



Studies on The Control of Three Species of Fungi Isolated From Sorghum with Extracts of Galic (*Allium sativum*) and Ginger (*Zingiber officinale*)

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ABSTRACT

Fungi contaminate grains and seeds with mycotoxins. The consumption of such mycotoxin contaminated grains by mammals cause mycotoxicoses. This has a great public health significance because the toxins are nephrotoxic, immunotoxic, tetratogenic and clastogenic.. These can cause death and disorder of central nervous system. They can also cause pulmonary system and intestinal tract disorder. Sorghum are important crops in Nigeria, Mali and Niger. The objective of this study include the isolation of three fungi associated with sorghum in storage, test for the presence of mycotoxin in stored sorghum, determination of antifungal activity of *Allium sativum* and *Zingiber officinale*. Results of the identification of fungi associated with stored sorghum showed that it contains *Aspergillus oryzae*, *Aspergillus flavus* and *Aspergillus niger*. Assay for mycotoxin showed that *Aspergillus oryzae* and *Aspergillus niger* produced aflatoxins B₁. Fumanisin B₁ and aflatoxinB₁ were produced by *Aspergillus flavus*. This finding will serve the purpose of alerting consumers on the dangers of consuming poorly stored sorghum. The results of this study also suggest that extracts of plants could prevent the growth of fungi in stored sorghum and also serve as a source of chemotherapeutic agents.

Keywords: Mycotoxins, aflatoxins, fungi, mycotoxicoses, nephrotoxic, fodder.

Introduction

Sorghum is an ancient cereal grain belonging to the grass family *Poaceae*. It is small, round and usually white, yellow, red brown or black. It is a group of highly variable small seeded grasses, widely grown around the world as cereal crops or grains for fodder and human food. They are highly tolerant to drought and other extreme weather conditions and have a similar nutrient content to other major cereals (Bennett and Klich, 2003). Sorghum are import crops in the semiarid

tropics of Asia and Africa (especially in India, Mali, Nigeria and Niger) with 95% of sorghum production in developing countries (McDonough *et al.*, 2000). The crop is favoured due to its productivity and short growing season under dry, high temperature conditions.

Fungi can cause infection in plants; hence they cause spoilage of food stuffs. They contaminate food with mycotoxins. Fungi grow in grains and seeds during storage and transport. This result in discolouration, musty odours, chemical and nutritional changes. These result in reduction in

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crop yield and quality with significant economic losses (Banso *et al.*, 2020a).

The consumption of such mycotoxin contaminated grains by mammals cause mycotoxicoses. This has enormous public health significance because the toxins are nephrotoxic, immunotoxic, tetratogenic and clastogenic. These are capable of causing death, disorder of central nervous, cardiovascular and pulmonary systems and intestinal tract (Bhot and Vasunthi, 2003) The toxins are relevant in human hepatoma and oesophageal cancer, increased susceptibility and diseases especially in children and childhood mortality (Beardall and Miller, 1994; Miller, 1996; Marasas, 2001). Storage fungi especially *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* species infect grains after harvest, they also grow on them during storage (Amadi and Adeniyi, 2009).

Mycotoxigenic fungi include *Aspergillus*, *Penicillium* and *Fusarium* but *Alternaria* among other are also important food contaminants. The fungi are also pathogenic to plants (Ramm *et al.*, 1994).

Mycotoxin control will result in economic gains as well as health improvement. Mycotoxins are toxic secondary metabolites produced by organisms of the fungus kingdom (Richard, 2007). They are capable of causing disease and death in both humans and other animals. The term mycotoxin is usually reserved for the toxic chemical products produced by Fungi that relatively colonize crops (Bennett and Klich, 2003).

Mycotoxins such as aflatoxin, citrinin, fumonisins, ochratoxin A, patulin, trichothecens, zearalenone and ergot alkaloids such as ergotamine are associated some human and animal diseases; they can be mutagenic, carcinogenic and immune-suppressive with both acute and chronic implications (Lewis *et al.*, 2005). Diseases associated with common mycotoxins are aflatoxicoses (aflatoxin), Balkan Endemic Nephropathy (BEN) (ochratoxin); equine Leukoencephalo malacia and porcine

pulmonary edema (fumonisin), haemorrhagic disease and alimentary toxic aleukia (ATA) (trichothecenes), estrogenic disease (zearalenone) (WHO, 2018).

Aflatoxin is about the most popular and widespread mycotoxin. The name mycotoxin was derived from the fact that it was originally found to be produced by *Aspergillus flavus* (Agrio, 1978), but is now known to be produced by other species of *Aspergillus*, Aflatoxin B1 is produced by *Aspergillus terreus*, though it may also be produced by *Aspergillus flavus* as well as *Aspergillus oryzae*. Aflatoxin B1 is the most toxic carcinogenic and most prevalent of the different aflatoxins (Amadi, 2009). The objective of this study is to alert consumers on the dangers of consuming poorly stores sorghum.

Materials and Methods

Plant material

Ginger (*Zingiber officinale*), garlic (*Allium sativum*) and sorghum were used in this study. They were purchased from new market, Bida Niger state.

Fungal screening

The fungi used in this study are *Aspergillus oryzae*, *Aspergillus flavus* and *Aspergillus niger*. The fungi were isolated from sorghum and identified after straining with lactophenol cotton blue. The organisms were maintained in potato dextrose agar slant and stored in the refrigerator until required.

Preparation of plant extracts

One hundred grams of dried powdered root of *Zingiber officinale* and *Allium sativum* were weighed separately into 400ml of extracting solvent (ethanol, methanol or chloroform) in different conical flasks. They were stirred intermediately over a period of 72 hours after which the extracts were filtered into different conical flasks (Banso *et al.*, 2021). The extracts obtained were evaporated to dryness using shaking water bath (Rolitech) at 80 °C.

Isolation of fungi associated with stored sorghum

The three *Aspergillus* species were isolated from stored sorghum by surface sterilizing the grains in NaOCl for 2 min and rinsed in two changes of sterile distilled water. The grains were blotted dry in between sterile Whatman No. 1 filter paper and plated on malt extract agar (MEA) at the rate of 10 grains per plate. Thirty grains was plated and incubated at room temperature at $25\pm 1^\circ\text{C}$. Subcultures were made from emerging colonies and pure cultures were used for subsequent studies.

Identification of fungi associated with stored sorghum

Pure fungal colonies were harvested and stained with lactophenol cotton blue on microscope slides for identification. The macro and microscopic identification was done following the identification key of Nelson *et al.* (1991).

Mycotoxin production

Malt extract was used. The medium was prepared routinely and sterilized. Twenty-five millimeters (25ml) of the broth was dispensed into sterile conical flasks. The inoculated flasks were incubated on shaker at room temperature of $25\pm 1^\circ\text{C}$ for 12 days (Banso *et al.*, 2023). The filtrate of the test isolates was assayed for the presence of mycotoxins.

Assay for mycotoxin

Thin layer chromatography (TLC) technique was used for detection of mycotoxins as described by Amadi and Adeniyi, 2009. Both the filtrates and the standards were spotted on the prepared TLC plates at a distance of 1.5cm from the lower edge and left for 1 minute to dry. The solvents used were chloroform, methanol (97:3 and 50:50 v/v) to detect the mycotoxins produced by the test organisms. The TLC plates were removed when the solvent reached a mark 1cm from the upper edge of the plates. The plates were left to dry up

and the solvent front marked across the plate. The colour spot was marked with pencil and visualized under UV lamp. The retention factors (RF) of the compounds/toxins in the filtrates were calculated. $R_f = \text{distance travelled by the solvent} / \text{distance travelled by the solvent}$.

Determination of antifungal properties of the extracts

The antifungal properties were determined using agar dilution method of Banso and Mann, 2006. In this method different volumes of the extracts were introduced into McCartney bottles containing appropriate volume of sterile malt extract agar. The malt extract agar was poured into different petri-dishes and allowed to solidify. The plates were inoculated with 5mm diameter of the fungal culture. Control experiments were performed without the extracts. The plates were incubated at $28\pm 2^\circ\text{C}$ for 48 hours. The antifungal properties were expressed in terms of diameter of growth (mm).

Statistical analysis

The data represents means of three replicates \pm standard deviation (SD). The results were subjected to analysis of variance and mean comparison were performed by Turkey's multiple range tests using SPSS version 20.0 (statistical package for social science, Inc Chicago II., United States). Differences between means were considered significant at $P < 0.05$.

Results and Discussion

Fungi associated with stored sorghum

The result of identification of fungi associated with stored sorghum showed that stored sorghum used in this study contain *A. oryzae*, *A. flavus*, *A. niger* (Table 1). *Aspergillus* species can contaminate many food commodities including maize, millet, sorghum and cassava (Bandyopadhyay *et al.*, 2007). Banso *et al.* (2020a) isolated *Aspergillus niger*, *Rhizopus stolonifer* and *Fusarium* species from orange and mango juices. Sorghum contaminated by fungi renders the grains unfit for human consumption,

discoloration and reduction of nutritional value (Sreenivasa *et al.*, 2010).

Table 1: Cultural and microscopic characteristics of fungal isolates from stored sorghum

Isolate	Cultural characteristics	Microscopic characteristics
<i>A. oryzae</i>	Surface colony is brownish yellow, cottony appearance initially white and then turns black. Reverse is white	Hyphae is hyaline and septate. The conidiophores ends at a round shaped vesicle.
<i>A. flavus</i>	Grey white colony, later turn black, reverse is orange yellow	The conidia is slightly rough, conidiophores is unbranched.
<i>A. niger</i>	Cottony white colony, turn black, reverse is white	Smooth coloured conidiophores and conidia are present

Myctoxin produced by fungi

Table 2 showed that *Aspergillus oryzae*, *Aspergillus niger* produced aflatoxins B₁. Fumanisin B₁ and aflatoxinB₁ were produced by *Aspergillus flavus*. The retention factor (R_f) recorded against *A. oryzae*, *A.niger* was 0.45 while values of 0.45 and 0.69 were recorded against *A. flavus* (Table 2). Many types of mycotoxins are produced by fungi (Heather *et al.*, 2006). Mycotoxins such as aflatoxin, citrinin, fumonisins, ochratoxin A, patulin, trichothecens, zearaleone and ergot alkaloids such as ergotamine are associated with a number of human and animal diseases; they can be mutagenic, carcinogenic and immune-suppressive with both acute and chronic implications (Lewis *et al.*, 2005).

Table 2: Mycotoxin produced by fungi isolated from stored grains

Fungi	Retention factor (R _f)	Mycotoxin
<i>A. oryzae</i>	0.45	Aflatoxin B ₁
	0.45	Aflatoxin B ₁
<i>A. flavus</i>	0.69	Fumanisin B ₁
<i>A. niger</i>	0.45	Aflatoxin B ₁

Antifungal properties of extracts of *A. sativum* and *Z. officinale*

The results of the antifungal properties of *A. sativum* and *Z. officinale* are shown in Tables 3 and 4. A value of 11.0±0.1mm was recorded against *A. oryzae* when ethanol extract of *A. sativum* was assayed against the fungi. The value recorded against the fungi when methanol extract of *A. sativum* was assayed against the fungi was 12.0±0.2mm while the mean diameter of growth recorded against the fungi when chloroform extract was assayed against the fungi was 13.0±0.1mm. The mean diameter of growth recorded against *A. niger* when ethanol extract of *A. sativum* was assayed against the fungi was 10.0±0.1 mm. A value of 11.0±0.1 mm was recorded against the fungi when methanol extract of the plant material was assayed against *A. niger* while a value of 13.0± 0.1 was recorded when chloroform extract was assayed against the fungi (Table 3). Exhibition of antifungal property by *A. sativum* and *Z. officinale* agree with the work of Banso *et al.* (2021) and Banso *et al.* (2020b) who reported that *Acalypha hispida* showed antimicrobial activity against bacteria and fungi. This suggests that extracts of plants could serve as substitutes for conventional preservatives; the extracts could also serve as a source of chemotherapeutic agents.

Table 3: Antifungal properties of *Allium sativum*

Organism	Mean diameter of growth (mm)±SD			
	Control	Ethanol	Methanol	Chloroform
<i>A. oryzae</i>	14.0±0.1	11.0±0.1 ^b	12.0±0.2	13.0±0.1 ^b
<i>A. flavus</i>	15.0±0.1	10.0±0.2 ^a	11.0±0.2 ^a	12.0±0.1 ^a
<i>A. niger</i>	15.0±0.1	10.0±0.1 ^a	11.0±0.1 ^a	13.0±0.1 ^a

SD = Standard deviation, significant level of difference from control; P^a<0.05, P^b>0.05

Table 4: Antifungal properties of extracts of *Zingiber officinale*

Organism	Mean diameter of growth (mm)±SD			
	Control	Ethanol	Methanol	Chloroform
<i>A. oryzae</i>	14.0±0.1	10.0±0.1 ^b	11.0±0.1 ^b	12.0±0.1 ^b
<i>A. flavus</i>	15.0±0.1	10.0±0.2 ^a	11.0±0.2 ^a	12.0±0.1 ^b
<i>A. niger</i>	15.0±0.1	10.0±0.2 ^a	11.0±0.2 ^a	12.0±0.1 ^b

SD = Standard deviation, significant level of difference from control; P^a<0.05, P^b>0.05

Conclusion

A. oryzae, *A. flavus* and *A. niger* grow in stored sorghum hence they contaminate sorghum with fungal poisonous secondary metabolites called mycotoxins. The consumption of such mycotoxin contaminated grains by animals and human beings cause mycotoxicoses. This has great public health significance. Mycotoxins are capable of causing death and disorder of central nervous system. They can also cause pulmonary system and intestinal tract disorder. Sorghum is important crops in Africa including Nigeria. The crop is favoured due to its productivity and short growing season under dry, high temperature conditions. Sorghum is mostly grown for fodder and human food. Extracts of *A. sativum* and *Z. officinale* exhibit antifungal property against the fungi used in this study. The findings could serve the purpose of alerting consumers on the dangers of consuming poorly stored sorghum. The results of this study also suggest that extracts of plants could prevent the growth of fungi in stored sorghum and also serve as a source of chemotherapeutic agents.

Conflict of interest

Authors have declared that no conflict of interest exist

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