport by flavonoids.

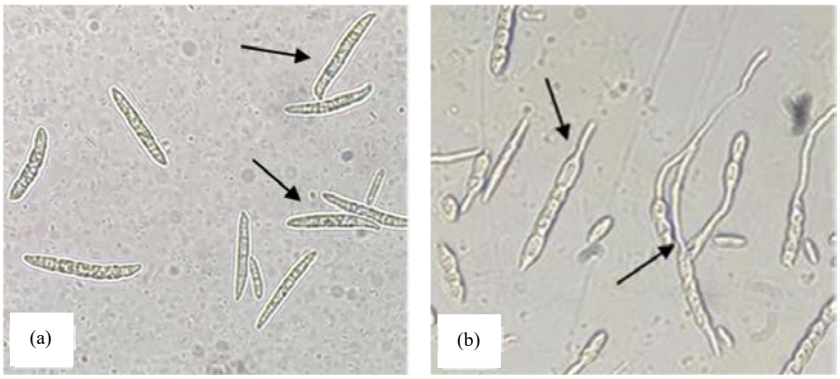

Fig. 4(a-b): Conidia of *Foc* (400X), (a) Non-germinated conidia and (b) Germinated conidia

Table 2: Germinated conidia of *Fusarium oxysporum* and the inhibition after 5 hrs of incubation

Code	Treatment	Number of germinated conidia	Inhibition (%)
A	NBE 0 ppm	58[c]	-
B	NBE 1,250 ppm	5.50[a]	90.52
C	NBE 2,500 ppm	23.25[b]	51.72
D	NBE 5,000 ppm	26[b]	59.91
E	NBE 10,000 ppm	28[b]	55.17
F	BE 20,000 ppm	6.75[a]	88.36
G	Mancozeb fungicide	0.75[a]	98.71
CV		18.75	

Same letter in column 3 within the table indicates no significant difference based on the Duncan's Multiple Range Test at 5%, NBE: Nano methanol extract from binahong leaves, BE: Methanol extract from binahong leaves and CV: Coefficient of variation

Tannins can damage and impair chitin synthesis, which is necessary for forming cell walls, while saponins can disturb the stability of the fungal cell membrane. Through their various mechanisms, terpenoids can also reduce the permeability of microorganism cell membranes[24].

The distortion of the hyphal shape, which appeared twisted and entangled in this study, was assumed to be influenced by the role of the secondary metabolites in Binahong. Terpenoids in Binahong can disrupt the process of fungal cell membrane and wall formation, leading to thinning of the cell wall[7].

***Fusarium oxysporum* conidial germination:** Following treatment with nano methanol extract derived from Binahong leaves, the *Foc* conidia were malformed. In the treatment with nano methanol extract of Binahong leaves, conidia appeared tubular with a longer size compared to normal conidia in the control (Fig. 4). The germinated conidia of *Foc* were longer than normal, which was believed to be influenced by the nano Binahong leaf methanol extract. The germinated conidia had a tubular shape and were longer than normal conidia[25].

The nano methanol extract of Binahong leaves at the tested concentrations (1,250, 2,500, 5,000 and 10,000 ppm was able to inhibit the *Foc* conidia germination. After 5 hrs, the number of germinated conidia in the control was 58, much higher than in the nano methanol extract of Binahong leaves treatments which were 6.75-28 (Table 2). Nano methanol extract of Binahong leaves caused the second highest inhibition at 90.52% after mancozeb at 98.71%. The highest inhibition of conidial germination (90.52%) among the extract treatments was observed at the concentration of 1,250 ppm.

However, among all the treatments tested, the fungicide mancozeb caused the highest inhibition in the germination of *Foc* conidia. The phenomenon may relate to the mode of action of mancozeb, which plays a crucial role in inhibiting conidial germination. Mancozeb can prevent respiration, essential for germination. Moreover, mancozeb can affect lipid metabolism[26].

The decrease in inhibition of conidial germination occurred along with the decrease in extract concentration. Similar results were observed in the study by Yulia *et al.*[9], where the increase in the concentration of water non-nano extract of Binahong leaves caused the decrease in the number of germinated *Colletotrichum capsici* conidia. This could be because the extract contained much sugar, especially at higher concentrations, which the fungus used as nourishment. The higher nutrient contents promoted more germinated conidia.

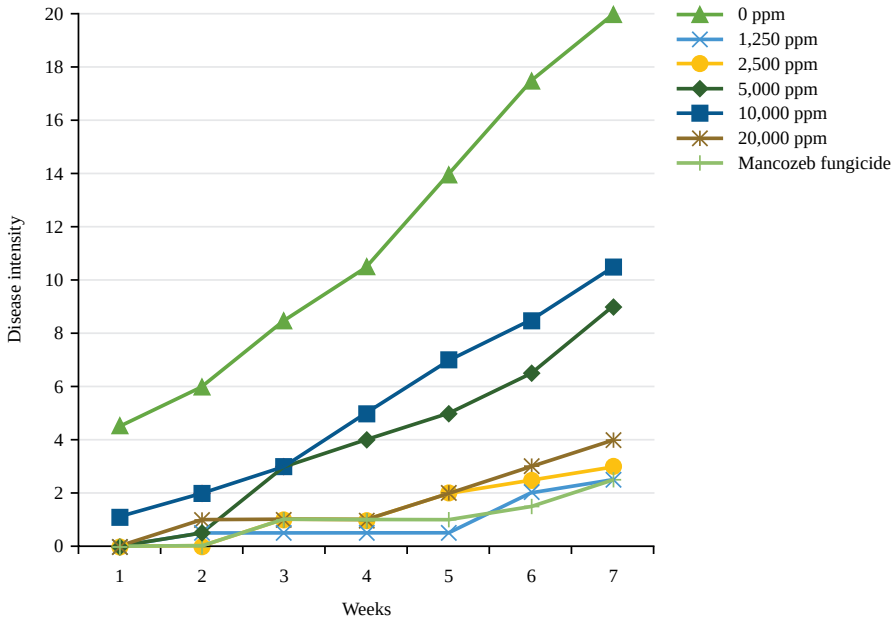

Fig. 5: *Fusarium* wilt disease intensity on shallots

Table 3: *Fusarium* wilt disease incidence on shallot at 32 days after inoculation

Code	Treatments	Disease incidence (%)	Inhibition (%)
A	NBE 0 ppm	35.0^{b}	-
B	NBE 1,250 ppm	10.0^{a}	71.43
C	NBE 2,500 ppm	10.0^{a}	71.43
D	NBE 5,000 ppm	20.0^{ab}	42.86
E	NBE 10,000 ppm	25.0^{ab}	28.57
F	BE 20,000 ppm	12.5^{a}	64.29
G	Mancozeb fungicide	7.5^{a}	78.57
CV		61.56	

Same letter in column 3 indicates no significant difference based on the Duncan's Multiple Range Test at 5%, NBE: Nano methanol extract of binahong leaves, BE: Methanol extract of binahong leaves and CV: Coefficient of variation

Factors influencing conidial germination include temperature, humidity, acidity (pH) and nutrients[27]. Inhibition may also be associated with the secondary metabolite content in Binahong leaf extract. Terpenoid and steroid compounds found in Binahong can damage the plasma membrane, thereby inhibiting conidial germination[27].

The higher inhibition observed in nano Binahong leaf methanol extract compared to non-nano Binahong methanol extract at 20,000 ppm might be related to the smaller particle size of the extract, which enhanced the activity of the compounds present. Smaller particle sizes can increase permeability, enhancing antioxidant activity[18].

Disease incidence and intensity of *Fusarium* wilt on shallot: At 32 days after inoculation on shallot seed bulbs, the disease incidences in the treatments of nano methanol extract of Binahong leaves at concentrations of 2,500 and 1,250 ppm were significantly different from the control (Table 3).

The fungicide mancozeb caused the highest suppression of *Fusarium* wilt disease incidence among the treatments at 78.57%, whereas the lowest was caused by nano methanol extract of Binahong leaves at 10,000 ppm i.e., 28.57%.

The treatment with nano methanol extract of Binahong leaves suppressed the intensity of *Fusarium* wilt disease (Fig. 5). The low increase in disease development observed in the first to third observations on the treatment of nano methanol extract of Binahong leaves was attributed to the influence of the application of Binahong leaf extract.

The suppression of disease intensity by the treatment with nano Binahong leaf methanol extract was due to the active compounds in Binahong that increased the resistance of shallot plants to *Foc* infection.

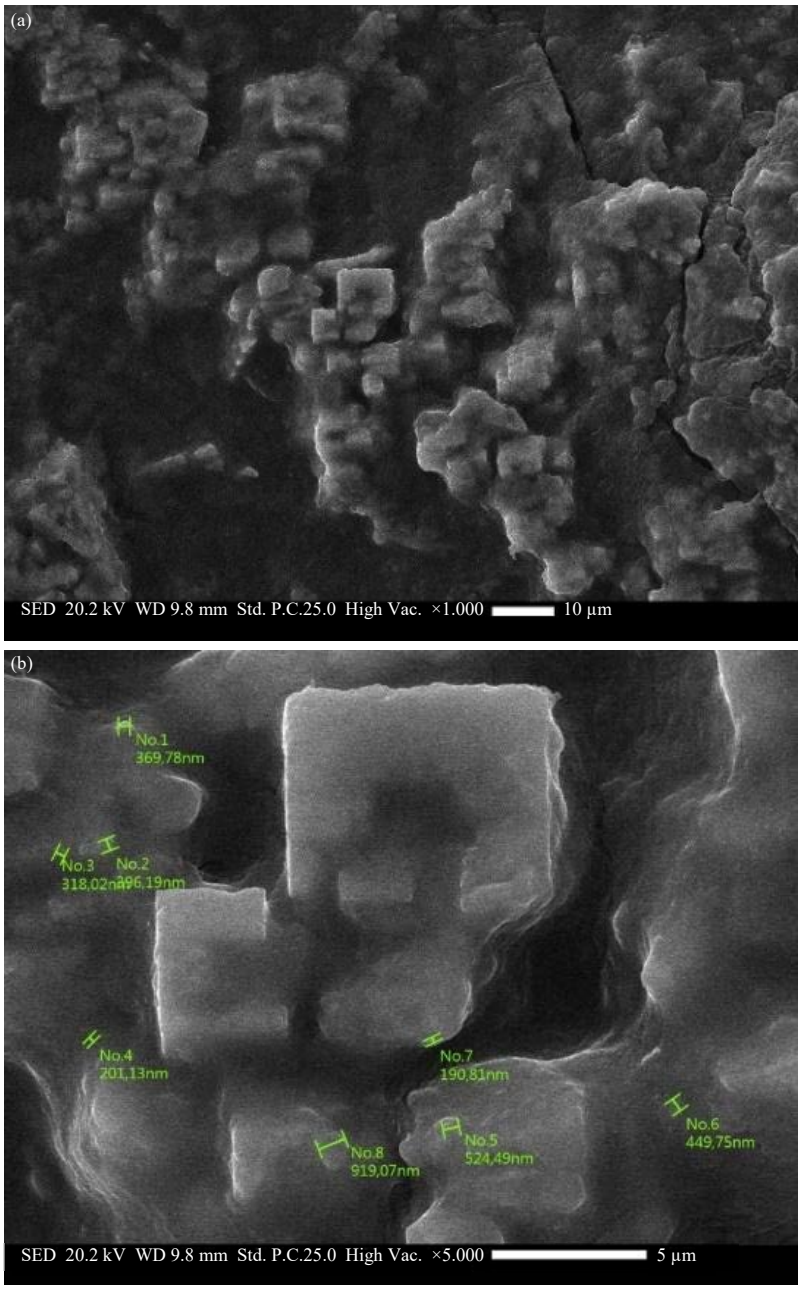

Fig. 6(a-b): Scanning Electron Microscope (SEM) of methanol extract nano Binahong, (a) Magnification 1,000x and (b) Magnification 5,000x with particle size shown

Table 4: AUDPC and the suppression of *Fusarium* wilt disease progress on shallots

Code	Treatment	AUDPC	Disease progress suppression (%)
A	NBE 0 ppm	206.25[b]	-
B	NBE 1,250 ppm	15.75[a]	92.36
C	NBE 2,500 ppm	24.00[a]	88.36
D	NBE 5,000 ppm	70.50[a]	65.82
E	NBE 10,000 ppm	93.90[a]	54.47
F	BE 20,000 ppm	30.00[a]	85.45
G	Mancozeb fungicide	17.25[a]	91.64
CV		65.38	

Same letter in column 3 indicates no significant difference based on the Duncan's Multiple Range Test at 5%, NBE: Nano methanol extract from binahong leaves, BE: Methanol extract from binahong leaves and CV: Coefficient of variation

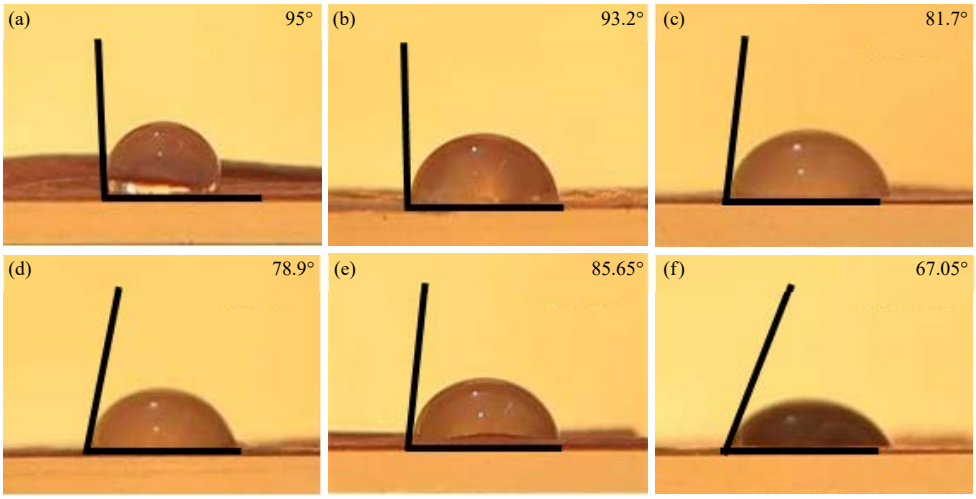

Fig. 7(a-f): Contact angles on the surface of the shallot bulb peel, (a) Water, (b) Nano Binahong leaf methanol extract (NBE) 1,250 ppm, (c) NBE 2,500 ppm, (d) NBE 5,000 ppm, (e) NBE 10,000 ppm and (f) Non-nano methanol extract of Binahong leaf 20,000 ppm

Binahong flavonoids, which are phenolic compounds, have antifungal activities and function as antioxidants to strengthen the plant's resistance to pathogen infections[28]. The increased resistance of shallot plants was likely attributed to soaking the shallot seed bulbs in the extract before planting. Biopesticide-treated shallot bulbs before planting increased the resistance and lowered the risk of smut disease on shallot plants[29].

The AUDPCs of all treatments range from 15.75 to 93.90, were significantly different from the control, which was 206.25 (Table 4). Nevertheless, nano methanol extract of Binahong leaves at 1.250 ppm caused the most significant disease progress suppression at 92.36%, while the 10,000 ppm of nano extract only 54.47%.

The high value of AUDPC indicates a low suppression of *Fusarium* wilt disease. The suppression of *Fusarium* wilt disease intensity was influenced by the absorption and spread of the nano Binahong leaf methanol extract on the seed bulb and plant surface. The contact angles of nano Binahong leaf methanol extract on the peel of the shallot surface were 67.05°-93.2°, smaller than that of water (111.45°).

The contact angle affects the absorption and spread of the extract on the shallot peel. Hydrophilic extracts (<90°) can adhere and spread well on the surface of shallot peel, thus improving their ability to suppress *Fusarium* wilt disease intensity. Insecticide solutions containing profenofos with the surfactant diethanolamide (DEA), which has a contact angle of less than 90°, can adhere and spread well on soybean leaves[30]. Hydrophilic materials (<90°) allow the material to flow and spread easily on the surface, whereas hydrophobic materials (>90°) repel water and make it difficult to spread on the surface[31].

Scanning electron microscope of nano Binahong leaf methanol extract: Upon conducting SEM observation, it was shown in Fig. 6a-b that the methanol extract derived from nano Binahong leaves displayed a diverse range of non-uniform shapes and particle sizes, providing detailed insights into the material's structural characteristics. Agglomeration or clumping of the extract can occur due to small particles sticking together to form agglomerates. The agglomeration causes larger particle sizes to be seen in the SEM. Agglomeration is suspected to be related to the non-uniform distribution of particle sizes in the extract.

Contact angle of the nano Binahong leaf methanol extract on shallot peel: The contact angle observed on the surface of shallot bulb peel, applied with nano Binahong leaf methanol extract, revealed apparent variations. Figure 7a-f indicates that the nano Binahong leaf methanol extract treatments had smaller contact angles than the control.

The treatments with nano Binahong leaf methanol extract at a concentration of 1,250, 2,500, 5,000 and 10,000 ppm had contact angles of 85.65°, 78.9°, 81.7° and 93.2°, respectively. The non-nano methanol extract of the Binahong leaf at 20,000 ppm had a contact angle of 67.05°.

Contact angle affects the absorption capacity of the extract on shallots. The absorption capacity of nano Binahong leaf methanol extract on shallot increases with the decrease of the contact angle. Nano Binahong leaf methanol extract at 10,000, 5,000 and 2,500 ppm had contact angles less than 90°, indicating hydrophilic properties. A contact angle less than 90° is hydrophilic, while an angle more than 90° is hydrophobic[32].

CONCLUSION

The results showed that the nano methanol extract of Binahong leaves was better in inhibiting the *in vitro* growth of *Fusarium oxysporum* and disease incidence and intensity compared to non-nano Binahong leaf extract. Therefore, the amount of raw Binahong leaf material needed to produce nano-sized extract would be smaller than the non-nano extract. With the same effectiveness, the nano extract would be more efficient. At 1,250 ppm, the nano extract caused the highest inhibition in colony growth and conidia germination i.e., 37.77 and 90.52%, respectively, compared to the non-nano extract i.e, 26.66 and 88.36%, respectively. Additionally, it could suppress the incidence and intensity of *Fusarium* wilt disease and also reduced the development of *Fusarium* wilt disease on shallots by 92.36%.

SIGNIFICANCE STATEMENT

Fusarium wilt, caused by *Fusarium oxysporum* f. sp., *cepae*, is a serious disease in shallot cultivation that can cause significant yield losses and is typically managed using synthetic fungicides, which raise environmental and health concerns. This study found that nano methanol extract of Binahong (*Anredera cordifolia*) leaves effectively inhibited fungal growth and suppressed disease severity, even at much lower concentrations than the non-nano extract. The enhanced efficacy is attributed to the nano formulation, which increases the bioavailability of active compounds. These findings highlight the potential of nano-biological fungicides as a sustainable and eco-friendly alternative for managing plant diseases in agriculture.

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