

Mycobiota and Fungal Metabolites in Improved Groundnut Varieties in Nigeria

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Abstract

The study investigated the incidence of mycobiota, *Aspergillus* spp metabolites and aflatoxin biosynthesis precursors in 60 samples of seeds and haulms of improved groundnut genotypes in Nigeria. Culturing of the infected seeds was done using the agar plate method and mycobiota identification was done using conventional cultural and microscopy method. The occurrence of *Aspergillus* toxins, and aflatoxin biosynthesis precursors were evaluated using liquid chromatography-mass spectrometry (LC-MS/MS). Three identified species and four unidentified species of fungi were isolated from the seeds with *Aspergillus niger* (47.19%) and *A. flavus* (17.82%) having higher incidence more than others. The seed samples from ICGX 86024 and ICGX 01276 had the highest and lowest fungal incidence respectively. No aflatoxin B₁, ochratoxin A or fumonisins were detected in the seeds and haulms samples of the improved groundnut despite the incidence of *A. flavus*. Fifteen fungal metabolites were found including eight *Aspergillus* metabolites and seven aflatoxin biosynthesis precursors. From our result, STC which is involved in aflatoxin biosynthesis pathway was below limit of detection and thus aflatoxin formation in the groundnuts might be interrupted. This report is foremost in elucidating the aflatoxins biosynthesis precursors in seeds and haulms of improved groundnut varieties in Northern Nigeria.

Keywords: Aflatoxin precursors, *Aspergillus* metabolites, groundnut, haulm, seeds, incidence, mycobiota.

1. Introduction

Groundnut (*Arachis hypogaea* L.) is a valuable oilseed and food legume crop in the savannah and semi-arid regions of the world. It is a commercial crop for local markets as well as for export in many developed and developing countries (Guchi, 2015a). Major groundnut producing areas in Nigeria are in the Central, Eastern and Western Northern zones (Ntare, 2007; Ifeji *et al.*, 2014; Vabi *et al.*, 2018). The production of quality marketable seeds especially from local varieties and some improved ones are constrained by *Aspergillus* species (Guchi, 2015b). The fungus often causes quantitative losses and produces highly poisonous chemical substances called aflatoxins. Aflatoxins are produced by *Aspergillus* species which are soil-borne fungi that occur Worldwide (Granados-Chinchilla, 2017; Gruber-Dorninger *et al.*, 2017).

In an attempt to improve groundnut v

arieties for resistance against fungal foliar diseases and aflatoxins, breeding techniques and recombinant DNA technology are now employed (Dwivedi 2003; Bhatnagar-Mathur, 2015). The International Crops Research Institute

for the Semi-Arid Tropics (ICRISAT) in West Africa has bred and released groundnut varieties that are high yielding in terms of the pods and fodders and also free of aflatoxins.

Though some improved groundnut cultivars were bred to resist aflatoxigenic fungi and aflatoxins formation by the crop breeders, there are several other minor mycotoxins or metabolites that could be produced in their seeds or in the haulms. There is however paucity of multi-mycotoxin research-based on such roughages within animal feed chain in developing countries like Nigeria. This investigation was therefore carried out to determine the incidence of mycobiota, the profile of *Aspergillus* metabolites and aflatoxin biosynthesis precursors in the seeds and haulms of improved groundnut varieties in Nigeria using multi-mycotoxins LC-MS/MS. The study has risk assessment significance and also gives plausible explanation to how improved groundnut varieties with the presence of *Aspergillus flavus* resist aflatoxin biosynthesis.

2. Materials and Methods

2.1. Experimental field

The experimental groundnut varieties were grown on farmers' participatory lines trial at location T19, Institute of Agricultural Research (IAR) Experimental Field,

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Ahmadu Bello University, Samaru Zaria, Kaduna State, Nigeria (Long. 7° 38' and 7° 50'E and Lat. 11° 01' and 11 10'N) with altitude of 686m above sea level under rain-fed conditions in the Northern Guinea Savannah of Nigeria. The mean daily air temperature of the area during the planting season was 24.3°C, while the mean annual rainfall was 1201 mm. The soil texture of the field was sandy loam and with a mean soil pH of 5.5. The unit plot size was 5m x 3m, and they were laid out in a randomized complete block design (RCBD) with each treatment replicated thrice. The plant spacing was 30 cm and 15 cm while plot to plot and replication to replication distance was 0.5 and 1.0 m, respectively. Total area of the farm was 17m x 37m i.e. 629m². The seeds of eight groundnut genotype (Short duration - ICGV-IS 07999, ICGV 94379 and ICGV 86024; Medium duration - ICGV 01276, ICGV-IS-09992, ICGX 24, ICGV-1S-09926 and ICGV 08540) were collected from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Also seeds of two local checks – medium-maturing SAMNUT 22 and early-maturing SAMNUT 24 collected from IAR&T were all sown on the flat at the mean depth of 3.5 cm on the 3rd week of July, 2018. Each of the plots was applied with basal dose of 20:40:20 NPK fertilizer at 400 kg ha⁻¹.

2.2. Sample collection

The haulms of each groundnut variety from each plot were separately collected and properly packed in polystyrene bags and then labelled accordingly. Harvested dried unshelled samples were collected in brown envelopes from the farm store in IAR, Samaru, before being transferred to and kept in the Mycotoxin and Pesticidal Residue laboratory of Department of Crop Protection, ABU, Zaria. Prior to commencing laboratory analysis procedures on the samples which was within a month of harvest, they were stored at 4° C to prevent further fungal growth and metabolite production within the samples (Garcia-Cela *et al.*, 2020). Each sample was divided into two portions. The samples from one portion were used for mycological analysis. The second portion was separately packed in zip lock and transported in to University of Natural Resources and Life Sciences Vienna (BOKU), Tulln, Austria for multi-mycotoxins analysis.

Identification of fungal species using Agar plate method. The Potato Dextrose Agar (PDA) was prepared following the method of Giorni *et al.*, 2019. It was then autoclaved, allowed to cool and poured aseptically in the Petri dishes. When the PDA was about to gel, four seeds from each groundnut genotypes were aseptically plated in the Petri dishes. The experimental design was complete randomized design and replicated 4 times. The individual colony of fungi species growing on the agar was examined after seven days of plating. The identification of fungi was carried out based on the colony characters and morphology of the fungus under stereoscopic microscope and by referring to the production of food-borne fungi manual (Joanne *et al.*, 2008).

2.3. Analysis of mycotoxin

2.3.1. Reagents

Liquid chromatographic grade of methanol (CH₃COOH) and acetonitrile were purchased from Merck (Germany) and VWR (Belgium) respectively. The Mass Spectrometry grade ammonium acetate and standards for

fungi metabolite were brought from Sigma-Aldrich (Austria). Decontamination of water was carried out consecutively through reverse osmotic pressure and ultra analytic system was purchased from Veolia water (UK). A total of 34 working solutions were made and kept at -20 °C in the refrigerator (Labcold®, FKvsl 4113, Fishers Scientific, UK) but were brought to 25 °C before use. Fresh final working solution was mixed accordingly for the spiking experiment.

2.4. Groundnut Extraction

Each grain and haulm samples were milled using a cyclone pulverizer which has one millimetre square sieve (Cyclotech, Sweden) before being homogenized. Five grams each were measured into the centrifuge tube (0.05 L polypropylene). Twenty millilitres of the separation solvent (acetic acid/water/acetonitrile 1:20:79, v/v/v) were added before being vortexed using laboratory rotary shaker (Model GFL 3017, Germany). Ratio of the dilution of the sample with the solvent was 1:1 and 5 millilitres of the dilution obtained from the extract was shot into the LC-MS/MS.

2.5. LC-MS/MS Parameters

Analysis of the extracts was achieved with a QTrap 5500 multimycotoxin LC-MS/MS system (Applied Biosystem, California, United State of America) furnished with Turbolon spray ESI source and High Performance Liquid Chromatography (Agilent, Germany). Other liquid chromatography/Mass Spectrometry protocols applied for chromatographic separation, identifying analytes that are positive, quantification of liquid standard identification were as described by Malachova *et al.*, 2015. The method precision was tested through proficiency testing organized by Bureau Interprofessionel des Etudes Analytique (BIPEA) (Gennevilliers, France) in accordance with ISO 13525:2015. All the 60 results of the groundnut were between -2 < x < 2 which was a satisfactory range.

Maximum and median concentration (µgkg⁻¹) of toxins and metabolites were determined from the data collected for each of the samples analysed. The fungi isolated from the seeds of the improved groundnut varieties were tabulated based on their species. The mean percentage of seeds infected with fungi per treatment were separated with Tukey's test at 5% level of probability.

3. Results

3.1. Mycobiota load in the groundnut seeds

The three *Aspergillus* species detected in the groundnut seed samples were *Aspergillus niger*, *A. flavus* and *A. fumigatus* (Table 1, Figures 1 and 2). Other four genera observed were *Penicillium* spp, *Rhizopus* spp, *Fusarium* spp and *Sclerotium* spp as shown in Table 1. The percentage of SAMNUT 22 (100%), SAMNUT 24 (96.67%), ICGV-1S-09992 (96.67%) and ICGV 94379 (100%) seeds infected with fungi were significantly higher ($p \leq 0.05$) than other varieties. There were total of 303 fungal isolates recovered from all samples, out of which 143 (47.19%) and 54 (17.82%) were *A. niger* and *A. flavus* respectively. The seed samples from ICGX 86024 and ICGX 01276 had the highest and lowest total fungal incidence respectively.

Table 1. Incidence of fungi isolated from culture plate of seeds of improved groundnut varieties at 5 Days After Inoculation

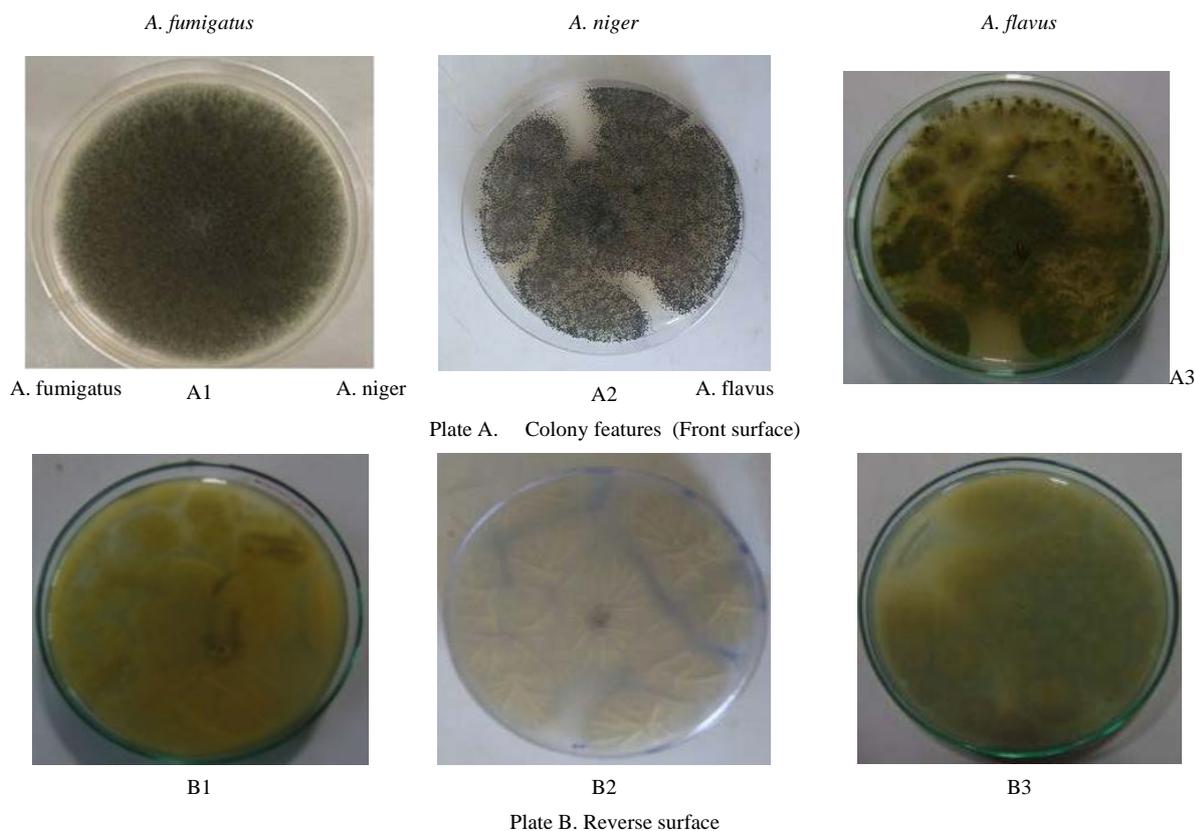
*Means with the same alphabet(s) within the column are not significantly different from one another using Tukey's test at 5 % probability level.

S/No	Variety /genotype	% of infected seeds	Incidence of fungal genera and species							Total fungi incidence
			<i>Aspergillus niger</i>	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>Fusarium sp.</i>	<i>Rhizopus sp.</i>	<i>Sclerotium sp.</i>	<i>Penicillium sp.</i>	
1	SAMNUT 22	100 ^{e*}	18	10	0	1	0	0	0	29
2	SAMNUT 24	96.67 ^e	10	8	0	8	6	6	0	38
3	ICGV-1S-09992	96.67 ^e	20	9	0	1	1	0	0	31
4	ICGX 24	46.67 ^a	7	2	0	3	12	0	1	25
5	ICGX 86024	100 ^e	19	8	0	7	0	2	0	36
6	ICGV -1S-09926	93.33 ^d	10	5	0	0	0	17	0	32
7	ICGV 01276	83.33 ^b	12	0	0	7	0	0	0	19
8	ICGV 07999	90.00 ^c	3	1	0	0	4	22	0	30
9	ICGV SM-08540	93.33 ^d	23	4	1	0	2	2	0	32
10	ICGV 94379	100 ^e	21	7	0	2	0	0	0	30
Total (%)			143	54	1	29	25	49	1	302
			(47.2)	(17.8)	(0.003)	(9.6)	(8.3)	(16.2)	(0.003)	(100)

Macroscopic observation of *A. fumigatus* showed blue grayish coloured ((Figure1, A1) and orange coloured in reverse also with powdery texture and wrinkle in reverse (B1). The colony morphology of *A. niger* observed reveals a black colour colony on the top (A2) and sulphur-yellow colonies on the reverse (B2). Downy and powdery textured and grow fast within 2 to 4 days. *A. flavus* colony was

white and yellowish green on the surface and powdery on the surface of the colony (A3); it was also pale yellow with downy texture on the base on reverse (B3). The mycelia grew filled the plate within 5 to 6 days.

The microscopic features of the three identified *Aspergillus* species is as shown in Figures 2 and Table 2.



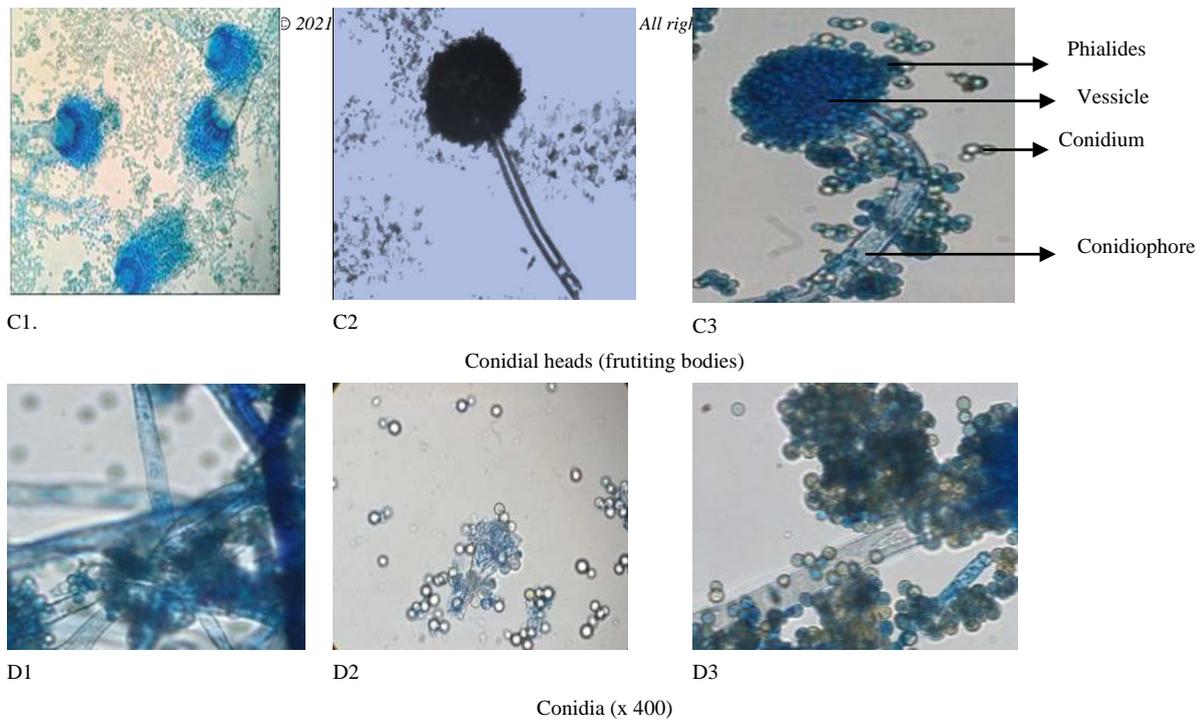


Figure 2. Microscopic features of the three identified *Aspergillus* species. Conidial heads (C1-C3) and conidia - x400 (D1-D3)

Table 2. Microscopic characteristics of the identified *Aspergillus* spp isolates

Fungus	Microscopic features							
<i>Aspergillus</i> species	Size (μm)	Stipes colour	Surface	Vesicle serration	Vessicle size	Metula covering	Shape	Conidia surface
<i>A. flavus</i>	400-800	Pale brown roughened	Quietly spherical	Biserate	30 - 40 μm)	2/3	Glubose ellipsoid	Smooth finely roughened
<i>A. niger</i>	400-3000	Slightly brown	Smooth walled	Biserate, large size	15.45 -20.45 μm	entirely	Glubose	Very rough, irregular
<i>A. fumigatus</i>	200 -400	Grayish near ape	Smooth walled	Uniserate, pyriform	15.45 - 20.45 μm	Upper2/3	Globose, small in columns	Smooth or spinose

3.2. Occurrence of fungal toxins and metabolites in groundnut seeds and haulms

Major mycotoxins such as aflatoxin B₁, ochratoxin A, fumonisin B₁, fumonisin B₂, fumonisin B₃, fumonisin B₄, fumonisin B₆ were below the limit of detection by LC-MS/MS analyser in all the seeds and haulms of the ten groundnut genotypes investigated. The occurrence of 8 emerging *Aspergillus* toxins, sterigmatocystin (STC) and other six biosynthesis precursors were detected in the groundnut and haulm samples. In addition, a bacterial toxin; monactin (0.017 $\mu\text{g kg}^{-1}$) was detected in the haulm of variety ICGV-1509926 (Tables 3 and 4). The only bacterial toxin detected was monactin found in the haulm of variety ICGV-1509926 with a low median concentration of 0.017 $\mu\text{g kg}^{-1}$.

No *Aspergillus* toxin and metabolites were detected in the seeds of ICGV-IS-09992 and ICGV-IS-09926. Also, no toxins and metabolites were detected in the haulms of ICGV-IS-09992. The seeds of ICGV-85024 had the highest co-occurrence of seven out of eight *Aspergillus* emerging toxins and metabolites, while in the haulms, the

highest co-occurrence of six metabolites was found in ICGV-1S-09926. Lower occurrence and concentration of *Aspergillus* toxins and metabolites were detected in the seeds of ICGV-01276, ICGV-SM 08540, SAMNUT 22 and SAMNUT 24 varieties than in their haulms (Table 3).

High median concentration of 3-Nitropropionic acid (2716.3 $\mu\text{g kg}^{-1}$) was detected in the seeds of ICGV-94379. Also, highest median concentration of 3-Nitropropanoic acid (3716 $\mu\text{g kg}^{-1}$) was detected in the haulms of the ICGV-94379 genotype.

Only the seeds of ICGV- IS-09926 had all the seven aflatoxin biosynthesis precursors including STC (Table 4). The median concentration level of STC in the seeds of ICGV-1S-09926 had a maximum concentration of 2.6 $\mu\text{g kg}^{-1}$ with a median of 0.87 μgkg^{-1} . The result also revealed that that 7 out of the 10 groundnut varieties' seeds were contaminated with versicolorin A, versicolorin C, and averufin (Table 3), but none of the aflatoxin biosynthesis precursors was detected in the haulms of ICGV- 07999, ICGV-24, ICGV-1S-09926, ICGV-85024.

Table 3. Maximum and median concentration ($\mu\text{g kg}^{-1}$)* of *Aspergillus* metabolites in groundnut seeds and haulms

Groundnut genotype/ <i>Aspergillus</i> metabolite	ICGV-07999		ICGV-24		ICGV-01276		ICGV-1S-09926		ICGV-1S-09992		ICGV-85024		ICGV-94379		ICGV-SM 08540		SAMNUT 22		SAMNUT 24	
	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm
3-Nitropropionic acid	0.4 (0.1)	1.0 (0.6)	0.3 (0.1)	-	-	-	-	-	-	-	0.3 (0.1)	8149 (2716)	8279 (3716)	-	0.4 (0.14)	-	-	-	-	-
gragillin	-	-	-	-	-	-	-	-	5533 (1844)	-	-	-	-	-	-	7279 (2426)	-	-	-	-
Malformin A	-	-	-	-	-	0.04 (0.01)	0.07 (0.02)	-	-	0.01 (0.003)	-	-	5.7 (1.8)	-	-	-	-	-	-	-
Malformin C	-	-	-	-	-	-	0.02 (0.02)	-	-	0.03 (0.01)	-	-	0.4 (0.14)	-	-	-	-	-	-	-
Kojic acid(KJ)	-	-	5.2 (1.7)	7.5 (3.2)	-	-	4.6 (1.5)	-	-	6.7 (2.2)	-	8149 (2716)	1889 (630)	-	6.6 (2.2)	-	-	-	-	6.1 (2)
Methyl funicone	-	-	0.7 (0.2)	0.1 (0.01)	-	0.2 (0.07)	0.8 (0.3)	-	-	0.3 (0.1)	-	-	-	-	1.16 (0.4)	-	0.04 (0.01)	-	0.5 (0.2)	
Emericellamide A	-	-	-	-	1516 (505)	1670 (605)	1156 (385)	-	-	2272 (757)	-	2.03 (0.7)	4.6 (0.2)	2748 (978)	2948 (998)	2663 (888)	2863 (890)	5642 (1881)	6642 (2881)	
Sydowinin A	-	-	-	1.21 (0.3)	-	-	-	-	-	1.13 (0.4)	-	-	-	-	-	-	-	-	-	

- = < limits of detection; sd = seed; hlm= haulm

Table 4. Maximum and median concentration ($\mu\text{g kg}^{-1}$) of sterigmatocystin (STC) and other aflatoxin biosynthesis precursors in groundnut seeds and haulms

G/nut genotype	ICGV-07999		ICGV-24		ICGV-01276		ICGV-1S-09926		ICGV-1S-09992		ICGV-85024		ICGV-94379		ICGV-SM 08540		SAMNUT22		SAMNUT24	
	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm	Sd	Hlm
Sterigmatocystin	-	-	-	-	-	-	2.6 (0.87)	-	-	-	-	-	-	-	-	-	-	-	-	-
Nidurufin	6.4 (2.1)	-	21.9 (1.0)	-	2.1 (0.8)	1.13 (0.38)	23.88 (7.96)	-	0.03 (0.1)	14.14 (4.7)	6.8 (2.3)	-	0.04 (0.01)	68.3 (22.8)	-	17.38 (5.75)	-	3.33 (1.1)	-	47.15 (16)
Versicolorin A	9.6 (3.2)	-	37.8 (13)	-	2.3 (0.8)	1.26 (0.4)	30.55 (10.2)	-	0.10 (0.33)	16.0 (5.30)	8.5(2.8)	-	4.4 (1.7)	74.6(25)	-	15.1 (5.02)	-	3.6(1.2)	-	48.3 (16.1)
Versicolorin C	26.1 (8.7)	-	91.8 (30.6)	-	4.8 (1.5)	3.7 (1.23)	84.3 (28.1)	-	0.19 (0.1)	51.99 (13.97)	25.4 (8.5)	-	5.4 (2.1)	213(70.9)	-	56.49 (18.9)	-	10.1 (3.4)	-	160 (53.3)
Averantin	0.4 (0.15)	-	1.6 (0.5)	-	0.9 (0.4)	0.07 (0.02)	1.66 (0.5)	-	-	1.1 (0.4)	0.4 (0.13)	-	-	3.56(1.2)	-	0.7 (0.2)	-	0.19(0.1)	-	2.9(0.9)
Averufin	3.4 (1.14)	-	13.4 (4.5)	-	1.6 (0.9)	0.44 (0.13)	12.61 (4.2)	-	0.08 (0.03)	9.11 (3.4)	3.29 (1.1)	-	0.05 (0.03)	25.4(8.5)	-	7.3 (2.41)	-	1.5 (0.03)	-	25.1 (8.4)
Norsolorinic acid	0.85 (0.3)	-	4.14 (1.4)	-	-	-	4.37 (1.4)	-	-	2.8 (0.95)	1.15 (0.4)	-	-	10.3(3.8)	-	1.7 (0.57)	-	0.5 (0.17)	-	8.1(2.7)

= < limits of detection; sd = seed; hlm= haulm; () = median concentration

4. Discussion

Several *Aspergillus* strains, their metabolites and aflatoxin biosynthesis precursors have been isolated from groundnut seeds and in feeds of ruminants such as cattle feed (Ranjbar *et al.*, 2011) and dairy goat feed from Brazil (da Silva *et al.*, 2015). Ruminant animals are often fed with groundnut haulms as roughages in order to contribute energy and nutrients to their diet (Granados-Chinchilla, 2017). *Aspergillus* species and aflatoxins have been found to be associated with the silages of corn and sorghum (Alonso *et al.*, 2013). Thus studies concerning safety of animals and humans that consume groundnut and/or their haulms should not be neglected.

Some isolated fungi from Jordan Desert leaves and fruits were identified as *Aspergillus*, *Alternaria*, *Rhizopus*, *Penicillium* and *Fusarium* at the genus level by using macroscopic and microscopic examinations depending on colony colour, shape, hyphae, conidia, conidiophores and

arrangement of spores (Alshaili and Bani-Hasan, 2017) as was carried out in this study. They further molecularly identified the isolated fungi at species level as the extracted fungal DNA was amplified by PCR using specific internal transcribed spacer primer (ITS1/ITS4).

A. niger had the highest incidence (47.20%) of fungal species isolated from the seeds in this study. It is reported that there are up to 145 different secondary metabolites isolated and detected from *A. niger* (Nielson *et al.*, 2009). These include intricate compounds such as naphtho- γ -pyrones, nigragillin, ochratoxin A and fumonisin B₂. *A. flavus* with the second highest incidence (17.8%) is popularly known for being a producer of carcinogenic aflatoxins. About 46 secondary metabolites have been identified in *A. flavus*, and these non-regulated fungal metabolites include 3-NPA, aflatrem, cyclopionic acid aflavorin, aflavozole, aflavine, aspergillilic acid, cladospirin, aspergillilic acid, cladospirin and gliotoxin (Adetunji *et al.*, 2014; Cary *et al.*, 2018). Other toxic regulated compound produced by *A. flavus* is kojic acid

which is implicated as a neurological poison and mitochondrial toxin (Ahuja, 2008).

The incidence of *Sclerotium* spp in the samples was 16.2%. Amber et al. (2012) reported that phenolic acids were detected by HPLC analysis of secondary metabolites in the fungus isolated from chickpea. They observed that this metabolite is responsible for severe collar rot infection in chickpea. The incidence of *Fusarium* spp in the sampled seeds was 9.6%. Nesic et al. (2014) identified 10 species of *Fusarium* and the mycotoxins produced by each of them. *F. culmorum* is known to be a producer of up to 10 mycotoxins which include fusarins, moniliformin, trichothecenes and zearalenone. Other *Fusaria* such as *F. graminearum*, *F. sporotrichioides*, *F. crookwellense* and *F. acuminatum* can produce up to eight mycotoxins each, while *F. equiseti* and *F. proliferatum* can produce six each, *F. verticillioides* - 4, *F. armeniacum* -3 and *F. pseudograminearum* - 2 (Berthiller et al. (2013).

The merits of LC-MS/MS based analytical method used in this study include allowing the sensitive concurrent determination of several fungal metabolites in many matrices (Sulyok et al., 2010; Berthiller et al., 2015; Malachova et al., 2018). LC-MS could tremendously assist in the discovery of recently produced mycotoxins (Varga, 2015), as well as hidden (Berthiller, 2013) or other altered forms (Rychlik, 2014) of mycotoxins in crop produce like groundnut.

Major mycotoxins like aflatoxin and their precursors were below level of detection in almost all the groundnut varieties investigated in this study. This is unlike other several previous studies on the natural occurrence of aflatoxins in groundnut samples from other countries. From the samples of stored peanut kernels collected from four different areas in Sudan, Bakhiet et al. (2011) using TLC techniques, reported AFB₁ concentration ranges of 17.57 - 404 µg kg⁻¹.

Eight *Aspergillus* metabolites in groundnut seeds and haulms were detected in this study. Nigragillin and Malformin C are being produced by *A. niger* (Burdock et al., 2001), Malformin A are produced by *A. ficuum*, *A. awamori* and *A. phoenicis* while Emericellamide A are produced by *A. nidulans* and Sydowinin A by *A. sydowinin* and *A. versicolor*. Kojic acid is known to be produced by *A. flavus* and *A. oryzae*, 3-NPA on the other hand is produced by *A. wentii*, *A. niger* and *A. oryzae*.

STC was not found in nine out of the 10 improved groundnuts varieties understudied. Out of the 10 analysed improved groundnut varieties, STC was only found in the seeds of ICGV-1S-09926 at low median concentration of 0.87 µg kg⁻¹. Sterigmatocystin is normally produced by *A. flavus*, *A. nidulans*, *A. parasiticus* and mainly *A. versicolor* (Versilovskis and De Saeger, 2010). It was reported to often occur where grains and cereal-based food are contaminated with aflatoxigenic fungi (Rank, 2011).

The outcome of this study suggested that the STC (and thus the aflatoxin) pathway is interrupted in the improved groundnut genotypes; thus, such genotypes where not able to produce aflatoxin. Chang et al., (2005) similarly reported that absence of interplay between *afIR* and *afII* contributes to non-aflatoxigenicity of *A. sojae*. They also reported a sequence stopping points in the aflatoxin biosynthesis gene cluster and flanking zones in non-aflatoxigenic *A. flavus* isolates. The non-occurrence of aflatoxins in most of the improved groundnut varieties

indicated that the use of breeding for resistance could be a better intervention needed in the mitigation of mycotoxin contamination in crops. This is, however, in addition to using cultural practice such as pre-and post-harvest managements, and chemical control methods.

STC is one of the precursors of aflatoxin B₁ and G₁ as indicated in a AF biosynthesis pathway as described by Jallow, 2015. Apart from STC being an aflatoxin precursor, it is with toxicity second to AFB₁ (Gao et al., 2015). The aflatoxin pathway really requires the presence of STC which often forms O-methylsterigmatocystin (OMST) with the help of O-methyltransferase. OMST could then be converted to AFB₁ & AFG₁ with the presence of cytochrome P-450 monooxygenase encoded by *ord-1* (Audebert et al., 2014; Bertuzzi et al., 2017; Wartu et al., 2017).

5. Conclusion

In this study identification of the fungal isolates up to species was impossible due to non-involvement of molecular characterization techniques. The producing fungus of some of the fungal metabolites detected could not be identified. Thus, molecular identification of fungi in further studies on this subject is necessary in future studies

The analysis indicated that there was no major mycotoxin detected in all the seeds and haulms of the ten groundnut genotypes despite the presence of *Aspergillus* spp. The improved groundnut varieties might have been bred to resist aflatoxin production, and thus were below detectable level. Planting of such improved varieties with good crop management practices can help reduce aflatoxin and other major mycotoxins contamination in groundnut before storage. In most of the groundnuts analysed, there were incidences of contamination of some minor mycotoxins and metabolites in the seeds more than their haulms.

The non-occurrence of aflatoxins in most of the improved groundnut varieties indicated that the use of breeding for resistance could be a better intervention needed in the mitigation of mycotoxin contamination in crops. This is, however, in addition to using cultural practice such as pre-and post-harvest managements, and chemical control methods. This report is believed to be the first in elucidating the aflatoxins biosynthesis precursors in seeds and haulms of improved groundnut varieties in Northern Nigeria. The result could serve as base information for groundnut breeders to produce a broad spectrum of mycotoxin-resistant groundnut.

Declaration of Interest Statement

There is no conflict of interests.

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