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Studies on the Control of Mycotoxin Producing Fungi Isolated from Sorghum Sold in Bida, Niger State Nigeria

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ABSTRACT: Sorghum is an important crop in Africa including Nigeria, Mali and Niger. Fungi contaminate grains including sorghum with fungal poisonous secondary metabolites called mycotoxins. The objectives of this study are the isolation of fungi associated with sorghum in storage and assay for the presence of mycotoxins in stored sorghum. Data obtained showed that stored sorghum used in this study contains *Rhizopus stolonifer*, *Aspergilus oryzae, Aspergilus flavus, Aspergilus niger, Aspergilus solani*, *Aspergilus terreus and Fusarium oxysposum. Rhizopus stolonifer* and *Fusarium oxysporum* produced *zearalenone* while *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus solani* and *Aspergillus tereus* produced aflatoxins B_1. Fumanisin B_1 and aflatoxinB_1 were produced by *Aspergillus flavus*. *Alium sativum* and *Zingiber officinale* exhibited antifungal activity against the test fungi. This research work will provide a long term economic impact in reducing mycotoxicoses which are acute and chronic toxic diseases caused by mycotoxins. The findings will also serve the purpose of alerting consumers on the dangers of consuming poorly stored sorghum.

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Mycotoxins are toxic secondary metabolites produced by organisms of the fungus kingdom (Richard, 2007) and 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, ochnatoxin A, patulin, trichothecens, zeareceonone 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). Diseases associated with common mycotoxins are aflatoxicoses (aflatoxin), Balkon Endemie Nephropathy (BEN) (ochratoxin); equine Leukoencephalo malacia and porcine pulmonary edema (fumonisin), haemorrhagic disease and alimentary toxic aleukia (ATA) (trichothecenes), estrogenic disease (zearalenone) (WHO, 2018).Mycotoxin control will result in economic gains as well as health improvement in Bida, Niger State. 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 terrous*, 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).Nigeria has experienced high recorded aflatoxin exposure levels in humans and has also reported the highest number of cases of hepatocellular carcinoma (HCC-liver cancer)

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attributable to aflatoxins (Liu and WU, 2010) in the whole world. Hot and humid conditions usually favour mould growth and mycotoxin production, hence mycotoxins occur more frequently in hot and humid areas. The significant economics and health hazards caused by fungal and mycotoxin especially in developing countries that have poor food storages is of great concern, so to ensure a healthy food supply thereby minimizing consequences to food security, trade, animal and human health; there is need to alert consumers of the dangers of consuming poorly stored sorghum and control the growth of mycotoxin producing fungi in sorghum sold in Bida, Niger State, Nigeria.

MATERIALS AND METHODS

Study Area: Bida lies within latitude $9^{o}\ 4^{1}$ 46.844^{1}N and longitude $6^{0}\ 0^{1}$ 34.9272^{1}E. It has a bimodal rainfall distribution averaging between 900mm and 1500mm annually and maximum temperatures hovering between 27^{0}C and 35^{0}C.

Sample collection: A total of 200 samples of sorghum were collected in the month of August, 2022. Three areas were selected for study site from new market, post office market and small market. In every market sorghum grains in store and market were sampled from five locations, each approximately 10 meters from the previous sampling location. At each location, 1kg of millet with or without visible signs of fungal growth was arbitrarily selected from the store. Forty (40) grains were taken from each location 3g each was separated to be used for fungal isolation, while 1g from each sample for mycotoxin analysis was separated and placed in separate polyethylene bags for mycotoxin analysis. To prevent further postharvest accumulation of mould prior to analysis; all the samples were stored at 4^{0}C in a freezer. All samples were homogenized in a blender; each sample was placed in a Vertis homogenizer model no 6-105 and milled for 3-5 minute, after milling the powered samples was transferred into new polyethylene bags. The blender cup was washed and disinfected with 70% ethanol before blending the next sample to avoid cross contamination.

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

Test organisms/fungal screening: The fungi used in this study are *Rhizopus stolonifer, Aspergillus orizae*, *Aspergillus flavus*, *Aspergillus niger, Aspergillus solani, Aspergillus terries* and *Fusarium oxysporium*. 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 (Banso *et al*., 2020b).

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. The extracts obtained were evaporated to dryness using shaking water both (Rolitech) at 80^{0}C (Banso *et al*. 2020a).

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 will be 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± 1°C for 12 days. 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. 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. Rf= distance travelled by the silent.

Determination of minimum fungicidal concentration (MFC): The content of the tubes that showed no visible fungal growth in the minimum inhibitory concentration experiments as earlier reported by Banso *et al*. (2020a) was cultured into freshly prepared malt extract agar (MEA) plates and assayed for the fungicidal effect of the extracts. The plates containing the test fungi were incubated at 28±2°C for 72 hours. The lowest concentration of the extract that does not yield any visible fungal growth on the solid medium

was regarded as the minimum fungicidal concentration.

*Comparison of minimum fungicidal concentration of ethanolic extract of Z. officinale with pure antibiotis:*The experiment was performed as described for the determination of minimum fungicidal concentration except that ethanolic extract of *Z. officinale* and two pure antibiotics – Nystatin (Vardhman export) and Griseofulvin (Clarion medicals Ltd), were used in the experiment. Various concentrations (5%, 10%, 15%, 20% 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, and w/v) of the antibiotics were obtained, using sterile water. The lowest concentration of the extract or antibiotics that does not yield any colony growth on the solid medium after the incubation period was regarded as the MFC. The result of the MFC of *Z.officinale* extract was compared with those of Greseofulvin.

Data analysis: The results were subjected to analysis of variance and mean comparisons was performed by Turkey's multiple range tests using SPSS version 20.0 (statistical package for social sciences, Inc, Chicago IL, 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 *R.stolonifer, A.oryzae, A. flavus, A. niger, A. solani, A. terreus* and *F. oxysporum* (Table 1). This agrees with the report that *Aspergillus*, *Penicillim Fusarium* and *Alternaria* among others are important food contaminants (Amadi, 2009).

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

Isolate	Cultural characteristics	Microscopic characteristics
R. stolonifer	Colony white initially and turns grey to yellowish brown. The reverse is white	The thallus is white cottony, branched mycelium, rhizoids and stolons present.
A. oryzae	Surface colony is brownish yellow, cottony appearance initially white and then turn black. Reverse is white	Hyphae is hyaline and septate. The conidiophores ends at a round shaped vesicle.
A. flavus	Grey white colony , later turn black, reverse is orange yellow	The conidia is slightly rough, conidiophores is unbranched.
A. niger	Cottony white colony, turn black, reverse is white	Smooth coloured conidiophores and conidia are present
F. solani	White cottony colony, turn bluish brown, underside is white milk brown, colonies loose and slimy.	Hyphae is septate and hyaline, microconidia are oval hyaline and smooth.
A. tarreus	Surface colony brown becomes dark with age.	Septate hyphae, short smooth conidiophores, conidiophores enlarged at the tip forming a swollen visicle.
F. oxysporum	White cottony mycelium and a dark undersurface, microconidia are oval in shape	Microconidia are slightly curved, non septate and pointed at the tip

Mycotoxin produced by fungi: Table 2 showed that *Rhizopus stolonifer* and *Fusarium oxysporum* produced zearalenone while *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus solani* and *Aspergillus tereus* produced aflatoxins B_1. Fumanisin B_1 and aflatoxinB_1 were produced by *Aspergillus flavus*. The retention factor (R_f) recorded against *A. oryzae*, *A.niger*, *R.solani* and *A.tereus* was 0.45 while a value of 0.41 was recorded against *F. oxysporum* and *R. stolonifer*. The retention factors recorded against A. flavus were 0.45 and 0.69 (Table 2). In a similar report Rouaa *et al*. (2021) demonstrated that *Aspergillus favus* produced Aflatoxin B_1, B_2 and G_2 in wheat, maize rice and peanuts. Mycotoxigenic fungi are common in agricultural regions. They grow on a wide range of crops and produce mycotoxins under different conditions (Richard *et al*., 2003).

Table 2: Mycotoxin produced by fungi isolated from stored grain

Fungi	Retention factor (R_f)	Mycotoxin
R. stolonifer	0.41	Zearalenone
A. oryzae	0.45	Aflatoxin B_1
A. flavus	0.45	Aflatoxin B_1
	0.69	Fumanisin B_1
A. niger	0.45	Aflatoxin B_1
A. solani	0.45	Aflatoxin B_1
A. terreus	0.45	Aflatoixin B_1
F. oxysporum	0.41	Zearalenone

Minimum fungicidal concentration (MFC) of ethanol extracts of A. sativum and Z. officinale: The results of minimum fungicidal concentration of *A. sativum* and *Z. officinale* are shown in Table 3. The minimum fungicidal concentration recorded against *R. stolonifer*, *A. oryzae*, *F .oxysporum* and *A. solani* was 50mg/ml when ethanol extract of *A. sativum* was assayed against the fungi while values of 40mg/ml

was recorded against *A. niger* and *A.terreus* (Table 3).Minimum fungicidal concentration values of 50mg/ml was recorded against *A. oryzae*, *A. niger* and *A. solani* when ethanol extract of *Z. officinale* was assayed against the fungi, however a fungicidal concentration value of 40mg/ml was recorded against *R. stolonifer*, *F. oxysporum* and *A. terreus* (Table 3). The results agree with report that microorganisms varied in their degree of susceptibility to a given agent (Banso *et al*., 2020a). Antifungal agent with high activity against fungus has a low minimum fungicidal concentration.

Table 3: Minimum fungicidal concentration (MFC) of ethanol extracts of *A. sativum* and *Z. officinale*

	A. sativum		*Z.officinale*	
Organism	MFC(mg/ml)	Control	MFC(mg/ml)	Control
R.stolonifer	50	NF	40	NF
A. oryzae	50	NF	50	NF
F.oxysporum	50	NF	40	NF
A.niger	40	NF	50	NF
F. solani	50	NF	50	NF
A.terreus	40	NF	40	NF

*Comparison of minimum fungicidal concentration (MFC) of ethanol extract of Z. officinale with those of Nystatin and Greseofulvin:*The results of minimum fungicidal concentration of ethanol extract of *Z. officinale* compared with Nystatin and Greseofulvin antifungals are shown in Table 4. The minimum fungicidal concentration of both antibiotics against *R. stolonifer, A. oryzae, F.oxysporum, A. niger, F.solani* and *A. terreus* were lower than those of ethanol extracts of *Z. officinale* against the organisms. The minimum fungicidal concentrations recorded against *R. stolonifer* and *A.oryzae* were 40mg/ml and 50mg/ml respectively when *Z. officinale* was assayed against then fungi. Minimum fungicidal concentration values ranging from 30mg/ml to 45mg/ml were recorded against nystatin while values ranging from 25mg/ml and 45mg/ml were recorded against greseofulvin (Table 4). The results show that there is no significant difference in the values recorded against *Z. officinale* and nystatin.

Table 4: Minimum fungicidal concentration (MFC) of ethanol extract of *Z. officinale* compared with Nystatin and Greseofulvin

	Z.officinale		Nystatin			Greseofulvin
Organism	MFC(mg/ml)	Control	MFC(mg/ml)	Control	MFC(mg/ml)	Control
R. stolonifer	40	NF	30	NF	25	NF
A.oryzae	50	NF	40	NF	25	NF
F.oxysporum	40	NF	35	NF	30	NF
A.niger	50	NF	40	NF	45	NF
F.solani	50	NF	45	NF	40	NF
A.terreus	40	NF	30	NF	30	NF

NF = No fungicidal activity,

Conclusion: 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. 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. Fungi 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. 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 A. *sativum*and *Z. officinale* could prevent the growth of fungi in stored sorghum.

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