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Results should be presented concisely. Only in exceptional cases will it be permissible to present the same set of results in both Table and figure. In discussion, point out the significance of the results and place the results in the context of other work and theoretical background. Results and Discussion part could be written separately if author so wish.

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PHYTOCHEMICAL AND ANTIFUNGAL ANALYSIS OF MAHOGANY LEAF EXTRACT AGAINST FUNGI IN SPOILT WATERMELON FRUITS

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ABSTRACT

Watermelon is an important fruit vegetable cultivated globally for its diverse health and nutritional benefits. However, its production is constrained by fungal pathogens causing significant damages and crop yield losses. This study identified the fungi species in the spoilt watermelon fruits and examined the efficacy of Mahogany leaf extracts (5, 10 and 15% concentrations) against the fungi isolates. The fungi were isolated using ager plate method and pure isolates were identified in accordance with a mycological atlas. Cold maceration technique was used for the extraction of plant material while qualitative method was adopted to determine the phytochemical constituents of the leaf extracts. In-vitro antifungal assay using food poisoned technique was used to evaluate the effectiveness of the leaf extracts against the fungi isolates. The results revealed the presence of three fungi species viz; Aspergillusniger, Fusariumoxynoporum, and Penicilliumcitrinum. Among the pathogens, Aspergillusniger had the highest percentage of occurrence (51.51%), followed by Fusarium oxynoporum (33.33%) while Penicilliumcitrinum had the lowest (15.15%). The phytochemical screening showed the presence of tannins, alkaloids, glycosides, flavonoids, balsams, steroids, and terpenoids in Mahogany leaf extracts. The extracts significantly ($p<0.05$) affected the mycelial growth of the pathogens with 15% leading to the least mycelial growth, recording 24.3$\pm$0.7, 20.7$\pm$0.9 and 15.4$\pm$0.8 mm for Aspergillus niger,Penicillium citrinum and Fusarium oxynoporumcompared to the control with 93.7$\pm$2.4, 91.3$\pm$1.6 and 95.3$\pm$1.2 mm respectively. The highest effect on growth inhibition (%) was observed with 15% concentration on Fusarium oxynoporum (83.8%) followed by Penicilliumcitrinum (77.2%) while the least was recorded with 5% concentration on Aspergillusniger (40.9%). The results demonstrated the great potential of Mahogany (Khaya senegalensis (Desr.)A. Juss.) leaf extracts as a natural and eco-friendly antifungal agent against post-harvest fungal infestationin watermelon (Citrullus lanatus Thunb) fruits. They are thus recommended to be incorporated in the control regime of these pathogens to prevent contamination and to improve the quality of the fruits.

Keywords: Watermelon, Fungi pathogens, leaf extract, anti-fungal effect and fruits spoilage.

Introduction

Watermelon (*Citrullus lanatus*Thunb; Family: Cucurbitaceae) is an important fruit vegetable widely cultivated in different parts of the world, characterized with a high water content, making it a refreshing and hydrating option, especially in tropical regions of the world (Alhaji *et al*., 2020). It is a significant source of lycopene, a powerful antioxidant associated with reduced risks of cancer and heart disease, and citrulline, an amino acid that improves blood flow and reduces muscle soreness

(Alhindi *et al*., 2016; Abubakar *et al.*, 2019). Economically, watermelon is a valuable cash crop for farmers in many tropical and subtropical regions, contributing significantly to the global fruit market (Sharma *et al*., 2020). It is rich in vitamins such as vitamin C, which supports immune function, and vitamin A, which promotes healthy vision and healthy skin (Abubakar *et al*., 2019).

Watermelon production faces significant challenges due to fungal pathogens attack and diseases which result in significant yield losses of up to 50% and reduced fruit quality (Sharma *et al*., 2020). The heavy reliance on chemical fungicides has resulted in serious issues including fungicide resistance, environmental degradation, and harmful residue accumulation in food, raising health concerns for consumers and contributing to ecological imbalances (Ahmad *et al*., 2005). For farmers, this results in increased production costs due to the frequent application of fungicides and lower profits from reduced crop yields. Additionally, the long-term environmental impact of chemical fungicide use has prompted policy makers to seek more sustainable agricultural practices (Yusuf *et al*., 2020).

In response to these limitations, there is a growing interest in exploring natural, eco-friendly, and sustainable alternatives using natural products such as plant extracts, which contain bioactive compounds like limonoids and flavonoids that exhibit strong antifungal properties (Olmo *et al*., 1997; Tizhe *et al*., 2019). These natural extracts offer a safer and environmentally responsible approach to managing fungal pathogens while reducing the ecological footprint of agricultural practices (Xu *et al*., 2020). Mahogany extract contains bioactive compounds such as limonoids, flavonoids, and tannins, which have been found to exhibit potent antifungal properties against pathogens like *Fusariumoxysporum* and *Colletotrichumorbiculare*, common causes of crop diseases (Yusuf *et al*., 2020). These natural compounds work by disrupting the cellular processes of the fungi, inhibiting their growth without the harmful side effects associated with synthetic chemicals. One of the key advantages of natural fungicides is their environmental sustainability. Since they are derived from plant sources, they are biodegradable and pose minimal risk to the environment, unlike chemical fungicides that can persist in the soil and water. Furthermore, natural fungicides are less likely to contribute to the development of resistant fungal strains, as they often contain multiple bioactive compounds that target pathogens through various mechanisms, making it harder for fungi to adapt (Khattak *et al*., 2013). This makes them a more sustainable long-term solution for disease management in agriculture. Therefore, investigating mahogany leaf extracts against watermelon pathogens addresses a critical gap in sustainable agricultural practices (Kumar *et al*., 2019).

This research thus, explores the phytohemical composition and the effect of Mahogany leaf extracts against fungal pathogens in spoilt watermelon fruits in Aliero local government area of KebbiState, Nigeria. This will benefit farmers by reducing post-harvest losses and input costs. Moreover, promoting natural fungicides aligns with global agricultural policies aimed at reducing chemical inputs and protecting the environment, contributing to healthier food systems and safeguarding public health (Sharma *et al*., 2020).

Materials and methods

Study Area

Aliero local government area is located at approximately latitudes 11º 03' S, 12º 47'N and longitudes 3º 6'W and 4º 27’E. It has a total area of 412 square kilometre and is bordered in the east by Tambuwal Local government area of Sokoto state in the North West by BirninKebbi local government area in the South West by Jega local government area and has a

population size of 125,785 inhabitants. The mean annual temperature and rainfall vary considerably but usually stand at 42^0C and 500mm respectively (Abubakar *et al*., 2020).

Sample Collection and Processing

A total of twenty (20) spoilt watermelon fruits were obtained from four different locations in Aliero town using clean polythene bags. The samples were transported to the Laboratory, Department of Plant Science and Biotechnology Kebbi State University of Science and Technology, Aliero for the mycological analysis.

Sterilization of Glasswares

Glasswares that were used in the study including petri dishes, forceps, flasks, razor and blade etc were sterilized by autoclaving at 121°C for 15 minutes (Aminu *et al.*, 2017). This process eliminates potential contaminants and ensures the integrity of the experimental results.

Media Preparation

The media used for experiments werePotato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA), which were prepared according to the manufacturer's instructions. PDA and SDA were autoclaved at 121°C for 15 minutes before use.

Sample Preparation, Inoculation and Incubation

Each of the infected samples was washed and surface sterilized in 1% commercial bleach for one minute. These were then rinsed in three successive changes of sterile distilled water and blotted dry with sterile filter paper. From each sample, five pieces of segments measuring $3mm^3$ from the advancing margins of rotted lesions was cut out with flame sterile scalpel and forceps, and plated on potato dextrose agar (PDA) and SDA in 90 mm Petri dishes (Oyeleke and Manga, 2008; Oyedeji *et al*., 2022). The plates were incubated at room temperature (28 ± 2°C) for seven days to allow fungal growth.

Purification of the Fungal Isolates

When fungal growth from the tissue was visible, fungi were sub cultured onto freshly prepared sterile media plates to obtain a pure culture for identification. Where there is a mixed culture, fungi were continuously sub cultured until pure isolates were obtained. Stock cultures of the pure isolates were prepared and preserved at 4°C in the refrigerator (Oyeleke and Manga, 2008; Kumar *et al*., 2020).

Identification of Fungal Isolates

The fungal isolates were subjected to certain comparative morphological studies by an image and analysis system using published descriptions in a mycological atlas contained in the Microbiology Laboratory of Department of Biological Sciences Kebbi State University Kebbi. This was followed by a slide mount of each isolate. The characteristics observed were matched with those available in the aforementioned mycological atlas and were identified accordingly (Abubakar *et al*., 2019).

Determination of Percentage Occurrence of the Isolates

This was done to determine the percentage occurrence of the different fungal isolates. The number of occurrence for each of the isolates was recorded and calculated using the formula of Carlson (2014) viz; % frequency =Number of identified fungi/Total number of fungi X 100.

Collection of Plant Materials and Preparation of the Extracts

Fresh leaves of Mahogany (*Khaya senegalensis*) were collected from the premises of Faculty of Sciences, KSUSTA, in a clean polyethylene bag and transported to the

laboratory, Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero. They were washed carefully to remove the dusts using tap water and then shade dried at room temperature (25–30 ℃) for ten days. The dried materials were crushed to powder using mortar and pestle and the fine powder was used to determine the phytochemical constituents of mahogany leaves using qualitative analysis.

Fresh plant materials were thoroughly washed in a 0.1% bleach solution (Clorox) for 3–5 minutes, rinsed three times with distilled water, and crushed using a mortar and pestle (Majeed *et al*., 2018; Khadem *et al*., 2022). Quantities of 25, 50, and 75 g of the crushed leaves were added to 500 mL of distilled water in labeled plastic containers, followed by the addition of 10 g of *Saccharomyces cerevisiae* yeast to enhance fermentation and molasses to provide microbial energy. The containers were sealed to ensure anaerobic conditions and allowed to ferment at room temperature for two weeks, and the gas by products released weekly in sterile conditions using a laminar airflow (Khadem *et al*., 2022). After fermentation, the mixtures were filtered through a muslin cloth to obtain liquid extracts, prepared in concentrations of 5%, 10%, and 15% (w/v), and stored refrigerated for later use (Abubakar and Koul, 2023).

Extraction Techniques

The extraction was performed using the cold maceration technique as outlined by Anas *et al*. (2023). A sterile conical flask was used to soak 40 g of ground leaf samples in 350 mL of distilled water for 24 hours. The mixture was then filtered through sterile filter paper (Whatman No. 1) and concentrated in a water bath at 100°C to remove any remaining water. The final extract was stored in a refrigerator for further analysis.

Qualitative Phytochemical Analysis of the Extracts

The phytochemical screening was carried out using standard procedures to determine for the presence of tannins, alkaloids, balsams, saponins, steroids, flavonoids, anthraquinones, glycosides, resins, carbohydrates, and tarpenes using standard procedures (Anas *et al.*, 2023).

In Vitro *Antifungal Assays*

The antifungal activity of mahogany leaf extracts was assessed using the poisoned food technique, a widely used *in vitro* method for evaluating antifungal properties. In this approach, different concentrations of the mahogany extract were incorporated into PDA medium before it solidified. After solidification, a small disc of fungal mycelium from a pure culture was placed at the center of each plate. A control plate, which did not contain the botanical extract, was also inoculated. All treatments were replicated three times and arranged in a completely randomized design. The plates were then incubated at 25°C for 7 days. Fungal colony growth was measured by recording the diameter of the colony at regular intervals. The percentage inhibition of fungal growth was calculated by comparing the growth in the treated plates to that in the control plates, which lacked the extract. The formula used to calculate the percentage of mycelial inhibition (%MI) was:

$$\%MI = [(Mc - Mt) / Mc] \times 100$$

Where Mc = mycelial growth in the control plate, Mt = mycelial growth in the treated plate, and %MI = percentage mycelial inhibition (Moses *et al.*, 2015)

Statistical Analysis

The data generated were processed and subjected to descriptive statistics using percentages so as to provide summary description of the subject using descriptive statistical tools by means of tables. The data on

the efficacy of different concentration of the leaf extracts was analyzed using analysis of variance (ANOVA) in OPSTAT software and significance level of $p < 0.05$ was considered statistically significant.

Results

Fungi Isolates Identified From the Spoilt Watermelon Fruits

The mycological analysis revealed the presence of three fungi species in the rotten watermelon fruits in the study area (Table 1). These include *Aspergillus niger, Fusarium oxynoporum,* and *Penicillium citrinum. Fusarium oxynoporum* was isolated in the samples from Mallamawa and Rariya markets only, *Aspergillus niger* and *Penicillium citrinum* in samples from Nasarawa, Rariya and Kaura markets only, while all the pathogens were found in samples from Rariya market.

Cultural and Morphological Characteristic of the Identified Fungi Species

The result in Table 2 presents the three (3) fungal isolates with their cultural and morphological features as identified from the four locations of the study area.

Percentage of Occurrence of fungal Isolates in the Spoilt Watermelon Fruits

The percentage of occurrence of the fungal isolates in the spoilt watermelon fruits in the study area is presented in Table 3. *Aspergillus niger* had the highest percentage of occurrence (51.51%), followed by *Fusarium oxynoporum* (33.33%) while *Penicillium citrinum* had the lowest (15.15%).

Phytochemical constituents of Mahogany leaf extracts

The results on the qualitative phytochemical screening of mahogany leaf extracts are presented in table 4. The phytochemicals detected include tannins, alkaloids, glycosides, flavonoids, balsams, steroids, and terpenoids. However, Saponins and Anthraquinones were not detected.

Effect of the Leaf Extract against the Fungi Isolates

The effect Mahogany leaf extract at various concentrations on the growth of pathogens is shown in Table 5. The results showed that the extracts significantly ($p< 0.05$) affect the mycelial growth of the pathogens with 15% concentration causing the least mycelia growth on all the fungi isolates examined recording 24.3 ± 0.7, 20.7 ± 0.9 and 15.4 ± 0.8 mm for *Aspergillus niger, Penicillium citrinum* and *Fusarium oxynoporum* respectively (Table 5). The highest effect on growth inhibition (%) was observed with 15% concentration on *Fusarium oxynoporum* (83.8%) followed by *Penicillium citrinum* (77.2%) while the least was found with 5% concentration on *Aspergillus niger* (40.9%) (Fig1). It was observed that the antifungal effectiveness of these extracts in culture is concentration dependent.

Table 1: Number of Fungal Species Isolated from the Spoilt Watermelon in Different Markets of Aliero Town

Sample Collection Location	Fungi Identified
Nasarawa	*Aspergillus niger* and *Penicillium citrinum*
Mallamawa	*Fusarium oxynoporum*
Kaura	*Penicillium citrinum, Aspergillus niger*
Rariya	*Aspergillus niger Penicillium citrinum* and *Fusarium oxynoporum*

Table 2: Cultural and Morphological Characteristic of the Isolated Fungi

Fungi Isolates	Cultural and Morphological Features
Aspergillus niger	Colonies consist of a compact white or yellow basal felt covered by a dense layer of dark brown to black conidial heads. Conidial heads are large globose, dark brown. Conidiophores stripes are smooth walled, hyaline or turning dark towards the vesicle. It has a size of 400-3000µm
Fusarium oxynoporum	Bean shape, slightly curved. The color of the colony is purple with cottony mycelium and a dark purple under surface. The reverse color is red to purple. Growth in 5-6 days. It has a size of 3-12µm.
Penicillium citrinu M	Velvety to woolly colonies on media often having musty odor, with colors ranging from white to greenish-blue or blue-green as conidia develop. Presence of has septate, hyaline hyphae, and erect, branched conidiophores arranged in a penicillate structure, bearing spherical to ellipsoidal conidia that are roughened and form in chains. Conidia measure about 3–5 µm in diameter and give the colonies a powdery appearance.

Table 3: Percentage Occurrence of the Fungal Isolates in the Spoilt Watermelon Fruits

Fungi species	No. of Isolates	Occurrence (%)
Aspergillus niger	17	51.51
Penicillium citrinum	5	15.15
Fusarium oxynoporum	11	33.33
Total	**33**	**100**

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Table 4: Phytochemical Constituents in Mahogany (*Khaya senegalensis*) Leaf Extracts

S/NO	Phytochemicals	Presence/absence
1	Tannins	+
2	Alkaloids	+
3	Baslams	+
4	Saponins	-
5	Steroids	+
6	Anthraquinones	-
7	Terpenoids	+
8	Flavonoids	+
9	Glycosides	+

Key: + = Detected, - = Not detected

Table 5: Effect of Mahogany Leaf Extracts on the Mycelial Growth of the Pathogens.

Fungi	Mycelial growth (mm) (Mean±SE)				C.V (%)	S.D
	0%	5%	10%	15%		
Aspergillusniger	93.7±2.4	55.3±0.9	38.0±1.0	24.3±0.7	4.6	2.0
Penicilliumcitrinum	91.3± 1.6	48.0±2.1	28.7±1.2	20.7±0.9	5.7	2.2
Fusariumoxynoporum	95.3±1.2	52.3±0.3	36.3±2.9	15.4±0.8	5.6	2.3

Keys: SE= standard error, C.V= coefficient of variance, S.D= standard deviation

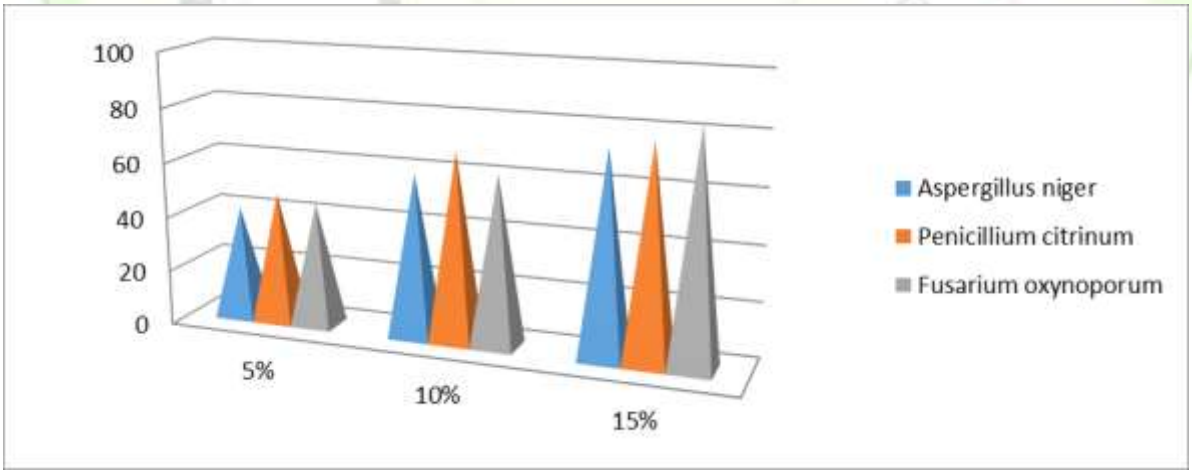

Fig 1: Effect of Mahogany Leaf Extracts on Growth Inhibition (%) of the Examined Fungi Isolates

Discussion

The current study identified three fungi species from the spoilt watermelon fruits, with *Aspergillus niger* being the most frequent (51.51%) and *Penicillium citrinum* the least (15.15%). The findings aligned partially with the results of Odelade and Oladeji (2020), who reported three fungal species, including *Fusarium oxysporum*, *Streptomyces spp.*, and *Aspergillus flavus*, from spoilt watermelon in

Ogbomoso, Nigeria. The overlap in species includes *Fusarium oxysporum*, and the total number of species is consistent with the present study. In contrast, Abubakar *et al*. (2020) identified nine fungal species from watermelon in Jega LGA, Kebbi State, Nigeria with only *Aspergillus niger* being a common species in the respective studies. Similarly, Ezekiel and Sombie (2014) reported eleven fungal pathogens on watermelon fruits in Ogun State, Nigeria, indicating higher fungal diversity in their study compared to the current report. These studies underscored the diversity of fungal species found on spoilt watermelon fruits in various regions of Nigeria. The higher number of fungal species identified in other studies may be due to environmental factors such as high temperatures and humidity, which promote fungal growth and accelerate fruit spoilage (Aladele *et al.*, 2008). The high sugar content, abundance of nutrients, and low pH of fruits made them particularly vulnerable to fungal infestation (Singh and Sharma, 2007).

Among the collection sites, Mallamawa had the highest fungal load (60.0%), while Kaura recorded the lowest (20.0%), likely reflecting differences in fruit handling, packaging, transportation, and storage practices (Aladele *et al*., 2008). Previous studies have also shown that *Aspergillus* species contribute significantly to the spoilage of watermelon and other fruits (Al Hindi *et al*., 2011; Tafinta *et al.*, 2013; Amadi *et al*., 2014; Udoh *et al*., 2015; Aminu and Ali, 2017). The fungi isolated from freshly cut watermelon fruits appear to exploit damaged or weakened surfaces to initiate rotting. Furthermore, the widespread use of poorly ventilated polythene sheets to cover unsold fruits fosters conditions that favor the growth of rot-causing pathogens, particularly yeasts (Aminu and Ali, 2017).

The global emphasis on reducing pesticide residues in food and the growing demand for organic agricultural products have spurred increasing interest in organic farming among crop scientists and farmers (Abdulla-Al-Mamun *et al*., 2019; Chaitra *et al*., 2023). Phytochemical analysis of mahogany (*Swietenia macrophylla)* leaf extracts revealed the presence of various bioactive compounds, including tannins, alkaloids, glycosides, flavonoids, balsams, saponins, steroids, and terpenoids, all of which are known for their antimicrobial properties. The study demonstrated that the extracts exhibited concentration-dependent inhibitory effects on mycelial growth, with higher concentrations leading to greater inhibition (Olmo *et al*., 1997; Paritala *et al*., 2015). These findings align with Durai*et al*. (2016a; 2016b), who identified similar phytochemicals in *S. macrophylla* extracts and reported their effectiveness against fungal species such as *Fusarium sp.*, *Helminthosporium sp.*, and *Alternaria sp.*, as well as bacterial pathogens like *Staphylococcus aureus* and *Escherichia coli*. Differences in phytochemical composition between studies could be attributed to environmental conditions and the specific plant parts used.

Additionally, compounds like methyl-1α-acetoxy derivatives, quercitrin, and rutin in mahogany extracts have been shown to possess strong antimicrobial activity against various pathogens (Olmo *et al*., 1997; Paritala *et al*., 2015). Goun *et al*. (2003) tested *S. mahagoni* seed extracts using methylene chloride and methanol and found them effective against seven microbial species, including molds such as *Rhizoctoniasolani* and bacterial pathogens like *E. coli* and *S. aureus*. However, methanol extracts were less effective, showing activity only against *R. solani*. Similarly, Haque *et al*. (2009) evaluated chloroform and ethyl acetate extracts of *S. mahogany* leaves and bark, finding significant antibacterial activity against pathogens like *Bacillus subtilis*, *Salmonella paratyphi*, and *Pseudomonas aeruginosa*. These results highlight the significant antimicrobial potential of *S. macrophylla* against both plant and human pathogens,

suggesting its potential application in sustainable agriculture and antimicrobial applications.

Conclusion

This study linked post-harvest spoilage of watermelon to the activity of pathogenic fungi, including *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium citrinum*. Aqueous extracts of mahogany leaves, tested at different concentrations, were found to significantly inhibit the mycelial growth of these fungi. The results indicate that the effectiveness of the extracts increases with concentration, suggesting their potential as a natural solution for preventing post-harvest deterioration in watermelon. Integrating these extracts into a comprehensive fungal control strategy could help extend the shelf life of watermelons and other fruits, reducing spoilage and promoting sustainable post-harvest management.

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