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Contamination of common spices by aflatoxigenic fungi and aflatoxin B_1 in Algeria

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Abstract

Spices are usually produced in areas where the climatic conditions are favourable to growth of toxigenic fungi and production of mycotoxins. This study assesses the occurrence of aflatoxigenic fungi and aflatoxin B_1 (AFB_1) in spices marketed in Algeria. A total of 44 spice samples (4 for each type of spice) composed of aniseed, black pepper, caraway, cinnamon, coriander, cumin, ginger, red pepper, saffron, sweet cumin, and sweet pepper were collected from four popular markets located in Algeria. Mycological analysis of the spice was by dilution plating while AFB_1 contamination levels were determined by high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) after post-column derivatisation. The commonly isolated fungi were species of *Aspergillus* (56.4%), *Penicillium* (25.1%), *Mucor* (12.8%) and *Eurotium* (5.7%). Species belonging to *Aspergillus* section *Flavi* represented 28.9% of the total Aspergilli. The aflatoxin producing ability of isolates belonging to *Aspergillus* section *Flavi* was determined on coconut agar medium and confirmed by thin layer chromatography and HPLC-FLD. Ninety-four isolates (38.4%) of the 245 *Aspergillus* section *Flavi* examined produced aflatoxins. The most frequent chemotypes (84%) correspond to isolates able to produce both aflatoxin B and cyclopiazonic acid followed by the producers of only aflatoxin B. Twenty-three (63.9%) of the 36 spices contained AFB_1 at levels ranging from 0.10 to 26.50 µg/kg. Two saffron (24.34 and 26.50 µg/kg) and two sweet cumin (14.65 and 19.07 µg/kg) samples were above the Algerian regulatory limit of 10 µg/kg. This work represents the first report about the occurrence of aflatoxigenic fungi and AFB_1 in the common spices in Algeria.

Keywords: *Aspergillus*, HPLC-FLD, mycotoxins

1. Introduction

Spices are products of plant origin used for thousands of years to season and to add flavour or colour to dietetic preparations, and have no nutritional value. The most countries that produce spices include India (74% of the world market), followed by Bangladesh (6%), Turkey (5%) and China (5%) (http://faostat.fao.org). Because of their processing (harvesting techniques, drying, storage) and environmental conditions, spices are among the most contaminated food products with toxigenic moulds and mycotoxins, especially aflatoxigenic fungi and aflatoxins (AF) (El Mahgubi *et al.*, 2013; Hammami *et al.*, 2014; Ozbey and Kabak, 2012). In Algeria, due to climatic conditions characterised by high temperature and inadequate storage, spices are very susceptible to aflatoxin contamination. Aflatoxins have been clearly identified as toxic, mutagenic, teratogenic, and carcinogenic compounds. *Aspergillus flavus* and *Aspergillus parasiticus* are the main producers of AFs: aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) (Varga *et al.*, 2011). In the EU, an acceptable level of aflatoxins for spices has been set at 5 µg/kg for AFB_1 and 10 µg/kg for aflatoxins in combination (AFB_1 + AFB_2 + AFG_1 + AFG_2) (FAO, 2004). However, the Algerian regulation has set the maximum acceptable AFB_1 and AFs levels at 10 µg/kg for human foods and 20 µg/kg for animal feeds.

To the best of our knowledge, contamination of aflatoxigenic fungi and AFs in common spices marketed in Algeria has not been previously reported. Therefore, the aim of this study was to investigate the natural occurrence of aflatoxigenic fungi and AFB_1 present in spices used widely in Algeria for the preparation of processed foods. A total of 44 spice samples (4 for each type of spice), commercialised in Algeria, including aniseed (*Pimpinella anisum* L.), black pepper (*Piper nigrum* L.), caraway (*Carum caraway* L.), cinnamon (*Cinnamomum zeylanicum*), coriander (*Coriandrum sativum* L.), cumin (*Cuminum cyminum* L.), ginger (*Zingiber officinale* Rosc.), red pepper (*Capsicum frutescens* L.), saffron (*Crocus sativus* L.), sweet cumin (*Foeniculum vulgare* Mill.) and sweet pepper (*Capsicum annuum* L.) were analysed for aflatoxigenic fungi by standard mycological analysis techniques and for AFB_1 by high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD) and post-column derivatisation.

2. Materials and methods

Samples collection

Forty-four samples of spices were chosen on the basis of their availability in the market and popularity of usage, and were collected randomly from locally popular markets stalls in four cities (Algiers: 36°46'N 3°13'E, Batna: 35°33'N 6°10'E, Biskra: 34°51'N 5°44'E and Oran: 35°41'N 0°37'W) in Algeria during May 2012. The collected samples (4 for each type of spice) included cumin, coriander, black pepper, caraway, red pepper, sweet pepper, aniseed, sweet cumin, saffron, cinnamon and ginger. Spices sampling was done in accordance with sampling provision described on European Regulation no. 401/2006 (EC, 2006). One hundred grams of samples were ground to a fine powder using a Waring blender (Waring, Torrington, CT, USA) at high speed for a short period to avoid heating of the sample.

Standard and reagents

All reagents (potassium chloride, phosphoric acid and hydrochloric acid) were of pro analysis grade. All solvents (methanol, acetonitrile, *n*-hexane and chloroform) were of HPLC grade. They were purchased from Merck (Darmstadt, Germany). Deionised water was used for the preparation of all aqueous solutions and for HPLC. Standard toxins, aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2), 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 (Saint Quentin Fallavier, France). The working solutions were prepared according to the AOAC procedure (AOAC, 2000).

Mycological analyses of spice samples

Dilution plating was used as the enumeration technique (Pitt and Hocking, 1997). Ten grams of each sample were added to a sterile 250 ml Erlenmeyer flask containing 90 ml of sterile water supplemented with 0.1% of Tween 80. This mixture was stirred for 10 min using a magnetic stirrer, and then decimal dilutions of 10^{-2}, 10^{-3} and 10^{-4} fold were made. Aliquots of 100 µl of each dilution were spread (in triplicate) on the surface of the dichloran rose-bengal chloramphenicol agar medium (Sigma Chemicals) (King *et al.*, 1979). Incubation took place at 28 °C for 5-7 days in the dark. Isolates of *Aspergillus* section *Flavi* were also cultured on *Aspergillus flavus-parasiticus* agar (Sigma Chemicals) for 3-5 days at 28 °C, in the dark, to confirm group identification by colony reverse colour. Stock cultures of the representative isolates were maintained for further examination in 20% glycerol at -20 °C.

The colonies of each glycerol tube were sub-cultured on 90 mm diameter petri-dishes containing 15 ml of malt extract agar (Sigma Chemicals) and Czapek-Dox agar (Sigma Chemicals). Cultures were incubated for 7 days at 25 °C, in the dark, and then analysed for colony colour, presence and size of sclerotia, head seriation and conidial morphology. For micro-morphological observations, the isolates were examined under the microscope (10×, 40× and 100× magnification). Identification was performed according to the taxonomic keys and guides available for the *Aspergillus* genus (Pitt and Hocking, 1997; Klich, 2002; Samson *et al.*, 2004).

The production of sclerotia by *Aspergillus* section *Flavi* was examined following the procedures described by Pildain *et al.* (2008). The isolates tested were grown on Czapek yeast agar (CYA) at 28 °C away from light. The production of sclerotia was followed periodically for three weeks. The diameter of sclerotia (average, 50 to 60 sclerotia per colony) was measured under light microscope using a 500 µm gridded mesh plate.

Aflatoxins and cyclopiazonic acid production in *Aspergillus* section *Flavi*

For a preliminary screening of aflatoxin 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 colouring 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 (LOD) was 50 ng/ml.

Aflatoxigenic isolates were tested for 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 and ammonium hydroxide (45:35:20, v/v/v). After pulverisation of the plates with Ehrlich's reagent, the CPA was detected under daylight as an intense purple spot. The LOD of the TLC technique was 1 µg/ml.

Analysis of aflatoxin B_1 in spices samples

Extraction of aflatoxin B_1 from samples

Aflatoxin B_1 levels were determined according to the methodology proposed by Nguyen *et al.* (2007) and Riba *et al.* (2010). A sub-sample of 20 g of thoroughly homogenised spices was finely powdered and added to 20 ml of 4% potassium chloride solution acidified to pH 1.5 with sulphuric acid. The mixture was homogenised and extracted with 180 ml acetonitrile on an orbital shaker (unimax 2010; Heidolph, Saffron Walden, UK) for 20 min and filtered through Whatman no. 4 filter paper (Whatman International Ltd., Maidstone, UK).

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 deionised 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 (Laboratora 4000; Heidolph, Schwabach, Germany) 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, Göttingen, Germany). Aflatoxin B_1 quantification was determined using HPLC (Ultimate 3000; DIONEX-Thermofisher scientific, Courtaboeuf, France). A post-column derivatisation electrochemically generated bromine (Coring Cell; Sigma-Aldrich) and a fluorescence detector (Spectra Physic 2000; Ultimate 3000, RS module; DIONEX-Thermofisher scientific) 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 × 4 mm, 3 µm particle size; Atlantic labo ics, Bruges, France) preceded by a C18 pre-column (Ultrasep 10 × 4 mm; Atlantic labo ics). 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.

Recovery experiments

Recovery experiments were performed by spiking AFB_1-free spices (20 g of ground sample) with two concentration levels (5 and 20 µg/kg) with AFB_1. Spiking was carried out in triplicates and a single analysis of a blank sample was also carried out. Aflatoxin B_1 concentrations were determined by HPLC analysis using the previously described method.

3. Results and discussion

Occurrence of fungi in the spices

A total of 44 samples of 11 different spices samples were analysed (Table 1). The density of the total fungal flora ranged from 450±113 to 2,010±1,494 cfu/g. The most contaminated spices are ginger, sweet pepper, red pepper, sweet cumin and saffron. However, aniseed, black pepper, coriander, caraway, cumin and cinnamon are relatively less contaminated as shown in Table 1. The commonly isolated fungi were species of *Aspergillus* (56.4%), *Penicillium* (25.1%), *Mucor* (12.8%) and *Eurotium* (5.7%) (Figure 1). The contamination frequencies of the spices by *Aspergillus* spp. varied between 16.3 and 86.4%. Highest frequencies were recorded in saffron (86.4%), ginger (82.6%), black pepper (71.4%), sweet pepper (69.6%) and red pepper (61.4%). The mean occurrence of *Aspergillus* in the sweet cumin, coriander, caraway, cumin and cinnamon was 59.7, 58.6, 47.4, 34.9 and 31.6%, respectively. However, aniseed was contaminated less frequently by *Aspergillus* spp. (16.3%).

Most investigations in regions with warm climates have highlighted the prevalence of fungal species of the genus *Aspergillus* in spices. Our results are consistent with those reported by El-Kady *et al.* (1992), Hashem and Alamri (2010) and El Mahgubi *et al.* (2013). However, considerable heterogeneity was observed in the density of fungal flora in samples belonging to the same origin and location. Indeed, the quality of foods of plant origin after harvest is influenced by a wide variety of biotic and abiotic factors (Magan and Aldred, 2005). *Aspergillus* section *Flavi* isolates were present in 37 (88.0%) of 44 analysed samples with high incidence in saffron (64.1%) and ginger (58%). Red pepper, caraway and cumin showed contamination frequencies of 24.2, 20.7 and 18.9%, respectively. However, relatively low frequencies were recorded in black pepper, cinnamon and aniseed. Several studies showed that *Aspergillus* section

Table 1. Occurrence of moulds[1], *Aspergillus*, *Aspergillus* section *Flavi*, and aflatoxigenic isolates in 44 samples spices collected from markets in Algeria.

Origin of spices	Spices (n=4)	Total fungal flora ± SD (cfu/g)[2]	*Aspergillus* (%)[3]	*Aspergillus* section *Flavi* (%)[4]	Number of isolates tested	Number of aflatoxigenic isolates (%)[5]
Algiers	aniseed (*Pimpinella anisum* L.)	800±370	16.3	06.4	5	5 (100)
	red pepper (*Capsicum frutescens* L.)	1,175±678	61.4	24.2	20	4 (20.0)
	sweet cumin (*Foeniculum vulgare* Mill.)	1,031±457	59.7	31.1	20	8 (40.0)
	sweet pepper (*Capsicum annuum* L.)	1,646±100	69.6	36.8	46	8 (17.4)
Batna	black pepper (*Piper nigrum* L.)	719±71	71.4	16.3	6	1 (16.7)
	caraway (*Carum caraway* L.)	565±322	47.4	20.7	7	5 (71.4)
	coriander (*Coriandrum sativum* L.)	752±312	58.6	30.3	12	7 (58.3)
Biskra	cumin (*Cuminum cyminum* L.)	550±330	34.9	18.9	7	1 (14.3)
Oran	cinnamon (*Cinnamomum zeylanicum* L.)	450±113	31.6	11.2	2	0 (0.0)
	ginger (*Zingiber officinale* Rosc.)	2,010±1,494	82.6	58.0	74	41 (55.4)
	saffron (*Crocus sativus* L.)	1,015±658	86.4	64.1	46	14 (30.4)
Total					245	94 (38.4)

[1] The commonly isolated fungi were species of *Aspergillus*, *Penicillium*, *Eurotium* and *Mucor*.
[2] SD = standard deviation.
[3] Calculated as a percentage of the total fungi.
[4] Calculated as a percentage of the total *Aspergillus*.
[5] For a preliminary screening of aflatoxin production, cultures were observed for fluorescence on coconut agar medium under long-wave UV-light (365 nm) after 3, 5 and 7 days and then confirmed by thin layer chromatography (TLC). The limit of detection of the TLC method for aflatoxin B and G was 50 ng/ml.

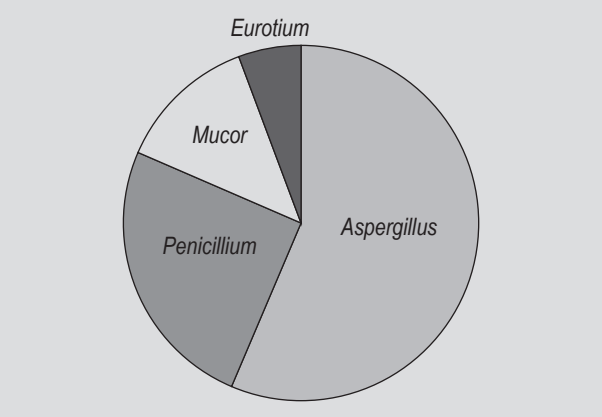

Figure 1. Incidence of the commonly isolated fungi (%) isolated from spices commercialised in Algeria.

Flavi is predominant over other species in different commercialised spices (Garrido *et al.*, 1992; Hashem and Alamri, 2010; Kong *et al.*, 2014).

Aflatoxins, CPA, sclerotia production and chemotypes of *Aspergillus* section *Flavi*

The incidence of aflatoxigenic strains is shown in Table 2. Our results showed that the ability to produce AFs varied considerably from one isolate to another. It is known that typical *A. flavus* strains produce AFB and CPA, but this production is extremely variable (Richard *et al.*, 1992). Among 245 isolates, 94 (38.4%) were aflatoxigenic with variable incidence (Table 1). Analysis of aflatoxin production by fluorescence in CAM showed a good correlation with the TLC results. Indeed, all strains producing blue fluorescence pattern on CAM with brilliant orange-yellow reverse coloration under daylight showed an intense blue and green fluorescence spot on TLC plates for AFB and AFG, respectively. 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).

Based on mycotoxin production patterns (AFB, AFG and CPA) and sclerotia size, the 94 aflatoxigenic strains were classified into seven chemotypes (Table 2). The majority of the aflatoxigenic strains (84%) produced CPA. Many authors highlighted a positive correlation between the production of AFB and CPA. Thus, Pildain *et al.* (2008) pointed out that CPA is produced not only by strains producing AFB like *A. flavus* and occasionally *Aspergillus pseudotamarii*, but also by *Aspergillus minisclerotigenes* and *Aspergillus parvisclerotigenus* that produce both AFB and AFG. The chemotypes I, II, III and IV (70% of the total aflatoxigenic strains) with yellow-green colonies and smooth to finely rough globose conidia represents the morphotype of typical

Table 2. Chemotype patterns of aflatoxigenic strains isolated from spices collected from markets in Algeria based on aflatoxins and cyclopiazonic acid producing ability, and on sclerotia size.

Chemotype	Mycotoxin[1]			Sclerotia[2]	Number of isolates	Percentage (%)[3]
	AFB_1	AFG_1	CPA			
I	+	–	+	–	42	44.5
II	+	–	+	L	28	29.8
III	+	–	–	L	2	2.0
IV	+	–	–	–	7	7.3
V	+	–	+	S	10	10.6
VI	+	+	+	S	4	4.1
VII	+	+	–	–	1	1.2

[1] AFB_1 = aflatoxin B_1; AFG_1 = aflatoxin G_1; CPA = cyclopiazonic acid; + = detected; – = non-detected.
[2] The large strain (L) having sclerotia >400 mm in diameter and the small strain (S) with sclerotia <400 mm.
[3] Percentage of the 94 aflatoxigenic isolates.

A. flavus (Samson *et al.*, 2004). Giorni *et al.* (2007) found that 70% of *A. flavus* isolated from maize in Italy were aflatoxigenic amongst which half were CPA producers. In addition, differences in AFs production by the toxigenic isolates may be in relation to the size of sclerotia (Criseo *et al.*, 2001). According to Hua (2003), all of the isolates producing sclerotia of type 'S' were aflatoxigenic, whereas isolates producing sclerotia of type 'L' were the most frequent and includes producers and non-producers of AFs.

The chemotype I (44.5%) and chemotype II (29.8%) were the majority strains. The isolates belonging to the chemotype V and VI with small 'S' sclerotia (<400 µm), were stronger aflatoxin producers than large 'L' sclerotia (>400 µm) isolates. The isolates of chemotype V could belong to the atypical *A. flavus*. However, the chemotype VI are related to *A. minisclerotigenes* or *A. parvisclerotigenus*. The type 'S' was rare (Giorni *et al.*, 2007) and encountered frequently in regions with high temperatures and low rainfall (Cardwell and Cotty, 2002). These authors suggest that the production of sclerotia of type 'S' is a form of adaptation to climatic fluctuations. The isolate belonging to the chemotype VII had distinctly darker green colonies and rough conidia, and produced AFB and AFG but not CPA; hence they may be related to *A. parasiticus*.

The results of recovery of aflatoxins are summarised in Table 3. The average recoveries were between 66.2 and 102.6%. 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 LOD and limit of quantification (LOQ) were determined by spiked spices samples with 5 µg/kg of AFB_1, based on signal-to-

Table 3. Recoveries of aflatoxin B_1 from spiked non-contaminated spices samples fortified with 5 and 20 µg/kg (n=3).

Spices	Spiking level (µg/kg)	Mean recovery (%) ± RSD (%)[1]
Aniseed	5	78.4±6.5
	20	81.1±12.5
Black pepper	5	72.1±19.0
	20	82.2±8.7
Caraway	5	75.7±9.1
	20	77.4±11.8
Cinnamon	5	71.6±9.5
	20	78.5±13.2
Coriander	5	81.6±8.6
	20	81.1±8.5
Cumin	5	70.1±6.8
	20	83.1±10.5
Ginger	5	76.4±9.1
	20	77.8±15.5
Red pepper	5	66.2±12.8
	20	68.3±9.5
Saffron	5	89.3±15.1
	20	102.6±8.5
Sweet cumin	5	92.6±13.6
	20	98.2±9.6
Sweet pepper	5	75.7±12.1
	20	84.1±11.5

[1] RSD = relative standard deviation.

noise ratio of 3:1 for the LOD and 10:1 for the LOQ. The LOD was established in 0.05 µg/kg. The LOQ was 0.1 µg/kg.

Aflatoxins content in spices

Of the 36 spices samples analysed by HPLC-FLD, 23 (63.9%) were contaminated with AFB_1 at concentrations ranging from 0.2 to 26.50 µg/kg (Table 4). The high levels of AFB_1 (26.50, 24.34, 19.07 and 14.65 µg/kg) were found in saffron and sweet cumin, respectively. These levels of AFB_1 are higher than the maximum limits set by Algerian regulations (10 µg/kg). In aniseed, black pepper, caraway, cinnamon, coriander, cumin, ginger, red pepper and sweet pepper, AFB_1 was detected with levels ranging from 0.10 to 3.44 µg/kg, lower than limit as recognised in Algeria (FAO, 2004). The occurrence of mycotoxins in spices differs geographically and depending on the climatic conditions. The presence of AFB_1 in widely varying amounts in spices has been reported by many authors (El Mahgubi *et al.*, 2013; Kong *et al.*, 2014; Ozbey and Kabak, 2012; Prelle *et al.*, 2014; Zinedine *et al.*, 2006).

Little data were reported about the contamination of saffron by mycotoxins. In Portugal, Martins *et al.* (2001) reported aflatoxin contamination of about 40% (2.0 to 2.75 µg/kg) in saffron. Aziz *et al.* (1998) reported the absence of aflatoxins in 5 samples analysed in India. The results reported in the literature concerning cumin are very different. Thus, contamination level of this spice in Morocco, a neighbouring country with the same climate as Algeria, is 57% (8 of 14 analysed samples) and up to 0.18 µg/kg (Zinedine *et al.*, 2006). In Portugal, AFs occurred in 42.9% of analysed cumin (1.25 to 2.3 µg/kg; Martins *et al.*, 2001). Kursun and Mutlu (2010) reported that AFs contamination varied from 4.55 to 8.57 µg/kg in cumin samples from Turkey. However, no AFs were detected in analysed cumin sampled in India (Aziz *et al.*, 1998), Ireland (O'Riordan and Wilkinson, 2008) and Turkey (Bircan, 2005). Furthermore, Bircan (2005) suggested that cumin is not suitable for AFs production. Additionally, Juglal *et al.* (2002) noted that the absence of AFs in the cumin and cinnamon may be due to inhibition of the aflatoxigenic fungi by essential oils and other aromatic substances produced by these plants.

Table 4. Occurrence of aflatoxin B_1 in spices samples (n=36) collected from markets in Algeria and analysed by high-performance liquid chromatography coupled with fluorescence detection.

Spices	Number of positive samples/total number of analysed samples	AFB_1 (µg/kg)[1]
Aniseed	3/4	0.14-0.66
Black pepper	0/2	ND
Caraway	2/2	0.10; 1.60
Cinnamon	1/2	0.20
Coriander	3/4	0.10-0.79
Cumin	0/2	ND
Ginger	4/4	0.10-2.60
Red pepper	2/4	0.19; 3.44
Saffron	2/4	24.34; 26.50
Sweet cumin	2/4	19.07; 14.65
Sweet pepper	4/4	0.10-3.17
Total	23/36	0.10-26.50

[1] The limit of detection was 0.05 µg/kg and the limit of quantification was 0.1 µg/kg; ND = not detected.

However, Madhyastha and Bhat (1985) reported that the red pepper and ginger are good substrates for growth and production of AFs. The screening for AFB_1 in black pepper, cumin and cinnamon proved negative. Our results are in accordance with those reported by many authors. For example, Elshafie *et al.* (2002) reported the contamination of 105 samples of spices (cumin, cinnamon, cloves, black pepper and turmeric) by *A. flavus*; however, AFs were not detected in the spices samples. Bartine and Tantawi-Elaraki (1997) found that growth of toxigenic strains of *A. flavus* was very low on black and white pepper, which was associated with the absence of AFB_1.

For black pepper, our results agree with those reported by Romagnoli *et al.* (2007) and Cho *et al.* (2008), who reported the absence of AFs in 11 and 2 samples analysed in Italy and Korea, respectively. In Turkey, Colak *et al.* (2006) reported contamination of 8.3% samples at 9.8-10.3 µg/kg. In Hungary, Fazekas *et al.* (2005) reported that 1/5 of samples were contaminated (0.46 µg/kg). In Morocco, 47% of the samples were contaminated (Zinedine *et al.*, 2006).

For red pepper, Sugita-Konishi *et al.* (2010) were found that only one sample of 6 (16.7 µg/kg) was contaminated with AFs in Japan. However, in Turkey, Aydin *et al.* (2007) reported high levels of AFB_1 contamination in red pepper with levels of contamination up to 40.9 µg/kg. Red pepper, in particular, appears to be quite a susceptible product for AF formation as a result of unsuitable processing conditions. Furthermore, few reports about ginger, sweet cumin and caraway contamination by mycotoxins were reported. For example, only AFB_1 were detected in ginger (0.18 µg/kg) (Cho *et al.*, 2008). However, 10/12 (86%) of analysed samples in Morocco were contaminated (0.63-3.50) (Zinedine *et al.*, 2006).

Concerning the cinnamon, several authors reported the absence of AF in this spice. For example, AFs were not found in any of cinnamon powder samples (n=17) in spices marketed in Turkey (Ozbey and Kabak, 2012). AFs were also not detected in cinnamon powder samples marketed in Japan (Hitokoto *et al.*, 1978), India (Saxena and Mehrotra,

1989), Bahrain (Musaiger *et al.*, 2008), Korea (Cho *et al.*, 2008) and Ireland (O'Riordan and Wilkinson, 2008). These results indicate that cinnamon is likely not to be a good substrate for growth of mycotoxin-producing fungi and mycotoxin accumulation.

Despite the high incidence of aflatoxigenic isolates, aniseed, caraway, coriander and ginger had a low content of AFB_1 (<3 μg/kg). The presence of mycotoxigenic fungi in food samples does not ultimately lead to the production of the respective mycotoxin. Many factors including storage and environmental conditions play a key role in the metabolism of secondary metabolites such as mycotoxins. This hypothesis may also explain in part no relationship between the incidence of mycotoxigenic isolates and the presence of the mycotoxins in a food as observed in our case. In this survey, a combination of mycological and AFs analysis were used to preliminarily assess the contamination of common spices marketed in Algeria. Our results show that there is a high risk potential for contamination of these products by aflatoxigenic fungi and AFB_1, especially in saffron and sweet cumin.

4. Conclusions

In conclusion, our study showed that all analysed spice samples were contaminated by moulds; the most contaminated spices were ginger, sweet pepper, red pepper, sweet cumin and saffron. Most of isolated fungal species belonged to *Aspergillus* sections *Flavi* and *Nigri*. A considerable variation in the production of AFs was observed among the tested isolates. Furthermore, the quantification of aflatoxins in spices samples showed a wide range of contamination by AFB_1. The present data suggest that some spices can represent a source of exposure to the carcinogenic mycotoxin, AFB_1. For these reasons, it is necessary to take measures to produce better quality spices. For example, the improvement of post-harvest procedures such as drying techniques and storage conditions could be useful to minimise fungal growth and prevent mycotoxin contamination. Packing can also enhance hygienic condition and participate to the supply of healthy spices.

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