

Seed-Borne Fungi of Groundnuts (*Arachis Hypogaea*) and Their Management with Ginger (*Zingiber Officinale*) Extract In Makurdi, Nigeria

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ABSTRACT

Experiments were conducted at the Crop and Environmental Laboratory of the Joseph Sarwuan Tarka University (formerly, Federal University of Agriculture), Makurdi to assess the occurrence of seed-borne fungi on nine varieties (Kampala, Kwankwaso, Jawunde, Samnut 21, Samnut, 22, Samnut 23, Samnut 24, Samnut 25 and Samnut 26) of groundnut (*Arachis hypogaea* L.) and to test the effect of ginger (*Zingiber officinale*) extract on *Aspergillus niger* and *Fusarium verticillioides*. Detection of seed-borne fungi was done by standard blotter methods while mycelial growth of *A. niger* and *F. verticillioides* were recorded on potato dextrose agar (PDA) culture medium. All the experiments were carried out in Completely Randomized Design replicated as appropriate. The fungi detected were *Aspergillus flavus*, *Aspergillus niger*, *Fusarium verticillioides*, *Fusarium solani* and *Botryodiplodia theobromae*. *A. flavus* and *A. niger* were the most predominant fungi encountered. *A. niger* was significantly ($p < 0.05$) higher in Kampala variety and was lowest ($P < 0.05$) in SAMNUT 24 and SAMNUT 26. Occurrence of *Fusarium verticillioides* was significantly ($P < 0.05$) lower in SAMNUT 24 compared with the other varieties except SAMNUT 26 while *Fusarium solani* was significantly ($P < 0.05$) higher in Jawunda followed by Kwankwaso, and Kampala varieties. There was no significant difference in the occurrence of *B. theobromae* amongst the varieties tested. Mycelial growth of *A. niger* was significantly ($P < 0.05$) lowest at 30 % w/v, ginger amended medium giving an inhibition rate of 91.4% compared with the control. Ginger extract at 30% w/v gave 100% inhibition of mycelial growth in *F. verticillioides* compared with the control. It is concluded that groundnut varieties are infected by various fungal organisms and ginger (*Zingiber officinale*) extract reduced the growth of *A. niger* and *F. verticillioides* infecting groundnuts

Keywords: Groundnut, variety, seed-borne fungi, *Aspergillus*, *Fusarium*, plant extract, ginger .

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) belongs to the genus *Arachis* in the family *Leguminosae*. Groundnut has prospects as a major source of income to farmers as it serves as cash crop in most rural communities (Awoke, 2003, Taru *et al.*, 2010). Groundnut is the thirteenth most important food crop and fourth most important oilseed crop of the world (Radha *et al.*, 2011). It contains 48-50% oil, 26-28% protein and 11-27% carbohydrate and also contain minerals and vitamins that supplement the dietary requirement of humans and livestock (Asibuo *et al.*, 2008; Mukhtar, 2009).

Seed is the most important input in agriculture, hence seed quality need to be given due attention (Aggrawal, 2005; Ntare *et al.*, 2008). Groundnut seeds carry a variety of microorganisms among which fungi are the most notable. Seed borne fungi cause a variety of damage which include seed abortion, shrunken seeds, seed rot, seed necrosis, seed discoloration and reduced seed germination (Shetty, 1988). Fungi growing on stored grains reduce the germination rate, carbohydrate, protein, total oil content, increase moisture content and also enhance other biochemical changes of grains (Bhattacharya and Raha, 2002, Bilgrami, *et al.*, 1976). Most seed infections occur in the field before harvest and these infections are influenced by weather conditions between flowering and maturity (Mehrota and Aggrawal, 2006). Warm humid conditions during this period often results in heavy pod and seed infection. It has been reported that seed-borne transmission of pathogens is

responsible for the perpetuation of plant diseases leading to drastic yield reduction (Oluma and Nwankiti, 2007). Hundreds of seed-borne fungi, both pathogenic and saprophytic have been isolated and identified (Mehrotic and Aggrawal, 2006; **Gebisa and G/Tsadik** 2024).

Many seed borne pathogens are found outside the embryo in the seed coat, pericarp or endosperm. Others are transmitted as seed contaminants on the seed surface. Some seed borne pathogens can be found both inside and outside the embryo (Aggrawal, 2005). A few of these pathogens grow directly into the young plant and cause systemic infection during seed germination (**Chaudhari et al.**, 2024). A high proportion of these pathogen, however may have a period of saprophytic growth and/or sporulation in the soil surrounding the seed before they infect the plant and cause diseases (Jensen and Hanne, 1988). Some common seed borne fungi include; *Aspergillus flavus*, *Aspergillus niger*, *Fusarium verticillioides*, *Fusarium solani* and *Lasiodiplodia theobromae* (**Chaudhari et al.**, 2024).

Some plant extract have been known to have medicinal and antimicrobial properties (Jabeen 2006; Lalitha, et al., 2010). Plant extracts are environmentally friendly, relatively safe and are biodegradable compared with synthetic chemicals (Sukanya et al., 2011; Khan and Nasreen, 2010; Enikuomelin, 2005; Gurjar et al. 2012). The use of plant extract on stored groundnut seeds have been shown to possess potent antifungal, antibacterial, insecticidal and nematicidal activity (Amvam et al., 1998; Isman, 1999; Nguefack et al., 2005; Nguefack et al., 2007); Oka et al., 2000). The extract of ginger has not been tested on seed-borne fungi on the SAMNUT varieties of groundnuts in Benue State. This work was therefore carried out to determine the fungal organism associated with SAMNUT varieties of groundnut and determine the effect of seed treatment with ginger extract on their occurrence.

MATERIALS AND METHODS

Experiments were conducted at the Crop and Environmental Protection Laboratory of the College of Agronomy, Joseph Sarwuan Tarka University (formerly, Federal University of Agriculture), Makurdi to determine the occurrence of seed-borne fungi on groundnut (*Arachis hypogaea* L.) and to study the effect of seed-treatment with ginger (*Zingiber officinale*) extract on *A.niger* and *F. verticilloides*

Assessment of the occurrence of Seed-borne fungi of groundnut

Fifty seed samples each from nine groundnut varieties consisting of Kampala, Kwankwaso, Jawunde, SAMNUT 21, SAMNUT 22, SAMNUT 23, SAMNUT 24, SAMNUT 25, SAMNUT 26 were collected after 24 months of storage of groundnut seeds harvested from a field experiment. The seeds were tested for occurrence of seed-borne fungi using standard blotter method. The groundnut seeds were sterilized in 10 % Sodium hypochlorite for 1 minute and rinsed in three changes of sterile distilled water (SDW). Ten seeds were placed on moist blotter in sterilized 9 cm diameter Petri dishes moistened with 10 ml SDW and incubated for seven days at ambient conditions of light and temperature. The treatments (varieties) were arranged in completely randomized design, replicated 10 times (10 plates per variety). The incubated seeds were observed for fungal growth and fungi detected were sub-cultured on PDA to obtain pure cultures. Isolated fungi were identified using identification manual by combining morphological and molecular data to enhance accuracy in species-level identification at the Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria. Number of seeds infected by each fungi detected in each of the varieties were recorded,

morphological characteristics of the each fungi encountered were described seven days after incubation and percentage fungal infection was computed. Percentage data were subjected to square root transformation before analysis of variance (ANOVA) using GENSTAT (17th Edition) 2014. Significantly different mean were separated using Fisher's Least Significant Difference (FSLD) at 5% level of probability (Obi, 2002).

Effect of Ginger (*Zingiber Officinale*) Concentrations on seed-borne *Aspergillus niger* and *Fusarium verticilloides* isolated from groundnut.

Fresh ginger materials were purchased from Makurdi North-Bank market, peeled and rinsed with sterile distilled water (SDW). Various weights of ginger consisting of 10 g, 20 g, 30 g were ground using a blender

and infused with 100 mls of SDW in 250 ml conical flask and filtered using double layer cheese cloth to give 10, 20 and 30 % w/v concentration respectively. Two fungi; *Aspergillus niger* and *Fusarium verticilloides* earlier isolated were used as test fungi. The treatment were various concentrations of ginger extracts consisting of 0 % w/v, 20 % w/v, 30 % w/v, and 40 % w/v set up in completely randomized design and replicated three times.

Media amendment with plant extracts and inoculation with test fungi

The extracts prepared were used to amend PDA (Potato Dextrose Agar) at the various concentrations (0, 20, 30, 40 % w/v), The control (0 % w/v) was PDA without extract. The amended media was prepared by adding 4 grams of PDA (Lab M) to 100ml plant extracts of the various concentrations. The flasks were autoclaved at 121°C for 15 minutes (Nduagu *et al.*, 2008) after which they were removed and allowed to cool on a laminar airflow chamber. When the media were cooled to about 40°C, 100 mg/l of streptomycin sulphate was added to prevent bacterial contamination. The media were then poured into 9 cm Petri dishes and allowed to solidify (Obagwu *et al.*, 1997).

The amended media and the control were inoculated at the centre with mycelial discs (5mm diameter) taken from advancing edges of 7 days-old pure culture of the test fungi (*Aspergillus niger* and *Fusarium verticilloides*) using a cork borer. The inoculated media were incubated at ambient conditions of light and temperature (30±2°C) for six days after which diameter of the test fungi were measured using a meter rule along two diagonal lines drawn on the reverse side of each Petri dish. Percentage reduction in mycelial growth were computed using the formula

$$Pr = \frac{P_1 - P_2}{P_1 \times 100} \quad Pr = \frac{P_1 - P_2}{P_1 \times 100} \quad \text{----- Equation 1}$$

Where;

P_r = % reduction in mycelial growth,

P_1 = mycelial growth in the untreated medium (control),

P_2 = mycelial growth on the treated medium.

and the performance of the amendments were rated as follows:

≤ 0 % inhibition (Not effective),

> 1- 20 % inhibition (Slightly effective),

> 21-50 % inhibition (Moderately effective),

> 51- < 100 % inhibition (Effective) ,

100 % inhibition (Highly effective) (Okigbo *et al.*, 2009)

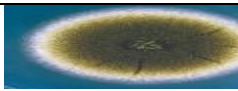

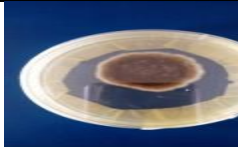



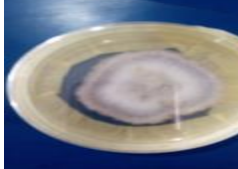


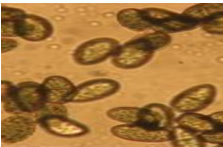
The data were subjected to analysis of variance (ANOVA) using GENSTAT (17th edition). and means were separated using Fisher's Least Significant Difference (FSLD) at 5% level of probability (Obi.). 2002

RESULTS

The morphological characteristics of seed-borne fungi encountered and detailed description of the colony are presented in Table 1. The fungi encountered included, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium verticillioides* , *Fusarium solani*, and *Botryodiplodia theobromae*. Table 2 shows the occurrence of the seed borne fungi detected on groundnut, in this trial. *Aspergillus flavus* and *A. niger* were the most predominant fungi encountered. While there was no significant difference in the occurrence of *A. flavus* on the groundnut varieties tested, *A. niger* was significantly ($p < 0.05$) higher in Kampala and was lowest ($P < 0.05$) in SAMNUT

24 and SAMNUT 26. Occurrence of *Fusarium verticilloides* was significantly ($P < 0.05$) lower in SAMNUT 24 compared with the other varieties except SAMNUT 26. The highest occurrence of *F. verticilloides* was on the variety Kampala. *Fusarium solani* was significantly ($P < 0.05$) higher in Jawunda followed by Kwankwaso, and Kampala varieties. *F. solani* was not detected in SAMNUT 22, 24, 25 and 26 and there was no significant difference in the occurrence of *B. theobromae* among the varieties tested.

Table 1: Morphological and microscopic presentation of fungi isolated from groundnut

Macro/Microscopic Characteristics	Appearance on PDA	Photomicrograph	Fungi
Colonies consist of dense felt yellow green conidiophores			<i>Aspergillus flavus</i>
Colony bears abundant and erect and usually crowded conidial structures, carbon black but sometimes deep brown black. Conidia heads are split into two or more loose to reasonable well defined columns. Conidiophores are smooth and hyaline.			<i>Aspergillus niger</i>
Morphological description White aerial mycelia grows rapidly and often become tinged with purple sometimes. Sporodochia may be present or absent; when present they may be tan to orange discrete sporodochia. Microscopic description Microconidia abundant and primarily singled celled, oval to club-shaped. Macroconidia are present, though sometimes rare; Their appearance varies from slightly sickle-shaped to almost straight.			<i>Fusarium verticillioides</i>
Morphological description Produces colonies that are white and cottony. Microscopic description Microconidia are oval or cylindrical, hyaline and smooth. Some may be curved. Macroconidia are slightly curved, hyaline and broad often aggregating in fascicles.			<i>Fusarium solani</i>
Morphological description The mycelium is hyaline and well branched. Main hyphae is found to be up to 6–8 μm wide. Average radial growth of the oomycete at 25°C on PDA is 11 mm per day.			<i>Botryodiplodia theobromae</i>

Microscopic description			
Sporangia are globose to somewhat cylindrical, measuring 15–55 µm in diameter and 65 µm in length.			

Table 2: Occurrence (%) of Seed borne fungi isolated from nine groundnut varieties in Makurdi, Nigeria

Groundnut Varieties	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Fusarium verticillioides</i>	<i>Fusarium Solani</i>	<i>Botryodiplodia theobromae</i>
KAMPALA	9.8	6.4	4.4	1.2	0.4
KWANKWASO	10.0	6.2	4.2	1.8	0.4
JAWUNDE	10.2	6.0	4.2	2.8	0.6
SAMNUT 21	9.0	5.6	4.0	0.4	0.0
SAMNUT 22	9.8	5.8	4.2	0.0	0.0
SAMNUT 23	9.4	5.6	4.0	0.4	0.0
SAMNUT 24	8.6	4.6	3.4	0.0	0.0
SAMNUT 25	9.4	5.2	4.0	0.0	0.2
SAMNUT 26	9.4	4.8	3.6	0.0	0.0
F-LSD(0.05)	Ns	1.26	0.90	0.70	Ns
Cv(%)	9.69	17.30	16.85	14.61	27.58

The effect of concentration of ginger on the mycelial growth of *Aspergillus niger* isolated from groundnut seed in Makurdi is presented in Table 3. Mycelial growth of *A. niger* was significantly ($P<0.05$) lowest at 30 % w/v in ginger amended medium giving an inhibition rate of 91.4 %. All the treatments significantly reduced mycelial growth compared with the control. The growth inhibition trends in *A. niger* showed that 30 % w/v ginger > 20 % w/v ginger > 10 % w/v ginger > control (no extract) at 6 days after inoculation.

Table 4 show the effect of different concentrations of ginger extracts on mycelia growth of *Fusarium verticillioides* isolated from groundnut in Makurdi. Ginger concentration at 30 % w/v completely inhibited (100 %) the growth of *Fusarium verticillioides* after 6 days of incubation having its mycelial growth significantly ($P<0.05$) lowest (0.00cm) compared with the other treatments. All the treatments tested significantly ($P<0.05$) reduced mycelial growth of *Fusarium verticillioides* compared with the control. The growth inhibition trends in *Fusarium verticillioides* showed that 30 % w/v > 20 % w/v > 10 % w/v ginger > control (no extract) at 6 days after inoculation.

Table 3: Effect of three concentrations of Ginger (*Zingiber officinale*) extract on the mycelia growth of *Aspergillus niger* 6 days after inoculation

Treatment	Mycelial growth (cm)	Inhibition	Rating
10 % w/v ginger	4.95	3.96	Moderately effective
20 % w/v ginger	3.68	54.59	Effective

30 % w/v ginger	0.67	91.74	Highly effective
Control	8.11	0	Not effective
FLSD	0.64		
CV	7.80		

Table 4: Effect of three concentrations of Ginger (*Zingiber officinale*) extract on the mycelia growth of *Fusarium verticillioides* 6 days after inoculation

Treatment	Mycelial growth (cm)	Inhibition	Rating
10 % w/v ginger	4.97	36.55	Moderately effective
20 % w/v ginger	3.68	53.06	Effective
30 % w/v ginger	0.00	100.00	Highly effective
Control	7.83	0	Not effective
FLSD	0.42		
CV	5.40		

DISCUSSION

This study established the presence of several seed-borne fungal organisms in stored groundnut varieties, with *Aspergillus flavus*, *A. niger*, *Fusarium verticillioides*, *F. solani*, and *Botryodiplodia theobromae* being predominant. The differential occurrence of these pathogens among the groundnut varieties suggests inherent varietal resistance, particularly noted in SAMNUT 24 and SAMNUT 26, which recorded the lowest incidence of *A. niger* and *F. verticillioides*. This aligns with previous reports indicating that varietal resistance significantly affects seed-borne pathogen load (Ntare *et al.*, 2008; Ekhuemelo and Yaaji, 2017; Chaudhari *et al.*, 2024).

The antifungal efficacy of ginger (*Zingiber officinale*) extract observed in this study corroborates findings from earlier studies that have demonstrated the broad-spectrum antifungal potential of plant-based extracts (Ademe *et al.*, 2013; Gurjar *et al.*, 2012; Nikiema *et al.*, 2024). The extract exhibited concentration-dependent activity against both *A. niger* and *F. verticillioides*, with the 30 % w/v treatment achieving complete inhibition of *F. verticillioides* growth and over 91 % inhibition of *A. niger*. This high efficacy can be attributed to the presence of active phytochemicals in ginger such as gingerol, shogaol, and zingerone, which have been reported to disrupt fungal cell membranes and inhibit enzymatic activity critical to fungal growth (Nakamura *et al.*, 1996; Bahraminejad, 2012; Iwuagwu *et al.*, 2019).

The inhibition pattern observed agrees with findings by Nguefack *et al.* (2007), who reported similar suppression of fungal pathogens in rice using essential oils. Navoda and Anupama (2022) tested extracts of **Aloe vera**, **garlic** (*Allium sativum*), **neem** (*Azadirachta indica*), and **ginger** (*Zingiber officinale*) against seed-borne pathogens including *Aspergillus flavus* and *A. niger* isolated from **peanut** (*Arachis hypogaea*) and other legumes. **Ginger extract** showed highest antifungal activity, comparable to the fungicide Captan 50% (WP). Additionally, **neem and ginger aqueous extracts** promoted seed germination and seedling vigor; **Aloe vera** was least effective in these measures. The effectiveness of ginger extract further reinforces the suitability of botanical fungicides as safer and eco-friendly alternatives to synthetic chemicals, which often pose environmental and health hazards (Isman, 1999; Sukanya *et al.*, 2011; Navoda and Anupama, 2022).

The complete inhibition of *F. verticillioides* by 30 % ginger extract is particularly significant due to the toxigenic potential of *Fusarium* species, which produce harmful mycotoxins affecting human and animal health (Bhattacharya and Raha, 2002). The suppression of such fungi through natural, biodegradable agents provides a valuable tool for improving post-harvest seed health and food safety, particularly in resource-limited communities in many rural settings in Africa.

While the study demonstrates promising results, the findings are limited to in vitro conditions. Therefore, further research should investigate the in-vivo efficacy and potential phytotoxic effects of ginger extract on groundnut seeds, as well as explore synergistic interactions with other plant extracts for enhanced antifungal spectra (Suprpta, 2012; Cosoveanu *et al.*, 2013).

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