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To cite this article: S. Barakat & K. M. Swaileh (2022) Fungal contamination, aflatoxigenic fungi and levels of aflatoxin B1 in spices marketed in the West Bank of Palestine, Food Additives & Contaminants: Part B, 15:4, 245-253, DOI: [10.1080/19393210.2022.2085330](https://doi.org/10.1080/19393210.2022.2085330)

To link to this article: <https://doi.org/10.1080/19393210.2022.2085330>



Published online: 06 Jun 2022.



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# Fungal contamination, aflatoxigenic fungi and levels of aflatoxin B1 in spices marketed in the West Bank of Palestine

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## ABSTRACT

Ninety-seven spices of seven different types were collected from different retailers in the West Bank of Palestine and were analysed for fungal contamination, specifically aflatoxigenic *Aspergillus* spp. and Aflatoxin B1 (AFB1) levels. *Aspergillus* was found in 89% samples analysed. Ground red chilli had the highest average number of fungal colonies. In decreasing order, mixed spices (57%), cardamom (53%), red chilli (52%), chicken spices (50%), sumac (47%) and pepper (38%), were contaminated with *Aspergillus* species. *Aspergillus niger* and *A. flavus* were dominating *Aspergillus* species in 37% and 23% of food samples analysed, respectively. Of the 11 tested isolates, 82% were identified as aflatoxin-producers. AFB1 was detected in 40.2% of the samples analysed with a mean value of  $2.09 \pm 3.20$  µg/kg. Red chilli powder followed by chicken spices and cardamom recorded the highest levels (6.98, 3.55 and 1.48 µg/kg, respectively). Twenty-two of the spices (23%), were above the European Union's maximum limit of 5 µg/kg.

## ARTICLE HISTORY

Received 5 March 2022  
Accepted 30 May 2022

## KEYWORDS

Spices; fungal contamination; *Aspergillus*; aflatoxin B1; ELISA; West Bank; Palestine

## Introduction

Being ubiquitous in a wide range of food and feedstuff, aflatoxins are among the major mycotoxins found in nature and among the most toxic natural products being produced by certain fungi of the genus “*Aspergillus*”. As secondary metabolites, aflatoxins are not needed for the normal growth of fungi nor for their function (Detroy et al. 1971). Hence, their production depends on environmental and developmental factors like moisture, light, temperature and pH (Georgianna et al. 2008). There are, at least, 16 types of aflatoxins, of which aflatoxins B1, B2, G1 and G2 are commonly found in contaminated food and feed, namely (Weidenborner 2001). Among the known species of aflatoxin-producing fungi, two species of the genus *Aspergillus*, section Flavi, are the most common. Producers of aflatoxins B1 and B2, which commonly occur in food samples include *A. flavus*, *A. parasiticus*, *A. nomius*, *A. arachidicola* and *A. minisclerotigenes* while those that commonly occur in food samples and produce aflatoxins G1 and G2 consist of *A. parasiticus*, *A. nomius*, *A. arachidicola* and *A. minisclerotigenes* (De Saeger 2011).

Due to its high toxicity, aflatoxin B1 (AFB1) is the most well-studied aflatoxin. It is the most widely distributed toxic aflatoxin and is considered the most potent natural carcinogen (Ellis et al. 1991). AFB1 was classified as a “group I carcinogen” by the International

Agency for Research on Cancer (IARC 2012). The toxicity spectrum of aflatoxins and mainly AFB1 is not only restricted to acute or even chronic carcinogenicity and hepatotoxicity but also expands to teratogenicity, genotoxicity and immunotoxicity causing a syndrome called aflatoxicosis (Groopman et al. 1988; Bondy and Pestka 2000). The maximum limits as reported by the Food and Agriculture Organization of the United Nations (FAO 2004) for aflatoxins contamination types AFB1, AFB2, AFG1 and AFG2 in consumer food products, namely, “nuts, peanuts, maize flour, figs and their products and other foods” should be no more than 5 µg/kg for AFB1 and 15 µg/kg for the sum of aflatoxins B1, B2, G1 and G2 (Vincenzi et al. 2011).

Worldwide (Goldblatt 1969), aflatoxin-producing fungi were recorded in a variety of foods including cereals (corn, rice, wheat, oat and barley), nuts (peanuts, pistachio, walnuts and hazelnuts) and spices (red chilli, black pepper, ginger, cardamom, saffron, cinnamon, thyme, anise). Hacibekiroglu and Kolak (2013) showed that sumac and cardamom were contaminated with aflatoxin. Tosun and Arslan (2013) showed that among the spices analysed ginger, sumac, black pepper and red pepper were contaminated. Jeswal and Kumar (2015) found red chilli, black pepper, turmeric, coriander, cumin, fennel, caraway, fenugreek and dry ginger all contaminated.

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South Asia and South-East Asia are considered areas with the highest occurrence of aflatoxins in foodstuff. Among 17,000 food samples analysed, 78% of the samples contained detectable amounts of mycotoxins (Schatzmayer and Streit 2013). The devastating outcome of economic losses due to mycotoxins affects approximately 25% of the World's food crops annually (Bryden 2007). On a global scale, risk assessment analysis has estimated that about 25% of the hepatocellular carcinoma cases could be attributed to aflatoxin exposure (Liu and Wu 2010). In 2004, at the rural areas of East Kenya, residents have grown maize contaminated with moulds that lead to the most recent outbreak of aflatoxicosis (Azziz-Baumgartner et al. 2005). In Malaysia, 95 packed food products, which included rice-based, wheat-based, corn-based, oats-based, oilseeds, nuts and spices were screened for AFB1 and 72.6% of the tested products were positive, with *A. flavus* being the dominant occurring species (Reddy et al. 2011). In Morocco, 55 samples of spices, including paprika, ginger, cumin and pepper were analysed for AFB1, with average contaminations of 2.88, 0.63, 0.03 and 0.09 µg/kg, respectively (Zinedine et al. 2006).

In Palestine, studies evaluating mycotoxins in foodstuff are limited and rather new, providing insufficient baseline data for the area and for a variety of food types. A recent study analysed 51 samples of rice, wheat flour and nuts, for the presence of aflatoxin and 45% of the samples tested positive, with means reaching 3.4, 2.8 and 4.5 µg/kg, respectively, all collected from the northern part of Palestine (Salman and Mudalal 2022). One study, in the form of an unpublished thesis, screened 50 samples of spices and medicinal herbs for aflatoxins (AlMosleh 2002). Results indicated that 48 of the samples contained aflatoxins, 13 of these even over 20 ppb. Therefore, the present study aims at analysing fungal and aflatoxin contamination in a variety of 7 spices marketed in the West Bank of Palestine.

## Materials and methods

### Sampling

A total of 97 samples (500 g/sample) of commonly consumed spices of 7 types were purchased randomly from markets in the West Bank of Palestine (Table 1). Each sample was obtained from a larger lot consisting of units weighing approximately 30 kg. Samples were placed in plastic bags and stored in a refrigerator at 5°C. Upon receipt and prior to analysis, samples were mixed well by inversion and one subsample was placed in a plastic bag for AFB1 analysis and a second subsample was placed in

**Table 1.** Fungal contamination of seven categories of spices collected from markets in the West Bank of Palestine.

Spices	No. of samples	Fungal colonies (CFUs/g)		
		Min	Max	Mean±SE
Cardamom	13	400	455,000	91,172 ± 62,235
Red Chilli	14	400	2,500,000	322,535 ± 201,055
Chicken spices	14	400	68,194	10,560 ± 4,492
Ginger	14	400	15,500	5,059 ± 1,448
Mixed spices	14	400	27,500	10,483 ± 4,117
Pepper	14	400	9,500	4,413 ± 1,147
Sumac	14	400	9,500	4,960 ± 1,947
Overall average				85,555 ± 46,462

a sterile resealable polybag for fungal assessment, stored refrigerated until analysis within 1 week to prevent any additional fungal contamination.

### Screening for fungal contamination

Samples were processed under standard aseptic conditions to prevent external contamination. Fungal isolation method was based on that of Reddy et al. (2009 and 2011). In sterile containers, samples were accurately weighed and thereafter suspended, as 5 g of homogenised solid sample, in peptone water of pH 7.2 (0.1%; Oxoid, Basingstoke, UK), adjusting the final volume to 50 mL with an initial dilution of 1:10. Thereafter, the mixture was shaken for 5 min using an Orbital Shaker (Marshall Scientific, Hampton, NH, USA) and allowed to settle for 2–3 min before the wash liquid was serially diluted in peptone water (0.1%) in preparation for plating on Rose Bengal Potato Dextrose Agar “R-PDA” (Sigma-Aldrich, St. Louis, MO, USA). Petri dishes containing the R-PDA were spread with 0.2 ml of each diluted sample suspension in duplicates. Blank negative controls were spread with sterile peptone water.

Cultures were incubated at 25 ± 2°C and examined at day 3 for fungal colony count before overgrowth of heavily contaminated samples. Cultures were left untouched or carefully handled to prevent overestimation of colony forming units (CFUs) due to the fragile nature of fungi capable of forming satellite colonies (Ganguli and Deshmukh 2007). After 7 days of incubation, the plates were examined using a standard colony counter (BOECO, Hamburg, Germany) for presence of yeasts and filamentous moulds. Countable plates of 8.5 cm Petri dishes, presenting 15 to 150 fungal colonies, were enumerated (Deak and Beuchat 1996). The results were expressed in colony forming unit per gram or millilitres of sample (CFU/g or/ml).

Visual examination took account of morphological characteristics of fungal colonies and microscopic features that were then identified using the available taxonomic keys (Larone 2002; Malloch 2017). Microscopic

images of fungi were prepared by the scotch tape method using lactophenol cotton blue (Larone 2002). The images were captured via Moticam X camera (Motic Instruments Inc., Richmond, BC, Canada).

### Screening for aflatoxigenic *Aspergillus* isolates

Green *Aspergillus* isolates from samples were recovered to obtain pure cultures by subculturing in Petri dishes containing PDA supplemented with chloramphenicol (0.025 mg/l) both supplied by Sigma Aldrich (St. Louis, MO, USA). After 7 days of incubation, cultures were examined for purity of isolate. The isolates were subcultured in separate sterile glass tubes containing PDA slanted agar for 7 days at a temperature of 25 °C in the dark, which were maintained in sterile mineral oil, tightly sealed with a screw cap under aseptic conditions and stored at – 20 °C.

Two media were used to identify aflatoxigenic isolates of *Aspergillus*; *Aspergillus* Differentiation Base Medium “ADBM” (HiMedia, Thane West – 400 604, India) and Coconut Agar Medium “CAM”. ADBM was used to differentiate aflatoxigenic *A. flavus* from other species of *Aspergillus* (Bothast and Fennell 1974; Salkin and Gordon 1975). ADBM media were prepared as specified by manufacturers and autoclaved at 121 °C for 15 min. Thereafter, media, supplemented with Chloramphenicol (0.025 mg/l), were poured in Petri dishes and left to cool down to room temperature before use.

The CAM media were prepared according to Davis et al. (1987) with a slight modification. “Briefly, 100 g of shredded coconut were placed in 300 ml of hot distilled water and homogenised for 5 min. The homogenate was filtered through four layers of cheesecloth and the pH of the clear filtrate was adjusted to pH 7 with 2N NaOH. Agar (0.2%) was prepared and autoclaved at 121 °C for 15 min. Chloramphenicol (0.025 mg/l) was added to the cooled media, which was poured into Petri dishes and left to cool down to room temperature before use.”

A loop of inoculum, mycelia and spore mass from the isolated *Aspergillus* subcultures were transferred, in duplicate, to freshly prepared ADBM and CAM under sterile conditions and incubated in a dark incubator at 28°C for 3 days. The plates containing ADBM were observed for the presence of an orange colour on the reverse of the colony, also named the Fluorescence-Based Method (FB method). Colonies plated on CAM were observed upside down on a UV transilluminator for fluorescence ability among isolates. In addition, the Ammonium Vapour Method (AV method) was employed by exposing the CAM cultures to ammonia vapour by adding one drop (300 µl) of ammonium hydroxide (25%; Sigma Aldrich,

St. Louis, MO, USA) according to Saito and Machida (1999). The formation of a pink colour on the reverse was recorded as positive for aflatoxin production.

### Quantitative assessment of aflatoxin B1

Aflatoxin B1 analysis of 97 samples was performed using a low matrix competitive AFB1 enzyme-linked immunosorbent assay (ELISA) kit (Helica Biosystems, Inc., Santa Ana, CA, USA). The manufacturer provided along with the antibody coated wells the following reagents: buffer, standard solutions, sample diluents, conjugate (Aflatoxin-HRP), substrate solution and stop solution. The pre-ground samples were weighed, 80% was passed through a 20 mesh (0.8 mm) screen and 20 g was weighed and placed in approximately 100 mL of 80% methanol (Sigma Aldrich, St. Louis, MO, USA). Thereafter, all samples were allowed to settle for 2–3 min before filtering the supernatant using a Whatman no. 1 fluted filter paper. Finally, 100 µL of the filtrate was diluted in 900 µL of the buffer. For ELISA, 100 µL of 6 standard solutions, of concentrations 0.02, 0.05, 0.1, 0.2 and 0.5ppm and 100 µL of prepared samples were added to the mixing wells containing 200 µL of sample diluents. Control samples (blanks) consisted of 100 µL sample diluents. After that, mixtures were transferred (in duplicates) to antibody coated wells and incubated for 30 mins. After automatically washing the wells three times with buffer (350 µL) using a microplate washer (Unilab, Ambala, India), 100 µL of conjugate (Aflatoxin-HRP) were added and further incubated in the dark for a further 30 min. The wells were then washed again three times, following addition of a substrate solution. The mixture was incubated at room temperature for 10 min. Finally, 100 µL stop solution was added and the colour intensity was read and recorded at an optical density of 450 nm using a Unilab microplate reader (Unilab, Fort Lauderdale, FL, USA). The limit of detection (LOD) of AFB1 as provided by the manufacturer of the ELISA kits was 0.14 µg/kg (0.28 ng/mL) and the limit of quantification (LOQ) was 0.43 µg/kg (0.86 ng/ml).

## Results and discussion

### Occurrence of fungi

Out of 97 samples, due to budget limitations only 64 non-specific sub-samples were assessed for fungal contamination, where all samples were contaminated with mould and/or yeast. Figure 1 shows a consortium of fungal species found in a sample.

Table 1 summarises the results of the fungal contamination of the 64 sub-samples. The average count of yeast and moulds in all spice samples analysed was 0.9



**Figure 1.** A 7-day old culture grown on R-PDA medium showing fungal diversity.

$\pm 0.5 \times 10^5$  CFUs/g. The highest CFU was recorded for the red chilli sample, with an average of  $3.2 \pm 2.0 \times 10^5$  CFUs/g, which may be due to possible pre-harvest, post-harvest and storage conditions. With regard to the average number of CFUs, spice types can be arranged as follows: red chilli > cardamom > chicken spices > mixed spices > ginger > sumac > pepper. Moreover, the results indicated that 16 samples out of 64 (25%) were beyond the maximum limits of  $1 \times 10^4$  CFUs/g set by the International Commission on Microbiological Specifications for Foods (ICSMF 1986).

In total, 7 genera of fungi were isolated using PDA medium and identified according to Malloch (2017) and Larone (2002) and any species, which were unknown using the basic guides for microscopic and macroscopic appearances were placed under an unidentifiable category. *Aspergillus* spp were the most common, contaminating and prevailing in 89% of the samples analysed and occurring 52% among species identified. Of the other identified species, *Rhizopus* spp., *Penicillium* spp., *Cladosporium* spp. and *Mucor* spp. occurred 12.2, 8.6, 7.2 and 4.1%, respectively. Rare species of *Trichoderma* was found in only one sample and compromised 0.5% of

the total fungal occurrence in samples. The remaining 15.0% was accounted for yeast and other species unidentifiable by general morphology.

By spice type, ground ginger (68%) was found to be the most frequently contaminated with *Aspergillus* species. In a decreasing order 57% of mixed spices, 53% of cardamom, 52% of red chilli, 50% of chicken spices, 47% of sumac and, 38% of pepper were contaminated with *Aspergillus* species (Table 2). *Aspergillus niger* and *A. flavus* were dominating *Aspergillus* species representing 37% and 23% of the identified isolates, respectively. Cardamom and pepper accounted for the highest number of fungal types (4.6 and 4 types of fungi/sample, respectively). This high average was followed by chicken spices, mixed spices, sumac, red chilli and ginger (3.6, 3.5, 3.4, 2.8 and 2.9 types of fungi/sample, respectively) (Table 3).

In a study that assessed 50 samples of 10 different spices from Saudi Arabia, 15 genera and 31 species of fungi were isolated with *Aspergillus*, *Penicillium* and *Fusarium* identified as the most common (Bokhari 2007). Jeswal and Kumar (2015) reported that in all spices from India assessed, *A. flavus* and *A. niger* were the most dominant.

### Recovery of green *Aspergillus* isolates

In the present study, 27 samples belonging to 7 different spice types were contaminated with green *Aspergillus* species (Table 3). These were mainly found in the red chilli powder and chicken spices constituting, respectively, about 30% and 26% of the total isolates recovered. In a study on Pakistani chilli samples, only *A. flavus* colonies were observed (Russell and Paterson 2007).

### Assessment of aflatoxigenicity

When found in suitable environmental conditions, *A. flavus* strains are able to produce aflatoxins and thrive at growing and sporulating (Reddy et al. 2009). The *A. flavus* isolates in this study, were analysed for aflatoxigenicity using ADBM and CAM medium, with the later medium utilised for the FB method and AV method. Of the green *Aspergillus* isolates, 11 were successfully

**Table 2.** Mean number of fungal types contaminating samples of a category of spices and percentages of infection (%) of different fungal types for each spice category.

Category spices (No. of samples as in Table 1)	Mean $\pm$ SD	<i>Aspergillus</i> spp	<i>Penicillium</i> spp	<i>Rhizopus</i> spp	<i>Cladosporium</i> spp	<i>Mucor</i> spp	<i>Trichoderma</i> spp	Yeast spp	Unidentified
Cardamom	4.6 $\pm$ 1.5	53	6	16	6	3	0	3	13
Chicken spices	3.6 $\pm$ 1.7	50	4	18	6	2	0	8	12
Chilli	2.9 $\pm$ 0.9	54	7	17	0	5	2	7	7
Ginger	2.8 $\pm$ 1.5	68	14	0	4	0	0	4	11
Mixed spices	3.5 $\pm$ 1.4	57	5	10	10	10	0	5	5
Pepper	4.0 $\pm$ 1.7	38	13	13	13	9	0	3	13
Sumac	3.4 $\pm$ 2.3	47	18	0	24	0	0	0	12

**Table 3.** Analytical results of green *Aspergillus* isolates in the spice samples.

Group No.	Sample type	No. of Sub-samples	No. of green <i>Aspergillus</i> isolates	Percentage of total isolates
1	Chicken spices	14	7	25.9
2	Red Chilli powder	14	8	29.6
3	Ground ginger	10	4	14.8
4	Mixed spices	6	1	3.7
5	Ground pepper	8	3	11.1
6	Cardamom	7	3	11.1
7	Sumac	5	1	3.7
	Total	64	27	100%

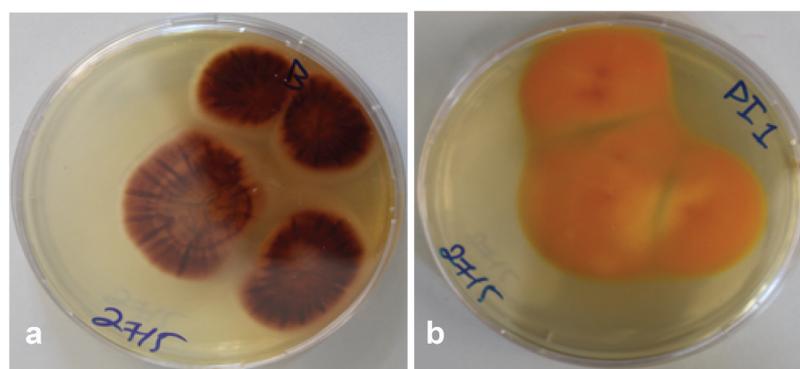
recovered and further processed to confirm identification of the aflatoxin-producing species by colony reverse colour formation on ADBM. This medium facilitates a rapid screening method to evaluate food spoilage and differentiate between *Aspergillus* species in general (Bothast and Fennell 1974; Beuchart 1984). Of the isolated species, 82% were identified with a bright orange reverse colour on the ADBM inferring the isolates being aflatoxic compared to the atoxic species as seen in Figure 2.

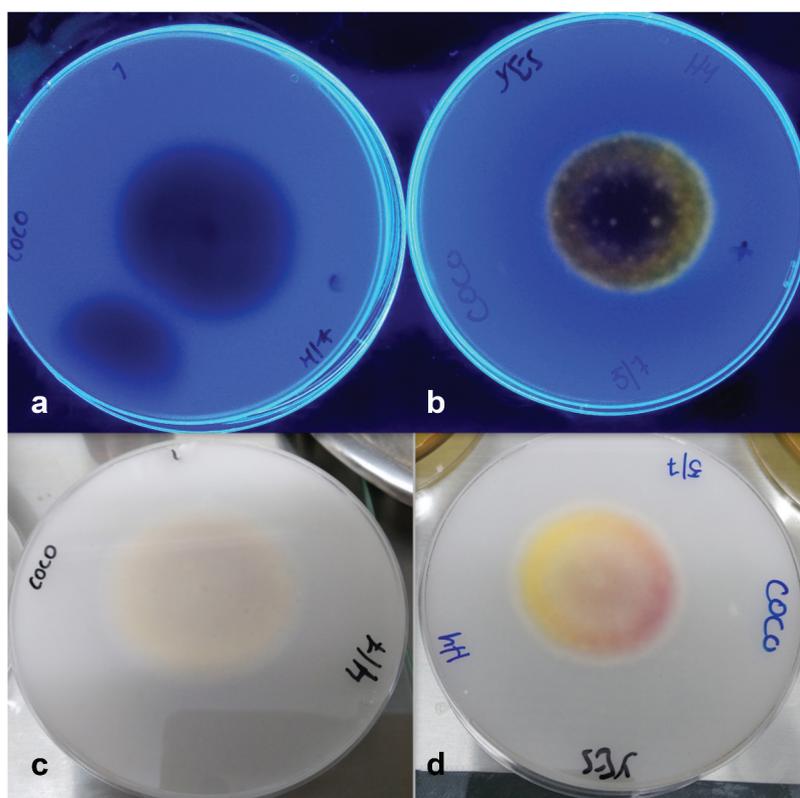
After growing isolates on CAM, colonies were exposed to UV light (365 nm) to distinguish the atoxic isolates (Figure 3a) from the aflatoxic isolates, as the latter displayed a characteristic beige ring around the colony (Figure 3b). Using the FB method, 7 isolates (64%) were found to produce aflatoxins (Table 4). In a third assessment, a characteristic pink colour displayed when aflatoxic strains were exposed to ammonium vapour (Figure 3c,d). When assessing toxigenicity of the 11 isolates by the AV method, 10 (91%) isolates were found to be toxic (Table 4). This overall percentage was similar to that obtained by ADBM results although some samples showed different results. Some isolates that showed negative toxigenicity by the FB method,

gave a weak pink colour in the AV method. Three isolates that showed negative or weak toxigenicity by the AV method gave a positive or negative result in the ADBM medium, showing the sensitivity of the ADBM medium to detect aflatoxicity.

The FB method on CAM was used as a preliminary step to differentiate between aflatoxic and atoxic strains that were capable of interfering with aflatoxin production when grown as co-inoculates in a culture (Degola et al. 2011). From 43 *A. flavus* isolates obtained from maize and soil, 23% were found to display fluorescence on CAM (Thathana et al. 2017). Another study showed that 65% of the *A. flavus* isolates from maize were aflatoxic through fluorescence on CAM (Okoth et al. 2012). In a study by Fani et al. (2014), which evaluated the toxigenicity of 524 isolates of *A. flavus*, false negatives reached 13 to 15% using the FB method on different media, in comparison to the AV method which gave no false negatives on CAM.

In the present study, the isolates found positive for aflatoxicity originated from red chilli, chicken spices, cardamom, ginger and mixed spices. Five (two sub-isolates from same sample) out of 11 (55%) of the aflatoxic species recovered from spices had shown positive results of AFB1 production with contents ranging between 1.7 and 13.5 µg/kg sample. Two red chilli powder samples, which contained the highest amount of AFB1, at levels of 12.5 and 13.5 µg/kg, evidently contained a toxic strain of *A. flavus*. These results support the notion of aflatoxic species being diverse in their ability to produce secondary metabolites (Frisvad and Larsen 2015). In addition, despite the low levels or even absence of AFB1 in the assessed food samples, the presence of the toxic strain alone sets a risk for further contamination at intolerable levels under conditions of improper food storage.

**Figure 2.** Two fungal strains grown on ADBM for 5 days. (a) Atoxic *Aspergillus* spp. And (b) Aflatoxic spp.; the orange pigmentation indicates aflatoxin production.



**Figure 3.** Aflatoxigenic assessment on CAM. (a) and (b) show the fluorescence based assay displaying the atoxigenic isolate (a) and the characteristic beige ring on the aflatoxigenic isolate (b). (c) and (d) represent the ammonia vapour test displaying the formation.

**Table 4.** Aflatoxigenic assessment of *A. flavus* isolates<sup>x</sup>.

Isolate	Food sample	ADBM	FB method	AV method
1	Cardamom 1	+	+	+
2	Cardamom 2	+	-	w
3	Chicken spices 1	-	-	w
4	Chicken spices 2	+	+	+
5	Red Chilli powder 1	+	-	+
6	Red Chilli powder 2	+	+	+
7	Ground ginger 1	+	+	+
8	Ground ginger 2	-	-	-
9	Ground ginger 3	+	+	+
10	Ground ginger 4	+	+	+
11	Mixed spices	+	+	+
Positive isolates (%)		81.8	63.6	90.9

<sup>x</sup>Isolates were assessed in duplicate. (-) negative for aflatoxin production, (+) positive for aflatoxin production and (w) weak aflatoxin production.

### Aflatoxin concentrations in spices

In this study 97 samples were screened for AFB1 contamination and the results of the positive samples including percentages, mean of AFB1 levels and the range of contamination for each spice type are presented in Table 5. From these, 39 (40.2%) were positive for AFB1 with concentrations ranging between 0.4 to 13.5 µg/kg and a mean value of  $2.09 \pm 3.20$  µg/kg. The standards precision was <10.0% (coefficient of variance). Variation of AFB1 levels were observed among the spices and spice

types. The highest concentration of AFB1 was found in chilli samples with a mean of  $6.98 \pm 4.09$  µg/kg ranging from 4.15 to 13.5 µg/kg. Chilli samples accounted for the highest number of contamination incidences, with 78.6% of the samples being contaminated. Chicken spices accounted for the second highest, ranging from 2.20–8.05 µg/kg with a mean of  $3.55 \pm 3.19$  µg/kg. About 23% of the positive samples had AFB1 levels above the EU maximum limit of 5 µg/kg. It is also worth noting that 18% of the contaminated spices assessed in the present study (data not presented), originated from the same local retailer, indicating poor handling, sanitation and prolonged storage of the food commodities from this unconventional retail store. Improper storage conditions and lengthened

**Table 5.** AFB1 levels (µg/kg) in the analysed spice samples.

Spices	No	Positives	Percentage	Mean± SD	Range
Cardamom	13	5	38.5	1.48 ± 2.44	0.65–6.20
Chicken spices	14	9	64.3	3.55 ± 3.19	2.20–8.05
Chilli	14	11	78.6	6.98 ± 4.09	4.15–13.5
Ginger	14	6	42.9	0.80 ± 1.48	0.40–5.25
Mixed spices	14	3	21.4	0.84 ± 2.02	1.05–6.90
Pepper	14	3	21.4	0.25 ± 0.57	0.70–2.00
Sumac	14	2	14.3	0.69 ± 1.90	3.05–6.65
Total	97	39	40.2	2.09 ± 3.20	0.40–13.50

exposure to dust and environmental pollutions are poor hygiene practices at typical open markets, causing contamination risks of food products.

In a study assessing spices, Zinedine et al. (2006) reported average contaminations of AFB1 in black pepper, ginger, red paprika and cumin were 0.09, 0.63, 2.88 and 0.03 µg/kg, respectively with the highest record owing to red paprika reaching 9.68 µg/kg. In an Irish study, a sample of chilli powder recorded the highest concentration of aflatoxin reaching 27.5 µg/kg (O’Riordan and Wilkinson 2008). In a study in Turkey, 58 of 93 organic spices were found positive for AFB1 with 41 samples found above the maximum limits of the EU (Tosun and Arslan 2013). In another study, 105 samples were analysed where red chilli flake, red chilli powder, black pepper and cumin samples were positive for aflatoxin, reaching percentages of 79.2, 63.6, 30.4 and 21.1%, respectively (Ozbey and Kabak 2012). Khazaeli et al. (2017) found that 30.8% of 120 analysed herb and spices samples were tainted with aflatoxins and at ranges between 0.7–57.5 µg/kg, fairly exceeding the EU maximum limit. These high levels may be due to the common practice of sun drying in post harvesting, where food commodities, especially chilli peppers, may be placed on a material distributed one layer above soil (Iqbal et al. 2011) increasing contamination risks.

## Conclusions

This study reveals the contamination levels of *Aspergillus spp.*; and AFB1 found in spices consumed by the average consumer in Palestine. Of the spices sampled, 89% were contaminated with *Aspergillus spp.* where 55% of the tested isolates were aflatoxigenic. Approximately 40% of the spices sampled contained AFB1 with 23% of those samples exceeding the maximum limits of the EU. These potential toxic levels and impending accumulation of aflatoxin assures the evident risk of an exponential increase in levels if no appropriate mitigation measures are considered.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

This work was supported by Birzeit University as a master thesis research for Sanade Barakat under the supervision of Prof. K. M. Swaileh.

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