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***Full Length Research***

# Efficacy of Select Botanical Extracts on the Control of the Fungal Pathogen *Pythium aphanidermatum* in Cucumber Cultivation

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**ABSTRACT**

*Pythium aphanidermatum*, a highly aggressive soil-borne oomycete, is a major pathogen causing root and stem rot, leading to substantial crop losses in warm and humid environments. Given the growing concerns regarding the environmental hazards and resistance associated with synthetic fungicides, the exploration of botanical extracts as sustainable, eco-friendly alternatives is gaining traction. In this study, methanolic extracts from five plants—Ginger (rhizome), Bitter kola (seeds), Neem (leaves), Aloe vera (leaves), and Moringa (leaves)—were evaluated for their antifungal efficacy against *P. aphanidermatum*. Pathogen isolation was conducted using serial dilution and direct methods, with culture media including Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Czapek Dox Agar (CDA). Pathogenicity was confirmed via Koch's postulates, establishing *P. aphanidermatum* as the causal agent of root and stem rot. Growth assessments revealed that PDA supported the highest pathogen growth rate (2.85 cm/day), followed by CDA (2.38 cm/day) and MEA (1.78 cm/day). In vitro assays demonstrated that botanical extracts, tested at concentrations ranging from 40,000 to 80,000 ppm, significantly inhibited *P. aphanidermatum* growth, with Bitter kola exhibiting the highest inhibition (85.9%), followed by Ginger (83.6%), Neem (80.7%), Moringa (73.1%), and Aloe vera (67.6%). Synthetic fungicides, Benlate and Mancozeb, showed higher inhibition at 150 ppm (89.7% and 88.5%, respectively), but the botanical extracts provide a viable, lower-risk alternative. Phytochemical analysis of the extracts revealed the presence of alkaloids (2.09-5.45%), saponins (0.40-2.92%), flavonoids (0.83-4.70%), and tannins (0.28-5.09%), except for tannins in Moringa oleifera, while proximate analysis indicated variations in ash (1.91%–3.39%), protein (0.53%–3.19%), fibre (2.84%–9.60%), moisture (6.40%–27.0%), and carbohydrate content (75.21%–80.60%). These findings suggest that botanical extracts, with their rich phytochemical profiles, offer significant potential as sustainable biocontrol agents for *P. aphanidermatum*, contributing to safer and more environmentally friendly crop protection strategies.

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## INTRODUCTION

The widespread use of synthetic fungicides has historically played a crucial role in managing plant fungal infections due to their broad-spectrum efficacy, cost-effectiveness, and reliability. However, growing concerns about theirenvironmental toxicity, development of resistant pathogen strains, and health risks, particularly in regions with limited regulatory oversight, have sparked a shift towards more sustainable pest management approaches (Ahmad et al., 2024; Matthews, 2020; Tudi et al., 2021). Integrated pest management (IPM) strategies, which seek to minimize chemical interventions, have increasingly been adopted to address these challenges (Banwo and Adamu, 2003; Shrestha et al., 2024).

In Nigeria, cucumber (Cucumis sativus) production is severely hampered by fungal pathogens, with Pythium aphanidermatum identified as a particularly aggressive species causing root and stem rot under warm, wet conditions. This pathogen, along with Fusarium oxysporum, contributes to significant yield losses, often exceeding 50% in affected regions (Muhammad et al., 2019; Shutt et al., 2021). Conventional fungicides, including azoxystrobin and fosetyl-aluminum, are effective against Pythium diseases but pose challenges related to environmental safety, pathogen resistance, and cost (Matić et al., 2019; Nwankiti et al., 1990). These factors underscore the need for environmentally friendly, accessible, and cost-effective alternatives. Botanical extracts offer promising potential as biocontrol agents due to their rich phytochemical profiles, demonstrated efficacy against various plant pathogens, and minimal environmental impact (Doherty and Roberts, 2022; Chaudhary et al., 2023). Previous studies have shown that plant-derived compounds such as those from neem (Azadirachta indica), ginger (Zingiber officinale), and bitter kola (Garcinia kola) exhibit significant antifungal activity, providing a basis for their use in pest management strategies (Stoll, 1992; Singh et al., 2010; Tegegne et al., 2008). Despite these promising findings, there remains limited localized research on the application of these botanicals in controlling Pythium aphanidermatum in sub-Saharan Africa.

This study aims to evaluate the antifungal efficacy of methanolic extracts from ginger rhizomes, bitter kola seeds, and leaves of aloe vera, neem, and moringa against P. aphanidermatum. It further seeks to compare their effectiveness with conventional fungicides (Benlate and Mancozeb) to highlight their potential role in eco-friendly crop protection. By addressing the need for sustainable, effective solutions, this research contributes to the growing body of knowledge on integrated disease management strategies tailored to local agricultural challenges.

The objectives of this study were to evaluate the antifungal efficacy of methanolic extracts from ginger, aloe vera, bitter kola, neem, and moringa against Pythium aphanidermatum under both in vitro and in vivo conditions. The study also aimed to compare the effectiveness of these botanical extracts with conventional fungicides—specifically Benlate and Mancozeb—by assessing their performance in laboratory-based inhibition tests and greenhouse trials. Additionally, the phytochemical composition of the selected botanical extracts was analyzed to determine the potential roles of individual compounds in fungal inhibition.

The research further investigated how temperature and relative humidity influence the growth and development of P. aphanidermatum in controlled environments. Ultimately, the study sought to explore the viability of using botanical extracts as partial alternatives to synthetic fungicides within an integrated disease management strategy for cucumber cultivation.

## MATERIALS AND METHODS

### Plant Material Collection and Preparation

Fresh leaves of Azadirachta indica (Neem), Aloe vera (Aloe), and Moringa oleifera (Moringa) were collected from Ekpoma environ, while fresh seeds of Garcinia kola (bitter kola seeds) and Zingiber officinale (ginger rhizomes) were purchased from a local market in Ekpoma, Edo State, Nigeria. They were authenticated at the Herbarium of Plant Science and Biotechnology Department, Faculty of Life Sciences, Ambrose Alli University, Ekpoma. All plant samples were washed with distilled water and separately oven-dried at 40°C for 7 days until a constant weight was achieved.

### Extraction Method

The dried leaves (neem, moringa, aloe) were ground into fine powder. For these samples, 500 g of each was soaked in 500 ml of methanol for 24 hours, filtered through four-fold cheesecloth, and concentrated. For the ginger rhizomes and bitter kola seeds (both dense plant tissues), 250 g of each were sliced thinly, air-dried, and then soaked in 500 ml of methanol for 48 hours. These were filtered and concentrated similarly. The resulting extracts were diluted to obtain concentrations of 40%, 60%, and 80% (i.e., 40,000 ppm, 60,000 ppm, and 80,000 ppm).

### Phytochemical Analysis

### Qualitative and Quantitative Phytochemical Determination

Phytochemical analysis of the plant materials was conducted to determine the levels of alkaloids, flavonoids, saponins, and tannins, using methods adapted from Krishnalah et al. (2009), and Edeoga et al. (2005). For alkaloid determination, 5 g of the plant powder was soaked in 200 ml of 10% ethanoic acid in ethanol for 4 hours. The mixture was filtered and concentrated to one-quarter of its original volume using a water bath. Concentrated ammonium hydroxide was then added dropwise to precipitate the alkaloids, which were collected on a pre-weighed filter paper, dried, and weighed. The percentage alkaloid content was calculated by difference. Saponin content was determined by extracting 5 g of the sample in 100 ml of 20% ethanol at 55°C for 4 hours. After filtration and re-extraction, the combined extracts were concentrated to 40 ml, partitioned with diethyl ether, and extracted with n-butanol. The n-butanol layer was washed with sodium chloride solution, evaporated, and the residue was weighed to determine saponin content. For flavonoids, 10 g of powdered sample was repeatedly extracted with 80% methanol.

The resulting filtrate was evaporated to dryness, and the residue was weighed to estimate the flavonoid content. Tannin determination involved boiling 1 g of the plant sample in 50 ml of distilled water for 30 minutes. The mixture was filtered, and appropriate reagents were added for quantification.

### Proximate Analysis

Proximate analysis of moisture, crude protein, ash, fibre, and carbohydrates was

carried out using the standard methods of the Association of Official Analytical Chemists (A.O.A.C., 1990). Moisture content was determined by drying 2 grams of each fresh sample to a constant weight in a crucible at 105°C. The dry matter obtained was used to determine the other parameters. Crude protein was determined using the Kjeldahl method, while crude fibre was determined by digesting 2 grams of the sample in 1.25% sulfuric acid and sodium hydroxide. The ash content was measured by incinerating 2 grams of the sample in a muffle furnace at 550°C for 5 hours. Nitrogen-free extracts (NFE), representing soluble carbohydrates, were determined mathematically.

**Media Preparation and Fungal Isolation**

**Isolation and Identification of Pythium aphanidermatum**

Fungal pathogen was isolated from diseased cucumber seedlings on Potato Dextrose Agar (PDA), and pure cultures were identified morphologically and microscopically.

**Evaluation of Media on Fungal Growth**

Three agar media—PDA, Malt Extract Agar (MEA), and Czapek-Dox Agar (CDA)—were tested for supporting fungal growth. A 5 mm plug from a 7-day-old culture was inoculated into each medium. Radial growth was measured daily for 5 days at 27 ± 2°C, and average growth rate was calculated (Jiménez-Pérez et al., 2022).

**Evaluation of Different Agar Media on Fungal Growth**

The linear growth of Pythium aphanidermatum was evaluated on three different agar media: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Czapek-Dox Agar (CDA). A 5 mm disc from a 7-day-old fungal culture was inoculated onto each medium and incubated at 27 ± 2°C for five days. Radial growth was measured daily, subtracting the initial inoculum radius (1 cm) from the total growth. The average daily growth rate was calculated by dividing the radial growth by the number of incubation days (Jiménez-Pérez et al., 2022). Each medium was replicated three times, and the mean growth rate was reported.

**Evaluation of Temperature and Relative Humidity on Fungal Growth**

The impact of temperature and relative humidity on the growth of Pythium aphanidermatum was studied. Five temperature levels (20 ± 2°C, 25 ± 2°C, 30 ± 2°C, 35 ± 2°C, and 40 ± 2°C) were used for incubation in a Gallenkamp incubator. The same 5 mm mycelial blocks from 7-day-old cultures were inoculated and observed for five days. Growth values were recorded, and the average growth per replicate was calculated. Relative humidity levels were achieved using different salt and sugar solutions in desiccators, with the corresponding humidities being: P2O5 (0%), MgCl2.6H2O (32.5%), glucose (55%), NaCl (75%), and KCl (85%). Distilled water provided 100% humidity.

**Evaluation of Plant Extracts in Inhibiting Pythium Growth**

The plant extracts used were derived from Azadirachta indica (Neem leaves), Garcinia kola (bitter kola seeds), Zingiber officinale (ginger rhizomes), Aloe vera (Aloe leaves), and Moringa oleifera (Moringa leaves). The extracts were prepared following the methods of Ali et al. (2025), Epidi et al. (2005), Ojo and Olufolaji (2005), and Amadioha (2003). Each plant sample was washed, oven-dried for seven days, and homogenized. Five hundred grams of each were extracted in 500 ml of methanol and

filtered after 24 hours using a four-fold cheesecloth. The extracts were prepared in concentrations of 40%, 60%, and 80% (equivalent to 40,000 ppm, 60,000 ppm, and 80,000 ppm).

**In Vitro Assay of Plant Extracts**

The efficacy of plant extracts on fungal inhibition was evaluated by determining the effects of extract concentrations on radial fungal growth (Iwuagwu et al., 2018; Eksteen et al., 2001; Amadioha, 2003). PDA media were amended with 1 ml of the plant extracts before solidification. The control plates were prepared by adding 1 ml of sterile distilled water to the PDA. A 5 mm disc from a 7-day-old culture of Pythium was placed in the centre of each plate. All treatments were replicated three times and incubated at 27 ± 2°C for seven days. Growth measurements were recorded along four radii, and percentage inhibition of mycelial growth was calculated using the formula by Allagui and Amara (2024):

$$\% \text{ inhibition} = \frac{dc - dt}{dc} \times \frac{100}{1}$$

Where:

dc = average diameter of fungal colony in control plates

dt = average diameter of fungal colony in treated plates.

**In Vivo Assay of Plant Extracts**

Cucumber seeds were sown in pots containing sterilized field soil at a rate of three seeds per pot. Five days post-germination, the plants were inoculated with a sporangial suspension (3 x 104 sporangia/ml) and treated with plant extracts. Control plants were inoculated with the sporangial suspension and watered with distilled water. Three replicate pots per treatment were arranged in a completely randomized block design. Disease incidence was calculated as:

$$\% \text{ incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times \frac{100}{1}$$

**Effects of Fungicides on Mycelial Growth**

The in vitro effects of fungicides (Benlate and Mancozeb) on Pythium were assessed by adding fungicide solutions at concentrations of 50, 100, and 150 ppm to PDA plates. A 5 mm plug of actively growing fungal culture was placed in the centre of each fungicide-amended plate, and cultures were incubated at 27 ± 2°C. Growth was measured daily, and percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{Co - Ct}{Co} \times \frac{100}{1}$$

Where:

Co = diameter of fungal growth in control plates

Ct = diameter of fungal growth in treated plates.

**STATISTICAL ANALYSIS**

All data were subjected to statistical analysis using ANOVA at a 5% significance level. Means were separated using Honestly Significant Difference (HSD), and standard error was used for descriptive statistics. SPSS version 15.0 was used for the analysis.

**RESULTS**

**Growth Studies of Pythium aphanidermatum on Different Media**

The mycelial growth of Pythium aphanidermatum varied significantly across the media tested. The fastest and most robust growth was observed on Potato Dextrose Agar (PDA), with an average radial growth

rate of 2.85 cm/day, completely covering the medium by the seventh-day post-inoculation. Czapek-Dox agar supported the second-highest growth rate at 2.38 cm/day. In contrast, Malt Extract Agar exhibited the slowest growth, with an average mycelial expansion of 1.78 cm/day (Figure 1).

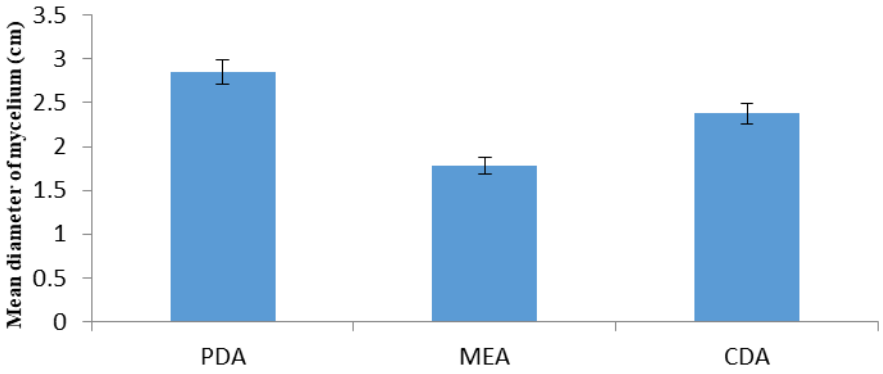



**Figure 1: Effects of three media on the mycelial growth of *Pythium aphanidermatum*. Results are means of three replicates taken with standard errors.**

**Effect of Temperature and Relative Humidity on Mycelial Growth of *Pythium aphanidermatum***

Temperature played a crucial role in the growth of *Pythium aphanidermatum*. The optimal temperature for radial mycelial growth was 30°C, where the growth rate peaked at 2.95 cm/day. A temperature of 25°C resulted in a similar growth rate of 2.49 cm/day, showing no significant difference from 30°C at the 5% probability level. Growth diminished substantially at 20°C and 40°C, with rates of 0.10 cm/day and 0.24 cm/day, respectively (Figure 2). Relative humidity also impacted growth, with higher levels promoting faster expansion. At 27°C and 100% relative humidity, the highest growth rate of 7.50 cm/day was observed. Lower relative humidity levels of 55.7% and 32.5% corresponded with reduced growth rates of 3.0 cm/day and 1.23 cm/day, respectively (Figure 3).

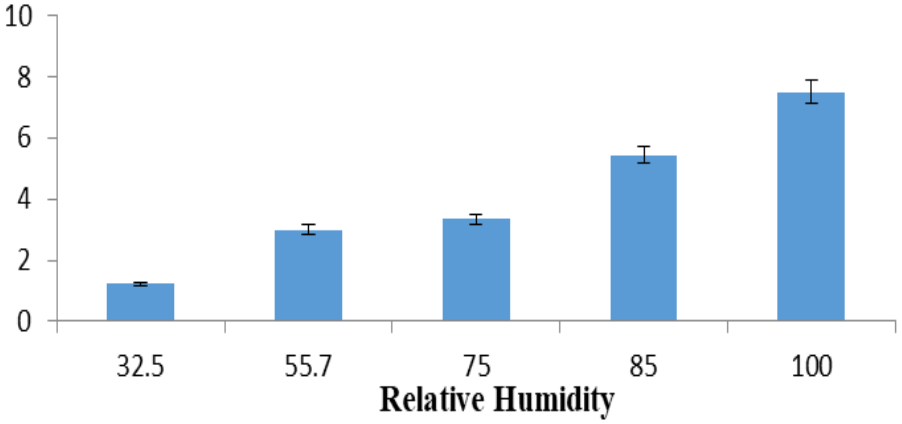



**Figure 2: Effect of temperature on mean mycelial growth of *Pythium aphanidermatum* Results are means of three replicates taken with standard errors.**

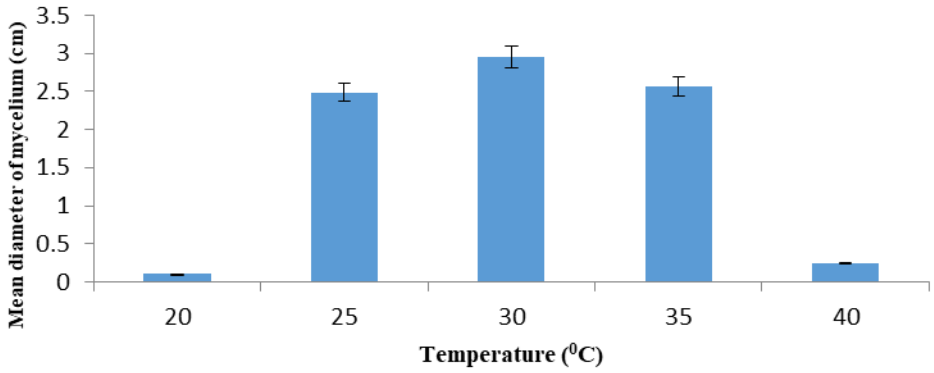



**Figure 3: Effect of Relative humidity on mean mycelial growth of *Pythium aphanidermatum***

*Results are means of three replicates taken with standard errors.

**Inhibitory Effects of Rhizome Ginger Extract on Mycelial growth on PDA**

Ginger rhizome extract demonstrated significant inhibitory effects on P. aphanidermatum mycelial growth at all tested concentrations. The highest inhibition (83.6%) occurred at 80,000 ppm, followed by 74% at 60,000 ppm and 58.3% at 40,000 ppm. The inhibitory effect was positively correlated with increasing concentrations of the extract (Figure 4).

**Inhibitory Effects of Aloe vera Leaf Extract on Mycelial growth on PDA**

Aloe vera extract exhibited the lowest inhibitory potential among the tested plant extracts. At 80,000 ppm, the highest inhibition was 67.6%, with a slight decrease at 60,000 ppm (67.6%) and a more notable reduction at 40,000 ppm (50.0%). No significant difference was observed between the 80,000 ppm and 60,000 ppm concentrations (Figure 4).

**Inhibitory Effects of Azadirachta indica Leaf Extract on Mycelial growth on PDA**

Neem leaf extract effectively inhibited mycelial growth at all concentrations. The highest inhibition (80.7%) was observed at 80,000 ppm, significantly higher than the inhibition at 60,000 ppm (69.9%) and 40,000 ppm (59.6%). There were significant differences between the three concentrations (Figure 4).

**Inhibitory Effects of Moringa oleifera Leaf Extract on Mycelial Growth**

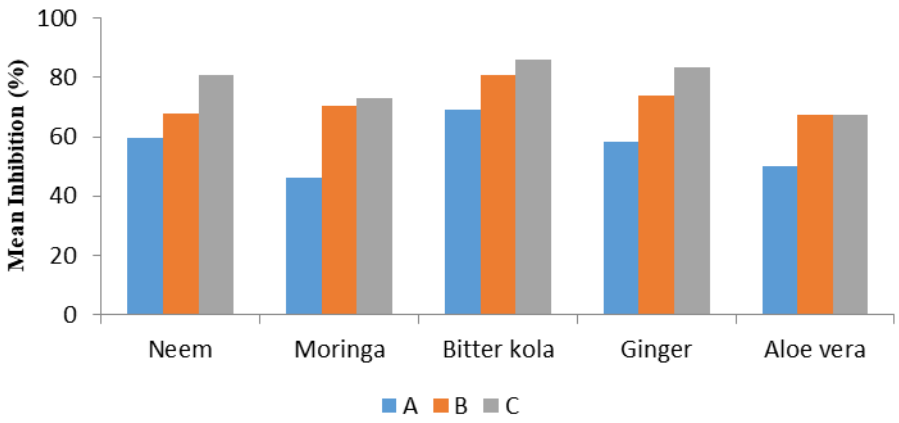
Moringa oleifera extract also showed inhibitory effects on the mycelial growth of P. aphanidermatum across all concentrations. The inhibition (73.1%) was at 80,000 ppm, followed by 70.5% at 60,000 ppm and 46.2% at 40,000 ppm. There was a significant difference between the 40,000ppm concentration and the higher concentrations, but no significant difference between 60,000 ppm and 80,000 ppm (Figure 4).

**Inhibitory Effect of Bitter Kola Seed Extract (Garcinia kola) on Mycelial Growth on PDA**

Bitter kola seed extract exhibited the highest inhibition of P. aphanidermatum among all the plant extracts tested. At 80,000 ppm, inhibition was 85.9%, with 60,000 ppm showing 80.8% inhibition and 40,000 ppm recording 69.2% inhibition. The differences between the concentrations were significant, with bitter kola consistently showing the strongest inhibition (Figure 4).

**Comparative Analysis of Plant Extracts for P. aphanidermatum control (in vitro)**

All five plant extracts—bitter kola, ginger, neem, moringa, and Aloe vera—inhibited the growth of P. aphanidermatum, with bitter kola demonstrating the greatest efficacy, followed by ginger, neem, moringa, and Aloe vera (Figure 4).




Legend: A - 40,000 ppm; B - 60,000 ppm; C - 80,000 ppm

**Figure 4: Effect of the plant extracts on the pathogen at different concentrations**

**Inhibitory Effects of Benlate on Mycelial Growth of *P. aphanidermatum* on PDA**

Benlate, a fungicide, was highly effective in inhibiting P. aphanidermatum mycelial growth, with 150 ppm showing the highest inhibition (89.7%), followed by 100 ppm (81.0%) and 50 ppm (74.6%). No significant difference was found between 150 ppm and 100 ppm (Figure 5).

**Inhibitory Effects of Mancozeb on Mycelial Growth of *P. aphanidermatum* on PDA**

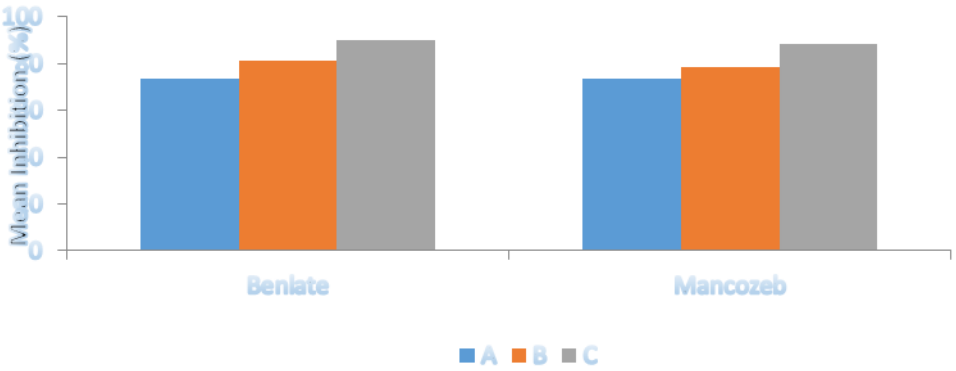
Mancozeb also inhibited mycelial growth in a concentration-dependent manner, with 150 ppm providing 88.5% inhibition, 100 ppm showing 78.2%, and 50 ppm yielding 73.7% inhibition. No significant differences were observed between the various concentrations (Figure 5).

**Comparison of Benlate and Mancozeb in Mycelial growth inhibition of *P. aphanidermatum***

Both fungicides demonstrated strong inhibitory effects at all concentrations tested, with Benlate outperforming Mancozeb. However, no significant differences were noted across the concentrations of each fungicide (Figure 5).

**Efficacy of Plant Extracts in Reducing *P. aphanidermatum* Infection in Three Cucumber Cultivars (in vivo)**

In the greenhouse trials, plant extracts showed limited control of P. aphanidermatum compared to the in vitro results. Bitter kola extract remained the most effective, particularly in the Marketmore cultivar, which displayed the lowest infection rate of 10% (Table 1).




Legend: A- 50 ppm; B - 100 ppm; C- 150 ppm

**Figure 5: Comparison of the effect of the chemical fungicides on the pathogen at different concentrations.**

**Percentage Infection of P. aphanidermatum in Cucumber Varieties treated with Benlate and Mancozeb**

Both fungicides effectively reduced infection in the three cucumber varieties. Technisem showed the highest infection rates when treated with Benlate (33.3%-40.0%) and Mancozeb (40.0%), followed by Poisett, while Marketmore exhibited the lowest infection rates (Tables 2 and 3).

**Table 1: Efficacy of Five Plant Extracts on Percentage Infection on *Pythium Aphanidermatum* in three Cucumber Cultivars**

| Extract | | Technisem Treated | Control | Marketmore Treated | Control | Poin sett Treated | Control |
|---|---|---|---|---|---|---|---|
| | **Number of seedlings** | **20** | **20** | **20** | **20** | **20** | **20** |
| Ginger | No. of infected seedlings | 6 | 12 | 4 | 8 | 7 | 8 |
| | Infection % | 30 | 60 | 20 | 40 | 35 | 40 |
| Neem | Number of Infected seedlings | 8 | 10 | 4 | 7 | 8 | 8 |
| | Infection % | 40 | 50 | 40 | 35 | 40 | 40 |
| Moringa | No. of infected seedlings | 7 | 12 | 5 | 8 | 7 | 9 |
| | Infection % | 35 | 60 | 35 | 40 | 35 | 45 |
| Aloe | No. of infected seedlings | 8 | 11 | 4 | 8 | 8 | 9 |
| | Infection % | 40 | 55 | 20 | 40 | 40 | 45 |
| Bitter Kola | No. of infected seedlings | 4 | 10 | 2 | 8 | 6 | 8 |
| | Infection % | 20 | 50 | 10 | 40 | 30 | 40 |

**Percentage Infection of P. aphanidermatum in Cucumber Varieties treated with Benlate and Mancozeb**

Both fungicides effectively reduced infection in the three cucumber varieties. Technisem showed the highest infection rates when treated with Benlate (33.3%-40.0%) and Mancozeb (40.0%), followed by Poisett, while Marketmore exhibited the lowest infection rates (Tables 2 and 3).

**Phytochemical and Proximate Analyses of Plant Extracts**

Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, and tannins in all five plant extracts, except tannins, which were absent in Moringa oleifera (Table 4a). Quantitative analysis showed that bitter kola had the highest alkaloid content (5.45 ± 0.68), while Aloe vera had the lowest (2.09 ± 0.02) (Table 4b). Proximate analysis indicated that ginger contained the highest carbohydrate (80.6 ± 16), moisture (27.0 ± 0.3), and fibre (9.60 ± 0.12) contents, while bitter kola had the highest ash (3.39 ± 0.01) and protein (3.19 ± 0.01) levels (Table 4c).

**Table 2: Efficacy of Benlate on Percentage Infection on *Pythium amphidernatum* in three Cucumber cultivars.**

| Chemical | | Marketmore | | Poisett | | Technisem | |
|---|---|---|---|---|---|---|---|
| | | Treated | Control | Treated | Control | Treated | Control |
| Benlate | No. of Seedlings | 15 | 15 | 15 | 15 | 15 | 15 |
| | No. of infected seedlings | 2 | 6 | 3 | 5 | 5 | 6 |
| | Infection % | 13.3 | 40.0 | 20.0 | 33.3 | 33.3 | 40.0 |

**Table 3: Efficacy of Macozeb on percentage infection on *Pythium amphidernatum* in three Cucumber cultivars.**

| Chemical | | Technisem | | Marketmore | | Poisett | |
|---|---|---|---|---|---|---|---|
| | | Treated | Control | Treated | Control | Treated | Control |
| Mancozeb | No. of Seedlings | 15 | 15 | 15 | 15 | 15 | 15 |
| | No. of infected seedlings | 6 | 6 | 3 | 7 | 3 | 6 |
| | Infection % | 40.0 | 40.0 | 20.0 | 46.7 | 20.0 | 40.0 |

**Table 4a: Proximate Composition of the Five Plant Samples (%)**

| Composition | Ginger | Bitter kola | Aloe vera | Neem | Moringa |
|---|---|---|---|---|---|
| Moisture | 27.0 ± 0.32 | 6.40 ± 0.10 | 20.40 ± 0.22 | 8.50 ± 0.23 | 11.50 ± 0.20 |
| Ash | 3.20 ± 0.01 | 3.39 ± 0.01 | 1.91 ± 0.21 | 2.71 ± 0.21 | 2.61 ± 0.23 |
| Protein | 0.53 ± 0.1 | 3.19 ± 0.01 | 1.69 ± 0.24 | 1.58 ± 0.34 | 2.56 ± 0.20 |
| Fibre | 9.60 ± 0.12 | 2.84 ± 0.01 | 4.83 ± 0.36 | 5.92 ± 0.47 | 4.82 ± 0.36 |
| Carbohydrate | 80.60 ± 16 | 79.53 ± 0.82 | 75.21 ± 0.25 | 77.12 ± 0.35 | 80.21 ± 0.25 |

**Table 4b: Phytochemical Components of the Five Plant Samples**

| Phytochemical Content | Ginger | Bitter kola | Aloe vera | Neem | Moringa |
|---|---|---|---|---|---|
| Alkaloid | + | + | + | + | + |
| Saponin | + | + | + | + | + |
| Flavonoid | + | + | + | + | + |
| Tannins | + | + | + | + | - |

Key: + Present - Negative

**Table 4c: Phytochemical Contents of the Five Plant Samples (%)**

| Composition | Ginger | Bitter kola | Aloe vera | Neem | Moringa |
|---|---|---|---|---|---|
| Tannin | 0.43 ± 0.01 | 5.09 ± 0.03 | 0.28 ± 0.01 | 0.36± 0.02 | - |
| Flavonoid | 4.70 ± 0.42 | 1.10 ± 0.84 | 0.83 ± 0.01 | 3.18 ± 0.01 | 1.48± 0.34 |
| Alkaloid | 5.35 ± 0.78 | 5.45 ± 0.68 | 2.09 ± 0.02 | 4.00 ± 0.15 | 3.52± 0.36 |
| Saponin | 0.80 ± 0.01 | 2.92 ± 0.82 | 0.40 ± 0.01 | 1.00 ± 0.02 | 2.28 ± 0.01 |

* Values are means of three replicate results ± S.

## DISCUSSION

The phytochemical screening of Garcinia kola (bitter kola), neem (Azadirachta indica), Aloe vera, ginger (Zingiber officinale), and Moringa oleifera revealed the presence of alkaloids, saponins, and flavonoids across all samples, with tannins notably absent in moringa. Quantitative analysis showed that alkaloid content (5.45 ± 0.68%) was the highest among the constituents, while tannins were present in lower concentrations (0.28 ± 0.01%). Bitter kola exhibited the highest phytochemical content, which may explain its superior inhibitory effect on *Pythium aphanidermatum* in vitro inhibition studies. The proximate analysis revealed that carbohydrate content ranged from 0.53 ± 0.1% to 80.60 ± 16%, indicating that these plants are substantial sources of energy. The high carbohydrate levels are consistent with those reported by Madaki et al. (2016), though the moisture content of ginger (27.0 ± 0.32%) was lower than that reported for leafy vegetables by Laden et al. (1996). Notably, bitter kola's lower moisture content suggests better storability compared to the other plant species.

The discrepancies between the phytochemical and nutrient content found in this study and those reported by studies such as Abu et al. (2023) and Adesuyi et al. (2012) may stem from variations in

environmental conditions, as proposed by Evans (2009). Factors such as temperature, rainfall, and altitude are known to influence plant secondary metabolite production (Alami et al., 2024). Additionally, continuous rainfall may lead to the leaching of water-soluble substances, including alkaloids and glycosides, potentially accounting for differences in bioactive compound concentrations.

Previous studies have reported inconsistent findings on the optimal growth media for *P. aphanidermatum.* The results in this study, agree with findings of Kouo-N'Golo et al. (2024) that Potato Dextrose Agar (PDA) is highly favourable for mycelia growth and propagule production of *P. aphanidermatum*. This finding also aligns with earlier reports by Fernando and Linderman (1994), and Verma et al. (2023), which indicated that PDA supports the highest radial growth and sporangial production, outperforming Malt Extract Agar (MEA) and Czapek Dox Agar (CDA). These findings are further corroborated by Shahin and Shepherd (1979), who demonstrated that Alternaria solani also grows more robustly on PDA than on other media. This suggests PDA's suitability for the cultivation of *P. aphanidermatum* and potentially other related pathogens.

Temperature and relative humidity play a critical role in the development and spread of *P. aphanidermatum*. Optimal fungal growth was observed between 25 ± 2°C and 35 ± 2°C, with a peak at 30 ± 2°C, which is consistent with the ambient temperatures during the cucumber growing season in Ekpoma, Edo State, Nigeria. This finding is in agreement with Choi et al. (2020). Also, Ogundana (1971), reported similar temperature requirements for the root rot pathogen in cowpeas. The alignment of laboratory and field conditions likely contributes to the pathogen's persistence and high virulence in cucumber crops during the rainy season.

Relative humidity, particularly during the wettest months of July through September, significantly enhanced the germination and severity of *P. aphanidermatum*. This observation mirrors the findings of Butler and Jadlov (1991), Halo et al. (2024), and Nowicki (2013), who reported that high atmospheric moisture promotes disease initiation in numerous crops. The high incidence of root rot observed during these months underscores the importance of rainfall, humidity, and reduced sunlight in driving disease establishment and progression in cucumber plants.

The antifungal efficacy of methanolic plant extracts was demonstrated both in vitro and in vivo, with all five plant extracts significantly inhibiting the growth of *P. aphanidermatum*. Bitter kola exhibited the highest antifungal activity, achieving 85.9% inhibition at 80,000 ppm, followed by ginger, neem, moringa, and Aloe vera. The concentration-dependent fungitoxicity observed is consistent with the work of Nahed (2007), highlighting the potential of these botanical extracts as sustainable biofungicides for managing root and stem rot in cucumbers (Thangaraj et al., 2023). Variation in fungitoxicity across the extracts may be attributed to the differential solubility of active compounds in methanol or the presence of fungitoxic inhibitors (Seepe et al., 2021).

At higher concentrations (80,000 ppm), a significant increase in inhibition was observed, with bitter kola consistently demonstrating superior efficacy compared to the other plant extracts. The trend of

increasing inhibition with concentration mirrors the results reported by Ojo and Olufolaji (2005) and Umana et al., (2016), reinforcing the potential of these plant extracts as alternatives to synthetic fungicides. Moreover, the findings from this study underscore the need for further research into the isolation and characterization of the active phytochemicals responsible for this antifungal activity.

Chemical fungicides, Benlate and Mancozeb, were also evaluated and demonstrated potent antifungal activity both in vitro and in greenhouse trials. Shah et al. 2023 reported similar findings. Benlate was particularly effective, inhibiting mycelial growth by 80.3% at 100 ppm and 89.3% at 150 ppm, outperforming Mancozeb at equivalent concentrations. This contrasts with earlier findings by Wokocha and Ebenebe (1980), who reported minimal fungicidal efficacy at higher concentrations. The superior performance of Benlate could be due to its higher solubility and more efficient site-specific mode of action. Benlate's mechanism, which involves interference with spindle formation during mitosis, has been well-documented suggesting its suitability for controlling soil-borne fungi like *P. aphanidermatum* (Bai et al., 2024; Richmond and Phillips, 1975)

While Benlate demonstrated high efficacy at low concentrations in in vitro studies, its application at higher concentrations may be required for effective in vivo control, particularly when used as a soil drench. However, the risk of phytotoxicity at elevated concentrations warrants caution. The systemic activity of Benlate, enabling its translocation via the xylem and phloem, may make it particularly effective for soil-drench applications, as noted by Lobna (2006).

This study underscores the potential of both botanical extracts and chemical fungicides for the management of *P. aphanidermatum* in cucumber cultivation. The results provide a compelling argument for the integration of plant-derived biofungicides into disease management strategies, reducing reliance on synthetic fungicides while maintaining efficacy in disease control.

## CONCLUSION

The phytochemical screening of ginger (Zingiber officinale), aloe (Aloe vera), bitter kola (Garcinia kola), neem (Azadirachta indica), and moringa (Moringa oleifera) at varying concentrations demonstrated their antifungal properties, with potential for controlling cucumber root and stem rot. Among the botanicals tested, bitter kola extract exhibited the highest percentage of fungal inhibition, positioning it as the most effective. Furthermore, fungicides, particularly Mancozeb at a higher concentration (40%), proved highly effective in both in vitro and in vivo trials, significantly reducing the level of infection.

This study highlights the potential of botanical extracts as viable alternatives to synthetic fungicides in managing cucumber diseases across the three test locations. While these botanicals present a promising substitute, it is essential to recognize that they may not entirely replace synthetic fungicides in all cases. Nevertheless, the integration of plant-based products into disease management strategies could substantially reduce reliance on chemical

fungicides, providing a more sustainable, eco-friendly, and cost-effective approach for farmers, especially in Nigeria.

The accessibility, affordability, biodegradability, and ease of extraction of these botanicals make them especially advantageous for resource-limited farmers who may not have access to synthetic fungicides. Moving forward, it is crucial to continue research in this area, integrating the use of botanicals with other pest and disease control methods to develop comprehensive, sustainable agricultural practices. Such efforts will contribute to reducing chemical dependence, minimizing environmental impact, and improving overall crop production.

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