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Evaluating the Antifungal Effectiveness of Three Plant Extracts in Controlling Panama Wilt in Banana Plants

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Authors' contributions

This work was carried out in collaboration among all authors. All authors contributed to the experimental designing, conducting, data collection of experiment, data analysis and manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to evaluate the antifungal potential of *Mikania micrantha*, *Senna alata*, and *Datura metel* extracts against *Fusarium oxysporum*, the causal agent of Panama wilt in banana and to determine the optimal concentration of the most effective extract for fungal suppression.
Study Design: Laboratory-based experimental research using in-vitro assays to assess the antifungal activity of selected plant extracts.
Place and Duration of Study: The study was conducted at the Laboratory of the Institute for Agro-technology and Rural Sciences, University of Colombo, Sri Lanka, from February to July 2024.
Methodology: The fungal pathogen was isolated from infected banana plant roots and cultured on Potato Dextrose Agar (PDA). Aqueous extracts of the plants were prepared and incorporated into

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PDA media. In Experiment 1, fungal colonies were exposed to five treatments: control (no application), *M. micrantha* extract, *D. metel* extract, *S. alata* extract, and a commercial fungicide. In Experiment 2, different concentrations (25%, 50%, 75%, and 100%) of the most effective extract from Experiment 1 were tested against *Fusarium oxysporum*. Fungal growth was monitored for seven days, and colony diameter measurements were statistically analyzed using SAS software, with Duncan's Multiple Range Test at a 5% significance level.

Results: Significant differences ($p < 0.05$) in fungal growth inhibition were observed among treatments. *D. metel* extract demonstrated the highest antifungal activity with a *F. oxysporum* growth inhibition of 68.4%, second only to the commercial fungicide (82.6%). The inhibitory effect was dose-dependent, with 100% *D. metel* extract achieving the highest suppression of *F. oxysporum* growth (77.9%), closely approaching the efficacy of the commercial fungicide.

Conclusion: *D. metel* extract demonstrated strong antifungal potential against *F. oxysporum*, making it a promising alternative to chemical fungicides for managing Panama disease in bananas. Further research is needed to explore its field efficacy, mode of action, and possible integration with other biocontrol strategies for enhanced disease management.

Keywords: Antifungal effects; banana wilt; Datura metel extract; Fusarium oxysporum; Mikania micrantha extract; Senna alata extract.

1. INTRODUCTION

Banana (*Musa* spp.) is one of the most widely consumed fruits globally and remains a dominant commodity in the international fruit market, with production spanning over 135 countries (FAO, 2024). In recent years, both the cultivated area and production volume have increased, driven by the fruit's growing popularity and economic significance. In addition to being a major cash crop, bananas are extensively cultivated in tropical and subtropical regions for local consumption (Khan and Nasreen, 2010). However, banana cultivation is highly vulnerable to fungal diseases, including Mycosphaerella leaf spots, Black and Yellow Sigatoka, Eumusae leaf spot, Mycosphaerella speckle, and Panama disease (Molina et al., 2008). These fungal infections can cause severe yield losses, reaching up to 90% in some cases (Khan and Nasreen, 2010). Among them, Panama disease is considered the most devastating, as it can lead to complete crop failure in severely affected plantations (Fernando et al., 2013; Ploetz, 2015).

Panama disease, also known as Fusarium wilt of banana, was the first banana disease to spread globally (Ploetz and Churchill, 2011). This lethal fungal disease is caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc). The global banana industry initially faced a major threat from *F. oxysporum* f. sp. *cubense* Race 1 (FocR1) (Ploetz, 2015). While this challenge was mitigated through the introduction of resistant Cavendish banana varieties, a more virulent strain, Tropical Race 4 (FocTR4) also referred to as *Fusarium odoratissimum* (Dale et al., 2017; Fones et al., 2020) has since emerged. Spreading rapidly across multiple continents, FocTR4 poses a significant threat to the global Cavendish banana industry, jeopardizing commercial production on a large scale (Fones et al., 2020; Ploetz, 2015).

Once established in a field, *Fusarium oxysporum* f. sp. *cubense* (Foc) can persist in the soil indefinitely, surviving for up to 30 years as chlamydospores within infected plant material or the roots of alternative host plants (Gnanasekara et al., 2015). The fungus enters banana plants through the roots and colonizes the vascular tissues, specifically the xylem vessels, where it obstructs the transport of water and nutrients. This disruption triggers external symptoms, including progressive yellowing and wilting of leaves, which initially appears along the outer margins before spreading inward. The disease progresses from older leaves to younger ones, eventually causing the affected foliage to collapse at the petiole. Additionally, the outer leaf sheaths of the pseudostem develop characteristic longitudinal splits (Yin et al., 2011; Guo et al., 2013).

Internally, infected plants exhibit a distinct discoloration of vascular tissues, transitioning from light yellow to dark brown. This discoloration first appears in the outer or older leaf sheaths and gradually extends into the pseudostem (Minerdi et al., 2008; Yin et al., 2011). As the infection advances, severe vascular blockage leads to plant death, resulting in significant economic losses in banana cultivation (Guo et al., 2013).

Different strategies to control Foc are currently being used including using biological control agents (Taping et al., 2023; Jin et al., 2024; Shukla et al., 2024), removing infected tissues and maintain crop sanitation (Pegg et al., 2019), developing resistant varieties (Dale et al., 2017; Naim et al., 2018), and introducing biosecurity protocols to reduce the spread of pathogen (Bubici et al., 2019). However, none of these methods have been identified as particularly effective, rather than controlling the pathogenic fungi with fungicides (Steinberg et al., 2020; Cannon et al., 2022).

As there are increasing concerns over the negative effects of using fungicides to human health and to the environment, they are not recommended for the control of the disease (Akila et al., 2011). Consequently, researchers are increasingly turning to alternative, eco-friendly management strategies that can mitigate the disease's impact while promoting sustainable agricultural practices. Plant-based approaches have emerged as a promising alternative, with botanical extracts showing considerable potential in managing Fusarium wilt. These natural solutions leverage secondary metabolites such as phenols, alkaloids, and terpenoids, which serve as chemical defense agents against plant pathogens (Tripathi and Singh, 2015). Botanical fungicides offer multiple advantages, including the ability to either directly act on pathogens or induce systemic resistance in host plants, thereby reducing disease development (Abdullahi et al., 2018).

Under the given circumstances, current research aims to explore innovative, sustainable approaches to combating Panama disease, focusing on the potential of plant extracts as an eco-friendly management strategy. By investigating the antifungal properties of various botanical substances, this study seeks to contribute to the development of more sustainable and environmentally responsible methods for protecting banana crops against this devastating fungal threat.

2. MATERIALS AND METHODOLOGY

2.1 Isolation and Morphological Identification of Fungal Pathogen

The causal organism was isolated from infected banana plant roots showing disease symptoms. The roots were washed, sectioned, and surface sterilized using 30% alcohol. Then the root fragments were kept on the culture medium Potato-Dextrose-Agar (PDA) aseptically to grow the pathogen at 27 ± 2 ^{0}C. The pure fungal cultures were then maintained on PDA plates for further investigations (Fig. 1). The pathogen was identified by microscopic examination based on the morphological characteristics (Gnanasekara et al., 2015).

2.2 Preparation of Plant Extracts

Three medicinal plants were selected based on their documented medicinal properties (Table 1). Healthy and fresh plant materials were collected, washed under running tap water and dried under shade. Then 50 g of from each of the sample was ground into a paste using a mortar and pestle, with 200 ml of distilled water serving as the extraction solvent. The resulting mixture was then filtered through a whatman no. 1 filter paper to separate the liquid extract from the plant residue and stored aseptically in sealed containers.

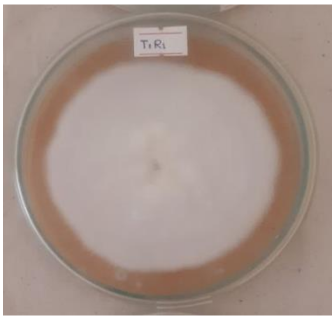


Fig. 1. Isolated *Fusarium oxysporum* colony on PDA media

2.3 Preparation of Plant Extract Culture Media

PDA media supplemented with 100 ml of the plant extract was prepared separately and poured into petri dishes under sterile conditions. After solidification the plant extract culture media was inoculated with isolated *Fusarium oxysporum* pathogen incubated at 27 ± 2 ^{0}C for 24 hours.

2.4 Experiment 1: Evaluation the Efficiency of Antifungal Activity of Different Plant Extracts against *F. oxysporum*

The experiment was conducted with five treatments and three replications, where each replicate contained three experimental units. The

Table 1. List of plants used in the study

Botanical name	Family	Part used	Reference for antifungal properties
Mikania micrantha	Solanaceae	Leaves	Devkota and Sahu, 2020
Senna alata	Fabaceae	Leaves	Ezemba, 2021; Saptarini et al., 2024
Datura metel	Solanaceae	Leaves	Rinez et al., 2013; Shah et al., 2022

treatments were as follows; T1: Control (No applications), T2: *Mikania micrantha* extract, T3: *Datura metal* extract, T4: *Senna alata* extract and T5: Commercial fungicide.

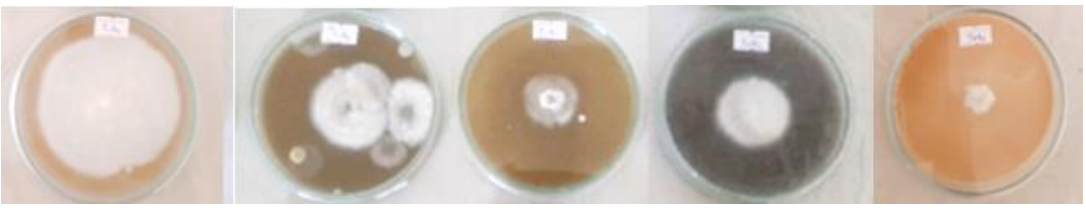
2.5 Experiment 2: Determination of Optimal Extract Concentration

Experiment 2 aimed to investigate the effect of concentration of the best plant extract identified in experiment 1 on controlling the growth of *F. oxysporum*. The experiment contained five treatments with three replicates per treatment, each containing three experimental units. Treatments were as follows; T1: Control (No applications), T2: 25% of plant extract, T3: 50% of plant extract, T4: 75% of plant extract, T5: 100% of plant extract.

2.6 Culture Maintenance in Experiments and Calculation of Inhibition Percentage of the Fungal Growth

In both experiments, petri dishes were stored at room temperature following the initial 24-hour period in the incubator. The identity of *Fusarium oxysporum* was confirmed, and the growth of the fungus was observed daily. Data on fungal growth were observed (Figs. 2 and 3) and colony diameter measured for 7 consecutive days.

Percentage inhibition of pathogen under in-vitro condition was calculated by the following formula described by Wonglom et al., (2019) in each experiment for seven days.



T_1 T_2 T_3 T_4 T_5

Fig. 2. Visual observations for fungal growth in experiment 1 at day 7
(T1: Control (No applications), T2: Mikania micrantha extract, T3: Datura metal extract, T4: Senna alata extract and T5: Commercial fungicide)

T1 T2 T3 T4 T5

Fig. 3. Visual observations for fungal growth in experiment 2 at day 7
(T1: Control (No applications), T2: 25% of D. metal plant extract, T3: 50% of D. metal plant extract, T4: 75% of D. metal plant extract, T5: 100% of D. metal plant extract)

$$\text{Inihibtion } \% = \frac{\text{Avarage diameter of control colony} - \text{Avarage diameter of treatment colony}}{\text{Avarage diameter of control colony}} \text{ x } 100$$

2.7 Data Collection and Analysis

For both experiments, colony diameter was measured and the inhibition of fungal mycelium growth was calculated. All the data were subjected to statistical analysis using SAS statistical software. Mean separation was done using DMRT at 5% significance level.

3. RESULTS AND DISCUSSION

Banana is one of the most important food and fruit crops worldwide, but its production is increasingly threatened by Fusarium wilt. Traditional control methods, such as chemical treatments against *Fusarium oxysporum* f. sp. cubense (Foc), have been employed; however, they have proven inadequate in effectively managing the disease while also posing significant environmental hazards (Akila et al., 2011). Consequently, there is a growing need for environmentally friendly approaches to control fusarium wilt in banana cultivation (Durgeshlal et al., 2019). In recent years, the search for natural antifungal agents derived from plants has gained considerable attention as a sustainable alternative to chemical pesticides. Efforts are being made to identify bioactive compounds in plants that can serve as effective antifungal agents with minimal environmental impact. The mechanism of fungal growth inhibition can be attributed to the sophisticated biochemical interactions of plant-derived compounds. Phytochemicals extracted from plant sources offer a promising solution by providing safer, non-toxic, and more efficient alternatives for managing fungal pathogens (Akila et al., 2011; Gnanasekara et al., 2015).

Previous research has reported different plant oil extracts like *Metasequoia glyptostroboides* (Bajpai et al., 2007). *Cymbopogon citratus* (Guimarães et al., 2011), *Syzygium aromaticum* and *Cinnamomum verum* (Monteiro et al., 2013) can use to suppress the *F. oxysporum*. Importantly, Oliveira et al. (2008) found that *Lippia sidoides* oil provided fungal control comparable to synthetic fungicide, carbendazim. Further studies have confirmed the effectiveness of various aqueous plant extracts against Fusarium species. *Acacia nilotica* (Kubara et al., 2018), *Azardiachta indica, Eucalyptus globulus, Artemisia annua, Ocimum sanctum* (Mengane and Kamble, 2014), *Azadirachta indica* (Gnanasekara et al., 2015) *Syzygium aromaticum*, and *Cinnamomum verum* (Monteiro et al., 2013; Saththivel and Vinujan, 2024). Addition to the *In-vitro* experiments, In-vivo experiments also confirm the possibility of using plant extracts to suppress fusarium wilt. In one comprehensive study, Huang et al. (2012), who examined the effectiveness of *Allium tuberosum* in managing Fusarium wilt, observing disease incidence reductions ranging from 58% to 79% across different banana varieties under controlled greenhouse conditions. These findings highlight the potential of plant-based biocontrol agents as sustainable alternatives to synthetic fungicides, offering an environmentally friendly approach to managing fungal diseases in agriculture.

In present work, antifungal efficacy of aqueous plant extracts from *Mikania micrantha*, *Senna alata*, and *Datura metel* was assessed against *Fusarium oxysporum*, the causal agent of Fusarium wilt in banana. The results revealed significant differences (P<0.05) in fungal colony measurement (Fig. 4) and in percentage inhibition of the fungal mycelium growth under in-vitro conditions (Table 2) across the different treatments over a seven-day observation period.

Among the tested plant extracts, *M. micrantha* (T2) and *S. alata* (T4) exhibited relatively weak antifungal activity, as evidenced by limited inhibition of mycelial growth. In contrast, *D. metel* (T3) demonstrated a substantial inhibitory effect (68.4^{b} at the seventh day of observations), significantly suppressing fungal colony expansion. The pronounced morphological abnormalities and structural distortions observed in *F. oxysporum* treated with *D. metel* extract suggest that its antifungal activity may be linked to multiple mechanisms, including oxidative stress induction, fungal cell membrane degradation, and disruption of essential metabolic processes (Rinez et al., 2013; Shah et al., 2022).

The effectiveness of *D. metel* extract against *F. oxysporum* aligns with previous studies highlighting the antifungal potential of plant-based bioactive compounds. Akharaiyi (2011) identified a diverse range of phytochemicals in *D. metel*, including saponins, flavonoids, tannins, glycosides, phenols, alkaloids, steroids, and terpenoids, many of which have been recognized

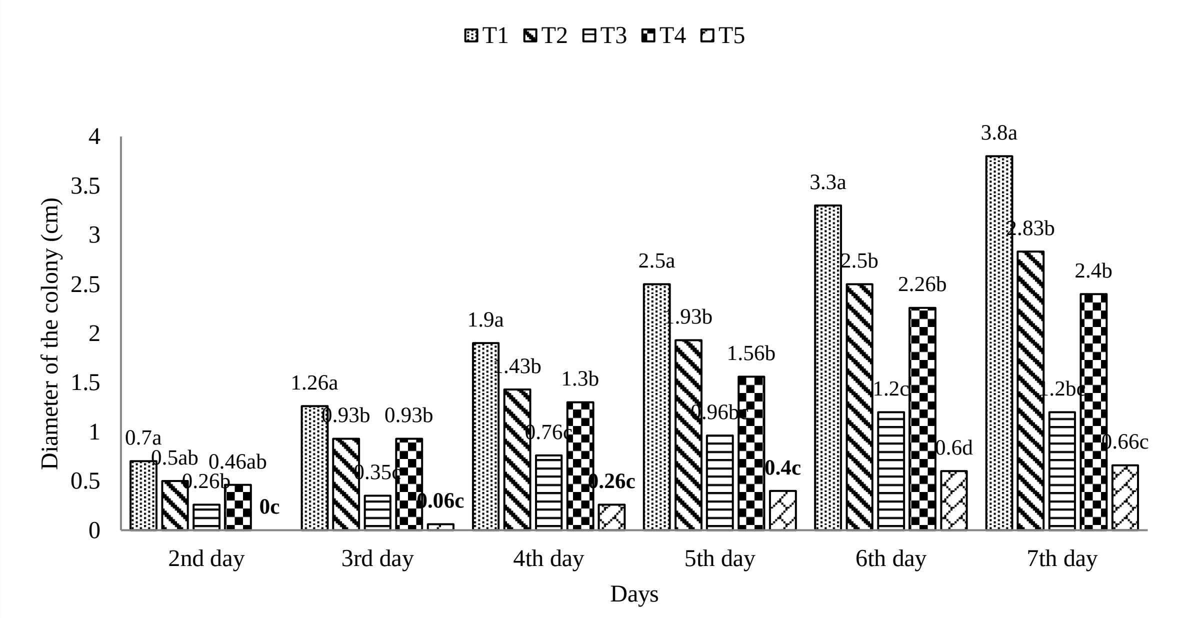


Fig. 4. Diameter of the *Fusarium oxysporum* colony in experiment 1

Means followed by same letter are not significantly different (P<0.05)

Table 2. Effects of different plant extracts on inhibition percentage of fungal mycelium growth in experiment 1

Treatments	2nd day	3rd day	4th day	5th day	6th day	7th day
T1 – Control (no applications)	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
T2 - *Mikania micrantha*	28.6^{c}	26.2^{c}	24.7^{c}	22.8^{c}	24.2^{c}	25.5^{c}
T3 - *Datura metal*	62.9^{b}	72.2^{b}	60.0^{b}	61.6^{b}	63.6^{b}	68.4^{b}
T4 - *Senna alata*	34.3^{c}	26.2^{c}	31.6^{c}	37.6^{c}	31.5^{c}	36.8^{c}
T5 - Commercial fungicide	100.0^{a}	95.2^{a}	86.3^{a}	84.0^{a}	81.8^{a}	82.6^{a}

Means followed by the same alphabets in a same column are not significantly difference according to Duncan's test at 0.05 level

for their antifungal properties (Sparg et al., 2004). Previous research supports these findings. Mohana Pradeep, 2020 investigated the environmentally friendly management of *Fusarium oxysporum* f. sp. using various plant extracts, including *D. metel*, custard apple, aloe, castor, and *Vitex negundo* on chili plants, and confirmed the efficacy of *D. metel* extract in inhibiting the growth of the *F. oxysporum* pathogen under in vitro conditions. These results further support the potential application of *D. metel* as a natural antifungal agent.

These findings indicate that *D. metel* possesses strong antifungal properties, ranking second only to the commercial fungicide. The results highlight the importance of plant extracts to be use as sustainable alternatives for commercial fungicides available. This is in line with the reports of Fernando et al. 2013 who stated that, the extracts of *Cinnamomum zeylanicum* and *Syzygium aromaticum* achieved best values in controlling Fusarium wilt disease of banana, obtaining values equal to the fungicide treatment used. Similarly, Abdullahi et al., (2018) found that *Acacia nilotica* and *Lawsonia inermis* leaves extracts compete favorably with the mancozeb fungicide.

Based on the results of the first experiment, *D. metel* extract was selected for further investigation to assess its antifungal efficacy at varying concentrations. The second experiment revealed a concentration-dependent suppression of fungal colony growth. As the concentration of *D. metel* extract increased, a progressive reduction in *F. oxysporum* colony diameter was observed. The 100% concentration exhibited the most significant inhibition, effectively limiting fungal growth (Fig. 5).

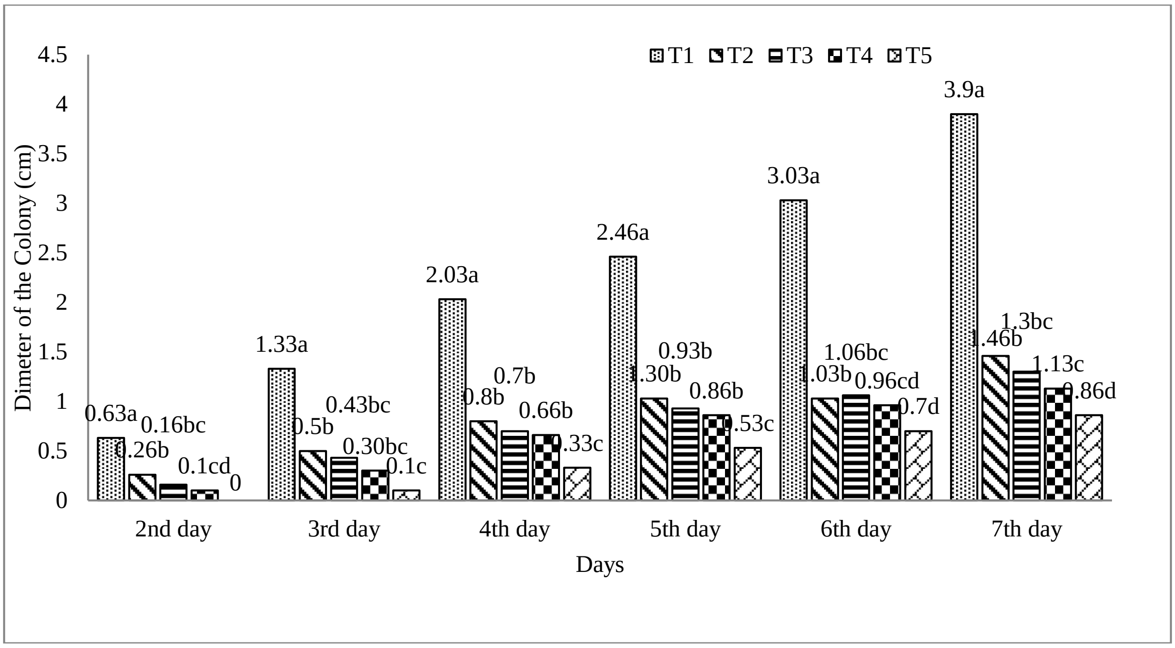


Fig. 5. Diameter of the colony in experiment 02

Means followed by same letter are not significantly different (P<0.05)

These findings suggest that *D. metel* contains potent antifungal compounds that exert a dose-dependent effect on fungal development. The increased inhibition at higher concentrations highlights the potential of this plant extract as a natural antifungal agent with practical applications in plant disease management. The ability of *D. metel* to suppress fungal growth at high concentrations further supports its suitability as an alternative to chemical fungicides, offering a more sustainable and environmentally friendly approach to controlling Fusarium wilt in banana cultivation.

4. CONCLUSION

The study revealed a significant antifungal potential of *Datura metal* extract against *Fusarium oxysporum*. Its concentration-dependent inhibition, significant morphological impact and performance comparable to commercial fungicides indicated it as an alternative, environmentally friendly fungal management solution. Future investigations should explore its mode of action, field efficacy, and potential synergistic effects with other biocontrol agents to enhance its effectiveness in agricultural applications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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