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Comparative *in Vitro* Antifungal Activity of *Azadirachta Indica* A. Juss. And *Anacardium Occidentale* L. Leaf Extracts Against Fungal Pathogens of *Amaranthus Hybridus*

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ABSTRACT
Fungal pathogens significantly affect the cultivation of *Amaranthus hybridus*, leading to reduced yield and quality. Environmental and health concerns associated with synthetic fungicides have increased interest in plant-based biofungicides as safer alternatives. This study evaluated the antifungal activity of aqueous leaf extracts of *Azadirachta indica* and *Anacardium occidentale* against *Fusarium oxysporum*, *Alternaria tenuissima*, and *Aspergillus flavus*. To obtain the extracts, 100 g of dried leaf powder was macerated in 1000 mL of distilled water. Antifungal activity was assessed using the poisoned food technique at 5%, 10%, and 15%. All treatments were replicated three times. The triplicates were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at $p \leq 0.05$. *Azadirachta indica* demonstrated a clear but moderate concentration-dependent antifungal activity with all tested fungi. The highest inhibition was shown against *Fusarium oxysporum* (28.6 ± 2.5% at 15% concentration). *Anacardium occidentale* showed mild antifungal activity, with *Alternaria tenuissima* showing no observable inhibition at the tested concentrations. These findings indicate that *A. indica* demonstrates moderate but considerable antifungal potential for managing fungal diseases of *Amaranthus hybridus.* Further *in vivo* studies are recommended to validate field efficacy.

Keywords: *Azadirachta indica*, *Anacardium occidentale*, Antifungal activity, Biofungicides, *Amaranthus hybridus*, Poisoned food technique

INTRODUCTION
Plant diseases caused by pathogenic microorganisms such as fungi, bacteria, viruses, and nematodes significantly reduce crop productivity worldwide, with annual yield losses ranging from 20% to 40% (Ayaz *et al.*, 2023). Among these pathogens, fungal diseases are particularly destructive to vegetable crops, causing considerable losses in both yield and quality (Zishan *et al.*, 2024).

For a very long time, synthetic fungicides have been widely used to control plant diseases. Their excess use, however, has raised serious concerns about environmental pollution, risks to human health, and the development of pathogen resistance. Consequently, there has been an increasing interest in the development of safe and ecologically friendly alternatives such as plant-derived biofungicides (Hu *et al*., 2023). Amaranthus hybridus is an important leafy vegetable widely cultivated in tropical and subtropical regions. It is valued for its high nutritional content, which includes proteins, vitamins, essential phytochemicals, and minerals. In many African countries, including Nigeria, the leaves are consumed almost every day, mainly as vegetables, and they contribute significantly to dietary nutrition (Mepha *et al.*, 2007: Dutta *et al.* 2024)

Despite its nutritional and economic importance, its production is often threatened by fungal pathogens that reduce it in yield and market value.

Several fungal pathogens have been reported to infect *A. hybridus*, including *Fusarium oxysporum*, *Alternaria tenuissima*, and *Aspergillus flavus*. These organisms cause diseases such as root rot, damping-off, and leaf spots, which lead to a significant reduction in yield (Parveen and Khatun, 2022). In extreme cases, losses of crops above 80% have been reported in infected fields (Edosa *et al.*, 2020).

As a result of the increase in demand for ecologically friendly plant disease management strategies, plant-based biofungicides have gained attention as potential alternatives to synthetic fungicides. Biofungicides derived from plant extracts contain bioactive compounds capable of inhibiting fungal growth through different mechanisms, which include the disruption of cell membranes, inhibition of spore germination, and interference with fungal metabolism (Kumar *et al.*, 2021).

Azadirachta indica (neem) is widely recognized for its antimicrobial properties. The plant contains several bioactive compounds, including azadirachtin, nimbin, salannin, and quercetin. These compounds have shown strong antifungal and antimicrobial activities (Wylie and Merell, 2022). They interfere with fungal growth processes, disrupt membrane integrity, inhibit spore germination, and inhibit metabolic activity (Sudan *et al.*, 2020). *Anacardium occidentale* (cashew) also contains phytochemicals such as phenolics, tannins, saponins, and terpenoids. These compounds exhibit antimicrobial and antifungal activities. Previous studies, such as that of Quejada *et al*. (2024), have shown that extracts of *Anacardium occidentale* can inhibit the growth of several fungal pathogens, particularly *Fusarium* and *Colletotrichum* species. Despite the increasing interest in botanical fungicides, limited studies have specifically investigated the antifungal potential of *Azadirachta indica* and *A. occidentale* against fungal pathogens associated with *A. hybridus*. Therefore, this study aimed to evaluate the comparative antifungal activities of aqueous leaf extracts of *Azadirachta indica* and *Anacardium occidentale* against *Fusarium oxysporum, Alternaria tenuissima*, and *Aspergillus flavus.* The ability of the plant extract to demonstrate antifungal activity on the fungal pathogens of Amaranthus will be a safer, more sustainable, and eco-friendly alternative to synthetic fungicides.

MATERIALS AND METHODS

Study Area and Sample Collection

This research was conducted in the laboratory of the Department of Botany, Lagos State University, Ojo, Lagos State, Nigeria.

The fungal isolates used in this study (*Aspergillus flavus*, *Fusarium oxysporum*, and *Alternaria tenuissima*) were obtained from the Plant Pathology Laboratory, Department of Botany, Lagos State University, Ojo. Fresh leaves of *A. indica* (Voucher number LSH 001176) were collected from the Lagos State University botanical garden, while leaves of *A. occidentale* (Voucher number LSH 001269) were obtained from a local garden in Ifo, Ogun State, Nigeria. Dr Kehinde Omolokun, a taxonomist in the Department of Botany, Lagos State University, authenticated both plant species.

Preparation of Plant Extracts

The collected leaves were thoroughly washed with distilled water to remove debris and impurities. The clean plants were then air-dried on the laboratory bench for 14 days away from direct sunlight and under sterile conditions. This was done to preserve phytochemical constituents. After drying, the leaves were ground into fine powder using an electric blender under sterile conditions. For extraction, 100 g of each powdered leaf sample was macerated in 1000 mL of distilled water. The mixture was covered with aluminum foil and allowed to macerate for 72 hours. The extracts were filtered successively. First, through a muslin cloth, followed by a Whatman No. 1 filter paper to remove particulate matter. The extraction procedure was carried out following standard aqueous maceration methods described by Ngatsi *et al.* (2023). The resulting filtrates were stored in clean, labelled containers. This was refrigerated at 4°C and used within 24 hours of preparation to preserve bioactive constituents.

Preparation and Sterilization of Culture Media

Potato Dextrose Agar (PDA) was prepared by dissolving 39 g of dehydrated PDA powder in 1000 mL of distilled water (Singh *et al.,* 2022).

The medium was thoroughly mixed and sterilized by autoclaving at 121°C for 15 minutes at 15 psi. After sterilization, the medium was allowed to cool to approximately 45°C. Then, 0.05 g/L of chloramphenicol was added to suppress bacterial contamination. The medium was aseptically poured into sterile Petri dishes and allowed to solidify. Control plates consisted of PDA without any plant extract and were used to determine baseline fungal growth.

Antifungal Assay

The antifungal activity of the aqueous leaf extracts was evaluated using the poisoned food technique (Ngatsi *et al.*, 2023). A stock solution of each extract was prepared at 100 g/1000 mL (w/v). Working concentrations of 5%, 10%, and 15% (v/v) were obtained by adding appropriate volumes of the stock extract to molten PDA at approximately 45°C before solidification. Approximately 20 mL of the prepared medium was poured into sterile Petri dishes and allowed to solidify. Fungal discs of 5 mm diameter, cut from the margins of actively growing cultures, were placed at the centre of each plate.

Data Collection and Statistical Analysis

Radial mycelial growth was measured after 5 days of incubation under controlled laboratory conditions at 28 ± 2°C. The experiment was laid out in a completely randomized design (CRD), with all treatments performed in triplicate. Percentage inhibition of radial growth was calculated using the following formula:

$$\% \text{ Inhibition} = [(C - T) / C] \times 100$$

Where C = radial growth in the control (mm) and T = radial growth in the treated plate (mm).

Data were expressed as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at $p \leq 0.05$. All analyses were conducted using SPSS software version 23.

RESULTS AND DISCUSSION

The antifungal activities of aqueous leaf extracts of *A. indica* and *A. occidentale* against the selected fungal pathogens were evaluated at concentrations of 5%, 10%, and 15%.

A. indica exhibited moderate concentration-dependent antifungal activity across all tested fungal isolates. The highest inhibition was recorded against *F. oxysporum* (28.6 ± 2.5% at 15%), followed by *A. tenuissima* (22.2 ± 2.0%) and *A. flavus* (21.4 ± 1.8%) at the same concentration (Table 1; Figure 1). Significant differences ($p \leq 0.05$) were observed among the tested concentrations for each fungal isolate.

Table 1: Antifungal Activity of *A. Indica* Leaf Extracts against Selected Fungal Pathogens at Varying Concentrations

Fungal Isolate	5% Concentration	10% Concentration	15% Concentration
Aspergillus flavus	3.6 ± 0.5^c	14.3 ± 1.2^b	21.4 ± 1.8^a
Fusarium oxysporum	7.1 ± 0.7^c	10.7 ± 1.0^b	28.6 ± 2.5^a
Alternaria tenuissima	6.7 ± 0.6^c	13.3 ± 1.1^b	22.2 ± 2.0^a

* Values represent mean ± standard deviation of triplicate determinations. Means with different superscripts within the same row differ significantly at $p \leq 0.05$ according to Tukey's HSD test.

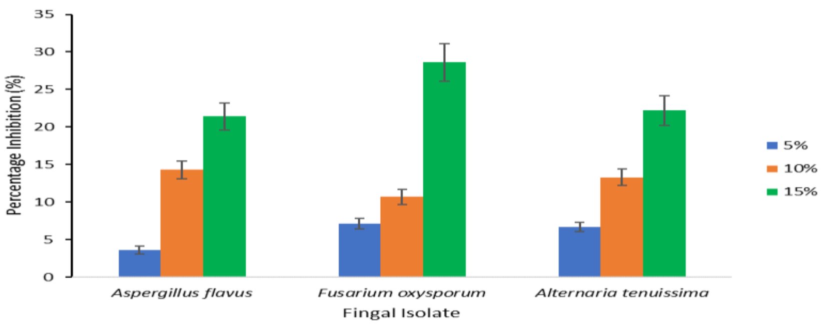

Figure 1: Percentage Inhibition of Radial Mycelial Growth of Fungal Isolates by A. Indica Leaf Extracts at Different Concentrations. Values Represent Mean ± SD (N = 3)

In contrast, *A. occidentale* showed relatively low antifungal activity. Mild inhibition was observed against *F. oxysporum* and *A. flavus* at higher concentrations, while *A. tenuissima* showed no observable inhibition at any of the tested concentrations (Table 2; Figure 2). Overall, *A. indica* demonstrated significantly stronger antifungal properties compared to *A. occidentale* under the experimental conditions. Representative culture plates are shown in Figure 3-5.

Table 2: Antifungal Activity of *A. Occidentale* Leaf Extracts against Selected Fungal Pathogens at Varying Concentrations

Fungal Isolate	5% Concentration	10% Concentration	15% Concentration
Aspergillus flavus	0.0 ± 0.0^{c}	3.6 ± 0.5^{b}	10.7 ± 1.0^{a}
Fusarium oxysporum	1.8 ± 0.3^{c}	5.4 ± 0.8^{b}	10.7 ± 1.1^{a}
Alternaria tenuissima	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}

* Values represent mean ± standard deviation of triplicate determinations. Means with different superscripts within the same row differ significantly at $p \leq 0.05$ according to Tukey's HSD test.

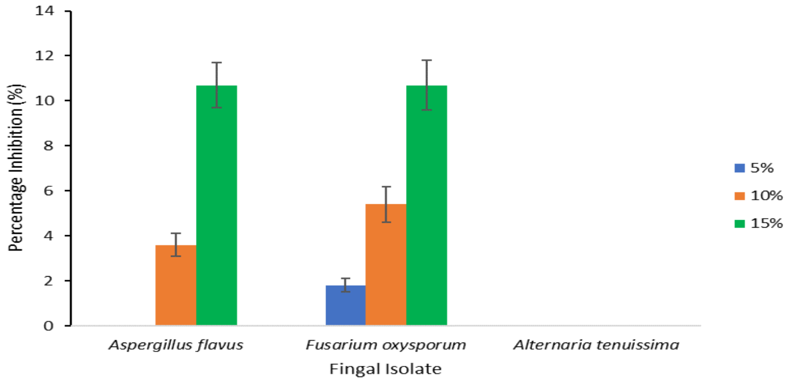

Figure 2: Percentage inhibition of radial mycelial growth of fungal isolates by *A. occidentale* leaf extracts at different concentrations. Values represent mean ± SD (n = 3)

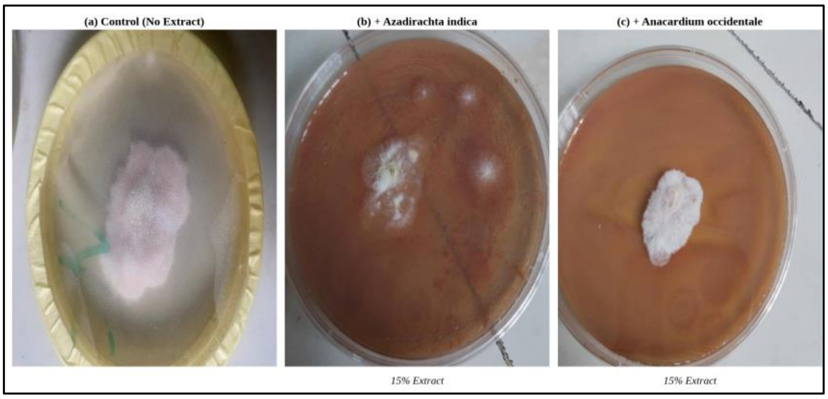

Figure 3: Representative culture plates of *F. oxysporum* showing: (a) control — PDA without extract; (b) PDA + *A. indica* 15% extract; (c) PDA + *A. occidentale* 15% extract. Visible reduction in mycelial growth in treated plates compared to control indicates antifungal activity

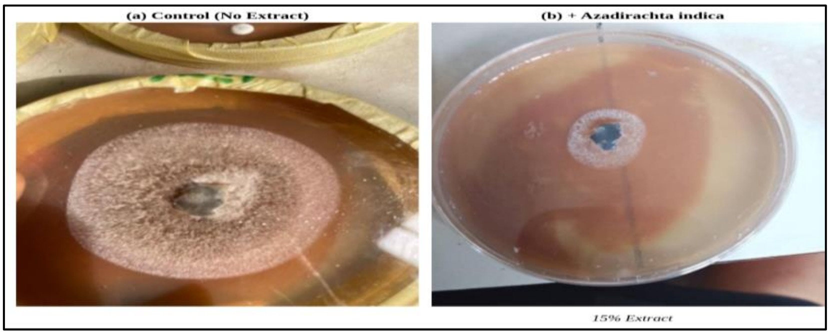

Figure 4: Representative Culture Plates of *A. Tenuissima* Showing: (A) Control — PDA without Extract; (B) PDA + *A. Indica* 15% Extract. No *A. Occidentale* Treated Plate Is Shown As No Measurable Inhibition Was Observed At Any Tested Concentration

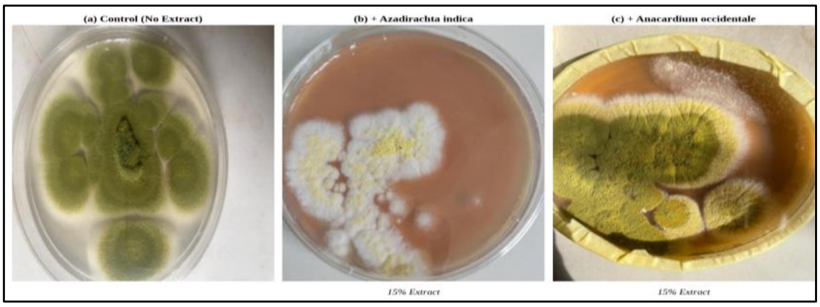

Figure 5: Representative Culture Plates Of *Aspergillus Flavus* Showing: (A) Control — PDA Without Extract; (B) PDA + *A. Indica* 15% Extract; (C) PDA + *A. Occidentale* 15% Extract. Reduced Sporulation and Colony Size Are Visible In Treated Plates Relative to the Control

Discussion

The observed concentration-dependent inhibitory activity of A. indica may be attributed to its rich phytochemical profile, which includes azadirachtin, nimbin, salannin, and quercetin. Such a dose-responsive pattern suggests that increasing extract concentration enhances the availability of bioactive compounds responsible for antifungal activity. These findings are consistent with those of Grillo *et al*. (2026), which demonstrated that ethanolic and aqueous leaf extracts of A. indica inhibited the growth of several fungal isolates, including dermatophytes of the genus *Trichophyton*, thereby supporting the broader antifungal potential of the plant beyond its established activity against phytopathogens.

The concentration-dependent inhibitory pattern observed is consistent with a dose-responsive suppression mechanism, as further discussed in relation to phytochemical composition.

The moderate antifungal activity of *A. indica* may be attributed to its rich phytochemical profile, which includes azadirachtin, nimbin, salannin, and quercetin. These compounds are known to interfere with fungal growth processes, which include disrupting their membrane integrity, inhibiting spore germination, and interfering with metabolic activity (Sudan *et al.*, 2020; Akinyemi *et al.*, 2023). The findings of this study are consistent with those of Ngatsi *et al.* (2023), who reported that neem extracts significantly inhibited the growth of *F. oxysporum*. Likewise, Gong *et al.* (2024) reported that neem-derived compounds reduce fungal colony growth and spore viability across several fungal species. The inhibition values recorded in the present study (up to 28.6%) are comparable to those reported in related work; Ngatsi *et al.* (2023) documented 25–40% inhibition of *F. oxysporum* using neem seed extracts. Akinyemi *et al.* (2023) also reported values of 20–35% using neem leaf preparations. The difference in inhibition values across studies may be attributed to many factors. These factors include the solvent used for extraction, plant part used, source of fungal isolate, and experimental conditions like inoculum size and incubation period. The visual evidence in Figures 3 - 5 further corroborates these quantitative findings.

The relatively higher antifungal activity of *A. indica* compared to *A. occidentale* may be attributed to differences in phytochemical composition and extractability. The antifungal constituents of *A. occidentale* are predominantly phenolic compounds that are more efficiently extracted with organic solvents such as methanol or ethyl acetate (Attah *et al.*, 2021). The use of aqueous extraction in this study may therefore have restricted the recovery of active antifungal compounds from cashew leaves, thereby limiting their observed efficacy. It should be noted that the comparative interpretation between the two species is constrained by this solvent-specific difference in extraction efficiency. Direct comparison of biological activity should therefore be made with caution.

Notably, *A. tenuissima* showed no observable inhibition at any tested concentration of *A. occidentale* extract. While this may reflect intrinsic tolerance mechanisms, whereby *Alternaria* species are reported to deploy enzymatic pathways capable of detoxifying plant phytochemicals (Sharma *et al.*, 2023), it is also plausible that higher concentrations or alternative solvents would yield measurable inhibition given the limited concentration range evaluated. Figure 4 visually corroborates this finding. These results underscore the need for targeted antifungal strategies rather than reliance on a single plant extract or extraction system.

From an applied perspective, the results suggest that neem-based formulations could be integrated into sustainable plant disease management systems. Botanical biofungicides, such as neem extracts, offer advantages, including biodegradability, reduced toxicity to non-target organisms, and compatibility with integrated pest management systems (Kumar *et al.*, 2021). However, field-level validation remains necessary to confirm efficacy under variable environmental conditions.

CONCLUSION

This study demonstrated that aqueous leaf extracts of *A. indica* demonstrated moderate but significant concentration-dependent antifungal activity against *F. oxysporum*, *A. tenuissima*, and *A. flavus*, while *A. occidentale* shows limited effectiveness under aqueous extraction conditions. The findings support the potential application of neem-based extracts as biofungicides in sustainable agriculture. Further studies involving alternative extraction methods, *in vivo* field evaluations, and expanded concentration ranges are necessary to optimize the practical utility of these plant-derived antifungal agents. These findings highlight the potential of locally available plant resources as cost-effective and environmentally sustainable biofungicides, particularly in resource-limited agricultural systems.

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