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Review

Occurrence and legislation of mycotoxins in food and feed from Morocco

Abdellah Zinedine [a,*], Jordi Mañes [b]

[a] Laboratory of Food Toxicology, National Institute of Health (INH), 27 Avenue Ibn Battouta, P.O. Box 769, Rabat-Agdal, Morocco
[b] Laboratory of Toxicology and Food Chemistry, Faculty of Pharmacy, University of Valencia, Avenue Vicent Andrés Estellés, s/n, 46100 Burjassot, Spain

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ABSTRACT

Mycotoxins are natural food and feed contaminants, mainly produced by moulds of genera *Aspergillus*, *Penicillium* and *Fusarium*. The number of mycotoxins known to exert toxic effect on human and animal health is constantly increasing as well as the legislative provisions taken to control their presence in food and feed. Morocco, a North African country, surrounded by the Mediterranean Sea and Atlantic Ocean, has a climate characterized by high humidity and high temperature which favor growth of moulds. This paper gives an overview about the contamination levels and the occurrence of some mycotoxins (e.g. aflatoxins, ochratoxin A, and *Fusarium* toxins) in cereals, bread, milk, spices, wine, olives, poultry feeds, dried fruits and nuts; the average of contaminated samples was often above 50%. A section on mycotoxin regulations by Moroccan authorities is discussed with a comparison with international and European limits. Recent data about the contamination of foods and feed from Morocco by mycotoxins are considered in this review. Finally, the paper gives a last part with conclusions and principal prospectives and recommendations that should be undertaken by authorities and scientists during monitoring of mycotoxins in food and feed produced and/or commercialized in Morocco.

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* Corresponding author. Tel.: +212 37771902; fax: +212 37772067.
E-mail address: zinedineab@yahoo.fr (A. Zinedine).

1. Introduction

Mycotoxins are natural food and feed contaminants, mainly produced by moulds of genera *Aspergillus, Penicillium* and *Fusarium*. The number of mycotoxins known to exert toxic effect on human and animal health is constantly increasing as well as the legislative provisions taken to control their presence in food and feed (Zinedine et al., 2006). Extensively considered mycotoxins are *Aspergillus* toxins (aflatoxins and ochratoxin A) and *Fusarium* toxins.

Morocco, a North African country, surrounded by the Mediterranean Sea and Atlantic Ocean, with about 4500 km of coasts, has a climate characterized by high humidity and high temperature which favor growth of moulds. Seventy percent of total population of the country lives in coastal cities. High contamination of the raw material and the non controlled conditions of processing are existing problems. Many kinds of food are still home made. A risk management program such as the HACCP system is not yet applied in most of the food processing unit. Mycotoxin problems associated with food have not yet been deeply studied for acquiring the necessary information. Regulatory issues are not available in the field of food exhibition and retailing. These factors and others which cannot be known because of the lack of studies and investigations in Morocco are causing many food-borne illnesses which cannot be identified and treated by health instructors or providers. According to Pr Benazzouz (Ibn Sina Hospital of Rabat, personnel communication), the prevalence of liver carcinoma in Moroccan patients is constantly increasing as well as the diseases with unknown etiology. Indeed, serological analysis reported that about 10% of confirmed patients with a liver carcinoma are not infected by B and C hepatitis Virus.

Mycotoxins usually enter the body via ingestion of contaminated foods, but inhalation of toxigenic spores and direct dermal contact are also important routes. Currently, more than 400 mycotoxins are identified in the world. Considering their heat stability, these substances constitute a potential risk for human and animal health. The chemical and biological properties of the mycotoxins are varied and their toxic effects are extremely variable. These effects are carcinogecity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity and immunotoxicity. Mycotoxins are not only dangerous for the health of consumers, but they deteriorate also the marketable quality of the contaminated products, thus involving strong economic losses.

Even if, since 1945, Ninard and Hinterman (when they were working at the Institute of Hygiene of Morocco) observed acute intoxications of pigs with mouldy feeds (Lafont, 1970), little investigations were undertaken to evaluate the contamination of foodstuffs by toxigenic fungi and also little information are available in this field. Indeed, the first preliminary studies were reported from the Agronomy and Veterinary Hassan II institute during the 1990th, where some Moroccan agricultural products including cereals were found contaminated by AFB1 and OTA (Tantaoui-Elaraki, Benabdellah, Majdi, Elalaoui, & Dahmani, 1994) using Thin Layer Chromatography technique. Recently, a main effort has been

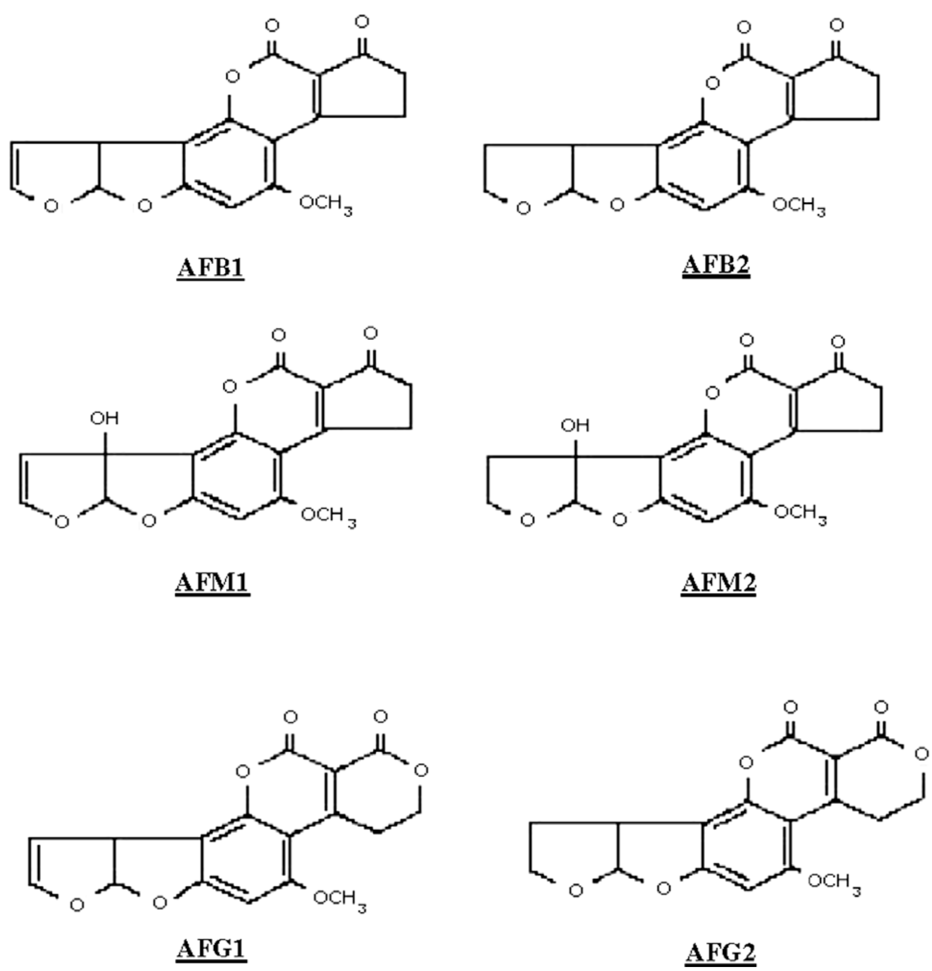

Fig. 1. Structure of aflatoxins.

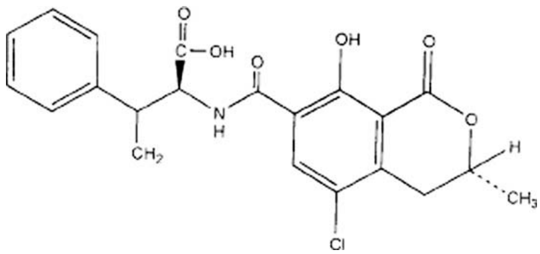

Fig. 2. Structure of OTA.

deployed by Moroccan scientists with the aim to assess the knowledge on the contamination of foodstuffs marketed in the country by mycotoxins, to compare the results found with data already published in some countries from the Mediterranean area and finally to study thereafter the potential impact of the contamination by evaluating of the human exposure to these toxins. The purpose of this paper is to give a general review of the principal researches carried out on the occurrence of mycotoxins in food available in Morocco, while insisting on the experience of the Laboratory of Food Toxicology of the National Institute of Health (INH) as the official central reference laboratory of the Ministry of Health for the control of toxic substances in food and the environment.

2. Aflatoxins

Aflatoxins (AFs, Fig. 1) are widely distributed toxins produced by strains of *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Kurtzman, Horn, & Hesseltine, 1987). *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins (Creppy, 2002). AFs are of great concern because of their detrimental effects on the health of humans and animals, including carcinogenic, mutagenic, teratogenic and immunosuppressive effects (Eaton & Gallagher, 1994). Aflatoxin B1 (AFB1) is the most potent hepatocarcinogen known in mammals and is classified by the International Agency of Research on Cancer as Group 1 carcinogen (IARC, 1993). AFs outbreaks affecting a large geographical area and causing over 123 deaths were reported in Kenya in 2004 and 2005. Epidemiological studies from this case showed a relationship between the outbreak and the local methods of harvesting, storing and preparing maize. Contamination of maize with AFs was found up to 1000 μg/kg (Centers for Disease Control, 2004).

Aflatoxin M1 (AFM1) is the main monohydroxylated derivative of AFB1 forming in liver by means of cytochrome P450-associated enzymes. AFM1 is classified as a Group 1 like carcinogenic to humans (IARC, 2002). Since milk is a major commodity for introducing AFs in the human diet, evidence of hazardous human exposure to AFM1 through dairy products has been shown by several studies. The maximum limits fixed by the Moroccan regulations project in liquid milk and milk for children (under 3 years) for AFM1 are 0.05 μg/L and 0.03 μg/kg, respectively (Food, 2004).

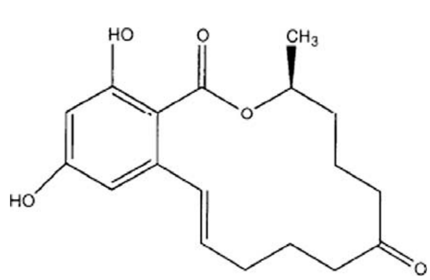

Fig. 3. Structure of ZEA.

3. Ochratoxin A

Ochratoxin A (OTA, Fig. 2), chemically known as N-{[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl]-carbonyl}-3-phenyl-L-alanine, is a mycotoxin described the first time by Van der Merwe, Steyne, Fourie, Scott, & Theron, 1965. Abarca, Accensi, Bragulat, Castella, and Cabañes (2003) reported that OTA is an ubiquitous secondary fungal metabolite primarily produced by the genera of *Aspergillus* (e.g., *A. ochraceus*) and *Penicillium* (e.g., *P. verrucosum*). OTA has been widely detected in cereals (barley, wheat, maize, oat) (Speijers & Van Egmond, 1993; Trucksess, Giler, Young, White, & Page, 1999), green coffee (Leoni, Valente-Soares, & Oliveira, 2000), grape juice (Zimmerli & Dick, 1995), and wine (Filali et al., 2001; Miraglia & Brera, 2002).

OTA has been implicated in a human disease of kidney referred to as Balkan endemic nephropathy, characterized by tubule interstitial nephritis and associated with high incidence of kidney, pelvis, ureter and urinary bladder tumors in some Eastern European countries (Pfohl-Leszkowicz, Petkova-Bocharova, Chernozemsky, & Castegnaro, 2002). OTA has been classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer (IARC, 1993). The Joint Committee FAO/WHO of Experts on Food Additives (JECFA) has established the provisional tolerable weekly intake (PTWI) of OTA at 100 ng/kg of body weight (bw) corresponding to approximately 14 ng/kg bw/day (JECFA-Joint FAO/WHO Expert Committee on Food Additives, 2001). Recently, the European Food Safety Authority (EFSA) has proposed a new safety value of 120 ng OTA/kg bw as a Tolerable Weekly Intake, which corresponds to a TDI of 17.1 ng/kg bw (European Food Safety Authority-European Food Safety Authority-European Food Safety Authority-European Food Safety Authority-EFSA, 2006).

In North African countries the most suspected foods susceptible to be contaminated by OTA are domestic and imported cereals such as wheat and sorghum, olives, poultry products, and spices (Grosso et al. 2003). Data published suggest the evidence association of elevated exposure to OTA with cases of human nephropathies in Tunisia and Egypt (Maaroufi et al., 1995; Wafa et al., 1998). In Morocco, about 2 million of people suffer from chronic diseases of kidney including chronic renal insufficiency and chronic interstitial nephropathy especially in young from both sexes. However the etiology of the diseases is not well established. A preliminary survey reported that the Moroccan population could be exposed to OTA (Filali et al., 2002). Indeed, 60% of the Moroccan human plasma sampled was positive for OTA (61.5% in the male and 56% in the female population), and an average concentration of 0.29 ng/mL (0.31 ng/mL in males, 0.26 ng/mL in females).

4. Zearalenone

Zearalenone (ZEA, previously known as F-2 toxin, Fig. 3) is a nonsteroidal oestrogenic mycotoxin biosynthesized through a polyketide pathway by a variety of *Fusarium* fungi in temperate and warm countries (Zinedine, Soriano, Moltó, & Mañes, 2007a). Fungi-producing ZEA contaminate corn and also colonize, to a lesser extent, barley, oats, wheat, sorghum, millet and rice.

ZEA is of a relatively low acute toxicity after oral administration in mice, rats and guinea pigs. Previous studies have demonstrated the potential for ZEA to stimulate growth of human breast cancer cells containing estrogen response receptors (Ahamed, Foster, Bukovsky, & Wimalasena, 2001; Yu, Zhang, Wu, & Liu, 2005). ZEA has been shown to be immunotoxic and genotoxic, and to induce DNA-adduct formation *in vitro* cultures of bovine lymphocytes (Lioi, Santoro, Barbieri, Salzano, & Ursini, 2004), DNA fragmentation and

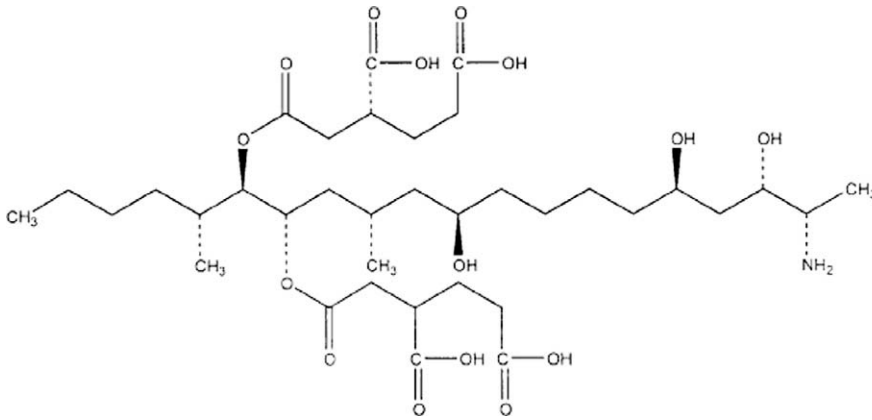

Fig. 4. Structure of FB1.

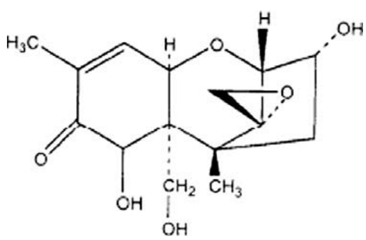

Fig. 5. Structure of DON.

micronuclei production in cultured DOK, Vero and Caco-2 cells (Abid-Essefi et al., 2003; Abid-Essefi et al., 2004), in Vero monkey kidney cells and in bone marrow cells of mice (Ouanes et al., 2003).

5. Fumonisisns

FB were first described and characterized in 1988 (Gelderblom et al., 1988). FB1 (Fig. 4) is the diester of propane-1,2,3-tricarboxylic acid. FB1 is the most toxic and has been shown to promote tumours in rats (Abel & Gelderblom, 1998) and cause equine leukoencephalomalacia (Marasas et al., 1988), porcine pulmonary oedema (Harrison, Colvin, Greene, Newman, & Cole, 1990), nephrotoxicity and liver cancer in rats, atherogenic effects in vervet monkey, medial hypertrophy of pulmonary arteries in swine, atherosclerosis in monkeys, immunosuppression in poultry, and brain haemorrhage in rabbits (Soriano, González, & Catalá, 2005).

Bhat, Shetty, Amruth, and Sudershan (1997) reported a possible case of acute exposure to FB1 involved 27 villages in India, where consumption of unleavened bread made from moldy sorghum or corn, containing up to 64 mg/kg FB1, was associated with an outbreak of human disease in India involving gastrointestinal symptoms (transient abdominal pain, borborygmus, and diarrhea). Fumonisins are possibly carcinogenic to humans and according to the International Agency for Research on Cancer, they are class 2B carcinogens (WHO-IARC, 1993). A provisional maximum for tolerable daily intake (PMTDI) is fixed for fumonisins B1, B2 and B3 alone or in combination, of 2 μg/kg bw/day on the basis of the NOEL of 0.2 mg/kg bw/day and a safety factor of 100 (Creppy, 2002).

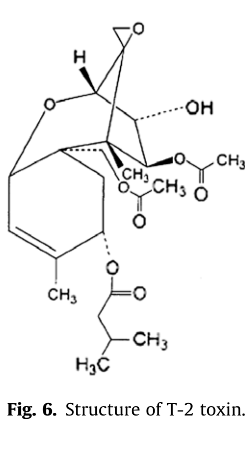

Fig. 6. Structure of T-2 toxin.

6. Trichothecens

The term trichothecene is derived from trichothecin, which was the one of the first members of the family identified. All trichothecenes contain a common 12,13-epoxytrichothene skeleton and an olefinic bond with various side chain substitutions (Bennett & klich, 2003). The trichothecenes constitute a family of more than sixty sesquiterpenoid metabolites produced by a number of fungal genera, including *Fusarium, Myrothecium, Phomopsis, Stachybotrys, Trichoderma* and *Trichothecium.*

Deoxynivalenol (DON, Fig. 5), also called vomitoxin, is a mycotoxin of the type B trichothecenes, which are epoxy-sesquiterpenoids. DON occurs predominantly in grains such as wheat, barley, and maize and less often in oats, rice, rye and sorghum. DON may have adverse health effects after acute, short-term, or long-term administration. After acute administration, DON produces two characteristic toxicological effects: decrease in feed consumption (anorexia) and emesis (vomiting). Although less toxic than many other major trichothecenes, the toxin DON is the most prevalent and is commonly found in barley, corn, rye, safflower seeds, wheat, and mixed feeds. The NOEL is 0.1 mg/kg bw/day (Creppy, 2002).

T-2 and HT-2 toxins are type A trichothecenes, which are closely related epoxy, sesquiterpenoids. Surveys have revealed the presence of T-2 and HT-2 toxins in grains such as wheat, maize, oats, barley, rice, beans, and soya beans as well as in some cereal-based products. T-2 and HT-2 toxins have been reported to be produced by *Fusarium sporotrichioides, Fusarium poae, Fusarium equiseti,* and *Fusarium acuminatum* (Creppy, 2002).

Outbreaks of acute poisoning in which the effects reported included nausea, vomiting, pharyngeal irritation, abdominal pain and distension, diarrhoea, bloody stools, dizziness and chills were reported. It has been postulated that T-2 toxin (Fig. 6) is associated with a human disease called alimentary toxic aleukia (ATA) that affected a large population in the Orenburg district of the former USSR during the 2nd World War. The symptoms of the disease include inflammation of the skin, vomiting, and damage to hematopoietic tissues. The acute phase is accompanied by necrosis in the oral cavity, bleeding from the nose, mouth, and vagina, and central nervous system disorders (Bennett & Klich, 2003).

7. Mycotoxin regulation

Mycotoxin-producing mold species are extremely common, and they can grow on a wide range of substrates under a wide range of environmental conditions. Mycotoxins can enter the food chain in the field, during storage, or at later points. Mycotoxin problems are exacerbated whenever shipping, handling, and storage practices are conducive to mold growth. The end result is that mycotoxins are commonly found in foods. Several authors classified mycotoxins as the most important chronic dietary risk factor, higher than synthetic contaminants, plant toxins, food additives, or pesticide residues. The economic consequences of mycotoxin contamination were demonstrated.

Since the discovery of the aflatoxins in 1960, regulations have been established in many countries to protect consumers from the harmful effects of mycotoxins that may contaminate foodstuffs, as well as to ensure fair practices in food trade. Various factors play a role in decision-making processes focused on setting limits for mycotoxins. These include scientific factors to assess risk (such as the availability of toxicological data), food consumption data, detailed knowledge about possibilities for sampling and analysis, and socio-economic issues (Van Egmond, Schothorst, & Jonker, 2007).

Since 1974, several countries have established or proposed maximum limits of mycotoxins in foods. In March 1999, the Food and Agriculture Organization organized with the collaboration of the World Health Organization, the 3rd international conference on mycotoxins. The conference was organized to sensitize authorities to the potential risk of contamination on human and animal health, and the economic effects of the contamination, to promote the harmonization of regulations and to recommend strategies for the prevention of the contamination by mycotoxins. Several national and international organizations and agencies have special committees and commissions that set recommended guidelines, develop standardized assay protocols, and maintain up to date information on regulatory statutes.

In Africa, fifteen countries were known to have specific mycotoxin regulations. These countries cover approximately 59% of the inhabitants of the continent. For the majority of the African countries, specific mycotoxin regulations (probably) do not exist. The fact that countries have no specific regulatory limit for mycotoxins does not mean that the problem is ignored. Several of these countries recognize that they have problems due to mycotoxins and that regulations should be adopted (Food, 2004).

In Morocco, no mycotoxins regulations in food and feed are still in force. However, a mycotoxin regulation project was prepared by the interdepartmental committee for food control and the repression of frauds (CIPCARF). This project is not yet adopted. This project envisages the regulations of mineral and organic contaminants in food and feed and set maximum permissible limits of mycotoxins in certain food products intended for human and animal consumption. The proposed limits for cereals intended for human consumption are 5, 30, and 200 μg/kg for AFB1, OTA, and ZEA, respectively; for milk and dairy products, the proposed limits are 0.05 and 0.03 μg/kg AFM1 for adults and children up to 3 years, respectively. A maximum limit for patulin in fruits and juices is also under discussion and the proposed level is 50 μg/kg. As for animal feeds, legal limits will be set only for AFB1, at 50 μg/kg for ovine and bovines, and 20 μg/kg for poultry and pigs.

Even if, according to Food (2004), Morocco had the most detailed mycotoxin regulations, in comparison with some African countries, some proposed limits remain high (case of aflatoxins and ochratoxin A in cereals) and require a revision, others mycotoxin limits were not proposed (case of fumonisins and trichothecenes) and needs to be introduced before the final adoption of this project. A comparison between the limits proposed by the Moroccan project for mycotoxin regulation in foods and the European regulations EC No. 1881/2006 and EC No. 1126/2007 (European Commission, 2007) is presented in Table 1, while the regulatory limits proposed for AFB1 in animal feeds are presented in Table 2.

8. Mycotoxins in food and feed from Morocco

8.1. Cereals and derivatives

8.1.1. Wheat, barley and corn

In Morocco, cereals represent a staple food for population, therefore bearing high social, economic and nutritional relevance. Moreover, cereals contribute to 12% approximately of the agricultural output and Moroccan households spend 25% of their food expenditure for this kind of products. On average, Morocco consumes 6 million tons of cereals each year. In addition, by 2020 the Moroccan population will require 8.5 million tons of cereals for the national consumption. Due to drought the country has endured during the last two decades, cereal yield production has been dramatically reduced in the range of 25–85% (INRA-National Institute of Agronomy Research of Morocco, 2002), leading to extensive importation from other countries. Thus, Morocco imports cereals for various countries particularly from France, USA, Canada, Brazil, Russia and Australia. It was reported that approximately 25% of cereals produced in the world are contaminated by mycotoxins (Devegowda, Raju, & Swang, 1998).

Preliminary surveys showed that Moroccan agricultural products including cereals appeared to be contaminated with spores of toxigenic strains of *Aspergillus*. Later, a series of analysis supported by the Direction Frauds Repression (Ministry of Agriculture) between 1991 and 1992 showed that a corn sample was found contaminated with 18 μg/kg of AFB1 (Tantaoui-Elaraki et al., 1994) using TLC technique.

Recently, a study that we had carried out on the contamination of 60 samples of grains of cereals commercialized in Morocco, showed that 40%, 40% and 55% of analyzed samples of corn, wheat and barley are respectively contaminated by OTA (Zinedine et al., 2006). In barley, OTA levels varied between 0.04 and 0.8 μg/kg, with an average concentration of 0.17 μg/kg. In corn samples, the highest value was 7.22 μg/kg while the average value was 1.08 μg/kg. In barley and wheat samples, ZEA and FB1 were above the limit of detection. Corn samples were found contaminated also by FB1 (50% of positive samples) with an average value of 1930 μg/kg, the highest value being 5960 μg/kg. This value exceeds the MRL value (1000 μg/kg) set by European regulation in corn (European Commission, 2006). Fifteen percent of corn samples showed a ZEA concentration higher than the limit of detection (LOD) with an average value and a maximum level of 14 and 17 μg/kg (Zinedine et al., 2006).

In another investigation, corn flour commercialized in retail markets of Rabat, was surveyed for the presence of aflatoxins, results showed an incidence of contamination of 80% with a maximum value of 11.2 μg/kg (Zinedine et al., 2007d). Ten percent of analyzed samples exceeded the MRL (4 μg/kg) set for total AFs by the European legislation. On average, Morocco consumes one mil-

Table 1
Comparison between European limits set for mycotoxins (Regulation CE No. 1881/2006 and Regulation CE No. 1126/2007) and maximum limits proposed by the Moroccan project in human foods

Mycotoxin	European limits		Moroccan limits	
	MRL (µg/kg)	Commodity	MRL (µg/kg)	Commodity
AFB1	2	Cereals and cereal products	10	All foods, wheat brain
	0.1	Baby foods including cereals	1	Peanuts, pistachios, nuts, almonds, vegetable oils in pasta, children foods
	5	Spices	3	Wheat meal
			5	Vegetable oils, cereals, wheat meal (complete)
AFs	4	Cereals and cereals products	–	–
	10	Spices		
AFM1	0.05	Liquid milk	0.05	Milk (product)
			0.03	Milk (product) for infant under 3 years
	0.025	Baby Milk	0.5	Milk powder
			0.03	Milk powder for infant under 3 years
OTA	2	Wine	30	Cereals
	5	Cereals		
	3	Cereal products		
	10	Dried raisins		
	10	Soluble coffee		
	0.5	Cereal products for babies		
ZEA	100	Cereals	200	Cereals, vegetable oils
	200	Corn		
	50	Bread		
	20	Cereal products for babies		
Fumonisins (B1 + B2)	4000	Unprocessed maize	–	–
	1000	Maize and maize-based foods intended for direct human consumption		
	800	Maize-based breakfast cereals and maize-based snacks		
	400	Processed maize-based foods and baby foods for infants and young children		
PAT	50	Fruits juices	50	Apple juices (products)
	10	Baby foods		
DON	1250	Unprocessed cereals other than durum wheat, oats and maize	–	–
	1750	Unprocessed durum wheat and oats and maize		
	750	Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption		
	500	Bread, pastries, biscuits, cereal snacks and breakfast cereals		
	200	Processed cereal-based foods and baby foods for infants and young children		

AFs: total aflatoxins, MRL: maximum residues limits, DON: deoxynivalenol, PAT: patulin, OTA: ochratoxin A and ZEA: zearalenone.

Table 2
Maximum limits proposed for AFB1 in animal feeds from Morocco

Mycotoxin	MRL (µg/kg)	Feed commodity
AFB1	50	Simple feedstuffs (except peanuts, copra, cottonseed, babassu, maize and their products)
		Complete feedstuffs for cattle, sheep and goats (except for dairy animals, calves and lambs)
		Complementary feedstuffs for cattle, sheep and goats (except for dairy animals, calves and lambs)
	30	Complementary feedstuffs for pigs and poultry (except young animals)
	20	Peanuts, copra, cottonseed, babassu, maize and their products
		Complete feedstuffs for pigs and poultry (except young animals)
	10	Complete feedstuffs for calves and lambs
		Other complete feedstuffs
		Other complementary feedstuffs, especially dairy animals
	5	Complete feedstuffs for dairy animals

lion tons of corn each year. Morocco imports corn exclusively from the US and Argentina (ONICL, 2005). Corn is among the commodities with high risk of mycotoxin contamination, whilst other cereals like wheat are resistant or only moderately susceptible to mycotoxin contamination in the field. Fungal growth and toxin production in corn have been found to depend on several interacting factors that stress corn plants. Stress factors include low moisture content of the soil, high daytime maximum temperatures, high nighttime minimum temperatures, and nutrient-deficient (Abbas et al., 2002).

The co-occurrence of OTA and the toxin DON and the associated toxigenic fungi in wheat grain from Morocco was studied by Hajjaji et al. (2006). Authors reported that few samples were contaminated by the two mycotoxins (two samples for OTA and seven for DON). The main isolated fungi belong to the *Aspergillus, Penicillium and Fusarium* genus; only two strains of *A. alliaceus* and 14 strains of *A. niger* were able to synthesize OTA.

8.1.2. Rice

The first study on the presence of OTA in rice commercialized in Morocco reported that OTA contaminate 90% of total samples analyzed. Levels of contamination in positive samples ranged between 0.02 and 32.4 µg/kg, where the average of OTA in positive rice samples is 4.15 µg/kg (Zinedine et al., 2007b). The maximum limit for OTA in cereals set by the Moroccan project is 30 µg/kg. This value is higher than maximum residue level (MRL) set by European regulations for OTA in cereals (5 µg/kg). Fifteen percent of total analyzed samples of rice exceeded the MRL of OTA set by the EU regulations.

In Morocco rice cultivation fluctuates vastly depending especially on climatic conditions. On a potential of 25,000 ha in the Gharb plain, the harvested area varies from 500 to 13,000 ha. On average the Moroccan population consumes 60,000 ton each year (2 kg/person/year). Due to drought the country has endured over the last two decades, rice yield production decreased dramatically from 44,000 ton in 1993 to 2500 ton in 1995, leading to extensive importation from other countries. Rice (*Oryza* sativa L.) is an important food crop worldwide, along with wheat and corn, and has been a major food in several countries. Park, Choi, Hwang, and Kim (2005) have reported that rice is naturally contaminated with *A. ochraceus* spores. Rice is an aquatic plant and is usually harvested at very high moisture levels (35–50%). Therefore, mycotoxin-producing moulds could contaminate the grain and produce important quantities of OTA during storage. Furthermore, rice is a better substrate for the characterization of OTA producing *A. ochraceus* strains.

8.1.3. Bread

In Morocco, the amount of cereal consumed is very high and bread is the food most consumed by the population. Wheat bread is essentially home made however baker's yeast is now more frequently used than traditional starter. Nowadays, Moroccan's lifestyle has changed due to the new working conditions. Consequently, a change of food consumption habits has been developed with the increase in bread consumption. The presence of OTA in bread consumed in Morocco has recently been reported by Zinedine, Juan, Idrissi, and Mañes (2007e). Results of this study showed the contamination of 48 out of 100 total analyzed samples with OTA. Levels of OTA in positive samples ranged between 0.14 and 149 μg/kg, where the average level of OTA in positive samples was 13 μg/kg. The highest frequency of positive samples (61.5%) and the most contaminated bread sample (149 μg/kg) were found in the Casablanca area. In this survey, 26% of total samples exceeded the maximum limit (3 μg/kg) set for OTA in cereal products by EU legislation.

Among cereal derived products, bread is of significant importance because it provides more nutrients to the population than any other single food and it is particularly important as a source of carbohydrates, proteins and vitamins. Wheat is utilized mainly as flour (whole grain or refined) for the production of a large variety of leavened and flat breads, and for the manufacture of a wide variety of other baking products. Some *durum* wheat is milled into flour to manufacture medium-dense breads in Mediterranean and Middle Eastern countries and some into coarse durum grain grits used to produce couscous (cooked grits) in Arabic countries. Bread is a product of daily consumption and highly demanded. The World Health Organization (WHO) recommends a 250 g/day intake which corresponds to 90 kg/person/year.

Mycotoxin exposure mainly occurs via the food chain. It was generally demonstrated that the main contributors to OTA intake are cereals and cereal products. Several authors have indicated bread as one of the main sources of daily intake of OTA.

OTA daily intake was estimated from this study. Given that bread consumption in Morocco is estimated to 210 kg/year/person (i.e., 577 g/day/person). For an adult (60 kg bw), the estimated daily intake of OTA was calculated to be 126 ng/kg bw/day. This value is seven times higher than the Tolerable Daily Intake (17.1 ng/kg bw/day) set by the European Food Safety Authority (EFSA, 2006) and nine times higher than the value set by the FAO/WHO Committee of Experts on Food Additives (14 ng/kg bw/day) (JECFA, 2001). Theses results showed that Moroccan population is highly exposed to damaging effects of OTA and the exposure could be related to cases of nephropathy widely reported in the country especially in young from both sexes of the population. However, this hypothesis needs to be confirmed especially by determination of OTA in biological fluids (blood, urine, breast milk etc.) in healthy individuals and from patients having renal dysfunctions (chronic renal insufficiency, chronic interstitial nephropathy etc.).

8.2. Pasteurized milk

In Morocco, the dairy sector has been spreading since 1975 when the Ministry of Agriculture set forth a plan to develop dairy production. This plan allowed production to increase from 581×10^6 L in 1975 to 2.1×10^9 L in 2000. However, the projected levels of production have not been reached. The Moroccan consumption per capita of milk remains below the projections. Recently the Moroccan government has set up a new dairy plan for the period 2000–2020 based especially on the improvement of milk quality and safety from the production to the commercialization. Milk and dairy products produced in Morocco were widely investigated since the 1980 for their microbiological and physicochemical properties; however, little information on the presence of AFs in milk is known. As far as we know, we have published the first study on the natural occurrence of AFM1 in pasteurized milk produced in Morocco (Zinedine et al., 2007c). This study was conducted on milk produced from February to April. According to Dragacci and Fremy (1993), highly-contaminated samples with AFM1 were found during winter. High AFM1 concentrations were obtained by Bakirci (2001) from milk samples from March, April and early May, these had higher AFM1 concentration. The seasonal variation of AFM1 contamination in milk seems to be because cows receive less concentrated feed in summer when they are grazing. A relationship between AFM1 occurrence level in milk and AFB1 content of feed was reported by Van Egmond (1989).

From this study, fifty four samples of pasteurized milk produced by five different industrial units from Morocco were surveyed for the presence of AFM1 using immunoaffinity columns and liquid chromatography coupled to fluorescence detection. Confirmation of AFM1 identity in positive samples was based on the formation of AFM1 hemi-acetal derivative (AFM2a) after derivatization with trifluoracetic acid (Zinedine et al., 2007c). Results showed that the incidence of AFM1 contamination in pasteurized milk samples was very high (88.8%) with a mean contamination value of 0.0186 μg/L. AFM1 levels in analyzed samples ranged from 0.001 to 0.117 μg/L. Results showed also that 7.4% of total samples exceeded the maximum level of 0.05 μg/L set by the European regulations for AFM1 in liquid milk. These results indicate indirectly that feeds for cows in Morocco were contaminated with AFB1. It should be mentioned that in Morocco, mouldy bread, which is improper for human consumption, is often kept in plastic bags and left in front of houses. This bread is generally gathered by retailers for farms and used as feeds to nourish animals especially cow's to increase milk production. This fact would probably increase the contamination of cow's milk with mycotoxin especially AFM1.

The weighted mean AFM1 concentrations in milk in European, Latin American, Far Eastern, Middle Eastern and African diets has been reported by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001) to be 0.023, 0.022, 0.36, 0.005 and 0.0018 μg/L, respectively. Thus, the observed mean AFM1 concentration in Moroccan milk samples was 10 times higher to that in the African diet, and lower than those reported for the European, Latin American and Far Eastern diets.

Human exposure to AFM1 is due to the consumption of contaminated milk and dairy products; its daily intake could be highly variable in the world. Infants represent the most exposed population due to their high consumption either of bovine milk and related by-products in their diet, or from breast milk where the mycotoxin can be excreted. The dietary intake of AFM1 was estimated from data on the concentration of AFM1 in milk reported by many countries and established by JECFA (2001). The intake of AFM1 from milk was calculated to be 6.8 ng/person/day for the European diet, 3.5 ng/person/day for the Latin American diet, 12 ng/person/day for the Far Eastern diet, 0.7 ng/person/day for the Middle Eastern diet, and 0.1 ng/person/day for the African diet. In Morocco, The average consumption of milk is about 64 L/person/year (i.e., 175 mL of milk/person/day). Given that until now there is not official data on milk intake in Morocco, AFM1 daily intake estimated in this study was only for orientation purposes. Therefore, AFM1 estimated daily intake from this study was 3.26 ng/person/day. This value is similar to that in the Latin American diet and is considered approximately 32 times higher than the dietary intake value estimated by JECFA for the African diet (Zinedine et al., 2007c).

8.3. Spices

In general, spices constitute a natural medium not favorable for the growth of moulds and the production of mycotoxins. Essential oils of clove and cinnamon showed an inhibiting effect on the growth of *A. parasiticus* and *F. moniliforme*. The addition of the extracts of clove to spices showed a considerable inhibition of the production of aflatoxins. Spices, such as pepper, paprika, cumin, ginger, and saffron are extensively used in Morocco to flavoring food as well as for medication and are highly valuable due to their preservative and antioxidant properties. Spices are largely produced in countries where tropical climates (high ranges of temperature, humidity and rainfall) are favorable to mycotoxin contamination. Furthermore they are usually dried on the ground in the open air in poor hygienic conditions that even more promote growth of moulds and production of mycotoxins.

In Moroccan kitchen, spices are used in particular for the preparation of delicious foods like "Tagines", "Tangia", "Couscous", "Rfissa", etc., and this with the aim to develop an adored tastes or a particular color. Considering their antimicrobial properties, some spices are also used in traditional process for the conservation of some home made meat products such as "Kaddid" and "Kliaâ" especially rural area. It is important to clarify that the control services of frauds repression in Morocco observed that some spices (ginger and paprika) especially commercialized by traditional retailers markets are fraudulently added with other products like wheat flour while insisting on a voluntary addition of artificial colorants to get the normal color of spice. This fat that is normally forbidden by Moroccan authorities, deteriorate the quality of spices and could possibility favorite the growth of moulds and mycotoxin production. A study on the natural occurrence of aflatoxins in 55 samples of spices commercialized in Morocco was reported by Zinedine et al. (2006). The higher levels of contamination were found in red paprika, with 100% of positive samples and an average concentration of 2.88 and 5.23 μg/kg for AFB1 and total aflatoxins, respectively. Among spices the maximum value was from red paprika sample (9.68 μg/kg). In this survey, incidence of AFs in paprika and ginger was higher than in cumin and pepper. These findings are in agreement with Bartine & Tantataoui-Elaraki, 1997 who demonstrated that growth of toxigenic strains of *A. flavus* is weak both in curcumin and in black and white pepper.

8.4. Dried fruits and nuts

Moroccan population consumes huge amounts of dried fruits directly or as ingredients included in special foods especially prepared during the 'Ramadan' fasting month and festival days like "Chebbakia", "Sellou", "Sefouf", and "Zammita". Almost all nuts such as pistachio, walnuts and peanuts consumed in Morocco are imported and little is known about their quality. Consequently, there is an importance to study the presence of mycotoxins, since there is a lack of information in the literature about their occurrence in these products. In Morocco, traditional techniques for the transformation and conservation of fruits are still used. Theses practices are very optimal conditions (especially temperature, humidity and fruits damages) for mould growth and mycotoxin production. The natural drying, which may consists in direct exposition of the fruit to the sun, is widely used especially in rural area.

Fresh Fruits (raisins, figs, etc.) having reached a sufficient degree of maturity are gathered and transported to drying places such as the terrace of house or a piece surrounded to prevent the access of animals. These surfaces of drying are in general exposed to a maximum sunning and are papered with herbs to avoid the contact with the ground. Fruits are spread out over these surfaces without preliminary treatment. After drying, fruits are collected and stored. During the process of fruits drying, the sugar is concentrated as the moisture content decreases resulting in an almost selective medium for xerotolerant moulds such as *A. niger* section nigri species. Among black aspergilli, *A. carbonarius* is the most important as OTA producing isolate observed more frequently. Other black aspergilli including the *A. niger* aggregate and *A. aculeatus* have also been found to produce OTA on grapes. The incidence of AFs and OTA in dried fruits and nuts could be avoided or at least decreased if good agricultural and manufacturing practices from harvesting to processing were used. It should be mentioned that the project for mycotoxin regulations did not set limits for AFs and OTA in dried fruits and nuts.

8.4.1. Aflatoxins in dried fruits and nuts

Juan et al. (2008) reported that dried fruits and nuts commercialized in Rabat could be contaminated with AFs. Theses authors analyzed 100 samples of pistachio, peanuts, dried raisins, figs and walnuts for the presence of AFs. Results from this study showed a weak contamination of peanut (only one positive sample out of 20). Levels of AFB1 and AFT are 0.17 and 0.32 μg/kg, respectively. In dried raisins, samples were found contaminated by AFB1, concentrations ranged between 3.2 and 13.9 μg/kg even if several authors reported that dried raisins do not seem to be a satisfactory substrate for *A. flavus* growth and AFs production. Dried figs contained only AFB1 (0.28 μg/kg) and AFG1 (0.28–32.9 μg/kg). According to Pitt and Hocking (1997), *A. flavus* and *A. niger* were reported as being the most common species in dried figs which was explained by their high sugar content.

Walnut and pistachio were found more contaminated with AFB1 levels ranging from 0.56 to 2500 μg/kg and from 0.04 to 1430 μg/kg, respectively. *Pistacia* sp. (*P. vera* and *P. atlantica*) was introduced into Morocco by the National Institute of Agronomic Research since 1950 and it is now cultivated on an area of about 120 ha, few production amounts of two varieties (*Achouri* and *Mateur*) were obtained. Thus, huge amounts of pistachio are imported by country.

8.4.2. OTA in dried fruits and nuts

The presence of OTA in dried fruits and nuts from Morocco was studied by Zinedine et al. (2007b). Authors have reported that the incidence of OTA in dried raisins, dried figs, walnuts, and peanuts were 30%, 65%, 35%, and 25%, respectively, while pistachio samples were free of OTA.

The averages for OTA in positive samples of peanut, dried figs, dried raisins and walnut are 0.68, 0.33, 0.96 and 0.11 μg/kg, respectively.

8.5. Beverages

Beverages (wine, fruits juices and beer) produced in Morocco were analyzed by Filali et al. (2001) for their content of OTA. The results from 30 wine samples, 20 red, 7 white and 3 rosé reported that

OTA concentrations in the wines ranged from 0.028 to 3.24 μg/l with an overall median of 0.65 μg/l. The median concentration of OTA in white and rosé wines was found to be 0.117 μg/l whereas that in the red wines was 0.912 μg/l. The concentrations of OTA in the red wines ranged from 0.04 to 3.24 μg/l and those in the white and rosé wines from 0.028 to 0.540 μg/l. The red wines were thus more contaminated than white and rosé ones. The EU regulation set the acceptable limit for OTA in wine at 2 μg/l (European Commission, 2006). Thus, one sample containing 3.24 μg/l was above this limit. The results from analysis of 14 samples of various fruit juices (cocktail, orange, mango, peach, pineapple, clementine and grapefruit) show that only one sample (grapefruit juice) was contaminated, with a concentration of 1.16 μg/l. In analyzed beers, OTA was not detected. Almost all the grapes produced in Morocco are used for the wine industry. Grape juices are imported from Europe in very limited amounts and should not have a significant influence on the daily intake of OTA by the Moroccan population. This and the consequent prevalence of OTA in human blood would be rather influenced by the OTA contents in cereals, beans, dried fruits, poultry and olives as has been already reported in other countries of Northern African Tunisia and Algeria (Creppy, 1999).

8.6. Olives

The production of olives in Morocco is about 6.9% of the global world production. The traditional harvest method used, and the long storage of fruits at ambient temperatures (18–28 °C) before processing may result in a severe loss and a poor quality of olives. Micro-organisms involved in post-harvest alterations of the fruits before the fermentation processes were studied by Asehraou, Mohieddine, Faid, and Seghrouchni (1997). Many mould strains, in particular *Aspergillus* and/or *Penicillium*, are able to develop on olive and produce OTA and/or citrinin and/or type B aflatoxins after harvest, during drying and storage of olives (El Adlouni, Tozlovanu, Naman, Faid, & Pfohl-Leszkowicz, 2006).

In Morocco, black table olives are prepared by an old process which may consist of drying and salting. The harvested black olives are filled in bags and salted (solid salt is sprinkled on the fruits while filling them in the bags). These bags are arranged one on the other and a heavy material (stone) is deposited on the top bag. The bitter black liquid is driven out under the action of weight and salt. A survey of the most frequent micro-organisms showed a low microbial load except for the yeasts and moulds. The most representative microbiota of black olives was species of moulds which may be associated with food poisoning due to their mycotoxins (Asehraou, Faid, & Jana, 1992). On some occasions, phenomenally high concentrations of OTA have been reported in black olives. Maaroufi et al. (1995) reported the contamination of one sample of black olives from Tunisia with a high level of OTA of 46,830 μg/kg (i.e., 46.83 ppm). The occurrence of toxigenic moulds in black olives processed by the non controlled traditional method is possible. Olives are among the commodities with high risk of

Table 3
Mycotoxins in food and feed from Morocco

Matrix	*N*	Mycotoxin	Incidence (%)	Average (range) μg/kg or μg/L	% of samples > EMRLs	References
Cereals and derivatives						
Wheat grain	20	OTA	40	0.42 (0.04–1.73)	–	Zinedine et al. (2006)
	17	OTA	11.7	Up to 30.6	2	Hajjaji et al. (2006)
	17	DON	41.1	Up to 128	–	
Wheat flour	17	AFs	17	0.07 (0.03–015)	–	Zinedine et al. (2007d)
Barley	20	OTA	55	0.17 (0.04–0.80)	–	Zinedine et al. (2006)
Corn	20	AFs	80	0.83 (0.23–11.2)	10	Zinedine et al. (2007d)
	20	OTA	40	1.08 (0.05–7.22)	5	Zinedine et al. (2006)
	20	ZEA	15	14 (11–16.5)	–	
	20	FB1	50	1930 (10–5960)	10	
Bread	100	OTA	48	13 (0.14–149)	26	Zinedine et al. (2007e)
Rice	20	OTA	90	4.15 (0.02–32.4)	15	Zinedine et al. (2007b)
Pasteurized milk	54	AFM1	88.8	0.018 (0.001–0.117)	7.4	Zinedine et al. (2007c)
Dried fruits						
Raisins	20	AFB1	20	10.7 (3.2–13.9)	20	Juan et al. (2008)
	20	OTA	35	0.96 (0.05–4.95)	–	Zinedine et al. (2007b)
Pistachio	20	AFB1	45	158 (0.04–1430)	20	Juan et al. (2008)
	20	OTA	–	–	–	Zinedine et al. (2007b)
Figs	20	AFB1	5	0.28	–	Juan et al. (2008)
	20	OTA	65	0.33 (0.03–1.42)	–	Zinedine et al. (2007b)
Peanut	20	AFB1	5	0.17	–	Juan et al. (2008)
	20	OTA	25	0.68 (0.10–2.36)	–	Zinedine et al. (2007b)
Walnuts	20	AFB1	30	360 (0.56–2500)	20	Juan et al. (2008)
	20	OTA	35	0.11 (0.04–0.23)	–	Zinedine et al. (2007b)
Black olives	25	OTA	36	1.43 (0.62–4.8)	–	Zinedine et al. (2004)
	10	OTA	100	(Up to 1.02)	–	El Adlouni et al. (2006)
	10	AFs	100	(Up to 0.5)	–	
	10	CIT	80	(<LOD)	–	
Spices						
Pepper	15	AFs	93	0.21 (0.04–0.55)	–	Zinedine et al. (2006)
Cumin	14	AFs	57	0.05 (0.01–0.18)	–	
Ginger	12	AFs	86	1.47 (0.03–9.10)	–	
Red paprika	14	AFs	100	5.23 (1.30–9.68)	–	
Animal feed						
Poultry feeds	315	AFB1	–	(20–5625)	–	Kichou and Walser (1993)
Poultry feeds	21	AFB1	66.6	1.26 (0.05–5.38)	–	Zinedine et al. (2007d)
Beverages						Filali et al. (2001)
Wine	30	OTA	100	0.65 (0.028–3.24)	3	
Beer	5	OTA	–	–	–	
Fruits juices	14	OTA	7.1	1.16	–	

AFs: total aflatoxins, CIT: citrinin, DON: deoxynivalenol, OTA: ochratoxin A, EMRLs: European maximum residues limits and *N*: number of analyzed samples.

mycotoxin contamination. Gourama and Bullerman (1988) isolated toxigenic strains of *A. flavus* and *A. ochraceus* that produced aflatoxins and ochratoxins from 'Greek-style' black olives produced in Morocco. A survey of the contamination of black olives commercialized in Morocco with mycotoxins reported that OTA was detected in 36% of total analyzed samples. OTA concentrations ranged from 0.62 to 4.8 μg/kg with an overall median of 1.43 μg/kg (Zinedine et al., 2004). More recently, Roussos et al. (2006) isolated strains of *A. flavus* and *A. niger*, from spoiled olive and olive cake of the 2003 and 2004 olive oil production campaigns in Morocco, that produced AFB1 and OTA. El Adlouni et al. (2006) reported the presence of OTA, citrinin and AFs in black olive "Greek style" purchased from supermarkets and retail markets and concluded that the simultaneous presence of these toxins increases toxic risks and should spur authorities to control the conservation of olives especially after harvest.

8.7. Poultry feeds

The presence of moulds and mycotoxins in poultry feeds results in the raw materials used in their production. Moulds and mycotoxin production of the raw materials occur during the pre-harvest (field produced fungi) and/or the post-harvest (storage produced fungi) periods. Lozada (1995) reported that during these periods, temperature and humidity play a role in the growth of fungi and mycotoxin production. Moreno and Suarez (1986) reported the contamination of poultry feeds with *A. flavus* and *A. parasiticus* that produced AFB1 and AFB2. Mixed poultry feeds presented a high total mould count reflecting the mould flora of raw materials, the most frequent and abundant fungi were *A. flavus* and *Penicillium* spp. (Bauduret, 1990).

In general, there is a lack of investigations on the presence of mycotoxins in animal feed. In the literature, only two papers reported the contamination of poultry feeds with aflatoxins. The first was reported by Kichou and Walser, 1993. The authors, by using semi-quantitative ELISA and TLC methods, analyzed 315 samples poultry feeds and their ingredients (corn, sorghum, wheat bran, soybean meal, cottonseed meal, sunflower meal). The level of contamination ranged from 20 to 200 μg/kg, except for four samples that contained high levels of AFB1 (2000–5625 μg/kg). These highly-contaminated samples were associated with clinical aflatoxicosis in broiler chickens. The second survey of Zinedine et al. (2007d) concerned a few samples ($n = 21$) purchased from retailer markets in Rabat. Results showed that the percentage of contamination by AFs is about 66.6%, while the contamination levels of poultry feeds samples ranged between 0.05 and 5.38 μg/kg for AFB1 (see Table 3).

9. Conclusions and recommendations

In this paper, most investigations on the occurrence of mycotoxins in Moroccan food and feed are presented. As found, Moroccan population could be exposed to risks of theses toxic substances particularly from the consumption of based food such cereals, dried fruits, milk and bread. Levels of contamination were sometimes above the MRLs set by European regulations in food. The contamination is due to the fact that may be some food safety and quality standards (good agricultural practices (GAPs), good manufacturing practices (GMPs), and the hazard analysis and critical control point (HACCP) system) are not applied and performed in most of Moroccan food units to control growth of moulds and mycotoxin production during harvesting, distribution and storage periods.

Because European countries are the first partner of Morocco in the field the agricultural products exchange, the Moroccan project for mycotoxin regulations needs to be harmonized with EU regulations. For this, the revision of this project by Moroccan authorities is a priority since some proposed values are high and some other limits in other commodities are absent and needs to be introduced.

The contamination level of some mycotoxin (e.g., OTA, FB1, AFs), found in some analyzed matrices, was quite high and the average of contaminated samples was often above 50%. This situation should spur Moroccan authorities to devise prevention measures and set programs for surveillance of mycotoxins in food and feed. More research about the presence of *Fusarium* toxins (DON, T-2 and HT-2 toxins, ZEA, and FB1) and a survey of a large number of food samples (cereals products, raw and UHT milk, infants cereals, animal feeds, oils) will be needed to fully assess this situation. It is also important to evaluate the exposure of Moroccan population to mycotoxins by determining the presence of these toxins in biological fluids such as serum and breast milk.

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