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A B S T R A C T

Wheat and its derivatives are a very important staple food for North African populations. The aim of this study was to analyze populations of *Aspergillus* section *Flavi* from local wheat based on aflatoxins (AFs), cyclopiazonic acid (CPA) and sclerotia production, and also to evaluate AFs-contaminated wheat collected from two different climatic regions in Algeria. A total of 108 samples of wheat were collected during the following phases: pre-harvest, storage in silos and after processing. The results revealed that among the *Aspergillus* species isolated, those belonging to section *Flavi* were predominant. Of the 150 strains of *Aspergillus* section *Flavi* isolated, 144 were identified as *Aspergillus flavus* and 6 as *Aspergillus tamarii*. We showed that 72% and 10% of the *A. flavus* strains produced AFs and CPA, respectively. Among the 150 strains tested, 60 produced amounts of AFB1 ranging from 12.1 to 234.6 μg/g of CYA medium. Also, we showed that most strains produced large sclerotia. AFB1was detected by HPLC in 56.6% of the wheat samples and derived products (flour, semolina and bran) with contamination levels ranging from 0.13 to 37.42 μg/kg.

1. Introduction

Contamination of food and feed with mycotoxins represents a high risk for human and animal health. Mycotoxins can cause acute or chronic intoxication and damage to humans and animals after ingestion of contaminated food and feed (Moss, 1996). Furthermore, mycotoxins are responsible for generating huge economic losses in the producing countries (Bhat and Vasanthi, 2003). Pittet (1998) reported that 25–40% of cereals consumed in the world are contaminated by these toxic compounds. Mycotoxins are secondary metabolites produced by some species of mold genera such as *Aspergillus, Penicillium* and *Fusarium*, which enter the food chain in the field, during storage, or later, under favorable conditions of temperature and humidity.

Aflatoxins (AFs) are the most potent natural carcinogens known (JECFA, 1997), affecting animal species, including humans. Four aflatoxins are commonly produced in foods, aflatoxins B1, B2, G1 and G2. These mycotoxins are produced by *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius, Aspergillus pseudotamarii*, *Aspergillus bombycis*, *Aspergillus toxicarius*, *Aspergillus minisclerotigenes*, *Aspergillus parvisclerotigenus* and *Aspergillus arachidicola* in *Aspergillus* section *Flavi* (Samson et al., 2006; Pildain et al., 2008). The most important aflatoxin producers from a public health point of view are *A. flavus* and *A. parasiticus*. Aflatoxin B1 (AFB1) is often found at the highest concentrations in contaminated food and feed. The most pronounced contamination has been encountered in corn, peanuts, cottonseed and other grain crops (Gourama and Bullerman, 1995).

In North African countries, the foods most susceptible to aflatoxin contamination are locally produced or imported cereals such as wheat. This crop is a staple in dry Mediterranean regions of North Africa, where its consumption in the form of couscous, pasta, macaroni, spaghetti, bread, and frik is a cultural tradition. The mycobiota of wheat and wheat products was found to be dominated by *Aspergillus* section *Nigri* and *Flavi* species (Riba et al., 2008). However, up to now, there has been no systematic study on contamination by AF producing species either on the levels of AFs in wheat consumed in Algeria or on its derivatives. Therefore, the aim of our study was to identify and screen *Aspergillus* section *Flavi* isolates for AFs and CPA production, and to evaluate the rates of contamination with aflatoxins in wheat destined for human consumption.

Abbreviations: AFs, aflatoxins; AFB1, aflatoxin B1; AFPA, *Aspergillus flavus* parasiticus agar; CAM, coconut agar medium; CPA, cyclopiazonic acid; CYA, Czapek yeast extract agar; CZ, Czapek-Dox agar; DRBC, dichloran rose-bengal chloramphenicol agar; HPLC, high performance liquid chromatography; TLC, thin layer chromatography; MEA, malt extract agar; PDA, potato dextrose agar.

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2. Materials and methods

2.1. Study area

The samples were collected from Mitidja and Sétif, two representative regions of the climate of different wheat-producing regions of Algeria. In addition, more than 80% of Algerian wheat is produced in these two areas. Because of its proximity to the Mediterranean sea, Mitidja (latitude, 36°43′N; longitude 4,°03′E; altitude, 200 m) has a high mean annual rainfall (700 mm). By contrast, Sétif (latitude, 36°11'N; longitude, 5°25′E; altitude, 1081 m) has a much lower mean rainfall (400 mm). These two regions are characterized by a sub-humid and a semi-arid climate, respectively. All together, 108 samples of wheat and its derived products, destined for human consumption, were collected from these two regions: during the seasons of 2004 (85 samples) and 2006 (23 samples).

2.2. Sample collection

The samples were collected during the following phases of production: pre-harvest (field samples), storage in silos and during processing (in the form of unclean and clean wheat, flour, semolina and bran). The sample collection data are summarized in Table 1.

For the field (pre-harvest) wheat (variety Waha), 27 (seven samples from Mitidja region and 20 samples from Sétif region) and 23 samples (13 samples from Mitidja region and 10 samples from Sétif region) were collected at the maturity stage in July 2004 and July 2006, respectively. The samples were collected along the diagonals of six 1-hectare parcels. Each sample was composed of 40–50 ears, randomly collected from four to five sampling points which were approximately 15–20 m apart.

Thirty-four samples of durum wheat (varieties Waha and Vitron) were collected from silos. In February 2005, 10 and 14 samples of durum wheat were collected after 6 months of storage in silos from Mitidja and Sétif, respectively. Ten samples of wheat stored in silos for 12 months were collected from Mitidja in September 2004. The stored wheat was harvested by combine harvester in many local fields in July 2004. For each sample, a sub-sample of 300–400 g was taken through the "trench-type" silo in a transect at three levels (low, middle and high) and combined to give a sample of about 1 kg per bin (cylindrical bins of corrugated metal).

From flour and semolina mills, 24 samples (12 per mill) were collected during the mills' routine intake sampling procedure. From each mill, three samples of 1 kg each were taken at four levels along the production chain: soft wheat (variety HD1220), durum wheat (variety Waha) stored for 9 months in mill bins, clean wheat and products (flour, semolina and bran). The cleaning of wheat consists of eliminating impurities from the grain, hydrothermal treatment and grain sorting. At the moment of collection, the moisture content of durum wheat and soft wheat grain stored in a silo was 12% w/w, whereas that of the clean wheat, flour and semolina ranged from 13% to 14% w/w.

After collection in paper bags, the samples were ground to a fine powder using a Waring Blendor at high speed for a short period to avoid overheating of the samples. Aliquots of 100–200 g were used for the analysis of mycoflora, and the remaining was stored at −20 °C for the aflatoxins analysis.

2.3. Reagents

All reagents (potassium chloride, phosphoric acid, hydrochloric acid, ammonium hydroxide, β-cyclodextrin) were of PA grade. All organic solvents (methanol, acetonitrile, 2-propanol, *n*-hexane, chloroform and ethyl-acetate) were of HPLC grade. Deionized water was used for the preparation of all aqueous solutions and for HPLC. Standard toxins, aflatoxins (AFs) 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). All other solvents and reagents were of analytical grade purchased from Merck, Germany.

2.4. Fungal isolates and culture conditions

Dilution plating was used as the enumeration technique (Pitt and Hocking, 1997). Ten grams of each sample were added to 90 ml of 0.1% peptone dissolved in water. This mixture was then shaken on a rotary shaker for approximately 15 min and diluted 10^2, 10^3 and 10^4 fold. Aliquots consisting of 0.1 ml of each dilution were spread (in triplicate) on the surface of the Dichloran Rose-Bengal Chloramphenicol Agar medium (DRBC; King et al., 1979) which was composed of: glucose 10 g/L; peptone 5 g/L; K_2HPO_4 1 g/L; $MgSO_4{\cdot}7H_2O$ 0.5 g/L; agar 15 g/L; Rose Bengal 25 mg/L; dichloran (2,6 dichloro-4-nitroaniline) 2 mg/L and chloramphenicol 100 mg/L. All Petri-dishes were incubated for 3–7 days at 28 °C in the dark and under normal atmosphere. One of the three sets of dilutions averaging between 10 and 60 colonies per Petri-dish was selected for enumeration. The results were expressed as average cfu/g. Stock cultures were maintained on PDA tubes and then stored at 4 °C for subsequent characterization and taxonomic identification procedures.

Table 1
Distribution of *Aspergillus* and *A. flavus* isolated from samples of pre-harvest and stored wheat and its derivatives collected in 2004 and 2006.

Samples origin	Mitidja region				Sétif region			Flour mill (Soft wheat)				Semolina mill (Durum wheat)			
Samples source	Field[a]		Stored wheat		Field[a]		Stored wheat	Soft wheat	Clean wheat	Flour	Bran	Durum Wheat	Clean wheat	Semolina	Bran
	2004	2006	Silo 1[b]	Silo 2[c]	2004	2006	Silo 3[b]								
Number of samples	7	13	10	14	20	10	10	3	3	3	3	3	3	3	3
Number of *Aspergillus* ± SD	255 ± 42	75 ± 12	496 ± 146	902 ± 98	792 ± 243	89 ± 44	393 ± 97	429 ± 128	405 ± 144	261 ± 67	184 ± 86	630 ± 244	510 ± 98	171 ± 39	276 ± 76
(%)[c]	38	25.7	74	70.6	72	31.2	81.8	66	73.6	95	52.6	84	85	57	69
Number of *A. flavus* ± SD	46 ± 12	15 ± 2	174 ± 23	367 ± 76	204 ± 90	14 ± 5	126 ± 41	124 ± 8	99 ± 14	24 ± 7	15 ± 5	158 ± 32	143 ± 55	10 ± 2	4 ± 10
(%)[d]	18	20	35	40.7	25.7	16	32	29	24.5	9	8	25	28	6	16
(%)[e]	6.8	5.1	26	28.7	18.5	5	26.2	19.1	18.0	8.6	4.2	21.0	23.8	3.4	11.0

ND: not detected.
SD: standard deviation.
[a] Wheat grains freshly harvested in July 2004 and 2006.
[b] Wheat grains stored for 6 months in a silo (2004 harvest).
[c] Wheat grains stored for 12 months in a silo (2004 harvest).
[e] Calculated as a percentage of the total fungi.
[d] Calculated as a percentage of the total *Aspergillus*.

2.5. Morphological characterization of the isolates

The colonies of *Aspergillus* section *Flavi* from each PDA tube were sub-cultured on 9 cm diameter Petri dishes containing 20 ml of Malt Extract Agar (MEA) and Czapek-Dox agar (CZ) (per liter): sucrose 30 g, K_2HPO_4 1 g, $NaNO_3$ 2 g, KCl 0.5 g, $MgSO_4{\cdot}7H_2O$ 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 and Agar 20 g). Cultures were incubated for 7 days, in the dark, at 25 °C and then analyzed for colony color, presence and size of sclerotia, head seriation and conidial morphology. For micromorphological observations, the isolates were examined under the microscope (10×, 40× and 1000× magnification). Identification was performed according to the taxonomic keys and guides available for the *Aspergillus* genus (Pitt and Hocking, 1997; Klich, 2002). All isolates were also cultured on *Aspergillus flavus* parasiticus Agar (AFPA) for 3–5 days at 25 °C, in the dark, to confirm group identification by colony reverse color. All isolates were also cultured on CZ at 42 °C, and colony diameters were measured after 7 days of incubation (Ehrlich et al., 2007).

2.6. Mycotoxigenic ability of the isolates

2.6.1. Aflatoxins detection

2.6.1.1. Detection by fluorescence on coconut agar medium. A preliminary screen for aflatoxin production was performed on the basis of emission of blue fluorescence after UV light excitation at 365 nm after growth of the isolates on Coconut Agar Medium (CAM) supplemented with 0.3% β-cyclodextrin (Davis et al., 1987; Fente et al., 2001). One hundred grams of shredded coconut was homogenized for 5 min with 300 ml of hot distilled water. The homogenate was filtered through four layers of cheesecloth, and the clear filtrate was adjusted to pH 7.0 with 2 N NaOH. Agar was added (20 g/l), and the mixture was sterilized by autoclaving at 120 °C for 15 min. The isolates were inoculated by the application of a mass of conidia to the central point on a 60 mm diameter Petri dish containing 10 ml of CAM. The cultures were incubated at 28 °C for a period of 7 days, and for the appearance of colonies with brilliant orange-yellow reverse coloration under daylight and blue fluorescence under long wavelength (365 nm) UV light was periodically verified. A blank consisting of sterilized, non-inoculated CAM medium, incubated under the same conditions, was used as control.

2.6.1.2. Detection by TLC. Thin layer chromatography (TLC) was used as a screening method to identify the positive samples essentially as described by Calvo et al. (2004). For this, four cores (16-mm diameter) corresponding to 2 g of fungal biomass were collected from each replicate of CAM cultures and placed in a 50-ml Falcon tube. AFs were extracted from these samples by adding 5 ml of chloroform three consecutive times. Extracts were allowed to dry and then re-suspended in 500 μl of chloroform. A volume of 15 μl of each extract was applied on a silica gel G_{60} plate (20 × 20 cm, 0.25 mm thick, Merck 5721, Germany), along with standard AFs mixture (containing AFB1 and AFG1 at 0.5 μg/ml each and AFB2 and AFG2 at 0.15 μg/ml each). The plates were developed in a benzene/glacial acetic acid (95:5, v/v) solvent system. After development, the plates were dried and observed under short (254 nm) and long wavelengths (365 nm). The detection limit was 0.05 μg/g for all the AFs. The AFs were detected as an intense blue and green fluorescence spot for AFB and AFG, respectively, with the R_f values of 0.8, 0.6, 0.5 and 0.4 for AFB1, AFB2, AFG1 and AFG2, respectively.

2.6.1.3. Detection by HPLC. High performance liquid chromatography (HPLC) was used to confirm the identity of the AFs and to quantify them. AFs production by isolated fungal strains was determined using HPLC following the methodology described by Bragulat et al. (2001). After 7 days of growth of isolates on Czapek yeast extract agar (CYA) at 28 °C, three agar plugs (10 mm in diameter) were removed, with a cork borer, from the central area of each colony. Plugs were weighted and introduced into 3 ml vials and extracted with 1 ml of methanol for 1 h. The extracts were centrifuged at 13,000 rpm for 10 min at 4 °C and filtered through a 0.45 μm hydrophilic PVDF filter (Millipore).

The presence of aflatoxins was detected by high performance liquid chromatography (HPLC) using a post-column derivatization electrochemically generated bromine (Kobra cell) and a fluorescence detector (Spectra physic 2000) (λ = 362 nm excitation, λ = 435 nm emission). The HPLC column used was a reverse phase RP C18 ProntoSil analytical column (250 × 4 mm, 3 μm particle size) preceded by a C18 pre-column (Ultrasep 10 × 4 mm) and the flow rate was 0.5 ml/min. For post-column derivatization, 119 mg potassium bromide and 350 μl of 4 M nitric acid were added to 1 l of the mobile phase (20:20:60 (v/v) acetonitrile/methanol/water), as suggested in the Kobra cell instruction manual. The system was run isocratically, with a flow rate of 0.5 ml/min; the elution times for AFB1, AFB2, AFG1 and AFG2 were 30.2, 24.1, 22.5 and 21.4 min, respectively. The chromatograms were analyzed with Class-LC10 software version 1.6 (Shimadzu). The limit of detection was 0.005 μg/kg for AFB1 and AFG1, and 0.02 μg/kg for AFB2 and AFG2.

2.6.2. Cyclopiazonic acid detection

The isolates were tested for cyclopiazonic acid (CPA) production on CYA medium following the method described by Pildain et al. (2004). All strains were inoculated on 90 cm diameter Petri dishes and incubated at 28 °C. After 10 days, 2 g of fungal biomass was extracted by adding 5 ml of chloroform, three consecutive times. Extracts were allowed to dry and then re-suspended in 500 μl of methanol, and filtered through a 0.45 μm hydrophilic PVDF filter (Millipore).

Crude extract samples were applied to TLC on silica gel plates as above. 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 TLC. Twenty microliter of standard and each extract (re-dissolved in 500 μl of methanol) were applied to a silica gel TLC plate, 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 (40:30:20, v/v) (Fernandez Pinto et al., 2001). After pulverization of the plates with Ehrlich's reagent, the CPA was detected under daylight as an intense purple spot with an R_f of 0.5. The detection limit of the TLC technique was 1 μg/g.

2.7. Analysis of AFs in wheat and wheat products

2.7.1. Extraction of AFs

The extraction of AFs from wheat samples was performed according to El Adlouni et al. (2006). Briefly, 20 g of fine powdered samples were 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 No. 4 Whatman paper.

2.7.2. 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. To the lower phases, 50 ml deionized water and 100 ml chloroform were added. The mixture was shaken for 10 min. 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. To the pooled chloroform extracts, 50 ml of 5% sodium bicarbonate was added and shaken for 10 min. 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 method previously described (Section 2.6.1.).

2.7.3. Determination of the rate of AFB1 recovery

The rate of AFB1 recovery was determined by spiking an AFB1-free sample (50 g of ground wheat) with an equivalent of 0.5, 5.0, 10 and 20 μg/kg of AFB1, from a 10 μg/ml stock solution of AFB1 dissolved in methanol. Spiking was carried out in triplicates and a single analysis of a blank sample was also carried out. After allowing the methanol solvent to evaporate overnight, AFB1 was extracted as described above. The percentage of AFB1 recovery was calculated and taken into account for the determination of AFB1 levels in analyzed samples.

3. Results

3.1. Distribution of isolates of Aspergillus section Flavi

The fungal strains isolated from 108 samples (pre-harvest, in a state of storage and from flour and semolina mills) collected in 2004 and 2006 are shown in Table 1. The results revealed the dominance of *Aspergillus* species from all the samples analyzed with the mean percentage of 64.5%. Regarding *Aspergillus* section *Flavi* isolation, the mean percentage found was 22.5% of the total *Aspergillus* and 15.1% of the total fungi. The other species isolated belonged to the *Aspergillus* section *Nigri*, *Circumdati* and *Terrei*. Colonization of wheat by species belonging to *Aspergillus* section *Flavi* was higher in stored samples (26%, 26.2% and 28.7%) compared to the field samples (5%, 5.1%, 6.8% and 18.5%). The soft and durum wheat from mills contained levels of these species ranging from 3.4% to 23.8% with a mean of 13.6% of the total fungi. These species were isolated at a low frequency from wheat products (flour, semolina and bran).

3.2. Identification of strains of Aspergillus section Flavi

Among the total strains of *Aspergillus* section *Flavi* isolated from wheat and its derivatives (flour, semolina and bran), 150 were chosen for their AFs, CPA production and sclerotia characterization.

Aspergillus strains belonging to the section *Flavi* were identified preliminarily at the species level, based on morphological characteristics (mainly colony color on Czapek-Dox agar and conidia morphology). Of the 150 strains of *Aspergillus* section *Flavi* collected from wheat, 144 were identified as presumptive *A. flavus* and 6 as *Aspergillus tamarii*. The 144 isolates of *A. flavus* displayed typical morphological features of yellow-green colonies with smooth to finely rough globose conidia. The reverse sides of colonies of *A. flavus* strains were of a bright orange color on *Aspergillus flavus* parasiticus agar (AFPA) plates and could grow at 42 °C. By contrast, the 6 isolates identified as *A. tamarii* were of brown-to-dark-brown color and produced rough conidia. Moreover, colonies of *A. tamarii* could not grow at 42 °C.

3.3. Aflatoxin production by isolates of Aspergillus section Flavi

The capacity for producing AFs was determined for 150 isolates collected in 2004 and 2006 from wheat and its derivatives. Initially, aflatoxin production was screened on Coconut Agar Medium (CAM), and the results showed that 45 isolates (30%) were aflatoxigenic. HPLC analysis revealed that among the 150 strains tested, 108 (72%) were AFB1 producers. The results concerning the percentage of AFB1-producing ability of *Aspergillus* section *Flavi* strains on CYA medium are shown in Table 2.

Isolates of *Aspergillus* section *Flavi* were randomly classified into five groups according to their capacity for producing AFB1, which ranged from 0.02 to 234 μg/g of medium (Table 2). Twenty-three isolates (15%) presented high aflatoxigenic capacity with mean levels of AFB1 ranging from 103 to 234.6 μg/g, and 37 (25%) were moderately aflatoxigenic (12.1–95.7 μg/g of medium) as demonstrated by HPLC analysis. Furthermore, we observed that a total of 40 strains (27%) were able to produce small quantities of AFB1 in the 0.02–0.09 μg/g range. Similarly, we observed that the high producers of AFB1 (greater than 100 μg/g) were also AFB2 producers (4–125 μg/g). However, none of the 42 isolates (28%) produced AFB1 at the detectable limit (5 μg/g) and no AFG production was detected in any of the 150 isolates analyzed.

Table 2
Occurrence and AFB1-producing ability of 150 isolates of *Aspergillus* section *Flavi* isolated from samples of pre-harvest, stored wheat and its derivatives collected in 2004 and 2006.

AFB1 (μg/g)[a]	No. of strains	Percentage (%)
<0.005	42	28
0.005–0.1	40	27
0.11–10.0	8	5
10.1–100	37	25
>100	23	15

[a] The amounts of AFB1 were calculated after 7 days of growth on CYA at 28 °C and analyzed by HPLC.

Table 3
Chemotype patterns of *Aspergillus* section *Flavi* strains on aflatoxins, cyclopiazonic acid and sclerotia producing ability.

Chemotype	Mycotoxins			Sclerotia	No. of strains	Percentage (%)[b]
	AFB1	AFGI	CPA			
I	–	–	–	–	45	30
II	+	–	–	–	45	30
III	–	–	–	+	30	20
IV	+	–	–	+	15	10
V	+	–	+	+[a]	6	4
VI	+	–	+	–	3	2
VII	–	–	+	–	4	2.7
VIII	–	–	+	+	2	1.3

AFB1: aflatoxin B1; AFG1: aflatoxin G1; CPA: cyclopiazonic acid.
[a] Only one strain (chemotype V) was able to produce the S-type sclerotia.
[b] Percentage of the 150 isolates.

3.4. Identification of chemotypes in Aspergillus section Flavi

Based on mycotoxin production patterns (AFB1, AFG and CPA) and formation of sclerotia, the 150 strains were classified into eight chemotypes (Table 3). Chemotype I was represented by 45 isolates (30%) non-producing of any mycotoxins or sclerotia; chemotype II was represented by 45 isolates (30%), which produced only AFB1; chemotype III was represented by 30 isolates (20%), which only present sclerotia; chemotype IV was represented by 15 isolates (10%), which produced both AFB1 and sclerotia; chemotype V was represented by six isolates, which were able to produce AFB1, CPA and sclerotia, including one isolate which produced small sclerotia (<400 μm); chemotype VI was represented by three isolates, which produced both AFB1 and CPA; chemotype VII was represented by four isolates, which were able to produce only CPA; and chemotype VIII was represented by only two isolates, which produced CPA and sclerotia.

3.5. Aflatoxin content in wheat and wheat products

The incidence and level of AFB1 contamination in pre-harvest and stored wheat are summarized in Table 4. AFB1 was detected in 30 of the 53 samples examined (or an incidence of 56.6%). The incidence of contamination in 2004 and 2006 was 53.3% and 60.9%, respectively, with concentrations ranging from 0.13 to 37.42 μg/kg. The high level of AFB1 (37.4 μg/kg) was found in sample wheat stored for 12 months. In the processing chain, AFB1was detected only in bran of flour mill (3.37 μg/kg) and semolina (1.18 μg/kg). Of the 53 samples analyzed, five (9.4%) were above the legal limit established by EU regulations (5 μg/kg) (European commission, 2006) and two samples were above the legal limit as recognized in Algeria (10 μg/kg) (FAO, 2004).

4. Discussion

Wheat is one of the world's most important food crops. Foods made from wheat and its derivatives are a major part of a diet

Table 4
Incidence and range of AFB1 level in samples of pre-harvest and stored wheat collected from the Mitidja and Sétif regions (Algeria) in 2004 and 2006.

Samples origin	Mitidja region				Sétif region		
Samples source	Field		Stored wheat		Field		Stored wheat
Season	2004	2006	Silo 1	Silo 2	2004	2006	Silo 3
Number of samples analyzed	3	13	6[a]	5[a]	2[b]	10	6[a]
Number of positive samples	2	8	5	4	1	6	2
Amount of AFB1 in positive samples (μg/kg)	1.35; 3.41	0.22–13.96	0.31–4.62	1.69–37.42	0.87	0.21–7.0	0.13; 0.44

[a] Each sample represents one bin of silo.
[b] Each sample represents the 10 pooled sub-samples collected in one land parcel.

for over a third of the world's people. In Algeria, climatic conditions characterized by high humidity and temperature and inadequate storage practices contribute to the potential for significant exposure of the Algerian population to AFs. In our previous study (Riba et al., 2008), species of *Fusarium*, *Penicillium*, *Alternaria* and *Mucor* and especially *Aspergillus* (belonging to section *Flavi* and *Nigri*) were the major fungal species most commonly isolated from Algerian wheat. The presently available data confirm that the genus *Aspergillus* displays a worldwide distribution, particularly in subtropical and warm temperate regions such as North Africa (Hocking and Pitt, 2003).

Among all the strains screened, we could not find any AFG producer. This observation suggests the absence of AFG-producing species (*A. parasiticus*, *A. nomius*, *A. bombycis*, *A. toxicarius*, and *A. arachidicola*) in the analyzed samples. On the other hand, Ehrlich et al. (2007) reported that *A. pseudotamarii* is characterized by a brown to dark-brown color, and is not able to grow at 42 °C. However, our results showed that all the AF-producers have yellow-green colonies and are able to grow at 42 °C. This observation suggests the absence of this species in our samples. In our study, the one strain producing small sclerotia produce only AFB and CPA. This strain does not obviously belong to *A. minisclerotigenes* or to *A. parvisclerotigenus*, which produce both AFB and AFG (Pildain et al., 2008). Thus, we can conclude that *A. flavus* is the only aflatoxigenic fungus in Algerian wheat. It is known that *A. flavus* strains are commonly associated with warmer geographical regions. Indeed, several studies reported the predominance of this species in wheat samples originating from Argentina (Vaamonde et al., 2003), Australia (Berghofer et al., 2003), Egypt (Abdel Hafez et al., 1990; Mazen et al., 1984), Iran (Ghiasian et al., 2004), and Turkey (Baydar et al., 2005). In addition, although aflatoxin-production ability has been detected in a variety of species of the *Aspergillus* genus, inside and outside the *Flavi* section, *A. flavus* and *A. parasiticus* remain the most important and representative AF-producers occurring naturally in food commodities.

Of the 150 strains examined, 108 (72%) produced AFB1 in amounts ranging from 0.02 to 234.6 μg/g of CYA medium. 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). For example, the incidence of aflatoxigenic *A. flavus* strains was higher in peanuts (69%) than in wheat (13%) (Vaamonde et al., 2003). In addition, among *Aspergillus* isolates, there is great variation in aflatoxin production especially within the most common aflatoxin-producing species, *A. flavus* (Abbas et al., 2005).

Our strains were classified into eight different chemotypes, based on patterns of mycotoxin (AFB1, AFG and CPA) and sclerotial production. The results obtained demonstrate a great variability in the AFB and CPA-producing potential of *A. flavus*. According to Pitt (1993), *A. flavus* isolates produce AFB1 and AFB2, CPA alone, AFB1, AFB2 and CPA or neither. Our results showed that only 10% of isolates are able to produce CPA. We observed that only 9 isolates (6%) of *A. flavus* were able to simultaneously produce AFB1 and CPA. In contrast, Frisvad et al. (2005) reported that production of CPA is correlated with AFB1 production. Giorni et al. (2007) showed that 70% of *A. flavus* isolated from Italian maize are producers of AFs, and that half of them are CPA producers.

Our results showed that only 21 aflatoxigenic strains (14%) are able to produce sclerotia (chemotypes IV and V). Several authors suggested that the size and formation of sclerotia are correlated to the aflatoxigenicity of isolates (Cotty, 1989; Criseo et al., 2001). It is currently known that all S-type strains, with small sclerotia (<400 μm in diameter) are aflatoxigenic. However, L-type strains, with larger sclerotia (>400 μm in diameter), usually include both aflatoxigenic and non-aflatoxigenic strains. Many authors reported that the S-type strains are rarely isolated (Vaamonde et al., 2003; Barros et al., 2005; Giorni et al., 2007) and are usually obtained from geographical regions characterized by high temperatures and low rainfall (Cardwell and Cotty, 2002).

Of the 53 samples of wheat and wheat products analyzed, 57.1% were contaminated by AFB1 and two samples were not safe for human consumption according to the national limits (10 μg/kg). Our results are in line with those reported by Perenzin et al. (2001) who found that 62% of wheat samples collected from experimental field plots in northern Italy (Lombardy) were contaminated by aflatoxins. In contrast, Jiménez and Mateo (2001) reported that although aflatoxigenic fungi were found at high levels in wheat, no aflatoxins were found in 165 samples collected from markets in Spain. Although the number of samples analyzed was limited, our results revealed a relatively high contamination of wheat grain comparing to wheat products. Therefore, it can be hypothesized that during processing, the quantity of mycotoxins is reduced as observed for the species belonging to *Aspergillus* section *Flavi*. Zinedine et al. (2007) reported that the incidence of AFB1 in wheat flour commercialized in Morocco was about 17.6%, and that levels of contamination ranged from 0.03 to 0.15 μg/kg. According to Behfar et al. (2008), none of 32 wheat flour samples was contaminated by aflatoxins. Some food processing methods have been shown to result in reduction or elimination of aflatoxins (Murphy et al. 2006). These results reveal the widespread occurrence of aflatoxigenic strains of *A. flavus* in Algeria, and highlight the importance of the post-harvest care of grains. Thus, whenever there is a problem in the storage or in the processing of wheat and wheat-based feeds that allows fungal growth, the risk of mycotoxin contamination should be taken into account. More investigations on levels of AFs in different food products are necessary to provide data on the exposure of the Algerian population to AFs.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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