Mycotoxin Research
https://doi.org/10.1007/s12550-022-00465-z

ORIGINAL ARTICLE

Mycotoxin levels and characterization of natural anti-fungal phytochemicals in pearl millet (*Pennisetum glaucum*) from Nigeria's six agroecological zones

Hadiza Kudu Muhammad[1,2,3] · Hadiza Lami Muhammad[1,3] · Patrick Berka Njobeh[2] · Maurice Monjerezi[4] · Limbikani Matumba[5] · Hussaini Anthony Makun[1,3]

Received: 21 February 2022 / Revised: 8 July 2022 / Accepted: 25 July 2022

Abstract

This study reports levels of multiple mycotoxins across Nigeria's six agro-ecological zones and corresponding levels of natural anti-fungal phytochemicals present in pearl millet (PM). 220 representative composite samples of PM were collected for mycotoxin analysis using ultrahigh performance liquid chromatography-mass spectrometry (UHPLC-MS), and 24 were randomly selected for determination of metabolites using gas chromatography-high resolution time of flight-mass spectrometry (GC-HRTOF-MS). In total, 15 mycotoxins were detected, all with levels below the European Union (EU) permissible limits and level of aflatoxins only up to 1.34 µg/kg. This is in sharp contrast to high levels of mycotoxins reported in maize samples from the same agroecological zones. Phytochemical analysis of the same samples identified a total of 88 metabolites, 30 of which are known anti-fungal properties from other previously published studies. The most common of these include methyl ester, bis (2-ethylhexyl) phthalate, and ç-tocopherol. The number of anti-fungal metabolites recovered from each sample ranged from 3 to 17 and varied widely in both number and composition across the agroecological zones. The anti-fungal metabolites may probably make PM less susceptible to fungal proliferation compared to other grains. Hence, it is worth exploring for possible sources of biological control products from PM.

Keywords Mycotoxins · Phytochemicals · Anti-fungal metabolites · Pearl millet · Nigeria

Introduction

Pearl millet (PM) is a drought-resistant grain crop that is extensively cultivated in the tropics (Jones et al. 1995). PM is the 6th most important cereal worldwide, sustaining one third of the world's population (FAOSTAT 2018). In 2015 and 2016, India, China, Niger, Mali, Nigeria, Sudan, and Burkina Faso were ranked as the highest producers, with a total production of approximately 11 million metric tons per year. In Africa, Nigeria was ranked as the 3rd largest producer of PM, after Mali and Niger, with a total production of 1.4 million tons of PM in 2016 (FAOSTAT 2018).

✉ Hadiza Kudu Muhammad
hadiza.muhammad@futminna.edu.ng

Hadiza Lami Muhammad
hadizalami@futminna.edu.ng

Patrick Berka Njobeh
pnjobeh@uj.ac.za

Maurice Monjerezi
mmonjerezi@unima.ac.mw

Limbikani Matumba
alimbikani@gmail.com

Hussaini Anthony Makun
hussaini.makun@futminna.edu.ng

[1] Food and Toxicology Research Group (FTRG), Department of Biochemistry, Federal University of Technology, P.M.B. 65, Minna, Nigeria

[2] Department of Biotechnology and Food Technology, Doornfontein Campus, University of Johannesburg, P.O. Box 17011, Gauteng 2028, South Africa

[3] Africa Centre of Excellence for Mycotoxin and Food Safety, Federal University of Technology Minna, Minna, Nigeria

[4] Department of Chemistry, University of Malawi, P.O. Box 280, Zomba, Malawi

[5] Food Technology and Nutrition Group-NRC, Lilongwe University of Agriculture and Natural Resources (LUANAR), P.O. Box 143, Lilongwe, Malawi

Published online: 03 August 2022

In Nigeria, PM is predominantly cultivated in the northern states of Kaduna, Yobe, Kano, and Borno. The promotion of the cultivation of PM can contribute to efforts towards improving food security and incomes for rural households because it has some advantages over other grain crops. PM does well in marginally fertile soils and tolerates unpredictable rainfall, and its cultivation does not require much labor and other farm inputs (Dudhate et al. 2018). PM is an important component in weaning food preparations, animal feed, nutraceutical, and special foods that enhance health (Adeyeye et al. 2016; Dias-Martins et al. 2018).

Widespread mycotoxin contamination of food, which is a threat to food safety, has been reported across sub-Saharan Africa, such as maize from Nigeria (Atehnkeng et al. 2016), Cameroon (Njobeh et al. 2009), Malawi (Matumba et al. 2015a), and Zimbabwe (Hove et al. 2016); wheat from South Africa (Mashini and Dutton 2006); cassava and yams from Nigeria (Chilaka et al. 2018); and beans and peanuts from Cameroon (Njobeh et al. 2009). However, several studies carried out over four decades across Nigeria and have shown low susceptibility of PM to fungal and mycotoxin contamination (Okonkwo and Nwokolo 1978; Okoye and Ekpenyong 1984; Makun et al. 2010, 2013; Osamwonyi and Wakil 2012; Ezekiel et al. 2014; Chilaka et al. 2016). Studies at the International Institute for Tropical Agriculture (IITA) show that PM could be 8 folds less predisposed to aflatoxins (AFs) as compared to maize and 4 folds less than sorghum. AFs in PM fall in the range of 2.6 to 8.1 µg/kg which is much less than AFs in maize (range: 1.1 to 480 µg/kg) and sorghum (1.6 to 90 µg/kg) (Bandyopadhyay et al. 2007). Lower susceptibility of PM and other types of millet to infection by potentially toxigenic fungi and contamination by mycotoxins compared to maize and sorghum has also been demonstrated in studies from other countries, e.g., USA (Wilson et al. 2006), Guinea (Bandyopadhyay et al. 2007), Ethiopia (Chala et al. 2014), and Uganda (Alpert et al. 1971). Nevertheless, Houissa et al. (2019) reported an exceptional case of Tunisian PM with high contamination of several secondary metabolites, although not in comparison with other grains. It has been hypothesized that the high resistance of PM to mycotoxigenic fungi is probably due to its less surface area, harder seed coat, and higher concentrations of natural inhibitory compounds than those for other grains (Ezekiel et al. 2014; Ajani and Vali 2017). Nonetheless, there is a paucity of studies that comprehensively report on the profile of anti-fungal phytochemicals in PM in relation to their mycotoxin contamination in Nigeria and similar agroecology in sub-Saharan Africa. Therefore, the present study was carried out to profile anti-fungal compounds and mycotoxins in PM across all the agroecological zones (AEZs) in Nigeria.

Materials and methods

Sampling and sample preparation

Nigeria is categorized into six AEZs based on climatic and environmental factors. PM was sampled from all districts in all the AEZs where it is mostly cultivated. These AEZs are Derived Savannah (DS), South Guinea Savannah (SGS), North Guinea Savannah (NGS), Sudan Savannah (SS), Sahel Savannah (SHS), and Mid Altitude (MA). Annual rainfall ranges from 100 to 1800 mm across the country, with a maximum temperature range of 25–43 °C across the sampling sites as documented by Udoh et al. (2000) and Atehnkeng et al. (2008) and presented in supplementary Table s1.

Two-hundred twenty representative PM samples were collected through purchases and donations from 32 districts in the indigenous PM growing areas in Nigeria. Sampling was done according to Makun et al. (2011) and European Commission Regulation (EC 2006) with some modifications. Briefly, samples were collected from separate batches by mixing thoroughly the grains in local storage facilities and market containers from the top, middle, and bottom of individual containers to get more consistency and a good representative sample. Fifty grams were sampled at each site and transported to the laboratory for further analysis. Each sample was homogenized using vortex homogenizer (model No 6–105) and milled for 3–5 min, after which the powdered samples were transferred into new polyethylene bags. The miller cup was washed and disinfected with 70% ethanol after each use to avoid cross-contamination. To prevent further post-harvest accumulation of molds prior to analysis, all the samples were stored at 4 °C in a freezer until analyzed.

Stock and working standard preparation

For experimental purposes, a combination of standard stock solutions was freshly prepared for calibration purposes and spiking experiments on the least contaminated samples. Standard stock solutions were prepared into two groups: the AFs mix, which consisted of aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2) in acetonitrile at a concentration of 50 µg/kg each, and the multi-mix group, also in acetonitrile, consisting of Fumonisin B1 (FB_1), Fumonisin B_2 (FB_2), Fumonisin B_3 (FB_3), zearalenone (ZEN), α-ZEN, and β-ZEN at concentrations of 2500 µg/kg each; deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), alternariol monomethyl ether (AME), HT-2, and T-2 at 5000 µg/kg each;

and 79 OTA at 200 µg/kg. The working standard solutions were prepared by diluting the stock solutions with acetonitrile making five standard concentrations to calibrate the instrument and establish external calibration curves.

Validation of UHPLC-MS method for multi-mycotoxin extraction

Validation parameters including linearity of the curve (quantification), accuracy (recovery), and sensitivity, i.e., limit of detection (LOD) and limit of quantification (LOQ) were estimated for all the mycotoxins tested according to method of Sofie et al. (2010). Method validation was carried out to ascertain the accuracy of the results of the multi-mycotoxins in PM samples following the method described herein. For quantification purposes, external calibration curves were established based on serial dilutions as presented in supplementary Table s2. Linear calibration curves generated for the mycotoxin standards were considered satisfactory when correlation coefficients (r^2) were greater than 0.99.

The apparent recovery experiments were carried out in triplicates on three least contaminated samples by spiking 5 g of each with 100 µl of multi-mycotoxin standards with known concentrations. Subsequently, spiked samples were mixed and kept for 12 h in a fume cupboard at room temperature to establish equilibrium between the sample matrix and the toxins. Mycotoxins from spiked samples were extracted as described after overnight establishment. From each spiked sample, 5 µl of the extract was injected into the UHPLC system. Each analyte detected was quantified by comparing its peak area on the calibration plot to that of equivalent mycotoxin standard. Recovery was calculated by taking the percentage of the concentration (peak area) measured from spiked sample per toxin concentration used for spiking the sample. OTA, $FB_{1,}$ and DON were not detected, but the recovery rates for the remaining 15 mycotoxins (Supplementary Table s3) are within the allowable limits of the recovery and RSD recommended by Codex and the Association of Official Analytical Chemists (AOAC 1995). The Codex recommends 60–120% of recovery rates of mycotoxins and the guideline for the recoveries by AOAC is 70–125% and RSDr below 15% of mycotoxins (Codex 2008; AOAC 1995).

Mycotoxin extraction and clean-up

Extraction of 15 mycotoxins from PM grain was done according to Abia et al. (2013) and Sofie et al. (2010), while multi-mycotoxin screening via UHPLC-MS, the analytical method, and validation parameters previously optimized by Rubert et al. (2012) were adopted.

Sample preparation for profiling of anti-fungal compounds

Twenty-four representative PM samples were randomly selected and two extraction solvents of 80% methanol and acetronitrile to chloroform to water (60:20:20, v/v/v) according to Hori and Fujinaga (1985) were used for extracting the metabolites. The extracts of PM samples were subsequently analyzed on the GC-HRTOF-MS. The collected GC-HRTOF-MS dataset was converted to mzML format using the LECO ChromaTOF-HRT software and then processed (peak-picking and alignment) on the XCMS Open-Source Tool (https://xcmsonline.scripps.edu). The resulting peak list showed 6270 variables with corrected peak retention times (min), mass-to-charge ratios (m/z), and integrated peak areas. The processed data were then imported to SIMCA 14.1 software (Umetrics, Umea, Sweden) for downstream multivariate statistical analyses. Metabolites were identified based on their mass spectra and retention time using the NIST, Mainlib, and Feihn metabolomic libraries.

Results

UHPLC-MS method performance

Mean recoveries of mycotoxins in spiked samples are provided in supplementary Table s3. The relative standard deviations (RSD) of the recoveries were generally low (≤ 5.0) for all types of mycotoxins which demonstrated the methods were well under control during the analytical sessions. The LOD and LOQ for all tested mycotoxins are also presented in supplementary Table s3, with 15-AcDON having the least sensitivity (LOQ = 14.665 µg/kg with LOD at 4.885 µg/kg).

Natural occurrence of mycotoxins in PM samples from the six agroecological zones of Nigeria

The findings in the present study show that PM samples were contaminated with multiple mycotoxins, but at low concentrations. In total, 15 individual mycotoxins (8 groups) were detected (Table s3), and their distribution is summarized in Fig. 1 (according to the AEzs). AF concentrations ranged from non-detectable to 3.8 µg/kg, but levels were below the EU regulatory limits of 2.0 and 4.0 µg/kg, respectively, for FB_1 and total aflatoxins ($FB_1 + FB_2 + FG_1 + FG_2$) in food meant for human consumption (EC 2010). Likewise, all the samples from across Nigeria were found to contain fumonisins ($FB_2 + FB_3$) below the EU permissible limit of 1000.0 µg/kg (EC 2007), with a maximum level of 204.7 µg/kg. Similarly, the maximum concentrations for AcDON, ZEN, OTB, T-2, HT2-toxin, and AME were 19.5, 40.0, 0.3, 158.5, 4.3, and 16.7 µg/kg, respectively. NGS, which is a

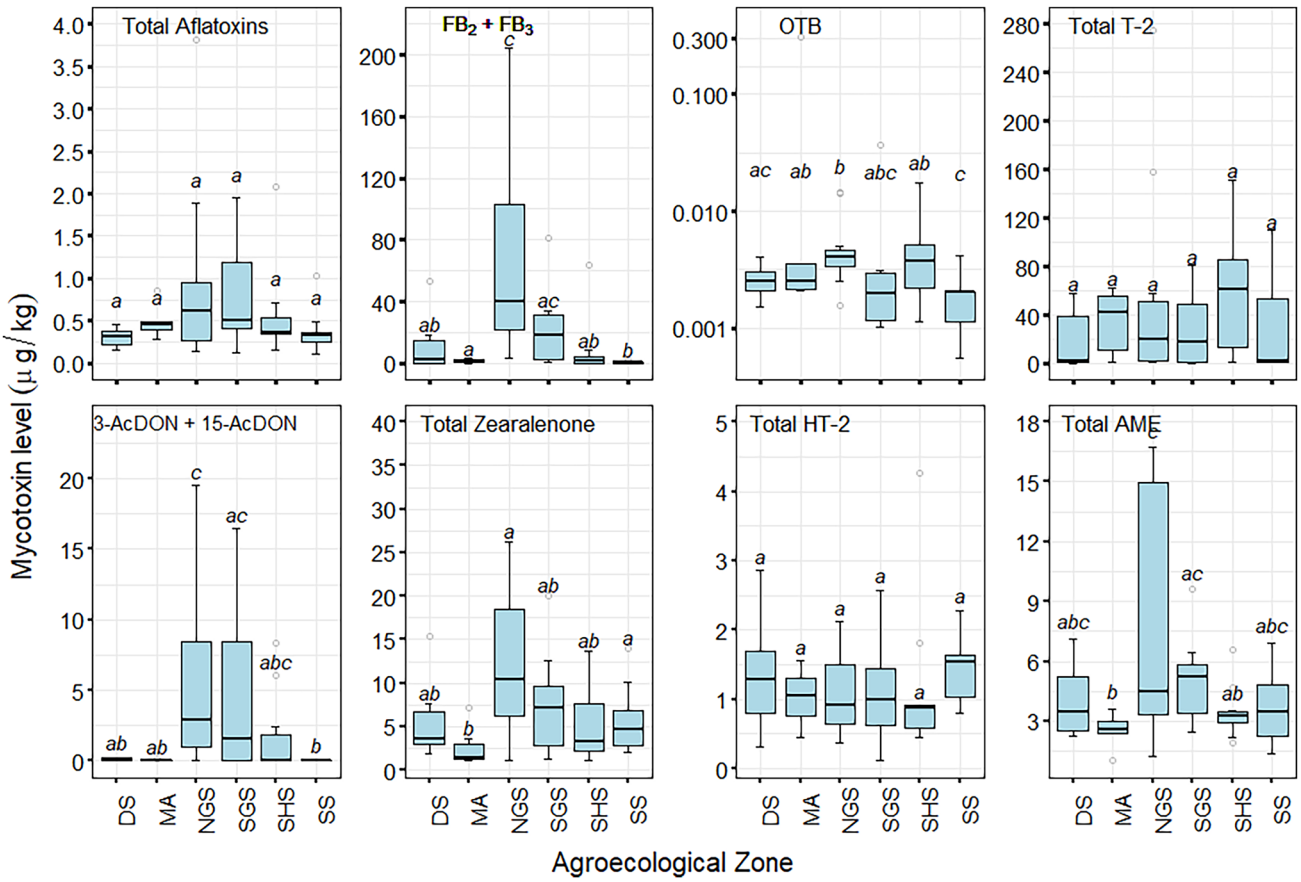

Fig. 1 Distribution of levels of total aflatoxins, total fumonisins, total deoxynivalenol, total zearalenone, total ochratoxins, total T-2, total HT-2 toxins, and total AME in PM samples across the six agroecological zones. Agro-ecological zones with different letters have significant differences ($p = 0.05$). The dots, error bars, and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively

dry AEZ had the highest concentrations for $FB_2 + FB_3$, total ZEN, and AME with mean levels at 66.4, 13.3, and 7.8 µg/kg, respectively. Meanwhile, for the other mycotoxins analyzed, values vary among the different AEZs with no significant differences ($p > 0.05$) as shown in Fig. 1.

The anti-fungal compounds in PM samples from the six agroecological zones of Nigeria

There were diverse profiles of compounds with known anti-fungal activity in PM samples, in addition to other metabolites. The retention time and % area (conc) of biochemical component identified in each PM sample is given in supplementary Table s4. A total of 36 out of 88 (40%) compounds identified in PM samples have confirmed anti-fungal properties (Table 1). The common anti-fungal metabolites reported across the AEZs are 9,12-octadecadienoic acid, methyl ester, (E, E)-hexadecanoic acid, tridecanoic acid, bis (2-ethylhexyl) phthalate, and ç-tocopherol (vitamin E) present. Out of the 24 samples analyzed, 19 samples had 9,12-octadecadienoic acid, methyl ester, and (E, E)-hexadecanoic acid; 13 samples had tridecanoic acid; and 16 samples had bis (2-ethylhexyl) phthalate while 21 had ç-tocopherol present (Table 1).

The presence of fatty acid compounds and vitamin E (as the only vitamin) in PM from all the AEZs sampled is like other millet varieties such as finger millet and barnyard millet (Sharma et al. 2016; Opoku et al. 1981). Another common metabolite with anti-fungal property is bis (2-ethylhexyl) phthalate, although this is the first report of its presence in PM. Each sample across the six AEZs had several anti-fungal metabolites ranging from 3 to 17, with the highest number of metabolites in DS, while the least number of anti-fungal metabolites were recovered from NGS. The distribution of anti-fungal metabolites across the six AEZs increased across the zones in the following order NGS (22) < MA (28) < SGS (28) < SS (33) < SHS (34) < DS (38) as shown in Fig. 2.

Table 1 Volatile biochemical component and their biological activity of PM samples

	Group and name	Formula	Positive samples ($n = 24$)	Bioactivity	Reference
Acidic					
1	Formic acid, 2,6-dimethoxyphenyl ester	$C_9H_{10}O_4$	1	Anti-fungal	Hassan et al. (2015)
Amide and derivatives					
2	9-Octadecenamide (Z)-	$C_{18}H_{35}NO$	4	Anti-fungal and Antibacterial	Mohammed et al. (2016)
Aromatic hydrocarbon					
3	3-Acetyl-2,5-dimethylthiophene	$C_8H_{10}OS$	4	Antibacterial and Anti-fungal	Lakshmi and Latha (2017)
4	3-Butene-1,2-diol, 1-(2-furanyl)-	$C_8H_{10}O_3$	2	Flavouring Antibacterial and Anti-fungal	Vega-Avila et al. (2012)
Diazirine					
5	3-Methyl-1,2-diazirine	$C_2H_4N_2$	3	NF	
	Esters and Phenolic esters				
6	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	5	Anti-fungal and Antioxidant	Varsha et al. (2015)
Fatty alcohol					
7	(Z)6, (Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$	2	Anti-fungal	Umaiyambigai et al. (2016)
8	1-Dodecanol	$C_{12}H_{26}O$	1	Anti-fungal	Carolina et al. (2011)
9	9,12-Octadecadien-1-ol (Z, Z)-	$C_{18}H_{34}O$	5	Anti-fungal and antimicrobial	Krishnaveni et al. (2014)
Fatty acid and their esters					
10	2,3,4-Trimethylpentanoic acid	$C_8H_{16}O_2$	2	Anti-fungal	Pohl et al. (2011)
11	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	2	Anti-fungal	ASC (2018)
12	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	7	Antioxidant, anti-fungal and anti-microbial activities	Elaiyaraja and Chandramohan (2016)
13	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	9	Anti-fungal, Antimicrobial and Nematicidal	Ali et al. (2017)
14	9,12-Octadecadienoic acid, methyl ester (E, E)-	$C_{19}H_{34}O_2$	19	Anti-fungal	Vimalavady and Kadavul (2013)
15	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	19	Anti-fungal, Antioxidant and Antibacterial	Agoramoorthy et al. (2007)
16	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	13	Antibacterial and Anti-fungal	Chandrasekaran et al. (2011)
17	Undecanoic acid, methyl ester	$C_{12}H_{24}O_2$	2	Anti-fungal	ASC (2018)
18	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	8	Anti-fungal	Gyung et al. (2010)
Furfural					
19	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo-	$C_{12}H_{16}O_5$	1	Antimicrobial activity	Obidi et al. (2013)
20	5-Hydroxymethylfurfural	$C_6H_6O_3$	9	Anti-fungal	Fabien et al. (2013)
21	Benzofuran, 2,3-dihydro-	C_8H_8O	6	Anti-fungal and Antiproliferative	Sabithira and Udayakumar (2017)
Other hydrocarbons and derivatives					
22	9,12-Octadecadienal	$C_{18}H_{32}O$	1	NF	
23	1,3,5,7-Tetroxane	$C_4H_8O_4$	2	NF	
Ketones					
24	Cholest-4-en-3-one	$C_{27}H_{44}O$	1	Antibacterial, and Anti-fungal	Zhu et al. (2018)
Phthalate compounds					
25	Bis (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	16	Antibacterial, Anti-fungal and anticancer	El-Sayed (2012)
26	Diisooctyl phthalate	$C_{24}H_{38}O_4$	2	Anti-fungal	Sultan et al. (2010)
27	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	7	Antimicrobial	Waheed et al. (2018)
Phytosterol					
28	á-Sitosterol	$C_{29}H_{50}O$	4	Anti-fungal	Mbambo et al. (2012)

Table 1 (continued)

	Group and name	Formula	Positive samples ($n = 24$)	Bioactivity	Reference
Pyrazole					
29	1H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl-	$C_7H_{14}N_2$	2	Antiviral, anticancer, anti-nflammatory, antibacterial and anti-fungal activities	Kumar et al. (2013)
30	2-Pyrazoline, 1-butyl-5-methyl-	$C_8H_{16}N_2$	2	Antibacterial and anti-fungal	Prabhakar et al. (2016)
Quinoline					
31	6,8-Dichloro-1,2,3,4-tetrahydro-2-methyl-4-[hydroxy-[2-hexahydropyridyl] methyl] quinolone	$C_{16}H_{22}C_{l2}N_2O$	6	Antimicrobial	Chemical Book (2018)
Siloxane/ TBDMS derivatives					
32	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	2	Antibacterial, anti-fungal and antioxidant	Gupta and Kumar (2017)
33	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	6	Anti-fungal	Sherazi et al. (2016)
34	tert-Butyldimethylsilylamine	$C_6H_{17}NSi$	3	Anti-fungal	Kankate et al. (2019)
35	5,5-Dimethyl-1,3-dioxane-2-ethanol, TBDMS derivative	$C_{14}H_30O_3Si$	1	Anti-fungal, Anticancer and antibacterial	Zablotskaya et al. (2018)
Sugar/Sugar alcohol					
36	á-l-Arabinopyranoside, methyl	$C_6H_{12}O_5$	2	Anti-fungal	Arif et al. (2009)
Vitamin					
37	ç-Tocopherol	$C_{28}H_{48}O_2$	21	Anti-viral and Anti-fungal	Baran and Thomas (2009)

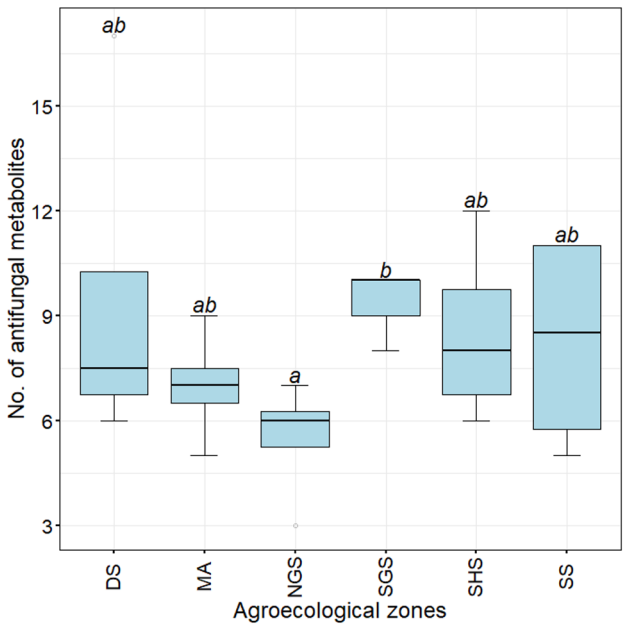

Fig. 2 Levels of anti-fungal metabolites in PM from the six agroecological zones of Nigeria. Agroecological zones with different letters have significant differences ($p = 0.05$). The dots, error bars, and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively

Discussion

The results of this study show a wide distribution of 15 mycotoxins in PM samples from Nigeria's PM growing AEZs (Fig. 1), but at levels below guideline values in food meant for human consumption (EC 2007, 2010). The low levels of mycotoxin contamination in Nigeran PM agree with earlier studies on Nigerian millet (Bandyopadhyay et al. 2007; Chala et al. 2014; Ezekiel et al. 2012), and they are in sharp contrast to much higher levels reported in Tunisian PM samples (Houissa et al. 2019). In this study, OTA was not encountered, which agrees with the other previous investigations about millet from Nigeria (Bandyopadhyay et al. 2007; Chala et al. 2014; Ezekiel et al. 2012). FB1, the most common of the fumonisins, was also not detected, which may suggest a host-specific variation in infection by toxigenic fungi and contamination by mycotoxins in PM leading to uncommon occurrence ratios of FB_1:FB_2:FB_3, as reported for aflatoxins in maize and groundnuts from Malawi (Matumba et al. 2015b).

Notwithstanding the generally low mycotoxin levels detected in this study, the distribution of mycotoxins in PM shows a subtle variation in mycotoxin concentrations across the AEZs (Fig. 1). NGS, amongst the AEZs studied, showed to be more predisposed to mycotoxin contamination, recording higher mean concentration for FBs,

ZEN, AcDON, and AME compared to other AEZs studied (Fig. 1). These findings corroborate with those from other studies in Nigeria, which reported mycotoxin levels differ across AEZs, with NGS having the highest concentrations (Atehnkeng et al. 2016; Chilaka et al. 2016). The variation of mycotoxin levels, as observed in these studies, is linked to high mycotoxin production potential of fungi in warmer climates (Shephard et al. 1996), which is characteristic of climatic conditions in NGS region.

This study shows a high profile of antifungal metabolites present in PM samples drawn from different AEZs in Nigeria. Out of the 88 different metabolites identified in PM samples, 40% of them have confirmed anti-fungal properties (Table 1). The effect of anti-fungal compounds on mycotoxins was reported by Azzouz and Bullerman (1982) and Vijayalakshmi et al. (2014) where some herbs and spices with anti-fungal properties inhibiting the growth of mycotoxigenic fungi. Although the study did not deduce the contribution of agricultural practices and morphological factors such as surface area and hard seed coat, it is likely that the broad spectrum of anti-fungal metabolites found in the present study may play a significant role in the resistance of PM to mycotoxin contamination. This might be one of the factors responsible for the low mycotoxin recorded in PM compared to the relatively higher levels of mycotoxins in other crops from the same AEZs, such as maize (Atehnkeng et al. 2016), yam and cassava (Chilaka et al. 2018), rice (Makun et al. 2011), and sorghum (Garba et al. 2017). The other crops do not have a high profile of antifungal metabolites reported. Even PM samples from NGS, an AEZ known to consistently register high mycotoxin levels (compared with other AEZs) in other grains including maize and sorghum (Atehnkeng et al. 2016; Chilaka et al. 2016) had low levels of mycotoxins. The number and distribution of the antifungal metabolites vary across the AEZs (Fig. 2). Coincidentally, the AEZs with this risk factors (NGS) registered lower median and mean number of anti-fungal compounds (Fig. 2), rendering weaker resistance to fungal proliferation. This may point to an effect of climatological parameters (e.g., drought stress), the ability of the plant to produce the right compounds, such as the case of phytoalexin (Grayer and Kokubun 2001), and/or their interaction, that require further research and/or consideration. Generally, anti-fungal metabolites act by destroying the pathogenic fungal cell. Studies have shown the difficulty to simplify the mechanism of anti-fungal action by plant secondary metabolites However, there are some correlations between the kind of secondary metabolite and its anti-fungal mechanism of action, but many of the compounds act via more than one mechanism of action (Freiesleben and Jäger 2014). Phenolic, nitrogen, and sulphur compounds were the major compounds found in PM in this study (Table 1).

The low mycotoxin contamination in PM shown in this study, coupled with its high nutritional value (Dias-Martins et al. 2018), renders it a top candidate for complimentary food for children who are vulnerable to toxins (Landrigan 2004) and require nutritious foods for development. Moreover, PM is adaptable to different AEZs and is drought tolerant (Dudhate et al. 2018). However, PM has a disadvantage of low yield compared to maize and rice (Muchow 1989), but it could be integrated in a systematic crop rotation, which would help to reduce vulnerability to pests and diseases, as well as susceptibility to mycotoxin contamination in this high yielding grain (Jianbo et al. 2016). This study contributes to the understanding the prevalence of mycotoxin contamination in PM in Nigeria and some of the reasons for low susceptibility of PM to mycotoxin concentration. This work suggests that the presence of anti-fungal metabolites in PM is one of the reasons for the low level of mycotoxin concentration. Thus, in addition to PM grain size, and compatibility, which have been previously reported, the presence of various anti-fungal metabolites, which suppress fungi colonization and subsequent production of mycotoxins, probably offers another significant reason for the low levels of mycotoxins reported in PM.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12550-022-00465-z.

Acknowledgements We appreciate the technical assistance from the Department of Biotechnology and Food Technology, University of Johannesburg, Gauteng, South Africa. We also appreciate Dr. Felix F. Fru of the Department of Plant and Soil Science, Faculty of Natural & Agricultural Sciences, University of Pretoria, for his technical support. This work was partly funded by the Tertiary Education Fund (TETFUND), Nigeria (TETFUND/FUTech/2014/49/IBIR).

Author contributions **Hadiza Kudu Muhammad:** Data collection and curation, investigation, methodology, validation, writing—original draft, writing—review & editing; **Hadiza Lami Muhammad**, **Patrick Berka Njobeh**, **and Hussaini Anthony Makun**: Conceptualization, methodology, resources, supervision, validation, and writing—review & editing; **Maurice Monjerezi** and **Limbikani Matumba**: Validation, visualization, and writing—review & editing. The authors read and approved the final manuscript.

Declarations

Ethics approval All the experiments carried out in this work comply with the current laws of federal republic of Nigeria and South Africa where the work was performed.

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Abia WA, Warth B, Michael S, Rudolf K, Angele NT, Njobeh PB, Mike FD, Paul FM (2013) Determination of multi-mycotoxin occurrence in cereals, nuts, and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). Food Control 31(2):438–453. https://doi.org/10.1016/j.foodcont.2012.10.006

Adeyeye SAO (2016) Fungal mycotoxins in foods: a review. Cogent Food Agric 2(1):1213127. https://doi.org/10.1080/23311932.2016.1213127

Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu MJ (2007) Antibacterial and anti-fungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz J Microbiol 38(4):739–742. https://doi.org/10.1590/S1517-83822007000400028

Ajani J, Vali PK (2017) Screening of millets for the natural occurrence of aflatoxin B_1 and their susceptibility in samples collected during pre-harvesting, harvesting and post-harvesting conditions. Indian J Adv Chem Sci 5(4):340–343

Ali A, Javaid A, Shoib A (2017) GC-MS Analysis and anti-fungal activity of methanolic root extract of *chenopodium album* against *Sclerotium rolfsii*. Planta Daninha 5:1–5

Alpert ME, Hutt MSR, Wogan GN, Davidson CS (1971) Association between aflatoxin content of food and hepatoma frequency in Uganda. Cancer 28(1):253–260. https://doi.org/10.1002/1097-0142(197107)28:1%3c253::AID-CNCR2820280151%3e3.0.CO;2-G

AOAC – Association of Official Analytical Chemists (1995) Sub-committee on feed additives and contaminants; AOAC: Rockville, MD, USA pp 41–42

Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R (2009) Natural products–antifungal agents derived from plants. J Asian Nat Prod Res 11(7):621–638. https://doi.org/10.1080/10286020902942350

ASC - Acme Synthetic Chemical (2018) Undecanoic acid. Last consolidated version available from: http://acmechem.com/undecanoic-acid. Accessed 28 Sept 2018

Atehnkeng J, Donner M, Ojiambo PS, Ikotun B, Augusto J, Cotty PJ, Bandyopadhyay R (2016) Environmental distribution and genetic diversity of vegetative compatibility groups determine biocontrol strategies to mitigate aflatoxin contamination of maize by *Aspergillus flavus*. Microb Biotechnol 9:75–88. https://doi.org/10.1111/1751-7915.12324

Atehnkeng J, Peter SO, Matthias D, Ikotun T, Richard AS, Peter JC, Bandyopadhyay R (2008) Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. Int J Food Microbiol 122:74–84. https://doi.org/10.1016/j.ijfoodmicro.2007.11.062

Azzouz MA, Bullerman LB (1982) Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. J Food Prot 45(14):1298–1301. https://doi.org/10.4315/0362-028X-45.14.1298

Bandyopadhyay R, Kumar M, Leslie JF (2007) Relative severity of aflatoxin contamination of cereal crops in West Africa. Food Addit Contam 24(10):1109–1114. https://doi.org/10.1080/02652030701553251

Baran R, Thomas L (2009) Combination of fluconazole and alpha-tocopherol in the treatment of yellow nail syndrome. J Drugs Dermatol 8(3):276–278

Carolina HP, Johan LFK, Vuyisile ST (2011) Anti-fungal free fatty acids: A Review. 1St ed. A. Mendez-Vilas: Science against microbial pathogens: Communicating Current Research and Technical Advances, 61-71

Chala A, Taye W, Ayalew A, Krska R, Sulyok M, Logrieco A (2014) Multimycotoxin analysis of sorghum (*Sorghum bicolor* L. Moench) and finger millet (*Eleusine coracana* L. Garten) from Ethiopia. Food Control 45:29–35. https://doi.org/10.1016/j.foodcont.2014.04.018

Chandrasekaran LM, Senthilkumar A, Venkatesalu V (2011) Antibacterial and anti-fungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum*. Eur Rev Med Pharmacol Sci 15:775–780

Chemical Book (2018) Quinoline compound https://www.chemicalbook.com/ProductCatalog_EN/111116-4.htm. Quinoline Chemical Book. Accessed 26 Sept 2018

Chilaka CA, De Boevre M, Atanda O, De-Saeger S (2018) Prevalence of Fusarium mycotoxins in cassava and yam products from some selected Nigerian markets. Food Cont 84(5):226–231. https://doi.org/10.1016/j.foodcont.2017.08.005

Chilaka CA, Boevre MD, Atanda OO (2016) Occurrence of *Fusarium* mycotoxins in cereals crops and processed products (ogi) from Nigeria. Toxins 8:342. https://doi.org/10.3390/toxins8110342

Codex - Codex Alimentarius Commission (2008) Proposed draft code of practice for the prevention (reduction) of mycotoxin contamination in cereals, including annexes on ochratoxin A, Zearalenone, Fumonisins and Tricothecenes. Codex Committee on Food Additives and Contaminants, Thirty-fourth Session. Codex Alimentarius Commission /RCP 51–2003

Dias-Martins AM, Pessanha KLF, Pacheco S, Rodrigues JAS, Carvalho CWP (2018) Potential use of pearl millet (*Pennisetum glaucum* (L.) R. Br.) in Brazil: food security, processing, health benefits and nutritional products. Food Res Int 109:175–186. https://doi.org/10.1016/j.foodres.2018.04.023

Dudhate A, Shinde H, Tsugama D, Liu S, Takano T (2018) Transcriptomic analysis reveals the differentially expressed genes and pathways involved in drought tolerance in pearl millet [*Pennisetum glaucum* (L.) R. Br]. PLoS ONE 13(4):e0195908. https://doi.org/10.1371/journal.pone.0195908

EC European Commission (2006) Commission regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Off J Eur Union L70:12–34

EC European Commission (2007) Commission Regulation (EC) Nº 1126/2007 of 28 September 2007 amending Regulation (EC) Nº 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. Off J Eur Union L 255:14–17

EC- European Commission (2010) Commission regulation (EU) no 165/2010 of 26 February 2010, amending regulation (EC) no 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxin. Off J Eur Union L 50:8–12

El-Sayed MH (2012) Di-(2-ethylhexyl) Phthalate, a major bioactive metabolite with antimicrobial and cytotoxic activity isolated from the culture filtrate of newly isolated soil *Streptomyces* (*Streptomyces mirabilis* Strain NSQu-25). World Appl Sci J 20(9):1202–1212

Elaiyaraja A, Chandramohan G (2016) Comparative phytochemical profile of *Indoneesiella Echioides* (L) Nees leaves using GC-MS. J Pharmacogn Phytochem 5(6):158–171

Ezekiel C, Sulyok M, Warth B, Krska R (2012) Multi-microbial metabolites in fonio millet (acha) and sesame seeds in Plateau State. Nigeria Eur Food Res Technol 235:285–293. https://doi.org/10.1007/s00217-012-1755-2

Ezekiel CN, Udom IE, Frisvad JC, Adetunji MC, Houbraken J, Fapohunda SO, Samson RA, Atanda OO, Agi-Otto MC, Onashile OA (2014) Assessment of aflatoxigenic Aspergillus and other fungi in millet and sesame from Plateau State. Nigeria Mycology 5(1):16–22. https://doi.org/10.1080/21501203.2014.889769

Fabien G, Benjamin K, Sébastien P, Veronique N, Marc P (2013) Selective oxidation of 5-hydroxymethylfurfural to 2,5-diformylfuran over intercalated vanadium phosphate oxides. RSC Adv 3:9942–9948. https://doi.org/10.1039/C3RA41890A

FAOSTAT - Food and Agriculture Organization of the United Nations Statistics (2018) Agricultural bulletin board on data collection,

dissemination and quality of statistics. Last consolidated version available from: www.faostat3.fao.org
Freiesleben SH, Jäger AK (2014) Correlation between plant secondary metabolites and their anti-fungal mechanisms–a review. Med Aromat Plants 3:154. https://doi.org/10.4172/2167-0412.1000154
Garba MH, Makun HA, Jigam AA, Muhammad HL, Patrick BN (2017) Incidence and toxigenicity of fungi contaminating sorghum from Nigeria. World J Microbiol 2(1):053–063
Grayer RJ, Kokubun T (2001) Plant–fungal interactions: the search for phytoalexins and other anti-fungal compounds from higher plants. Phytochemistry 56(3):253–263. https://doi.org/10.1016/S0031-9422(00)00450-7
Gupta D, Kumar M (2017) Evaluation of in vitro antimicrobial potential and GC–MS analysis of *Camellia sinensis* and *Terminalia arjuna*. Biotechnol Reports 13:19–25. https://doi.org/10.1016/j.btre.2016.11.002
Gyung JC, Kyoung SJ, Yong HC, Ju HY, Jin-Ceol K (2010) Anti-fungal activity of lower alkyl fatty acid esters against powdery mildews. Plant Pathol J 26(4):360–366. https://doi.org/10.5423/PPJ.2010.26.4.360
Hassan R, El-Kadi S, Sand M (2015) Effect of some organic acids on some fungal growth and their toxins production. Int J Adv Biol 2(1):1–11
Hori T, Fujinaga T (1985) Analytical use of solvent extraction with acetonitrile/water/chloroform and 1-propanol/water/cyclohexane mixtures. Talanta 32(8):735–743. https://doi.org/10.1016/0039-9140(85)80176-4
Houissa H, Lasram S, Sulyok M, Šarkanj B, Fontana A, Strub C, Krska R, Schorr-Galindo S, Ghorbel A (2019) Multmycotoxin LC-MS/MS analysis in pearl millet (*Pennisetum glaucum*) from Tunisia. Food Control 106:106738. https://doi.org/10.1016/j.foodcont.2019.106738
Hove M, De Boevre M, Lachat C, Jacxsens L, Nyunga LK, De Saeger S (2016) Occurrence and risk assessment of mycotoxins in subsistence farmed maize from Zimbabwe. Food Cont 69:36–44. https://doi.org/10.1016/j.foodcont.2016.04.038
Jianbo Q, Fei D, Minzheng Y, Xu J, Jiarong S (2016) Effect of preceding crop on *Fusarium* and mycotoxin contamination on Wheat grains. J Sci Food Agric 96(13):4536–4571. https://doi.org/10.1002/jsfa.7670
Jones E, Liu C, Gale MD, Hash C, Witcombe J (1995) Mapping quantitative trait loci for downy mildew resistance in pearl millet. Theor Appl Genet 91:448–456. https://doi.org/10.1007/bf00222972
Kankate RS, Gide PS, Belsare DP (2019) Design, synthesis, and antifungal evaluation of novel benzimidazol tertiary amine type of fluconazole analogues. Arab J Chem 12(8):2224–2235. https://doi.org/10.1016/j.arabjc.2015.02.002
Krishnaveni M, Dhanalakshmi R, Nandhini N (2014) GC-MS analysis of phytchemicals, fatty acid profile, antimicrobial activity of gossypium seeds. Int J Pharm Sci Rev Res 27:273–276
Kumar V, Kaur K, Gupta GK, Sharma AK (2013) Pyrazole containing natural products: synthetic preview and biological significance. Eur J Med Chem 69:735–753. https://doi.org/10.1016/j.ejmech.2013.08.053
Lakshmi PN, Latha HS (2017) Synthesis, antimicrobial evaluation and molecular properties prediction of *Chalcones* n. World J Pharm Pharm Sci 6(12):1895–1905
Landrigan PJ (2004) Children as a vulnerable population. Int J Occup Med Environ Health 17(1):175–177
Makun HA, Adeniran AL, Mailafiya SC, Ayanda IS, Mudashiru AT, Ojukwu UJ, Jagaba AS, Usman Z, Salihu DA (2013) Natural occurrence of `ochratoxin A in some marketed Nigerian foods. Food Control 31(2):566–571. https://doi.org/10.1016/j.foodcont.2012.09.043
Makun HA, Anjorin ST, Moronfoye B, Adejo FO, Afolabi OA, Fagbayibo G, Balogun BO, Surajudeen AA (2010) Fungal and aflatoxin contaminations of some human food commodities in Nigeria. Afr J Food Sci 4(4):127–135
Makun HA, Dutton M, Njobeh P, Phoku J, Yah C (2011) Incidence, phylogeny and mycotoxigenic potentials of fungi isolated from rice in Niger State, Nigeria. J Food Saf 31:334–349. https://doi.org/10.1111/j.1745-4565.2011.00305.x
Mashini K, Dutton MF (2006) The incidence of fungi and mycotoxins in South Africa wheat and wheat-based products. J Environ Sci Health Part B 41(3):285–296. https://doi.org/10.1080/03601230500357348
Matumba L, Van Poucke C, Njumbe EE, Jacobs B, De Saeger S (2015a) Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 32(6):960–969. https://doi.org/10.1080/19440049.2015.1029535
Matumba L, Sulyok M, Njoroge SMC, Emmanuel Njumbe Ediage EN, Van Poucke C, De Saeger S, Krsk R (2015b) Uncommon occurrence ratios of aflatoxin B_1, B_2, G_1, and G_2 in maize and groundnuts from Malawi. Mycotoxin Res 31:57–62. https://doi.org/10.1007/s12550-014-0209-z
Mbambo B, Odhav B, Mohanlall V (2012) Anti-fungal activity of stigmasterol, sitosterol and ergosterol from *Bulbine natalensis* Baker (Asphodelaceae). J Medicinal Plants Res 6(38):5135–5141. https://doi.org/10.5897/JMPR12.151
Mohammed GJ, Al-Jassani MJ, Hameed IH (2016) Anti-bacterial, anti-fungal activity and chemical analysis of Punica grantanum (Pomegranate peel) using GC-MS and FTIR spectroscopy. Int J Pharmacogn Phytochem Res 8(3):480–494
Muchow RC (1989) Comparative productivity of maize, sorghum, and pearl millet in semi-arid tropical environment I. Yield Potential Field Crop Res 20(3):191–205. https://doi.org/10.1016/0378-4290(89)90079-8
Njobeh PB, Dutton MF, Koch SH, Chuturgoon AA, Stoev SD, Mosonik JS (2009) Simultaneous occurrence of mycotoxins in human food commodities from Cameroon. Mycotoxin Res 26(1):47–57. https://doi.org/10.1007/s12550-009-0039-6
Obidi OF, Adelowotan AO, Ayoola GA, Johnson OO, Hassan MO, Nwachukwu SCU (2013) Antimicrobial activity of orange oil on selected pathogens. Int J Biotechnol 2(6):113–122
Okonkwo PO, Nwokolo C (1978) Aflatoxin B1: sample procedure to reduce levels in tropical foods. Nutrit Rep Int 17(3):387–395
Okoye ZSC, Ekpenyong KI (1984) Aflatoxins B_1 in native millet beer brewed in Jos suburb. Trans R Soc Trop Med Hyg 78(3):417–418. https://doi.org/10.1016/0035-9203(84)90146-9
Opoku AR, Ohenhen SO, Ejiofor N (1981) Nutrient composition of millet (*Pennisetum typhoides*) grains and malt. J Agric Food Chem 29(6):1247–1248. https://doi.org/10.1021/jf00108a036
Osamwonyi UO, Wakil SM (2012) Isolation of fungal species from fermenting pearl millet gruel and determination of their antagonistic activities against indicator bacterial species. Niger Food J 30(1):35–42. https://doi.org/10.1016/S0189-7241(15)30011-4
Pohl CH, Kock JLF, Thibane VS (2011) Anti-fungal free fatty acids: a review. In: Mendez-Vilas A (ed) Science against microbial pathogens: communicating current research and technical advances, 1st edn. FORMATEX, Pp 61–71
Prabhakar W, Babu KS, Ravindranath LK, Reddy PGP, Latha J (2016) Synthesis and anti-bacterial, anti-fungal activity of novel pyrazoline derivatives containing phenothiazine ring. Int J Chem Stud 4(2):51–58
Rubert J, Soler C, Mañes J (2012) Occurrence of fourteen mycotoxins in tiger nuts. Food Control 25(1):374–379. https://doi.org/10.1016/j.foodcont.2011.11.004
Sabithira G, Udayakumar R (2017) GC-MS Analysis of methanolic extracts of Leaf and Stem of *Marsilea minuta* (Linn.). J Altern

Complement Med 3(1):1–13. https://doi.org/10.9734/JOCAMR/2017/30871
Sharma A, Sood S, Agrawal PK, Kant L, Bhatt JC, Pattanayak A (2016) Detection and assessment of neuratical in methanolic extract of finger millet (*Eleusin coracana*) and Barnyard millet (*Echinochloa frumentacea*). Asian J Chem 28(7):1633–1637
Shephard GS, Thiel PG, Stockenström S, Sydenham EW (1996) Worldwide survey of Fumonisin contamination of corn and corn-based products. J AOAC Int 79(3):671–687. https://doi.org/10.1093/jaoac/79.3.671
Sherazi AZ, Jabeen K, Iqbal S, Yousaf Z (2016) Management of *Ascochyta rabiei* by *Chenopodium album* extracts. Planta Daninha 34(4):675–680. https://doi.org/10.1590/S0100-83582016340400007
Sofie M, Van Poucke C, Detavernier C, Dumoultn F, Van Velde MDE, Schoeters E, Van Dyck S, Averkieva O, Van Peteghem C, De Saeger S (2010) Occurrence of mycotoxins in feed as analysed by a multi-mycotoxin LC-MS/MS method. J Agric Food Chem 58(1):66–71. https://doi.org/10.1021/jf903859z
Sultan MZ, Moon SS, Park K (2010) Natural phthalate derivatives from the bacterium *Burkholderia cepacia* K87. J Sci Res 2(1):191–195
Udoh JM, Cardwell KF, Ikotun T (2000) Storage structures and aflatoxin content of maize in five agro-ecological zones of Nigeria. J Stored Prod Res 36:187–201. https://doi.org/10.1016/S0022-474X(99)00042-9
Umaiyambigai D, Saravanakumar K, Raj GA (2016) Phytochemical profile and anti-fungal activity of leaves methanol extract from the *Psydrax dicoccos* (Gaertn) Teys. & *Binn. Rubiaceae* Family. Int J Pharmacol Phytochem Ethnomed 7:53–61. https://doi.org/10.18052/www.scipress.com/IJPPE.7.53
Varsha KK, Devendra L, Shilpa G, Priya S, Pandey A, Nampoothiri KM (2015) 2,4-Di-tert-butyl phenol as the anti-fungal, antioxidant bioactive purified from a newly isolated *Lactococcus sp*. Int J Food Microbiol 211:44–50. https://doi.org/10.1016/j.ijfoodmicro.2015.06.025
Vega-Avila E, Tapia-Aquila R, Reyes-Chilpa R, Guzmán-Gutiérrez SL, Pérez-Flores J, Velasco-Lezama R (2012) Actividad antibacteriana y antifúngica de *Justicia spicigera*. Rev Latinoam Quím 40(2):75–82
Vijayalakshmi A, Sharmila R, Gowda NKS, Sindhu G (2014) Study on antifungal effect of herbal compounds against mycotoxin producing fungi. J Agric Technol 10(6):1587–1597
Vimalavady A, Kadavul, (2013) Phytocomponents identified on the various extracts of stem of *Hugonia mystax L.* (*Linaceae*). Eur J Exp Biol 3(1):73–80
Waheed A, Chohan MM, Ahmed D, Ullah N (2018) The first report on the in vitro antimicrobial activities of extracts of leaves of *Ehretia serrata*. Saudi J Biol Sci 26(6):1253–1261. https://doi.org/10.1016/j.sjbs.2018.05.025
Wilson JP, Jurjevic Z, Hanna WW, Wilson DM, Potter TL, Coy AE (2006) Host-specific variation in infection by toxigenic fungi and contamination by mycotoxins in pearl millet and corn. Mycopathologia 161(2):101–107. https://doi.org/10.1007/s11046-005-0170-7
Zablotskaya A, Segal I, Kazachonokh G, Popelis Y, Shestakova I, Nikolajeva V (2018) Silyl modification of biologically active compounds. 14. Organosilicon lipid-like derivatives of hydroxyethyl tetrahydro(iso)quinoline and thiazole with antitumour, antibacterial and antifungal properties. SILICON 10:1129–1138. https://doi.org/10.1007/s12633-017-9585-2
Zhu Y-Z, Liu J-W, Wang X, Jeong I-H, Ahn Y-J, Zhang C-J (2018) Anti-BACE1 and antimicrobial activities of steroidal compounds isolated from marine *Urechis unicinctus*. Mar Drugs 16(3):94. https://doi.org/10.3390/md16030094