



Occurrence and Pathogenicity of Fungal Pathogens in Guinea Corn, Millet and Wheat Seeds and their Control with Extracts of Garlic (*Allium sativum*) Cloves and Pawpaw (*Carica Papaya*) Seeds

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Received: June 23, 2025

Published: July 28, 2025

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Abstract

This study investigated the prevalence, pathogenicity, and in vitro control of fungal pathogens associated with wheat, sorghum, and millet seeds using garlic (*Allium sativum*) cloves and pawpaw (*Carica papaya*) seed extracts. Extracts were tested at concentrations of 0g/200 mL, 50g/200 mL, 100g/200 mL, and 150g/200 mL, with experiments conducted in a completely randomized design, replicated three times, and incubated at $28 \pm 2^\circ\text{C}$ for five days. The fungal pathogens identified included *Aspergillus niger*, *A. flavus*, and *Lasiodiplodia theobromae*, with *A. niger* showing the highest frequency in sorghum (53.33%), millet (58.33%), and wheat (68.75%), while *L. theobromae* had the lowest frequency in these cereals (sorghum: 26.67%, millet: 16.67%, wheat: 12.50%). Pathogenicity tests revealed that *A. niger* and *L. theobromae* were highly pathogenic to wheat and very highly pathogenic to sorghum and millet. Garlic and pawpaw extracts effectively inhibited the radial growth of these fungi, with the highest concentration (150g/200 mL) exhibiting the greatest antifungal activity. Significant differences ($P \leq 0.05$) were observed for growth inhibition across incubation periods, extract types, and fungal pathogens. The findings suggest that garlic and pawpaw extracts at optimized concentrations can serve as eco-friendly alternatives for managing cereal-associated fungal pathogens, particularly wheat, sorghum, and millet. Formulating these extracts into sustainable seed treatment technologies could help mitigate post-harvest losses, enhance food security, and align with environmentally sustainable agricultural practices.

Keywords: Botanical Extracts; Food Security; Garlic Pathogenicity Test; Pawpaw; Post-Harvest; Seed-Borne Fungi

Introduction

Cereal crops such as guinea corn (*Sorghum bicolor*), millet (*Pennisetum glaucum*), and wheat (*Triticum aestivum*) are staple food sources globally, particularly in sub-Saharan Africa, where they contribute significantly to food security and livelihoods. In Nigeria, these staple cereals such as guinea corn (*Sorghum bicolor*), millet (*Pennisetum glaucum*), and wheat (*Triticum aestivum*) constitute a significant portion of the diet and serve as important sources of income for millions of rural farmers. Despite their importance, fungal infections during cultivation, storage, and processing threaten

the quality and yield of these crops. This challenge is exacerbated in regions like Dutsin-Ma, Katsina State, where the climatic conditions favor fungal proliferation, necessitating urgent and sustainable intervention strategies. Pathogenic fungi, including species of *Fusarium*, *Aspergillus*, *Penicillium*, and *Colletotrichum*, have been identified as major contributors to seed-borne diseases which not only compromise seed viability but also, lead to reduced germination rates, nutritional degradation, and the production of harmful mycotoxins [1,2]. Fungal pathogens are among the most destructive agents in cereals, causing significant pre- and post-harvest

losses. Effective management of these pathogens is thus critical for improving crop productivity, ensuring food safety, and protecting public health.

Traditional control measures often involve chemical fungicides, which, while effective, pose environmental and health risks, including residual toxicity and the emergence of resistant fungal strains and high costs [3,4]. Consequently, there is an increasing demand for eco-friendly and sustainable alternatives, such as plant-based biofungicides [5,6]. Garlic (*Allium sativum*) and pawpaw (*Carica papaya*) are well-documented for their antifungal properties, attributed to bioactive compounds like allicin in garlic and papain in pawpaw [7,8]. The use of plant-based biofungicides, derived from natural extracts with antifungal properties, has gained increasing attention as a viable alternative to synthetic chemicals [9]. Plant such as Garlic (*Allium sativum*) and pawpaw (*Carica papaya*) are two plants with well-documented antimicrobial activities that offer promising potential for controlling fungal pathogens. Garlic contains bioactive compounds such as allicin, ajoene, and sulfur compounds, which exhibit broad-spectrum antifungal activity [8]. Similarly, pawpaw seeds are rich in phenolic compounds, flavonoids, and proteolytic enzymes, which have shown efficacy against a range of fungal species [7]. The integration of these plant extracts into seed treatment protocols represents a sustainable and eco-friendly approach to disease management in cereal crops.

This study investigates the occurrence, pathogenicity, and control of fungal pathogens affecting guinea corn, millet, and wheat seeds in Dutsin-Ma, Nigeria. The objectives include identifying the prevalent fungal pathogens affecting these cereals, evaluating their pathogenic potential, and assessing the antifungal efficacy of garlic and pawpaw seed extracts. Furthermore, the research seeks to contribute to sustainable agricultural practices, addressing the challenges of food security and post-harvest losses in rural communities by supporting the development of sustainable seed treatment technologies that align with local agricultural practices and environmental conservation goals.

Materials and Methods

Study area

The study was conducted at Federal University Dutsin-Ma, Katsina State, Nigeria. The study area is characterized by a semi-arid climate with temperatures ranging from 25°C to 40°C, an average annual rainfall of 600-800 mm, and relative humidity conducive to fungal growth. The selection of Dutsin-Ma was based on its agricultural significance and reported challenges with diseases and cereal storage.

Collection of diseased seed samples

Guinea corn (*Sorghum bicolor*), millet (*Pennisetum glaucum*), and wheat (*Triticum aestivum*) seeds were collected from farms and stores in Dutsin-Ma. Each sample was collected in sterile polythene bags to prevent contamination. Samples were taken to the laboratory for isolation and identification of pathogens.

Preparation of Potato Dextrose Agar (PDA) medium

Potato Dextrose Agar (PDA) was prepared by dissolving 3.8 g of powdered PDA in 120 mL of sterile water in a conical flask then heated on hot plate and stirred until fully dissolved. The conical flask was sealed with cotton wool and foil paper. The PDA solution was then autoclaved at 121°C for 15 minutes and allowed to cool down to 45-50°C in water bath. Exactly 0.12 g of Streptomycin Sulphate powder was added into the medium to suppress the growth of bacterial. Exactly 20 mL of the molten PDA was poured into sterile Petri dishes and allowed to solidify before inoculation of the prepared plant tissues.

Sterilization and inoculation of seed samples

Seed samples of guinea corn, millet, and wheat were surface-sterilized using 5% Sodium hypochlorite solution, rinsed in three successive changes of distilled water, and air-dried [10] before inoculation of the diseased tissues on PDA. Four seeds of each sample were plated on PDA medium in Petri dishes and the plates were incubated at $28 \pm 2^\circ\text{C}$ for 5 days until fungal colonies appeared.

Isolation and identification of fungi

Fungal colonies that emerged were sub-cultured on freshly prepared PDA for another 5 days to obtain pure cultures. Morphological characteristics such as colony color, texture, and sporulation and microscopic features such as conidia shape and size were used to identify fungi based on standard manuals [11].

Determination of frequency of occurrence of the pathogen

The frequencies of the isolated pathogens were determined by counting the number of times each isolate occurred out of the total number of isolates grown on PDA during the period of isolation on each crop. This was expressed as a percentage of the total number of all isolates on each crop as described in the equation below.

$$\% \text{ frequency of occurrence} = \frac{a}{b} \times \frac{100}{1}$$

a = Number of times each isolate was encountered on a particular crop.

b = Total number of times the different isolates were encountered on a particular crop.

Pathogenicity test

Healthy seeds of guinea corn, millet, and wheat were surface-sterilized with 5% sodium hypochlorite solution, then rinsed in three successive changes of sterile distilled water to remove residual bleach, air-dried and inoculated with spore suspensions (1×10^6 spores/mL) of isolated fungi. The spore suspensions (1×10^6 spores/mL) were mixed with 20 mL of PDA and allowed to solidify. Seeds were incubated on the solidified PDA and covered with moist filter paper in Petri dishes for 7 days at $28 \pm 2^\circ\text{C}$. Control seed plates were treated with sterile distilled water only and observed under same condition. Symptom development was monitored throughout the incubation period using a scale developed for the experiment as follows

Grade	Scale (%)	Symptoms	Classification of isolates
0	0-20	No or little infection	No Infection
1	21-40	Presence of infection	Slight infection
2	41-60	Infection conspicuously present	Moderate infection
3	61-80	Infection present in large quantity	High infection
4	81-100	Whole seed completely infected	Very high infection

Table 1: Scale for pathogenicity test.

Preparation of plant extracts

Precisely 0g, 50g, 100g, and 150g of pawpaw seed and garlic clove powders were accurately weighed using an electric weighing machine. Then, 200 mL of sterile water was measured with a measuring cylinder and poured into separate conical flasks containing the respective amounts of pawpaw seed and garlic clove powders. The mixtures were thoroughly stirred and allowed to settle for 24 hours. After settling, they were filtered to obtain extracts with concentrations of 0 g/200 mL, 50 g/200 mL, 100 g/200 mL, and 150 g/200 mL for both pawpaw seed and garlic clove extracts.

Antifungal assay

The antifungal activity of garlic and pawpaw extracts was evaluated using the poisoned food technique. Exactly 5 mL of each extract concentration 0 g/200 mL, 50 g/200 mL, 100 g/200 mL, and 150 g/200 mL of garlic and pawpaw was separately amended in 15mL of PDA and left to solidify before inoculation. Fungal plugs (5 mm diameter) of each of the isolated fungi were placed at the center of each plate. Plates were incubated at $28 \pm 2^\circ\text{C}$ for 5 days. Fungal growth was measured (diameter of colonies) using a transparent ruler in both the treated and the control plates for 5 days from the point of inoculation to the distal growth margin [6].

Statistical analysis

Data on fungal occurrence, pathogenicity, and inhibition rates were analyzed using analysis of variance (ANOVA) at a significance level of $P < 0.05$. The means were compared using Duncan’s Multiple Range Test (DMRT).

Results

Identification of fungi

Fungi identified from wheat, sorghum and millet seeds were *Aspergillus niger*, *A. flavus* and *Lasidioplochia theobromae*. Cultures of these fungi grown on Potato Dextrose Agar after 5 days of incubation are presented in figure 1-3 below.

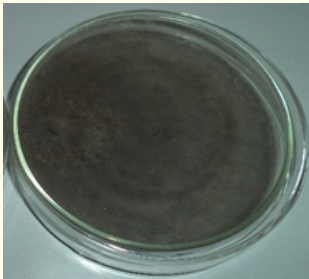


Figure 1: Culture of *A. niger* grown on PDA after 5 days of inoculation.

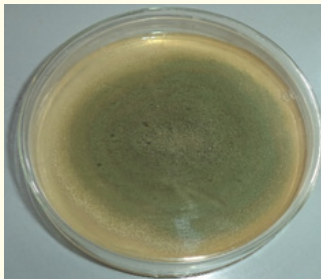


Figure 2: Culture of *A. flavus* grown on PDA after 5 days of inoculation

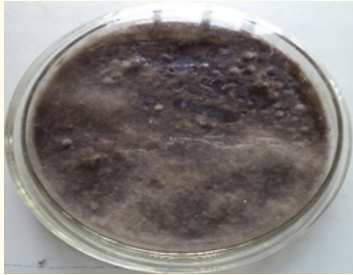


Figure 3: Culture of *Lasidioplochia theobromae* grown on PDA after 5 days of inoculation.

Frequency of fungal isolates

Occurrence of fungi showed that in sorghum seeds, *A. niger* had the highest frequency of eight (8) while *A. flavus* was the least occurred with frequency of only three (3). In Wheat *A. niger* was the fungus with the highest occurrence of seven (7) compared with *L. theobromae* with the least frequency of two (2). Similarly, millet recorded the highest frequency of eleven (11) isolates of *A. niger* compared with only two (2) of *L. theobromae*. This result indicated that *A. niger* is the most frequently occurring fungus in wheat, millet and sorghum in the study area as shown in table 2.

Crop	Fungus	Frequency	Percentage Frequency (%)
Sorghum	<i>Aspergillus niger</i>	8	53.33
	<i>A. flavus</i>	3	20.00
	<i>Lasidioplochia theobromae</i>	4	26.67
Total		15	100
Wheat	<i>A. niger</i>	7	58.33
	<i>A. flavus</i>	3	25.00
	<i>L. theobromae</i>	2	16.67
Total		12	100
Millet	<i>A. niger</i>	11	68.75
	<i>A.flavus</i>	3	18.75
	<i>L.theobromae</i>	2	12.50
Total		16	100

Table 2: Fungi Isolated from Sorghum, Wheat and Millet and their frequencies and percentage frequencies.

Pathogenicity test of *L. theobromae* in sorghum, wheat and millet

Pathogenicity test of *L. theobromae* conducted on healthy sorghum, wheat and millet showed that the fungus was able to elicit infection on the healthy seeds grown on PDA amended with spore suspension of *L. theobromae*. Visible fungal growth on the seeds on the PDA culture plates indicated that the seeds of wheat, sorghum and millet were severely infected as seen in figures 4-6.



Figure 4: Pathogenicity of *L. theobromae* on sorghum grown on PDA.



Figure 5: Pathogenicity of *L. theobromae* on wheat grown on PDA.



Figure 6: Pathogenicity of *L. theobromae* on millet grown on PDA.

The levels of infectivity and growth ranged from highly pathogenic in wheat to very highly pathogenic in sorghum and millet, respectively based on the result of pathogenicity test obtained and compared to the scale developed to classify the pathogenicity of the fungus identified as shown in table 3.

Seeds	Grade	Scale	Symptoms interpretation	Classification of isolates
Wheat	3	61-80	Large quantity of seeds were infected by pathogens	Highly pathogenic
Sorghum	4	81-100	Whole seeds completely infected by pathogen	Very highly pathogenic
Millet	4	81-100	Whole seeds completely infected by pathogen	Very highly pathogenic

Table 3: Pathogenicity test of *L. theobromae* innoculated into healthy sorghum, millet and wheat seeds after 5 days on PDA.

Pathogenicity test of *A. niger* in sorghum, wheat and millet

Pathogenicity test of *A. niger* inoculated into healthy sorghum, wheat and millet seeds show that the fungus was able to cause infection within the 5 days of incubation on PDA. Symptoms of infections were observed on all the seeds with level of infectivity ranging from one crop seed to another as presented in figures 7-9.

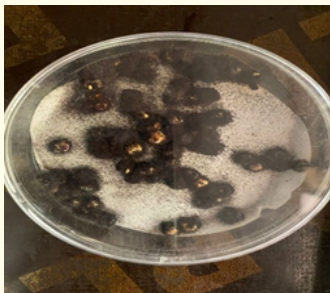


Figure 7: Pathogenicity of *A. niger* on sorghum grown on PDA.



Figure 8: Pathogenicity of *A. niger* on wheat grown on PDA.

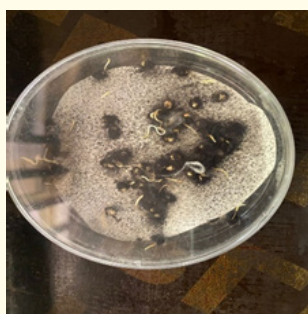


Figure 9: Pathogenicity of *A. niger* on millet grown on PDA.

Growth of the fungus and severity of infectivity ranged from highly pathogenic in wheat to very highly pathogenic in sorghum and millet, respectively based on the scale used to classify the virulence of pathogenicity of the fungus according to the pathogenicity test result as presented in table 4.

Effect of concentration of garlic extract on growth inhibition of *A. niger*

The result presented in table 5 shows that growth of *A. niger* decreased significantly as the concentration of garlic extract increased. At 150 g/200 mL, the fungus exhibited the lowest growth across all days compared to the control (0.00 g/200 mL) and to the other concentrations.

Effect of garlic extract on growth inhibition of *L. theobromae*

Table 6 shows that the growth of *L. theobromae* was significantly reduced with increasing concentrations of garlic extract. However, radial growth increased with longer incubation period. The highest growth was observed in the control (0.00 g/200 mL), while the least growth occurred at the highest garlic concentration (150 g/200 mL).

Seeds	Grade	Scale	Symptoms interpretation	Classification of isolates
Wheat	3	61-80	Large quantity of seed were infected by pathogens	Highly pathogenic
Sorghum	4	81-100	Whole seeds completely infected by pathogen	Very highly pathogenic
Millet	4	81-100	Whole seeds completely infected by pathogen	Very highly pathogenic

Table 4: Pathogenicity test of *A. niger* innoculated into healthy sorghum, millet and wheat seeds after 5 days on PDA.

Conc (g/200 mL)	Days after inoculation and radial growth (mm)				
	1	2	3	4	5
0.00	5.0000 ^a	18.0000 ^a	31.0000 ^a	40.0000 ^a	52.0000 ^a
50.00	1.5000 ^{cd}	11.0000 ^b	21.0000 ^b	31.0000 ^a	42.5000 ^a
100.00	2.0000 ^{bc}	10.0000 ^b	13.0000 ^c	18.0000 ^b	26.5000 ^b
150.00	3.0000 ^b	9.0000 ^b	15.8333 ^c	15.5000 ^b	22.5000 ^b
SEM	0.4269	1.1415	2.2491	3.0252	3.6323

Table 5: Effect of garlic extract on *A. niger* grown on PDA 5 days of inoculation.

Values followed by the same letter in each column indicate no significant difference (using Duncan's Multiple Range Test, DMRT).

Conc (g/200 mL)	Days after inoculation and radial growth (mm)				
	1	2	3	4	5
0.00	5.0000 ^a	18.0000 ^a	31.0000 ^a	40.0000 ^a	51.5000 ^a
50.00	2.5000 ^b	3.0000 ^b	4.5000 ^b	5.5000 ^b	5.5000 ^b
100.00	1.5000 ^{bc}	2.0000 ^b	2.5000 ^b	2.5000 ^b	2.5000 ^b
150.00	1.0000 ^c	1.5000 ^b	2.0000 ^b	2.0000 ^b	2.0000 ^b
SEM	0.4885	2.1071	3.7004	4.8112	6.3048

Table 6: Effect of garlic extract on *L.theobromae* growth on PDA after 5 days of inoculation.

Values followed by the same letter in each column indicate no significant difference (using Duncan's Multiple Range Test, DMRT).

Effect of Pawpaw Seed extract on growth inhibition of *A. niger*

Result presented in table 7 shows increasing concentrations of pawpaw seed extract reduced the growth of *A. niger*. The control (0.00 g/200 mL) recorded the highest fungal growth, while the lowest growth was observed with 150 g/200 mL of extract, especially on Day 5.

Significant differences among the extract concentrations were observed on each day of incubation.

Effect of Pawpaw Seed extract on growth inhibition of *L. theobromae*

Result in table 8 shows that the growth of *L.theobromae* decreased significantly with increasing concentrations of pawpaw seed extract. The control (0.00 g/200 mL) consistently recorded the highest fungal growth, while the lowest growth occurred at 150 g/200 mL of extract, especially on Day 5. Significant differences were observed among concentrations on each day of incubation.

Conc (g/200 mL)	Days after inoculation and radial growth (mm)				
	1	2	3	4	5
0.00	5.0000 ^a	18.0000 ^a	31.0000 ^a	40.0000 ^a	51.5000 ^a
50.00	3.0000 ^b	10.0000 ^b	22.0000 ^b	30.0000 ^b	42.5000 ^a
100.00	2.0000 ^b	13.5000 ^b	20.0000 ^b	22.5000 ^c	26.5000 ^b
150.00	3.0000 ^b	12.0000 ^b	17.0000 ^b	20.5000 ^c	23.5000 ^b
SEM	0.3718	0.9791	1.6719	2.3415	3.4826

Table 7: Effect of pawpaw seed extract on *A. niger* growth on PDA after 5 days of inoculation.

Values followed by the same letter in each column indicate no significant difference (using Duncan's Multiple Range Test, DMRT).

Conc (g/200 mL)	Days after inoculation and radial growth (mm)				
	1	2	3	4	5
0.00	5.0000 ^a	18.0000 ^a	31.0000 ^a	40.0000 ^a	51.5000 ^a
50.00	1.0000 ^b	10.0000 ^b	20.5000 ^b	31.5000 ^a	45.0000 ^a
100.00	.0000 ^b	3.5000 ^c	5.5000 ^c	8.5000 ^b	18.0000 ^b
150.00	.0000 ^b	2.0000 ^c	5.0000 ^c	9.0000 ^b	13.0000 ^b
SEM	0.6336	1.9415	3.3274	4.1939	5.0370

Table 8: Effect of pawpaw seed extract on *L. theobromae* growth on PDA after 5 days of inoculation.

Values followed by the same letter in each column indicate no significant difference (using Duncan's Multiple Range Test, DMRT).

Discussion

The results of the study revealed that *A. niger*, *A. flavus* and *L. theobromae* are the major fungal pathogens causing infections in cereal crops like guinea corn, millet, and wheat in the study area. These pathogens significantly affect the productivity and quality of the crops both in the field and in storage. This result agreed with the findings obtained by [12] isolated fungal species like *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., and *Alternaria* spp. in these crops. According to [13] the fungi are not only capable of reducing seed viability but also have the ability to pose health challenges as a result of secretion of toxic substances like mycotoxins, aflatoxins and fumonisins. These fungi pathogens are commonly found in semi-arid regions like Dutsin-Ma, Nigeria, even on leguminous crops like cowpea [14] and on vegetables like tomato and pepper ([15,16]. The high frequency of fungal occurrence observed in the study area may be exacerbated by suboptimal post-harvest storage practices by peasant farmers and in some instances due to high humidity levels [17].

Pathogenicity tests of *A. niger* and *L. theobromae* conducted using wheat, sorghum and millet showed that the fungi elicited infections in all seed-types with more severe infection in sorghum and millet compared to wheat. This may be due to the highly aggressive and virulent nature of the pathogens on the substrate resulting in severe infection [18]. It has been found on millet and wheat that infections by these pathogens have resulted in reduced seed vigour and germination rate by 40-60% [19].

The study revealed that period of incubation and concentration of extracts from *Allium sativum* (garlic) and *Carica papaya* (pawpaw) had significant inhibitory effect due to their antifungal properties. Results have shown that garlic contained bioactive compounds such as allicin, ajoene, and diallyl disulfide, which disrupt fungal cell membranes and inhibit spore germination [20]. Similarly, finding by [21] showed that a 10% aqueous extract of garlic inhibited the growth of *A. flavus* and *Fusarium oxysporum* in wheat by over 80%. The effectiveness of pawpaw seeds on the other hand depends on the presence phytochemical compounds alkaloid, flavonoid, papain which contain antifungal properties. Research by [22] showed that as the concentrations of pawpaw seed extracts

increased, fungal growth in millet and guinea corn reduced by 70% yielding better results. It has further being demonstrated that the combination of garlic and pawpaw extracts produced a synergistic effect, enhancing antifungal efficacy of the extracts. According to, [23] the combination of 5% garlic extract with 5% pawpaw seed extract reduced the pathogenicity of *Fusarium* spp. in millet by 85%. Such combinations could be effective in the management of mixed fungal infection observed by a farmer.

It is therefore critical in reducing fungal infections in cereal crops which will ultimately improve food security in Nigeria. Appropriate and effective use of botanical extracts of garlic and pawpaw can reduce post-harvest losses associated with pathogenic attack, enhance seed viability for a long period of time, and reduce over dependence on synthetically hazardous chemicals, which are often costly and non-biodegradable in the environment.

Conclusion

The study has shown that *Aspergillus niger*, *A. flavus*, *Lasiodiplodia theobromae* are major pathogens of sorghum, millet and wheat. The result further revealed that *A. niger* occurred more frequently in all the crops compared to the other pathogens. The occurrence and pathogenicity of fungal pathogens in guinea corn, millet, and wheat seeds present significant challenges to food security in Dutsin-Ma, Nigeria. However, botanical extracts from garlic and pawpaw offer a promising, eco-friendly solution for fungal control. The use of these extracts could enhance antifungal effectiveness, reduce economic losses, and promote sustainable agricultural practices in the region.

Conflict of Interest Disclosure

The authors of this research article declare that there were no conflicts of interest.

Funding Acknowledgement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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