Fungal Contaminants of Spices Used in Tunisia

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Abstract

Spices are used extensively not only to flavor foods but also for their medicinal properties. To date, no data are available regarding the nature of the toxigenic fungal species that may contaminate these products marketed in Tunisia. The aim of this study was to investigate the natural occurrence of fungi in commonly consumed spices collected from different military catering. A total of 213 samples representing five kinds of spices (Curcuma, red pepper, coriander, caraway and cumin) were tested for the total fungal count following a standardized dilution method. Fungal isolates were identified according to morphological and microscopic characteristics. Fungal contamination showed a large variation, about 88 % of total spices samples were infected with fungi. The highest level of contamination ($\geq 5 \log_{10}$ CFU/g) was observed in red pepper and caraway samples. Three fungal genera were identified: *Aspergillus spp* (36.1%), *Mucor spp* (28.2%) and *Penicillium spp* (1%). *Aspergillus* genus was present in 50% of curcuma samples and 48% of coriander samples while it was too low in cumin samples with 16.6%. A total of 95 strains of five *Aspergillus* species were identified with the dominance of *Aspergillus niger* (61%) and *Aspergillus flavus* (30%). *Aspergillus niger* were present in all spices kinds while *Aspergillus flavus* prevalence was 32% in curcuma samples, 12% in coriander samples, 10% in red pepper samples and less present in cumin and caraway samples (0.5%).

Keywords

Spices, Fungi, *Aspergillus*, Tunisia

1. Introduction

Spices are natural products obtained from parts of several plant species, they are widely used in food preparation. In Tunisia, as in all Mediterranean countries, spices are frequently used to flavor foods but also for their medicinal properties. As with many other agricultural products, spices and herbs may be exposed to a wide range of microbial contamination during pre- and post-harvesting. Moreover, the contamination may occur during processing storage, distribution, sale and/or use [1]. Fungi are considered to be the main contaminants of spices [2]. In addition, because of possible neglects during processing, food containing spices are more likely to be deteriorated and could be toxic to humans due to mycotoxins that are produced by some fungal species [3]. Spices are frequently heavily contaminated with xerophilic storage moulds and bacteria [4-5]. *Aspergillus* and *Penicillium* are the most frequent fungal contaminants of spices [3]. Other species of moulds are frequently isolated from spices, including *Scopulariopsis* and *Sporendonema* [6]. *Aspergillus*, *Penicillium*, and *Fusarium* genera are the most common fungi contaminant of spices [3]. In Tunisia, there is not yet any work which is interested in the study of the fungal flora which contaminates spices but rather there are many publications about mycotoxins in spices.

Ghali *et al.* [7] reported that the contamination frequency of Ochratoxin A in spices commercialized in Tunisia such as, red pepper, cumin and black pepper was 57.1%. More recently, they found that about 69.2% of spice samples contained aflatoxins with a high occurrence of Aflatoxin B1 [8].

Therefore, the aim of this study was to investigate the natural occurrence of toxigenic fungi in commonly consumed spices in Tunisia and give baseline information on their safety and to help us conduct further studies.

2. Materials and Methods

2.1. Sampling

Samples from five types of spices (curcuma, red pepper, coriander, caraway and cumin), widely used in Tunisian food preparation were collected. A total of 213 dried and ground spices samples from different military caterings covering the whole country were collected in sterile identified polyethylene bags and then placed in clean containers of inert material, protected against contamination and damage resulting from transport.

2.2. Mycological study

2.2.1. Total moulds counts

All media and reagents were purchased from Biokar Diagnostics (Allonne, France). Serial dilutions method, according to the standard [9] was used to determine total fungal counts on Sabouraud chloramphenicol Agar medium (Peptic digest of Meat 10 g/l, Glucose 20 g/l, Chloramphenicol 0.5 g/l, Bacteriological agar 15 g/l, pH of the ready-to-use media at 25°C: 5.7 ± 0.2). Ten grams of each sample were added to 90 ml of Tryptone Salt Broth (Tryptone 1 g/l, Sodium chloride 8.5 g/l, pH of the ready-to-use media at 25°C: 7.0 ± 0.2) in 250 ml sterile glass bottle with sterile glass beads of 5 mm diameter to facilitate the manual homogenization for 30 min. The spice-water suspension was allowed to stand for 10 min with intermittent shaking before being plated then tenfold serial dilution were prepared. Petri dishes containing 15 ml of Sabouraud medium were surface inoculated with 100 μ L portion of each dilution in duplicates. The serial dilution method was used to determine total fungal counts. Plates were incubated at $25 \pm 1^\circ\text{C}$ for 7 days and examined for the growth of moulds daily. Fungal colonies were examined visually and microscopically and colonies counted as colony forming units per gram (CFU/g). Pure colonies of fungal isolates were obtained by sub-culturing on similar media at $28 \pm 1^\circ\text{C}$ and were characterized based on their macroscopic and microscopic characteristics. The fungal isolates were transferred to sterile plates for purification and identification, only fungal species with medical significance were identified.

2.2.2. Fungal contamination levels

Scale with five levels of contamination was established which covers a range from Level 1 corresponding to a total fungal count $< 2 \log_{10}$ CFU/g to level 5 corresponding to a total fungal count $> 5 \log_{10}$ CFU/g (Table 1).

3. Results

A total of 213 samples of five different spices samples were analysed for fungi contamination by dilution plating. The majority (88%) of spices samples were contaminated with at least one fungus. All red pepper samples were contaminated with fungi (Figure 3). The highest level of fungal contamination (level 5) was observed in six samples of red pepper and one sample of coriander (Table 1, Figure 1). The level 3 of contamination was frequent in the samples of red peppers, caraway and coriander which present (30 %) of total samples (Figure 1). Cumin samples are the less contaminated by fungi since 38 % of samples were at level 1. Three principal fungal genera were identified namely: *Aspergillus spp* (36%), *Mucor spp* (28%), *Penicillium spp* (1%) (Figure 2). *Aspergillus* was frequent in curcuma (50%), coriander (46%), caraway (39%) and red pepper (26%) of samples, while it was low in cumin samples (16.6%) (Figure 3). However, *Penicillium* was detected only in tow samples of red pepper and caraway (1%) (Fig.3). A total of 95 strains of five *Aspergillus* species were identified: *Aspergillus niger* (58 strains), *Aspergillus flavus* (29 strains), *Aspergillus nidulans* (5 strains), *Aspergillus versicolor* (2 strains) and *Aspergillus terreus* (1 strain) (Table 2). *Aspergillus niger* and *Aspergillus flavus* were the most frequent species with respectively 60% and 30% of total identified species. *Aspergillus niger* was present in coriander and caraway (36%), in curcuma (32%), in red pepper (26%) while it was low in cumin (14%) (Figure 4). On the other hand, *Aspergillus flavus* was highly present only in curcuma (32%), coriander (12%) and red pepper (10%), while it was less present in cumin and caraway samples (0.5%).

Figure 1. Distribution of fungal contamination levels by kinds of spices.

Figure 2. Main fungal genera present in spices samples.

Figure 3. Identification of fungal flora present in spices samples.

Figure 4. Contamination of spices samples by *Aspergillus flavus* and *Aspergillus niger*.

Table 1. Scale used to classify total fungal contamination into five levels

Contamination levels	L1	L2	L3	L4	L5
Fungal counts (CFU/g)	$<2 \log_{10}$	$\geq 2 \log_{10} < 3 \log$	$\geq 3 \log_{10} < 4 \log$	$\geq 4 \log_{10} < 5 \log$	$\geq 5 \log_{10}$

Table 2. Number of *Aspergillus* species isolated from spices samples

Isolated <i>Aspergillus</i> species					
Spices	<i>A. flavus</i>	<i>A. niger</i>	<i>A. nidulans</i>	<i>A. terreus</i>	<i>A. versicolor</i>
Curcuma	16	16	3	1	1
Red pepper	4	10	0	0	0
Coriander	5	16	2	0	1
Caraway	2	13	0	0	0
Cumin	2	3	0	0	0
Total	29	58	5	1	2

4. Discussion

The high contamination of spices with fungi was given by several researchers such as Punam and Dhiraj [10] who revealed the wide range of fungal contamination in spices. As well as the high fungal contamination of red pepper which was reported by Bokhari [11] when the red pepper samples were relatively the richest in total number of fungi (average of 5 samples was 2,682.4 colonies per g fresh weight) and those reported by Azzoune *et al.* [12] that the most contaminated spices are ginger, sweet pepper, red pepper, sweet cumin and saffron.

30% of total samples were at the Level 3 of fungal contamination ($\geq 3 \log_{10} \leq 4 \log_{10}$ CFU /g) (Figure 1). This level was usually reported [13]. Recently, Makhlof *et al.* [14] found that the overall mean fungal contamination was 7.6×10^3 CFU/g, and most spices displayed a moderate mean fungal load that ranged from 10^3 to 4×10^3 CFU/g.

38% of cumin samples are at level 1 of fungal contamination which is similar to results reported by Bhat *et al.* [15] that no fungi were found in the cumin seed samples sold in retail shops in the city of Bombay. One year later, Garrido *et al.* [16] evaluated the fungi contaminated of 33 different commercial spices and reported that only nutmeg, anise, tarra-gon and cumin showed very low fungal contamination. This result was later confirmed by Azzoune *et al.* [12] when they found that aniseed, black pepper, coriander, caraway, cumin and cinnamon, consumed in Algeria, are relatively less contaminated by fungi.

Three principal fungal genera were identified: *Aspergillus* spp, *Mucor* spp, *Penicillium* spp. Our results were consistent with those reported by Azzoune *et al.* [12] that the commonly isolated fungi were species of *Aspergillus* (56.4%), *Penicillium* (25.1%), *Mucor* (12.8%) and *Eurotium* (5.7%). Confirmed later by Makhlof *et al.* [14] when identification of the fungal genera found in spices showed that *Aspergillus* was the most frequent genus (76%), followed, in decreasing order, by *Mucorales* (36%), *Penicillium* (30%) and *Fusarium* (7.5%).

Srivastava and Chandra [17] studied the mycobiota of 4 kinds of spices (coriander, cumin, fennel and fenugreek) in India and reported that *Aspergillus* followed by *Fusarium* were the most frequent genera. Bhat *et al.* [15] tested the microbial profile of cumin seeds and chilli powder sold in retail shops in the city of Bombay and reported that *Aspergillus* was the predominant genus in chilli powder samples. Also, they reported that no fungi were found in the cumin seed samples examined. El-Kady *et al.* [18] isolated 38 genera and 81 species from 120 samples of 24 kinds of spices collected from different places at Assiut Governorate, Egypt. They reported that the predominant genera were *Aspergillus* (25 species) and *Penicillium* (7 species) of which *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum* and *Penicillium corylophilum* were the most commonly occurring.

The incidence of *Penicillium* genus in studied spices was too low (1%) (Figure 3) comparing with results reported by Azzoune *et al.* [12] and Makhlof *et al.* [14] when *Penicillium* incidence was 25.1% and 30% respectively.

The dominance of both *Aspergillus niger* and *Aspergillus flavus* species with respectively 60% and 30% of prevalence in all spices samples was in agreement with results found by Aiko and Mehta [19] when *Aspergillus niger* was found to be the most commonly occurring contaminant and was isolated from 42 (66.66%) samples. Same results reported by Hashem and Alamri [20], when *Aspergillus niger* and *Aspergillus flavus* had the highest occurrence remarks and emerged between 93% and 46.7% of samples, respectively. However, Garrido *et al.* [16] reported that *Aspergillus flavus* was found to be a predominant component of the spices mycobiota. El Mahgubi *et al.* [21] also observed a wide-

spread contamination of paprika, cumin and pepper marketed in Morocco by *Aspergillus flavus* with 57% of isolates.

Aspergillus flavus specie was less present in cumin and caraway samples (0.5 %). This can be explained by the effect of the matrix composition which can promote the growth of *Aspergillus flavus* in the turmeric, coriander and red pepper samples, and inhibit it in the caraway and cumin samples. Studies have revealed that secondary metabolites of many plants are able to prevent the growth of fungi [22]. Moghadam *et al.* [23] indicated that Cinnamon, Clove, Thyme, Zataria, Cumin and Caraway essential oils have antifungal activity against *Aspergillus ochraceus*.

5. Conclusion

Mycological study carried out on 213 samples, representing five kinds of spices (Curcuma, red pepper, coriander, caraway and cumin) show that fungal contamination was very high. *Aspergillus* was the most frequent genus detected with the dominance of two species *Aspergillus niger* and *Aspergillus flavus*. Cumin samples are the least contaminated by fungi including *Aspergillus* genera which can be explained by the ability of this spice to inhibit fungi.

Following these results, other additional studies will be carried out such as the search for mycotoxins in these spices, the study of the antifungal activity of cumin and the explanation of the low incidence of the *Penicillium* genera in the spices consumed in Tunisia.

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References

- [1] McKee, L. H. (1995). Microbial contamination of spices and herbs: A review. LWT—Food Science and Technology, Volume 28, Issue 1, 1-11.
- [2] Kneifel, W. and Berger, E. (1994). Microbial criteria of random samples of spices and herbs retailed on the Austrian market. J. Food Prot., 57, 893-901.
- [3] Kocić-Tanackov, S. D., Dimić, G. R., and Karalić, D. (2007). Contamination of spices with moulds potential producers of sterigmatocystine. Acta Periodica Technologica, (38), 29-35.
- [4] Dimić, G., Škrinjar, M., Došen-Bogićević, V. (2000). Moulds, potential producers of sterigmatocystin in spices. Tehnologija Mesa, 2000, Vol. 41, No.4/6, pp. 131-137 ref.31.
- [5] Romagnoli, B., Menna, V., Gruppioni, N., and Bergamini, C. (2007). Aflatoxins in spices, aromatic herbs, herbs-Teas and medicinal plants marketed in Italy. Food Control, 18: 697-701.
- [6] Zinedine, A., Brera, C., Elakhdari, S., Catano, C., Debegnach, F., Angelini, S., De Santis, B., Faid, M., Benlemlih, M., Minardi, V., and Miraglia, M. (2006). Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. Food Control, 17: 868-874.
- [7] Ghali, R., Hmaïssia-khlifa, K., Ghorbel, H., Maaroufi, and Hedili, A. (2008). Incidence of aflatoxins, ochratoxin A and zearalenone in tunisian foods. Food Control, Volume 19, Issue 9, pp. 921-924.
- [8] Ghali, R., Hmaïssia-khlifa, K., Ghorbel, H., Maaroufi, K., and Hedili, A. (2010). Aflatoxin determination in commonly consumed foods in Tunisia. Journal of the Sciences of Food and Agriculture, 90: 2347-2351.
- [9] Norme Française, N. F. (2002). Microbiology of food animal feeding stuffs—Enumeration of yeasts and moulds by colony-count technique at 25 °C - Routine method. NF.V08-059 November2002.
- [10] Punam Jeswal and Dhiraj Kumar. (2015). Mycobiota and Natural Incidence of Aflatoxins, Ochratoxin A, and Citrinin in Indian Spices Confirmed by LC-MS/MS. International Journal of Microbiology, v. 2015, p. 8.
- [11] Bokhari, F. M. (2007). Spices mycobiota and mycotoxins available in Saudi Arabia and their abilities to inhibit growth of some toxigenic fungi. Korean Soc. Mycol., 35(2): 47-53.
- [12] Azzoune, N., Mokrane, S., Riba, A., Bouras, N., Carol, V., Sabaou, N., and Mathieu, F. (2015). Contamination of common spices by aflatoxigenic fungi and aflatoxin B1 in Algeria. Quality Assurance and Safety of Crops & Foods, Wiley-Blackwell, 2015, 8(1), pp. 137-144.
- [13] Dimić, G. R., Kocić-Tanackov, S. D., Tepić, A. N., Vujičić, B. L., and Šumić, Z. M. (2008). Mycopopulation of spices. Acta Periodica Technologica, (39), 1-9.
- [14] Makhoulouf, J., Carvajal-Campos, A., Querin, A., Tadrist, S., Puel, O., Lorber, S., and Bailly, S. (2019). Morphologic, molecular and metabolic characterization of *Aspergillus* section Flavi in spices marketed in Lebanon. Scientific Reports, 9(1), 1-11.
- [15] Bhat, R., Geeta, H., and Kulkarni, P. R. (1987). Microbial profile of cumin seeds and chilli powder sold in retail shops in the

- city of Bombay. *J. Food Protect.*, 50: 418-419.
- [16] Garrido, M. D., Jordano, R., Martinez, P., Jordal, M., and Pozo, R. (1988). Fungal contamination of commercial spices. *Alimentaria*, 25(189), 81-84.
- [17] Srivastava, R. K. and Chandra, S. (1985). Studies on seed mycoflora of some spices in India: Qualitative and quantitative estimations. *International Biodeterioration*, 21: 19-26.
- [18] El-Kady, S., El-Maraghy, S. M., and Mostafa, E. M. (1992). Contribution of the mesophilic fungi of different spices in Egypt. *Mycopathologia*, 120: 93-101.
- [19] Aiko, V. and Mehta, A. (2016). Prevalence of toxigenic fungi in common medicinal herbs and spices in India. *3 Biotech*, 6(2), 159.
- [20] Hashem, M. and Alamri, S. (2010). Contamination of common spices in Saudi Arabia markets with potential mycotoxin-producing fungi. *Saudi Journal of Biological Sciences*, 17(2), 167-175.
- [21] El Mahgubi, A., Puel, O., Bailly, S., Tadriss, S., Querin, A., Ouadia, A., Oswald, I. P., and Bailly, J. D. (2013). Distribution and toxigenicity of *Aspergillus* section *Flavi* in spices marketed in Morocco. *Food Control*, 32: 143-148.
- [22] Ynalvez, R. A., Compean, K. L., Addo-Mensah, A. (2018). Qualitative Determination of the Secondary Metabolites and Evaluation of the Antimicrobial Activity of Leaf Extracts from Different Plant Families (Boraginaceae, Fabaceae, Lamiaceae and Lauraceae) against Microorganisms of Clinical Importance. *Journal of Pharmaceutical Research International*, 2018, 1-12.
- [23] Moghadam, Z. A., Hosseini, H., Hadian, Z., Asgari, B., Mirmoghtadaie, L., Mohammadi, A., Shamloo, E., and Javadi, N. (2019). Evaluation of the antifungal activity of cinnamon, clove, thymes, zataria multiflora, cumin and caraway essential oils against Ochratoxinogenic *Aspergillus ochraceus*. *Journal of Pharmaceutical Research International*, v. 26, n. 1, p. 1.