Mycobiota and Mycotoxins of Nuts and Some Dried Fruits from Saudi Arabia

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Abstract: Twelve samples of edible nuts and dried fruits purchased from markets in Mekka and Al-Dawadmy regions were mycologically analyzed. Using two types of solid media namely dichloran rosebengal chloramphenicol (DRBC) and dichloran 18% glycerol (DG18) it was possible to isolate 23 fungal species belonging to 12 genera. Common fungi included *Aspergillus flavus*, *A. niger* and *Penicillium citrinum*. Other fungi such as *A. fumigatus*, *A. terreus*, *A. sydowii*, *Eurotium amstelodami*, *Paecilomyces variotii* and *Trichoderma harzianum* were moderately encountered. Out of 40 strains of *Aspergillus* 16 (40%) were able to produce mycotoxins. Aflatoxins B1 and B2 were produced by 8 out of 20 *A. flavus* strains (100 – 600 µg/Liter of culture medium). These toxins in addition to Aflatoxins G1 and G2 were produced by one isolate of *A. parasiticus* (200 µg/L). Ochratoxin A was extracted from cultures of *A. niger*, *A. barasiliensis*, *A. aculeatus* and *A. sclerotioniger* (100-200 µg/L). Fortunately, no aflatoxins or ochratoxins were detected in chloroform extracts of nuts and other dried fruits.
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1. Introduction

Worldwide, nuts are esteemed and highly priced food delicacy because of their pleasant taste and flavor in addition to their content of proteins and antioxidants. Different kinds of edible nuts including almonds, peanuts, hazelnuts, pistachios, walnuts and cashew in addition to many dried fruits are frequently imported to Saudi Arabia for consumption by citizens and visitors. Nuts are among the crops that can be contaminated by mycotoxins which represent a major problem in several countries, North America (Bhatnagar *et al*., 2004), Brazil (Pacheco *et al*., 2010), Asia (Pitt and Hocking, 2004; Bonjar, 2004) and Africa (Bankole *et al*., 2006). Aflatoxins are a group of secondary metabolites produced mainly by *A. flavus* and *A. parasiticus* (Kurtzman *et al.*, 1987) in several crops. They are potent hepatotoxins and their carcinogenicity has forced governments and regulatory agencies to set very low tolerance levels in food (Van Egmond, 2002). In the European Union (EU Commission Regulation, 2006), the lowest maximum limit for aflatoxin B_1 other than for products for infants is set as 2.0 µg/kg for products such as groundnuts (peanuts), tree nuts, dried fruit and its processed products, cereals and products derived from cereals, including processed cereal products. The highest maximum limit for aflatoxin B_1 is 12.0 µg/kg for food stuffs such as almonds, pistachios and apricot kernels. With regards to the total aflatoxins, the lowest EU limit other than food for infants is 4.0 µg/kg for products such as groundnuts (peanuts) and tree nuts, dried fruit and its processed products, cereals and products derived from cereals, including processed cereal products. While the highest limit is set for groundnuts, almonds, pistachios, apricot kernel, hazelnuts and Brazil nuts at 15.0 µg/kg (these limits apply to the products to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs). As mentioned in Naturland (2000) brazil nuts are one of the foodstuffs that must be examined for a possible aflatoxin infestation. If more than 4 microgram/kg of aflatoxin B1, B2, G1 and G2 are measured, or the aflatoxin B1 content alone exceeds 2 µg, the nuts can no longer be sold. One problem for the producers is that the fungus responsible for causing aflatoxin can infiltrate the hard outer shell to infest the nut without being noticeable from the outside. Moreover, very few laboratories exist in the growing regions, which would be capable of monitoring the harvest before it is actually shipped. The only way of ensuring that aflatoxin cannot be produced is to strictly adhere to the proper measures for collecting, storage and transport, as well as adopting a careful and hygienic method of processing (Naturland ,2000).

2.Material and Methods

Collection of Samples:

Twelve samples of dried fruits and seeds (250 grams each) were collected from different markets in Mekka and Al-Dawadmy, Saudi Arabia. Samples included bean seeds, corn grains, powdered coffee, cardamom fruits, pistachio, hazelnut and dates

Isolation media:

Two types of media were chosen for the mycological analysis of the dried fruits as

recommended by Pitt and Hocking (2009) and Samson *et al*. (2004). These media are:

Dichloran Rosebengal Chloramphenicol agar (DRBC):

The medium contains (gram/liter): Peptone 5 gm., Glucose 10 gm., KH_2PO_4 1gm, $MgSO_4.7H_2O$ 0.5 gm., Dichloran (2, 6-dichloro-4-nitroaniline) solution (0.2% (w/v) in ethanol). 1ml , Rose Bengal 0.025 gm., Chloramphenicol 0.1 gm., Agar 15 gm. , Distilled water 1000 ml. Final pH should be 5.6. Ingredients are mixed, heated to dissolve agar and sterilized by autoclaving at 121°C for 15 min. The medium is allowed to cool to 45 ± 1°C in a water bath prior to pour plating. DRBC agar is especially useful for analyzing sample containing "spreader" molds (e.g. *Mucor*, *Rhizopus*, etc.), since the added dichloran and rosebengal effectively slow down the growth of fast-growing fungi, thus readily allowing detection of other yeast and mold propagules, which have lower growth rates.

Dichloran 18% Glycerol agar (DG18):

It comprises (gram/liter): Peptone 5 gm. , Glucose 10 gm., KH2PO4 1 gm. , $MgSO_4.7H_2O$ 0.5 gm., Dichloran (0.2% in ethanol) 1ml , Glycerol 220 gm., Chloramphenicol 0.1 gm., Agar 15 gm., distilled water 1000 ml. The medium was autoclaved at 120°C for 20 min. The final pH was adjusted to 5.6. This medium is used as a general purpose mold enumeration medium and is preferred when the water activity ($a_{w)}$ of the analyzed food is 0.95 or lower. The low water activity of this medium reduces interference by bacteria and fast-growing fungi.

Mycological analysis:

The dilution plate method described in Pitt and Hocking (2009) was employed for this purpose. Proper dilution rate was ranging from 1/20 to 1/200 (Weight/volume). Cultures in 5 replicates per sample were prepared and incubated at 28^0C for 7-10 days after which the growing fungal colonies were counted, identified and isolated in pure cultures.

Identification of fungal cultures:

Macro- and microscopic morphological features were used to identify the different fungal isolates contaminating the dried food samples. The following references were consulted to check correct identification: Raper and Fennell (1965), Pitt (1979), Moubasher (1993), de Hoog *et al*. (2000), Samson *et al*. (2004) and Pitt and Hocking (2009).

Determination of aflatoxins and ochratoxins:

Extraction of toxins from nuts and dried fruits:

The natural occurrence of mycotoxins in the different samples of nuts and dried fruits was estimated according to methods recommended by AOAC (1984). Toxin extraction was performed using chloroform : water (10:1 v/v) mixture. The obtained crude extracts were purified by column chromatography containing anhydrous sodium sulphate (15 g) and silica gel (10 g). Defatting of samples was done with n-hexane. Extracts were air dried and kept in dark vials till chromatographic analysis.

Extraction of mycotoxins from fungal cultures:

The different strains of Aspergillus Section Flavi (*A. flavus* and *A. parasiticus*) and Section Nigri (*A. brasiliensis*, *A. niger* and *A. sclerotioniger*) were cultivated in autoclaved Potato dextrose broth (50 ml/flask) for 7 days at 28^0C under stationary conditions. For each fungal strain the mycelium was homogenized using liquid nitrogen and remixed with the flask contents (broth medium with possibly secreted toxins). Mycotoxin extraction was done as mentioned above and the purified dry extract was kept in dark vials till chromatography.

Qualitative estimation of aflatoxins:

Thin layer chromatography (TLC) is one of the most widely used separation techniques in mycotoxin analysis. Since 1990, it has been considered as the AOAC (1984) official method of choice to identify and quantitate aflatoxins at levels as low as 1 ng /g. Precoated silica gel plates type 60 f254 TLC, (E, Merk, Germany) were used. Rectangular glass jar (30x15x30 cm) was used for developing chromatoplates. A suitable volume of solvent mixture (chloroform: methanol, 97:3 v/v) was placed in the bottom of the jar so that the starting spots on the plates would be 1 cm above the upper surface of the solvent mixture. Chromatographic plates (20x20 cm) were activated by heating 1 h at 120°C in a hot air oven, and removed immediately to a desiccator to cool. Parallel starting spots, 2 cm from each side of the plate and 1.5 cm apart, were made with micropipets from chloroform extracts with authentic reference aflatoxins and ochratoxins. Spots were left to dry in air. Prepared plates were then transferred to the chromatographic jar, developed to a suitable distance (10 cm), and removed. The solvent front was marked and the plates were dried in air. Spots were viewed under UV light (366 nm) and the outline of each fluorescent spots was marked by sharp pin.

Rf values, colors, and intensities of the unknown spots were compared with those of the authentic reference mycotoxins (El-Bazza *et al*., 1982).

Quantitative Determination of Aflatoxins and ochratoxins

The dilution-to-extinction (Coomes *et al*., 1965) and comparison of standards (AOAC, 1984) techniques were used for estimation of aflatoxins concentrations.

3. Results and Discussion

Fungi contaminating nuts and dried fruits

The mycological analysis of nuts and dried fruits samples revealed the isolation of 23 fungal species belonging to 12 genera on both DRBC and DG18 as shown in tables (1, 2 and 3). Unidentified sterile mycelia were also obtained from coffee and cardamom samples. The number of fungal species was markedly higher on DRBC than on DG18 (500 versus 306 colonies per gram in all samples). However the spectrum of isolated fungal species was slightly lower on the former medium than on the latter (16 versus 19 species)

On DRBC medium, the broadest spectrum of fungal species was obtained from cardamom fruits (11 species) with the main contaminants (based on counts) being *A. flavus*, *A. terreus*, *Penicillium citrinum* and *P. islandicum*. Powdered coffee was also rich in fungal species (9 species) with *A. flavus*, *A. niger*, *A. terreus* and *Paecilomyces variotii* being the major contaminants (Table 1).

On both isolation media, *Aspergillus* was the most dominant genus. It was contaminating all nut and dried fruit samples contributing 82 % and 61.1% of the total fungal counts on DRBC and DG18 respectively (Table 3). On DRBC medium the highest count of *Aspergillus* was obtained from pistachio husks (86 colonies/gram) which were heavily contaminated with *A. flavus* (80 colonies/gram) as shown in table (1).

The genus *Aspergillus* was represented by 8 species of which *A. flavus* and *A. niger* were the commonest. *A. flavus* was found contaminating all samples analyzed on DRBC contributing 48.4% of *Aspergillus* count and 39.6% of total fungal population. On DG18 *A. flavus* was observed on 66.6% of samples matching 38.5% and 23.5% of *Aspergillus* and total fungal count respectively (Table 3).

Aspergillus niger appeared on 100% and 75% of samples matching 27.0% and15.7% of total fungal population on DRBC and DG18 respectively. *A. niger* was found in relatively high counts on cashew and dates.

Aspergillus fumigatus was more frequently isolated on DRBC than on DG18 (66.6% versus 25%) sharing in the fungal count with 4.6% and 3.26% respectively.

Aspergillus terreus was moderately encountered (3 samples on each medium) appertaining to 9.4% and 14.1% of total fungal count on DRBC and DG18 respectively. *A. terreus* appeared in relatively high counts on corn grains (32 colonies/gram) but its count was as low as 8 and 7 colonies on coffee and cardamom respectively.

Aspergillus sydowii occurred generally in low counts on both isolation media and was less frequent on DG18(8.3% of samples) than on DRBC (25%).

The remaining *Aspergillus* species (*A. candidus*, *A. ustus* and *A. wentii*) appeared in low counts as well as in low incidence on DG18 and were completely missed on DRBC.

The osmophilic fungus *Eurotium amstelodami* was moderately encountered on DG18 (25% of samples) contributing 10.5% of total fungi.

Paecilomyces variotii occurred in low counts on 25% of samples cultured on DRBC but it was less frequent on DG18.

Penicillium was highly encountered on both medium types (50 – 58% of samples) matching 11.6 and 23.5% of total fungi on DRBC and DG18 respectively. Among the 4 *Penicilllium* species identified, *P. citrinum* was the basic constituent with counts and incidences close to its genus.

Trichoderma harzianum a in low counts on 41.6% and 25% of samples cultured on DRBC and DG18 respectively.

The remaining fungal genera and species were less frequently isolated from the different nut and dried fruit samples as shown in table (1-3)

It is worthy to mention that data of the present investigation confirm to a great extent the earlier reports on fungi contaminating edible nuts in Saudi Arabia. Differences in the fungal counts and composition of genera and species among samples are expected due to variations in geographical localities from which nut samples were collected. Types of isolation media, techniques for mycological analysis and the number of samples tested are all factors leading to variations in the final reports on these fungi. Zohri and Abdel-Gawad (1993) recorded a wide range of moulds representing several genera and species from 5 seed samples of each almond, cashew nut, chestnut, hazelnut, pistachio nut and walnut collected from different markets in Ar'Ar,. The total counts of fungi were widely fluctuated between 1960-7704 and 1948-7434 colonies/g dry seeds on glucose-Czapek's and glycerol agar media respectively. During that study 20 genera, 53 species and 2 varieties of fungi were isolated. The prevalent fungi on the 2 agar media were *Aspergillus flavus*, A. *niger* and *Penicillium chrysogenum*. On glucose-Czapek's agar, *Rhizopus stolonifer* and *Aspergillus flavus* var. *columnaris* were isolated from all 6 kinds of nut, *A. parasiticus* from 5 kinds and *A. fumigatus* from 4 kinds with high frequencies. *Eurotium* species were completely absent on glucose-Czapek's agar but they were isolated in high frequency from all kinds of nut on glycerol agar medium.

Date fruits analyzed in the present study produced only four species of *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus*) which often occurred in low counts (Tables 1 &2). Gherbawy *et al*. (2012) studied the mycological profile of the retail date fruits distributed in different markets at Taif, Saudi Arabia in addition to the presence of aflatoxins and ochratoxin A. They isolated 22 fungal species belonging to 12 genera from 50 different date samples. *Aspergillus flavus*, *A. niger*, *Penicillium chrysogenum*, and *Rhizopus stolonifer* were the most prevalent species among isolated fungi. Toxicity test using Artimia larvae indicated that seven out of 18 isolates of *A. flavus* had aflatoxins potentials, while nine out of 36 isolates of *A. niger* were ochratoxigenic.

In the present work cashew nuts were contaminated with eight fungal species of which *A. flavus*, *A. niger* and *P. citrinum* occurred in relatively high counts. Nearly similar findings were reported from Brazil by Freire and Kozakiewicz (2005) who investigated the mycobiota of cashew kernels and found that members of *Aspergillus* and *Penicillium* were the dominant. Species potentially toxigenic such as *Alternaria alternata, Aspergillus clavatus*, *A. flavus, A. parasiticus, A. ochraceus, A. ustus, Penicillium citrinum* and *P. oxalicum* were frequently isolated. There are other comprehensive studies on the occurrence of filamentous fungi in cashews traded in diffrent countries. In Thailand, major fungi isolated comprise *A. flavus* (60%), *Nigrospora oryzae* (58%), *A. niger* (53%), *Chaetomium globosum* (47%) and *Eurotium chevalieri* (40%) (Pitt *et al.,* 1993). Similar findings have been made with Brazilian cashews, although *A. niger* had been the dominant species (60%). As for *A. flavus*, the second most frequently isolated species (21%), all isolates were non-sclerotial forms. Dominant penicillia were *Penicillium brevicompactum* and *P. glabrum* (13% and 8%,respectively). Fungi have also been reported in different cashew processing units of India (Krishnaswami *et al.,* 1973). A wide range of fungi, representing several genera and species, has been reported associated with deterioration of cashew kernels in Saudi Arabia and Nigeria (Zohri, and Abdel-Gawad, 1993; Adebajo and Diyaolu, 2003). In Nigeria, Adebajo and Diyaolu (2003) examined thirty-two samples of retail cashew nuts from Lagos. They noticed that pH values (5.1-6.3) of all the samples were conducive for fungal growth and mycotoxin production. Moisture content levels ranged between 4.1 and 6.8%. Fifteen samples had moisture contents up to or above 5.8%, the highest level estimated to be 'safe' for the storage of the nuts. Fourteen fungal species, mostly toxigenic and belonging to 5 genera were isolated. Seven species were from genus *Aspergillus,* 3 from *Penicillium,* 2 from *Rhizopus* and one each from *Mucor* and *Syncephalastrum.* The most predominant isolates were: *A. niger, A. restrictus, A. flavus*, *A. fumigatus* and *Aspergillus* sp. The mean and range of total fungal counts (CFU/g) in samples were: 3,368 (180 to 16, 300). At acceptable fungal levels of 103 and 104/g, only 14 and 28 samples, respectively, were deemed fit for human consumption. All the species recovered induced detectable loss in weights of the milled nuts, though to varying extents and would be expected to cause considerable spoilage of the nuts.

More recently, Suleiman (2010) studied the fungi associated with cashew nuts biodeterioration in Nigeria. He isolated *Trichoderma viride*, *Cephalosporium* sp and *Aspergillus niger*. He also noticed that symptoms of deteriorated cashew nuts include shrinking and chlorosis. Offensive odour was peculiar to an advance infection, usually with *Aspergillus niger*.

ycological analysis of raisins showed contamination with *A. flavus*, *A. niger A. sydowii* and P. *citrinum* (Tables1&2). Reports from Yemen Republic (Al-Ghalibi *et al*., 2008) revealed the prevalence of *A. niger* on dry raisins. *A. flavus* was less frequently isolated. *Penicillium* was isolated in moderate frequency on 1 and 20% sucrose Czapek's agar media, but in low frequency on Sabouraud dextrose agar medium. Their results revealed also that 3 out of 7 samples of raisins were contaminated with aflatoxins at levels ranging from 2.68 to 11.56 µg Kg^{-1}). In Armenia, Hakobyan *et al*.(2010) were able to make mycological analysis of forty-one samples of Armenian made and eleven samples of imported raisins collected in several markets in Yerevan. They isolated and identified thirty two species of filamentous fungi from *Aspergillus*, *Penicillium, Alternaria, Trichoderma* and *Syncephalastrum* genera. Species belonging to *Aspergillus* have a very high frequency of occurrence, 65.2% with species from *Nigri* section showing the highest occurrence (66.7% of all isolated fungi belonging to *Aspergillus*). *A. carbonarius* and *A. nige* were the dominant among fungi from section *Nigri*. Both Armenian and imported samples of raisin had a high contamination level by these fungi which are potential producers of ochratoxin A. In Armenian samples two more ochratoxigenic species belonging to *Aspergillus* section *Nigri*: *A. sclerotioniger* and *A. lacticoffeatus* were detected but their frequency of occurrence was low. Thirty seven strains of *A. flavus* were isolated with 92% of them from Armenian raisins.

Pistachio nuts examined in the present work showed contamination with various fungal species including *A. flavus*, *A. fumigatus*, *A. niger*, *Paecilomyces variotii*, *Penicillium citrinum*, *Rhizopus*

stolonifer, and *Trichoderma harzianum*. Most of these fungi were reported to be isolated from pistachio nuts in other countries. In Iran, Heidarian *et al*. (2006) made an extensive investigation of one hundred and eighty-four dry pistachio samples randomly collected from storages and pistachio selling stores in different areas of Kerman province. The identified fungi were *Scytalidium thermophilum*, *Ulocladium alternariae*, *Alternaria tenuissima*, *Alternaria* sp., *Acremonium* sp., *Aspergillus niger*, *A. flavus*, *A. parasiticus*, *A. phoenicis*, *A. terreus*, *A. puniceus*, *Aspergillus* sp., *Rhizopus* sp. and *Penicillium* sp. In a subsequent Iranian study, Khosravi *et al.* (2007) evaluated the natural occurrence of fungal contamination in stored nuts including pistachio, peanut, hazelnut and almond selected randomly from different regions of Tehran. Mycological analyses revealed that the most frequent isolated fungi from different nuts were *Aspergillus* (32.2%), *Penicillium* (30.3%), *Mucor* (17.1%), *Fusarium* (18.2%), *Paecilomyces* (6.9%) and yeast (5.1%).

Mycotoxins in nut samples and dried fruits:

All tested samples were devoid of detectable amounts of aflatoxins and ochratoxins. The inability to detect mycotoxins in these samples might be due to the few number of representative samples and the possibility of infestation with non toxinogenic strains of *A. flavus* or *A. niger*. Surface contamination with potentially toxinogenic fungi does not always mean the presence of their toxins. It is well known that toxin production depends on several factors including water activity, temperature, food substrate and strain of the mold (Tajkarimi *et al.,* 2011). These results are in harmony with those reported by some investigators in different countries. In Brazil, Bittencourt *et al.* (2005) mentioned that no aflatoxin was found in the 60 samples of corn meal and flour obtained from Sao Paulo Market. A market research of various food products (cereal and cereal products, nuts and nut products, spices, dry fruits and beverages) in Qatar in 2002, revealed no detected levels of aflatoxin contamination in rice and wheat (Abdulkadar *et al.,* 2004). It is extremely important to refer to the study of Deabes and Al-Habib (2011) who surveyed the toxigenic fungi and aflatoxins in 30 samples of nuts collected from Al-Qassim region, Saudi Arabia. They reported that percentages of positive samples with aflatoxins were 80, 80, 60, 40, 40, and 20% for pistachio, peanuts, walnuts, almonds, hazelnuts and cashews. They also found that concentrations of aflatoxins B1 were ranging from a minimum of 0.3 µg/kg in cashews to a maximum of 140 µg/kg in peanuts. Zohri and Abdel Gawad (1993) were able to detect aflatoxins B1 & G1 in 3 out of the 5 samples tested of chestnut at concentrations ranging between 20 to 60 micrograms/kg. All other samples of almond, cashew nut, hazelnut, pistachio nut, and walnut that were analyzed were mycotoxin free. Alwakeel and Nasser (2011) made a comprehensive study on mycobiota and mycotoxin content of forty samples of edible nuts and dried seeds randomly collected from different locations in Al-Riyadh region, Saudi Arabia. They found a predominance of *Aspergillus niger* and *A. flavus* in all medium types. Aflatoxin B1 (8.5 µg mL-1) was detected in peanuts containing *A. flavus*. Aflatoxins B1 (1.7 µg mL-1) and B2 (1.7 µg mL-1) were detected in sunflower seeds containing *A. terreus*. T2 toxin (2.8 mg mL-1) was detected in pumpkin seeds containing *Stachybotrys chartarum* and DAS (2.4 µg mL-1) was detected in a salted peanut sample containing *Trichothecium roseum*. The authors suggested that Government authorities for food safety consumption should continue to monitor and set appropriate guidelines and information initiatives for public knowledge on the safety of these agricultural products whole year round. Potentially micotoxigenic species were frequently isolated from cashews in Brazil although only small amounts of mycotoxins, specially G2 aflatoxin had been detected in substandard samples (Freire *et al.,* 1999).

Mycotoxins produced by fungi contaminating nuts and dried fruits:

Testing the ability of 40 different fungal isolates to produce mycotoxins revealed that 16 (40%) were able to produce either aflatoxins (*A. flavus* and *A. parasiticus*) or ochratoxins (*A. niger*, *A. aculeatus* and *A. barasiliensis* and *A. scletioniger*).to produce Ochratoxin A as shown in table 4. Aflatoxin and ochratoxin produers represent 22% and 17.5% of total isolates respectively. In Algeria, Fernane *et al* (2010) tried to evaluate fungal contamination and aflatoxin (AF) and ochratoxin A (OTA) occurrence in 31 pistachio samples and to study the mycotoxigenic capacities of the isolates. The most frequently found fungi were *Penicillium* spp. (38%), *Aspergillus* section Nigri (30%) and *A. flavus* (22%). They observed that 56.5% of *A. flavus* isolates were able to produce aflatoxins B1 and B2 and one of uniseriate isolate of section Nigri was ochratoxin A producer, whereas OTA production capacity was detected in 33.3% of the *Aspergillus* section Nigri biseriate. At least one of the potentially ochratoxigenic species was found in 64.5% of samples.

Thus, far, the overall findings presented here show that retail nut samples as packaged and sold in Saudi Arabia are susceptible to fungal deterioration and possibly mycotoxin contamination especially during storage. On the other hand, this contamination could be due to long-term storage, marketing under non-hygienic conditions of the food products in the

poor environmental conditions including high moisture and temperature. We suggest that monitoring fungal contaminations and mycotoxins in nuts can be simplified using predetermined profiles of nuts mycoflora for each exporting country.

Table (1): Counts (colonies /gram fresh sample) of fungal genera and species isolated from food samples on DRBC medium.

	Bean seeds	Corn grains	Powdered coffee	Cardamon	Almond	Cashew	Nuts	Pistachio seeds	Pistachio Husks	Raisin	Dates	Dates
Aspergillus	33	41	25	23	9	40	27	24	86	27	24	50
flavus	20	3	7	12	7	14	5	14	80	21	14	1
fumigatus	8	3	1	1		1	6		2			1
niger	5	3	9	2	2	25	16	10	3	5	10	48
sydowii				1					1	1		
terreus		32	8	7								
Eurotium amstelodami							2					
Emericella nidulans			1									
F. proliferatum			1	1								
Mortierella alpine				1								
Paecilomyces variotii		1	8						2			
P. citrinum		1		22	1	19		1		3		
P .duclauxii				1								
P. islandicum				10								
Rhizopus stolonifer								1				
Syncephalastrum racemosum			1				1					
Trichoderma harzianum	1				2	3	1	1				
Sterile mycelium			1	4								
Total count per sample	34	43	37	62	12	62	31	27	88	30	24	50
Number of genera	2	3	6	5	3	3	4	4	2	2	1	1
Number of species	4	6	9	11	4	5	6	5	5	4	2	3

Table (2): Counts (colonies /gram fresh sample) of osmophilic fungal genera and species isolated from food samples on DG18 medium.

	Bean seeds	Corn grains	Powdered coffee	Cardamon	Almond	Cashew	Nuts	Pistachio seeds	Pistachio husks	Raisins	Dates
Aspergillus	15	44	6	7	7	11	3	16	22	29	17
A. candidus				1							
A. flavus	7	11	3		4			12	8	17	10
A. fumigatus	8	1	1				1				
A. niger			2		3	9	2	4	4	12	2
A. sydowii									10		
A. terreus		32		6							5
A. ustus						1					
A. wentii						1					
Eurotium amstelodami		17	14	1							
Fennellia flavipes									1		
Fusarium oxysporum	2										
F. proliferatum			1								
Paecilomyces variotii		1	2								
P. citrinum	1		1	6	2	50	2			8	
P. pinophilum				2							
Phoma glomerata						1					
Rhizopus stolonifer			1								
Syncephalastrum racemosum			2								
Trichoderma harzianum	1				2		1				
Total count per sample	19	62	27	16	11	62	6	16	23	37	17
Number of genera	4	3	7	3	3	3	3	1	2	2	1
Number of species	5	5	9	5	4	5	4	2	4	3	3

Table (3): Counts (colonies /gram in all samples), frequency of occurrence (out of 12 samples)of glucophilic– (on DRBC) and osmophilic (on DG18) fungal genera and species isolated from food samples.

Fungal species	DRBC		DG18	
	TC	F &OR	TC	F & OR
Aspergillus	409	12 H	187	12 H
A. candidus Link	00	---	1	1 L
A. flavus Link	198	12 H	72	8 H
A. fumigatus Fresenius	23	8 H	11	3 M
A. niger van Tieghem	138	12 H	48	9 H
A. sydowii (Bainier & Sartory) Thom & Church	3	3 M	10	1 L
A. terreus Thom	47	3 M	43	3 M
A. ustus (Bainier) Thom &Church	00	--	1	1 L
A. wentii Wehmer	00	--	1	1 L
Eurotium amstelodami Mangin	2	1 L	32	3 M
Emericella nidulans (Eidam) Vuillemin	1	1 L	00	--
Fennellia flavipes Wiley & Simmons	00	--	1	1 L
Fusarium oxysporum Schlechtendal	00	--	2	1 L
F. proliferatum (Matsushima) Nirerberg	2	2 L	1	1 L
Mortierella alpina Peyronel	1	1 L	00	--
Paecilomyces variotii Bainier	11	3 M	3	2 L
Penicillium	58	6 H	72	7 H
P. citrinum Thom	47	6 H	70	7 H
P. duclauxi Delacroix	1	2 L	00	--
P. islandicum Sopp	10	1 L	00	--
P. pinophilum Hedgcock	00	--	2	1 L
Phoma glomerata (Corda) Wollenweber	00	--	1	1 L
Rhizopus stolonifer (Ehrenberg) Vuillemin	1	1 L	1	1 L
Syncephalastrum racemosum Cohn ex Schroeter	2	2 L	2	1 L
Trichoderma harzianum Rifai	8	5 M	4	3 M
Sterile mycelium	5	2 L	00	--
Total count in all samples	500		306	
Number of genera	11		10	
Number of species	17		19	

TC = Total count, F = Frequency out of 13 samples, OR = Occurrence Remark, H = High Occurrence= 6-12 cases, M = Moderate Occurrence= 3-5 cases, L = Low Occurrence= 1, 2 cases.

Table (4): Mycotoxins produced by fungi isolated from dried fruits

AUMC No.	Name	Source	Mycotoxins detected	Approximate concentration (µg/L medium)
9001	*A. flavus*	Dates	Aflatoxin B1	300
			Aflatoxin B2	300
9002	*A. parasiticus*	Cardamon	Aflatoxin B1	200
			Aflatoxin B2	200
			Aflatoxin G1	200
			Aflatoxin G2	200
9003	*A. flavus*	Cardamon	Aflatoxin B1	300
			Aflatoxin B2	300
9004	*A. flavus*	Dates	Aflatoxin B1	300
			Aflatoxin B2	300
9005	*A. flavus*	Pistachio	Aflatoxin B1	100
9006	*A. flavus*	Coffee	Aflatoxin B1	500
			Aflatoxin B2	500
9007	*A. flavus*	Pistachio husks	Aflatoxin B1	600
			Aflatoxin B2	600
9008	*A. flavus*	Raisin	Aflatoxin B1	300
			Aflatoxin B2	300
9009	*A. flavus*	Raisin	Aflatoxin B1	300
			Aflatoxin B2	300
9010	*A. barasiliensis*	Cardamon	Ochratoxin-A	100
9011	*A. niger*	Coffee	Ochratoxin-A	200
9012	*A. aculeatus*	Cashew	Ochratoxin-A	100
9013	*A. barasiliensis*	Pistachio	Ochratoxin-A	200
9014	*A. niger*	Nuts	Ochratoxin-A	200
9015	*A. barasiliensis*	Nuts	Ochratoxin-A	200
9016	*A. sclerotioniger*	Dates	Ochratoxin-A	200
9017	*A. flavus*	Bean	-	-
9018	*A. flavus*	Almond	-	-
9019	*A. flavus*	Corn	-	-
9020	*A. flavus*	Coffee	-	-
9021	*A. flavus*	Corn	-	-
9022	*A. flavus*	Bean	-	-
9023	*A. flavus*	Pistachio	-	-
9024	*A. flavus*	Nut	-	-
9025	*A. barasiliensis*	Nut	-	-
9026	*A. niger*	Dates	-	-
9027	*A. niger*	Almond	-	-
9028	*A. barasiliensis*	Bean	-	-
9029	*A. niger*	Pistachio	-	-
9030	*A. barasiliensis*	Nut	-	-
9031	*A. niger*	Cashew	-	-
9032	*A. niger*	Cardamom	-	-
9033	*A. aculeatus*	Bean	-	-
9034	*A. barasiliensis*	Coffee	-	-
9035	*A. flavus*	Pistachio husks	-	-
9036	*A. flavus*	Coffee	-	-
9037	*A. flavus*	Cardamom	-	-
9038	*A. flavus*	Cardamom	-	-
9039	*A. niger*	Dates	-	-
9040	*A. niger*	Cashew	-	-

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