Research Note

Occurrence of Toxigenic Fungi and Aflatoxin Potential of *Aspergillus* spp. Strains Associated with Subsistence Farmed Crops in Haiti

JUNIOR ARISTIL, GIOVANNI VENTURINI,* AND ALBERTO SPADA

Dipartimento di Scienze Agrarie e Ambientali, Produzione, Territorio, Agroenergia, Università degli Studi di Milano, via G. Celoria 2, 20133 Milano, Italy

ABSTRACT

Subsistence farming and poor storage facilities favor toxigenic fungal contamination and mycotoxin accumulation in staple foods from tropical countries such as Haiti. The present preliminary study was designed to evaluate the occurrence of toxigenic fungi in Haitian foodstuffs to define the mycotoxin risk associated with Haitian crops. The objectives of this research were to determine the distribution of toxigenic fungi in the Haitian crops maize, moringa, and peanut seeds and to screen *Aspergillus* section *Flavi* (ASF) isolates for production of aflatoxins B_1 and G_1 in vitro. Maize, moringa, and peanut samples were contaminated by potential toxigenic fungal taxa, mainly ASF and *Fusarium* spp. The isolation frequency of *Aspergillus* spp. and *Fusarium* spp. was influenced by locality and thus by farming systems, storage systems, and weather conditions. Particularly for ASF in peanut and maize samples, isolation frequencies were directly related to the growing season length. The present study represents the first report of contamination by toxigenic fungi and aflatoxin in moringa seeds, posing concerns about the safety of these seeds, which people in Haiti commonly consume. Most (80%) of the Haitian ASF strains were capable of producing aflatoxins, indicating that Haitian conditions clearly favor the colonization of toxigenic ASF strains over atoxigenic strains. ASF strains producing both aflatoxins B_1 and G_1 were found. Understanding the distribution of toxigenic ASF in Haitian crops and foodstuffs is important for determining accurate toxicological risks because the toxic profile of ASF is species specific. The occurrence of toxigenic fungi and the profiles of the ASF found in various crops highlight the need to prevent formation of aflatoxins in Haitian crops. This study provides relevant preliminary baseline data for guiding the development of legislation regulating the quality and safety of crops in this low-income country.

Key words: Aflatoxins; *Arachis hypogaea*; *Aspergillus* spp.; *Moringa oleifera*; *Zea mays*

Mycotoxins are toxic secondary metabolites produced by fungi belonging to several genera, including *Aspergillus, Fusarium,* and *Penicillium*. Mycotoxins represent a health problem particularly in tropical countries with high ambient temperatures and high relative humidity *(46)*. Among toxigenic fungi (TF), *Aspergillus* section *Flavi* (ASF) has been widely studied for its production of mycotoxins such as aflatoxins (AFs) *(39)*. AFs mainly occur in the forms of aflatoxin B_1 (AFB1), B_2 (AFB2), G_1 (AFG1), and G_2 (AFG2). AFB1 is one of the most potent naturally occurring mutagens and carcinogens known *(17)*. Daily consumption of foods contaminated with AFB1 can result in stunting in children, immune suppression, cancer, and reduced life expectancy especially in tropical countries where AF regulations are not in force and crops are consumed without monitoring *(36)*. ASF and AFs are found in a wide range of crops, mainly maize (*Zea mays*) and peanuts (*Arachis hypogaea*) but also spices and oilseed *(2, 13, 16, 20)*. Genotypes, drought, insect activity, delayed harvest, and

slow and inadequate drying are major causes of ASF and AF contamination *(10, 28)*. Colonization of maize and maize-derived products by TF has been reported in various Latin American countries such as Argentina *(3),* Brazil *(31),* and Ecuador *(26)*. TF and AFs also frequently contaminate Latin American peanuts *(47)*. In a tropical country such as Haiti with widespread subsistence farming and limited storage facilities, fungal growth and mycotoxin contamination may proliferate. In a previous study, Haitian maize and peanuts were highly contaminated by high concentrations of AFs *(35)*. AF exposure was confirmed by monitoring an AF biomarker in urine samples from Haitian adults and children *(14)*. However, no previous study has been conducted on the occurrence of TF in Haitian crops to provide insights on fungal ecology in Haiti. The objectives of this preliminary study were to (i) determine the distribution of *Aspergillus* spp. and *Fusarium* spp. (FUS) associated with Haitian maize, moringa seeds (*Moringa oleifera*), and peanut seeds, (ii) evaluate the AF contamination of samples, and (iii) screen ASF isolates for production of AFB1 and AFG1 in vitro.

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* Author for correspondence. Tel: +39 0250316791; Fax: +39 0250316781; E-mail: giovanni.venturini@unimi.it.

TABLE 1. *Isolation frequency of* Aspergillus *spp. (ASF, ASN, and ASC),* Fusarium *spp. (FUS), and* Penicillium *spp. (PEN) in maize, moringa seed, and peanut seed samples from Haiti*

Host	Sample no.	Farmer	Isolation frequency (%)[a]				
			ASF	ASN	ASC	FUS	PEN
Maize	1	A	48.0 ABC	28.0 BC	ND	33.3 AB	10.7
	2	B	9.3 A	6.7 A	ND	69.3 BC	24.7
	3	C	25.3 AB	6.8 AB	ND	70.0 C	24.0
	4	D	34.0 AB	23.3 ABC	ND	50.7 AB	45.3
	5	E	97.3 C	58.0 E	ND	50.8 ABC	45.3
	6	F	80.7 BC	51.3 DE	ND	32.7 AB	42.0
	7	G	31.3 AB	20.7 ABC	ND	31.3 A	53.3
	8	H	46.7 ABC	39.3 CD	ND	26.0 A	32.0
	9	I	40.0 AB	33.3 CD	ND	26.7 A	37.3
Moringa seed	10	J	80.7 C	12.7	10.7	ND	2.7
	11	K	54.7 B	12.0	10.6	ND	17.3
	12	L	1.3 A	ND	ND	14.7	9.3
Peanut	14	M	4.0 A	60.0 C	ND	ND	ND
	15	N	46.7 B	11.3 A	ND	ND	26.0
	16	O	28.0 A	38.0 B	ND	ND	ND
	17	P	16.7 A	22.0 A	ND	1.3	2.7
	18	Q	6.7 A	10.7A	ND	ND	2.7

[a] For each host, within a fungal taxon, values with different letters are significantly different at $P < 0.05$ (Ryan-Einot-Gabriel-Welsch F test). Absence of letters indicates no significant difference. ND, not detectable.

MATERIALS AND METHODS

Study region and sampling procedure. Eighteen samples were collected from farmers in southern Haiti (Sud Department, Les Cayes Arrondissement) during February 2015. The local agricultural products that were collected were maize kernels ($n = 9$), moringa ($n = 3$), and peanut seeds ($n = 6$). All samples (not less than 1 to 2 kg) were taken directly from the homesteads of different farmers (Table 1). During the sampling session, short interviews were conducted with growers concerning the crop production process. Samples were transported to the Mycology Laboratory (Department of Agricultural and Environmental Sciences, University of Milan, Milan, Italy) for mycological and AF analyses. Three representative subsamples (about 50 g) were randomly removed from each sample and stored at 4°C until analyzed.

Mycological analyses and fungal identification. The analysis of the viable mycobiota associated with samples was estimated using a direct plating technique after surface disinfection *(27, 41)*. All fungal colonies were screened according to macro-morphological and cultural criteria and assigned to genera *(27, 33, 44)*. Colonies identified as *Aspergillus, Fusarium,* and *Penicillium* were grown in pure culture by monoconidial isolation. *Fusarium* isolates were identified to section or species complex on the basis of their macro- and micromorphology *(22)* as well as colonies of *Aspergillus* spp. *(34, 39, 42)*. Data on fungal incidence were recorded as the percentage of kernels or seeds infected by each fungal taxon.

Determination of aflatoxigenic potential of ASF strains. The ability of 49 arbitrarily selected ASF monoconidic strains to synthesize AFs was assessed following the procedure described by El Mahgubi et al. *(12)*.

AF analysis. Samples were analyzed for total AFs using a commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kit (Ridascreen Aflatoxin Total, R-Biopharm

Rhône, Glasgow, UK). AF extraction and analysis were performed according to the manufacturer's instructions. The recovery rates were obtained in spiked samples, corresponding to the standard AFB1 (0.050 and 1.35 μg/kg) for each analyzed crop. The recovery rates for maize, moringa, and peanuts were approximately 85, 92, and 95%, with mean coefficients of variation of approximately 10, 15, and 6%, respectively.

Statistical analysis. The SPSS statistical package for Windows, v. 23.0 (SPSS, IBM, Armonk, NY) was used for all statistical analyses. Normal distribution and homogeneity of variances were verified, and a one-way analysis of variance (ANOVA) was used applying the Ryan-Einot-Gabriel-Welsch F test to detect significant differences between isolation frequency (IF) means for each fungal taxon. When data did not meet the requirements for parametric tests, they were analyzed with a Kruskal-Wallis nonparametric ANOVA and Mann-Whitney test for pairwise comparisons. Contingency tables were produced with AF chemotype frequencies, which were then compared using the chi-square test or a two-tailed Fisher's exact test, when appropriate.

RESULTS

Mycological analyses of the Haitian samples revealed variable levels of fungal contamination concerning both the quantitative and qualitative composition of the culturable mycobiota. Mycobiota of maize samples was composed mainly of potential toxigenic taxa such as *Aspergillus* spp., FUS, and *Penicillium* spp. (PEN) (90% of fungal isolates). Other fungi were isolated at low frequencies (10%). A similar mycobiota was observed for moringa seeds samples, with the majority of fungi (71%) belonging to *Aspergillus* spp., FUS, and PEN. Other fungi made up 28% of the total mycobiota isolated from moringa seeds. Peanut mycobiota was mainly composed of *Aspergillus* spp. (51%). Other

TABLE 2. *Mean isolation frequency of potential toxigenic taxa in maize and peanut samples from Haiti with different growing season length (GSLs)*

Host	GSL (mo)	Isolation frequency (%)			
		ASF	ASN	FUS	PEN
Maize[a]	3	30.3 A	34.0	47.2	40.4
	4	37.7 A	18.4	29.6	29.6
	5	89.0 B	6.7	49.3	24.0
Peanut	4	13.8	32.7	0.3	1.3
	5	36.7	11.3	ND	26.0
		$P = 0.034^b$	$P = 0.070$	ND	$P = 0.400$

[a] For maize samples, within a toxigenic taxon, means with different letters are significantly different at $P < 0.05$ (Kruskal-Wallis test). Means without letters are not significantly different.

[b] P values for a Mann-Whitney test comparing the means obtained from peanut samples with different GSLs. ND, not detectable.

fungi made up 39% of the total mycobiota, and 10% of the seeds were not contaminated.

The IFs of the potential TF taxa isolated from maize kernels differed among the samples. Only IFs measured for PEN were similar among samples (Table 1). ASF contamination was variable. Samples from farmers E and F were significantly more contaminated than was the sample from farmer B, but all other samples had similar ASF contamination. A similar pattern was observed for IFs measured for *Aspergillus* section *Nigri* (ASN); the sample from farmer B had the lowest IF, similar only to the sample from farmer C and significantly different from samples from farmers E and F. Isolated FUS mainly belonged to the *Fusarium fujikuroi* species complex (FFSC). Significant differences in FUS contamination were found among samples from farmers B and C and samples from farmers G, H, and I.

IFs for moringa seeds differed among samples only regarding ASF (Table 1). The sample from farmer L contained only ASF and FUS. Samples from farmers J and K contained strains belonging to *Aspergillus* section *Circumdati* (ASC).

Peanut samples were mainly contaminated by ASF and ASN, and the IFs for these taxa were the only values that were significantly different among samples (Table 1). The sample from farmer R was contaminated only with Mucoraceae strains (data not shown). All samples had similar ASF contamination except the sample from farmer N, which had the highest IF. The highest IFs for ASN were

TABLE 3. *Chemotype patterns of Haitian* Aspergillus *section* Flavi *strains isolated from maize, moringa seeds, and peanuts based on aflatoxin production*

Host	No. of tested isolates	No. of isolates with aflatoxin chemotype:				P^a
		I (AFB1)	II (AFB1+ AFG1)	III (AFG1)	IV (none)	
Maize	34	24	2	ND[b]	8	<0.001
Moringa seed	5	3	1	ND	1	0.449
Peanut	10	8	1	ND	1	0.007

[a] Two-tailed Fisher's exact test comparing number of isolates by aflatoxin chemotype.

[b] ND, not detectable.

found in samples from farmers M and O. FUS were isolated from only the sample from farmer P.

Among the inventoried agricultural variables, the growing season length (GSL) was the only one that had a significant effect on ASF occurrence in maize and peanut samples; ASF contamination was directly related to the crop cycle duration (Table 2). In maize samples, ASF was significantly higher in samples with a 5-month GSL than in samples with a 3- or 4-month GSL. For peanut samples, ASF was significantly higher in samples with a 5-month GSL than in samples with a 4-month GSL (Table 2).

The isolated fungal population consisted of strains of ASF ($n = 119$), ASN ($n = 66$), FUS ($n = 21$), and PEN ($n = 37$). Regarding monoconidic ASF strains, 56, 25, and 38 strains were isolated from maize, moringa, and peanut, respectively.

Based on AF production patterns, 49 ASF strains were classified into four chemotypes (Table 3). Chemotype I was represented by 35 ASF strains (71%) characterized by only AFB1 production. Chemotype II consisted in four ASF strains (9%) producing both AFB1 and AFG1. No strain belonged to chemotype III (producing only AFG1). Chemotype IV was consisted of the atoxigenic ASF strains ($n = 10$; 20%). In general, a majority (80%) of Haitian ASF strains were toxigenic compared with 20% atoxigenic strains ($\chi^2 = 33.11$; $P < 0.001$). No significant association was found between AF production profile and host of the Haitian ASF strains. Maize and peanut samples were mainly contaminated by chemotype I and II ASF strains and to a lesser extent by atoxigenic ASF strains. Moringa seeds were contaminated by a population of ASF strains with a homogenous chemotype pattern (Table 3).

Among the Haitian samples, the majority of maize (75%), peanut (80%), and moringa (100%) samples were positive for AFs. For the maize samples, the limits of detection of the method (signal-to-noise ratio, 3:1) and quantification (signal-to-noise ratio, 10:1) were 1.7 and 5.1 µg/kg, respectively. For moringa and peanut samples, limits of detection were 2.1 and 1.8 µg/kg, respectively, and limits of quantification were 6.5 and 5.6 µg/kg, respectively. AF contamination in maize samples ranged from nondetectable to 1,500 µg/kg, with a median of 95.4 µg/kg. AFs contamination in moringa and peanut seeds ranged from nondetectable to 700 and 500 µg/kg, respectively, with medians of 70.8 and 45.3 µg/kg, respectively.

DISCUSSION

This study is the first updated evaluation of the mycobiota colonizing Haitian maize, peanut, and moringa. The only peer-reviewed study on Haitian TF was carried out in 1983 and 1984 and focused only on maize (8). Recent studies have been conducted on AF contamination in Haitian maize samples (30, 35); however, mycological analyses and the toxigenic potential of isolated Haitian fungi have not been reported. The mycobiota found in Haitian samples was generally similar to that found in other nearby Latin American and Caribbean countries (1, 9).

In the Haitian maize samples, the high *Aspergillus* spp. prevalence is consistent with the well-demonstrated conducive conditions for *Aspergillus* spp. infection occurring frequently in tropical latitudes (19). The second potential TF taxon isolated from Haitian maize was FFSC, which includes prolific producers (*Fusarium verticillioides* and *Fusarium proliferatum*) of *Fusarium* toxins, e.g., fumonisins, frequently associated with maize worldwide (23, 25, 40). The differences among maize samples in fungal contamination levels seem to be due to the effect of location. Location could be considered the interaction of factors such as weather, agronomic practices, storage conditions, and fungal population characteristics (41). Local effects shaped the contamination patterns of *Aspergillus* spp. and FUS, similar to findings previously reported from studies of mycobiota in maize (11).

Climatic conditions and agronomic factors can influence the incidence of *Aspergillus* spp. (24) and FFSC (41) in crops. Among the inventoried practices, the only one that resulted in a significant effect on IFs was the GSL; GSL was positively correlated with ASF IF. Therefore, the prolonged presence of maize in the field, with climatic conditions and kernel moisture favorable to fungal growth, caused the increase in ASF contamination levels. Such results have been reported by several authors in other countries (7, 18, 45).

Maize samples in the present study were contaminated by AF at a similar level as reported previously in studies of Haitian maize (8, 30, 35). The median AF accumulation was more than 20 µg/kg, which is the U.S. Food and Drug Administration regulatory limit. The specificity, accuracy, and precision of the rapid ELISA did not permit a deep analysis of AF accumulation in samples compared with liquid chromatography methods, but the ELISA provided important baseline data for further analyses of AF contamination in Haitian crops. More work on the agronomic factors influencing the occurrence of TF in maize grown in Haiti is needed considering the optimal GSL and the typical Haitian bimodal rainfall pattern.

Evaluation of the general mycobiota in moringa seeds revealed that these seeds were highly contaminated with *Aspergillus* spp. This finding is the first report of TF contamination in moringa seeds. The prevalence of ASF in moringa seeds was high, and the consequent accumulation of AFs was verified in AF quantification assays. As the first report of the contamination of moringa seeds with AFs, the present study highlights safety concerns associated with these seeds, which people in Haiti commonly consume (21).

The general mycobiota found in Haitian peanut samples is similar to that previously reported, i.e., contamination with several fungal taxa (15, 29, 43, 47). The potential toxigenic mycobiota mainly consisted of *Aspergillus* spp. The prevalence of both ASF and ASN was influenced by local environmental factors (4). In the present study, the effect of harvest time on ASF contamination was significant. Harvesting peanuts without delay could be one of the best strategies, in addition to avoiding plant stress due to drought and pests, for reducing ASF infection and subsequent AF contamination of the seeds (28, 38).

A large percentage (80%) of wild Haitian ASF strains in this study were toxigenic, especially those isolated from maize and peanuts, in agreement with previous reports on Latin American ASF strains (3, 32). The most frequent AF chemotype was that producing only AFB1, confirming the dominance of this group in maize and peanuts (6, 37). Although colonization by different ASF species was not investigated in detail in the present study, Haitian conditions clearly favored toxigenic ASF strains over atoxigenic strains (11). Some ASF strains produced both AFB1 and AFG1. These results could lead to more research into the ecology and biodiversity of toxigenic Haitian mycobiota associated with crops. Species identification will allow researchers to determine whether such chemodiversity is confirmed, e.g., by finding other toxigenic species of ASF such as *A. nomius* or *A. parasiticus* in addition to the more ubiquitous *A. flavus*. Understanding the distribution of *A. flavus* and *A. parasiticus* in foodstuffs is important for determining accurate toxicological risks because each species might present a specific toxic profile (5).

In conclusion, the present study has provided for the first time information on the mycobiota of maize, peanuts, and moringa seeds in one of the main food production areas in Haiti. Constant monitoring of Haitian foodstuffs, particularly cereals and nuts, must be maintained given the frequency and levels of TF contamination found in the present study. In general, good agricultural practices can help reduce mycotoxins and the TF contamination in crops exposed to variable environmental conditions during growth in the field. More effort should be made to improve storage practices and food processing to minimize economic losses and reduce hazards to human health due to mycotoxin contamination. Further exploration of simple and accessible farming strategies for minimizing mycotoxin contamination in crops will be essential in Haiti where AF exposure risk is high and poverty is widespread.

REFERENCES

1. Almaguer, M., T. I. Rojas, F. J. Rodríguez-Rajo, and M. J. Aira. 2012. Airborne fungal succession in a rice field of Cuba. *Eur. J. Plant Pathol.* 133:473–482.

2. Amaike, S., and N. P. Keller. 2011. *Aspergillus flavus*. *Annu. Rev. Phytopathol.* 49:107–133.

3. Astoreca, A. L., A. M. Dalcero, V. F. Pinto, and G. Vaamonde. 2011. A survey on distribution and toxigenicity of *Aspergillus* section *Flavi* in poultry feeds. *Int. J. Food Microbiol.* 146:38–43.

4. Atayde, D. D., T. A. Reis, I. J. Godoy, P. Zorzete, G. M. Reis, and B. Corrêa. 2012. Mycobiota and aflatoxins in a peanut variety grown in different regions in the state of São Paulo, Brazil. *Crop Prot.* 33:7–12.

5. Atehnkeng, J., P. S. Ojiambo, M. Donner, T. Ikotun, R. A. Sikora, P. J. Cotty, and R. Bandyopadhyay. 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.

6. Barros, G. G., A. M. Torres, M. I. Rodriguez, and S. N. Chulze. 2006. Genetic diversity within *Aspergillus flavus* strains isolated from peanut-cropped soils in Argentina. *Soil Biol. Biochem.* 38:145–152.

7. Bilgrami, K. S., and A. K. Choudhary. 1998. Mycotoxins in preharvest contamination of agricultural crops, p. 1–43. *In* K. K. Sinha and D. Bhatnagar (ed.), Mycotoxins in agriculture and food safety. Marcel Dekker, New York.

8. Castor, L. L., C. J. Mirocha, and H. L. Chang. 1987. Aflatoxin occurrence in maize samples collected in Haitian markets. *Plant Dis.* 71:969–971.

9. Chiotta, M. L., M. L. Ponsone, M. Combina, and S. N. Chulze. 2016. *Aspergillus* and ochratoxin A in Latin America, p. 265–287. *In* N. Garg, S. M. Abdel-Aziz, and A. Aeron (ed.), Microbes in food and health. Springer International, Berlin.

10. Cleveland, T. E., P. F. Dowd, A. E. Desjardins, D. Bhatnagar, and P. J. Cotty. 2003. United States Department of Agriculture–Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest Manag. Sci.* 59:629–642.

11. Cotty, P. J., and R. Jaime-Garcia. 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *Int. J. Food Microbiol.* 119:109–115.

12. El Mahgubi, A., O. Puel, S. Bailly, S. Tadrist, A. Querin, A. Ouadia, I. P. Oswald, and J. D. Bailly. 2013. Distribution and toxigenicity of *Aspergillus* section *Flavi* in spices marketed in Morocco. *Food Control* 32:143–148.

13. Georgiadou, M., A. Dimou, and S. Yanniotis. 2012. Aflatoxin contamination in pistachio nuts: a farm to storage study. *Food Control* 26:580–586.

14. Gerding, J., N. Ali, J. Schwartzbord, B. Cramer, D. L. Brown, G. H. Degen, and H. U. Humpf. 2015. A comparative study of the human urinary mycotoxin excretion patterns in Bangladesh, Germany, and Haiti using a rapid and sensitive LC-MS/MS approach. *Mycotoxin Res.* 31:127–136.

15. Guezlane-Tebibel, N., N. Bouras, S. Mokrane, T. Benayad, and F. Mathieu. 2013. Aflatoxigenic strains of *Aspergillus* section *Flavi* isolated from marketed peanuts (*Arachis hypogaea*) in Algiers (Algeria). *Ann. Microbiol.* 63:295–305.

16. Hammami, W., S. Fiori, R. Al Thani, N. A. Kali, V. Balmas, Q. Migheli, and S. Jaoua. 2014. Fungal and aflatoxin contamination of marketed spices. *Food Control* 37:177–181.

17. International Agency for Research on Cancer. 1993. Aflatoxins, p. 245–396. *In* IARC monographs on the evaluation of carcinogenic risks to humans, vol. 56. Some naturally occurring substances: food items and constituents, heterocycles azoaromatic amines and mycotoxins. International Agency for Research on Cancer, Lyon, France.

18. Jones, R. K., H. E. Duncan, and P. B. Hamilton. 1981. Planting date, harvest date and irrigation effect on infection and aflatoxin production by *Aspergillus flavus* in field corn. *Phytopathology* 71:810–816.

19. Klich, M. A. 2007. *Aspergillus flavus*: the major producer of aflatoxin. *Mol. Plant Pathol.* 8:713–722.

20. Lanier, C., N. Heutte, E. Richard, V. Bouchart, P. Lebailly, and D. Garon. 2009. Mycoflora and mycotoxin production in oilseed cakes during farm storage. *J. Agric. Food Chem.* 57:1640–1645.

21. Leone, A., A. Spada, A. Battezzati, A. Schiraldi, J. Aristil, and S. Bertoli. 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: an overview. *Int. J. Mol. Sci.* 16:12791–12835.

22. Leslie, J. F., and B. A. Summerell. 2006. The *Fusarium* laboratory manual. Blackwell, Ames, IA.

23. Leyva-Madrigal, K. Y., C. P. Larralde-Corona, M. A. Apodaca-Sánchez, F. R. Quiroz-Figueroa, P. A. Mexia-Bolaños, S. Portillo-Valenzuela, J. Ordaz-Ochoa, and I. E. Maldonado-Mendoza. 2015. *Fusarium* species from the *Fusarium fujikuroi* species complex involved in mixed infections of maize in northern Sinaloa, Mexico. *J. Phytopathol.* 163:486–497.

24. Medina, A., A. Rodriguez, and N. Magan. 2014. Effect of climate change on *Aspergillus flavus* and aflatoxin B_1 production. *Front. Microbiol.* 5:348.

25. Mohammadi, A., M. Shams-Ghahfarokhi, F. Nazarian-Firouzabadi, R. Kachuei, M. Gholami-Shabani, and M. Razzaghi-Abyaneh. 2016. *Giberella fujikuroi* species complex isolated from maize and wheat in Iran: distribution, molecular identification and fumonisin B_1 in vitro biosynthesis. *J. Sci. Food Agric.* 96:1333–1340.

26. Pacin, A. M., H. H. L. González, M. Etcheverry, S. L. Resnik, L. Vivas, and S. Espin. 2003. Fungi associated with food and feed commodities from Ecuador. *Mycopathologia* 156:87–92.

27. Pitt, J. I., and A. D. Hocking. 2009. Fungi and food spoilage. Springer, Dordrecht, The Netherlands.

28. Pitt, J. I., M. H. Taniwaki, and M. B. Cole. 2013. Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of food safety objectives. *Food Control* 32:205–215.

29. Prakash, B., P. Singh, A. Kedia, A. K. Dwivedy, A. Singh, and N. K. Dubey. 2012. Mycoflora and aflatoxin analysis of *Arachis hypogaea* L. and assessment of *Anethum graveolens* L. seed and leaf essential oils against isolated fungi, aflatoxin production and their antioxidant activity. *J. Food Saf.* 32:481–491.

30. Resnik, S., M. L. Costarrica, and A. Pacin. 1995. Mycotoxins in Latin America and the Caribbean. *Food Control* 6:19–28.

31. Rocha, L. O., V. K. Nakai, R. Braghini, T. A. Reis, E. Kobashigawa, and B. Corrêa. 2009. Mycoflora and co-occurrence of fumonisins and aflatoxins in freshly harvested corn in different regions of Brazil. *Int. J. Mol. Sci.* 10:5090–5193.

32. Rocha, L. O., G. M. Reis, R. Braghini, E. Kobashigawa, J. de Araújo, and B. Corrêa. 2012. Characterization of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* section *Flavi* isolated from corn grains of different geographic origins in Brazil. *Eur. J. Plant Pathol.* 132:353–366.

33. Samson, R. A., J. Houbraken, U. Thrane, J. C. Frisvad, and B. Andersen. 2010. Food and indoor fungi, CBS laboratory manual series 2. CBS-Fungal Biodiversity Centre, Utrecht, The Netherlands.

34. Samson, R. A., C. M. Visagie, J. Houbraken, S. B. Hong, V. Hubka, C. H. Klaassen, G. Perrone, K. A. Seifert, A. Susca, J. B. Tanney, J. Varga, S. Kocsub, G. Szigeti, T. Yaguchi, and J. C. Frisvad. 2014. Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Stud. Mycol.* 78:141–173.

35. Schwartzbord, J. R., and D. L. Brown. 2015. Aflatoxin contamination in Haitian peanut products and maize and the safety of oil processed from contaminated peanuts. *Food Control* 56:114–118.

36. Shephard, G. S. 2008. Impact of mycotoxins on human health in developing countries. *Food Addit. Contam. A* 25:146–151.

37. Sweany, R. R., K. E. Damann, Jr., and M. D. Kaller. 2011. Comparison of soil and corn kernel *Aspergillus flavus* populations: evidence for niche specialization. *Phytopathology* 101:952–959.

38. Torres, A. M., G. G. Barros, S. A. Palacios, S. N. Chulze, and P. Battilani. 2014. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Res. Int.* 62:11–19.

39. Varga, J., J. C. Frisvad, and R. A. Samson. 2011. Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*. *Stud. Mycol.* 69:57–80.

40. Venturini, G., G. Assante, and A. Vercesi. 2011. *Fusarium verticillioides* contamination patterns in northern Italian maize during the growing season. *Phytopathol. Mediterr.* 50:110–120.

41. Venturini, G., S. L. Toffolatti, G. Assante, L. Babazadeh, P. Campia, E. Fasoli, D. Salomoni, and A. Vercesi. 2015. The influence of flavonoids in maize pericarp on *Fusarium* ear rot symptoms and fumonisin accumulation under field conditions. *Plant Pathol.* 64:671–679.

42. Visagie, C. M., J. Varga, J. Houbraken, M. Meijer, S. Kocsubé, N. Yilmaz, R. Fotedar, K. A. Seifert, J. C. Frisvad, and R. A. Samson. 2014. Ochratoxin production and taxonomy of the yellow aspergilli (*Aspergillus* section *Circumdati*). *Stud. Mycol.* 78:1–61.

43. Wagacha, J. M., C. Mutegi, L. Karanja, J. Kimani, and M. E. Christie. 2013. Fungal species isolated from peanuts in major Kenyan markets: emphasis on *Aspergillus* section *Flavi*. *Crop Prot.* 52:1–9.

44. Watanabe, T. 2010. Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC Taylor and Francis, Boca Raton, FL.

45. Wiatrak, P. J., D. L. Wright, J. J. Marois, and D. Wilson. 2005. Influence of planting date on aflatoxin accumulation in Bt, non-Bt, and tropical non-Bt hybrids. *Agron. J.* 97:440–445.

46. Williams, J. H., T. D. Phillips, P. E. Jolly, J. K. Stiles, C. M. Jolly, and D. Aggarwal. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am. J. Clin. Nutr.* 80:1106–1122.

47. Zorzete, P., A. C. Baquião, D. D. Atayde, T. A. Reis, E. Gonçalez, and B. Corrêa. 2013. Mycobiota, aflatoxins and cyclopiazonic acid in stored peanut cultivars. *Food Res. Int.* 52:380–386.