

Original Article

Prospective Study of Food Chain Mold Contamination in Rabat City

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ABSTRACT

Introduction: Contamination by molds is a major public health problem and a real danger for both humans and animals due to the synthesis of toxic metabolites (Mycotoxins) during their proliferation in foods of plant or animal origin. Their effects on health are very harmful and can go as far as death in the event of heavy contamination. Prolonged exposure to low doses can cause several pathologies including nephropathies, cancers, liver damage, hemorrhagic syndromes, and immunological and neurological disorders. **Material, Methods and Results:** In this work, the contamination of Moroccan foodstuffs with mycotoxins was studied. A total of 22 samples: 13 samples of spices, 7 samples of coffee, 1 sample of tea and 1 sample of chocolate, were purchased from different popular markets of Rabat. Mycological study of 22 of these samples showed a large number of fungal contaminants resulting in a 100% contamination of all our samples. Among them, it indicates the presence of the principal genus implicated in the production of mycotoxins: *Aspergillus* spp (77%), *Penicillium* spp (18%), and *Mucor* (90%). Detection of mycotoxins is performed by LC-MS/MS. The results showed a variation in level of aflatoxin B1 and ochratoxin A, without exceeding the maximum limits set by Moroccan regulations. **Conclusion:** The prevention of contamination of raw materials and the development of molds implies respect for good farming practices and storage conditions for food products. Risk management is the responsibility of governments and require total respect of international recommendations to reduce the impact on the economy and health population.

INTRODUCTION

Contamination of the food chain by mycotoxins has always attracted attention because of its economic consequences relating to their effects on humans, animal productivity and trade. Poisoning, which can occur by inhalation, skin contact or breast-feeding, can rarely be acute because of the small amounts that can be ingested with contaminated food, the chronic form is possible and linked to the cumulative effect of doses.

Several factors determine their biosynthesis such as temperature, exposure to light, CO₂ in the air, available nutrients and the presence of other competing microorganisms.

Morocco, because of its hot and humid climate and the eating habits of its population and its conditions of storage and handling of foodstuffs, constitutes a favorable ground for the development of toxinogenic molds and the consequent contamination of the food chain by mycotoxins, the best known of which are aflatoxins, ochratoxin A and trichothecenes.

The objective of this work is to report the study of fungal contamination carried out on 22 samples taken from various points of sale in the city of Rabat, capital of Morocco, and the results cultures of the identified mycotoxins.

MATERIAL AND METHODS

It is a prospective study carried out over a period of 4 months, at the Parasitology-Mycology Department of the Mohamed V Military Instruction Hospital in Rabat.

Material

In order to have a heterogeneity of samples, 22 food samples taken from different points of sale in the city of Rabat and in different forms: in bulk from the Medina, in bulk from a large area and in bags, were The subject of the mycological study was ground coffee, coffee in capsules and flavors, ground tea, chocolate powder and certain condiments including cumin, pepper, red chilli and ginger.

Methodology

Away from light and heat, the samples were placed in seven sterile jars numbered 1 to 7 and ABC according to the origin:

(1: Coffee, 2: Tea, 3: chocolate powder, 4: Cumin, 5: Pepper, 6: Red pepper, 7: Ginger)

(AT: bulk from the Medina; B: in bulk from a large area; VS: in a bag)

Sabouraud medium with chloramphenicol was used because its relatively acidic pH, favorable to mushroom cultivation and their morphological study.

The macroscopic study of the fungus colonies was made after an Incubation at 20 - 25 ° C temperature for a period of 5 to 7 days for the yeasts, and at 37 ° C or 30 ° C aerobically after several days up to to 21 days for filamentous fungi (Figures 1 and 2).

The macroscopic identification criteria were the Color of the colonies, their Texture, Topography, Speed of Growth and Fruiting Structures. The microscopic analysis of the fungal colonies was carried out by examining the preparations in the fresh state (between slide and coverslip) with Blue Cotton, carried out at objective 40. The various analyzes were carried out on the ultra-performance liquid chromatography system (Waters Acquity UPLC) coupled to a triple quadrupole mass spectrometer (TQD, Waters), controlled by MassLynx® software (version 4.1). The quantifications were made by the TrgetLynx® application. This system was denoted LC-MSMS.

The reagents used included Acetonitril (mass spectrometry grade), Ultrapure water (Resistivity: 18.2 MΩ.cm

at 25 ° C, Conductivity: 0.055 μS/cm at 25 ° C) produced by a Milli-Q system (Millipore; France), and the 150 ml Erlenmeyer flask.

During this study, the following mycotoxins were sought: Aflatoxins B1, B2, G1, G2 And Ochratoxin A.

RESULTS

The mycological study identified 100% fungal contamination, either the 22 food products analyzed. The strains isolated belonged to the genera *Aspergillus*: 77% (*Asp niger*: 59% *Asp flavus* 9%, *Asp fumigatus* 4.5% *Asp nidulans* 4.5%), *Penicillium* 18% (*Mucorale* 90% the most frequent, *Cladosporium herbarum*: 4.5%, *boveria*: 4.5%, *paecilomyces variotii*: 4.5%) (Graphic 1), (Table 1).

The results of the mycological study of all the samples made it possible to identify the fungal species found in the different samples taken and to perform the mycotoxin assay (Table1).

The foodstuffs were polycontaminated for ground coffee DU, coffee-capsule ES, *Vanilio* capsule NES coffee, tea, chocolate, cumin, pepper, red pepper, ginger, unlike *cosi* capsules NES, *Robusta* uganda, *Valluto* Decaffeinato and *Sachertorte* which were monocontaminated. (Graphic 2)

Chromatogram had permitted the Extraction of cultures of mycotoxins in the 22 treated samples; LC-MS/MS coupling analysis consisted of a first chromatographic detection by liquid chromatography with a strip detector diodes (LC-DAD), which allows to directly measure the absorbance over several lengths wave at a time, and a second detection by mass spectrometry of the molecules separated beforehand by chromatography (Graphic 3, 4), (Table 2).

DISCUSSION

Foodborne illnesses, consequences of contamination by various agents, in particular pathogenic microorganisms including molds, constitute a major public health problem.

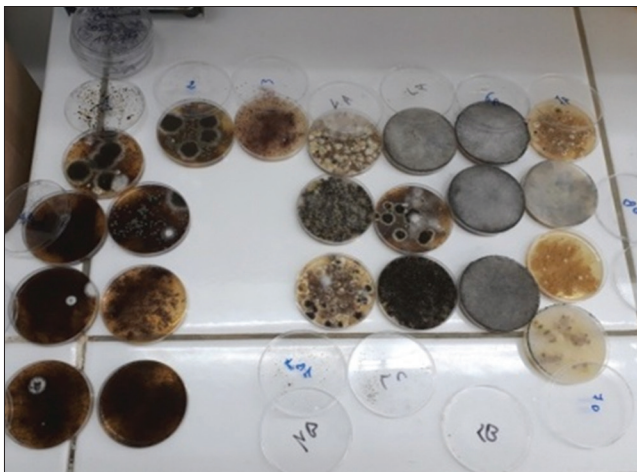


Figure 1. Mold present in the different samples after 7 days of incubation

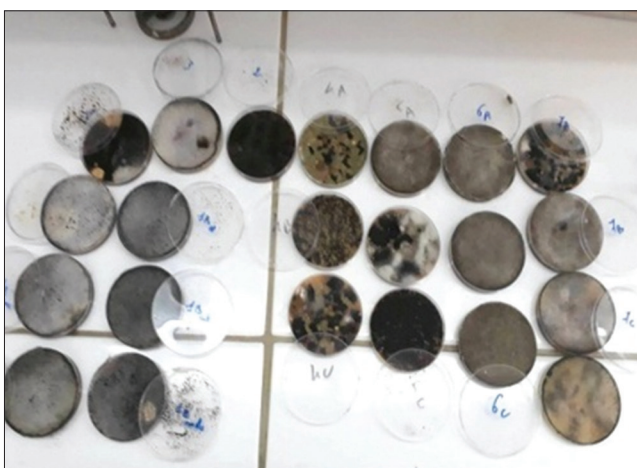
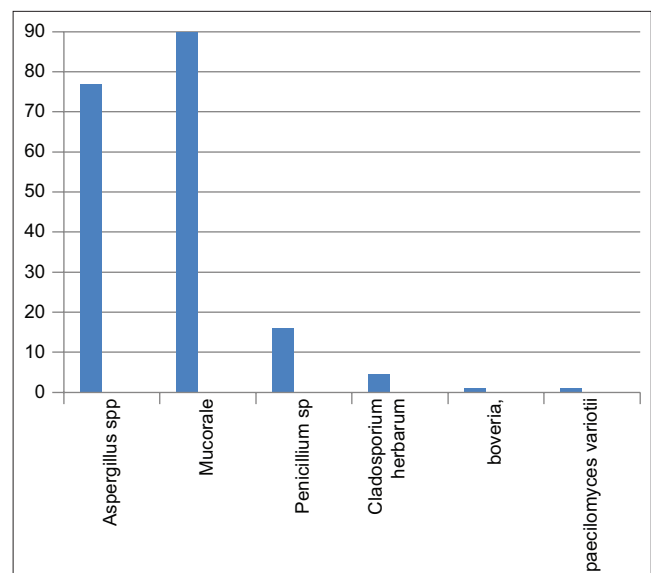


Figure 2. Mold present in the different samples after 10 days of incubation



Graphic 1. Percentage of positive samples based on species for all samples processed

Table 1. Results of the mycological study of the 22 treated samples

Samples	The molds								
	Asp Niger	Asp, flavus	Asp. fumigatus	Asp. nidulans	Penicillium SP	Mucor-ral	Clado-sporium herbarum	Beau-veria,	paecilo-myces variotii
Ground coffee DU	+	-	-	-	-	+	-	-	-
capsule ES	+	-	-	-	-	+	-	-	-
Capsule NES Vanilio	-	+	-	-	+	+	-	-	-
Capsule NES cosi,	-	-	-	-	-	+	-	-	-
Capsule NES Robusta uganda	-	-	-	-	-	+	-	-	-
Capsule NES VallutoDecaf	-	-	-	-	-	+	-	-	-
Capsule NES Sachertorte	-	-	-	-	-	+	-	-	-
Tea	+	-	-	-	+	-	+	-	-
Chocolate						+	+	-	-
Cumin (AT)	+	-	-	+	-	+	-	-	-
Cumin (B)	+	-	-	-	-	+	-	-	-
Cumin (C)	+	+	+	-	-	+	-	+	-
	Asp. niger	Asp. flavus	Asp. fumigatus	Asp. nidulans	Penicillium Sp	Mucor-rale	Clado-sporium herbarum	Handsome-veria	paecilo-myces variotii
Pepper (A)	+	-	-	-	+	-	-	-	-
Pepper (B)	+	-	-	-	-	+	-	-	-
Pepper (VS)	+	-	-	-	-	+	-	-	-
Red pepper (A)	+	-	-	-	-	+	-	-	-
Red pepper (B)	+	-	-	-	-	+	-	-	-
Red pepper (VS)	+	-	-	-	-	+	-	-	-
Ginger (A)	+	-	-	-	+	+	-	-	-
Ginger (B)		-	-	-	-	+	-		+
Ginger (C)	+	-	-	-	-	+	-	-	-
Ginger (Rhizome)	-	-	-	-	-	+	-	-	-

1 : Coffee 3 : Chocolate powder 4 : Cumin 6 : Red pepper

2 : Tea 5 : Pepper 7: Ginger

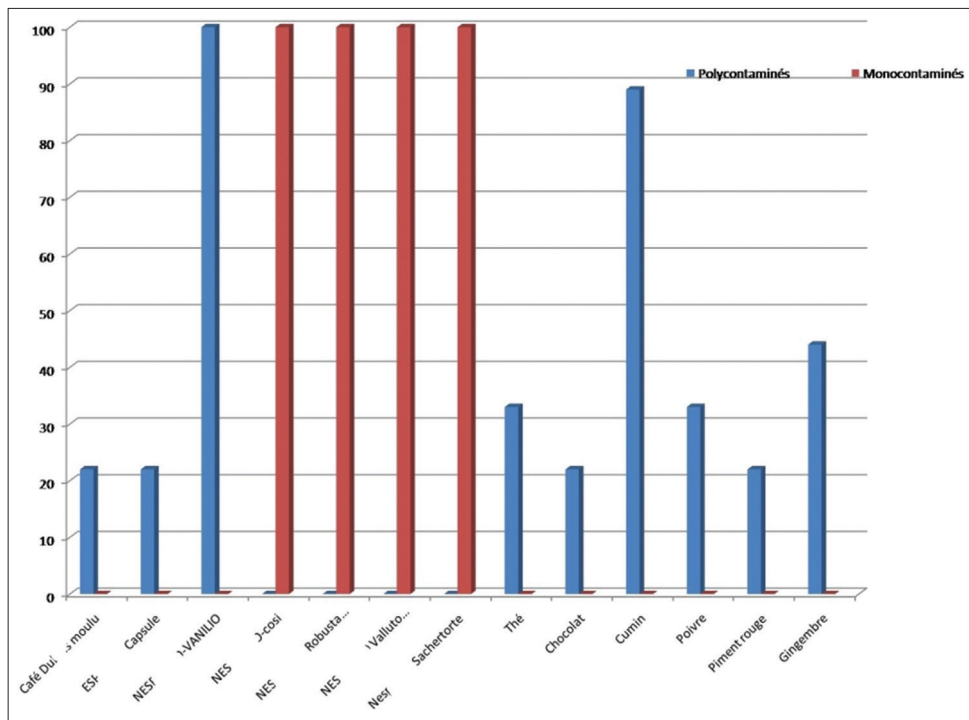
A, B, C: The different origins of each sample: (AT : bulk from the Medina; B: in bulk from a large area; VS: in a bag)

Mycotoxins, of which there are more than 300 types, constitute the toxic secondary metabolites which develop on the plant in the open field or during storage, in all regions of the world (1). The most common are aflatoxins, ochratoxins, trichothecenes, zeralenone, fumonisins, ergot alkaloids, citrine, patulin and sterigmatocystin(2). Their production is random and generally responds to environmental factors such as temperature, humidity, the nature of the substrate or the presence of other molds. A mycotoxins can be produced by different strains of mold and a strain

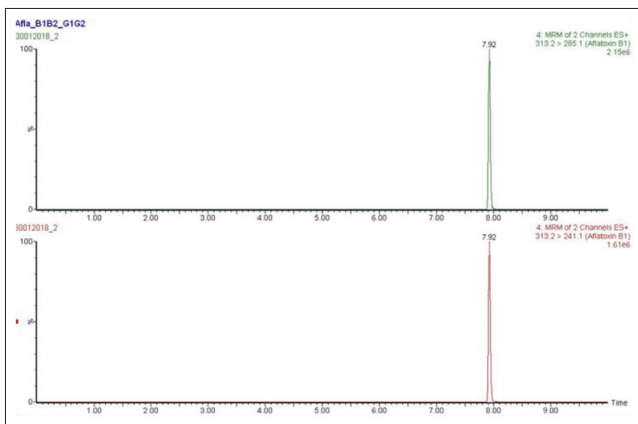
can produce several different toxins, depending on climatic conditions(3).

Their effects on health are very harmful and can go as far as death in the event of heavy contamination. Prolonged exposure to low doses can cause several pathologies including nephropathies, cancers, liver damage, hemorrhagic syndromes, and immunological and neurological disorders (4, 5).

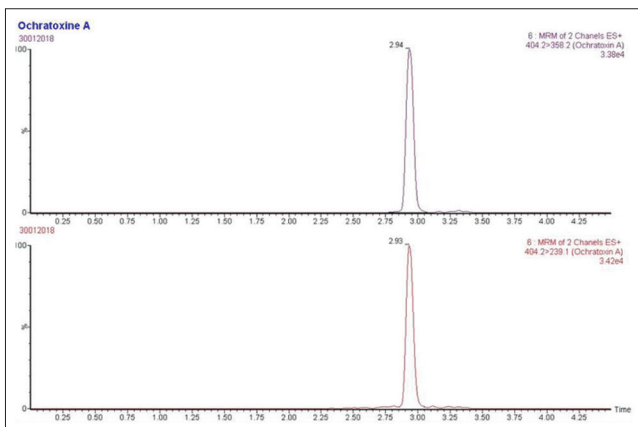
The direct economic consequences are the drop in the production yield of farm animals (contaminated by food), non-marketable food (deterioration of organoleptic



Graphic 2. Percentage of poly contaminated samples, mono contaminated for the different samples



Graphic 3. Aflatoxins aspect on chromatogram



Graphic 4. Ochratoxine A aspect on chromatogram

characteristics) or the destruction of excessively contaminated food. The molds of the genus *Aspergillus*, *penicillium* and *Fusarium* are of greatest concern to humans (6).

Thus, the contamination of foodstuffs intended for human and animal consumption poses a major problem of availability and safety of the world food supply (FAO). To this end, several recommendations and national and international programs have been established in order to reduce the impact on the economy and health through preventive and curative measures.

The results of our study agree with those of the literature, by highlighting the fungal flora of the different substrates and the dosage of two types of mycotoxins: Aflatoxin and Ochratoxin.

Our results varied according to the type and nature of the samples analyzed and the molds sought, the most common of which were *Aspergillus*, *Mucorale* and *Penicillium*.

The nine fungal species identified were mainly: *Mucorale*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Penicillium*, *Cladosporium herbarum*, *Beauveria*, *Paecilomyces variotii*.

For Coffee (brands DU, NES, ES), The main molds isolated were: *Aspergillus niger*, *A. flavus*, *Penicillium*, *Mucorale* (*Mucor* and *Rhizopus* sp). The most significant contamination was noted at the level of Vanilio capsules of the NES brand and also at the level of coffee DU and ES. Among the toxinogenic species identified, we found: *A. niger*, *Penicillium* sp, and *A. flavus*(7, 10). Our results are therefore similar to those of the literature.

The biodiversity of the fungal strains present seems to be linked to the geographical, ecological and human context (local traditional practices and unequally respected hygiene conditions). Suarez-Quiroz et al. obtained a similar result with identification of black *Aspergillus* (*A. niger* and *A. carbonarius*), the predominant group of molds isolated in Cameroonian coffee samples, much higher than that reported on Brazilian or Vietnamese coffee beans (8,

Table 2. Detection of the presence of mycotoxins in the 22 treated samples

Product	Mycotoxin detected	Concentration
Ground Coffee (DU brands)	Not detected	
Cafécapsules ES	Not detected	
Capsule NES Cosi	Not detected	
Capsule NES Sachertorte ...	Not detected	
Capsule NES Robusta uganda	Not detected	
Valluto capsule NES Decaffeinato	Not detected	
Capsule NES Vanilio	AFB1	0.004 µg/kg
Tea	AFB1	3.2 µg/kg
Chocolate	Not detected	
Cumin (A)	AFB1	0.07 µg/kg
Cumin (B)	AFB1	0.05 µg/kg
Cumin (C)	AFB1	0.03 µg/kg
Pepper (A)	AFB1	0.16 µg/kg
	OTA	1.43 µg/kg
Pepper (B)	AFB1	0.12 µg/kg
Pepper (C)	AFB1	0.14 µg/kg
Chili (A)	AFB1	3.11 µg/kg
Red pepper (B)	AFB1	3.25 µg/kg
Red Pepper (C)	AFB1	1.28 µg/kg
Ginger (A)	AFB1	1.31 µg/kg
Ginger (B)	AFB1	0.73 µg/kg
Ginger (C)	AFB1	1.12 µg/kg
Rhizome ginger	AFB1	1.73 µg/kg
	OTA	3.1 µg/kg

1 : Coffee 3 : chocolate (powder) 4 : Cumin 6 : Red pepper

2 : Tea 5 : Pepper 7: Ginger

A, B, C: The different origins of each sample: (AT : bulk from the Medina; B: in bulk from a large area; VS: in a bag)

11-13). According to Pitt and Hocking, the incidence of *Aspergillus*, like the genera *Fusarium* and *Penicillium*, increases in environments with high temperatures and low water activity, and where ideal conditions are found in the final stages of coffee processing during drying and during storage. The strong contamination observed can come from the contact of the coffee beans with the air and the ground, as well as from the ventilation conditions of the storage enclosure (14).

Regarding the contamination of tea samples, the molds found in our study were *Aspergillus niger* and *Penicillium*; *Cladosporium herbarum* has been found both in tea and in Chocolate powder where we also found the mucoral kind.

The contamination of the samples of the spices in our study made it possible to find several molds, in particular for the cumin which was the most contaminated especially

for the forms packaged in sachets, the molds found were *Aspergillus niger*, *A.fumigatus*, *A. flavus*, *A.nidulans*, *Penicillium sp*, *Beauveria* and *mucorales*. The form conditioned (in sachet) is the sample that contained the most mold (*Aspergillus niger*, *A.fumigatus*, *A. flavus*, *Penicillium sp*, *Beauveria et mucorales*), these findings were different from those found in previous studies in which the unconditioned forms had revealed the absence of molds, which had been explained by the possibility of the alteration of the antifungal activity during packaging, by the different nature of the cumin used, or by the possibility of post-contamination by the handlers(15).

For Pepper, *Aspergillus niger* and mucoral were detected in both packaged and loose forms in the medina, the latter also containing *penicillium*.

As for the samples of the Red Pepper, the presence of *Aspergillus niger* and mucoral was noted on the 3 types of samples as was the case for the samples of Ginger where the presence of mucoral was noted in all forms of ginger, and also of *Aspergillus niger* in conditioned ginger and in loose ginger (medina), the latter also experienced the presence of *penicillium* unlike loose ginger (large area) in which a new species was detected and which is *Paecilomyces variotii*.

However, in a work performed in Morocco (15), certain marketed spices (ginger and chilli) are fraudulently modified by the addition of other products such as wheat flour and an artificial coloring ensuring the normal color of the spice. The spices used as antioxidants and in traditional medicine, are endowed with preservative properties, they are produced by countries with a tropical or subtropical temperate and humid climate and experiencing heavy rainfall. Left on the ground in an ambient area during the drying phase, they are often contaminated after harvest, the conditions for mold growth and mycotoxin production being ideal.

In addition, the study carried out by Zineddine et al., on the incidence of aflatoxins in samples of spices marketed in Morocco, showed that chilli and ginger were more contaminated compared to pepper and cumin, however with a low contamination rate below international standards (16). This corroborates the conclusions reported by Tantaoui et al. who found that the growth of toxigenic mold strains of *A. flavus* was very low on curcumin, white pepper, and black pepper (17).

CONCLUSION

Food safety is the subject of particular attention by producers, governments and consumers, given the health risks for both humans and animals in which toxigenic fungi constitute a real danger, due to highly dangerous toxic substances which they secrete during their proliferation in foods of plant or animal origin. These mycotoxins with very variable physicochemical and toxicological compositions affect all stages of the food chain and can be sources in particular of carcinogenicity, genotoxicity, teratogenicity, hepatotoxicity and immunotoxicity.

The geographical position and the hot and humid climate of the Mediterranean rim and in Morocco more particularly, are all factors favoring the growth of molds and

the production of mycotoxins in coastal areas with a high concentration of the total population.

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