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Viability of Fungal Spores Isolated from Sorghum Grains Sampled from the Field, Market and Different Storage Facilities in the Six Agro-ecological Zones of Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MHG, HAM and BNP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BNP and AAJ managed the analyses of the study. Authors LMH and AYK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Nutritional quality, organoleptic attributes and food safety are usually compromised by the presence of Microscopic fungi or fungal spores in food and feeds that humans and animals solely rely on. It is therefore intended in this study to re appraise the viability of fungal spores in sorghum from different ecosystems in Nigeria with a view to establish the level of infection/contamination and also to establish a basis for predicting the possible mycotoxins that may likely be present in sorghum obtained from the study areas. Sorghum sample was collected from six Agro ecological zones of

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Nigeria. Each zone was transversely delineated into districts and five villages (at least 20 Km from each other) called locations were selected in each district. In each district, Sorghum grains in stores, bunches in the field and sorghum grains in the market were sampled from five locations, each approximately 20 km from the previous sampling location. The mycological analytical procedures were performed under aseptic condition. One gram of milled sample was weighed into a test tube and diluted in 9 ml of sterile Ringer's solution, vortexed and serially diluted further to $10^{-6.}$ One ml from each test tube was cultured by pour plate technique on Ohio Agricultural Station agar (OAESA) and potato dextrose agar (PDA) and incubated for 4-7 days at 25℃. Plates were counted for fungal colonies using a colony counter and the number of fungal colonies per gram of sample was calculated and expressed in colony forming units per gram of sample. The fungi species were isolated and subsequently identified using MEA/CYA media for Aspergillus and Penicillium species and PDA for the fusarium species. It was observed that: the count range in all the samples from all the ecological zones is far above the standard Mycological poor quality standard of 7 x 10^4. The highest value of 1.3x 10^8 and the lowest value of 6.7x 10^6 all which outrageously exceed the bad quality range were obtained. This is a clear indication that people subsisting on sorghum and sorghum based products are at a high risk of exposure to both Mycoses and Mycotoxicosis in all the Agro ecological zone of the country and the traditional storage system seems infective in curtailing fungal proliferation in the studied area.

Keywords: Sorghum; fungi; agro-ecological zones; colony forming unit; nutritional quality; Nigeria.

1. INTRODUCTION

Significant focus and resources have been allocated to increase food production over the past decade. For instance, when it was only 5% that was directed towards reducing losses, 95% of the research investments during the past 30 years were reported to have been committed on increasing productivity [1,2]. Global food security can be attained, not only through increasing agricultural productivity, but by also minimising losses and ensuring its safety alongside. Food production is currently being challenged by Limited land, water and increased weather variability due to climate change. There is therefore, need to approach this challenge through reductions in the post-harvests losses at farm, retail and consumer levels in order to achieve sustained food security.

Based on the UN, [3] projection, the current world population is expected to reach 10.5 billion by 2050, further adding to global food security concerns, it has also been estimated by Food and Agriculture Organization of the same U.N. that, about 1.3 billion tons of food are globally wasted or lost per year [4]. Based on these projections, food supplies would need to increase by 60% (estimated at 2005 food production levels) in order to meet the food demand in 2050 [5]. Food availability and accessibility can be increased when attention is focused on the entire value chain, such as: increasing production, improving distribution, and reducing the losses. Thus, reduction of post-harvest food losses is a critical component of ensuring future global food security.

A reduction in food loses also improves food security by increasing the real income for all the consumers [6]. This is due to the fact that, crop production contributes significant proportion of typical incomes in certain regions of the world (70 percent in Sub-Saharan Africa) and efforts geared at reducing food loss can directly increase the real incomes of the producers [6]. Food losses do not merely reduce food available for human consumption but also cause negative externalities to society through costs of waste management, greenhouse gas production, and loss of scarce resources used in their production.

It was indicated in a study by Institute of Mechanical Engineers that, the current agricultural practices use 4.9 Gha (global hectares or 4931 million hectares) of the total 14.8 Gha (14894 million hectares) of land surface on the earth [7]. The same agricultural practices in addition uses 2.5 trillion m^3 of water per year and consume over 3% of the total global energy [7]. Taking into account the estimated food losses of about 30-50% of total production, this translates to wasting 1.47-1.96 Gha of arable land, 0.75-1.25 trillion m^3 of water and 1% to 1.5% of global energy [7].

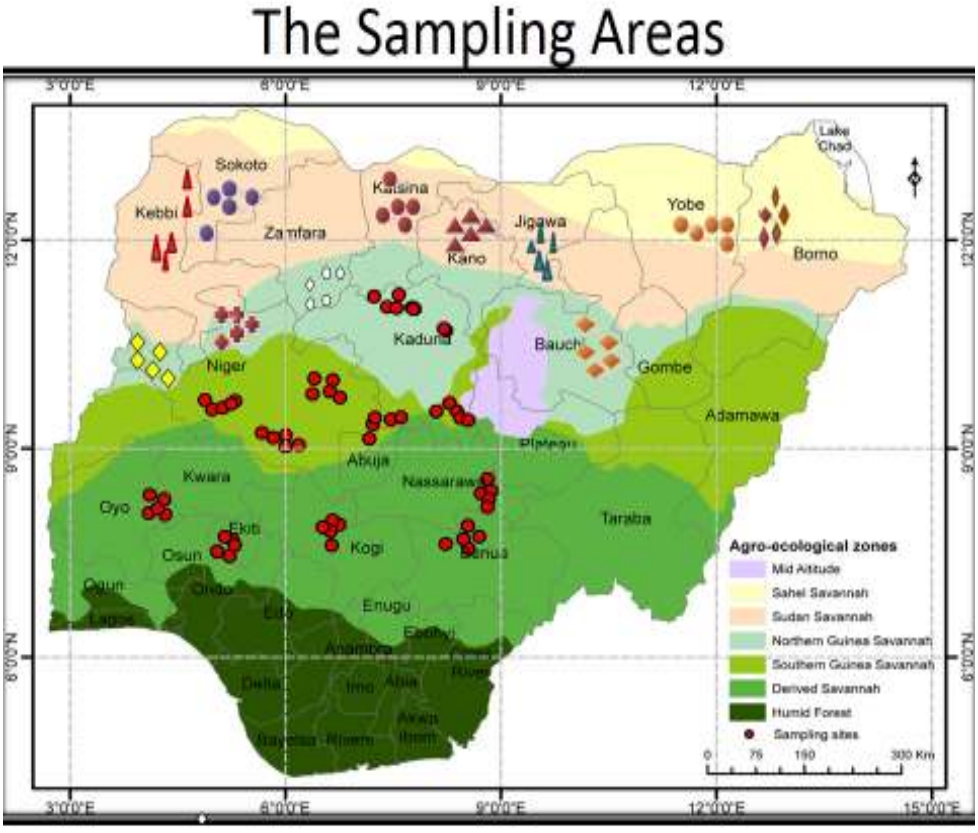
Given the significant role food loss reductions could have toward sustainably contributing to

global food security, it is important to regularly assess the viability of fungal spores at intervals so as to predict the possible loss that can be incurred and the possible health hazards that may be associated with such food(s) substance(s).

2. MATERIALS AND METHODS

2.1 Sampling

This was based on the method of Atenkengh et al. [8] with some modifications. Five districts were selected for the study, that is: Derived Savannah (DS) (Ado-Ekiti, Lafia, Lokoja, Makurdi, and Ogbomosho), the Southern Guinea Savannah (SGS) (Abuja, Akwanga, Bida, Minna, and Mokwa), the Northern Guinea Savannah (NGS) (Zaria, Kontagora, Kaduna, Bauchi and Rijau), the Sudan Savannah, (SS) (Sokoto, Daura, Kebbi, Dutse and Dawanau), and five districts (Goronyo, Sabon Birni, Baure, Kirikasamma and Guri/Nguru) were selected from the Sahel savannah SHS while Riyom, Toro, Langtang and Wase were selected from the Mid-Altitude In each district, Sorghum grains in stores and bunches in the field were sampled from five locations, each approximately 20 km from the previous sampling location. At each location, a single farmer who grew sorghum in the previous season was identified and 1 kg of sorghum with or without visible signs of fungal growth was arbitrarily selected from the farmer's store. Only Sorghum that had been in storage for up to 2 months were sampled from each farmer during the survey. This duration is long enough for mycotoxin to accumulate in fungi infected Sorghum grains [9]. All the samples were placed in bags, properly sealed and transported to our laboratory in Federal University of Technology, Minna. A total of 25 Kg (1 kg×five locations×five districts) were collected in the DS , SGS, NGS, Sudan savannah SS and SHS, while 20 kg was collected from the Mid- Altitude. Hundred grams was taken from each kg in each of the locations and these 100 g were composited and then sub lotted to the final 100 g which was used for fungal isolation. To prevent further postharvest accumulation of moulds prior to analysis, all the samples were properly sealed and stored at 4℃.



Map. 1
Modified from: Atenkengh et al., (2008) [8]

2.2 Spores Culture and Growth

The mycological analytical procedures [10] was performed under aseptic condition. One gram of milled sample was weighed into a test tube and diluted in 9ml of sterile Ringer's solution, vortexed and serially diluted further to 10^{-6}. One ml from each test tube was cultured by pour plate technique on Ohio Agricultural Station agar (OAESA) and potato dextrose agar (PDA) and incubated for 4-7 days at 25℃. Plates were counted for fungal colonies using a colony counter and the number of fungal colonies per gram of sample was calculated and expressed in colony forming units per gram of sample (CFU/g) as:

$$\text{CFU/g} = \frac{\text{Number of colonies}}{\text{Plating volume (1 ml)}} \text{ X reciprocal of DF}$$

Where DF = dilution factor

Isolated fungal colonies were further sub-cultured on PDA, Czapek yeast agar (CYA) and malt extract agar (MEA) according to Kaufman et al. [10] under aseptic conditions and incubated at 25℃ for 7 days. Pure fungal colonies were harvested and stained with lactophenol in cotton blue and mounted on microscope slides for identification. The macro- and microscopic identifications of *Fusarium* species was done following the identification keys of Pitt and Hocking, [11] and Nelson et al. [12]. Both the indentified and unidentified fungal isolates were sent to Inqaba Biotechnological Laboratories, Pretoria, South Africa for confirmation and further analysis and identification (In case of the unidentified samples). For preservation, isolates were sub-cultured on PDA slants for 7 days at 25℃ and stored at 4℃.

2.3 DNA Extraction, PCR and Sequencing

In a case where the morphological characteristics of individual fungal spp.were not sufficient for clear identification and depending on the relative importance of the fungus with respect to its potential to produce various mycotoxins, further analysis was performed. The technique involving the comparison of nucleic acid profiles of individual fungal species was, therefore, employed using a GeneAmp PCR System 9700 and an automated sequencer—ABI PRISM 3700 Genetic analyser according to Samson et al. [13].

Genomic DNA analysis (during confirmation and identification) was performed using a Fungal/Bacterial DNA extraction kit (Zymo Research Corporation, Southern California, USA). The freeze-dried cultures were allowed to stand 1 hour at room temperature and then DNA was extracted. In this case, about 60 mg of sample was mixed with 200 µl of phosphate buffer saline (PBS) contained in a 1.5 ml ZR Bashing BeadTM lysis tube. The lysis tube was then placed in a beater and processed for 5 mins, followed by centrifugation at 10,000 g for 1 min. The supernatant was transferred to a Zymo-SpinTM IV spin filter in a 1.5 ml Eppendorf tube and again centrifuged at 7,000 *g* for 1 min. The content was filtered into a collection tube and 1,200 µl of fungal/bacterial DNA binding buffer was added and vortexed. Extraction mixture (800 µl) was transferred to a Zymo-Spin™ IIC column in the collection tube which was again centrifuged at 10,000 *g* for 1 min with the supernatant discarded (X2). A 200 µl of DNA pre-wash buffer I aliquot was added to the Zymo-Spin™ IIC column in a new collection tube and centrifuged at 10,000 *g* for 1 min. The filtrate was discarded, while retaining the column, which was then placed into a new tube, 500 µl fungal/bacterial DNA wash buffer II was added to the Zymo-Spin™ IIC column and again centrifuged at 10,000 *g* for 1 min. The Zymo-Spin™ column was transferred to a sterile 1.5 ml Eppendorf tube and 100 µl DNA elution buffer was added directly to the column matrix. This was then centrifuged at 10,000 *g* for 30 secs to elute the DNA.

Identification of *fungal* spp. in question was done by isolating the translation elongation factor (TEF) 1α region following the sequence obtained from different databases. The primer sequences used were those described by O'Donnell and Cigelnik, [14] designed in conserved 5' and 3' regions. The primers were synthesized at a 0.01 µM scale and purified using reverse-phase cartridge purification (Inqaba Biotechnical Industries (Pty) Ltd, South Africa). These primers were resuspended in 2 µM TE buffer prepared from a stock solution concentration of 100 µM.

Therafter, PCR was performed using the Fermentas 2 X PCR mix (Fermentas Life Science, Lithuania). The PCR mix for each sample was made to consist of 25 µl of 2 X PCR mix,1µl of each 2 µM primer, 1 µl of DNA (final concentration of 10 µM), and was constituted to a final volume of 50 µl with nuclease free water. A

negative control, containing all of the reagents except the DNA was also prepared. The PCR was then performed using an Eppendorf 96-well Thermocycler (Eppendorf, USA). The PCR cycling conditions was set as: Pre-dwelling at 95^{o}C for 3 mins, 35 cycles denaturation at 95^{o}C for 1 min, annealation at 58℃ for 45 secs, extension at 72℃ for 1 min 30 seconds, post-dwelling at 72℃ for 10 minutes and hold at 4℃ and the samples were thereafter retrieved.

The preparation of 2% agarose gel was carried by dissolving 2 g of agarose (Fermentas Life Science, Lithuania) in 98 ml 1x TBE buffer (Fermentas Life Science, Lithuania) and then boiled. The solution was thereafter cooled to approximately 60℃. Ethidium bromide (3 µl) (Sigma-Aldrich, ST Louis, MO, USA) was then added to the solution and thoroughly mixed. The agarose solution was then poured into a casting chamber (Bio-Rad Laboratories, California, USA) and the combs with 10 wells were carefully inserted. The chambers of the running system (Bio-Rad Laboratories, California, USA) was filled with 1 X TBE buffer (Fermentas Life Science, Lithuania). Each PCR product (2 µl) previously obtained was mixed with 10 µl of 6 X orange loading dye (Fermentas Life Science, Lithuania) and loaded into the wells. The chamber was closed and run at 70 V for 15 minutes. The PCR product so formed was viewed using the Vacutec Gel documentation system and the product size was confirmed by comparing it to the Middle Range FastRuler (Fermentas Life Science, Lithuania).

PCR products obtained from the synthesis process were cleaned using shrimp alkaline phosphatase and *E. coli* exonuclease I (Fermentas Life Sciences, Lithuania). The purity of the DNA was confirmed by running a 2% agarose gel (as previously described). Automated DNA sequencing was performed at Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, RSA) using the SpectruMedix model SCE 2410 automated DNA sequencer (SpectruMedix, State College, PA). The sequencing reaction mixture (prepared by Inqaba Biotechnical Industries (Pty) Ltd, South Africa) included the ABI BigDye Terminator Cycle Sequencing kit version 3.1 dye (Applied Biosystems, Foster City, CA) and the same primers used in the original PCR reaction.

Samples were then analyzed on an ABI PRISM 3700 Genetic analyzer (AB, Applied Biosystems, Nieuwerkerk a/d Yssel, The Netherlands). The forward and reverse sequences were assembled using the programmes SeqMan and EditSeq from the LaserGene package (DNAStar Inc. Madison, WI). Alignments of the partial β-tubulin gene sequences Samples data were calculated using a software package BioNumerics (Applied Maths BVBA, Saint Martens-Latem, Belgium) and adjustments made manually with the aid of an eye to maximize homology.

Some reference sequences for the TEF 1 α coding region referred to by O'Donnelland Cigelnik, [14], and Geiser et al. [15] was used. These sequences, in FASTA format was obtained from the FUNGI ID v. 1.0 database Geiser et al. [15].

2.4 Statistical Analysis

Data analysis was performed by analysis of variance (ANOVA). The test of least-significant differences (LSD) was used to determine the significant differences between means. Analysis was conducted using PROC GLM in SAS (SAS Institute, Cary, NC, USA). Statistical significance was indicated by $p \leq 0.05$.

3. RESULTS

3.1 Description of Storage Facility

Store room = locally made/constructed facility from blocks or bricks with the side walls often plasteredand floor often enforced with concrete.

Rumbu (local silo) = A facility constructed from mud bricks with the base separated from the ground using stones. It always has a thatched roof.

Sealed rumbu = Mode of construction similar to rumbu but is sealed to the top instead of being roofed. A window like opening is provided as the only entrance to the facility.

Ruhewa: This has the shape and design as the rumbu but is entirely made from grasses instead of the mud brick and therefore more porous than the latter.β.

Table 1. Colonies forming unit (CFU/g) of fungi species from sorghum grains in the six agro-ecological zones of Nigeria

Agro-ecological zones	District	Colony forming unit (CFU/g) Mean ± SEM	Storage facility
Derived Savannah (DS)	DSD1F	$1.13 \times 10^{7} \pm 0.34 \times 10^{5}$	
	DSD1STR	$2.57 \times 10^{7} \pm 1.02 \times 10^{6}$	**Ordinary store room**
	DSD1MKT	$7.78 \times 10^{7} \pm 2.4 \times 10^{5}$	
	DSD2F	$7.2 \times 10^{6} \pm 3.6 \times 10^{6}$	
	DSD2STR	$4.87 \times 10^{7} \pm 2.6 \times 10^{6}$	**Store room**
	DSD2MKT	$6.63 \times 10^{7} \pm 0.5 \times 10^{7}$	
	DSD3F	$3.62 \times 10^{7} \pm 2.02 \times 10^{6}$	
	DSD3STR	$1.82 \times 10^{7} \pm 1.0 \times 10^{6}$	**Store room**
	DSD3MKT	$2.62 \times 10^{7} \pm 8.1 \times 10^{6}$	
	DSD4F	$5.8 \times 10^{6} \pm 9.6 \times 10^{5}$	
	DSD4STR	$5.55 \times 10^{7} \pm 4.9 \times 10^{6}$	**Store room**
	DSD4MKT	$7.5 \times 10^{6} \pm 1.3 \times 10^{6}$	
	DSD5F	$6.37 \times 10^{7} \pm 6.7 \times 10^{6}$	
	DSD5STR	$4.83 \times 10^{7} \pm 1.2 \times 10^{7}$	**Thatch Rhumbu/S/room**
	DSD5MKT	$1.12 \times 10^{7} \pm 12.7 \times 10^{6}$	
Southern Guinea Savannah (SGS)	SGSD1F	$8.97 \times 10^{7} \pm 4.6 \times 10^{6}$	
	SGSD1STR	$5.25 \times 10^{7} \pm 8.3 \times 10^{5}$	**Roof top and Store room**
	SGSD1MKT	$1.11 \times 10^{8} \pm 2{,}9 \times 10^{8}$	
	SGSD2F	$1.16 \times 10^{8} \pm 1.23 \times 10^{7}$	
	SGSD2STR	$4.17 \times 10^{7} \pm 3.4 \times 10^{6}$	**Store room**
	SGSD2MKT	$1.3 \times 10^{7} \pm 7.1 \times 10^{5}$	
	SGSD3F	$2.2 \times 10^{7} \pm 1.4 \times 10^{7}$	
	SGSD3STR	$2.7 \times 10^{7} \pm 0.54 \times 10^{7}$	**Store room**
	SGSD3MKT	$2.97 \times 10^{7} \pm 2.6 \times 10^{6}$	
	SGSD4F	$5.25 \times 10^{7} \pm 1.0 \times 10^{7}$	
	SGSD4STR	$4.38 \times 10^{7} \pm 3.7 \times 10^{6}$	**Store room**
	SGSD4MKT	$6.65 \times 10^{7} \pm 2.4 \times 10^{6}$	
	SGSD5F	$1.32 \times 10^{8} \pm 6.8 \times 10^{7}$	
	SGSD5STR	$2.58 \times 10^{7} \pm 1.9 \times 10^{6}$	**Rumbu with thatch roof**
	SGSD5MKT	$3.58 \times 10^{7} \pm 3.8 \times 10^{6}$	
Northern Guinea Savannah (NGS)	NGSD1F	$1.00 \times 10^{8} \pm 8.7 \times 10^{7}$	**Store room**
	NGSD1STR	$1.05 \times 10^{8} \pm 0.23 \times 10^{8}$	
	NGSD1MKT	$1.12 \times 10^{8} \pm 2.5 \times 10^{7}$	
	NGSD2F	$8.9 \times 10^{7} \pm 1.3 \times 10^{7}$	
	NGSD2STR	$1.4 \times 10^{7} \pm 4.3 \times 10^{6}$	**Rumbu/Store room**
	NGSD2MKT	$1.03 \times 10^{8} \pm 5.6 \times 10^{7}$	
	NGSD3F	$8.9 \times 10^{7} \pm 0.97 \times 10^{7}$	
	NGSD3STR	$6.88 \times 10^{7} \pm 2.8 \times 10^{6}$	**Rumbu/Ruhewa**
	NGSD3MKT	$1.05 \times 10^{8} \pm 6.9 \times 10^{7}$	
	GSD4F	$5.7 \times 10^{7} \pm 1.4 \times 10^{7}$	
	NGSD4STR	$9.4 \times 10^{7} \pm 3.8 \times 10^{6}$	**Rumbu/Store room**
	NGSD4MKT	$6.28 \times 10^{7} \pm 1.02 \times 10^{7}$	
	NGSD5F	$8.05 \times 10^{7} \pm 4.4 \times 10^{6}$	
	NGSD5STR	$1.02 \times 10^{8} \pm 6.1 \times 10^{7}$	**Underground Pit/Rumbu**
	NGSD5MKT	$6.08 \times 10^{7} \pm 0.56 \times 10^{7}$	
Sudan Savannah (SS)	SD1F	$3.2 \times 10^{7} \pm 1.6 \times 10^{7}$	**Sealed Rumbu/Ruhewa**
	SSD1STR	$3.33 \times 10^{7} \pm 0.54 \times 10^{7}$	
	SSD1MKT	$1.32 \times 10^{7} \pm 8.6 \times 10^{6}$	
	SSD2F	$2.35 \times 10^{7} \pm 0.24 \times 10^{7}$	
	SSD2STR	$2.08 \times 10^{7} \pm 7.4 \times 10^{6}$	**Sealed Rumbu/Ruhewa**
	SSD2MKT	$4.78 \times 10^{7} \pm 1.4 \times 10^{6}$	
	SSD3F	$2.88 \times 10^{7} \pm 5.9 \times 10^{6}$	
	SSD3STR	$1.9 \times 10^{7} \pm 0.34 \times 10^{7}$	**Rumbu/Ruhewa**
	SSD3MKT	$1.18 \times 10^{7} \pm 6.8 \times 10^{6}$	
	SSD4F	$6.13 \times 10^{7} \pm 1.4 \times 10^{7}$	
	SSD4STR	$7.25 \times 10^{7} \pm 0.67 \times 10^{7}$	**Sealed Rumbu/Ruhewa**
	SSD4MKT	$1.03 \times 10^{7} \pm 9.3 \times 10^{6}$	
	SSD5F	$4.55 \times 10^{7} \pm 3.8 \times 10^{6}$	

Agro-ecological zones	District	Colony forming unit (CFU/g) Mean ± SEM	Storage facility
	SSD5STR	$2.08 \times 10^{7} \pm 0.64 \times 10^{7}$	**Rhumbu/Underground str**
	SSD5MKT	$7.43 \times 10^{7} \pm 8.5 \times 10^{6}$	
Sahel Savannah (SHS)	SHSD1F	$1.02 \times 10^{7} \pm 0.65 \times 10^{7}$	
	SHSD1STR	$1.82 \times 10^{7} \pm 9.7 \times 10^{6}$	**Sealed Rumbu/Ruhewa**
	SHSD1MKT	$6.7 \times 10^{6} \pm \times 10^{7}$	
	SHSD2F	$3.4 \times 10^{7} \pm 8.4 \times 10^{6}$	
	SHSD2STR	$9.5 \times 10^{6} \pm 7.9 \times 10^{5}$	**Sealed Rumbu/Ruhewa**
	SHSD2MKT	$9.38 \times 10^{7} \pm 1.8 \times 10^{7}$	
	SHSD3F	$8.63 \times 10^{7} \pm 1.5 \times 10^{6}$	
	SHSD3STR	$1.62 \times 10^{7} \pm 0.57 \times 10^{7}$	**Store room/Ruhewa**
	SHSD3MKT	$1.8 \times 10^{7} \pm 4.9 \times 10^{6}$	
	SHSD4F	$1.28 \times 10^{7} \pm 0.65 \times 10^{7}$	
	SHSD4STR	$3.23 \times 10^{7} \pm 7.4 \times 10^{6}$	**Underground str/Ruhewa**
	SHSD4MKT	$2.37 \times 10^{7} \pm 0.8 \times 10^{7}$	
	SHSD5F	$1.53 \times 10^{7} \pm 5.3 \times 10^{6}$	
	SHSD5STR	$4.47 \times 10^{7} \pm 6.9 \times 10^{6}$	**Underground str/Ruhewa**
	SHSD5MKT	$8.75 \times 10^{7} \pm 1.23 \times 10^{7}$	
Mid- Altitude (MALT)	M-ALTD1STR	$5.3 \times 10^{7} \pm 7.4 \times 10^{6}$	**Store room**
	M-ALTD1MKT	$8.06 \times 10^{7} \pm 3.4 \times 10^{6}$	
	M-ALTD2F	$4.86 \times 10^{7} \pm 1.23 \times 10^{7}$	
	M-ALTD2STR	$7.9 \times 10^{7} \pm 1.9 \times 10^{7}$	**Thatched roofed Rumbu**
	M-ALTD2MKT	$3.13 \times 10^{7} \pm 0.56 \times 10^{7}$	
	M-ALTD3F	$3.60 \times 10^{7} \pm 2.8 \times 10^{6}$	
	M-ALTD3STR	$2.70 \times 10^{7} \pm 5.4 \times 10^{6}$	**Store room/ Rumbu**
	M-ALTD3MKT	$2.65 \times 10^{7} \pm 0.32 \times 10^{7}$	
	M-ALTD4F	$7.43 \times 10^{7} \pm 1.03 \times 10^{7}$	
	M-ALTD4STR	$2.9 \times 10^{7} \pm 4.8 \times 10^{6}$	**Store room/Rumbu**
	M-ALTD4MKT	$2.7 \times 10^{7} \pm 3.4 \times 10^{6}$	

DS = Derived Savannah, D1 = District 1, SGS = Southern Guinea Savannah, D2 = District 2
NGS = Northern Guinea Savannah, D3 = District 3, SS = Sudan Savannah, D4 = District 4
SHS = Sahel Savannah, D5 = District 5, MA = Mid- Altitude

Table 2. The identified fungal species and their partial β-tubulingene sequences from the six agro-ecological zones of Nigeria

Fungi	Gene sequence (β-tubulin) unless specified	Size (base pair) (bp)	Strain
Aspergillus flavus	5'gcttcgagtt agtatgcttt ggaccaagga actcctcaaa3'	493	CA 47
Aspergillus fumigatus	5'ggtaaccaaa tcggtgctgc tttctggtat gtcttgacct3'	556	UOA/HCPF10229
Aspergillus carbonarius	5'ctcaggcttt gtagcatgag tctagatgcc cattgttact3'	483	IHEM 1931
Aspergillus parasiticus	5'tttggaccag gtgaactcct ccaaagcatg atctcggatg3'	487	GC-3-3
Aspergillus oryzae	5'aaaatcggtg ctgctttctg gtatgtctca atgccttcga3'	480	Hb8
Aspergillus unguis	5'cttccgtagg tgaacctgcg gaaggatcat taccgagtgc3'	572	WJP S04
Aspergillus niger	5.ggtaaccaaa tcggtgctgc tttctggtac gtatacaact3'	552	UAO/HCPF4289
Apergillus ustus	5'gctttctggt acgtcgaaaa attccctcag cgattgtttg3'	310	CNM-CM4036
Aspergillus versicolor	5'tggtgcgtcg aaaatttcat ccatttcaga tggtatttcc3'	374	NRRL238
Neosartorya fischeri	5'ggggcggaat agctcgccga agggaccggc acggacagcg3'	477	FH 198
Aspergillus melleus	5'gtaaccaatc ggtgctgctt tctgggtagg ctacacgttg3'	577	CBS 54665
Aspergillus ochraceus	5'aaccaatcgg tgctgctttc tggtaagtct acacgttggc3'	563	CBS 94870
Emericella nidulans	5'tggtaaccaa atcggtgctg ctttctggtg agcgaaaatt3'	474	KCCM 60326
Aspergillus japonicum	5'ccaccaggca gaccatctcc ggcgaacatg gcctcgacgg3'	300	IHEM 5627
Sclerocleista ornata	5'aaagaaacca accgggattg cctcagtaac ggcgagtgaa3'	576	NRRL2256 (28S)
Aspergillus paradoxus	5'ggcgggcccg cccttgtggc cgccgggggg cttacgctcc3'	437	CBS643-95
Emericella quadrilineata	5'ggtaaccaaa tcggtgctgc tttctggtga gtcgaaaatt3'	443	FSU 9309
Penicillium citreonigrum	5'tggtacgtgc agacctggac aagatccatc aattgaggct3'	431	NRRL 35629
Penicillium restrictum	5'acgtgcagac ctgaaagatt catcaattga ggcttcgaga3'	394	-

Fungi	Gene sequence (β-tubulin) unless specified	Size (base pair) (bp)	Strain
Penicillium crustosum	5'ttttttttc gcgttgggna tcaattgaca ggttcctaac3'	382	CBS 110074
Penicillium implicatum	5'acaacgtaag tactactgat gacccattga gtatggatag3'	261	IFO 6098
Penicillium malodoratum	5'caaagagcct ggagttcatt tggctcgagt gatcagaatt3'	446	GR 77
Penicillium rogulosum	5'tggtacgtgc cacgcctccc tgggatatcc cgagcacaac3'	492	ZJ 01
Penicillium expansum	5'cgcgttgggt atcaattgac aatttactaa ctggattgca3'	429	Ps-3R
Penicillium janczewski	**5'accatgtgag tacaatatgt tggaattggc tgcttaagca3'**	214	NBRC 6103
Penicillium fellatum	5'tggtatgtgc agacctggac aagatccatc aattgaggct3'	433	NRRL 6172
Penicillium paxillii	5'cccacgtaag tgatactgac ctccatggga ttgaaaacat3'	218	GA4-77
Penicillium aurentiogresum	5'tggtatgtac agacttggac aagatccatc aattgaggct3'	433	NRRL 35614
Penicillium glabrum	5'tggtacgtgt tgcaaccacg atcaccaatt gatagcctgt3'	422	NRRL 35684
Penicillium nalgiovense	5'cagagctttt tttttcgcgt tgggtatcaa ttgacaagtt3'	395	NRRL 911
Paecilomyces variotii	5'tggtatgttg gaaatcaata ggagaaatga aagaaagagc3'	472	CBS 101075
Penicillium decumbens	5'atgaggtaag tgtatttata taccagctcg atggatctta3'	214	GA4-26
Fusarium oxysporum	3'tttctggcag accatctctg gcgagcacgg cctcgacagc3'	405	Oe 30
Fusarium graminaerum	5'tttccgcgct gtcagcgttc ctgagctcac ccagcagatg3'	351	-NA
Fusarium verticilloides	5'tttccgcgct gtcagcgttc ctgagttgac ccaacagatg3'	401	SBP48
Fusarium solani	5'acgccttcag cgctgtctcc gtccccgagc tcacccagca5'	273	FCW 33
Gibberala/Fusarium moniliforme	5'acctctcttt tttaagttcg tgctgtgctg ttgcacgcgt3'	564	FV 4773
Fusarium poae	5'cgaggaagat gctaacagtg tttatcaggg taaccaaatc3'	210	ITOA 2354
Fusarium acuminatum	5'tgcttgctgt gttgctgcgc gttggagctg cctttgcgcc3'	502	R 6934
Fusarium chlamydosporum	5'gacataccta tacgttgcct cggcggatca gcccgcgccc3'	425	CanS-26
Fusarium proliferatum	5'cttcagggtt tccagatcac ccactccctc ggtggtggta3'	317	MAAF 236459
Fusarium subglutinans	5'tggcaaacca tctctggcga gcacggcctc gacagcaatg3'	291	ITEM-4408
Fusarium avenaceum	5'ctttctggca gaccatctct ggcgagcatg gccttgacag3'	303	IS-10992
Fusarium sambucinum	5'ttaccgagtt taaactccca aacccctgtg aacatacctt3'	501	S-166A
Fusarium trincinctum	5'gtgcccctga ttctccccgc tgggtggtag cagctcaaca3'	636	S-419
Fusarium equiseti	5'tgatgctaac agtgtttatt agggtaacca aattggtgct3'	401	CBS448 84
Fusarium decemcellulare	5'gaacctgcgg agggatcatt accgagttta caactcccaa3'	511	DO 58
Fusarium dimerium	5'atgttgcctc ggcggatcag cccgctcccc gtaaaacggg3'	360	CBS221.76
Fusarium longipes	5'gtctcgacct gtccccagtg atgctaacaa tgtttattag3'	363	IFM-50036
Fusarium lateritium	5'atgttgcctc ggcggatcag cccgctcccc gtaaaacggg3'	485	Fu-10-44145
Alternaria alternata	5'ctcccctcac cagccgcggt gcccactcct tccgcgccgt3'	422	CIDEFI-180
Alternaria infectoria	5'gggatcatta cacaataaca aggcgggctg gacacccccc3'	530	IS-0211ARD13M2
Curvularia lunata	5'gatcattaca caatacaata tgaaggctgt ccgcagctgg3'	537	FMR-11692
Curvularia pallescens	5'cacaattaaa atatgaaggc ccttcaaacc ggctggatta3'	490	ZM10239-2
Endomyces fibuliger	5'tatctggttg atcctgccag tagtcatatg cttgtctcaa3'	2371	S-8014
Phoma sorghina	5'cctagagttg taggctttgc ctgctatctc ttacccatgt3'	456	SA05 03
Absidia cocorymbifera	5'agcagcaaag tgcgataatt attgcgactt gcattcatag3'	391	YNLF-35(8SrRNA)
Rhizomucor pussillus	5'atcattaaaa agttgtggaa atcgtggtga cctctattgg3'	563	CNM-CM4227
Rhizomucor stolonifer	5' agttgtggaa cctctattgg atcattaaaa atcgtggtga 3'	1372	CcGMU Scafold7
Candida krusei	5'tatctggttg atcctgccag tagtcatatg cttgtctcaa3'	1765	MUCL 29849
Schizosaccharomyces pombe		-NA	-NA
Rhodontonila mucilaginosa	5'tccgtaggtg aacctgcgga aggatcatta gtgaatatag3'	603	WM-03556
Rhizomucor vuil	5'cctgtgctggactgccacactgctcacatcgcctgcaagttcaacg ag3'	458	Ole 06.11e5

4. DISCUSSION AND CONCLUSION

While diseases caused by most of the pathogenic organisms are acute in nature, reverse seems to be the case with Mycoses or Mycotoxicosis caused by the fungi or fungal secondary metabolites. In the latter case, manifestation always (or in most cases) appeared to be on chronic basis. Survival and viability of fungal spores is solely dependent on the prevailing environmental conditions of which temperature, humidity, moisture, water activity

(a_w), and gas tension forms major part. Findings made from this work revealed that: while almost all the fungal species identified infecting sorghum thus far were also found in the previous work of [16,17,18,19,20,21] in Nigeria, and in Burkina Faso [22,23], Sudan [24,25] Cameroon [26], Zimbabwe [27], South Africa [20,28], Tanzania [29], Americas [26,27] Brazil [30], Argentina [22] India [27,31], South east Asia [32], Europe and Oceania [33] and Australia [22], what is of major concern is the very high viability of the fungal spores in the studied areas. This is clearly indicative of the fact that, the storage facility currently in use in almost all the Agro ecological zones in Nigeria have very little impact in creating an unconducive environment for proliferation of fungal spores. What this may imply by extrapolation is the reduction in the nutritional value of food and feedstuff as well as elaborating several mycotoxins. Also, mycotoxins may be carried over into meat and eggs when poultry and other animals are fed with contaminated feed made from sorghum from these storage facilities [34]. According to Gimeno, [35] samples can be qualified as good (count range < 3.10^4 CFU/g), regular (count range $3.104–7.10^4$ CFU/g), and bad (>7.10^4 CFU/g), but the result obtained thus far in our studies clearly reveals that none of the samples from the six Agro ecological zones is within the "good" or even the "regular" range. This should be a serious source for concern due the fact that: sorghum is third most consumed food crop after rice, maize in the cities in these six agro ecological zones and the second most consumed food crop after maize in the rural areas of the same zones. Fungi wether the toxigenic or atoxigenic strains like other microorganisms will assimilate and utilize the most readily available nutrients in the materials they grow upon for their survival and because of their saprophytic nature may gradually cause spoilage that may result in the loss of 5 to 100% of the nutrients in the crop [36]. A careful view of Tables 1 revealed that of the five storage facilities employed across the Agro ecological zones, the "Sealed rumbu" storage facility used mostly in the Sahel savannah Agro ecological zone proved to be most effective as market sample from district1 (D1) and a stored samples from district2 (D2) all where the type of storage facility is employed gives a CFU/g values of 6.7 x 10^6 and 9.5 x 10^6 respectively. This is an indication that, improvement on this method coupled with improved agricultural practices will certainly improve the safety of the sorghum samples and other related crops across these Agro ecological zones. Conversely, it is petinent to point out that, in the District1 and District 5 of the Southern guinea savannah and District 1 of the Norhern guinea savannah zones where sorghum are stored on the "Roof top" and a "Semi porous store room" respectively, the higher values (CFU/g) of 1.1x 10^8(SGS D1), 1.32X 10^8(SGSD5) and 1.1 x 10^8(NGS D1) were obtained respectively. This is also clear pointer that such storage facilities employed particularly the former employed in SGS need to be abandoned while greater improvement has to be made in the later.

The fungal species that were identified to strain level and comfirmed through Polymerase chain reaction (PCR) (Table 2) might be (depending on the genetic make up) Toxigenic or Atoxigenic. In whatever category they happen to fall, the consequences to be encountered still remains to be reckon with because if not for the additional mycotoxins that will be produced by the toxigenic strains all other effects such as: lost of viability of the crop, lost of nutrients, lost of organoleptic properties, lost flavour and discolouration of the commodity will be inflicted on the sorghum grains from the various econiches studied. For the strains that eventually prove to be toxigenic, will produce series of secondary metabolites that may appear (depending on their toxicity and organ(s) affected) to be carcinogenic, oestrogenic, teratogenic, nephrotoxic, hepato- toxic etc.

The different fungal species thus far isolated and confirmed and the ability some of these to produce varieties of secondary metabolites (mycotoxins) and their ability to cause mycotoxicosis and some (from the identified strain) that are capable of causing mycoses, it become even more imperative for the authourities concerned such as: NAFDAC and SON, the Non Governmental Organisation (NGO's) and Mycotoxicology Society of Nigeria (MSN) to embark on aggressive campaign to enlighten the farmers on the observed insufficiencies and deficiencies in their storage facilities and the possible mitigation strategies to be adopted as a short term majors while the long term mitigation majors may include a synergy of effort between select crop protectionists, mycotoxicologists, and building engineers on how to come up with a storage facility that is effective, affordable, accessible and available to farmers. Modification of the existing ones for increased efficiency might also go a long way to mitigate the problem(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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