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Antifungal Efficacy of Plant-Derived Essential Oils against *Aspergillus flavus* Isolates from Maize in Makurdi, Nigeria

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Abstract

Maize contamination by *Aspergillus flavus* and its aflatoxins poses a persistent threat to food safety and public health in Nigeria. This study isolated and identified *Aspergillus* species from maize obtained in three major markets in Makurdi, Benue State, using morphological characterization and the ammonia vapor test. Essential oils were extracted from lemon peels, banana peels, turmeric rhizomes, neem leaves, and lemongrass through Soxhlet extraction, purified by distillation, and evaluated for antifungal activity using the disc diffusion method. Results revealed that all tested oils inhibited fungal growth to varying degrees, with turmeric and neem extracts exhibiting the strongest inhibition zones, while banana peel oil showed the least effect. Neither the positive control (Flucostat I.V.) nor the negative control (Tween-80) produced inhibition, confirming that the observed antifungal activity originated from the plant extracts. These findings demonstrate the promise of locally available botanicals as eco-friendly antifungal agents for managing *Aspergillus* contamination in maize systems.

Keywords

Aspergillus flavus, Antifungal Activity, Essential Oils, Disc Diffusion Assay, Maize Storage

1. Introduction

Essential oils are volatile aromatic compounds naturally synthesized by plants as

part of their secondary metabolism. They are typically stored in specialized structures such as secretory glands, ducts, or trichomes and encompass diverse chemical classes, including terpenes, aldehydes, ketones, phenolics, and esters. For centuries, these oils have been valued in traditional medicine, food preservation, and cultural practices because of their distinctive fragrance and therapeutic potential. In recent decades, scientific research has increasingly focused on their broad-spectrum antimicrobial, antifungal, antioxidant, and insecticidal activities, with growing recognition of their potential as eco-friendly alternatives to synthetic chemicals in agriculture and food systems [1] [2]. Their natural origin, rapid biodegradability, and reduced toxicity to humans and animals confer advantages over conventional fungicides and synthetic preservatives, which often accumulate as residues in food and the environment.

Maize (*Zea mays* L.) is one of the most important cereals worldwide and is particularly central to food security, nutrition, and livelihoods in sub-Saharan Africa. In Nigeria, maize is a staple crop consumed across rural and urban populations, serving as a key ingredient in human diets, animal feed, and various industrial applications. However, maize production and storage are challenged by fungal contamination, which threatens both yield and safety. Among the most destructive fungal species infecting maize is *Aspergillus flavus*, a ubiquitous soil-borne pathogen capable of colonizing kernels before and after harvest. The consequences of *A. flavus* contamination are two-fold: reduction of grain quality and the production of aflatoxins, a class of secondary metabolites that are among the most potent naturally occurring carcinogens [3] [4]. Aflatoxin contamination has been recognized as a major public health problem in Africa. Consumption of contaminated maize has been linked to episodes of acute aflatoxicosis, such as outbreaks in Kenya that resulted in high mortality rates [5] [6]. Chronic exposure, even at sub-lethal levels, is associated with hepatocellular carcinoma, immune suppression, growth retardation in children, and increased susceptibility to infectious diseases [7] [8]. Economically, aflatoxin contamination leads to rejection of maize in international markets, reduced farmer incomes, and significant postharvest losses [9]. These challenges underscore the urgent need for effective and sustainable strategies to manage aflatoxin risks in maize-based systems. Currently, the use of synthetic fungicides remains a common approach to controlling *A. flavus* in stored grain. However, several challenges are associated with their application. Synthetic fungicides are costly, may cause toxicological risks to humans and livestock, and often leave residues that raise environmental concerns. Moreover, the repeated use of single-target fungicides accelerates the development of resistant fungal strains, reducing their long-term effectiveness [10]. These limitations highlight the importance of exploring natural, biodegradable antifungal agents with multiple mechanisms of action.

Essential oils represent promising candidates for managing fungal contamination in food and agricultural systems. Previous studies have shown that oils derived from plants such as citrus, neem, turmeric, and lemongrass can suppress

fungal growth and reduce aflatoxin production. Their antifungal mechanisms are diverse, ranging from disruption of cell membrane integrity and inhibition of ergosterol biosynthesis to induction of oxidative stress and interference with sporulation [11]-[13]. Importantly, essential oils often contain multiple bioactive compounds, which can act synergistically to inhibit fungal pathogens while reducing the likelihood of resistance development compared with single-compound synthetic fungicides [14].

Several essential oils of interest are readily available from local plant resources in Nigeria. Lemon peel oil, rich in limonene, citral, and other terpenoids, has been reported to possess strong antimicrobial and antifungal properties [15] [16]. Banana peel oil, though less studied, contains fatty acids and phenolic acids with moderate antimicrobial activity, and recent reports suggest potential antifungal effects against postharvest pathogens [17] [18]. Turmeric rhizome oil contains turmerones and curcuminoids, which have been documented to inhibit aflatoxigenic fungi by generating oxidative stress and suppressing spore formation [14] [19]. Neem leaf oil is well known for its broad-spectrum bioactivity, attributed to compounds such as azadirachtin and nimbin, which interfere with fungal cell wall synthesis and metabolism [12] [20] [21]. Lemon grass oil, dominated by citral and geraniol, has been widely studied for its potent antifungal efficacy, including inhibition of *A. flavus* growth and aflatoxin production in maize [13]. While these findings demonstrate the antifungal potential of essential oils, important gaps remain. Most studies have focused on individual oils or single plant species, without comparative evaluation of multiple locally available botanicals. Additionally, many investigations have been conducted under laboratory conditions without comprehensive chemical characterization of the oils to establish links between phytochemical composition and antifungal efficacy. Limited studies have simultaneously applied techniques such as gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FTIR) to profile the chemical constituents of oils and correlate them with biological activity. Moreover, there is a scarcity of research evaluating plant-derived oils obtained from agricultural by-products and wastes, such as lemon and banana peels, which could provide cost-effective and sustainable sources of antifungal agents.

This study was therefore designed to extract, characterize, and evaluate the antifungal activity of essential oils obtained from lemon peels, banana peels, turmeric rhizomes, neem leaves, and lemongrass against aflatoxigenic *Aspergillus flavus* isolated from maize. The chemical composition of the oils was determined using GC-MS and FTIR techniques, while antifungal activity was assessed through in vitro assays. The central hypothesis was that essential oils derived from these locally available plants, particularly those rich in terpenoids and phenolic compounds, would exhibit significant antifungal activity against *A. flavus* and could serve as effective, eco-friendly alternatives to synthetic fungicides. Specifically, it was expected that citrus and lemongrass oils would demonstrate stronger antifungal properties due to their high citral and limonene content, while turmeric, neem,

and banana peel oils would provide moderate to variable activity depending on their phytochemical profiles. By addressing these gaps, the study contributes to the growing body of evidence on natural antifungal agents.

2. Materials and Methods

Figure 1 shows the map of the study location. The study was conducted in Makurdi, the capital city of Benue State, Nigeria. Makurdi lies within the Benue Trough along the River Benue floodplain and is geographically located at approximately latitude 7˚44′ N and longitude 8˚32′ E, with an average elevation of 113 m above sea level. The area falls within the Southern Guinea Savannah ecological zone (Figure 1) and is characterized by a tropical climate with distinct wet and dry seasons. Agriculture constitutes the predominant economic activity in the region, with extensive cultivation of cassava, rice, maize, groundnut, yam, and other crops.

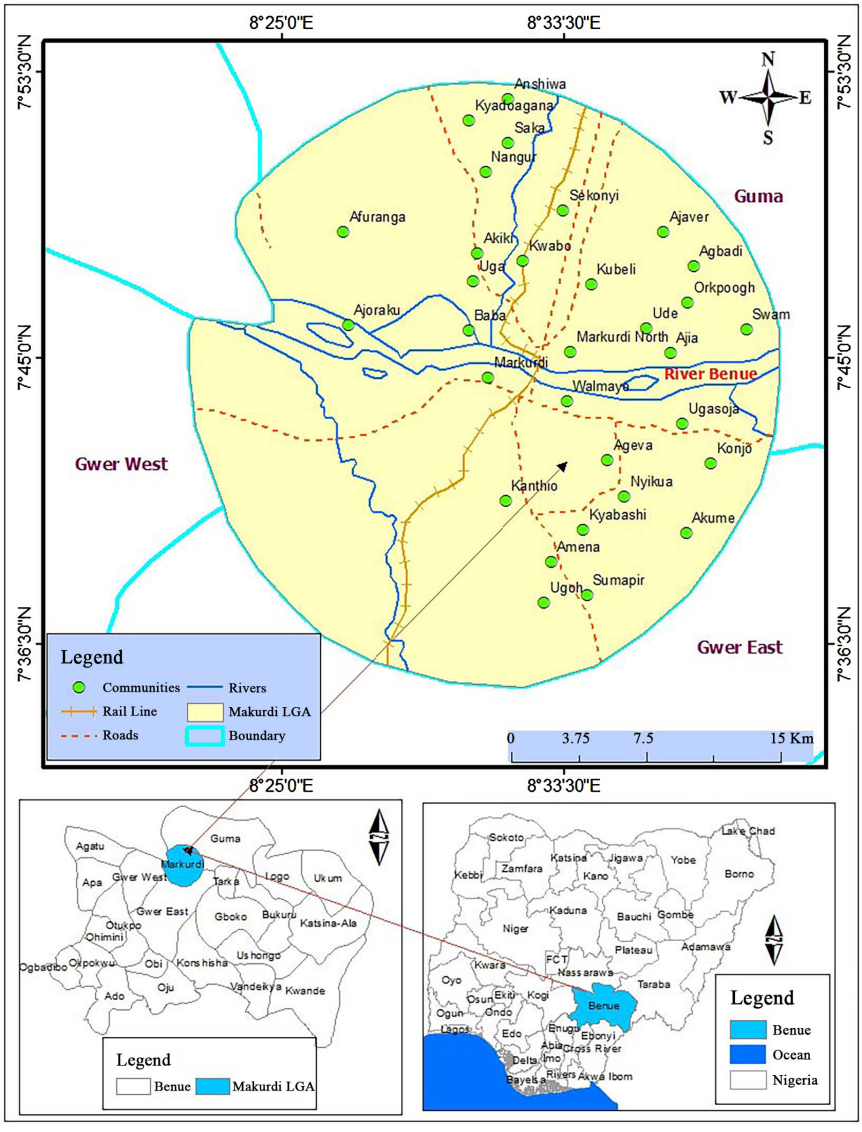

Figure 1. Map of Makurdi local government, Benue state.

2.1. Preparation of Plant Materials and Extraction

Although Soxhlet extraction with n-hexane was earlier reported, it was for all oil-rich substances. Essential oil extraction was carried out by hydrodistillation using a clevenger-type apparatus [15]. The mixture was heated on a thermostatically controlled heating mantle and allowed to boil gently for 3 - 4 hours. During distillation, the volatile oil components were vaporized along with water vapour, condensed in the condenser, and collected in the calibrated arm of the clevenger apparatus. Oils were stored in sterile vials until use.

2.2. Isolation and Identification of Fungal Isolates

Surface-sterilized maize seeds were plated on Potato Dextrose Agar (PDA) supplemented with ciprofloxacin and incubated at 27˚C ± 2˚C. Emerging colonies were purified and identified based on macroscopic and microscopic features, including colony color, conidial morphology, and vesicle structure, following the procedure of Temesgen and Sefawdin [22]. The toxigenic *Aspergillus flavus* isolate was confirmed using the ammonia vapor (AV) test described by Degola *et al.* [23] with slight modification. Three-day-old cultures grown on PDA were inverted, and 200 µL of 25% (v/v) ammonia solution was applied to the inner surface of each Petri-dish lid. Plates were sealed with parafilm and observed after 15 - 20 min for color change on the reverse colony surface. The appearance of a plum-red to pink coloration indicated aflatoxin-producing strains, whereas no color change denoted non-toxigenic isolates. All tests were conducted in triplicate to ensure consistency.

2.3. Antifungal Assay

Essential oils were emulsified in Tween-80 and tested by disc diffusion [24]. Sterile Whatman discs (6 mm) were impregnated with 0.1 mL oil and placed on PDA plates inoculated with standardized fungal suspensions. Flucostat I.V. served as positive control and 5% Tween-80 as negative control. Plates were incubated at 28˚C - 30˚C for 72 hours, after which inhibition zones were measured [25].

The fungal inocula used in this study were designated as C1, C2, and C3, representing *Aspergillus flavus* isolates obtained from maize samples obtained from North Bank Market (C1), Railway Market (C2), and Modern Market (C3), Makurdi, Benue State, Nigeria. Each isolate was processed and tested as an independent strain. Inoculum preparation and standardization were performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines for antifungal susceptibility testing of filamentous fungi (CLSI document M38). Briefly, conidia were harvested from 7-day-old cultures grown on potato dextrose agar medium and suspended in sterile normal saline containing 0.05% Tween 20. The resulting suspensions were allowed to settle to remove heavy hyphal fragments, and the upper homogeneous conidial suspension was adjusted spectrophotometrically to match the 0.5 McFarland turbidity standard, corresponding to an inoculum density of approximately 1×10^6 conidia/mL. The standardized inocula were used immediately for antifungal susceptibility testing.

2.4. Data Analysis

Zone diameters were recorded in millimeters and expressed as block means. Non-detectable inhibition was recorded as 0.00 mm [26]. All experiments were conducted in replicates, and inhibition zone diameters were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) to determine differences among treatments, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was considered at $p < 0.05$.

3. Results

Aspergillus flavus was successfully isolated from maize grains collected in all three markets, and ammonia vapor tests confirmed the presence of toxigenic strains (Figure 2). Essential oils extracted from the five botanicals inhibited fungal growth to varying degrees. Among them, turmeric and neem oils produced the largest inhibition zones, followed by lemon and lemongrass oils, while banana peel oil was the least effective. The positive control (Flucostat I.V.) showed no suppression of fungal growth, while the negative control (Tween-80) produced no inhibition, confirming that the antifungal activity originated solely from the plant extracts. Interestingly, the positive control (Flucostat I.V.) did not produce measurable zones of inhibition against *Aspergillus flavus* under the assay conditions used.

Figure 2. Pure cultures of *Aspergillus flavus* isolates.

The results of ammonium vapor test for the preliminary detection of aflatoxin is presented in Figure 3. The appearance of a plum-red to pink coloration indicated aflatoxin-producing strains, whereas no color change denoted non-toxigenic isolates.

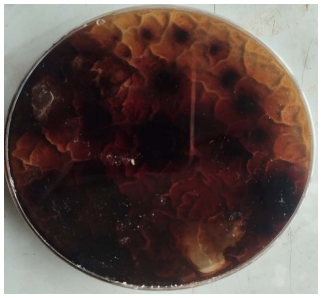

Figure 3. Ammonium vapor test showing plum red coloration (indicating toxigenic *A, flavus*) and dark colonies indicating non-toxigenic isolates.

The antifungal activity of the essential oils was first visualized on agar plates as zones of growth inhibition against *A. flavus* isolates. Representative agar plates illustrating these inhibition zones are shown in Figure 4. Turmeric and neem oils produced distinct zones of inhibition, while lemon and banana peel oils displayed weak or negligible activity. The negative control (Tween-80) and positive control (Flucostat I.V.) showed no inhibition.

Figure 4. Representative plate showing inhibition zones of neem leaves (N) and turmeric rhizomes (T).

Quantitative inhibition zone diameters (mm) obtained from replicate assays are summarized in Table 1, while a comparative bar chart illustrating mean inhibition zones is presented in Figure 5. Although several block means recorded zero inhibition values, turmeric and neem oils produced distinct inhibition zones in specific plates and blocks, resulting in higher overall mean inhibition values when data were pooled across replicates.

Table 1. Inhibition zone diameters (mm) for essential oils and controls.

Block (Plate mapping)	Plate	Negative control	Positive control	Lemon (mm)	Banana (mm)	Turmeric (mm)	Neem (mm)
Block 1 (B1: P1 = C1, P2 = C2, P3 = C3)	P1	0.00	0.00	0.00	0.00	11.00	0.00
	P2	0.00	0.00	5.00	0.00	7.00	4.00
	P3	0.00	0.00	0.00	0.00	11.00	6.00
	Block 1 mean	**0.00**	**0.00**	**1.67**	**0.00**	**9.67**	**3.33**
Block 2 (B2: P4 = C1, P5 = C2, P6 = C3)	P4	0.00	0.00	0.00	0.00	20.00	0.00
	P5	0.00	0.00	0.00	0.00	0.00	0.00
	P6	0.00	0.00	0.00	0.00	0.00	0.00
	Block 2 mean	**0.00**	**0.00**	**0.00**	**0.00**	**6.67**	**0.00**
Block 3 (B3: P7 = C1, P8 = C2, P9 = C3)	P7	0.00	0.00	0.00	0.00	6.00	4.00
	P8	0.00	0.00	0.00	0.00	15.00	10.00
	P9	0.00	0.00	0.00	0.00	0.00	0.00
	Block 3 mean	**0.00**	**0.00**	**0.00**	**0.00**	**7.00**	**4.67**

Continued

Block 4 (B4: P10 = C1, P11 = C2, P12 = C3)	P10	0.00	0.00	0.00	0.00	11.00	4.00
	P11	0.00	0.00	0.00	0.00	6.00	0.00
	P12	0.00	0.00	0.00	0.00	0.00	0.00
	Block 4 mean	**0.00**	**0.00**	**0.00**	**0.00**	**5.67**	**1.33**
	Overall mean (all blocks, n = 12 plates)	**0.00**	**0.00**	**0.42**	**0.00**	**7.25**	**2.33**

B = Block, P = Plate mapping.

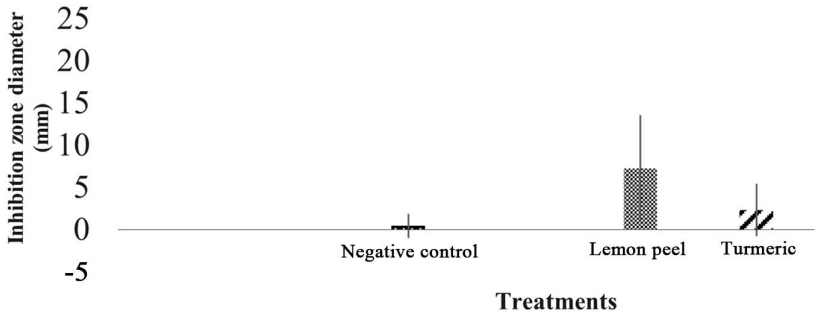

Figure 5. Comparative inhibition zones of essential oils and controls against *Aspergillus flavus*. Bars represent mean inhibition zone diameters (mm) ± SD (n = 12).

To further explain the observed antifungal activity of the plant-derived oil extracts, gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared (FTIR) analyses were conducted to determine their major chemical constituents and functional groups. Due to the large number of detected compounds, only the predominant constituents (top 3 - 5) with relatively high abundance and documented antifungal relevance are summarized in **Table 2**, while the key functional groups identified by FTIR analysis are presented in **Table 3**.

Table 2. Major GC-MS compounds identified in plant-derived oil extracts.

Plant source	Major compounds (top 3 - 5 only)	Relative abundance (%)	Reported bioactivity
Turmeric rhizome (*Curcuma longa*)	Dibutyl phthalate, 9, 12-Octadecadienoic acid, 1-Octadecene, Heptadecanoic acid	17.27, 15.66, 8.47, 7.14	Antifungal; membrane disruption
Neem leaves (*Azadirachta indica*)	Bis (2-ethylhexyl) phthalate, n-Hexadecanoic acid, 9, 12-Octadecadienoic acid	92.35, 0.59, 0.48	Strong antifungal; sporulation inhibition
Lemongrass (*Cymbopogon citratus*)	Bis (2-ethylhexyl) phthalate, n-Hexadecanoic acid, Phenolic derivatives, 9, 12-Octadecadienoic acid	18.09, 13.34, 5.92, 2.94	Antifungal; cell membrane damage
Lemon peel (*Citrus limon*)	Diisooctyl phthalate, Tumerone, Curlone	43.12, 29.93, 12.28	Antifungal; growth and sporulation inhibition
Banana peel (*Musa* spp.)	cis-Vaccenic acid, n-Hexadecanoic acid, Palmitoleic acid, Bis (2-ethylhexyl) phthalate	25.35, 23.30, 5.86, 5.80	Weak-moderate antifungal

Table 3. FTIR functional groups detected in plant-derived oil extracts.

Plant source	Key functional groups detected	Peak range (cm^{-1})	Biological interpretation
Turmeric rhizome (*Curcuma longa*)	O-H, C-H, C=O, C=C	3444, 2924 - 2871, 1736, 1626	Antifungal via oxidative stress induction and membrane disruption
Neem leaves (*Azadirachta indica*)	O-H, C-H, C=O, C-O	3401, 2918 - 2849, 1735, 1031	Inhibits fungal metabolism and toxin biosynthesis
Lemongrass (*Cymbopogon citratus*)	O-H, C-H, C=O, C=C	3334, 2916 - 2849, 1735, 1600 - 1650	Cell membrane damage and growth suppression
Lemon peel (*Citrus limon*)	O-H, C-H, C=O, C=C	3440, 2918 - 2849, 1735, 1597 - 1515	Sporulation inhibition and membrane destabilization
Banana peel (*Musa* spp.)	O-H, C-H, C=O, C=C	3078, 2920 - 2853, 1733, 1641 - 1517	Weak-moderate antifungal via lipid bilayer disruption

4. Discussion

The present study demonstrates that plant-derived essential oils exhibit promising antifungal activity against *Aspergillus flavus*, although their efficacy varied considerably across plant sources. Turmeric oil showed the highest inhibitory potential, while banana peel oil displayed the weakest activity. These differences are attributable to variations in chemical composition as revealed by GC-MS and FTIR analyses. Turmeric oil, dominated by turmerones and phenolic compounds, is known to disrupt fungal metabolism and downregulate aflatoxin biosynthesis genes, explaining its superior inhibition [13]. In contrast, banana peel oil, while rich in phenolics, yielded relatively weak activity, which aligns with reports suggesting that peel extracts may require synergistic formulation with other botanicals to achieve stronger antifungal effects [17]. The apparent presence of zero inhibition values in some experimental blocks reflects variability across replicates and inoculum sources rather than absence of antifungal activity, as measurable inhibition was consistently observed for turmeric and neem oils in multiple plates and concentrations. Neem and lemongrass oils also performed well, consistent with earlier studies reporting azadirachtin-rich neem extracts and citral-rich lemongrass oils as potent antifungal agents [2] [27]. The observed activity can be linked to disruption of fungal membranes, inhibition of spore germination, and interference with cellular redox balance [12]. Lemon peel oil showed moderate activity, likely due to its abundance of limonene and flavonoids, which act by destabilizing cell membranes and suppressing sporulation [28]. The chemical basis of antifungal efficacy observed here agrees with literature reports emphasizing the central role of terpenoids and phenolics in antifungal action. Disc diffusion assays further revealed variation across inoculum concentrations (C1 - C3), which were derived from maize samples collected at North Bank, Railway, and Modern markets. Differences in fungal load may explain the variability in inhibition patterns. For instance, lemon peel oil displayed clearer inhibition zones against isolates from North Bank and Railway markets than from Modern market. Turmeric oil

maintained relatively consistent inhibition across all concentrations, while neem and lemon oils occasionally produced irregular zones, likely due to differences in inoculum density, oil diffusion, and compound solubility. Such variability is commonly reported in essential oil assays, where viscosity, volatility, and polarity affect zone formation [1]. Overall, the antifungal potency ranked as follows: lemongrass and lemon peel > turmeric > neem > banana peel. This trend agrees with earlier findings reporting citrus and lemongrass oils as highly effective antifungal agents [29]. Interestingly, the synthetic antifungal control (Flucostat I.V.) did not completely suppress *A. flavus*, whereas natural oils produced measurable inhibition across isolates. This absence of measurable inhibition may be attributed to methodological limitations associated with disc diffusion assays for filamentous fungi. Systemic antifungals, particularly azole formulations, often exhibit reduced or inconsistent diffusion in solid agar media, leading to underestimation of antifungal activity. Standard susceptibility testing guidelines recommend broth-based methods rather than agar diffusion for reliable evaluation of antifungal efficacy against *Aspergillus* species. It could also be from the age of the inoculum. Therefore, the lack of inhibition observed for the positive control does not invalidate the assay but reflects known limitations of the disc diffusion method when applied to filamentous fungi [30]. The negative control (Tween-80 solution) showed no activity, confirming that inhibition resulted exclusively from plant-derived oils. Importantly, the multi-targeted nature of essential oils reduces the likelihood of resistance development compared with single-target synthetic fungicides [10].

Variability in inhibition zones across inoculum concentrations and market-derived isolates highlights the complexity of fungal response to antifungal agents. Such differences may reflect inherent genetic diversity within *A. flavus* populations, consistent with reports of strain-to-strain variation in aflatoxin production and antifungal susceptibility [6]. This underlines the need for multi-location trials and broader isolate screening to ensure consistent performance. While promising, the results should be interpreted within the limitations of in vitro assays. Laboratory-based inhibition zones may not fully reflect field conditions, where factors such as humidity, temperature fluctuations, and grain matrix effects influence efficacy. Furthermore, essential oil composition can vary with plant source, maturity, and extraction methods, affecting reproducibility [31]. Nevertheless, these findings support the growing body of evidence that botanicals represent viable eco-friendly antifungal tools.

The implications extend beyond food safety to farmer livelihoods and environmental sustainability. By utilizing locally available materials such as fruit peels and indigenous plants, farmers can generate low-cost antifungal agents while reducing agro-waste. This aligns with circular economy principles and consumer demand for chemical-free food systems [32] [33].

5. Conclusion

This study confirmed the antifungal potential of essential oils derived from lemon

peels, banana peels, turmeric rhizomes, neem leaves, and lemongrass against *Aspergillus flavus* isolated from maize. Among the tested botanicals, turmeric and lemongrass oils exhibited the strongest inhibition, neem and lemon peel oils demonstrated moderate activity, while banana peel oil was least effective. GC-MS and FTIR analyses revealed that antifungal activity was closely linked to the presence of bioactive compounds such as turmerones, citral, azadirachtin, and limonene. The findings highlight the value of plant-based extracts as sustainable alternatives to synthetic fungicides for aflatoxin management in maize. Although further field validation is needed, this work underscores the feasibility of harnessing locally available botanicals to improve food safety, protect public health, and enhance farmer resilience in Nigeria and similar environments.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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