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The Antifungal Potential of Seven Plant Species Against Selected Plant Pathogen

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ABSTRACT

The antifungal activities of methanol extract from seven plant species, namely *Plectranthus amboinicus* (Mexican mint), *Morinda citrifolia* (noni), *Clitoria ternatea* (butterfly pea), *Passiflora suberosa* (corkystem passionflower), *Azadirachta indica* (neem), *Moringa oleifera* (ben oil tree) and *Vernonia amygdalina* (bitter leaf) were assessed against six fungal plant pathogens using poison agar technique at concentrations of 5%, 10%, 15% and 20%. The *in vitro* study revealed that the effectiveness of these plant extracts varied in suppressing the growth of *Ganoderma boninense*, *Rhizoctonia solani*, *Rigidoporus microporous* (basidiomycetes), *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4), Pyricularia oryzae (ascomycetes), and *Phytophthora palmivora* (oomycetes). Notably, *A. indica* demonstrated complete inhibition of all pathogens across all concentration levels, except against Foc TR4 at 5%, where it achieved 48.05% inhibition. This highlights the broad-spectrum antifungal potential of *A. indica*, as it proved effective against all the selected fungal plant pathogens. Complete inhibition of Foc TR4 was achieved solely with extracts from *P. amboinicus*, *M. citrifolia*, *C. ternatea*, *A. indica* and *V. amygdalina* at concentrations of 10% and above, which indicate higher concentrations required for effective inhibition of Foc TR4. Nevertheless, most pathogens were effectively suppressed at 5% concentration, and *A. indica*, *V. amygdalina* and *C. ternatea* demonstrated inhibition against all tested pathogens starting at 10%. These findings suggest these plant species could be developed into bio-fungicides for controlling major plant diseases in Malaysia, with *A. indica* being the

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most promising candidate. Further field trials are needed to validate their commercial viability compared to synthetic fungicides.

Keywords: Antifungal potential, botanical plant extracts, plant diseases, plant protection, poison agar

INTRODUCTION

Malaysia's agriculture sector is crucial for sustaining its population and driving economic growth through produce exports, which contribute approximately 8% to the country's gross domestic product (GDP). The key crops planted in Malaysia include oil palm, rubber, cocoa, rice, and tropical fruits (International Trade Administration, 2024). Despite its fertile land and favourable climate, the sector contends with challenges such as pest and disease outbreaks, climate change impacts, extreme weather events, labour shortages, and rapid population growth. Among these, insect infestation and disease pose a considerable menace to global food security, trade and livelihoods. Annually, as much as 40% of global crop production is lost due to pests and diseases (Food and Agriculture Organization of the United Nations [FAO], 2024), with fungal diseases alone accounting for 70-80% of crop losses (Peng et al., 2021). For instance, rice blast and sheath blight caused by *Pyricularia oryzae* and *Rhizoctonia solani* account for approximately 30% of losses in global rice production (Nalley et al., 2016; Zhu et al., 2019). The economic impact of *Ganoderma* disease on oil palm is estimated at around RM 1.5 billion (Malaysian Palm Oil Board [MPOB], 2022). Meanwhile, the disease incidence of *Rigidoporus microporus* in Malaysia ranges from 5% to 40%, based on random sampling conducted in rubber plantations across five states (Andrew, 2020). Recent estimates suggest that tropical race 4 of the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc TR4) could potentially spread across 1.7 million hectares of banana plantations in 29 countries by 2040 if no significant interventions are instituted, with annual production losses projected at around 10 billion dollars (Scheerer et al., 2018). Furthermore, *Phytophthora palmivora* affects all parts of the cocoa tree, causing 20%–30% pod losses through black pod rot and killing up to 10% of trees annually through stem cankers (Guest, 2007; Perrine-Walker, 2020).

Fungicides are widely used by growers as a primary approach in disease management, with various active ingredients (a.i.) such as propiconazole, tricyclazole, azoxystrobin and tebuconazole that are commonly employed (Mohiddin et al., 2021). While chemical fungicides provide rapid action and accessibility at lower costs, their extensive use raises environmental and health concerns (Wong, Hamid et al., 2020). Developing fungal resistance to these chemicals urges ongoing efforts to explore alternative solutions. The persistence of fungicide residues in food and the environment further emphasises the need for safer alternatives (Baibakova et al., 2019). Decades of conventional agricultural practices have clearly had a significant environmental impact. Balancing the present-day imperative to enhance food production and supply without compromising the health of the

planet and the ability of future generations to meet their needs is essential (Çakmakçı et al., 2023). In response, sustainable agriculture has emerged as a solution to address these challenges (Coulibaly et al., 2021). Therefore, implementing and adopting best practices for managing fungal plant pathogens in crops while minimising environmental impact is critical for sustainable agriculture and ensuring food security.

Environment-friendly solutions are available, such as biological control, natural compounds, crop rotation, resistant varieties, cultural practices, Integrated Pest Management (IPM), soil amendments, and precision agriculture (Çakmakçı et al., 2023). Humans can minimise their reliance on synthetic chemicals through sustainable disease management practices, thereby reducing pollution and environmental harm while preserving biodiversity by safeguarding beneficial organisms and habitats typically affected by chemical pesticides. Additionally, they contribute to soil health and water quality preservation (Sofo et al., 2022). Despite potentially higher initial costs, adopting sustainable practices tends to reduce the dependence on costly chemical inputs and enhances crop resilience, ultimately boosting profitability. By prioritising the health and safety of agricultural workers and communities, sustainable farming also fosters resilience to environmental stresses and climate change. The improved soil health and biological control methods reduce the likelihood of disease outbreaks and crop failures and are vital for ensuring food security amidst evolving environmental conditions.

In recent decades, there has been a rapid surge in interest regarding the exploration of natural compounds derived from medicinal or botanical plants as potential substitutes for chemical fungicides (Hernández-Ceja et al., 2021; Kursa et al., 2022; Lee et al., 2022). For instance, Ali et al. (2024) highlighted the significant antifungal efficacy of methanol and ethanol extracts derived from *Azadirachta indica* seeds and leaves against various fungal pathogens such as *Colletotrichum coccodes*, *Cladosporium fulvum*, *R. solani* and *F. oxysporum*. Additionally, research by Ilondu (2013) demonstrated the antifungal properties of ethanolic leaf extracts from *V. ambigua*, *V. amygdalina* and *V. cinerea* against groundnut leafspot diseases caused by *Cercosporella apersica* and *Curvularia lunatus*. These extracts contain bioactive compounds, including flavonoids, terpenoids, glycosides, phenols, alkaloids, saponins and tannins, that have been shown to possess antimicrobial properties (Ali et al., 2024; Ilondu, 2013).

Despite that, there remains a gap in understanding the antifungal efficacy of plant extracts from families such as Lamiaceae, Rubiaceae, Fabaceae, Passifloraceae, Meliaceae, Moringaceae and Asteraceae against the important fungal plant pathogens in Malaysia. These pathogens, which affect major crops, include *Ganoderma* basal stem rot in oil palm (Isha et al., 2020), white root rot in rubber (Go et al., 2023), rice blast and sheath blight in rice (Wong, Surendran et al., 2020), cocoa pod rot and durian fruit rot caused by *Phytophthora* species (Latifah et al., 2018), and *Fusarium* wilt in banana (Clement et al., 2019). Addressing this gap, the present study assesses the crude extracts of seven plant

species for their antifungal activity under laboratory conditions against important plant pathogens, namely *G. boninense*, *R. solani*, *R. microporous*, Foc TR4, *P. oryzae*, and *P. palmivora*, which commonly affect the crops in Malaysia. This study explores safer and sustainable bio-fungicides as alternatives to synthetic chemicals for crop protection.

MATERIALS AND METHODS

Collection of Plant Materials

Leaf samples of *P. amboinicus*, *M. citrifolia*, *C. ternatea*, *P. suberosa*, *A. indica*, *M. oleifera* and *V. amygdalina* were collected from various sites within Universiti Putra Malaysia (UPM), located in Seri Kembangan, Selangor, Malaysia (Table 1). The collected leaves were brought to Makmal Serbaguna B, situated in the Department of Plant Protection, Faculty of Agriculture, UPM, for further processing.

Table 1
Location of plant material collection

Plant specimen (Common name)	Location of plant material
Plectranthus amboinicus (Mexican mint)	Ladang 10, Universiti Putra Malaysia
Morinda citrifolia (noni)	Institute of Bioscience, Universiti Putra Malaysia
Clitoria ternatea (butterfly pea)	Faculty of Agriculture, Universiti Putra Malaysia
Passiflora suberosa (corkystem passionflower)	Faculty of Agriculture, Universiti Putra Malaysia
Azadirachta indica (neem)	Taman Herba, Universiti Putra Malaysia
Moringa oleifera (ben oil tree)	Taman Herba, Universiti Putra Malaysia
Vernonia amygdalina (bitter leaf)	Taman Herba, Universiti Putra Malaysia

Fungal Cultures

The selected fungal plant pathogens, namely *G. boninense, R. solani, R. microporous, Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4), *P. oryzae* and *P. palmivora* used in this study were obtained from the Fungal Culture Collection Unit, Department of Plant Protection, Faculty of Agriculture, UPM. Each fungal strain was sub-cultured and maintained using different selected sterilised media: *R. solani, R. microporous*, Foc TR4 and *P. oryzae* on Potato Dextrose Agar (PDA) (Oxoid, UK), *G. boninense* on Malt Extract Agar (MEA) (Oxoid, UK) and *P. palmivora* on Corn Meal Agar (CMA) (Oxoid, UK). Subsequently, all culture plates were maintained in a culture chamber at a temperature of 26 ± 2 °C under laboratory conditions.

Preparation of Plant Crude Extracts

The leaf specimens of *P. amboinicus*, *M. citrifolia*, *C. ternatea*, *P. suberosa*, *A. indica*, *M. oleifera* and *V. amygdalina* were washed to eliminate dirt and soil particles. Subsequently, all

leaves were air-dried at room temperature (26 ± 2 °C) for three days before being grounded separately into a fine powder (<100 µm) using a grinder (Cross Beater Mill SK 100, Retsch, Germany). Following this, 50 g of the grounded leaves were separately macerated in 300 ml of methanol (Fisher Chemical™, USA) in a conical flask and stirred at 120 rpm for 24 hr using an orbital shaker (Forma 420 Orbital Shaker, Thermo, USA). Each mixture was filtered using Whatman No. 1 filter paper (Cytiva, UK) and concentrated using a rotary evaporator (R215W, Buchi, Switzerland) at 145 rpm (42 °C). The resulting dried extracts were collected in a beaker with 5 ml of methanol (Fisher Chemical™, USA) and stored in an airtight container at 4 °C, following the method described by Wong, Hamid, Shah et al. (2020).

Screening of Antifungal Activities

The poison agar technique, as described by Balouiri et al. (2016), was used to assess the antifungal efficacy of *P. amboinicus*, *M. citrifolia*, *C. ternatea*, *P. suberosa*, *A. indica*, *M. oleifera* and *V. amygdalina* against six selected fungal plant pathogens, namely *G. boninense*, *R. solani*, *R. microporous*, *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4), *P. oryzae* and *P. palmivora*. Stock solutions from the crude plant extracts were prepared and diluted in methanol (Fisher Chemical™, USA) at a ratio of 1:10, following the method described by Abu et al. (2017). Nineteen millilitres of sterilised PDA (Oxoid, UK) and 1 ml of plant extract were mixed together and plated onto a sterilised Petri dish (Brandon, USA) to achieve a 5% concentration. This procedure was repeated to attain concentrations of 10%, 15%, and 20%, with the amount of plant extract added into the sterilised PDA (Oxoid, UK), as detailed in Table 2. Each concentration had five replications, and 0% served as the negative control. The same step was repeated for other plant extracts.

Table 2
The quantity of methanol extract utilised to obtain various concentration levels for the poison agar of the tested plants

Percentage of plant extract (%)	The methanol extract (ml)	PDA agar (ml)	Final concentration (mg/ml)
5	1	19	100
10	2	18	200
15	3	17	300
20	4	16	400

The poison agar medium was prepared individually at 100, 200, 300, and 400 mg/ml concentrations for each crude extract. A fungal disc with a diameter of 5 mm diameter, obtained from the actively grown pure culture of the selected pathogens, was placed at the centre of Petri dishes (Brandon, USA) containing various concentrations of the crude

extracts, with five replications for each concentration (Yusoff et al., 2020). This process was repeated for the other crude plant extracts. Subsequently, all plates were incubated at 26 ± 2 °C. The antifungal activity of the tested plants against the pathogens was assessed using the percentage inhibition of diameter growth (PIDG). This involved measuring the diameter of mycelial growth daily until the Petri dishes (Brandon, USA) with 0% concentration of the tested plants were completely covered with pathogen mycelia. The mycelial growth of the pathogens on different treatment concentrations was observed and recorded daily, following the PIDG formula described by Wong et al. (2020c).

$$\text{PIDG (\%)} = \frac{D1-D2}{D1} \times 100$$

Where, D1 is the average mycelial growth in control plates, and D2 is the average mycelial growth in treatment plates.

Experimental Design and Statistical Analysis

The effect of plant extracts and their antifungal activities were conducted *in vitro* using a completely randomised design (CRD) with five treatments (0%, 5%, 10%, 15%, 20%), each replicated five times. Statistical analysis was conducted using SAS® Software (SAS Institute, North Carolina State University, USA, Version 9.4, 2012). Mean comparisons were determined using the Least Significant Difference (LSD) at a 5% probability level.

RESULTS

Effect of *P. amboinicus* Crude Extract on Mycelial Growth of Selected Fungal Plant Pathogens

The Percentage Inhibition of Diameter Growth (PIDG) of selected fungal plant pathogens treated with *P. amboinicus* (Mexican mint) extract at concentrations of 5%, 10%, 15% and 20% is presented in Figure 1. Mexican mint extract showed high efficacy against *G. boninense* and *R. microporous* (basidiomycetes), as well as *P. oryzae* (ascomycetes), achieving PIDG values of 100% across all concentrations tested (Figure 1). All pathogens were completely inhibited by the 10% concentration of *P. amboinicus* extract, except for *P. palmivora* (84.25%) within the oomycetes group. Furthermore, concentrations of 15% and 20% consistently displayed 100% suppression against all pathogens, with no significant difference observed (Figure 1).

Effect of *M. citrifolia* Crude Extract on Mycelial Growth of Selected Fungal Plant Pathogens

The antifungal activity of *M. citrifolia* (noni) methanol extract against selected fungal plant pathogens is presented in Figure 2. The results illustrated that *G. boninense*, classified

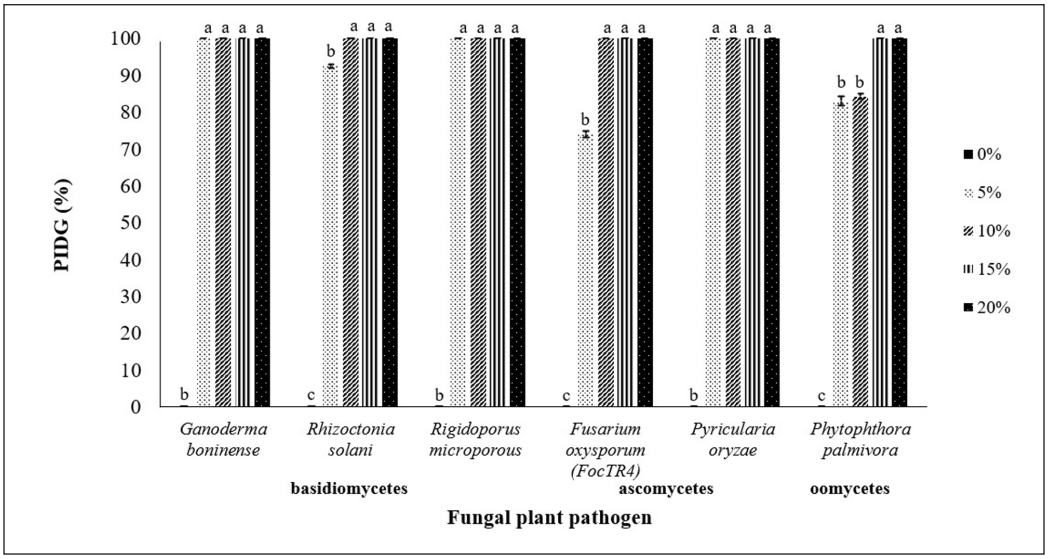

Figure 1. Percentage inhibition of diameter growth (PIDG) of *Plectranthus amboinicus* against selected fungal plant pathogens

Note. Measurements were taken at different intervals: Three days after inoculation (DAI) for *Rhizoctonia solani*; 8 DAI for *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) and *Phytophthora palmivora*; 9 DAI for *Ganoderma boninense*; and 11 DAI for *Pyricularia oryzae* and *Rigidoporus microporous*. Values are the means of five replicates. Means with the same letter are not significantly different at $p = 0.05$

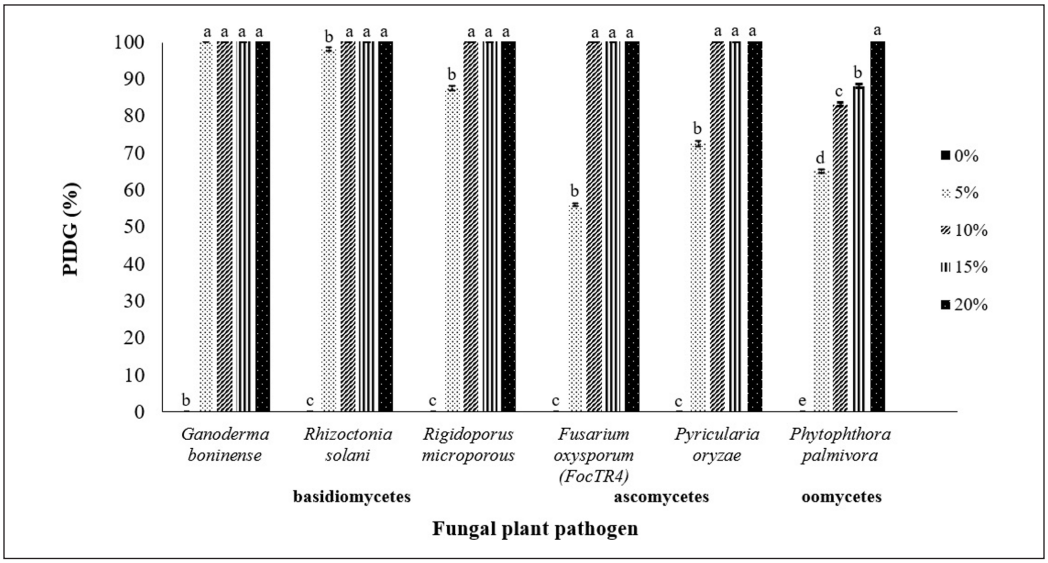

Figure 2. Percentage inhibition of diameter growth (PIDG) of *Morinda citrifolia* against selected fungal plant pathogens

Note. Measurements were taken at different intervals: Three days after inoculation (DAI) for *Rhizoctonia solani*; 8 DAI for *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) and *Phytophthora palmivora*; 9 DAI for *Ganoderma boninense*; and 11 DAI for *Pyricularia oryzae* and *Rigidoporus microporous*. Values are the means of five replicates. Means with the same letter are not significantly different at $p = 0.05$

under basidiomycetes, was the only pathogen effectively inhibited by noni extracts across all concentrations (ranging from 5% to 20%). Another two basidiomycetes (*R. solani* and *R. microporous*) and two ascomycetes (Foc TR4 and *P. oryzae*) were entirely inhibited by noni extract starting from 10% and above concentrations. Meanwhile, the most effective inhibition (100%) against *P. palmivora* (oomycetes) was achieved only with a 20% concentration, whereas for the concentrations of 10% and 15%, the PIDG values were recorded at 83% and 88%, respectively. At 20%, noni plant extract demonstrated the ability to suppress the mycelial growth of all tested pathogens (Figure 2).

Effect of *C. ternatea* Crude Extract on Mycelial Growth of Selected Fungal Plant Pathogens

Figure 3 depicts the antifungal efficacy of *C. ternatea* (butterfly pea) methanol extract against six selected fungal plant pathogens. Notably, butterfly pea extract significantly inhibited *G. boninense* (basidiomycetes) *and P. palmivora* (oomycetes) growth, achieving a PIDG of 100% across all concentrations. Additionally, *R. solani* and *R. microporous* (basidiomycetes), as well as Foc TR4 and *P. oryzae* (ascomycetes), revealed complete suppression by butterfly pea extract at concentrations of 10% and higher (Figure 3). This indicated that butterfly pea extracts effectively controlled all pathogens at 10%.

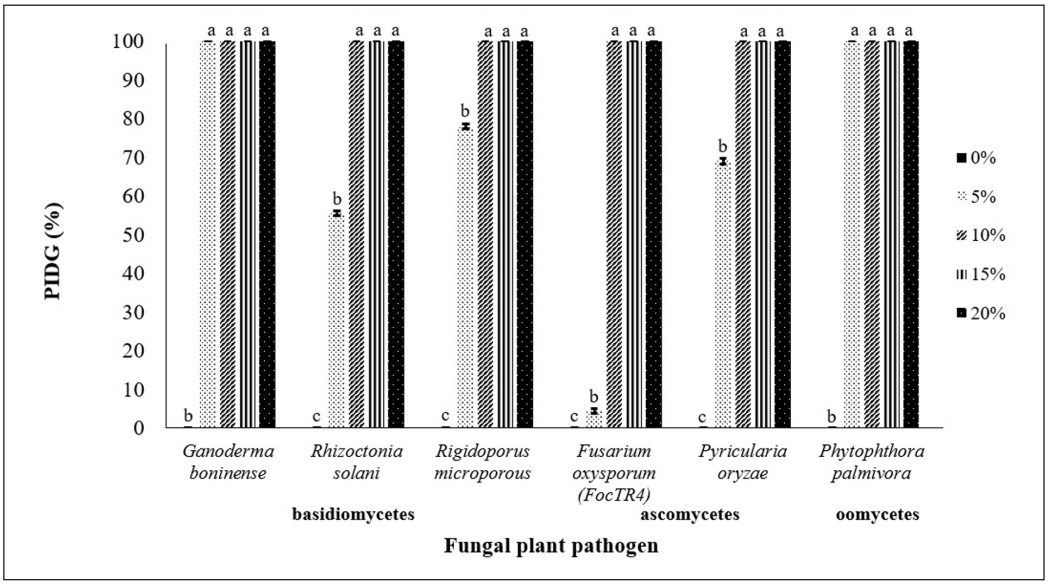

Figure 3. Percentage inhibition of diameter growth (PIDG) of *Clitoria ternatea* against selected fungal plant pathogens

Note. Measurements were taken at different intervals: Three days after inoculation (DAI) for *Rhizoctonia solani*; 8 DAI for *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) and *Phytophthora palmivora*; 9 DAI for *Ganoderma boninense*; and 11 DAI for *Pyricularia oryzae* and *Rigidoporus microporous*. Values are the means of five replicates. Means with the same letter are not significantly different at $p = 0.05$

Effect of *P. suberosa* Crude Extract on Mycelial Growth of Selected Fungal Plant Pathogens

The antifungal efficacy of *P. suberosa* (corkystem passionflower) methanol extract against the selected pathogen was revealed in Figure 4. Among the tested pathogens, *P. oryzae* (ascomycetes) and *P. palmivora* (oomycetes) were completely inhibited by corkystem passionflower extracts across all concentrations, as shown in Figure 4. Furthermore, complete inhibition of *R. solani* (basidiomycetes) occurred at concentrations above 10%, while *G. boninense* and *R. microporus*, also from the same class, were entirely inhibited at concentrations above 15%. Nevertheless, 5% and 10% *P. suberosa* extract against *G. boninense* still achieved a PIDG value of more than 80% (Figure 4). Conversely, corkystem passionflower extract exhibited the lowest effect against Foc TR4 (ascomycetes) across all concentrations, with significant differences observed between concentration levels (Figure 4).

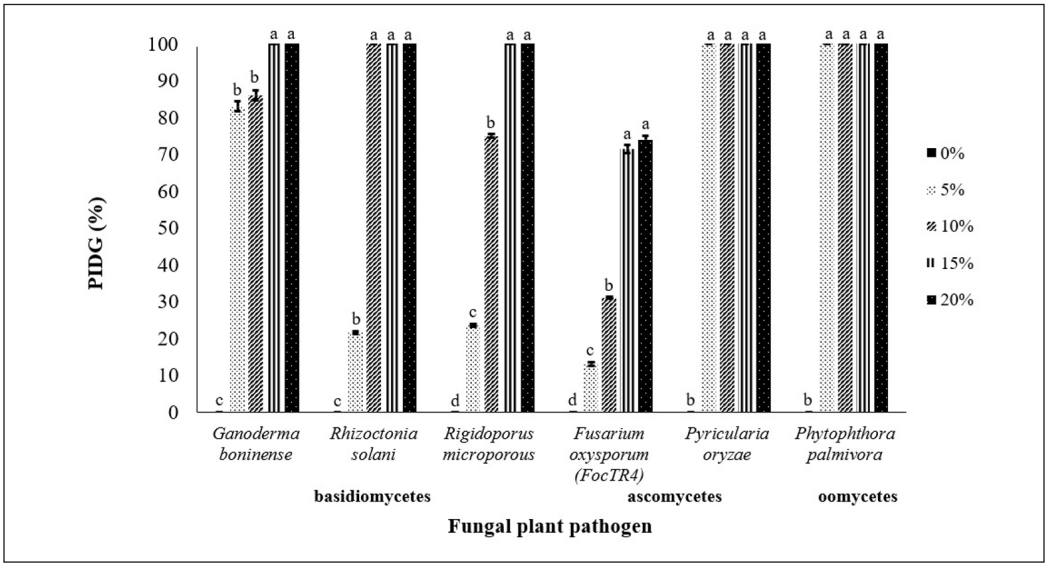

Figure 4. Percentage inhibition of diameter growth (PIDG) of *Passiflora suberosa* against selected fungal plant pathogens

Note. Measurements were taken at different intervals: Three days after inoculation (DAI) for *Rhizoctonia solani*; 8 DAI for *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) and *Phytophthora palmivora*; 9 DAI for *Ganoderma boninense*; and 11 DAI for *Pyricularia oryzae* and *Rigidoporus microporous*. Values are the means of five replicates. Means with the same letter are not significantly different at $p = 0.05$

Effect of *A. indica* Crude Extract on Mycelial Growth of Selected Fungal Plant Pathogens

Figure 5 shows the efficacy of *A. indica* (neem) extract against six selected pathogens. Neem extract exhibited high efficacy against all the tested pathogens, namely *G. boninense*, *R. solani* and *R. microporus* (basidiomycetes), as well as *P. oryzae* (ascomycetes) and *P.*

palmivora (oomycetes), except Foc TR4 (ascomycetes) at 5%. The plant extract achieved 100% inhibition against these pathogens at all concentrations, with no significant difference observed between concentration levels (Figure 5). Concerning Foc TR4 (ascomycetes), complete suppression by neem extract was attained at concentrations of 10% and above. *A. indica* demonstrated the ability to suppress mycelial growth of all the tested pathogens at 10% onwards (Figure 5).

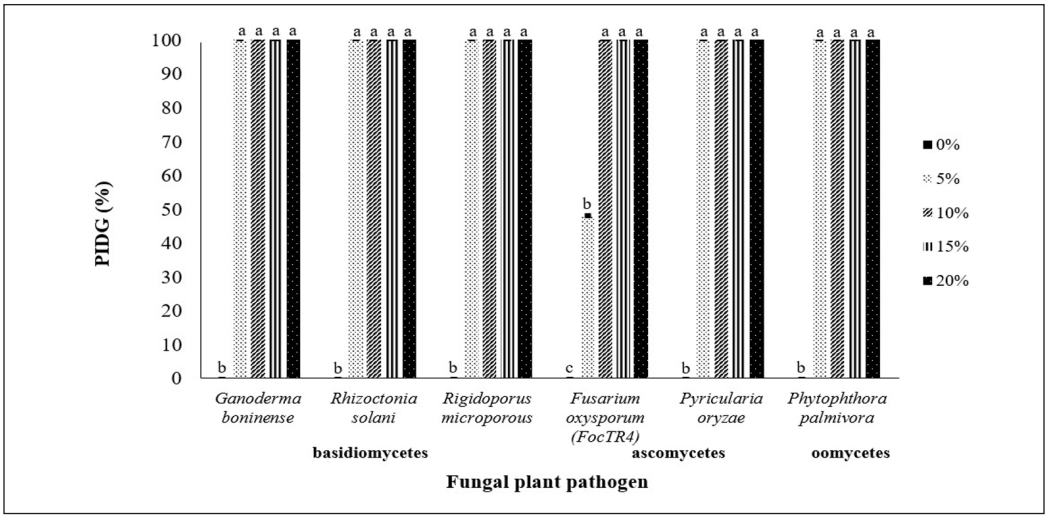

Figure 5. Percentage inhibition of diameter growth (PIDG) of *Azadirachta indica* against selected fungal plant pathogens

Note. Measurements were taken at different intervals: Three days after inoculation (DAI) for *Rhizoctonia solani*; 8 DAI for *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) and *Phytophthora palmivora*; 9 DAI for *Ganoderma boninense*; and 11 DAI for *Pyricularia oryzae* and *Rigidoporus microporous*. Values are the means of five replicates. Means with the same letter are not significantly different at $p = 0.05$

Effect of *M. oleifera* Crude Extract on Mycelial Growth of Selected Fungal Plant Pathogens

The results in Figure 6 demonstrated the variable antifungal activity of *M. oleifera* (ben oil tree) methanol extract against selected fungal plant pathogens at different concentrations. The methanol extract of the ben oil tree possessed high potential in suppressing the growth of *G. boninense* and *R. solani* (both basidiomycetes), achieving 100% inhibition across all concentrations (Figure 6). The results further indicate that the plant extract exhibited 100% inhibition at 10% and above concentrations against *R. microporus* (basidiomycetes) and *P. palmivora* (oomycetes). In contrast, the methanol extract of the ben oil tree did not entirely inhibit the growth of fungal species classified under ascomycetes, namely Foc TR4 and *P. oryzae*, across the concentrations. However, 10% and above concentrations of the plant extract could score PIDG more than 80% against both pathogens, with their highest PIDG values recorded at 93.25% and 85%, respectively, at 20% concentration (Figure 6).

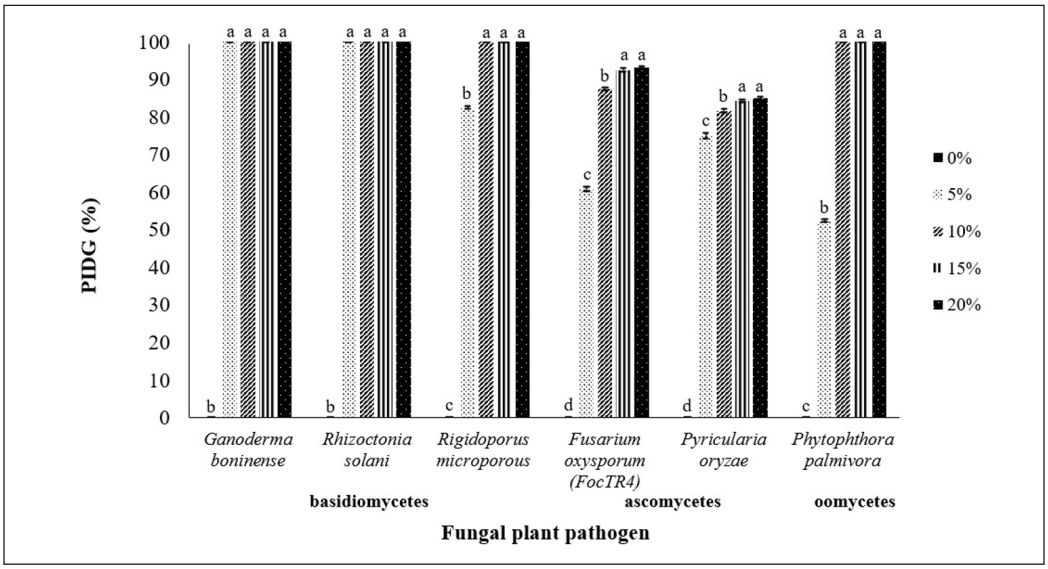

Figure 6. Percentage inhibition of diameter growth (PIDG) of *Moringa oleifera* against selected fungal plant pathogens

Note. Measurements were taken at different intervals: Three days after inoculation (DAI) for *Rhizoctonia solani*; 8 DAI for *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) and *Phytophthora palmivora*; 9 DAI for *Ganoderma boninense*; and 11 DAI for *Pyricularia oryzae* and *Rigidoporus microporous*. Values are the means of five replicates. Means with the same letter are not significantly different at $p = 0.05$

Effect of *V. amygdalina* Crude Extract on Mycelial Growth of Selected Fungal Plant Pathogens

Figure 7 reveals the antifungal activity of *V. amygdalina* (bitter leaf) against selected pathogens from three distinct groups: basidiomycetes, ascomycetes and oomycetes. Bitter leaf extract exhibited significant potential in inhibiting the growth of *G. boninense* and *R. solani* (both basidiomycetes), as well as *P. oryzae* (ascomycetes), achieving complete inhibition (100%) across all concentrations (5%, 10%, 15% and 20%). The growth of *R. microporous* (basidiomycetes), Foc TR4 (ascomycetes) and *P. palmivora* (oomycetes) was entirely inhibited by the bitter leaf extract at concentrations of 10% and above. Notably, bitter leaf extract exhibited the capacity to suppress the mycelial growth of all tested pathogens at concentrations of 10% and higher (Figure 7).

Comparison of the Antifungal Activity of Plant Extracts Against Selected Fungal Plant Pathogens at Five Concentration Levels

Table 3 summarises the antifungal activities of several plant methanol extracts against selected fungal plant pathogens. The growth of *G. boninense* was completely inhibited by nearly all plant extracts (*A. indica*, *V. amygdalina*, *C. ternatea*, *P. amboinicus*, *M. citrifolia* and *M. oleifera*) at concentrations as low as 5%, except for *P. suberosa*, which required

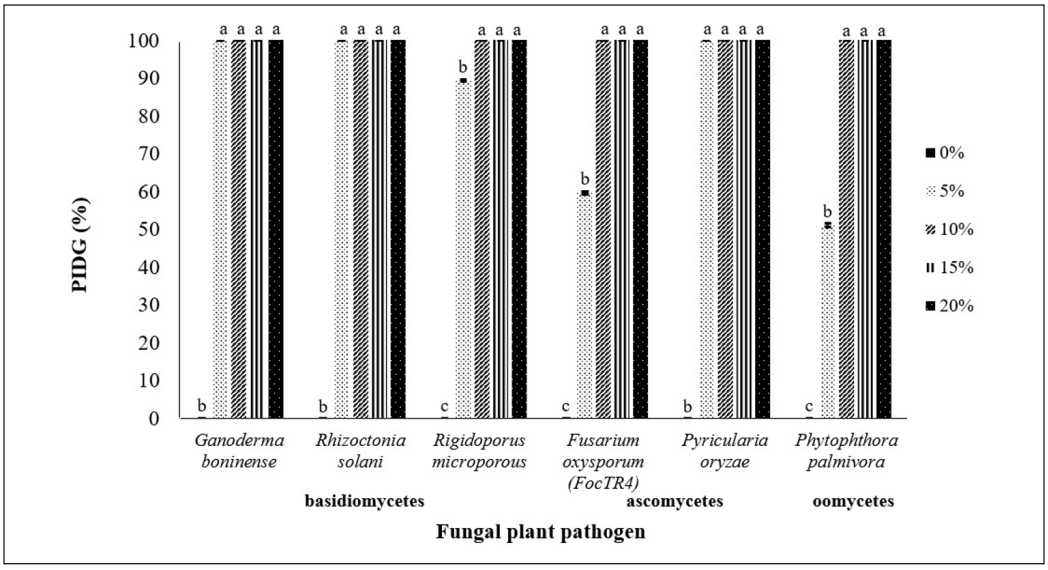

Figure 7. Percentage inhibition of diameter growth (PIDG) of *Vernonia amygdalina* against selected fungal plant pathogens

Note. Measurements were taken at different intervals: Three days after inoculation (DAI) for *Rhizoctonia solani*; 8 DAI for *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4) and *Phytophthora palmivora*; 9 DAI for *Ganoderma boninense*; and 11 DAI for *Pyricularia oryzae* and *Rigidoporus microporous*. Values are the means of five replicates. Means with the same letter are not significantly different at $p = 0.05$

a concentration of 15% or higher to achieve 100% inhibition. For *R. solani*, complete inhibition was observed with three plant extracts (*A. indica*, *V. amygdalina* and *M. oleifera*) at 5% concentration, while four other extracts (*C. ternatea*, *P. amboinicus*, *M. citrifolia* and *P. suberosa*) achieved 100% inhibition at 10% or higher. *R. microporous* was fully inhibited by 5% extracts of *A. indica* and *P. amboinicus*, while four other plant extracts (*V. amygdalina*, *C. ternatea*, *M. citrifolia* and *M. oleifera*) required a 10% concentration, and *P. suberosa* needed 20%. Complete inhibition of *P. oryzae* was achieved by the 5% extracts of *A. indica*, *V. amygdalina*, *P. amboinicus* and *P. suberosa*, followed by *C. ternatea* and *M. citrifolia*, achieving 100% inhibition at 10%, while *M. oleifera* at 20% resulted in only 85% inhibition (Table 3). The growth of *P. palmivora* was fully inhibited by *A. indica*, *C. ternatea* and *P. suberosa* at 5% concentration, followed by *V. amygdalina* and *M. oleifera* at 10%, *P. amboinicus* at 15%, and *M. citrifolia* at 20%. Regarding Foc TR4, nearly all plant extracts achieved 100% inhibition at a concentration of 10% or higher, except for *M. oleifera* and *P. suberosa*, which did not reach complete inhibition even at 20%, with PIDG values recorded at 74% and 93.25%, respectively (Table 3).

Comparing between plant species, the current findings indicate that higher concentrations (15% and 20%) generally resulted in better inhibition across all plant extracts. *A. indica* (neem) exhibited remarkable antifungal activity against all pathogens

Table 3
Maximum inhibition of seven methanol leaf extracts on the mycelial growth of selected fungal plant pathogens at different concentrations

	Fungal plant pathogen					
	Ganoderma boninense	***Rhizoctonia solani***	***Rigidoporus microporous***	***Pyricularia oryzae***	***Phytophthora palmivora***	***Fusarium oxysporum* (Foc TR4)**
Plant species	***PIDG %** (plant extract concentration)					
Azadirachta indica	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (10% and above)
Vernonia amygdalina	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (10% and above)
Clitoria ternatea	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (10% and above)
Plectranthus amboinicus	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (15% and above)	100.00 ± 0.00[a] (10% and above)
Morinda citrifolia	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (20%)	100.00 ± 0.00[a] (10% and above)
Moringa oleifera	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (10% and above)	85.00 ± 0.49[c] (20%)	100.00 ± 0.00[a] (10% and above)	93.25 ± 0.24[b] (20%)
Passiflora suberosa	100.00 ± 0.00[a] (15% and above)	100.00 ± 0.00[a] (10% and above)	100.00 ± 0.00[a] (15% and above)	100.00 ± 0.00[a] (5% and above)	100.00 ± 0.00[a] (5% and above)	74.00 ± 1.00[d] (20%)

*Values are the means of five replicates. Means with the same letter are not significantly different at $p = 0.05$

except Foc TR4 at 5%, achieving complete inhibition at 10% and above concentrations. This suggests that neem is a potent natural broad-spectrum antifungal agent, demonstrating maximum effectiveness even at relatively low concentrations (5%–10%). A similar trend was observed with *V. amygdalina* (bitter leaf), which displayed strong antifungal activity, particularly at concentrations of 10% or higher, achieving 100% inhibition against all fungal plant pathogens. However, at 5%, the extracts were less effective against *R. microporous*, *P. palmivora* and Foc TR4 (Table 3). *C. ternatea* (butterfly pea) also revealed promising results, with moderate antifungal activity at 5% but achieving 100% inhibition for all fungal plant pathogens at concentrations above 10%. *P. amboinicus* (Mexican mint) showed comparable efficacy, reaching complete inhibition at concentrations above 15%, with strong antifungal activity even at lower concentrations (Table 3). *M. citrifolia* (noni) exhibited good effectiveness at all concentrations, achieving full inhibition at 20%. Meanwhile,

M. oleifera (ben oil tree) revealed consistent antifungal performance, though it did not inhibit completely at 20% against *P. oryzae*. Lastly, *P. suberosa* (corkystem passionflower) demonstrated the mildest antifungal activity among the plants tested but still showed significant inhibition at higher concentrations.

DISCUSSION

In recent years, there has been a notable surge in the use of plant extracts as environmentally friendly treatments for plant diseases. Methanol has been recognised as the best solvent for extracting bioactive substances like phenolics, flavonoids, alkaloids, and terpenoids from plants (Truong et al., 2019). Despite limited research on the efficacy of the plant extracts used in this study against *G. boninense* and *R. microporus*, methanol typically yields higher concentrations of bioactive compounds compared to alternatives like ethanol or distilled water (Andleeb et al., 2020). The strong inhibitory effects observed in the plant extracts against *G. boninense* and *R. microporus* in this study are likely attributed to the efficacy of methanol extraction.

This study found that *A. indica* (neem), *M. oleifera* (ben oil tree) and *V. amygdalina* (bitter leaf) extracts achieved complete suppression of *R. solani* at all concentrations (5%, 10%, 15% and 20%). Ali et al. (2024) highlighted the susceptibility of *R. solani* to the methanol extract of A. indica, with an inhibition zone of 23 ± 00 mm. A. indica is also utilised in foliar spray at 3% neem oil concentration to manage sheath blight caused by *R. solani* (Kumar, 2020). Goss (2018) demonstrated significant suppression of *R. solani* and *F. solani* in lettuce through aqueous Moringa leaf, seed and bark extracts in greenhouse and field studies. Additionally, *V. amygdalina* methanol extract showed mild activity against *R. solani* (Ohigashi et al., 1991). Conversely, extracts from *P. amboinicus* (Mexican mint), *M. citrifolia* (noni), *C. ternatea* (butterfly pea) and *P. suberosa* (corkystem passionflower) inhibited *R. solani* by 100% at higher concentrations (10% onwards). Islam et al. (2023) reported that finotin, a protein from butterfly pea seeds, effectively inhibits various fungal pathogens, including *R. solani*. Meela et al. (2019) highlighted *P. suberosa*'s promising antifungal activity against multiple pathogens, including *R. solani*. The findings of this study align with those of Ali et al. (2024), Goss (2018), Islam et al. (2023), Kumar (2020), Meela et al. (2019) and Ohigashi et al. (1991), demonstrating the efficacy of these plant extracts against *R. solani* at different concentrations.

It was observed that *P. amboinicus* (Mexican mint), *M. citrifolia* (noni), *C. ternatea* (butterfly pea), *A. indica* (neem) and *V. amygdalina* (bitter leaf) extracts achieved 100% inhibition of Foc TR4 at concentrations ranging from 10% to 20%. However, *P. suberosa* (corkstem passionflower) and *M. oleifera* (ben oil tree) extracts did not achieve complete inhibition across all concentrations tested. Comparing these results to those of Malik et

al. (2021), M. oleifera has been reported to give the best results (59.33%), followed by *A. indica* (55.00%) in disease reduction at 40% concentration against *Fusarium oxysporum* f. sp. *capsica*. Meela et al. (2019) reported the promising antifungal activity of P. suberosa against various pathogens, including *F. oxysporum*. The findings reported by Meela et al. (2019) and Malik et al. (2021) contradict the results observed in this study for Foc TR4. These dissimilarities could arise from species diversity and varying virulence levels within *F. oxysporum*, a species complex with numerous formae speciales (f. sp.) and races specialised to infect specific host plants (Rana et al., 2017). Each forma specialis causes distinct diseases in different host plants, highlighting the pathogen's variability among these specialised forms. Overall, the efficacy of the plant extracts against Foc TR4 varied significantly across concentrations, suggesting the potential for increased efficacy at higher concentrations to inhibit Foc TR4 mycelial growth.

Based on the findings of this study, 100% suppression of *P. oryzae* was consistently achieved across all concentrations in the plant extracts of *P. amboinicus* (Mexican mint), *P. suberosa* (corkstem passionflower), *A. indica* (neem) and *V. amygdalina* (bitter leaf). This aligns with the findings by Agbowuro et al. (2020), who similarly reported the antifungal properties of neem extracts against rice blast disease caused by *P. oryzae*. However, *M. citrifolia* (noni) and *C. ternatea* (butterfly pea) extracts achieved complete inhibition at 10% and above concentrations, indicating high efficacy but slightly varied potency compared to the aforementioned extracts. Meanwhile, *M. oleifera* (ben oil tree) extract did not achieve complete inhibition across all concentrations tested but demonstrated inhibition above 75%. *M. oleifera* extracts have been reported by Ilanko et al. (2019) as weak inhibitors of bacterial growth when used alone but show significantly enhanced antibacterial efficacy when combined with conventional antibiotics.

Plant extracts of *C. ternatea* (butterfly pea), *P. suberosa* (corkstem passionflower) and *A. indica* (neem) achieved 100% suppression on *P. palmivora* across all concentrations tested. *M. oleifera* (ben oil tree) and *V. amygdalina* (bitter leaf) extracts showed full inhibition at 10%–20%, revealing their efficacy albeit at slightly higher concentrations. Yousaf et al. (2018) corroborated these findings, demonstrating that methanol leaf extracts of *A. indica* and *M. oleifera* effectively controlled the mycelial growth of *P. palmivora* at concentrations of 5%, 10% and 15%. Additionally, Chainanta et al. (2023) highlighted the potential of *V. amygdalina* ethyl acetate to suppress the growth of *P. palmivora*. In contrast, achieving complete inhibition of *P. palmivora* with *P. amboinicus* (Mexican mint) (15-20%) and *M. citrifolia* (noni) (20%) requires higher concentrations of methanol extracts. Yusoff et al. (2020) found that the concentration of the crude extract had the most significant impact on reducing mycelial growth and influencing the percentage of fungal inhibition in the modified PDA medium. This effect is likely related to the concentration of antifungal compounds in the crude extract.

CONCLUSION

The screening of seven plant extracts for fungitoxic potential showed promising results, with *A. indica* (neem), *V. amygdalina* (bitter leaf) and *C. ternatea* (butterfly pea) achieving complete inhibition against all tested fungal plant pathogens at concentrations of 10% and above (15% and 20%). Some pathogens required higher concentrations for effective suppression, particularly *Foc TR4*. Notably, *A. indica* demonstrated broad-spectrum efficacy, fully inhibiting five out of six pathogens at concentrations as low as 100 mg/ml, highlighting its potential to be developed as a versatile bio-fungicide. These findings suggest that these plant extracts could be eco-friendly alternatives to synthetic fungicides. Given the availability of these plant species in Malaysia, further research to isolate bioactive compounds and conduct field trials are recommended to assess their practical application as sustainable bio-fungicides to reduce reliance on agrochemicals.

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