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, Aspergillus oryzae, Aspergillus flavus, Aspergillus niger, Aspergillus solani, Aspergillus terreus and Fusarium oxysporum. Rhizopus stolonifer and Fusarium oxysporum produced zearalenone while Aspergillus oryzae, Aspergillus niger, Aspergillus solani and Aspergillus terreus produced aflatoxins B1, Fumonisin B1 and aflatoxin B1, were produced by Aspergillus flavus. Allium 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.

DOI: https://dx.doi.org/10.4314/jasem.v27i7.10

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Cite this paper as: BANSO, A; AJEIGBE, S.O; AJAYI, M. A (2023). Studies on the Control of Mycotoxin Producing Fungi Isolated from Sorghum Sold in Bida, Niger State Nigeria. J. Appl. Sci. Environ. Manage. 27 (7) 1403-1407

Dates: Received: 18 May 2023; Revised: 21 June 2023; Accepted: 04 July 2023 Published: 30 July 2023

Keywords: Mycotoxins; immunotoxic, aflatoxin; fumunosin B1, Aspergillus

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, ochratoxin A, patulin, trichothecens, zearaenone 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 Leukoencephal malacia and porcine pulmonary edema (fumonisins), 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 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). 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).
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° 4’ 46.844”N and longitude 6° 0’ 34.9272”E. It has a bimodal rainfall distribution averaging between 900mm and 1500mm annually and maximum temperatures hovering between 27°C and 35°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°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 officinale) 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 terris 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 intermittently 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°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 80°C for 72 hours. 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

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was regarded as the minimum fungicidal concentration.

Comparison of minimum fungicidal concentration of ethanolic extract of *Z. officinale* with pure antibiotics: 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 Griseofulvin.

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

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

**Mycotoxin produced by fungi:** Table 2 showed that *Rhizopus stolonifer* and *Fusarium oxysporum* produced zearalenone while *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus solani* and *Aspergillus terreus* produced aflatoxins B₁, Fumonisins B₁ and aflatoxinB₁ were produced by *Aspergillus flavus*. The retention factor (R<sub>s</sub>) recorded against *A. oryzae*, *A. niger*, *R. solani* and *A. terreus* 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 flavus* produced Aflatoxin B₁, B₂ and G₂ 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

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Retention factor (R&lt;sub&gt;s&lt;/sub&gt;)</th>
<th>Mycotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. stolonifer</em></td>
<td>0.41</td>
<td>Zearalenone</td>
</tr>
<tr>
<td><em>A. oryzae</em></td>
<td>0.45</td>
<td>Aflatoxin B₁</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>0.45</td>
<td>Aflatoxin B₁</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>0.45</td>
<td>Fumonisins B₁</td>
</tr>
<tr>
<td><em>R. solani</em></td>
<td>0.45</td>
<td>Aflatoxin B₁</td>
</tr>
<tr>
<td><em>A. terreus</em></td>
<td>0.45</td>
<td>Aflatoxin B₁</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>0.41</td>
<td>Zearalenone</td>
</tr>
</tbody>
</table>

**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

**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*, *Penicillium* *Fusarium* and *Alternaria* among others are important food contaminants (Amadi, 2009).

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.
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

<table>
<thead>
<tr>
<th>Organism</th>
<th>A. sativum MFC (mg/ml)</th>
<th>Z. officinale MFC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. stolonifer</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>F. oxysporum</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>A. niger</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>F. solani</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>A. terreus</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Z. officinale MFC (mg/ml)</th>
<th>Nystatin MFC (mg/ml)</th>
<th>Greseofulvin MFC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. stolonifer</td>
<td>40</td>
<td>NF</td>
<td>30</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>50</td>
<td>NF</td>
<td>45</td>
</tr>
<tr>
<td>F. oxysporum</td>
<td>40</td>
<td>NF</td>
<td>30</td>
</tr>
<tr>
<td>A. niger</td>
<td>50</td>
<td>NF</td>
<td>45</td>
</tr>
<tr>
<td>F. solani</td>
<td>50</td>
<td>NF</td>
<td>40</td>
</tr>
<tr>
<td>A. terreus</td>
<td>40</td>
<td>NF</td>
<td>30</td>
</tr>
</tbody>
</table>

**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.

**Acknowledgments:** This institution based research (IBR) with reference number TETF/DR&D/POLY/BIDA/2022/VOLII/SN6 was funded with institution based research fund (IBRF) obtained from TETFund. The authors acknowledge TETFund for funding this research work.

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