Advances in Bioscience and Clinical Medicine
ISSN: 2203-1413
www.abcmed.aiac.org.au

Original Article

# Prospective Study of Food Chain Mold Contamination in Rabat City

Maryem Iken[1]*, Noufissa Aloui[2], Leila Boumhil[3], Hafida Naoui[4], Badr Lmimouni[5]

[1]*Assistant professor,* [2]*Interne,* [3]*Specialist,* [4]*Professeur,* [5]*Professor, Parasitology and Mycology laboratory, Mohamed V military teaching Hospital, University Mohamed V Rabat, Morocco*

**Corresponding Author:** Maryem Iken, E-mail: bousiken@yahoo.fr

**ARTICLE INFO**

*Article history*
Received: March 21, 2021
Accepted: September 01, 2021
Published: October 31, 2021
Volume: 9 Issue: 4

Conflicts of interest: None.
Funding: None.

**Key words:**
Mold,
Food,
Aspergillus,
Mycotoxins

**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-MSMS. The results showed a variation in levelsof 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 breastfeeding, 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 Mohammed 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)

Published by Australian International Academic Centre PTY.LTD.

http://dx.doi.org/10.7575/aiac.abcmed.v.9n.4p.6

(**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.

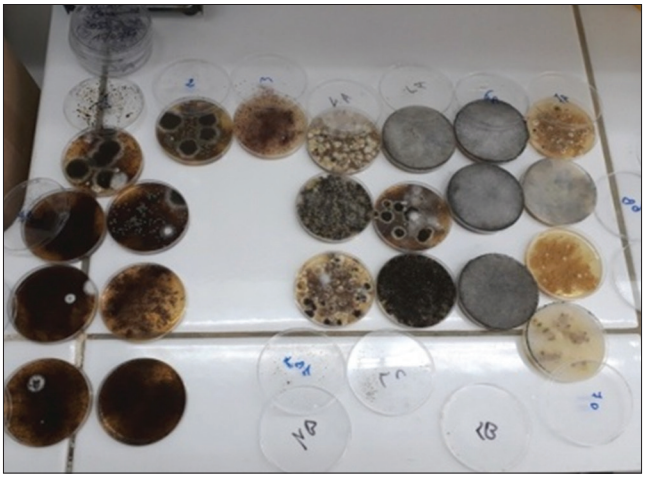


**Figure 1.** Mold present in the different samples after 7 days of incubation

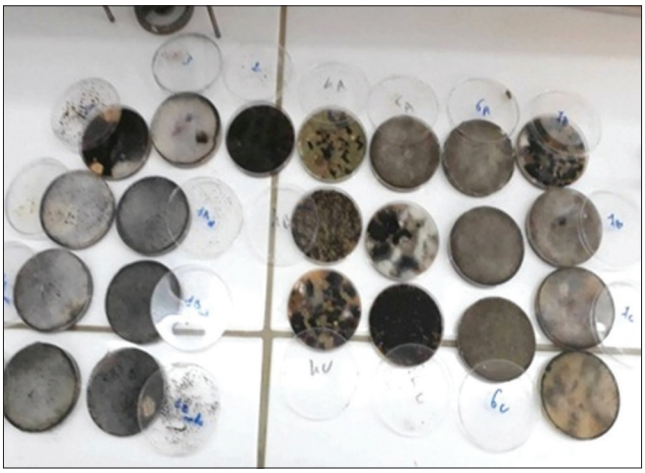


**Figure 2.** Mold present in the different samples after 10 days of incubation

## 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% (Mucoral 90% the most frequent, Cladosporium herbarum: 4.5%, beauveria: 4.5%, paecilomyces variotii: 4.5%) (Graphic 1), (Table 1).

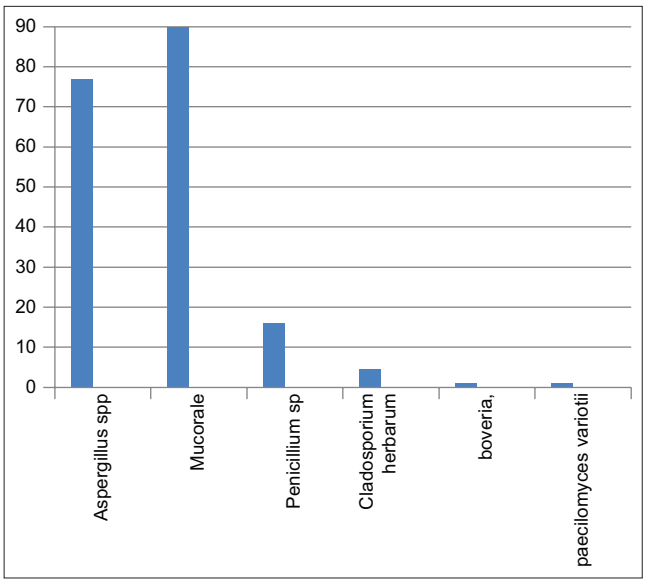
The results of the mycological study of all the samples made it possible to identify the fungal species found in the different samples taken and to perform the mycotoxin assay (Table1).

The foodstuffs were polycontaminated for ground coffee DU, coffee-caspule 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.




**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

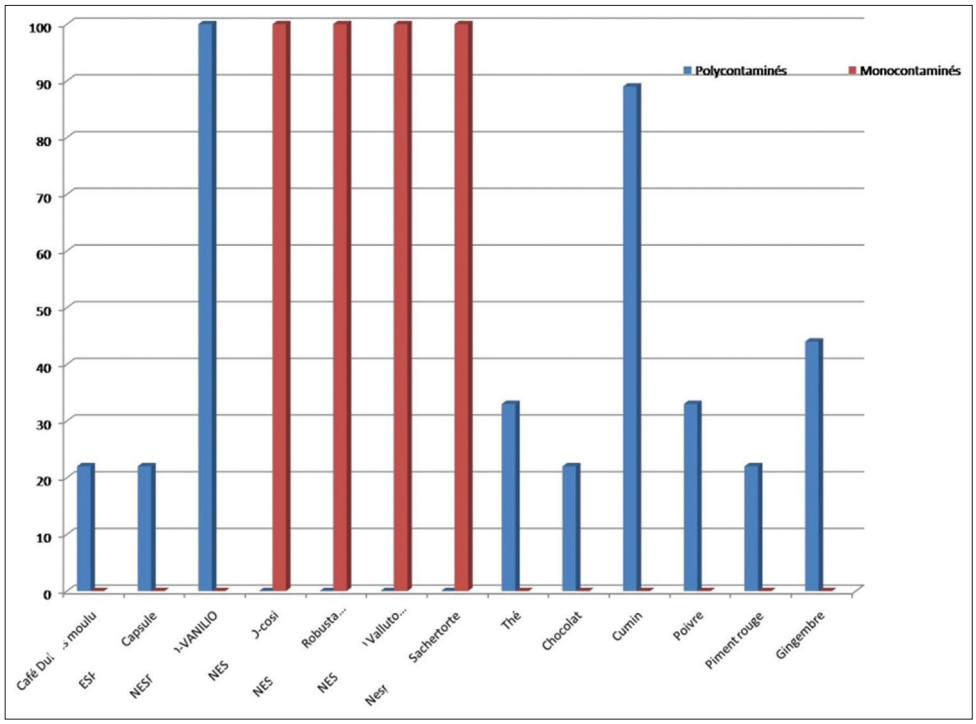
| Samples | The molds | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Asp Niger** | **Asp, flavus** | **Asp. fumigatus** | **Asp. nidulans** | **Peni-cillium SP** | **Muco-ral** | **Clado-sporium herbarum** | **Beau-veria,** | **paecilo-myces variotii** |
| Ground coffee DU | + | - | - | - | - | + | - | - | - |
| capsule ES | + | - | - | - | - | + | - | - | - |
| CapsuleNES 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** | **Peni-cillium Sp** | **Muco-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)

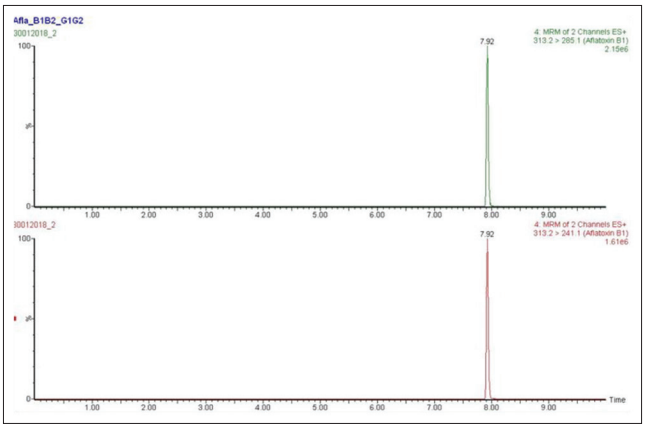
Mycotoxins, of which there are more than 300 types, constitute the toxic secondary metabolites which develop on the plant in the open field or during storage, in all regions of the world (1). The most common are aflotoxins, ochratoxins, trichothecenes, zeralenone, fumonisins, ergot alkaloids, citrine, 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).

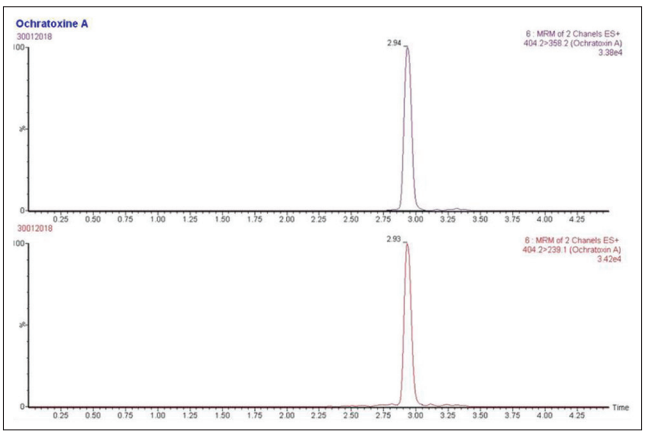
The direct economic consequences are the drop in the production yield of farm animals (contaminated by food), non-marketable food (deterioration of organoleptic




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




**Graphic 3.** Aflatoxins aspect on chromatogram




**Graphic 4.** Ocratoxine 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, Penicillum, 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 | 012 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 cumen which was the most contaminated especially for the forms packaged in sachets, the molds found were Aspergillus niger, A.fumigatus, A. flavus, A.nidulans, Penicillum sp, Beauveria and mucorales. The form conditioned (in sachet) is the sample that contained the most mold (Aspergillus niger, A.fumigatus, A. flavus, Penicillum 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.

**References**


1. Oliveri C, Catara V: Mycoflora and Biodiversity of Black Aspergilli in Vineyard Eco-Systems, in The Dynamical Processes of Biodiversity-Case Studies of Evolution and Spatial Distribution. InTech open. 2011; 259-276
2. Gimeno A, Martins ML: Micotoxinas, Micotoxicosis en animales y humanos. third Ed. Special nutriens. Miami. USA. 2011; 13-30
3. Richard JL et al: Mycotoxins: risks in plant, animal and human systems. CAST Task Force Report. 2003;139: p. 101-103.
4. Peraica M, Radica B, Lucica A, Pavlovica M: Toxic effects of mycotoxins in humans.Bulletin of the World Health Organization. 1999; 77 (9): p. 754-766.
5. Ruppol P, Delfosse P, Hornick J L: Contamination of the dairy sector by mycotoxins: a risk for public health in sub-Saharan Africa.Ann Med Vét. 2004; 148: p. 141-6.
6. Nganou D N: Mise au point d'outils moléculaires pour l'identification des flores fongiques ochratoxinogènes: application à la traçabilité du café Camerounais. Presse academique francophone. Ed Riga. Cameroun. 2016; 150-154.
7. Nielsen DS, Honhotl S, Tano-debra K, Jespersen L: Yeast populations associated with Ghanaian cocoa fermentations analyzed using denaturing gradient gel electrophoresis (DGGE).Yeast. 2005; 22 (4): p. 271-284.
8. Taniwaki MH, Hocking AD, Pitt JI, Fleet GH: Growth and mycotoxin production by food spoilage fungi under high carbon dioxide and low oxygen atmospheres.International journal of food microbiology. 2009; 132 (2-3): p. 100-108.
9. Silv C F, Schwan R F, Sousa Dias E S, Wheals A E: Microbial diversity during maturation and natural processing of coffee cherries of Coffea arabica in Brazil.International Journal of Food Microbiology. 2000; 60 (2-3): p. 251-260.
10. Noonim P, Mahakarnchanakul W, Nielsen K, Frisvad J, Samson R: Isolation, identification and toxigenic potential of ochratoxin A-producing Aspergillus species from coffee beans grown in two regions of Thailand.International Journal of Food Microbiology. 2008; 128 (2): p. 197-202.
11. Suárez-Quiroz M, González-Rios O, Barel M, Guyot B, Schorr-Galindo S, Guiraud JP: Study of ochratoxin A, producing strains in coffee processing.International journal of food science & technology. 2004; 39 (5): p. 501-507.
12. Martins ML, Martins HM, Gimeno A. Incidence of microflora and of ochratoxin A in green coffee beans (Coffea arabica). Food Addit Contam. 2003; 20(12):1127-31. doi: 10.1080/02652030310001620405.
13. Leong SL, Hocking AD, Scott ES: Aspergillus species producing ochratoxin A: isolation from vineyard soils and infection of Semillon bunches in Australia. J Appl Microbiol. 2007; 102(1):124-33. doi: 10.1111/j.1365-2672.2006.03056.x.
14. Pitt J I, Hocking AD: Fungi and food spoilage. Flight. Springer. 2009; 519.
15. Zinedine A, Idrissi L: Presence and regulation of mycotoxins in food in Morocco: Current situation and perspectives. 2004; Doctoral thesis. Sidi Mohammed Ben Abdallah University. www. technolabo.my.
16. Zinedine A, Juan C, Soriano J M, Moltó J C, Idrissi L, Mañes J: Limited survey for the occurrence of aflatoxins in cereals and poultry feeds from Rabat, Morocco. International Journal of Food Microbiology. 2007; **115**(1): p. 124-127.
17. Tantaoui-Elaraki A, Riba A, Oueslati S, Zinedine A: Search for mycotoxins in foodstuffs distributed in Morocco.Moroccan Journal of Agronomic and Veterinary Sciences. 2011; 14 (3): p. 11-16.