Trop J Nat Prod Res, December 2023; 7(12):5664-5669

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical and *In vitro* Antifungal Evaluation of Some Selected Leaf Extracts against Fungi Associated with *Sesamum indicium* (seeds) Spoilage within Dekina Local Government Area, Kogi East, Nigeria: A Molecular Approach

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ARTICLE INFO

Article history:
Received 10 January 2023
Revised 07 October 2023
Accepted 30 November 2023
Published online 01 January 2024

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ABSTRACT

Sesame (*Sesamum indicum* L.) is one of Nigeria's most important oil crops, effective storage is still a leading problem with the preservation of sesame. Fungal infestation of sesame is a major cause of spoilage. The present study aimed to evaluate the potential biocontrol agents of some selected plant extracts (*Cymbogogon citratus, Moringa oleifera, Azadirachta indica* (Neem), and *Reuwolfiea vomitoria*) against four sesame seed spoilage heterotrophic fungi (*Fusarium monoliforme, Aspergillus flavus, Alternaria sesame*, and *Penicillium* sp.). Infected seed samples of sesame were collected from different sesame farms within Dekina local government and its environs to isolate fungal pathogens. The *in-vitro* potential of the tested plant extracts was evaluated against four isolates of phytopathogenic heterotrophic fungi in an agar well diffusion method. A total of 281 heterotrophic fungi were isolated. *Alternaria sesame* (number of isolate (n) = 91) had the highest distribution, while *Fusarium monoliforme* had the lowest distribution (n = 19). Extract concentration was impassively proportional to antifungal activity across all the fungal isolates at P<0.005. The antifungal activity of some selected extracts *Cymbogogon citratus, Moringa oleifera, Azadirachta indica* (Neem), and *Reuwolfiea vomitoria*) on the fungal isolates associated with sesame seed within Kogi East and its environs showed moderate antifungal activity (mycelium growth between 20 -60% across all test isolates). It thus offers a choice alternative in the preservation and processing of sesame seed within the region and the country.

***Keywords*:** Sesame seed, antifungal, Phytochemical, plant disease, fungi, and molecular detection.

Introduction

Sesame (*Sesamum indicum* L.), often called Benniseed, is a plant in the Pedaliaceae family and one of the oldest oil seed crops ever grown by humans.

The *Ebira* people of Kogi Central, Nigeria, refer to benniseed by the original local name "gorigo." At the same time, the Hausas, Yorubas, and Igbos—three of the largest tribes in Nigeria—call it "Ridi," "Isasa," and "Ekuku," respectively. Other tribes in Nigeria, including the Tiv, who consume a lot of sesame seeds, call it by the Ishwa.[2]

Sesame seeds can be consumed, but their oil is the most valuable commodity in the world.[3]

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Citation: Opisa AN, Simon EC, Bello KE, David ZA, Adegoke AS, Lamidi Y, Zainab A, Patience A, Dorcas AR. Phytochemical and Invitro Antifungal Evaluation of Some Selected Leaf Extracts against Fungi Associated with *Sesamum indicium* (seeds) Spoilage within Dekina Local Government Area, Kogi East, Nigeria: A Molecular Approach. Trop J Nat Prod Res. 2023; 7(12):5664-5669. http://www.doi.org/10.26538/tjnpr/v7i12.44

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

One of the most significant barriers to optimum sesame production, along with a weak genetic base, is susceptibility to biotic and abiotic stresses.[4]

Among these critical diseases affecting sesame, fungi infestation has been recognized as the primary causes of the loss in sesame production in Nigeria.[5]

Alternaria, Curvularia, Fusarium, Penicillium, and *Rhizopus* species have been reported to be connected to sesame spoilage in the past.[6]

Farmers in Kogi State were upset over losing their stored sesame. Fungi are the primary cause of post-harvest losses, and Kogi farmers will experience a drop in sesame yield and market value if they are not adequately managed.[7] Even though sesame is essential to human nutrition, medicine, and culture, little is known about how infections damage sesame and its pathophysiology.

The post-harvest attack by pathogens during storage is one of the main elements working against Nigeria's sesame crop's ability to be produced at its best.[8] An excellent first step in developing a sesame management strategy will be to isolate and identify the fungus responsible for this loss. By controlling these fungi, the post-harvest loss of sesame seed in storage can be reduced. There have been a series of reports on the antifungal activity of *Cymbogogoncitratus, Moringa oleifera, Azadirachta indica* (Neem),and*Reuwolfiea vomitoria* in Nigeria .[8] This study aimed to determine the presence of fungi related to sesame seeds spoilage in the Dekina LGA and assess

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

the efficacy of using specific leaf extracts to curb and manage these fungi during storage.

Materials and Methods

Study Area

The study was conducted in Kogi State's Dekina Local Government Area in north-central Nigeria. The distance between Lokoja, the state capital, and Dekina is 89 kilometers. It is 80 kilometers (km) from the state capital, Lokoja, and is located between Latitude 70.30N and Longitude 60.42E.[7] According to the 1991 population census, the local government's estimated population of Dekina was around 1,000,000,000. The local government has sixteen communities, and the area has a tropical environment.[9] The Dekina people's primary occupation includes agriculture, fishing, and trade. Igala-speaking tribes from the ancient kwararafa empire make up the Dekina people.

Sample collection: sesame seeds

Sesame seeds showing visible signs and symptoms of spoilage/fungal infections were collected from farmers who have stored the dried seed in air tight bags in farm stores for 2-6 months in Abocho, Okabo, Ochadamu, and Agbeji within Dekina LGA between January – June, 2021 as described by Adebisi (2005).[10] Two hundred and forty seeds were collected from each village from ten farmers. The collection sites represented different geographic locations within the mentioned villages at varying elevations and with other climatic conditions. The collected sesame were identified by standard identification key to be food grade (white coloured sesame seed) and oil grade (Brown sesame seed) seeds and were transferred to the biological laboratory of the University of Nigeria Nsukka in sterile polyethylene bags for further screening.

Sample preparation

Sesame fungal pathogen isolation: A 1:10 dilution of the sample and diluent was made by suspending ten grams of pulverized sesame seed in 90 mL of sterile distilled water and vigorously mixing it. Following that, a modified Czapek Dox Agar (CDA) supplemented with 250 mg/ml of streptomycin was used to make a series of tenfold dilutions in the range of 10-1 to 10-5 and seeded onto it to prevent bacterial growth. Every plating was repeated in duplicate.[9]

For 3-6 days, plates were incubated at 28^{o}C, and the growth of the colonies was monitored every day. For purification, a PDA medium was used to culture and sub-culture sesame seed fungal pathogens. For subsequent testing, all pure cultures were kept in an agar slant at 4^{o}C.

Sesame fungal pathogen identification: These pathogens were identified based on their morphological, microscopic, and growth properties.[10] The factors that were used to categorize the sesame fungal infections included pigment production, colony color, structures that produced spores or conidia, and spore morphologies.[10] The features of the spore and mycelium were examined using a compound microscope with 400x magnification (Olympus Microscope, Germany). Following the guidelines outlined by Mathur and Kongsdal, these traits were used to identify the fungal isolates at the genus level. The identified genus of fungi was also subjected to the following molecular characterization steps:

-Nucleic acid extraction: Nucleic acid will be extracted from our maintained culture using a QIAmp DNA kit (Qiagen, Hilden, Germany) following the manufacturer's instruction and stored at - 20°c in aliquot until use.

-Detection of Fungal Genomes

The genome of the fungi was determined using established specific primers in a conventional PCR with varying PCR conditions, as reported in table 1.

The PCR amplicon will be analyzed on 1.5% tris-borate-EDTA (TBE) agarose gel electrophoresis and viewed in a UV light Transilluminator. Each sample will be tested at least in duplicate.

-Sequencing and Characterization

After purification of the amplified products, a bidirectional sequencing was conducted with 1st base Apical Scientific (Selangor, Malaysia). Sequencing results were edited by Bioedit software. The resulting sequences were blasted in a nucleotide blast cascade in NCBI to retrieve the standard fungi nomenclature. The Fungal genus genetic reference sequences for each fungus were recovered from NCBI GenBank. Phylogenetic trees were created using the Maximum Likelihood method (MEGA, V11). The reliability of phylogenetic trees was tested 1000 times by bootstrap.

Collection of plant leaves

After physical examination of the plant (*Cymbogogon citratus, Moringa oleifera, Azadirachta indica* (Neem), and *Reuwolfiea vomitoria*), healthy looking and uninfected leaves of the used plants were collected from the plant garden of Kogi State University, Anyigba. The leaves were washed under running water to remove dust particles and other particles before air drying them in the laboratory away from direct sunlight. All plants were identified using a standard identification key at Bioresources Development and Conservation Programme (BDCP) in Nsukka, Nigeria.

Preparation of leaf extracts

Fresh leaves (20 - 30 g) *of Cymbogogon citratus, Moringa oleifera, Azadirachta indica* (Neem), and *Reuwolfiea vomitoria* were air dried at room temperature (32 – 35 °C) away from direct sunlight for five days. The dried leaves were pulverized by a mortar and pestle and were further blended into powder using a tabletop blender at a moderate speed.[10]

A 20 g of the powdered leaves were added into a 180 ml of (70 %) ethanol in a Soxhlet extractor and was allowed to extract for 10-15 hours. The net extract yield was measured for the ethanolic extract. All extracts were stored at 4^{o}C till use.[10]

Phytochemical Analysis

The extracts underwent a phytochemical examination for the qualitative detection of alkaloids, flavonoids, steroids, volatile oil, glycoside, reducing sugar, tannins, and saponins.[10]

Plant extracts' impact on mycelial

Each of the plant extract concentrations was divided into two milliliters (2ml) portions and aseptically distributed into sterile Petri plates before being covered with Sabouraud Dextrose Agar (SDA) and gently rocked to combine the components. The latter procedure was repeated for concentrations of 80 mg/ml, 60 mg/ml, 40 mg/ml, and 20 mg/ml. [10]

A 2mm mycelial disc of the test fungi was sliced into the center of the SDA Petri dishes modified with varying concentrations of the plant extracts. All plates were incubated upside down at 28^{o}c for 72 hours. Negative control plates were with no extracts and only SDA, while a positive control plate was incorporated with 8.5mg/l of Benlate in place of the tested plant extract for quality check. The Diameter of mycelium growth was measured to the nearest millimeters. [11]

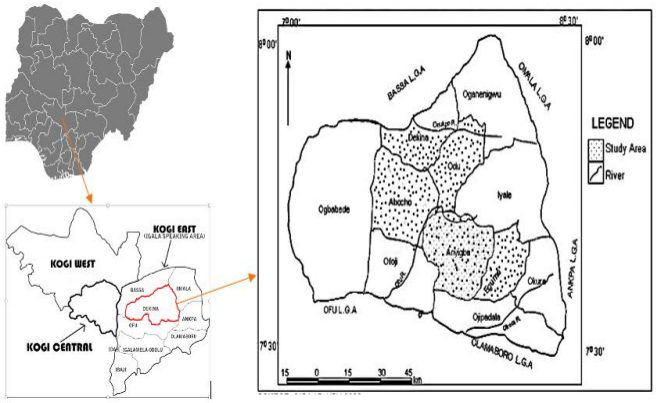

Figure 1: Map of Dekina showing the sample sites

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Statistical analysis

Descriptive statistic was used to present the antifungal activity and mycelium growth in percentages. Standard error was used to measure the error indexes in charts. IBM Statistical package for Social Science (SPSS) version 29 was used for the analysis. Chisquare was used to measure the level of association between the plant extracts and the level of fungal mycelium growth.

Result and Discussion

A total of four different heterotrophic fungi were isolated. *Alternaria spp* (n=91) had the highest distribution, while *Fusarium monoliforme* had the lowest distribution (n=19) (Figure 2). The representative organism of the four fungal groups was amplified (Figure 3), sequenced, and blasted against reference sequences from the GenBank. The phylogenetic tree construction reveals four different genera, as shown in Figure 4.

The effects of the ethanolic extracts of the tested plants against the fungi isolated in the study to be associated with sesame food spoilage were examined. The mycelium of Alternaria sesame was inhibited at a higher extract concentration. The percentage inhibition of Alternaria sesame mycelium decreases as the concentration reduces for *C. citratus* and *M. olifera* (Table 2). *Aspergillus flavus penicillium spp*and *Fusarium moniforme* demonstrated a similar pattern of mycelium inhibition when tested with varying concentrations of the extracts. The higher the concentration, the higher the inhibition index of the extracts on the fungal mycelium, as represented in Table 3-5.

The phytochemical screening of the collected plant samples revealed a variety of bioactive components, ranging from Tannin, Alkaloids, flavonoids, and others (Table 1). *M. olifera* had the highest proximate value for Reducing sugar, while *A. indica* had the highest Tannin and alkaloid content (Figure 5).

Cymbopogon citratus, *Moringa oleifera*, *Azadirachta indica*, and *Rauwolfia vomitoria* had their ethanolic leaf extracts photochemically screened to find tannins, alkaloids, flavonoids, saponins, phenols, and glycosides. However, the quantitative makeup of the bioactive substances varies between the various plant leaf extracts screened. The leaf extracts of numerous plants have been discovered to have similar bioactive components. [12-14]

The findings of this study demonstrate that the ethanolic plant leaf extracts of all four plants evaluated displayed intense levels of antifungal activity against all fungal species connected to sesame seeds in the four communities examined. According to Sultana and Ghaffar,[15] several of these fungi linked to the sources carried by sesame seeds are not strange.[16]

The findings of this investigation also demonstrate that all concentrations of all screened ethanolic plant leaf extracts were highly effective at preventing the mycelial proliferation of all isolated fungus connected to sesame seeds. But *Azadirachta indica* was more effective than other plant leaf extracts. This could be explained by a higher concentration of bioactive compounds in the leaves of A. indica compared to the other examined plants.

The biological activity of a variety of plant products, including plant leaf extracts, gum, resin, and essential oils, have been demonstrated to be extracted both in vitro and in vivo and are employed as bio-fungicide compounds.[17-19]

The primary benefit of employing plant leaf or other plant part extracts as antifungal medicines is their natural origin and low likelihood of infections acquiring resistance to them.[17] Gnananickam (2002)[20] asserts that they may have little adverse effects on plants' physiological functions and little to no environmental risks.[20]

This investigation revealed the antifungal properties of plant leaf extract on typical sesame seed infections. The findings of earlier studies on the antibacterial or antifungal properties of specific plant-based extracts [16-21] are consistent with this finding.[22]

Aspergillus flavor, Fusarium moniliforme, and Penicillium sp. have been identified as sesame seed-borne fungi, which raises significant health concerns because they are known to create highly potent mycotoxins that are harmful to people and animals like chickens if consumed.[23]

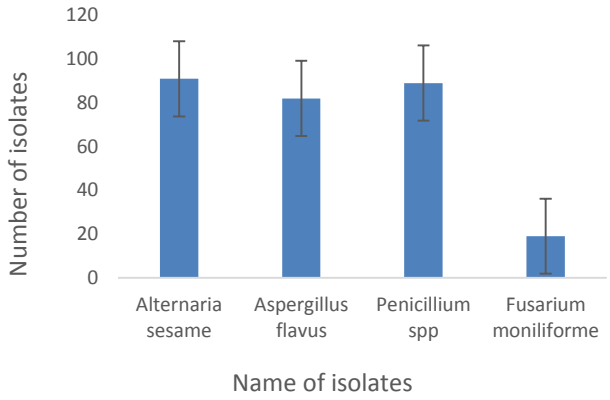
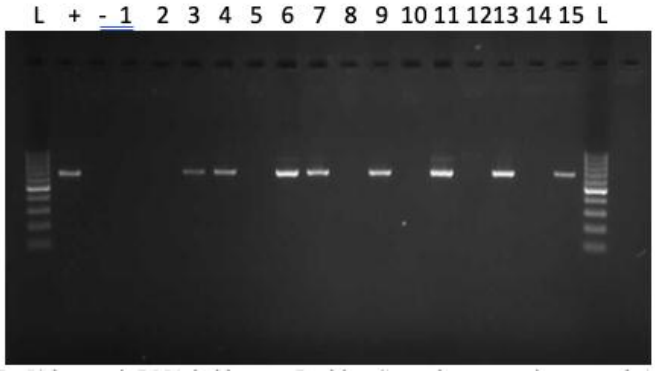


Figure 2: A bar chat showing the number of the different isolated fungi



L- 50 base pair DNA ladder, + = Positive Control, - = negative control, 1-15 = fungal sample well

Figure 3: An agarose gel image showing positive bands of Aspergillus flavus DNA amplification at 380 bp.

Table 1: Qualitative composition of phytochemicals in the test leaves

Phytochemical	*C. citratus*	*M. oleifera*	*A Indica*	*R. vomit*
Tannin	+	+	+	+
Alkaloid	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Phenol Reducing sugar	+	+	+	+
Glycosides Volatile oil	+	+	+	+

Key: + = present

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

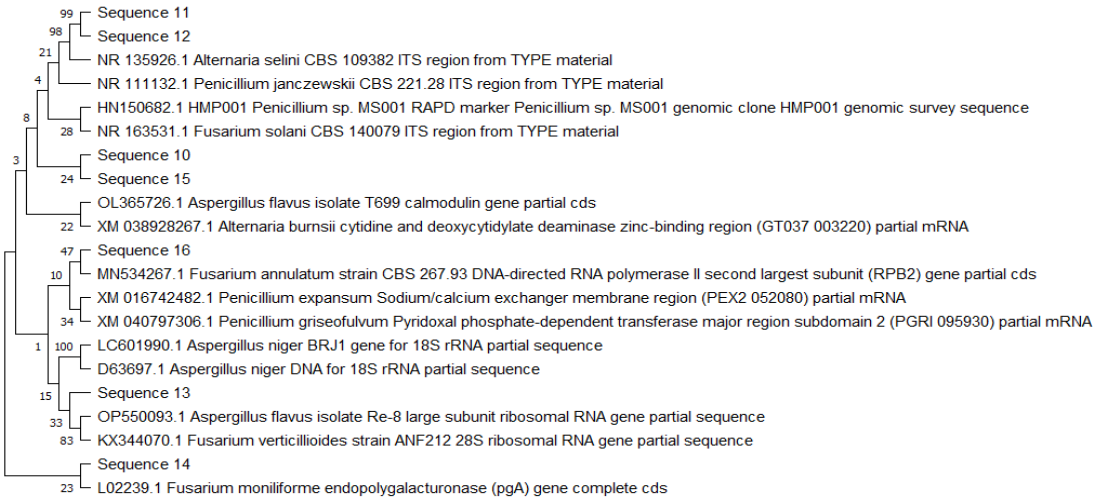

Figure 4: A phylogenetic tree showing the genetic related of isolated fungi (sequence 10 -16) based on their genetic relatedness

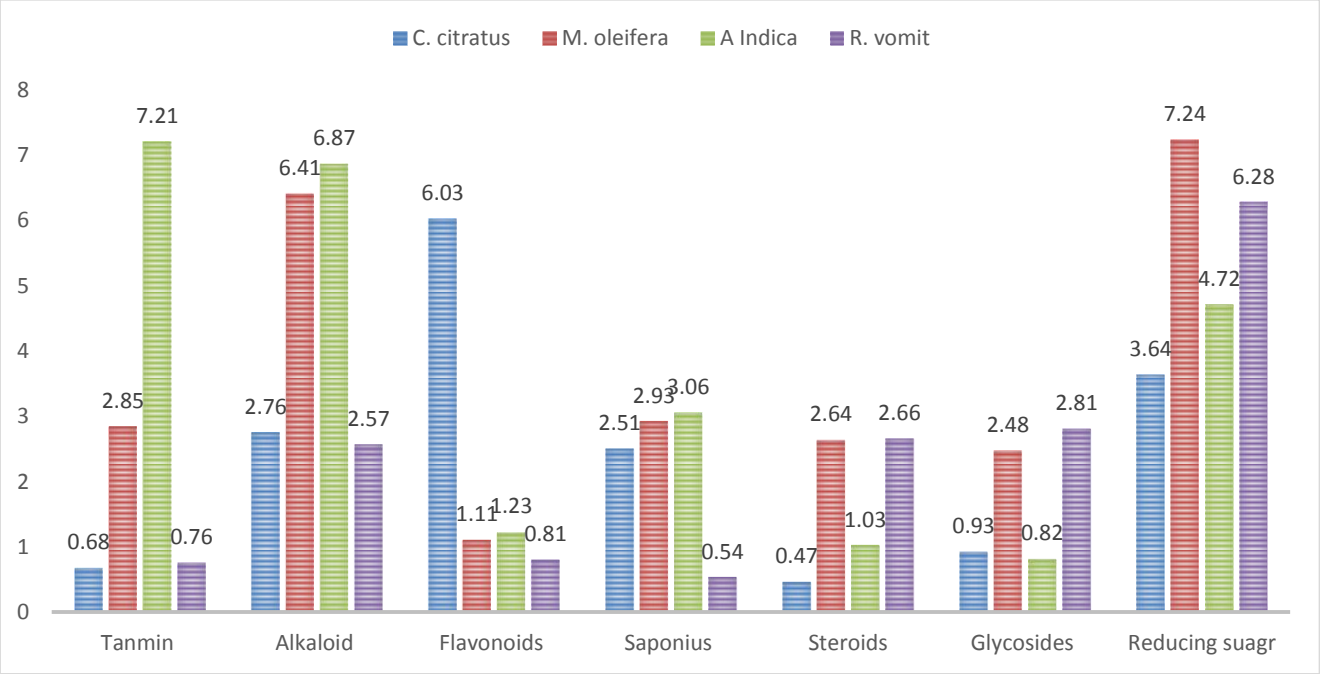

Figure 5: A multiple bar chart showing the quantitative mean values of the phytochemicals of the plant extracts.

Table 2: Effect of leaves ethanolic extract of *C citratus*, *M. Oleifera*, *A. Indica* *R. vomitoria* on mycelial Growth (mm) of *Alterania sesame*

Concentration (mg/ml)	*Cymbopogon citratus*		*Moringa oleifera*		*Azadirachtainidca indica*		*Rauwolifia vomitoria*	
	M.G (mm)	% I	M.G (mm)	% I	M.G (mm)	% I	M.G (mm)	% I
100	0.00	100	0.00	100	0.00	100	0.00	100
80	0.00	100	0.00	100	0.00	100	0.00	100
60	2.44	63.91	1075	89.69	1.27	82.19	1.98	72.80
40	3.49	48.37	3.26	55.21	2.64	62.97	2.65	59.62
20	4.83	28.55	4.48	38.46	3.83	46.28	3.22	52.37
Benlate	0.00	100	0.00	100	0.00	100	0.00	100
N.C	6.37	0.00	6.78	0.00	7.28	0.00	7.13	0.00
LSD P≤ 0.05	1.47		1.69		0.87		1.22	

Key: M.G-Mycelium Growth, I-Inhibition, N.C-Negative Control.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Effect of ethanolic extract of the leaves of *C citratus. M. oleifera, A. indica, and B.Vomitoria* on mycelial Growth (mm) of *Aspergillus flavus*

Concentration (mg/ml)	*Cymbopogon citratus*		*Moringa oleifera*		*Azadirachtainidica*		*Rauwolifia vomitoria*	
	M.G (mm)	**% I**	**M.G (mm)**	**% I**	**M.G (mm)**	**% I**	**M.G (mm)**	**% I**
100	0.00	100	0.86	89.49	0.00	100	0.74	89.26
80	1.27	82.19	0.93	83.92	0.00	100	1.98	72.80
60	2.54	65.72	2.39	65.31	1.27	81.56	2.43	61.66
40	3.86	52.14	3.41	50.62	2.53	60.83	3.55	58.63
20	4.87	34.72	3.86	47.11	3.07	45.63	4.05	38.92
Benlate	0.00	100	0.00	100	0.00	100	0.00	100
N.C	7.46	0.00	6.84	0.00	7.37	0.00	7.88	0.00
LSD P≤ 0.05	1.49		1.81		0.76		1.32	

Key: M.G-Mycelium Growth, I-Inhibition, N.C-Negative Control

Table 4: Effect of Ethanoic extract of the leaves of *C. citratus, M oleifera, A. Indica and R,Vonitoria* on mycelial Growth of *Penicillium spp*

Concentration (mg/ml)	*cymbopogon citratus*		*Moringa oleifera*		*Azadirachtainidica*		*Rauwolifia vomitoria*	
	M.G (mm)	**% I**	**M.G (mm)**	**% I**	**M.G (mm)**	**% I**	**M.G (mm)**	**% I**
100	0.00	100	0.00	100	0.00	100	0.00	100
80	0.54	91.46	0.47	93.28	0329	96.87	038	94.67
60	1.46	77.21	1.68	76.86	1.53	82.16	1.98	78.13
40	3.12	58.73	2.88	66.28	2.17	75.92	3.03	60.58
20	4.95	36.49	3.43	43.11	3.07	51.23	4.83	39.21
Benlate	0.00	100	0.00	100	0.00	100	0.00	100
N.C	6.88	0.00	7.21	0.00	6.76	0.00	7.08	0.00
LSD P≤ 0.05	1.03		1.18		0.59		1.22	

Key: M.G-Mycelium Growth, I-Inhibition, N.C-Negative Control.

Table 5: Effect of ethanolic extract of leaves of *S. atratus, M. oleifera, A. indica, and R. vomitoria* on mycelial growth (mm) of *Fusarium moniliforme*

Concentration (mg/ml)	*Cymbopogon citratus*		*Moringa oleifera*		*Azadirachtainidica*		*Rauwolifia vomitoria*	
	M.G (mm)	**% I**	**M.G (mm)**	**% I**	**M.G (mm)**	**% I**	**M.G (mm)**	**% I**
100	1.53	82.06	2.14	71.48	0.00	100	1.87	74.24
80	2.67	68.70	3.21	52.38	1.06	85.81	2.34	62.83
60	3.44	46.70	3.62	43.05	2.27	67.43	3.54	53.67
40	3.86	30.23	4.03	36.11	2.79	53.56	4.004	34.28
20	2.15	22.17	4.71	28.46	3.62	3507	4.97	21.38
Benlate	0.00	100	0.00	100	0.00	100	0.00	100
N.C	8.53	0.00	8.12	0.00	7.78	0.00	8.03	0.00
LSD P≤ 0.05	1.37		1.04		0.71		1.14	

Key: M.G-Mycelium Growth, I-Inhibition, N.C-Negative Control.

A. flavus also produces aflatoxins, which have decreased seedling elongation, impede chlorophyll production, inhibit various enzymes, and degranulate the endoplasmic reticulum.[24] In this regard, great care should be taken to prevent seed-borne fungus from growing on the seeds intended for eating and planting.

The fact that these plant leaf extracts outperform Benlate, a common chemical fungicide, in preventing the mycelial growth of sesame seed-borne fungus is relevant to the study's results. According to odd control tests,[25] benlate significantly reduces the fungal rot of yams and cocoyams.[26]

The fungicidal ability of these plants has been positively identified based on the findings above on the activity of plant leaf extracts on plant pathogenic fungi. Given that it has been demonstrated that plant leaf extracts can be used to prevent the spread of diseases that affect sesame and other seeds, their usage as preventative insecticides should be promoted[27] To reduce the harmful fungi in the soil, their leaf litter could be used as manure, as initially suggested by Okigbo *et al.*[28] The development of organic fungicides from those plants should therefore be the focus of future research because they are safer and more readily available in our communities than current chemical fungicides, which have adverse environmental effects.

Conclusion

The antifungal activity of some selected extracts on the fungal isolates associated with sesame seed within Kogi east and its environs shows

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moderate antifungal activity. It thus offers a choice alternative in the preservation and processing of sesame seed within the region and the country.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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