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Investigations on aflatoxigenic fungi and aflatoxins contamination in some nuts sampled in Algeria

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In this study, 40 nuts samples (almonds, pistachios, hazelnuts, peanuts and walnuts) available in the Algerian market were investigated for aflatoxigenic fungi and aflatoxins contamination. An evaluation of mould biodiversity revealed *Aspergillus* link to be the most frequent genus, while species belonging to the section *Nigri* (30.6%) and to the section *Flavi* (27.9%) were predominantly isolated. 78.6 and 52.4% of the 420 isolates of *Aspergillus* section *Flavi* examined produced cyclopiazonic acid (CPA) and aflatoxins (AFs), respectively. The majority of the aflatoxigenic strains (90.5%) were identified as *Aspergillus flavus*. In 90% of the nuts samples, aflatoxins were detected by HPLC with widely fluctuating contamination levels. Their concentrations ranged from 0.2 to 25.82 µg/kg. The highest levels were found in peanuts, pistachios and walnuts.

Key words: Aflatoxins, Algeria, *Aspergillus* section Flavi, nuts.

INTRODUCTION

Risk of *aflatoxin* (AFs) contamination hits top values in such commodities as nuts. *Aspergillus flavus* and *A. parasiticus* are the most important aflatoxigenic species naturally occurring in agricultural commodities (Pildain et al., 2008; Pitt and Hocking, 2009). These species are distributed worldwide in soil and air, and they can primarily colonize plants in the field and secondarily transfect harvested or stored plant products. Favorable temperature and humidity join inadequate storage practices contributing to mycotoxin contamination of foods and their spoilage. Toxigenic species diagnoses are important, since they could give an indication of the future potential due to the presence of mycotoxins.

Aflatoxins incidence in foods and feeds is closely monitored and regulated in more than 100 countries. Legal limits of *aflatoxin* B1 (AFB1) and total AFs for peanuts and nuts in Algeria are 10 and 20 µg/kg, respectively (FAO, 2004). The purpose of this study was to analyze the incidence and biodiversity of aflatoxigenic species belonging to *Aspergillus* section *Flavi*, as well as to survey current aflatoxins levels in Algerian commercialized nuts. Our aim was to achieve an evaluation of the health risk regarding the consumption of these products.

MATERIALS AND METHODS

Sample collection

A total of 40 nut samples (eight for each type of nut) composed of pistachios, roasted hazelnuts, shelled almonds, shelled peanuts and unshelled walnuts were purchased from retail shops and local markets of different locations of Algeria during March 2010. Almonds and peanuts samples were taken from retail already shelled. For pistachios, the shell is ajar, therefore it is highly

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vulnerable to contamination. 500 g of representative samples were taken from each collected sample after through mixing. The samples with shells (pistachios, roasted hazelnuts and walnuts) were manually shelled after disinfection with alcohol 70%. Finally, 200 g of each sample were finely powdered by a brief high speed Waring Blender in order to avoid sample overheating. Aliquots of 100 g of nut were used for the mycological analysis, and the remainder was stored at -20°C for the aflatoxins analysis.

Standard and reagents

All reagents (potassium chloride, phosphoric acid, hydrochloric acid) were of pro analysis (PA) grade. All solvents (methanol, acetonitrile, *n*-hexane, chloroform) were of high-performance liquid chromatography grade. They were purchased from Merck (Darmstadt, Germany). Deionized water was used for the preparation of all aqueous solutions and for HPLC. Standard toxins, aflatoxins (AFB1, AFB2, AFG1, AFG2) and cyclopiazonic acid (CPA), and Ehrlich's reagent (1 g of 4-dimethyl-aminobenzaldehyde in 75 ml ethanol and 25 ml concentrated HCl) were supplied by Sigma Chemicals (France). The working solutions were prepared according to the AOAC procedure (AOAC, 2000).

Isolation and identification of aflatoxin-producing fungi

Fungal isolation and culture conditions

Dilution plating (surface-spread method) (Pitt and Hocking, 1997) was used for colony counting. 10 g of each milled nuts sample were homogenized in 90 ml 0.1% peptone-water solution for 30 min in an orbital shaker. Serial decimal dilutions up to 10^{-4} were made and 0.1 ml aliquots were inoculated in triplicate onto the Dichloran Rose-Bengal Chloramphenicol Agar medium (DRBC) (King et al., 1979). All Petri dishes were incubated for 3 to 7 days at 28°C in the dark. Stock cultures of the representative strains were maintained for further examination in 20% glycerol at - 20°C.

Morphological characterization of the isolates

For each isolate, spores were suspended in 500 µL of 0.2% agar, and this suspension was used for inoculations on 9 cm diameter Petri dishes containing 20 mL of Malt Extract Agar (MEA) (malt extract, 20 g; glucose, 20 g; peptone, 1 g; agar, 20 g; distilled water, 1000 ml; pH, 6.5) and Czapek-Dox Agar (CZ) (sucrose, 30 g; K_2HPO_4, 1 g; $NaNO_3$, 2 g; KCl, 0.5 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $ZnSO_4 \cdot 7H_2O$, 0.01 g; $CuSO_4 \cdot 5H_2O$, 0.005 g; Agar, 20 g; distilled water, 1000 ml; pH, 6.2). Cultures were incubated for seven days in the dark, at 25°C, and then analyzed for colony color, eventual presence, color and size of sclerotia, head seriation and conidial morphology. Identification followed the taxonomic keys and guides available for the *Aspergillus* genus (Pitt and Hocking, 1997). Isolates were also cultured on CZ medium at 42°C, and colony diameter was measured after 7 days of incubation (Ehrlich et al., 2007; Kurtzman et al., 1987).

Aflatoxigenic ability of the isolates

For a preliminary screening of aflatoxins production, strains were inoculated at a central point on a 6 cm diameter Petri dish containing 10 ml of Coconut Agar Medium (CAM) supplemented with 0.3% β-cyclodextrin (Fente et al., 2001), and incubated for 5 days in the dark at 28°C. Cultures were tested for 365 nm UV light fluorescence and for bright orange-yellow colony reverse coloring expression under daylight. Thin layer chromatography (TLC) was used as a screening method to confirm the positive samples essentially as described by Calvo et al. (2004). The limit of detection was 50 ng/ml. Aflatoxins quantification was determined using High Performance Liquid Chromatography (HPLC). A post-column derivatization electrochemically generated bromine (Coring Cell) and a fluorescence detector (Spectra Physic 2000) with 362 nm for excitation, and 435 nm for emission) were used. The HPLC column used was a reverse phase RP C18 ProntoSil analytical column (250 x 4 mm, 3 µm particle size) preceded by a C18 pre-column (Ultrasep 10 x 4 mm). The mobile phase consisted of distilled water, acetonitrile, methanol (6:2:2, v/v/v) with 119 mg/l of KBr and 110 µl/l of 65% HNO_3. The injection volume was 20 µl and flow rate was 1 ml/min.

Cyclopiazonic acid detection

The isolates were tested for cyclopiazonic acid (CPA) production on CYA medium following the method described by Pildain et al. (2004). To determine the detection limit, a series of different concentrations (0.5, 1, 10, 25 and 50 µg/ml) of CPA dissolved in methanol was prepared and a volume of 20 µl of each was applied to a silica-gel, which was previously impregnated with a solution of oxalic acid (2% in methanol) for 2 min and dried. The plates were run in the same direction with ethyl acetate, 2-propanol, ammonium hydroxide (45:35:20, v/v/v). After pulverization of the plates with Ehrlich's reagent, the CPA was detected under daylight as an intense purple spot. The detection limit of the TLC technique was 1 µg/g.

Extraction of AFs from nut samples

Aflatoxins levels were determined according to the methodology proposed by El Adlouni et al. (2006) and Nguyen et al. (2007). A sub-sample of 20 g of thoroughly homogenized nuts was finely powdered and added to 20 ml of 4% potassium chloride solution acidified to pH 1.5 with sulfuric acid. The mixture was homogenized and extracted with 180 ml acetonitrile on an orbital shaker for 20 min, and filtered through Whatman no 4 filter paper.

Purification of the extract

The *n*-hexane (100 ml) was added to the filtrate and shaken for 1 min. After separation, the upper phase (*n*-hexane) was discarded. 50 ml of deionized water and 100 ml of chloroform were added to the lower phases. The mixture was shaken for 10 min and, after separation, the lower phase (chloroform) was collected. The upper phase was re-extracted three times with 20 ml of chloroform using the above conditions. Then, 50 ml of 5% sodium bicarbonate was added and shaken for 10 min to the pooled chloroform extracts. The upper phase (bicarbonate) was collected, acidified to pH 1.5 with concentrated hydrochloric acid and allowed to stand about 20 min. The acidified solution was extracted three times with chloroform (100, 50 and 50 ml).

The pooled chloroform phases were evaporated to near dryness under vacuum using a rotary evaporator placed in a 40°C water bath. The extract was re-suspended in 1 ml of methanol, sonicated and filtered through a 0.2 µm Minisart cartridge (Sartorius AG Goettingen, Germany). The analysis was performed using the previously described method.

Recovery experiments

Recovery experiments were performed by spiking aflatoxin-free peanut, almond and pistachio samples (20 g of ground sample) with two concentration levels (5 and 20 µg/kg) with AFB1, AFG1, AFB2

Table 1. Occurrence of moulds*, *Aspergillus*, *Aspergillus* section *Flavi* and *Aspergillus* section *Nigri* in 40 samples nuts collected
from markets in Algeria.

Nuts (n=8)	Total number of fungi ± SD (cfu/g)	*Aspergillus* (%)[a]	*Aspergillus* section *Flavi* (%)[b]	*Aspergillus* section *Nigri* (%)[b]
Pistachios	$16 \times 10^4 \pm 13.7 \times 10^3$	99.3	34.0	55.3
Roasted hazelnuts	$6 \times 10^2 \pm 2.8 \times 10^2$	66.0	38.3	25.3
Shelled almonds	$38 \times 10^3 \pm 2.3 \times 10^3$	99.8	15.4	64.4
Shelled peanuts	$16 \times 10^3 \pm 1.3 \times 10^2$	57.0	38.1	5.0
Unshelled walnuts	$4 \times 10^3 \pm 3 \times 10^2$	18.0	13.8	3.1
Mean		68.0	27.9	30.6

SD, standard deviation; [a]Calculated as a percentage of the total fungi; [b] Calculated as a percentage of the total *Aspergillus*. *The commonly isolated fungi were species of *Aspergillus*, *Penicillium* and *Mucor*.

and AFG2. Spiking was carried out in triplicates and a single analysis of a blank sample was also carried out. Aflatoxins concentrations were determined by HPLC analysis using the previously described method.

RESULTS

Mycological analysis

The fungal strains isolated from 40 samples of nuts (pistachios, roasted hazelnuts, shelled almonds, shelled peanuts and unshelled walnuts) collected from Algiers retail shops and local markets are shown in Table 1. The mean counts of the fungal colonies ranged from 6×10^2 to 16×10^4 cfu/g. Pistachios, shelled almonds, shelled peanut and unshelled walnuts were highly contaminated by total fungi. The commonly isolated fungi were species of *Aspergillus*, *Penicillium* and *Mucor*.

The most repre-sented genus in contaminated samples was *Aspergillus*, which was isolated in all analyzed samples with the mean percentage of 68.0%. The species isolated belonged to the *Aspergillus* section *Nigri, Flavi, Circumdati* and *Terrei.* Highest frequencies were recorded in shelled almonds (99.8%) and pistachios (99.3%).

Distribution and morphological characterization of isolates of *Aspergillus* section Flavi

Regarding *Aspergillus* section *Flavi* isolation, the mean percentage found was 27.9% of the total *Aspergillus* statistic. Colonization by species belonging to *Aspergillus* section *Flavi* was higher in roasted hazelnuts (38.3%), shelled peanut (38.1%) and pistachios (34%) (Table 1). Based on morphological characteristics, we found three distinct morphotypes among the 420 isolates studied. The morphotype 1 (386 isolates, 92%) represents yellow-green colonies and smooth to finely rough globose conidia. The morphotype 2 was represented by four isolates (1%) with dark-green colonies and rough conidia. Colonies of the two morphotypes of isolates tinged of a bright orange died on the reverse side of *Aspergillus flavus/A. parasiticus* Agar (AFPA) plates and could grow at 42°C. The isolates of morphotype 3 (30 isolates, 7.2%) showed dark-brown color producing ornated and brown conidia on AFPA medium; these isolates could not grow at 42°C.

Aflatoxins production by isolates of *Aspergillus* section Flavi

The incidence of aflatoxigenic strains is shown in Table 2. Among 420 isolates, 220 (52.4%) were aflatoxigenic. The incidence of aflatoxigenic strains was 56.2, 51.8, 44, 40 and 30% for pistachios, roasted hazelnuts, shelled almonds, shelled peanuts and unshelled walnuts, respectively. Analysis of aflatoxins production by fluorescence in CAM showed a good correlation with the TLC results.

Indeed, we found that all strains producing blue fluorescence pattern on CAM with brilliant orange-yellow reverse coloration under daylight showed an intense blue and green fluorescence spot for AFB and AFG, respectively. All of the morphotype 2 (4 isolates) were found to be strongly aflatoxigenic. The isolates belonging to the morphotype 1 with small “S” sclerotia (< 400 µm), were stronger producers than large”L” sclerotia (> 400 µm) isolates. The isolates of morphotype 3 were non-aflatoxigenic.

Chemotypes in *Aspergillus* section Flavi isolates

Based on mycotoxin production patterns (AFB, AFG and CPA) and sclerotia size, the 420 strains were classified into seven chemotypes (Table 3). Chemotype I (30.7%) for CPA producers; (ii) chemotype II (19.3%) for non-producers with “L” sclerotia; (iii) chemotype III (39.3%) for AFBs and CPA producers; (iv) chemotype IV (1.2%) for

Table 2. Occurrence and aflatoxins-producing ability of 420 isolates of *Aspergillus* section *Flavi* isolated from nuts collected from markets in Algeria.

Nut	Number of strains	Number of aflatoxigenic strains[a] (%)
Pistachios	130	73 (56.2)
Roasted hazelnuts	85	44 (51.8)
Shelled almonds	150	66 (44)
Shelled peanuts	55	22 (40)
Unshelled walnuts	50	15 (30)
Total	420	220 (52.4)

[a] For a preliminary screening of aflatoxins production, cultures were observed for fluorescence on CAM under long-wave UV light (365 nm) after 3, 5 and 7 days and then confirmed by TLC. The limit of detection (LOD) of TLC method for AFB and AFG was 50 ng/ml.

Table 3. Chemotype patterns of *Aspergillus* section *Flavi* strains isolated from nuts collected from markets in Algeria based on aflatoxins and cyclopiazonic acid producing ability, and on sclerotia size.

Chemotype	Mycotoxin			Fluorescence on CAM and TLC (color)	Sclerotia number of strains		Percentage (%)[c]
	AFB1	AFG1	CPA				
I	-	-	+	-[a]	-	150	30.7
II	-	-	-	-	L[b]	95	19.3
III	+	-	+	++ (Blue)	-	193	39.3
IV	+	-	-	+ (Blue)	L	6	1.2
V	+	-	+	++ (Blue)	S[b]	38	7.9
VI	+	+	+	+++ (Blue; Green)	S	4	1.0
VII	+	+	-	+++ (Blue; Green)	-	4	1.0

AFB1, aflatoxin B1; AFG1, aflatoxin G1; CPA, cyclopiazonic acid. [a] +++, Strong signal; ++, medium signal; +, weak signal; −, not detected. The aflatoxigenic isolates produced amounts of AFs ranging from 0.50 to 2000 µg/g of CAM. [b]The large strain (L) having sclerotia >400 mm in diameter and the small strain (S) with sclerotia <400 mm.[c] Percentage of the 420 isolates.

AFB “L” sclerotia; (v) chemotype V (7.9%) for AFB, CPA and “S” sclerotia producers; (vi) chemotype VI (1%) for AFB, AFG, CPA and “S” sclerotia producers; (vii) chemotype VII (1%) for AFB and AFG producers. The four isolates belonging to the chemotype VII have a distinctly darker green colonies and rough conidia, which produce AFB and AFG but not CPA. Isolates belonging to chemotypes VI and VII produced greater amounts of AFs (up to 2 mg/g). The chemotype I, II, III, IV, V and VI represents the morphotype I with yellow-green colonies and smooth to finely rough globose conidia. The chemotype VII represents the morphotype 2. The isolates with dark brown (morphotype 3) were included in chemotype I.

Performance of the methods

The results of recovery of aflatoxins are summarized in Table 4. The average recoveries were between 72.6 and 91.8%. The performance characteristics were within the acceptable margins indicated in the Commission Regulation No. 401/2006 (EC, 2006) for methods of sampling and analysis for the official control of mycotoxins. The limit of detection and limit of quantification were determined by spiked nuts samples with 5 µg/kg of AFB1, AFG1, AFB2 and AFG2, based on signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ. The limit of detection was established in 0.05 µg/kg for AFB1 and AFG1, and 0.2 µg/kg for AFB2 and AFG2. The limit of quantification, were 0.1 µg/kg for AFB1 and AFG1, and 0.5 µg/kg for AFB2 and AFG2.

Aflatoxins content in nuts

Of the 40 nuts samples analyzed by HPLC, 36 (90%) were contaminated with aflatoxins (Table 5). AFB1 was detected with concentrations ranging from 0.2 to 20.52 µg/kg. The high levels of AFB1 (20.52, 8.72 and 6.34 µg/kg) and AFs (25.82, 13.45 and 8.76 µg/kg) were found in shelled peanuts, pistachios and unshelled walnuts, respectively. In almonds and roasted hazelnuts samples, only AFB1 was detected with levels ranging from 1.65 to

Table 4. Recoveries of aflatoxins B1, B2, G1 and G2 from spiked non-infected almond, peanut and pistachios samples fortified with 5 and 20 µg/kg.

Aflatoxin	Spiking level (µg/kg)	Mean recovery (%)[a] ± RSD (%)[b]		
		Almond	Peanut	Pistachio
AFB1	5	84.1 ± 2.5	86.4 ± 3.1	80.2 ± 4.1
	20	88.1 ± 4.9	87.8 ± 3.5	91.8± 2.9
AFB2	5	86.2 ± 2.7	86.2 ± 2.7	78.1 ± 2.9
	20	85.7 ± 2.1	88.3 ± 3.5	89.6 ± 1.9
AFG1	5	87.4 ± 1.8	72.6 ± 4.5	82.5 ± 4.7
	20	81.6 ± 2.5	89.3 ± 2.1	88.5 ± 2.5
AFG2	5	78.5 ± 3.2	75.7 ± 1.6	76.5 ± 5.3
	20	81.6 ± 5.6	79.2 ± 4.6	81.7 ± 2.8

[a]Number of replicates: N =3; [b] RSD, relative standard deviation.

Table 5. Occurrence of aflatoxins in nuts samples (n=40) collected from markets in Algeria and analyzed by HPLC.

Nuts sample	No. of samples (No. of positive samples)	Range of AFB1 (µg/kg)	Mean ± SD (µg/kg)	Range of AFs (µg/kg)	Mean ± SD (µg/kg)
Pistachios	8 (7)	0.28 - 8.72	4.45 ± 2.64	0.41 - 13.45	6.70 ± 3.40
Roasted hazelnuts	8 (7)	0.20 - 2.81	1.33 ± 0.88	0.20 - 2.81	1.33 ± 0.88
Shelled almonds	8 (8)	1.65 - 4.00	2.12 ± 1.56	1.65 - 4.00	2.12 ± 1.56
Shelled peanuts	8 (8)	0.20 - 20.52	6.30 ± 3.64	0.34 - 25.82	7.10 ± 3.80
Unshelled walnuts	8 (6)	0.20 - 6.34	3.42 ± 1.35	0.60 - 8.76	4.90 ± 2.44

The LOD were 0.05 µg/kg for AFB1and AFG1, and 0.2 µg/kg for AFB2 and AFG2. The LOQ were 0.1 µg/kg for AFB1 and AFG1, and 0.5 µg/kg for AFB2

4.00 µg/kg and 0.20 to 2.81 µg/kg, respectively. Of the 40 samples analyzed, two peanuts samples (11.26 and 20.52 µg/kg) were above limit as recognized in Algeria (10 µg/kg for AFB1 and 20 µg/kg for AFs) (FAO, 2004).

DISCUSSION

High contamination of the majority of nuts samples can be sustained by storage without packaging and marketing under non-hygienic conditions when temperatures reach 30°C. Our results show very high frequencies of *Aspergillus* particularly in shelled almonds (99.8%) and pistachios (99.3%). We recorded 66 and 57% in roasted hazelnuts and shelled peanut, respectively. In nuts, *Aspergillus* species, and particularly *A. flavus* and *A. Niger*, have been frequently reported (Ihejirika et al., 2005; Horn and Dorner, 2009; Rodrigues et al., 2013). Species belonging to *Aspergillus* section *Flavi*, and especially, *A. flavus* can act as an endophyte in peanuts or invade the peanut fruits (Horn and Dorner, 1998; Bankole et al., 2005). As reported by Bayman et al. (2002) and Rodrigues et al. (2013), we observed the dominance of the black *Aspergillus* strains in almonds (64.4%) and pistachios (55.3%). The association of *A. flavus* and *A. niger* group in nuts can indicate the co-occurence of mycotoxins, particularly AFs and ochratoxin A. This association between these species has been reported by Rodrigues et al. (2012). Because of the effect of roasting, roasted hazelnuts samples were contaminated with lower total number of fungi dominating by *Aspergillus* section *Flavi* (38.3%) and *Aspergillus* section *Nigri* (25.3%). Of the 420 strains examined by TLC technique, 220 (52.8%) aflatoxins producers were detected. The incidence of aflatoxigenic strains was 52.4% for nuts analyzed. The percentage of aflatoxigenic strains of *A. flavus* has been shown to vary with the nature of substrate and environmental factors (Horn, 2003; Klich, 2007). There are few studies concerning the aflatoxigenic contamination incidence in nuts comercialized in Algeria. Fernane et al. (2010a) found 56.5% aflatoxigenic isolates of *A. flavus*. For *A. flavus* isolated from pistachios samples taken in Spain, 70.8% were identified as aflatoxigenic (Fernane et al., 2010b). *Aspergillus* section *Flavi* isolates have been found to be extremely diverse in terms of aflatoxins, CPA and sclerotial production (Vaaamonde et al., 2003; Razzaghi-Abyaneh et al., 2006; Giorni et al., 2007). Strains belonging to chemotypes I, II, III and IV were classified as typical *A. flavus*. These strains represent 90.5% of a total

aflatoxigenic isolates. Thus, our results confirm that the most common aflatoxin-producing fungi belong to *A. flavus* group. Based on the sclerotia size, isolates belonging to chemotype V were classified as atypical *A. flavus.* Chemotype VI for AFB, AFG, CPA and "S" sclerotia producers have been classified as *A. minisclerotigenes* or *A. parvisclerotigenus* (Frisvad et al., 2005; Pildain et al., 2008). In this study, only four isolates of *A. parasiticus* and four isolates of "S" type strain producers AFB and AFG were isolated from peanut and pistachios. Peanuts are regarded as one of the major habitats for *A. parasiticus* (Barros et al., 2006). This species were recorded at high proportions (48% of all *Aspergillus* section *Flavi*) in portuguese almonds (Rodrigues et al. 2009, 2011). However, this species is more restricted geographically as compared to *A. flavus* (Frisvad et al., 2007).

The aflatoxins were found in 36 out of 40 (90%) nuts samples. The high levels of AFB1 (20.52 and 8.72 µg/kg) and AFs (13.45 and 25.82 µg/kg) were found in pistachios and shelled peanuts samples, respectively. In pistachio samples, the incidence of contamination with AFs was 87.5% (7 of 8). Only one pistachio sample analyzed (12.5%) exceeded the maximum limit (10 µg/kg) set by Algerian regulations. The incidence of contamination of pistachios reported by literature is variable. According to a report from Morocco, 45% of pistachio nut samples contained AFs (Juan et al., 2008). In Saudi Arabia, 34% pistachio nut samples were contamined with AFs and two samples contained 411 and 126 µg/kg of AFB1 (El tawila et al., 2013). In Qatar, 27.7% of analyzed pistachio nut samples were contaminated with AFs with levels up to 289 and up to 81.6 µg/kg (Abdulkadar et al., 2000). Pistachios could be contaminated with AFs in every stage, from maturity until storage (Georgiadou et al., 2012). Only AFB1 was detected in roasted hazelnuts and shelled almonds. Roasted hazelnut samples showed a weak contamination with AFB1compared to other nuts analyzed due probably to the effect of roasting. For almonds, few studies have been reported concerning AFs contamination of these nuts compared to the other nuts. Luttfullah and Hussain (2011) report that three out of 10 (30%) shelled almond samples from Pakistan were contaminated with with AFs. In Saudi Arabia, nine of 53 samples (17%) of almonds were contaminated with AFs (3.5 ± 3.8 µg/kg). On the other hand, almonds and walnuts are considered at lower AFs risk (Jelinek et al., 1989). Among the nuts analyzed in our study, peanut showed the highest levels and the highest mean contamination with AFs ranging from 0.34 to 25.82 µg/kg (mean 7.10 µg/kg). Peanuts are considered to be at high risk of contamination with AFs because they are frequently contaminated with *Aspergillus*, especially aflatoxigenic species. Recently, it has been reported that AFB1 was detected in 25% of raw peanut from China, ranging from 0.01 to 720 µg/kg (Ding et al., 2012). On the other hand, Juan et al. (2008) showed a weak contamination of the analyzed samples of peanut with AFs (5%). Mphande et al. (2004) reported that 78% of raw peanut from Botswana contained AFs at concentrations ranging from 12 to 329 µg/kg. Six out of 8 (75%) walnuts samples contained AFs ranging from 0.60 to 8.76 µg/kg. Literature available on the occurrence of AFs in walnut indicates variable levels of contamination. Deabes (2010) reported that concentration of AFs in walnut samples from Saudi Arabia ranged from 12 to 140 µg/kg of sample. From this country, El Tawila reported that 50% of walnut samples contained low amounts of AFs compared to groundnuts and pistachio. However, the contamination levels of AFs in walnut samples from Morocco ranged from 1.24 to 4320 µg/kg (mean 360 µg/kg).

Difference in climate conditions, methods of handling during harvesting, drying process and transferring leading to mechanical damages of nuts and inadequate drying after rewetting for dehulling are determinant for the final aflatoxins content. In conclusion, our results show high contamination by strains belonging to *Aspergillus* section *Flavi* and *Nigri.* Only two of 40 samples showed levels above "recognized" limits in Algeria, suggesting that, despite the extent of contamination, the risk is minimal. However, if storage conditions are more favorable for aflatoxigenic fungi, the aflatoxins levels may be more important. Finding a practical strategy to reduce the risk of aflatoxin contamination of food and feed is vital.

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