BJPS

Brazilian Journal of Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902019000118135

Article

Risk assessment of the occurrence of aflatoxin and fungi in peanuts and cashew nuts

Paula Kujbida[1,2], Patrícia Penido Maia[3], Ariadne Naama de Araújo[2], Leonardo Daniel Mendes[3], Mariana Lepri de Oliveira[3], Walicyranison Plinio Silva-Rocha[2], George Queiroz de Brito[2], Guilherme Maranhão Chaves[2], Isarita Martins[3]*

[1]Department of Pharmacy and Pharmaceutical Administration, Fluminense Federal University, Niteroi, RJ, Brazil,
[2]Department of Clinical and Toxicological Analysis, Federal University of Rio Grande do Norte, Natal, RN, Brazil,
[3]Laboratory of Toxicants and Drugs Analysis, Federal University of Alfenas, Alfenas, MG, Brazil

In the present study, the occurrence of fungi and aflatoxins (AFs) in peanut and cashew nut samples was investigated. Mycological analysis revealed the presence of fungi in 58.8% of samples, and assessment of AFs by chromatographic methods revealed that 52.9% were contaminated by AFs. AFB_1 was the principal component in all AF-contaminated samples, with a mean level of 14.0, and 1.08 µg/kg in peanut and cashew nut, respectively. Eleven samples (32.4%) exceeded the total AF maximum level (4 µg/kg) and 8 samples (23.5%) exceeded the AFB_1 (2 µg/kg) established by the European Commission. Our findings suggest that the incidence of AFs emphasizes the need for regular monitoring and a more stringent food safety system to control AFs at the lowest possible levels in peanuts and cashew nuts. The hypothetical dietary exposure suggests that the food products evaluated may significantly contribute to the overall human exposure.

Keywords: Aflatoxin. Peanut. Cashew nut. Fungi. Risk assessment.

INTRODUCTION

Aflatoxins (AFs), difuranocoumarins, are toxic secondary metabolites produced by filamentous fungi that can contaminate a number of raw food commodities with consequent impacts on public health and the agricultural economy (Hussein, Brasel, 2001; Bumbangi *et al*., 2016). These mycotoxins are highly hepatotoxic compounds and can cause both acute and chronic toxicity in humans and other animals (Peraica *et al.*, 1999; Wang, Lien, Lig, 2018; Nugraha, Khotimaha, Rietjensa, 2018). AFB_1, AFB_2, AFG_1 and AFG_2 are the main types of AFs naturally found in foods. Among those, AFB_1 is the most commonly found in food, is the most toxic, and was categorized as a Group 1 carcinogen by the International Agency for Research on Cancer in 1988 (IARC, 1993). Of note, *Aspergillus flavus*, *A. parasiticus* and *A. nomius* are the main fungal species of AF producers (Villa, Markaki, 2009). Nevertheless, other *Aspergillus* and *Penicillium* species such as *Penicillium puberulum* have been reported to be capable of producing AFs (Hodges *et al.*, 1964). Studies have shown the predominance of *Fusarium* spp., *Penicillium* spp., and *Aspergillus* spp. in Brazilian air samples (Gambale, 1998; Almeida *et al.*, 2002; Baquião *et al.*, 2012), and the predominant soil fungi identified in Brazilian peanut, corn, and Brazil nut plantations are *Penicillium* spp. and *Aspergillus* spp. (Almeida *et al.*, 2002; Zorzete *et al.*, 2011; Baquião *et al.*, 2012).

Delegations from countries in which the climatic conditions lead to relatively high AF contamination in food wish to have standards by which higher levels of contamination are permitted, allowing trade of their products in world markets. Additionally, when stringent international standards are used, the populations of these countries are placed at a higher risk because products with low levels of contamination are exported, leaving the more contaminated, lower-quality products for domestic consumption (CAC, 2001; Agyekum, Joly, 2017).

Nuts are known to be high-risk foods for AF contamination in Brazil, and these foods are considered

***Correspondence:** I. Martins. Laboratório de Análises de Toxicantes e Fármacos – LATF, Faculdade de Ciências Farmacêuticas, Universidade Federal de Alfenas--UNIFAL-MG. Rua Gabriel Monteiro da Silva 700 , 37130-000, Alfenas, MG, Brazil. Tel: +55-35-3701-9508. E-mail: isarita.sakakibara@unifal-mg.edu.br

the largest export product in that country (Milhome *et al.*, 2014). Efforts are made to implement good manufacturing practices throughout the peanut and cashew production chain in Brazil and thus to guarantee that all nuts exported meet the conditions and comply with the AF limits specified in importing countries. In 2001, the major Brazilian peanut industries launched the "Pro-Peanut" seal, a program of self-regulation with a focus on prevention of AFs, to promote the production of peanuts within the national and international quality standards (Pró-Amendoim, 2012). However, not all companies are part of the program, and AFs in peanut and peanut products have continued to be a problem in Brazil (Rodriguez-Amaya, Sabino, 2002). Furthermore, there are no programs for monitoring the level of mycotoxins in cashew nuts.

Cashew nuts are an extremely important source of income for the people living in Northeastern Brazilian (Damaceno Júnior, Bezerra, 2002). Exportation of cashew nuts has great economic importance, especially in the States of Ceará and Rio Grande do Norte, which accounted for almost all of the sales of cashew shelled in 2012 (IPECE, 2013). In addition to the roasted nuts, the local population and tourists also consume foods and drinks based on cashew apples, while the processed kernels are the principal exported commodity (Paula-Pessoa, Leite, Pimentel, 1995).

The present study evaluated the incidence of AFs and fungal contamination in peanut and cashew nuts commercially available in Natal, a city of the State of Rio Grande do Norte, located on the northeastern coast of Brazil. The research is of high importance to awareness of the need of the improve the system of inspection of AFs in food in Brazilian Northeast to ensure that the maximum allowable limits established are respected, thereby preventing economic and public health problems.

MATERIAL AND METHODS

Chemicals

The standards of AFB_1, AFB_2, AFG_1 and AFG_2 were purchased from Sigma (St. Louis, MO, USA). Methanol, chloroform, $CuSO_4$, and silica gel 60 on TLC were supplied by Merck (Darmstadt, Germany). Formic acid, Trifluoroacetic acid, Chloramphenicol, and Sodium chloride were purchased from Vetec (RJ, Brazil). Toluene, ethyl acetate, celite and hexane were from Isofar (RJ, Brazil), CAAL (SP, Brazil), Diacel (SP, Brazil) and Dinâmica (SP, Brazil), respectively. Sabouraud and Potato dextrose agar were purchased from Himedia (Mumbai, India), while Chromagar Candida was from Difco (Sparks, MD, USA).

Sampling

Peanuts and cashew nut sampling in Natal-RN-Brazil was conducted between May 2013 and July 2014. Thirty-four samples were randomly collected from grocery and supermarket shelves, and included 22 samples of peanuts or peanut products (peanut crumbly candy), and 12 samples of cashew nuts.

TLC determination of aflatoxins

TLC analysis was carried out as described by Rocha *et al.* (2008), based at AOAC (1999), with some modifications. Briefly, 40 g aliquots were taken from the samples, homogenized for 5 minutes in a blender with methanol (270 mL) and 4% KCl (30 mL), and then filtered through Whatman filter paper. Next, 150 mL of 10% CuSO4 and 5 g of celite was added to 150 mL of the filtrate, and the mixture was homogenized and filtered. The purified filtrate (150 mL) was transferred to a separating funnel, distilled water (150 mL) and chloroform (10 mL) were added, and the mixture was shaken vigorously for 3 minutes. The process was repeated twice more and the chloroform phases pooled to obtain the chloroform extract, which was evaporated in a water bath at 80ºC. Subsequently, the residue was resuspended in 500 µL of chloroform, and 10 µL was applied to a thin-layer chromatographic plate with a micro-syringe (Hamilton Microliter® Syringes, 10 µL), together with the aflatoxin standards. TLC was performed with toluene-ethyl acetate-formic acid (60:30:10) as the mobile phase. The plates were observed under ultraviolet light at 366 nm.

HPLC-FD determination of aflatoxins

The extraction, clean-up and derivation of aflatoxins were based on the method described by Ding *et al.* (2012) and VICAM (1999) with some modifications. In brief, 5 g of finely ground sample was extracted with 0.6 g of sodium chloride and 15 mL of a methanol: water solution (70:30 v/v) by ultrasonic bath (50°C) for 5 min. The extract was filtered using filter paper (Whatman No. 4, USA), and 4 mL of the filtrate was mixed with 2 mL petroleum ether. The mixture was mixed using a vortex for 30 s and then let stand to separate into two layers. The lower solution (3 mL) was collected, diluted with 8 mL pure water, mixed and filtered with an organic membrane (0.45 µm). The extracts obtained (8 mL) were applied to an Aflatest immunoafinity column (VICAM, Milford, USA) at a flow rate of one droplet every second. The column was then washed with distilled water (8

mL), and AFs were eluted with methanol (2 mL) into an amber vial. The eluate was evaporated to dryness under a stream of nitrogen gas. The purified extract was derivatized by adding 200 mL of hexane and 100 mL of trifluoroacetic acid, followed by 10 min incubation (40 °C). After evaporation to dryness under a stream of nitrogen, the dry residue was dissolved in a solution of water:acetonitrile:methanol (60:20:20, v/v/v; 1 mL) and filtered through a Millex PTFE 0.45 mm (Millipore, USA) for HPLC-FD quantitative analysis.

The HPLC equipment consisted of a Shimadzu LC-10ATvp (Kyoto, Japan) gradient system equipped with a Shimadzu SIL-10AF (Kyoto, Japan) auto-injector with a 20 µL loop and an LC-10AD pump. The column oven used was a Shimadzu CTO-10ASvp (Kyoto, Japan) operated at 25ºC. The detection was performed with an RF-10AXL fluorescence detector (Kyoto, Japan) set at 360 nm (excitation) and 440 nm (emission). Separation was achieved by isocratic elution carried out with a mobile phase composed of water:acetonitrile:methanol (60:20:20, v/v/v), at a flow rate of 0.5 mL/min, with a Supelcosil™ LC-18 (150 x 4.6 mm, 5 µm) column protected by a similar guard-column (40 x 4.6 mm). Data acquisition and treatment were performed using Class-VP software (Shimadzu).

Daily intake and risk assessment for AFB_1

The hypothetical dietary exposure for AFB_1 was calculated as follow: Exposure (ng/kg^{-1} body weight (bw)/day) = (contamination level X amount consumed)/bw (Ding *et al*., 2012).

To risk assessment, the dietary exposure for AFB_1 was calculated as follow: Exposure (ng/kg^{-1} bw/day) = [median contamination level (14.0 or 1.08 ng/g of peanuts or cashew nuts, respectively) X amount consumed]/bw (Villa, Markaki, 2009).

Fungal assay

To search for and isolate the fungal mycobiota, we applied the method of Silva *et al.* (2007) with slight modifications. Mechanically triturated samples (2 g) were resuspended in a saline solution (0.9 % NaCl; 1:10 dilution). Subsequently, the suspension was vortexed for 10 minutes and allowed to settle for 1 h. Next, 100 µL was seeded on the surface of Sabouraud Dextrose Agar (SDA) added to 50 mg/mL cloramphenicol plates and incubated at 25ºC for 3-5 days. Subsequently, colonies were macroscopically observed and purified for further identification.

The microscopic study of filamentous fungi was based on the general morphology of the colony, aspect, texture, color (pigment diffusion), diameter, days of culture and other morphological features inherent to each fungus (Riddell, 1950). Next, microcultivation on potato dextrose agar (PDA) was used for the microscopic analysis. Identification of filamentous fungi was based on a comparison of fungal structures (conidia, vesicles and phialids) (Barnett, Hunter, 1972; Nóbrega, Suassuna, 2004).

For yeast identification, yeast colonies were seeded onto the surface of CHROMagar *Candida* to check for purity and colony color. Petri dishes were incubated at 30°C, for 48–96 h. The isolated yeasts were identified according to classical methods (Yarrow, 1998).

RESULTS

1. Mycological analysis

In the present study, the most important toxigenic fungi were isolated from peanuts and cashews and were analyzed (Table I and Figure 1). The mycological analysis revealed the presence of fungi in 58.8% of samples, as follows: *Aspergillus* spp. (29.4%), *Rhodotorula* spp. (17.6%), *Penicillium* spp. (5.9%), *Candida albicans* (2.9%), and *Exophila* spp. (2.9%). In 14.7% of the samples, the isolated filamentous fungi did not produce sporulation, despite the use of PDA for the induction of conidiogenesis. Therefore, they were considered *Mycelia sterilia*. Climatic conditions of Northeast Brazil (high temperature and relative humidity) may contribute to the contamination and the growth of fungi.

2. Chromatography analysis

The samples of peanuts, and cashew nuts were first screened by TLC analysis for AFs. Of the 34 samples analyzed, 52.9% were contaminated with AFs (Table I). The levels of total AFs, as well as the four individual AFs, in samples selected for TLC analysis were quantified by HPLC-FD. The occurrence and distribution of AFs in peanut, and cashew nut samples are presented in Table II. A typical chromatogram obtained for AFs in this study is shown in Figure 2. AFs were detected in 18 out of 34 samples (52.9%) at maximum concentrations of 122.35 µg/kg for AFB_1, 130.91 µg/kg for AFB_2, 2.87 µg/kg for AFG_1, and 9.77 µg/kg for AFG_2. AFB1 was the principal component in all samples in which AFs were detected, with a mean level of 11.1 µg/kg.

TABLE I - Occurrence of fungi and aflatoxins in peanut and cashew samples marketed in Northeast Brazil (Natal, RN, Brazil) monitored by TLC analysis

Sample	Nº	Yeast fungi	Filamentous fungi	Aflatoxins detection
Peanuts	01	*Candida albicans; Exophiala* spp.	-	+
	02	*Rhodotorula* spp.	*Myceliasterilia*	n.d.
	03	*Rhodotorula* spp.	*Penicillium* spp.	n.d.
	04	-	-	n.d.
	05	-	-	n.d.
	06	-	-	+
	07	-	*Penicillium* spp.	+
	08	-	*Mycelia sterilia*	n.d.
	09	-	-	+
	10	-	-	n.d.
	11	-	-	+
	12	*Rhodotorula* spp.	-	+
	13	*Rhodotorula* spp.	-	+
	14	-	*Myceliasterilia*	n.d.
	15	-	*Aspergillus* spp.	n.d.
	16	*Rhodotorula* spp.	*Aspergillus* spp.	+
	17	*Rhodotorula* spp.	*Aspergillus* spp.	+
	18	-	*Aspergillus* spp.	+
	19	-	-	+
	20	-	-	+
	21[a]	-	-	+
	22[a]	-	-	+
Cashew nuts	23	-	-	n.d.
	24	-	*Aspergillus* spp.	+
	25	-	*Aspergillus* spp.	n.d.
	26	-	-	n.d.
	27	-	*Aspergillus* spp.	n.d.
	28	-	-	n.d.
	29	-	*Mycelia sterilia*	+
	30	-	*Aspergillus* spp.	n.d.
	31	-	-	n.d.
	32	-	*Mycelia sterilia*	+
	33	-	*Aspergillus* spp.	n.d.
	34	-	*Aspergillus* spp.	+

[-]absence of fungi growth; [+]presence of aflatoxins; [a]peanut crumbly candy; n.d.: not detected

3. AFB_1 intake estimates

Most agencies have not set a tolerable daily intake for AFs because no threshold can be assumed for genotoxic carcinogens (Cano-Sancho *et al.*, 2013). Nevertheless, Kuiper-Goodman (1998) estimated a Provisional Maximum Tolerable Daily Intake (PMTDI) of 1.0 ng AFB_1/kg bw for adults and children without hepatitis B virus infection. AFB_1 is the most potent natural carcinogen known, and risk assessment in of AFB_1 peanuts and cashew nuts examined in the present work was estimated for the consumption of 50 g by children (20 kg),

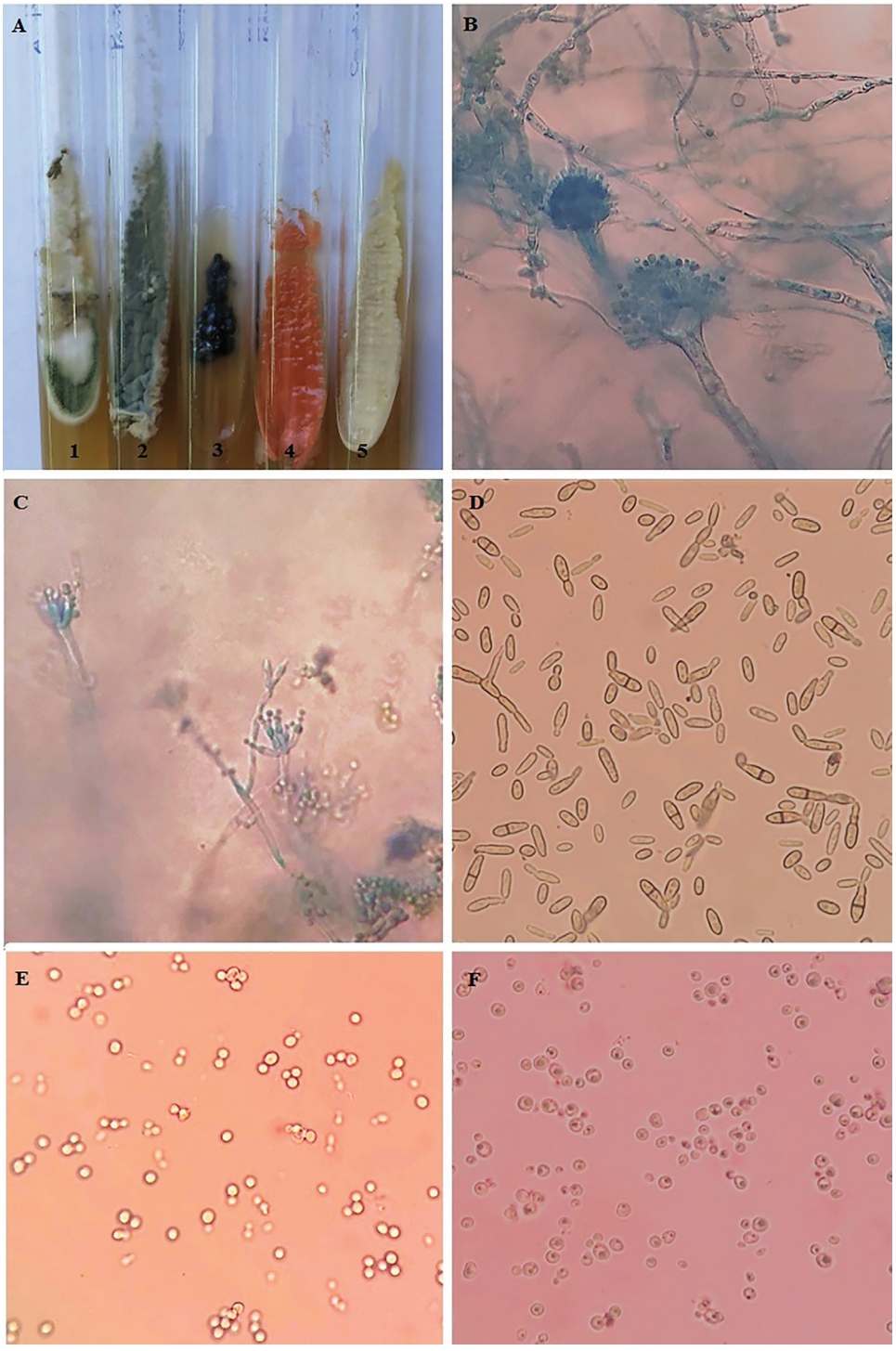

FIGURE 1 - Macromorphological and micromorphological aspects of fungi isolated from peanut, and cashew samples marketed in Northeast Brazil, after incubation on Sabouraud Dextrose Agar (SDA), at 25-30°C, for 3 to 5 days. A- Macromorphological aspects of fungal colonies. 1. *Aspergillus flavus* (yellow-green velvety colony with white reverse). 2. *Penicillium* sp. (grey-green furrowed colony with white reverse). 3. *Exophiala* sp. (wet, creamy, black yeast-like colony.) 4. *Rhodotorula* sp. (smooth to mucoid, salmon pink yeast colony) 5. *Candida albicans* (white-cream creamy colony) B- Micromorphological aspect of *Aspergillus flavus* (long conidiophores, hemispherical vesicle, biseriate or sometimes uniseriate phialides, smooth or slightly rough with long chains conidia). Micromorphological aspect of *Penicillium* sp. (conidia produced in chains from the tips of the phialides). Micromorphological aspect of *Exophiala* sp. (dematiaceous budding cells and torulose mycelium). Micromorphological aspect of *Rhodotorula* sp. (unicellular blastoconidia, lacking pseudohyphae and true hyphae). Micromorphological aspect of *Candida albicans* (blastoconidia; pseudohyphae and true hyphae may be present).

TABLE II - Levels of AFB_1, AFB_2, AFG_1, and AFG_2 (µg/kg) found in peanut and cashew samples marketed in the Northeast Brazil (Natal, RN, Brazil) monitored by HPLC-FD analysis

Sample type	Positive (incidence)	[a]B_1 (µg/kg)	[a]B_2 (µg/kg)	[a]G_1 (µg/kg)	[a]G_2 (µg/kg)	[b]$B_1 + B_2 + G_1 + G_2$ (µg/kg)
Peanuts (22)	14 (63.6%)	n.d. – 122.35	n.d. – 130.91	n.d. – 2.87	n.d. – 9.77	27.5 ± 44.7
Cashew nuts (12)	4 (33.3%)	n.d. – 2.79	n.d. – 4.16	n.d. – 0.41	n.d. – 0.44	3.3 ± 2.3

[a]Range; [b]Mean ± standard deviation; [n.d.]Not detect

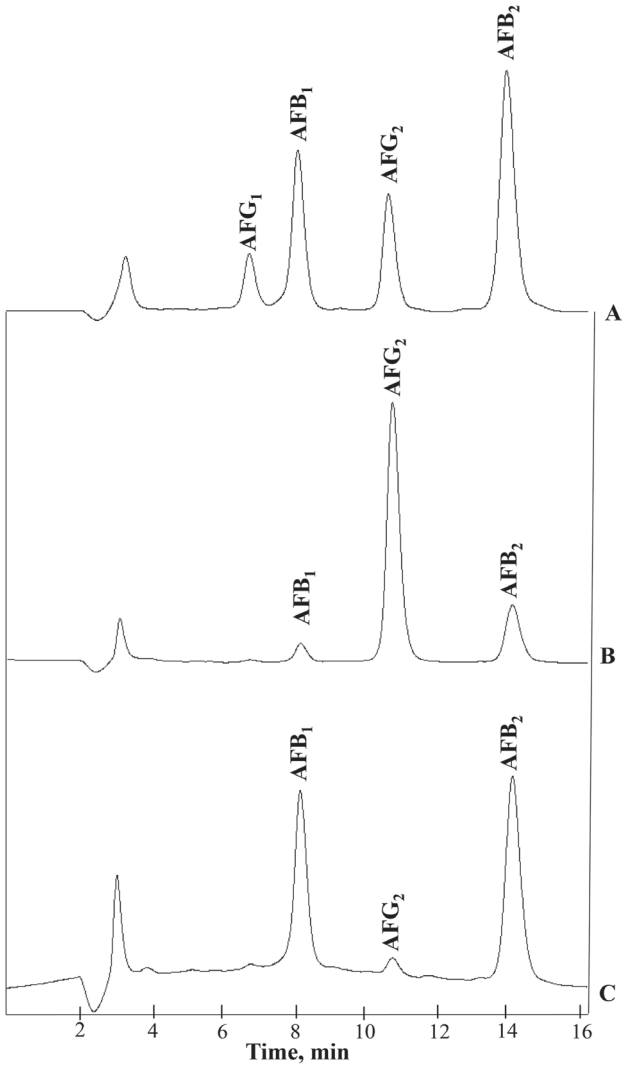

FIGURE 2 - Chromatograms showing peaks of AFB_1, AFB_2, AFG_1, and AFG_2: (A) AFs standard mixture, (B) peanut, and (C) cashew nut by HPLC-FD.

adolescents (50 kg) and adults (70 kg).

In Table III, the hypothetical dietary exposure of AFB_1 for consumers in Natal-RN-Brazil is shown. Consumption of 50 g from the most highly contaminated peanut and cashew nut samples (122.35 and 2.79 ng AFB_1/g, respectively) by a child (20 kg) led to an AFB_1 daily intake (305.88 and 6.98 ng AFB_1/kg bw, respectively) that is approximately 306-fold (peanuts) and 7-fold (cashew nuts) higher than the PMTDI (1 ng AFB_1/kg bw). Additionally, a consumption of 100 g by an adolescent (50 kg) displayed a daily intake of approximately 244-fold (peanuts) and 5-fold (cashew nuts) higher than the PMTDI, and an adult (70 kg) leads to an AFB1 daily intake approximately 174-fold (peanuts) and 4-fold (cashew nuts) higher than the PMTDI. Consumption of the less contaminated peanut or cashew nut sample (0.35 ng AFB_1/g) by children, an adolescent and an adult displayed a daily intake from 0.35- to 0.88-fold lower than the PMTDI. However, taking into account the consumption by an adult of 50 to 100 g of peanuts or cashew nuts contaminated with 20 or 10 ng/g (Brazilian legislation limit), respectively, the daily intake of AFB_1 is from 7.14 to 28.57 ng of AFB_1 kg/bw.

DISCUSSION

The isolation of *Exophiala* spp. has been previously reported in Brazil nuts (Freire, Kozakiewicz, 2005). However, to the best of our knowledge, this is the first report of the isolation of this genus of yeast from peanuts.

Interestingly too, we detected either yeasts or yeast-like fungi in 7 out of 34 (20.6%) of the samples. Despite the fact that AFs were never described for *Candida*, *Rhodotorula*, and *Exophiala*, all three genera are of medical interest (Hazen, 1995). Therefore, they should not be isolated from food that will be consumed by humans. Furthermore, *Candida albicans* belongs to the normal mycobiota of the gastrointestinal tract of humans and warm-blooded animals (Calderone, 2002). Therefore, the presence of this yeast in our samples indicated fecal contamination.

Notably, we found a positive correlation between the presence of filamentous fungi and AFs in 8 out of 34 samples (23.5%). The correlation between the level of substrates contaminated by fungi and the levels of AFs present was considered weak, suggesting that the presence of fungi in the samples does not necessarily

TABLE III - Estimated dietary exposure for AFB_1 (ng AFB_1/kg bw/day)

Age	Children	Adolescent	Adult
Body weight (kg)	20	50	70
Consumption of peanuts or cashew nuts (g)[a]	20 – 50	50 – 100	50 – 100
Mean 14.0 ng AFB_1/ g peanut	14.0 – 35.0	14.0 – 28.0	10.0 – 20.0
Mean 1.08 ng AFB_1/ g cashew nut	1.08 – 2.70	1.08 – 2.16	0.77 – 1.54
Most contaminated peanut sample: 122.35 ng AFB_1/ g	122.35 – 305.88	122.35 – 244.70	87.39 – 174.79
Most contaminated cashew nut sample: 2.79 ng AFB_1/ g	2.79 – 6.98	2.79 – 5.58	7.99 – 3.99
Less contaminated peanut/ cashew nut sample: 0.35 ng AFB_1/ g	0.35 – 0.88	0.35 – 0.70	0.25 – 0.50

[a]Estimated daily consumption. Provisional Maximum Tolerable Daily Intake (PMTDI): 1 ng AFB_1/kg bw/day.

indicate an AF contaminated sample (EMBRAPA, 2007). However, integrated management from field to food or feed processing is necessary to find means of preventing/ controlling fungal growth and reduce the adverse health effects of AF (Torres *et al.*, 2014).

The highest level of AF (AFB_1+AFB_2+AFG_1+AFG_2) contamination has been detected in samples of shelled peanuts, followed by cashew nuts with a total concentration of 133.74, and 5.85 µg/kg, respectively. According to the regulation limits of AFs in Brazil (ANVISA, 2011) (20 and 10 µg/kg in peanuts and cashew nuts, respectively), four peanut samples exceeded the regulatory limit, which was approximately 11.8% of all samples. However, 11 samples (32.4%) exceeded the total AF maximum levels (4 µg/kg) and 8 samples (23.5%) exceeded the AFB_1 maximum levels (2 µg/kg) established by the European Commission (2010).

In addition to the fact that the rejection of AF-contaminated consignments in European markets leads to economic losses, the control of the occurrence of AFs in foods is of great concern because these toxins have toxicological effects not only at high doses but also at low doses, as AFs have been shown to be potent human hepatocellular carcinogens at low levels of exposure. In the present study, 41.2% of samples had a level of total AFs in lower doses than that permitted in Brazil; therefore, even when released for consumption, continuous exposure to these types of food and other AF contamination increases the risk of developing chronic toxicity in consumers (Figure 3).

Thus, consumption of samples contaminated with AFs in the mean level (14.0 or 1.08 ng/g of peanuts or cashew nuts, respectively) would be within the range of permissible contamination (except for exposure of children to 50 g sample of peanuts), and 14- to 28-fold higher of ng AFB_1 kg/bw than the PMTDI.

In the present study, the daily intake for all ages for peanuts and cashew nuts contaminated with AFB_1 ranged from 0.25 to 305.88 and 0.25 to 6.98 ng AFB_1/kg bw per day (daily consumption of 20 to 100 g), respectively. However, the risk of exposure to AFs depends on the concentration of these toxins in the food, the amount consumed, the frequency of consumption and the consumers' preference. It is known that contamination with AFs in Brazil occurs in other popular foods such as rice (Silva *et al.*, 2008), Brazil nuts (Reis *et al.*, 2012), milk (Londoño *et al.*, 2013), dairy products (Iha *et al.*, 2011), maize (Sabino *et al.*, 1989; Manizan *et al.*, 2018) and corn products (Kawashima, Valente Soares, 2006). Therefore, even if cashew nut and peanut consumption is not daily, these foods can contribute to the daily exposure to low doses of AFs.

FIGURE 3 - Incidence of AFs showing the importance of regular monitoring and a more stringent food safety system to effectively control the AFs at the lowest possible levels in peanuts and cashew nuts.

CONCLUSION

Although legislation has set maximum concentrations of AFs in cashew nuts and peanuts in Brazil, our study showed that the incidence of AFs emphasizes the need for regular monitoring and a more stringent food safety system to effectively control the AFs at the lowest possible levels in peanuts and cashew nuts. Furthermore, the results suggest that citizens of the capital of Rio Grande do Norte, despite the low prevalence of hepatitis B surface antigen-positive individuals (virus that increases the risk of developing liver cancer), there is the possibility of ingestion of AFs in concentrations that result in exposure above the PMTDI, which can increase the risk of hepatocellular carcinoma.

ACKNOWLEDGEMENTS

This research was funded by a scholarship from the Pro-Rectory of Research at UFRN and UNIFAL-MG.

REFERENCES

Agyekum M, Jolly CM. Peanut trade and aflatoxin standards in Europe: Economic effects on trading countries. J Policy Model. 2017;39(1):114-128.

Almeida AP, Fonseca H, Fancelli AL, Direito GM, Ortega EM, Corrêa B. Mycoflora and fumonisin contamination in Brazilian corn from sowing to harvest. J Agric Food Chem. 2002;50(13):3877-3882.

Agência Nacional de Vigilância Sanitária. Resolução. ANVISA. - RDC n.º 7, de 18 de fevereiro de 2011 - Dispõe sobre Regulamento Técnico sobre limites máximos tolerados (LMT) para micotoxinas em alimentos, 2011. [cited 2018 Jan 18]. Available at: http://portal.anvisa.gov.br/documents/10181/2968262/(1)RDC_07_2011_COMP.pdf/61aa7690-68fa-4ecf-a0c9-8b4001117428.

AOAC. International Official Methods of Analysis. 16ª ed. Washington, DC: AOAC, 1999.

Baquião AC, Zorzete P, Reis TA, Assunção E, Vergueiro S, Correa B. Mycoflora and mycotoxins in field samples of Brazil nuts. Food Control. 2012;28(2):224-229.

Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. Minnesota: Burgess Publishing Company; 1972. 241p.

Bumbangi NF, Muma JB, Choongo K, Mukanga M, Velu MR, Veldman F, Hatloy A, Mapatano MA. Occurrence and factors associated with aflatoxin contamination of raw peanuts from Lusaka district's markets, Zambia. Food Control. 2016;68:291-296.

Codex Alimentarius Commission. CAC. Joint FAO/WHO food standards program, codex committee on food additives and contaminants. Thirty-third session. Dordrecht, The Netherlands: Codex Alimentarius Commission; 2001.

Calderone RA. Candida and candidiasis. Washington (D.C.): ASM Press; 2002.

Cano-Sancho G, Sanchis V, Marín S, Ramos AJ. Occurrence and exposure assessment of aflatoxins in Catalonia (Spain). Food Chem Toxicol. 2013;51:188-193.

Damaceno Júnior JA, Bezerra FC. Qualidade de pedúnculo de cajueiro-anão precoce cultivado sob irrigação e submetido a diferentes sistemas de condução e espaçamento. Rev Bras Frutic. 2002;24(1):258-262.

Ding X, Li P, Bai Y, Zhou H. Aflatoxin B1 in post-harvest peanuts and dietary risk in China. Food Control. 2012;23(1):143-148.

Embrapa. Micotoxinas: Importância na Alimentação e na Saúde Humana e Animal. 2007. [cited 2018 Jan 18]. Available at: http://www.cnpat.embrapa.br/cd/jss/acervo/Dc_110.pdf.

European Commission. Guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins. 2010. [cited 2018 Jan 18]. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_alfatoxins_guidance-2010_en.pdf.

Freire FCO, Kozakiewicz Z. Filamentous fungi, bacteria and yeasts associated with cashew kernels in Brazil. Rev Ciênc Agron. 2005;36(2):249-254.

Gambale W. Fungos Contaminantes. In: Zaitz C, Campbell I, Marques AS, Ruiz LR, Souza VM. (Editors). Compêndio De Micologia Médica. São Paulo: Medsi Editora Médica e Científica; 1998. p.113-121.

Hazen KC. New and emerging yeast pathogens. Clin Microbiol Rev. 1995;8(4):462-478.

Hodges FA, Zust JR, Smith HR, Nelson AA, Armbrecht BH, Campbell AD. Mycotoxins: aflatoxin isolated from penicillium puberulum. Science. 1964;145(3639):1439.

Hussein SH, Brasel JM. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology. 2001;167(2):101-134.

International Agency for Research on Cancer. IARC. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monographs on the Evaluation of carcinogenic Risks to Humans, 56, p. 245-395; 1993.

Iha MH, Barbosa CB, Okada IA, Trucksess MW. Occurrence of aflatoxin M1 in dairy products in Brazil. Food Control. 2011;22(12):1971-1974.

IPECE. Instituto de Pesquisa e Estratégia Econômica do Ceará, 2013. [cited 2018 Jan 18]. Available at: http://www.ipece.ce.gov.br/enfoque/EnfoqueEconomicoN60_07_03_2013.pdf.

Kawashima LM, Valente Soares LM. Incidência de fumonisina B_1, aflatoxinas B_1, B_2, G_1 e G_2, ocratoxina A e zearalenona em produtos de milho. Food Sci Technol. 2006;26(3):516-521.

Kuiper-Goodman T. Food safety: mycotoxins and phycotoxins in perspective. In: Miraglia M, van Edmond H, Brera C, Gilbert J. (Editors). Mycotoxins and phycotoxins-developments in chemistry: Toxicology and food safety (pp. 25-48). Fort Collins, Colo: Alaken Inc.; 1998.

Londoño VAG, Boasso AC, De Paula MCZ, Garcia LP, Scussel VM, Resnik S, Pacín A. Aflatoxin M_1 survey on randomly collected milk powder commercialized in Argentina and Brazil. Food Control. 2013;34(2):752-755.

Manizan AL, Oplatowska-Stachowiak M, Piro-Metayer I, Campbell K, Koffi-Nevry R, Elliott C, Akaki D, Montet D, Brabet C. Multi-mycotoxin determination in rice, maize and peanut products most consumed in Côte d'Ivoire by UHPLC-MS/MS. Food Control. 2018;87:22-30.

Milhome MAL, Lima CG, De Lima LK, Lima FAF, Sousa DOB, Nascimento RF. Occurrence of aflatoxins in cashew nuts produced in northeastern Brazil. Food Control. 2014;42:34-37.

Nóbrega FVA, Suassuna ND. Análise sanitária de sementes de amendoim (Arachis hypogaea L.) armazenadas em algumas áreas do estado da Paraíba. Rev Biol Ciênc Terra. 2004;4(2):1-13.

Nugrahaa A, Khotimaha K, Rietjensa IMCM. Risk assessment of aflatoxin B1 exposure from maize and peanut consumption in Indonesia using the margin of exposure and liver cancer risk estimation approaches. Food Chem Toxicol. 2018;113:134-144.

Paula-Pessoa PEF, Leite LA, Pimentel CRMS. Situação atual e perspectiva da agroindústria do caju. In: Araújo JPP, Silva VVS (Org.). Cajucultura: modernas técnicas de produção. Fortaleza: Embrapa-CNPAT; 1995. p.23-42.

Peraica M, Radić B, Lucić A, Pavlović M. Toxic effects of mycotoxins in humans. Bull World Health Org. 1999;77(9):754-766.

Pró-Amendoim. Garantia de qualidade. 2012. [cited 2018 Jan 18]. Available at: http://www.abicab.org.br/pro-amendoim-anuncia-90-de-produtos-em-conformidade-com-os-indices-de-seguranca/.

Reis TA, Oliveira TD, Baquião AC, Gonçalves SS, Zorzete P, Corrêa B. Mycobiota and mycotoxins in Brazil nut samples from different states of the Brazilian Amazon region. Int J Food Microbiol. 2012;159(2):61-68.

Riddell RW. Permanent stained mycological preparation obtained by slide culture. Mycologia. 1950;42(2):265-270.

Rocha MD, Maia PP, Rodrigues MAC, Martins I. Incidência de Aflatoxinas em Amostras de Amendoim e Paçoca Comercializadas na Cidade de Alfenas - MG, Brasil. Rev Bras Toxicol. 2008;21(1):15-19.

Rodriguez-Amaya DB, Sabino M. Mycotoxin research in Brazil: the last decade in review. Braz J Microbiol. 2002;33(1):1-11.

Sabino M, Prado G, Inomata EI, Pedroso MO, Garcia RV. Natural occurrence of aflatoxins and zearalenone in maize in Brazil. Part II. Food Addit Contam. 1989;6(3):327-331.

Silva JO, Cândido LMB, Novello D, Machado C. Ocorrência de aflatoxinas em arroz consumido por militares do Exército Brasileiro por cromatografia em camada delgada e cromatografia líquida de alta eficiência. Ciênc Agrotec. 2008;32(4):1238-1244.

Silva N, Junqueira VCA, Silveira NFA, Taniwaki MH, Santos RFS, Gomes RAR. Manual de Métodos de Análise Microbiológica de Alimentos. São Paulo: Varela; 2007.

Torres AM, Barros GG, Palacios SA, Chulze SN, Battilani P. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. Food Res Int. 2014;62:11-19.

VICAM. Aflatest instruction manual. Watertown, USA; 1999. p. 86.

Villa P, Markaki P. Aflatoxin B_1 and ochratoxin A in breakfast cereals from Athens market: Occurrence and risk assessment. Food Control. 2009;20(5):455-461.

Wang X, Lien KW, Lig MP. Probabilistic health risk assessment for dietary exposure to aflatoxin in peanut and peanut products in Taiwan. Food Control. 2018;91:372-380.

Yarrow D. Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP, Fell JW (Editors). The yeasts: a taxonomic study (pp 77-100). New York: Elsevier; 1998.

Zorzete P, Reis TA, Felício JD, Baquião AC, Makimoto P, Corrêa B. Fungi, mycotoxins and phytoalexins in peanut varieties, during plant grown in the field. Food Chem. 2011;129(3):957-964.

Received for publication on 02th March 2018
Accepted for publication on 28th May 2018