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Antifungal Effect of *Ocimum gratissimum* on Fungi Associated with Retailed Cashew Nuts in Lokoja, Kogi State

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Post-harvest handling practises predispose cashew nuts to fungal contamination, leading to the production of mycotoxins that are harmful to human and animal health. Consequently, effective conservation methods are essential to reduce mycotoxin prevalence. The emergence of resistance to synthetic preservatives and their associated side effects has prompted the exploration of natural botanicals as alternatives. This study evaluated the antifungal effect of *Ocimum gratissimum* on fungi associated with retailed cashew nuts. Plant material was collected from the Biological Garden of Federal University Lokoja and extracted using the cold maceration technique. The poisoned food technique was employed to assess the efficacy of the extract. Results revealed that cashew nut samples had a fungal load ranging from 1.86×10^6 CFU/ml to 3.10×10^6 CFU/ml. The identified fungal isolates included *Aspergillus flavus*, *Fusarium solani*, and *Candida tropicalis*. The highest zones of inhibition were observed at 100% extract concentration, with 51.1% inhibition against *Aspergillus flavus*, 70.3% against *Fusarium solani*, and 67.1% against *Candida tropicalis*. Variations in inhibition zones may be attributed to differences in the cellular composition of the fungal isolates. This study concludes that *Ocimum gratissimum* extract exhibits significant antifungal activity against the tested organisms. The study recommends the use of *Ocimum gratissimum* as a natural antifungal preservative for cashew nuts, given its bioactive compounds that inhibit fungal growth.

Keywords: Cashew nut, fungitoxicity, *Ocimum gratissimum*, antifungal, mould

Introduction

The utilisation of medicinal plants in complementary and traditional medicine for the treatment, prevention, and management of ailments has been a cornerstone of human health practices since antiquity (Ekweogu et al., 2019). The World Health Organization (WHO) estimates that 60% of the global population relies on herbal medicine, with approximately 80% of people in developing countries depending almost entirely on these remedies for their primary healthcare needs (Khan and Ahmad, 2019). Notably, some individuals prefer herbal medications over conventional drugs due to their accessibility, affordability, and the natural therapeutic properties of their active compounds (Anukwuorji et al., 2012). Over the past decade, medicinal plants and their bioactive constituents have attracted significant research interest for their potential in managing and preventing chronic and life-threatening conditions, including cancer, diabetes, stroke, and arthritis (Bernell and Howard, 2016), as well as offering alternative treatments for mental health disorders (Venuprasad et al., 2014) and addressing the healthcare needs of the elderly (WHO, 2019).

Beyond their role in treating diverse ailments, medicinal plants serve as a foundation for developing novel pharmaceuticals for both conventional and traditional medicine. *Ocimum gratissimum*, commonly known as scent leaf, holds promise as both a voluntary remedy for various illnesses and a potential source for new drug development (Tanko et al., 2018).

The cashew plant, a member of the Anacardiaceae family, is a small to medium-sized tree primarily cultivated for its nuts, which are highly prized in global markets (Cobley and Steele, 2016). Renowned for their pleasant flavour, cashew nuts are considered a premium food delicacy. However, post-harvest processing of cashew nuts is associated with a significant risk of fungal contamination, particularly by *Aspergillus* species, which produce harmful mycotoxins such as aflatoxins (Musangi et al., 2024).

While major cashew-producing nations have adopted advanced technologies and stringent regulations for post-harvest processing, packaging, and marketing, traditional peasant methods remain prevalent in Nigeria, despite the establishment of cashew processing factories and plantation-based cultivation (Esuruoso, 2014). These traditional practices increase the susceptibility of cashew nuts to mould contamination, especially

during retail. Typically, nuts are packaged in thin, hand-knotted polyethylene bags, further exacerbating the risk. Mycotoxins from contaminated cashews pose serious health risks, including liver damage and cancer, leading to increased healthcare costs and reduced marketability (Bacha et al., 2018). As mycotoxins increasingly contribute to human and animal pathology, there is an urgent need for effective conservation strategies to reduce their prevalence, mitigate fungal contamination of cashew nuts, and extend the shelf life of packaged products.

Materials and Methods

Study Area

The study was conducted in Lokoja, Kogi State, Nigeria, a significant hub for cashew nut sourcing in Nigeria. Its strategic location facilitates access to both local and international markets, supported by climatic conditions conducive to cashew cultivation and marketing.

Sample Collection

Fresh *Ocimum gratissimum* leaves were collected in clearly labelled Ziploc bags. The botanical identity was verified at the herbarium unit of the Department of Biology, Federal University Lokoja, before being sent to the laboratory for further examination. The leaves were cleaned under running tap water and air-dried at room temperature to a consistent weight. Cashew nuts, purchased from retail establishments, were transported to the laboratory for further analysis.

Preparation of Media

Potato dextrose agar (PDA; Biolab Hungary) was used as the growth medium. A total of 39 g of PDA powder was dissolved in 1000 mL of distilled water in a sterile conical flask. The mixture was autoclaved at 121°C. To inhibit the growth of undesirable microorganisms, 0.01% powdered streptomycin was added to the sterilised medium (Olaomi, 2023).

Sample Preparation

One gram of sterile cashew nut samples was aseptically ground into powder using a sterile mortar and pestle to ensure uniform sample consistency and facilitate accurate measurement of fungal contamination levels. The ground samples were aseptically transferred into a stock solution consisting of a test tube containing 9 mL of sterile distilled water, labelled as 10^{-1}. From this stock solution, 1 mL was taken with a sterile syringe and transferred to subsequent dilution test tubes until reaching the test tube marked 10^{-9}, to reduce the microbial load before isolation. Three replicates were prepared for each setup.

Fungal Isolation and Identification

Samples serially diluted from 10^{-3} to 10^{-9} were selected for fungal enumeration. A 0.5 mL aliquot of each diluted sample was placed in the centre of a sterile Petri dish, followed by the addition of 20 mL of cooled molten PDA agar, which was gently rocked to mix with the sample (Okigbo et al., 2014). Inoculated plates were incubated at 25°C ± 2°C, and fungal growth was observed. A small piece of mycelium, free of medium, was picked with a sterile inoculating needle, spread in a stain on a slide, and covered with a clean cover slip to avoid air bubbles (Copping, 2014). The morphological, microscopic, and cultural characteristics of the isolated fungi were identified as described by Barnett and Hunter (1998).

Preparation of Botanical Extract

A modified method by Okigbo et al. (2014) was used. Dried *Ocimum gratissimum* leaves were weighed at 25 g, 50 g, 75 g, and 100 g and soaked in 100 mL of sterile warm water at 45°C to produce four distinct concentrations of warm aqueous extracts: 25%, 50%, 75%, and 100%, respectively. The setups were left for 24 hours, then filtered using muslin cloth. The filtrates were collected in sterile conical flasks and sterilised in a water bath.

Determination of Antifungal Activity of the Extracts

The poisoned food technique (Okigbo et al., 2014) was used to evaluate the effect of the botanical extract on fungal growth. For each extract concentration, 2 mL was added to sterile Petri dishes, followed by approximately 7 mL of melted PDA medium. Plates were gently rocked to ensure even distribution of the extracts and allowed to cool and set. Control plates consisted of plain PDA medium without plant extract. A 7 mm diameter mycelial disc, taken from the colony edge of a 7-day-old culture of fungal isolates, was used to inoculate both the medium-extract and control plates. Three replicates were prepared for each extract concentration and control. Inoculated plates were incubated at 22°C ± 2°C. Radial growth was measured on the third, fifth, and seventh days post-inoculation using two predrawn perpendicular lines on the back of the culture plates to determine colony diameters. Percentage inhibition was calculated using the method described by Wokocha and Nneke (2011) as follows:

$$\text{Percentage inhibition} = \frac{R1 - R2}{R1} \times 100$$

where: $R1$ = furthest radial distance of pathogen in control plate, $R2$ = furthest radial distance of pathogen in treated plates containing extract.

Data Analysis

Zones of inhibition were measured, recorded, and presented in tabular format, indicating the concentrations and mean zones of inhibition. The tabulated data were analysed using Microsoft Excel, with histograms generated to illustrate percentage inhibitions, and interpreted as described by Okigbo et al. (2014).

Results

A total of three fungal isolates were recovered from ready-to-eat cashew nut samples. Identification and characterization of the isolates were based on characteristics such as growth rate,

colony diameter, pigmentation, hyphae form, and conidium form. The recovered isolates were *Aspergillus flavus*, *Candida tropicalis*, and *Fusarium solani*. Table 1 presents the fungal counts for each isolate from the cashew nut samples. Sample A had a fungal count of 2.42×10^6 CFU/ml, Sample B had 3.10×10^6 CFU/ml, Sample C had 1.86×10^6 CFU/ml, and Sample D had 2.62×10^6 CFU/ml. Sample B exhibited the highest fungal count, potentially due to its local packaging method, while Sample C had the lowest fungal count.

The *Ocimum gratissimum* extract was tested against the identified fungal isolates. Table 2 shows the zones of inhibition of radial growth for the fungal isolates in response to the botanical extract. Measurements were performed in triplicate, and the mean zones of inhibition were calculated. The extract demonstrated inhibitory effects on all tested isolates. The highest percentage inhibition was observed at 100% extract concentration, with 51.1% against *Aspergillus flavus*, 70.3% against *Fusarium solani*, and 67.1% against *Candida tropicalis*. The extract showed higher inhibition percentages against *Fusarium solani* and *Candida tropicalis* compared to *Aspergillus flavus*, possibly due to differences in the cellular composition of the test isolates.

Table 1 Average total fungal count of ready-to-eat cashew nut samples

Sample codes	Fungal count (CFU/ml)
A	2.42×10^6
B	3.10×10^6
C	1.86×10^6
D	2.62×10^6

This study identified *Candida tropicalis*, *Fusarium solani*, and *Aspergillus flavus* as fungal species associated with the deterioration of retailed cashew nuts. These organisms have been previously linked to the spoilage of stored cashew nuts (Okigbo et al., 2014). The deterioration likely originates in the soil and spreads during storage, particularly when infected nuts show no distinguishable external symptoms (Despreaux et al., 2011). Plant extracts have demonstrated fungicidal activity against various plant pathogens (Okemo et al., 2013) and have been applied as natural preservatives for agricultural products using different fungal isolates (Okigbo et al., 2014). However, the use of natural plant products to prevent spoilage of retailed cashew nuts has received limited investigation. This study establishes that *Ocimum gratissimum* effectively inhibits the mycelial growth of fungi responsible for cashew nut spoilage.

The fungicidal efficacy of plant extracts varies depending on the test fungus, extract concentration, and plant material used (Okigbo et al., 2014). The observed variation in fungitoxicity among the test isolates, as indicated by differing percentage zones of inhibition, may be attributed to the presence of active compounds such as alkaloids, tannins, and saponins in *Ocimum gratissimum*. Additional factors influencing the efficacy of these active principles include the plant's age, the extraction solvent, the extraction technique, and the timing of plant material harvesting. According to Olaomi (2023), antifungal compounds in plant extracts contribute to reduced pathogen deterioration, inhibition of radial growth, and suppression of spore germination in vitro, consistent with findings by Okigbo et al. (2015). Variations in the extracts' fungitoxic effects against pathogens may result from the solubility of active compounds in water or the presence of inhibitors to the fungitoxic principle. These observations align with the findings of Lee et al. (2011) and Qasem and Abu-Blan (2016).

The differential susceptibility of fungi to antifungal agents is evident from the varied zones of inhibition produced by the plant extracts against the test organisms. Several studies highlight the critical role of medicinal plant extracts in managing phytopathogenic fungi (Lee et al., 2011; Choi et al., 2014). Recent research has increasingly focused on the antifungal activity of various plants, with studies evaluating extracts or complex mixtures from advanced plants against filamentous fungi, unicellular fungi, and molds (Gonzalez-Lamothe et al., 2020). The antifungal potential demonstrated by *Ocimum gratissimum* in this study supports its traditional use in medicine. This efficacy is likely due to the presence of active phytochemicals, which continue to serve as a primary source of pharmaceutical agents in conventional medications.

Conclusion

The antimicrobial properties of *Ocimum gratissimum* substantiate its medicinal applications. This study concludes that the plant contains bioactive chemical compounds and exhibits significant antifungal activity against fungi associated with the spoilage of retailed cashew nuts. The observed antifungal effects are likely attributable to the bioactive constituents previously reported in the plant extract. Consequently, this study recommends the use of *Ocimum gratissimum* as an antifungal agent against certain fungal pathogens, given its bioactive components capable of inhibiting fungal growth. Additionally, it could serve as a biopreservative in food and beverages to control harmful fungal growth and as a food additive. Further research is needed to evaluate the antifungal activity of *Ocimum gratissimum* extract against other pathogenic fungi.

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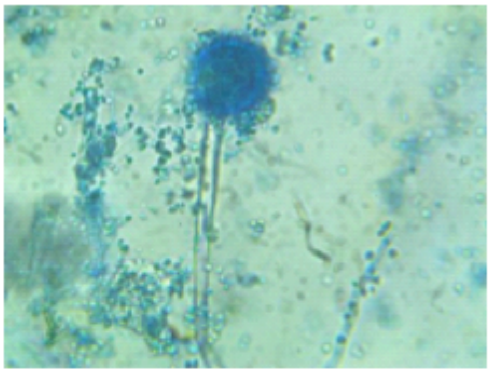


Plate I: *Aspergillus flavus*

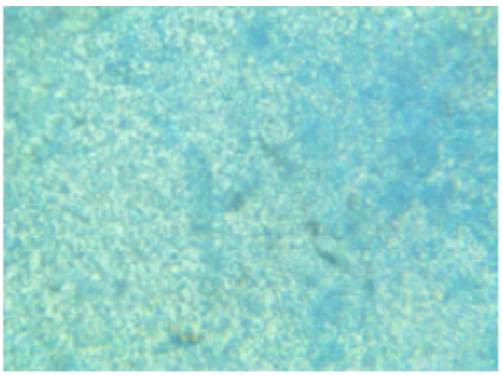
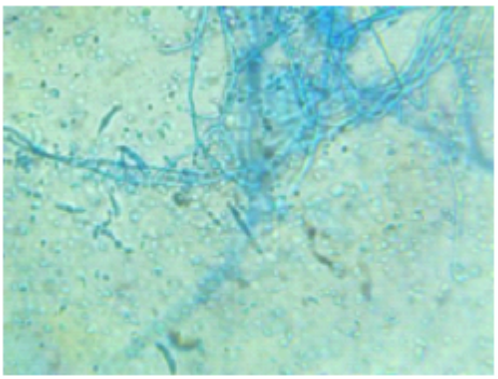


Plate II: *Candida tropicalis*



Plant III: *Fusarium solani*

Plate I: *Aspergillus flavus* **Plate II:** *Candida tropicalis* **Plate III:** *Fusarium solani*

Plate 1: *

in food products, animal feed and cereals in Tunisia. *Journal of Stored Produce Research*, *24*, 199–206.

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Table 2 Antimicrobial activity of *Ocimum gratissimum* extract to control fungi isolated from cashew nuts

Days	*Aspergillus flavus*				*Fusarium solani*				*Candida tropicalis*				C
	25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%	
1	2.5	2.0	1.5	1.0	1.50	1.15	1.10	1.00	1.17	1.22	1.20	1.00	4.0
2	3.0	2.5	2.0	1.5	1.58	1.37	1.42	1.15	1.60	1.50	1.45	1.50	4.5
3	3.5	3.0	2.5	2.0	1.63	1.80	1.75	1.20	1.80	1.70	1.60	1.64	4.8
4	4.0	3.5	3.0	2.5	1.67	1.89	1.80	1.38	1.85	1.90	1.70	1.85	5.1
5	4.5	4.0	3.5	3.0	2.00	1.95	1.95	1.95	1.96	1.90	1.85	1.90	5.4
6	5.0	4.5	4.0	3.5	2.10	2.25	2.00	1.98	2.00	2.00	1.94	1.93	5.8
7	5.5	5.0	4.5	4.0	2.40	2.50	2.30	2.00	2.00	2.00	2.00	1.95	6.2
Means (mm)	4.0	3.5	3.0	2.5	1.84	1.84	1.76	1.52	1.77	1.75	1.68	1.68	5.11
PI (%)	21.7	31.5	41.3	51.1	64.0	64.0	65.6	70.3	65.4	65.8	67.1	67.1	-

Table 3 *

C = Control; **PI** = Percentage Inhibition

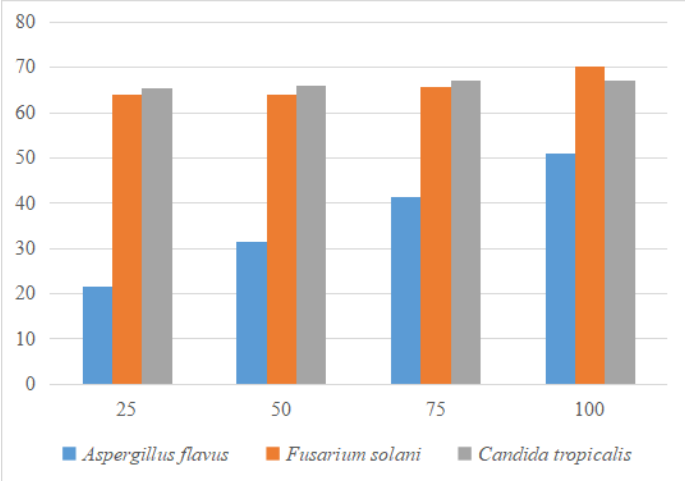

Figure 1 Percentage inhibitory activity of plant extract against tested isolates.

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