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Research Article

Incidence and toxigenicity of fungi contaminating sorghum from Nigeria

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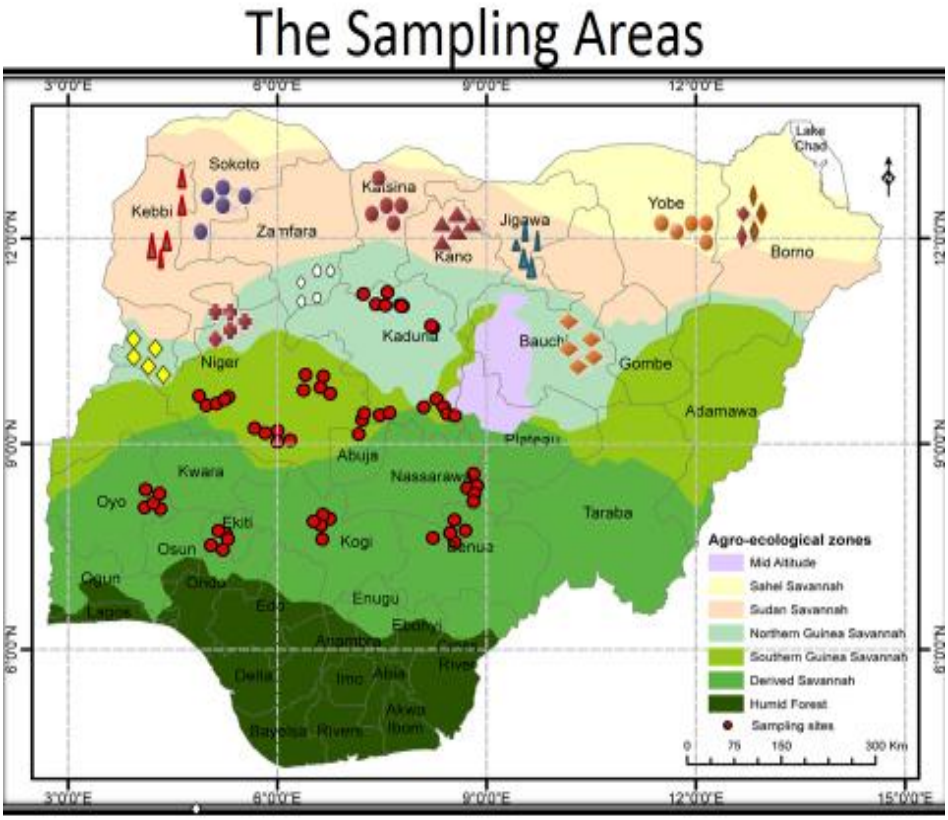
Each Agro ecological zone was transversely delineated into 5districts and five villages(at least 20 Km from each other) called "locations" were selected in each district. In each district, Sorghum grains in stores, bunches in the field and sorghum grains in the market were sampled from five locations, each approximately 20 km from the previous sampling location. The mycological analytical procedures were performed under aseptic condition. Plates were counted for fungal colonies using a colony counter and the number of fungal colonies per gram of sample was calculated as CFU/g. The fungi species were isolated and subsequently identified using MEA/CYA media for *Aspergillus* and *Penicillium* species and PDA for the fusarium species Toxigenicity studies on strains representing species of *Aspergillus*, *Penicillium, Fusarium* was carried out to determine their ability to produce aflatoxin B1 (AFB1); aflatoxin B2 (AFB2); aflatoxin G1 (AFG1); aflatoxin G2 (AFG2); OTA, ZEN, DON and FB1. A total of 701 isolates were recorded which consist of 67 confirmed fungal strains. *Aspergillus species* formed the majority with 346(49.6%) followed by the *Fusarium species* with 186(26.7%) then *Penicillium species* with 102(14.6%) while others such as *Cuvularia, Phoma, Alternaria, Rhizormucor* constitutes 67 (9.0%)strains of the total population.

Key Words: Mycotoxins, Toxigenicity, Agro-ecological zones, *Aspergillus, Penicillium, Fusarium*.

INTRODUCTION

Fungi are ubiquitous (omnipresent) plant and animals pathogens that are major spoilage agents of foods and feedstuffs (Pawlowska*et al.,* 2012).Presence of microscopic fungi or fungal spores in crops or feed made from the crop, most often compromise the nutritional quality, organoleptic attributes and the safety of the food and feeds that humans and animals solely rely on. Aside of their negative impact on the nutritional and organoleptic properties, moulds and fungi are known to be notorious synthesisers of mycotoxins. Consumption of a mycotoxin infected crop can cause acute to chronic toxic effects characterized by carcinogenic, mutagenic, teratogenic, and estrogenic properties. By extension, animal feed made from crops contaminated with mycotoxins may induce sanitary disturbances and mortality among animals and secondary contamination of human consumers via eggs, meat, and milk. Additionally, moulds like other microorganisms will assimilate and utilize the most readily available nutrients in the materials they grow upon and spoilage may result in the loss of 5 to 100% of the nutrients in the feed.

*Sorghumbicolo*r is a staple grain for over 750 million people in Africa, Asia and Latin America (CAC, 2011) that is traditionally grown mainly in the semi-arid tropics for human consumption and production of local alcoholic drinks and animal feeds. Nigeria is the largest sorghum producer in West Africa, accounting for about 71% of the total regional sorghum output (Ogbonna, 2011). Nigerian sorghum production also accounted for 35% of the African production in 2007 (FAO, 2012). The country is the third largest world producer after the United States and India (FAOSTAT, 2012). However, 90% of sorghum produced by United States and India is destined to animal feed, making Nigeria the world leading country for food grain sorghum production. It is



Adopted from Atenkengh (2008) with modification

estimated that annual economic losses encountered with sorghum and sorghum products in Asia and Africa due to mould are in excess of US $130 million (CAC 2012; Chandrashekar *et al*. 2000). The renewed focus on Sorghum is also because it is one of the most drought tolerable crops and its high water-use efficient characteristics makes it the crop of choice to boost food security in drought stricken regions of Africa and for the future against the anticipated water scarcity in the world (Zida, 2008).

This research work therefore aims to reappraise the level of fungal infection and the toxigenicity of the fungal strains isolated thereof from the studied samples

MATERIALS AND METHODS

Sampling

This was based on the method of Atenkengh *et al., (*2008) with some modifications. Five districts where sorghum is produced at both subsistence and commercial level 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, SabonBirni,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 and in the markets 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 1kg 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 (Olakojo and Akinlosotu 2004). All the samples were placed in bags, properly sealed and transported to our laboratory in Federal University of Technology, Minna. A total of 435samples was collected from the field, store, and market (each measuring 100g). A total of 100g was taken from each kg in each eco-niche of the locations and these 100g were composited and then sub slotted to the final 100g which was used for fungal isolation. To prevent further postharvest accumulation of moulds prior to analysis, all the samples were properly sealed and stored at4^{o}C.

SPORES CULTURE AND GROWTH

The mycological analytical procedures (Kaufman *et al.,* 1963) were 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^{o}C. 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:

CFU/g = Number of colonies x reciprocal of the dilution factor
Plating volume (1ml)

Isolated fungal colonies were further sub-cultured on PDA, Czapek yeast agar (CYA) and malt extract agar (MEA) according to Kaufman *et al.,* (1963) under aseptic conditions and incubated at 25^{o}C 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, (1997) and Nelson *et al.,* (1983). 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^{o}C and stored at 4^{o}C.

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 Gene Amp PCR System 9700 and an automated sequencer—ABI PRISM 3700 Genetic analyser according to Samson et al. (2004).
Genomic DNA analysis(during confirmation and identification) was performed using a Fungal/Bacterial DNA extraction kit (Zymo Research Corporation, Southern California, USA).
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, (1997) 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.
Thereafter, 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^{o}C for 45 secs, extension at 72^{o}C for 1 min 30 seconds, post-dwelling at 72^{o}C for 10 minutes and hold at 4^{o}C 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°C. 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 were viewed using the Vacutec Gel documentation system and the product size was confirmed by comparing it to the Middle Range Fast Ruler (Fermentas Life Science, Lithuania).
PCR products obtained from the synthesis process was 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 Spectru Medix 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 Big Dye 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, Nieuwerkerka/dYssel, The Netherlands). The forward and reverse sequences were assembled using the programmes SeqMan and EditSeq from the Laser Genepackage (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 Nirenberg and O'Donnell, (1998), and Geiser *et al.,* (2004) was used. These sequences, in FASTA format was obtained from the FUNGI ID v. 1.0 database (Geiser *et al.,* 2004).

Screening of Fungal Isolates for Mycotoxigenic Potentials:

This was carried out as described by Makun *et al.,* (2011), without modification. Briefly, each strain

representing the fungal species of *Aspergillus*, *Penicillium, Fusarium* from each of the agro ecological zone was further tested for toxigenicity to determine their ability to produce the following mycotoxins: aflatoxin B1 (AFB1); aflatoxin B2 (AFB2); aflatoxin G1 (AFG1); aflatoxin G2 (AFG2); OTA, ZEN, DON and FB1. These isolates were cultured individually on solid yeast extract sucrose agar (YES) agar in a 90-mm Petri dish and incubated at 25^{o}C for 28 days according to the method of Singh *et al*. (1991).Mycotoxins synthesized by each fungus were extracted by dissolving 5 g of isolate including the medium in 10 mL of dichloromethane (DCM). The crude extract obtained was filtered through a Whatman no. 2V filter paper and the filtrate put in a screwcap vial, dried under a stream of N2 gas and stored at 4^{o}C until analyzed. Methanol was used for fumonis in extraction. The clean-up method of Sydenham *et al*. (1992) was adopted for FB analysis.

A two-dimensional TLC technique (Patterson and Roberts 1979) as previously described was used for the detection of ZEN and DON

RESULTS

Fungi identified and their relative abundance in the six Agro-ecological zones of Nigeria

The prevalence of fungi in each agro-ecological zone is species dependent and largely depend on the prevailing environmental conditions therein. As shown in the table**3.1** below, *Aspergillus* and *Penicillium* species were predominantly found in the Northern guinea savannah and to some extent, the Sudan savannah while the *Fusarium* species were to a greater extent found to be predominant in the Derive and Southern guinea savannah. Other species such as *A. alternate, A. infectora, Endomycesfibuliger, Schizosaccharomycespombes* and the related species seems to have no distinct pattern of distribution.

Statistical Analysis

Data analysis were 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 $p \leq 0.05$.

DISCUSSION

Significant focus and resources have been allocated to increase food production over the past decades. Research investments (almost 95%) during the past 30 years were reported to have focused on increasing productivity and only 5% directed towards reducing losses (WFLO 2010; Kader 2005). To sustainably achieve the goals of food security, food availability needs to be also increased through reductions in the post-harvest losses at farm, retail and consumer levels. Findings made from our studies revealed that, 82.5% of the fungal isolates co-occurred in the samples from all the Agro-ecological zones. It is also clearly indicated from the data obtained in this study that, sorghum samples from Northern guinea savannah had the highest contamination by fungi species such as, *A.flavus, A. fumigatus, A. niger, A. ochraceus*(from the *Aspergillus* family), all the isolated *Penicillium species* with exemption of *P. aurentiogresum and P. paxillii* are found to be predominant in the derived savannah and southern guinea savannah respectively. Data obtained also indicate that, fungi of the *Fusarium* species constitute the highest fungal contaminants in the Southern Guinea savannah. The former observations and the latter all agreed with the findings made by Makun *et al.,* (2009) and Onyike and Nelson, (1999) that evaluated the fungal contamination of sorghum, in Niger State (Midlebelt region of Nigeria) and in Nigeria respectively.

Worthy of consideration is the high prevalence of two Aspergillus species (i.e. *A.flavus* and *A. parasiticus*) in all the the samples from the six Agro-ecological zones studied (Table 1); these are notorious in the production of a secondary metabolite (Aflatoxins). *A. flavus*that is widely found as commensal in the tissues of variety of plants, has the ability to thrive over a normal range of storage temperatures and at low water activity level. It can be said that it is difficult to imagine a commodity in which this fungus is not capable of growth if pre harvest, harvest and storage conditions are less than ideal (Pitt and Hockings, 1999). In the case of *A. parasiticus*, though, reported to be rare in occurrence, data from our study revealed its high occurance in all the zones sampled and this is a source for concern as the toxigenic strain of this fungus is capable of producing both B and G – type aflatoxins. When this finding (on *A. flavus and A. parasiticus*) is correlated with the result of the toxigenicity studies on Table 2, in which 69% and 72% of the test strains of *A. flavus* proved to be toxigenic for AflatoxinsB1and B2 and85,77,85 and 85% of the *A. parasiticus* strains proved to be effective producers of Aflatoxins B1,B2, G1 and G2 it could be concluded that, immediate mitigation strategies need to be put in place to avert food safety crisis in areas such as Nigeria and other sub Saharan Africa and Asia where 750million people subsists on sorghum and sorghum derived products and other developed nations such as USA and Mexico where it is grown for animal feed production. *Neosartoryafischeri* that was reported to be rare in cereals and more prevalent as a fruit spoilage agent (Frisvad and Samson,1991) has been found in samples from four of the six agro ecological zones, with the highest frequency of 64.7% in the Derived savannah from our studies. Worthy of note is the ability of this specie to produce Aflatoxins in our studies (Table 2), if contamination is ruled out, this may call for further and detailed investigation. The ability of other species: *Slerocleistaornata, and Emericellaquadrilineata* to produce a faint blue and

Table 1. Incidence, Relative frequency and relative density of fungi species isolated from sorghum in the six Agro-ecological zones of Nigeria

Fungi species	DS	SGS	NGS	SS	SHS	M-ALT	Relative density(RD%)	Kernel colour DS--------M--ALT
	Incidence a and Frequency(%)							
Aspergillusflavus (73)	09(12.33)	12(16.44)	27(36.98)	12(16.44)	08(10.96)	05(6.85)	21.98	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillusfumigatus (40)	1(2.5)	10(25)	15(37.50)	5(12.5)	4(10.00)	5(12.50)	11.56	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergilluscarbonarius(55)	3(5.50)	9(16.36)	15(27.27)	12(21.82)	12(21.82)	4(7.27)	15.90	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillusparasiticus(20)	11(55.00)	4(20.00)	3(15.00)	1(5.00)	0(0.00)	1(5)	5.78	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillusoryzae(16)	5(31.25)	5(31.25)	0(0.00)	2(12.50)	4(25.00)	0(0.00)	4.62	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillus unguis(08)	4(50.00)	0(0.00)	2(25.00)	0(0.00)	2(25.00)	0(0.00)	3.31	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillusniger(58)	7(12.07)	8(13.79)	25(43.10)	7(12.07)	6(10.34)	5(8.62)	16.76	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillususstus(12)	4(33.33)	2(16.67)	1(8.33)	2(16.66)	2(16.66)	1(8.33)	3.47	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillusversicolor(2)	1(50.00)	0(0.00)	1(50.00)	0(0.00)	0(0,00)	0(0.00)	0.58	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Neosartoryafischeri (17)	11(64,71)	1(5.88)	2(11.76)	0(0.00)	2(11.76)	0(0.00)	4.93	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR

Fungi species	DS	SGS	NGS	SS	SHS	M-ALT	Relative density(RD%)	Kernel colour DS--------M--ALT
Aspergillusmelleus (4)	0(0.00)	0)0.00)	0(0.00)	0(0.00)	4(100.00)	0(0.00)	1.16	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillusochraceus (21)	3(14.29)	3(14.29)	5(23.81)	3(14.29)	5(23.81)	2(9.52)	6.07	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Emericellanidulans (06)	2(33.33)	1(16.66)	2(33.33)	1(16.66)	1(16.66)	0(0.00)	1.73	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillusjaponicum(4)	0(0.00)	0(0.00)	2(50.00)	1(25.00)	1(25.00)	0(0.00)	1.16	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Sclerocleistaornate (5)	0(0.00)	2(40.00)	2(40.00)	0(0.00)	0(0.00)	1(20.00)	1.45	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Aspergillusparadoxus (5)	1(20.00)	0(0.00)	2(50.00)	0(0.00)	0(0.00)	2(50.00)	1.45	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR
Emericellaquadrilineata (3)	0(0.00)	0(0.00)	2(75.00)	1(25.00)	0(0.00)	0(0.00)	2.94	^{1}R, ^{2}R/W, ^{3}W, ^{4}Y, ^{5}M/W , ^{6}BR

Table 1. Cont.

Penicillium citreonigrum (22)	1(4.55)	4(18.18)	8(36.36)	6(27.30)	2(9.10)	1(4.55)	21.57	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium restrictum (2)	0(0.00)	0(0.00)	1(50.00)	0(0.00)	0(0.00)	1(50.00)	1.96	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium crustosum (2)	0(0.00)	0(0.00)	2(50.00)	1(25.00)	0(0.0)	1(25.00)	3.92	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium implacatum(5)	1(20.00)	0(0.00)	2(50.00)	1(25.00)	0(0.00)	1(20.00)	4.91	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium verrucosum(2)	0(0.00)	0(0.00)	1(50.00)	0(0.00)	0(0.00)	1(50.00)	1.96	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR

Fungi species	DS	SGS	NGS	SS	SHS	M-ALT	Relative density(RD%)	Kernel colour DS--------M--ALT
Penicillium rogulosum (2)	0(0.00)	0(0.00)	1(50.00)	0(0.00)	0(0.00)	1(50.00)	1.96	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penilliumexpansum (5)	1(20.00)	1(20.00)	2(40.00)	0(0.00)	0(0.00)	1(20.00)	4.90	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium janczewskii (4)	0(0.00)	1(25.00)	1(25.00)	2(50.00)	0(0.00)	0(0.00)	3.92	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium fellatum (2)	0(0.00)	0(0.00)	1(50.00)	1(50.00)	090.00)	0(0.00)	1.96	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium paxillii (11)	1(9.09)	4(36.36)	0(0.00)	2(18.18)	2(18.18)	0(0.00)	10.78	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium aurentiogresum(12)	4(33.33)	2(16.67)	2(16.67)	2(16.67)	0(0.00)	2(16.67)	11.76	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium glabrum (7)	1(14.29)	0(0.00)	3(42.86)	1(14.29)	0(0.00)	2(28.58)	6.86	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium nalgiovense (5)	0(0.00)	1(20.00)	3(60.00)	0(0.00)	0(0.00)	1(20.00)	4.90	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Paecilomycesvariotii (9)	0(0.00)	0(0.00)	6(66.66)	2(33.33)	0(0.00)	1(16.66)	8.82	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Penicillium decumbens(7)		1(14.29)	3(42.87)	0(0.00)	1(14.29)	0(0.00)	6,86	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumoxysporum (40)	1(2.50)	5(12.50)	11(27.50)	14(35.00)	5(12.50)	3(7.50)	21.51	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumgraminaerum (22)	5(22.73)	5(22.73)	9(40.91)	0(0.00)	0(0.00)	3(13.64)	11.83	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumverticilloides (27)	0(0.00)	6(22.22)	11(40.74)	4(14.81)	4(14.81)	2(7.41)	14.52	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR

Fungi species	DS	SGS	NGS	SS	SHS	M-ALT	Relative density(RD%)	Kernel colour DS--------M--ALT
Fusariumsolani(35)	5(14.29)	13(37.14)	5(14.29)	9(25.71)	3(8.57	0(0.00)	18.82	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR

Table 1. Cont.

Fusariummoniliforme (12)	1(8.33)	1(8.33)	2(16.66)	6(50.00)	1(8.33)	1(8.33)	6.45	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumpoae (15)	2(13.33)	3(20.00)	3(20.00)	3(20.00)	3(20.00)	1(6.67)	8.06	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumacuminatum (6)	1(16.66)	0(0.00)	3(50.00)	2(33,34)	0(0.00)	0(0.00)	3.23	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusarium Chlamydosporum (11)	1(9.09)	2(18.18)	4(36.36)	3(27.27)	0(0.00)	1(9.09)	5.91	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumproliferatum (8)	0(0.00)	3(37.50)	5(62.50)	0(0.00)	0(0.00)	0(0.00)	4.30	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumsubglitinans(3)	0(0.00)	3(100.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1.61	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumavenaeceum (3)	0(0.00)	1(33.33)	1(33.33)	0(0.00)	0(0.00)	1(33.33)	1.61	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumsambicinum (1)	0(0.00)	0(0.00)	0(0.00)	1(100.00)	0(0.00)	0(0.00)	0.54	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumtrincitum (1)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100.00)	0(0.00)	0.54	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumequiseti(1)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100.00)	0.54	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumdecemcellulare (1)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100.00)	0.54	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumdimerium(1)	0(0.00)	1(100.0)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0.54	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fusariumlongipes (1)	1(100.0)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0.54	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Fungi species	DS	SGS	NGS	SS	SHS	M-ALT	Relative density(RD%)	Kernel colour DS--------M--ALT
Fusariumlaterium(1)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100.0)	0(0.00)	0.54	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Alternaria alternate (15)	2(13.33)	5(33.33)	2(13.33)	1(6.66)	5(33.33)	0(0.00)	23.81	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Alternariainfectora (4)								[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Cuvularialunata(8)	0(0.00)	3(37.50)	1(12.50)	0(0.00)	4(50.00)	0(0.00)	12.70	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Cuvulariapallescens(1)	0(0.00)	1(100.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1.58	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Endomycesfibuliger(4)	0(0.00)	3((75.00)	0(0.00)	1(25.00)	0(0.00)	0(0.00)	6.35	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Phomasorghina(10)	2(20.00)	5(50.00)	2(20.00)	1(10.00)	0(0.00)	0(0.00)	15.87	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR

Table 1. Cont.

Absidiacorymbifera(5)	1(20.00)	1(20.00)	1(20.00)	0(0.00)	2(40.00)	0(0.00)	7.94	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Rhizomucorpussillus(11)	3(27.30)	5(45.45)	2(18.18)	0(0.00)	1(9.09)	0(0.00)	17.44	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Rhizopusstolonifer (7)	1(14.29)	3(42.86)	1(14.29)	1(14.29)	0(0.00)	1(14.29)	11.11	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Candida Krusei(1)	0(0.00)	1(100.00)	0(0.00)	0(0.000	0(0.00)	0(0.00)	1.57	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Schizosaccharomycespombe(1)	0(0.00)	1(100.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1.57	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR
Rhodotonilamucilaginosa(1)	1(0100.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1.57	[1]R, [2]R/W, [3]W, [4]Y, [5]M/W , [6]BR

DS = Derived savannah
SGS = Southern guinea savannah
NGS = Northern guinea savannah
SS = Sudan savannah
SHS = Sahel savannah
M- ALT = Mid altitude

Superscripts 1 – 6 stands for: DS, SGS, NGS, SS, SHS, M- ALT respectively
R = red, W = white, M = milk, Y = yellow, BR = brick red

Table 2. Toxigenicity of the Isolated *Aspergillus* Fungal species From Sorghum in Nigeria

Fungi species	**Number Tested**	**Strains positive for:**						
		Aflatoxins				**Ochratoxins**	**Frequency (%)**	**Unidentified Metabolites (UM)**
		B1	B2	G1	G2			
A*. flavus*	29	20(69)	21(72)	05(17)	00(0)	**-**	0.00	
A. parasiticus	13	11(85)	10(77)	11(85)	11(85)	**-**	0.00	
A. ochraceus	05	01(20)	00(0)	00(0)	00(0)	**-**	0.00	R/ UM
A. niger	29	02(7)	00(0)	08(28)	00(0)	24	82.76	5salmon
A. carbonarius	29	04(14)	01(4)	05(17)	00(0)	22	75.86	2 R/O
A. oryzae	04	01(25)	01(25)	00(0)	00(0)	02	50.00	2 G/UM
A. versicolor	01	00(0)	00(0)	00(0)	00(0)	00	0.00	
A. unguis	01	00(0)	00(0)	00(0)	00(0)	01	100.00	BR/UM
Neosartoryafischeri	01	01(100)	00(0)	00(0)	00(0)	**-**	0.00	
Slerocleistaornata	01	**+**	**+**	**+**	**+**	**-**	0.00	
Emericellaquadrilineate	01	**+**	**+**	**+**	**+**	**-**	0.00	
Penicillium verrucosum	01	**+**	**+**	**+**	**+**	**+**	0.00	

Key: UM = unidentified metabolite, R/O = red/ orange, G = green, BR = Brick red,
Digits 1-10 = Number of fungi species that produces the metabolite

Table 3. Toxigenicity of some Isolated *Fusarium* Fungi species From Sorghum in Nigeria

Fungi species	Number tested	Incidence for:					
		FB1	Freq (%)	ZEA	Freq (%)	DON	Freq (%)
Fusariumsolani	15	06	40.00	00	0.00	00	0.00
Fusariumoxysporum	18	05	27.78	12	66.67	00	0.00
Fusariumverticilloides	14	11	78.58	01	7.14	08	57.14
Fusariumproliferatum	05	04	80.00	01	20.00	00	0.00
Fusariumgraminaerum	09	00	0.00	07	77.78	07	77.78
Fusariumchlamydosporum	10	02	20.00	00	0.00	00	0.00
Fusariummoniliforme	08	06	75.00	06	75.00	06	75.00
Fusariumpoae	10	02	20.00	00	0.00	06	60.00
Fusariumacuminatum	06	01	16.67	00	0.00	00	0.00
Fusariumavenaeceum	02	01	50.00	00	0.00	00	0.00

green fluorescence in the Ultra violet source will probably reveal a lot when further investigated. *A. Niger* has been known for its wide industrial use and occasional production of ofochratoxin Abarca *et al*, (1994) Theochratoxin production has been confirmed by the result obtained in our study. However, the distinction between the two results was, while Abarca*et al.,*(1994) found 2 of 19 *A. niger* strains producing ochratoxin, our findings shows 24 of 29 (82.7%) to be producers of the mycotoxin (Table 2) Worthy of note also, is the fact that, of the numerous *Penicillium species* tested for toxigenicity, only extract from *P.verrucosum* appeared to fluoresce blue and green in the ultraviolet source signifying possible production of Aflatoxin B and G and also tested positive for ochratoxin.(Table 2).

Data obtained from our study revealed the presence of *Fusariumverticilloides, F. solani, F. poae and F. oxysporum*in all the six Agro-ecological zones which is in agreement with the finding made by Onyike and Nelson (1999), Atenkegh*et al.,* (2008), Makun*et al.,* (2011). However, *F. proliferatum* (A notorious FB1 producer) seems to prevail more in the SGS and NGS agro-ecological zones. The occurrence of the former species in all the Agro- ecological zones is a source for concern, due the fact that, finding from our study indicate the production of FB1, ZEA, DON by these strains (Table 3). Another issue of great concern is the high toxigenicity(FB1production) portrayed by *F. verticilloides and F. proliferatum* in which 78.5 and 80% of the strains tested prove to be effective FB1 producers (Table 3). Production of ZEA and DON by 77.7, 57.1 and 60.0% of the tested strains of *F. graminaerum, F. verticilloides and F. poae* is also an indicator that possible contamination of food and feeds made from this grains is very much possible.

Taking the data generated from this study together, it can be deduced that, sorghum as the fifth most cultivated crop in the world and one of the most promising crops of the future against anticipated water scarcity, is susceptible to contamination by variety of fungal species. Of the 63 fungal isolates (confirmed at molecular level), majority are known to produce variety of secondary metabolites that are known to have pathologic effects in both human and animals. Also, as indicated in tables 2 and 3, co-occurrence of mycotoxins cannot be ruled out in sorghum from almost all the six Agro-ecological zones of Nigeria, therefore periodic reappraisal of fungal and mycotoxin contamination is very necessary, considering the central role this crop plays in food security of the country. This is with a view to have a holistic data on the fungi distribution and predict possible mycotoxin contamination and device possible mitigation strategies to avert food sufficiency and safety crisis in the country and other regions where sorghum is extensively used as a source of food and feed.

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