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Microbial Contamination and Mycotoxins from Nuts in Riyadh, Saudi Arabia

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ABSTRACT

The occurrence of harmful aflatoxins from agricultural products varies with geographic location, farming practices and processing. To date, no data was reported from Saudi Arabia on mycotoxin content of nuts and edible seeds. Forty samples of edible nuts and dried seeds were randomly collected from different locations in Al-Riyadh, Saudi Arabia. Fungi were detected by seed-plate and dilutions plate method and were cultured on glucose-Czapek's agar, sucrose-Czapek's agar and starch yeast agar. Purified fungal isolates were identified morphologically. Mycotoxins were extractedusing chloroform and detected by thin layer chromatography. Bacterial analysis was done using total plate count method. There was a predominance of *A. niger* and *A. flavus* in all medium types. Aflatoxin B_1 (8.5 μg mL^{-1}) was detected in peanuts containing *A. flavus*. Aflatoxin B_1 (1.7 μg mL^{-1}) and B_2 (1.7 μg mL^{-1}) was detected in sunflower seeds containing *A. terreus*. T2 toxin (2.8 mg mL^{-1}) was detected in pumpkinseeds containing *Stachybotrys chartarum* and DAS (2.4 μg mL^{-1}) was detected in a salted peanut sample containing *Trichthecium roseum*. Four nut samples showed contamination with bacteria. Turkish pine seeds and American walnut had total plate counts of 12×10. Pakistani pine seeds and Iranian salted pistachio had TPC of 3×10. Listeria monocytogenes was isolated from American walnut samples. 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.

Key words: Aflatoxin, mycotoxin, *Aspergillus* species, osmophilic, osmotolerant fungi, glucose-Czapek's agar

INTRODUCTION

Mycotoxins are natural metabolism products of moulds which can have a toxic effect on humans and animals. They have most recently come to light over the toxic mold that has suddenly become an issue of the 21st century. Aflatoxins are the most toxic form of mycotoxins. Some types of food, such as dried fruit, spices and nuts, show an increased risk of aflatoxin release due to fungal infestation (Soubra *et al*., 2009; Wang and Liu, 2007). As mycotoxins are temperature-resistant they are usually not destroyed when the food is processed (Yazdanpanah *et al*., 2005).

Aflatoxins are detected occasionally in milk, cheese, corn, peanuts, cottonseed, nuts, almonds, figs, spices and a variety of other foods and feeds (Soubra *et al*., 2009; Wang and Liu, 2007; Pacheco and Scussel, 2007; Kenjo *et al*., 2007; Molyneux *et al*., 2007; Cheraghali *et al*., 2007; Abdulkadar *et al*., 2002). Milk, eggs and meat products are sometimes contaminated because of the animal consumption of aflatoxin-contaminated feeds. However, the commodities with the highest risk of aflatoxin contamination are corn, peanuts and cottonseed (Soubra *et al.*, 2009; Wang and Liu, 2007; Pacheco and Scussel, 2007; Kenjo *et al.*, 2007; Molyneux *et al.*, 2007; Cheraghali *et al.*, 2007; Abdulkadar *et al.*, 2002; Mahmoud *et al.*, 2001). Aflatoxins are detected in as much as 70% of corn products with more than 1000 μg kg^{-1} level of aflatoxin (Wang and Liu, 2007). In peanuts, aflatoxin level is recorded to as much as 28.4 μg kg^{-1} (Wang and Liu, 2007). Probably one of the worst mycotoxins (Aflatoxin) is the ones produced by at least three strains of *Aspergillus* found in nuts (Hedayati *et al.*, 2007). Aflatoxins are toxic metabolites produced by certain fungi in/on foods and feeds (Ehrlich *et al.*, 2007). They are probably the best known and most intensively researched mycotoxins in the world. Aflatoxin found in nuts is a carcinogenic toxin that has been linked to liver cancer in many countries (Wild and Gong, 2010; Caldas *et al.*, 2002). Aflatoxin also causes other problems, for most people it is believed that the levels are low enough to not be harmful to an individual who occasionally has a few nuts (Alwakeel, 2009). Aflatoxins have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world (Williams *et al.*, 2004). The occurrence of aflatoxins is influenced by certain environmental factors; hence, the extent of contamination will vary with geographic location (Mwanda *et al.*, 2005) agricultural and agronomic practices and the susceptibility of commodities to fungal invasion during preharvest, storage and/or processing periods (Park, 2002). Processing per se reduces the amount of aflatoxins in foods by as much as 80% (Park, 2002). For these reasons, though most countries have adopted measures to control levels of mycotoxins specifically aflatoxins in agricultural products, environmental conditions affecting storage and consumption make it difficult or impossible to attain low concentrations of this aflatoxins (Dorner, 2008). Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans. As it is realized that absolute safety is never achieved, many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed.

Numerous reports from many countries on the occurrence of mycotoxins have been published, however, none has been reported from Saudi Arabia. Considering the fact that nuts consumption is very high in Saudi Arabia, we deemed it necessary to conduct this study to investigate mycotoxins and bacterial contamination in edible nuts in this region.

MATERIALS AND METHODS

Collection of samples: Forty samples of edible nuts and dried seeds were randomly collected from different locations in Al-Riyadh, Saudi Arabia between May 2008 and July 2010. English and Scientific names of nuts and dried seeds are enumerated in Table 1. Each sample was placed in a sterile polyethylene bag, sealed and double-sealed with another bag for storage.

Isolation and identification of fungi: Fungi were detected using two methods. The first is seed-plate method as described by Seo *et al.* (2008). Four seeds were placed on the surface of sterile media. Five plates were used for each sample and each medium; the plates were incubated for 5-7 days at 25°C. The second method is the dilution plate method as used by Kenjo *et al.* (2007). Five gram seeds of each sample were placed in a 500 mL sterilized distilled water in Erlenmeyer flask and shaken for 15 min. One mL of seed suspension was placed into each Petri dish, 12-15 mL of melted and cooled medium was poured. Five plates were used for each sample and for each medium. *Glucophilic* fungi were cultured on glucose-Czapek's agar medium in which glucose

Table 1: English and scientific names of the tested nuts and dried seeds

English name	Scientific name
Peanut seeds (Salted)	*Arachis hypogaea*
Peanut seeds	*Arachis hypogaea* (unsalted)
Peanut seeds	*Arachis hypogaea* (unsalted)
Peanut seeds (Salted)	*Arachis hypogaea*
Peanut seeds with lemon	*Arachis hypogaea*
Peanut seeds (Salted)	*Arachis hypogaea*
Peanut seeds (Salted) in kernels	*Arachis hypogaea*
Salted Chick-pea	*Cicer arietinum*
Salted Turkish Chick-pea	*Cicer arietinum*
Chick-pea	*Cicer arietinum*
Turkish Chick-pea	*Cicer arietinum*
Turkish Pine seeds	*Pinus pinea*
Pakistani Pine seeds	*Pinus pinea*
Chinese Pine seeds	*Pinus armandii*
Sunflower seeds with lemon	*Helianthus annuus*
Salted sunflower seeds	*Helianthus annuus*
Iranian Pistachio	*Pistacia vera*
American Walnut	*Juglans major*
Cashew	*Anacardium occidentale*; syn. *Anacardium curatellifolium*
Karela seeds	Karela (*Momordica charantia*)
Karela seeds with Lemon	Karela (*Momordica charantia*)
Roasted pumpkin seeds (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
Roasted pumpkin seeds (salted) (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
Roasted pumpkin seeds (salted) (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
Roasted pumpkin seeds (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
Iranian Roasted pumpkin seeds (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
Egyptian Roasted pumpkin seeds (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
Afghani Roasted pumpkin seeds (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
Iranian Roasted pumpkin (salted) (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
Iranian Roasted pumpkin seeds (Pepita)	*Cucurbita maxima* or *Cucurbita moschata* with lemon
Chinese Roasted pumpkin seeds (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
Syrian Roasted pumpkin seeds (Pepita)	*Cucurbita maxima* or *Cucurbita moschata*
American Almond	*Prunus dulcis*
American Almond (not salted)	*Prunus dulcis*
American Almond	*Prunus dulcis*
Turkish Hazelnut	*Corylus colurna*
Syrian salted Pistachio	*Pistacia vera*
Iranian salted Pistachio	*Pistacia vera*
Iranian salted Pistachio	*Pistacia vera*
Syrian Pistachio	*Pistacia vera*

(10 g L^{-1}) replaced sucrose. To determine cellulose decomposition by fungal species, glucose was replaced by powdered cellulose (20 g L^{-1}) in cellulose-Czapek's agar as medium. *Osmophilic* and *osmotolerant* fungi were allowed to grow on sucrose-Czapek's agar which contained 200 g L^{-1} sucrose instead of glucose. Thermophilic and thermotolerant fungi were cultured on starch yeast agar (YpSS) which contained g L^{-1}: Soluble starch, 20; yeast extract, 4; KH_2PO_4, 1; $Mgso_4.7H_2O$, 0.5 and agar, 15 g. All types of media were supplemented with chloramphenicol (20 μg mL^{-1}) and Rose Bengal (30 ppm) as bacteriostatic agent. Pure cultures of fungi were kept in slant agar tubes which containing 0.5 g chloramphenicol.

Identification of fungal isolates: Purified fungal isolates were identified morphologically (based on macroscopic and microscopic characteristics) whenever possible, in the original Petri dishes culture (Kenjo *et al*., 2007; Seo *et al*., 2008).

Extraction of mycotoxins from samples: The samples were stored at 22°C for 1, 2, 3 and 4 months then extracted for the presence of aflatoxins B_1, B_2, T_2 Toxin and DAS. During these periods the rate of fungal growth was determined visually as described by Joosten *et al*. (2001). Twenty gram of each sample was defatted by extraction with cyclohexane for 10 h using a Soxhlet-type extractor. The defatted residue was extracted for another 10 h with chloroform. The chloroform extract was dried over anhydrous sodium sulphate, filtered and then evaporated under vacuum to near dryness. The residue was diluted with chloroform to 1 mL.

Detection and verification of mycotoxins: Caldas *et al*. (2002) did thin layer chromatographic technique of the clean extract on percolated silica gel plate type for the presence of mycotoxins according to standard procedures as used in the detection of mycotoxins in foods.

Simple configuration method of recorded mycotoxins on precoated silica gel plates was done. The TLC plates commonly used are normal phase silica gel plates. Some acidic metabolites like cyclopiazonic acid, citrinin and luteoskyrin can be useful to impregnate the plate with oxalic acid. This is simply done by dipping the plate in an 8% solution of oxalic acid in water or methanol followed by air-drying. After application the TLC-plate, a suitable TLC-procedure can be performed using the following solvents: TEF: Toluene/Ethyl Acetate/Formic acid (90%) 5:4:1, CAB: Chloroform/Acetone/Iso propanol 85:15:20 and CM: Chloroform/Methanol 97:3.

After elution and air drying in a dark fume hood, the TLC-plates are examined in visible light (VIS), long wave UV-light (UV-366) and short wave UV-light (UV-254) some metabolites are treated with 1/2 min in UV-254 followed by UV-366.

The following spray reagents are useful for visualizing and verification of secondary metabolites:

- **Spray 1:** 0.5% p-anisaldehyde in ethanol/acetic acid/conc. sulphuric acid 17:2:1 (most metabolites)
- **Spray 2:** 50% sulphuric acid in water (e.g., aflatoxins B_1 and B_2; verruculogen; viridicatins; cyclopiazonic; streigmatocystin; T-2 toxin
- **Spray 3:** $FeCl_3$ in butanol and heating for 5 min at 130°C (e.g., Aspergillic acid; kojic acid; penicillic acid; citrinin; verrucologen
- **Spray 4:** 20% $AlCl_3$ in 60% ethanol and heating for 5 min at 130°C (e.g., penitrem A; trichothecenes B; sterigmatocystin; gliotoxin; T-2 toxin
- **Spray 5:** NH_3 vapour in 1-3 min (mycophenolic acid; xanhomegnin; viomellien, penicillic acid; ochratoxin A; kojic acid; citrinin; patulin, diacetoxyscirpenol (DAS))

Extraction of mycotoxins from fungal isolates: Culture of selective 25 fungal isolates collected from the current study was examined. The tested samples were represented by 3 species of *Aspergillus* (*A. flavus* (2 isolates), *A. tamarii* (1 isolate) and *A. terreus* (1 isolate), 1 isolate of *Acremonium strictum*, one isolate each of *Curvularia ovoidae* and *Paecilomyces variotii*, 3 isolates of *Penicillium (Penicillium chrysogenum* (1 isolate) and *Penicillium purpurogenum* (2 isolates), two isolates of *Stachybotrys chartarum and* two species of *Trichoderma* (*Trichoderma harizianum* (1 isolate) and *Trichthecium roseum* (1 isolate).

Inocula were prepared from 7-days old culture of each isolate on PDA slope as spore suspensions in 0.2% aqueous tween 80 (v/v). Isolates were inoculated into 250 mL Erlenmeyer flasks each containing 50 mL Capek's liquid medium supplemented with 0.2% yeast extract and 1.0 peptone and incubated at 28°C for 10 days as static culture (PYCZ) (Youssef *et al*., 2008a).

After incubation, the control of each flask (medium+mycelium) was homogenized for 5 min in a high-speed blender with 100 mL chloroform. The extract procedure was repeated three times. The chloroform extracts were combined, washed, dried, filtered and concentrated to near dryness, cleaned and mycotoxins are detected as previously described by Nieminen *et al*. (2002).

Bacterial analysis: A total of 40 samples of nuts and dried seeds were analyzed for bacterial total plate count using a method employed by Freitas *et al*. (2009) and Hossain *et al*. (2004).

Data analysis: Data analysis was done using Total Count (TC of species/ 100 seeds), (TC%) Total Count of one fungi species/total count of all species×100, Incidence (I) is: (the occurrence of fungi in each number of a specimen of nuts) and Percentage Incidence (%I) was calculated by the fallowing equation: incidence (I)/ the number of nut specimens×100. This applies to all the tables (Youssef *et al*., 2008b).

RESULTS

In peanut samples, *Aspergillus flavus* was isolated in all 7 peanut samples with 34.3% total count and 184 counts/100 seeds. *Aspergillus niger* showed the highest total percentage count with 38.8% and 208 counts/100 seeds. *A. niger* was seen in 6 of 7 peanut samples (85.7%). Other fungal species isolated from peanut samples include; *Aspergillus tamarii*, *Aspergillus terreus*, *Mucor hiemalis*, *Paecilomyces variotii* . The percentage from the total count was (3%). The percentage from the total count of *Aspergillus versicolor*, *Cladosporium sphaerospermum* , *Microascus cinereus*, Neurospora crassa, *Penicillium chrysogenum*, *Penicillium glabrum*, *Rhizopus*, *Syncephalastrum racemosum*, *Trichotecium roseum* and unidentified yeasts was 1.5% (Table 2).

In 4 samples of chick peas, *A. niger* predominated with 45.7% total count followed by *A. flavus* (5.7%) and 2.9% total count each of *Emericella acrestata, Emericella nidulans, Penicillium purpurogenum* and *R. oryzae*. *R. oryzae* predominated with 42.1% total count in pine seeds samples together with 18.4% of *A. niger* and 13.2% of *A. flavus*. Similarly, *A. niger* and *A. flavus* and predominated in sunflower seed samples, hazelnut sample, walnut sample, karela seed samples, pumpkin seed samples, almond nut samples and pistachio samples. *R. oryzae* also was isolated in high total counts in walnut, cashew, kerala seeds, pumpkin, almond and pistachios. (Table 2).

In cellulose-Czapek agar, *A. niger* and *A. flavus* were the two predominant fungal specie which were isolated in peanuts, chick peas, pine seeds, hazelnuts, walnut, cashew, kerala seeds, pumpkin seeds, almond and pistachios. Pumpkin seeds contained the highest number of isolated fungal species (TC of 752 in 11 sample) followed by peanuts (TC of 384 in 7 sample), pistachios (TC of 368 in 5 sample) and chick peas (TC of 248 in 4 samples). The rest of the samples had total counts of less than 80 per sample (Table 3).

In 40% sucrose-Czapek agar, *A. niger*, *A. flavus* and *Eurotium montevidensis* were the three most predominant fungal specie which were isolated in peanuts, chick peas, pine seeds, hazelnuts, walnut, cashew, kerala seeds, pumpkin seeds, almond and pistachios (Table 4).

Table 2: Total Count (TC/100 seeds), Percentage Total Count (%TC), Incidence (I) and Percentage Incidence (%I) of fungal species isolated from 40 samples of nuts and dried seeds on glucose Czapek's agar at 25°C.

	Peanut (7 samples)				Chick pea (4 samples)				Pine seeds (3 samples)				Sun flower seeds (2 samples)			
Fungal species	TC	%TC	I out of 7	%I	TC	%TC	I out of 4	%I	TC	%TC	I out of 3	%I	TC	%TC	I out of 2	%I
Aspergillus flavus	184	34.3	7	100	16	5.7	2	50	40	13.2	2	66.7	8	12.5	1	50
A. niger	208	38.8	6	85.7	128	45.7	2	50	56	18.4	2	66.7	24	37.5	1	50
A. tamarii	16	3	2	28.6	0	0	0	0	0	0	0	0	0	0	0	0
A. terreus	16	3	1	14.3	0	0	0	0	0	0	0	0	8	12.5	1	0
A. sclerotioniger	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. versicolor	8	1.5	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium sphaerospermum	8	1.5	1	14.3	0	0	0	0	0	0	0	0	8	12.5	1	50
Colletotrichum coccoides	0	0	0	0	0	0	0	0	16	5.3	1	33.3	0	0	0	0
Curvularia ovoidea	0	0	0	0	0	0	0	0	8	2.6	1	33.3	0	0	0	0
Emericella acrestata	0	0	0	0	8	2.9	1	25	0	0	0	0	0	0	0	0
E. nidulans	0	0	0	0	8	2.9	1	25	8	2.6	0	0	8	12.5	1	50
Eurotium amstelodami	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Microascus cinereus	8	1.5	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Mucor hiemalis	16	3	2	28.6	0	0	0	0	0	0	0	0	0	0	0	0
Neurospora crassa	8	1.5	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces variotii	16	3	2	28.6	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium aurantiogriseum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. brevicompactum	0	0	0	0	0	0	0	0	8	2.6	1	33.3	0	0	0	0
P. chrysogenum	8	1.5	1	14.3	0	0	0	0	8	2.6	1	33.3	0	0	0	0
P. citrinum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. glabrum	8	1.5	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
P. purpurogenum	0	0	0	0	8	2.9	1	25	0	0	0	0	0	0	0	0
Rhizopus oryzae	8	1.5	1	14.3	8	2.9	1	25	128	42.1	3	100	0	0	0	0
Syncephalastrum racemosum	8	1.5	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Trichothecium roseum	8	1.5	1	14.3	0	0	0	0	8	2.6	1	0	0	0	0	0
Ulocladium consortiale	0	0	0	0	0	0	0	0	8	2.6	1	33.3	8	12.5	1	50
Unidentified yeasts	8	1.5	1	14.3	104	35	3	75	16	5.3	1	33.3	0	0	0	0
Total count	536				280				304				64			

	Hazelnut (1 sample)				Walnut (1 sample)				Cashew (1 sample)				Karela seeds (2 samples)			
Fungal species	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 2	%I
Aspergillus flavus	40	41.7	1	100	24	23.1	1	100	24	20	1	100	32	33.3	1	50
A. niger	16	16.7	1	100	72	69.2	1	100	0	0	0	0	48	50	1	50
A. tamarii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. terreus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. sclerotioniger	32	33.3	1	100	0	0	0	0	0	0	0	0	0	0	0	0
A. versicolor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. sphaerospermum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colletotrichum coccoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curvularia ovoidea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Emericella acrestata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Emericella nidulans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eurotium amstelodami	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Microascus cinereus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mucor hiemalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2: Continued

	Hazelnut (1 sample)				Walnut (1 sample)				Cashew (1 sample)				Karela seeds (2 samples)			
Fungal species	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 2	%I
Neurospora crassa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces variotii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium aurantiogriseum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. brevicompactum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. chrysogenum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. citrinum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. glabrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. purpurogenum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus oryzae	0	0	0	0	8	7.7	1	100	96	80	1	100	8	8.3	1	50
S. racemosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichothecium roseum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ulocladium consortiale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yeast	8	8.3	1	100	0	0	0	0	0	0	0	0	8	8.3	1	50
Total count	96				104				120				96			

	Pumpkin seeds (11 samples)				Almond (3 samples)				Pistachio (5 samples)			
Fungal species	TC	%TC	I out of 11	%I	TC	%TC	I out of 3	%I	TC	%TC	I out of 5	%I
Aspergillus flavus	56	10.2	5	45.5	64	40	2	66.7	112	33.3	3	60
A. niger	318	57.6	10	91	64	40	2	66.7	192	57.1	4	80
A. tamarii	0	0	0	0	0	0	0	0	0	0	0	0
A. terreus	8	1.5	1	9.1	0	0	0	0	0	0	0	0
A. sclerotioniger	32	5.8	1	9.1	0	0	0	0	0	0	0	0
A. versicolor	0	0	0	0	0	0	0	0	0	0	0	0
C. sphaerospermum	0	0	0	0	0	0	0	0	0	0	0	0
Colletotrichum coccoides	0	0	0	0	0	0	0	0	0	0	0	0
Curvularia ovoidea	0	0	0	0	0	0	0	0	0	0	0	0
Emericella acrestata	0	0	0	0	0	0	0	0	0	0	0	0
Emericella nidulans	0	0	0	0	0	0	0	0	0	0	0	0
Eurotium amstelodami	24	4.4	1	9.1	0	0	0	0	8	2.4	1	20
Microascus cinereus	0	0	0	0	0	0	0	0	0	0	0	0
Mucor hiemalis	0	0	0	0	0	0	0	0	0	0	0	0
Neurospora crassa	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces variotii	32	5.8	1	9.1	0	0	0	0	0	0	0	0
Penicillium aurantiogriseum	32	5.8	1	9.1	0	0	0	0	0	0	0	0
P. brevicompactum	0	0	0	0	8	5	1	33.3	8	2.4	1	20
P. chrysogenum	10	1.8	1	9.1	0	0	0	0	8	2.4	1	20
P. citrinum	8	1.5	1	9.1	0	0	0	0	0	0	0	0
P. glabrum	0	0	0	0	8	5	1	33.3	0	0	0	0
P. purpurogenum	8	1.5	1	9.1	0	0	0	0	0	0	0	0
Rhizopus oryzae	8	1.5	1	9.1	16	10	1	33.3	8	2.4	1	20
S. racemosum	0	0	0	0	0	0	0	0	0	0	0	0
Trichothecium roseum	0	0	0	0	0	0	0	0	0	0	0	0
Ulocladium consortiale	0	0	0	0	0	0	0	0	0	0	0	0
Yeasts	16	2.9	1	9.1	0	0	0	0	0	0	0	0
Total count	552				160				336			

Table 3: Total Counts (TC), Percentage Total Counts (%TC), Incidence (I)and Percentage Incidence (%I) of cellulose decomposing fungal species isolated from 40 samples of nuts and dried seeds on cellulose Czapek's agar at 25°C

	Peanut (7 samples)				Chick pea (4 samples)				Pine seeds (3 samples)				Sun flower seeds (2 samples)			
Genera and species	TC	%TC	I out of 7	%I	TC	%TC	I out of 4	%I	TC	%TC	I out of 3	%I	TC	%TC	I out of 2	%I
Absidia corymbifera	0	0	0	0	40	16.1	3	75	0	0	0	0	0	0	0	0
Acremonium strictum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alternaria alternata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus flavus	120	13.3	5	71.4	56	22.6	2	50	96	41.4	3	100	32	100	1	50
A. fumigatus	8	2.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. niger	160	41.7	5	71.4	136	54.8	4	100	24	10.3	2	66.7				
A. ustus	0	0	0	0	0	0	0	0	16	6.9	1	33.3	0	0	0	0
Cephaliophora irregularis	0	0	0	0	0	0	0	0	8	3.4	1	33.3	0	0	0	0
Cladosporium cladosporioides	0	0	0	0	0	0	0	0	8	3.4	1	33.3	0	0	0	0
Emericella nidulans	16	4.2	2	28.6	0	0	0	0	0	0	0	0	0	0	0	0
Eurotium amstelodami	0	0	0	0	8	3.2	1	25	0	0	0	0	0	0	0	0
Fusarium sambucinum	16	4.2	2	28.6	0	0	0	0	0	0	0	0	0	0	0	0
F. verticillioides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynascus sepedonium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neurospora crassa	8	2.1	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces variotii	8	2.1	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium oxalicum	16	4.2	2	28.6	8	3.2	1	25	8	3.4	1	33.3	0	0	0	0
Rhizopus oryzae	16	4.2	2	28.6	0	0	0	0	40	17.2	2	66.7	0	0	0	0
Scopulariopsis brevicaulis	0	0	0	0	0	0	0	0	8	3.4	1	33.3	0	0	0	0
Sporotrichum roseolum	0	0	0	0	0	0	0	0	8	3.4	1	33.3	0	0	0	0
Stachybotrys chartarum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichothecium roseum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichoderma harzianum	0	0	0	0	0	0	0	0	16	6.9	1	33.3	0	0	0	0
Ssterile mycelia	16	4.2	2	28.6	0	0	0	0	0	0	0	0	0	0	0	0
Total count	384				248				232				32			

	Hazelnut (1 sample)				Walnut (1 sample)				Cashew (1sample)				Karela seeds (2samples)			
Genera and species	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 2	%I
Absidia corymbifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acremonium strictum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alternaria alternata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus flavus					16	13.3	1	100	40	83.3	1	100	88	55	2	100
A. fumigatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. niger	96	100	1	100	88	64.7	1	100	8	5.6	1	100	72	45	1	50
A. terreus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. ustus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cephaliophora irregularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium cladosporioides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Emericella nidulans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eurotium amstelodami	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium sambucinum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. verticillioides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynascus sepedonium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neurospora crassa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3: Continued

	Hazelnut (1 sample)				Walnut (1 sample)				Cashew (1samples)				Karela seeds (2samples)			
Genera and species	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 2	%I
Paecilomyces variotii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium oxalicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus oryzae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scopulariopsis brevicaulis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sporotrichum roseolum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stachybotrys chartarum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichothecium roseum	0	0	0	0	16	11.8	1	100	0	0	0	0	0	0	0	0
Trichoderma harzianum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sterile mycelia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total count	96				120				48				160			

	Pumpkin seeds (11 samples)				Almond (3 samples)				Pistachio (5 samples)			
Genera and species	TC	%TC	I Out of 11	%I	TC	%TC	I Out of 3	%I	TC	%TC	I Out of 5	%I
Absidia corymbifera	32	4.3	2	18.2	0	0	0	0	0	0	0	0
Acremonium strictum	16	2.1	1	9.1	0	0	0	0	0	0	0	0
Alternaria alternata	8	1.1	1	9.1	0	0	0	0	0	0	0	0
Aspergillus flavus	96	12.8	2	18.2	72	33.1	3	100	152	41.3	4	80
A. fumigatus	144	19.2	5	45.5	0	0	0	0	0	0	0	0
A. niger	72	9.6	4	36.5	64	28.6	3	100	176	47.8	5	100
A. terreus	240	31.9	6	54.5	0	0	0	0	0	0	0	0
A. ustus	0	0	0	0	0	0	0	0	0	0	0	0
Cephaliophora irregularis	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium cladosporioides	48	6.4	3	27.3	0	0	0	0	0	0	0	0
Emericella nidulans	0	0	0	0	0	0	0	0	0	0	0	0
Eurotium amstelodami	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium sambucinum	0	0	0	0	0	0	0	0	0	0	0	0
F. verticillioides	8	1.1	1	9.1	0	0	0	0	0	0	0	0
Corynascus sepedonium	8	1.1	1	9.1	0	0	0	0	0	0	0	0
Neurospora crassa	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces variotii	0	0	0	0	8	3.6	1	33.3	0	0	0	0
Penicillium oxalicum	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus oryzae	8	1.1	1	9.1	80	35.7	2	66.7	40	10.9	2	40
Scopulariopsis brevicaulis	56	7.5	2	18.2	0	0	0	0	0	0	0	0
Sporotrichum roseolum	16	2.1	1	9.1	0	0	0	0	0	0	0	0
Stachybotrys chartarum	0	0	0	0	0	0	0	0	0	0	0	0
Trichothecium roseum	0	0	0	0	0	0	0	0	0	0	0	0
Trichoderma harzianum	0	0	0	0	0	0	0	0	0	0	0	0
Sterile mycelia	0	0	0	0	0	0	0	0	0	0	0	0
Total count	752				224				368			

Among the thermophilic/thermotolerant fungi, *A. fumigatus* and *A. niger* predominated in almost all tested samples of peanuts, sunflower seeds and cashew nuts. *E. nidulans* was isolated from chickpeas, pine seeds and walnuts (Table 5).

Collectively comparing the total fungal counts isolated from 40 samples of nuts and dried seeds showed the predominance of *A. niger* (range: 36.9% TC on 40% sucrose-Czapek agar to as much

Table 4: Total Counts (TC), Percentage Total Counts (%TC), Incidence (I)and Percentage Incidence (%I) of osmophilic fungal species isolated from 40 samples of nuts and seeds on 40% sucrose Czapek's agar at 25°C

	Peanut (7 samples)				Chick pea (4 samples)				pine seeds (3 samples)				Sun flower seeds (2 samples)			
Fungal species	TC	%TC	I out of 7	%I	TC	%TC	I out of 4	%I	TC	%TC	I out of 3	%I	TC	%TC	I out of 2	%I
Alternaria alternata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus flavus	104	20.3	4	57.1	40	11.9	2	50	104	34.2	3	100	40	38.5	2	100
A. niger	144	28.1	5	71.4	96	28.6	3	75	152	50	3	100	16	15.4	1	50
A. proliferans	0	0	0	0	8	2.4	1	25	0	0	0	0	0	0	0	0
A. terreus	8	1.6	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium cladosporioides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eurotium amstelodami	40	7.8	3	42.9	72	21.4	3	75	8	2.6	1	33.3	24	23.1	1	50
Eurotium montevidensis	120	23.4	4	57.1	32	9.5	2	50	40	13.2	1	33.3	0	0	0	0
E. repens	0	0	0	0	8	2.4	1	25	0	0	0	0	0	0	0	0
E. ruber	0	0	0	0	8	2.4	1	25	0	0	0	0	0	0	0	0
E. ubrum	48	9.4	3	42.9	72	21.4	2	50	0	0	0	0	0	0	0	0
E. umbrosus	0	0	0	0	0	0	0	0	0	0	0	0	24	23.1	1	50
Mucor hiemalis	16	3.1	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces variotii	8	1.6	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium oxalicum	8	1.6	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus oryzae	8	1.6	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Sterile mycelium	8	1.6	1	14.3	0	0	0	0	0	0	0	0	0	0	0	0
Total count	512				336				304				104			

	Hazelnut (1 sample)				Walnut (1 sample)				Cashew (1 sample)				Karela seeds (2 samples)			
Fungal species	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 2	%I
Alternaria alternata	0	0	0	0	0	0	0	0	0	0	0	0	8	9	1	50
Aspergillus flavus	24	25	1	100	24	21.4	1	100	72	429	1	100	16	18.2	1	50
A. niger	72	75	1	100	88	78.6	1	100	0	0	0	0	24	27.3	1	50
A. proliferans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. terreus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium cladosporioides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eurotium amstelodami	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. montevidensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. repens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. ruber	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. rubrum	0	0	0	0	0	0	0	0	0	0	0	0	40	45.5	1	50
E. umbrosus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mucor hiemalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces variotii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium oxalicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus oryzae	0	0	0	0					96	57.1	1	100	0	0	0	0
Sterile mycelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total count	96				112				168				88			

	Pumpkin seeds (11 samples)				Almond (3 samples)				Pistachio (5 samples)			
Fungal species	TC	%TC	I out of 11	%I	TC	%TC	I out of 3	%I	TC	%TC	I out of 5	%I
Alternaria alternata	0		0	0	0	0	0	0	0	0	0	0
Aspergillus flavus	80	11.4	7	63.6	24	18.8	0	0	112	37.8	3	60

Table 4: Continued

	Pumpkin seeds (11 samples)				Almond (3 samples)				Pistachio (5 samples)			
Fungal species	TC	%TC	I out of 11	%I	TC	%TC	I out of 3	%I	TC	%TC	I out of 5	%I
A. niger	88	12.5	7	63.6	88	68.8	2	66.7	152	51.4	4	80
A. proliferans	0	0	1	9.1	0	0	0	0	0	0	0	0
A. terreus	0	0	2	18.2	0	0	0	0	0	0	0	0
Cladosporium cladosporioides	32	4.6	0	0	0	0	0	0	16	5.4	2	40
Eurotium amstelodami	110	15.6	3	27.3	0	0	0	0	0	0		0
E. montevidensis	176	25	6	54.5	0	0	0	0	0	0	0	0
E. repens	0	0	0	0	0	0	0	0	0	0	0	0
E. ruber	0	0	0	0	0	0	0	0	0	0	0	0
E. rubrum	202	28.7	2	18.2	0	0	0	0	0	0	0	0
E. umbrosus	16	2.3	2	18.2	0	0	0	0	0	0	0	0
Mucor hiemalis	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces variotii	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium oxalicum	0	0	0	0	0	0	0		0		0	
Rhizopus oryzae	0	0	0	0	16	12.5	2	66.7	16	5.4	1	40
Sterile mycelium	0	0	0	0	0	0	0	0	0	0	0	0
Total count	704				128				296			

Table 5: Total Count (TC), Percentage Total Count (%TC), Incidence (I) and Percentage Incidence (%I) of thermophilic and/or thermotolerant fungal species isolated from 40 samples of nuts and seeds on YPSS Medium at 45°C

	Peanut (7 samples)				Chick pea (4 samples)				pine seeds (3 samples)				Sun flower seeds (2 samples)			
Genera and species	TC	%TC	I out of 7	%I	TC	%TC	I out of 4	%I	TC	%TC	I out of 3	%I	TC	%TC	I out of 2	%I
Absidia corymbifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus flavus	0	0	0	0	0	0	0	0	8	3.6	1	33.3	0	0	0	0
A. fumigatus	32	57.1	1	14.3	32	23.6	1	25	96	42.9	1	33.3	96	1	1	50
A. niger	24	42.9	1	14.3	88	64.7	3	75	96	42.9	1	33.3	8	1	1	50
A. terreus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Emericella nidulans	0	0	0	0	8	5.9	1	25	8	3.6	1	33.3	0	0	0	0
Rhizomucor pusillus	0	0	0	0	8	5.9	1	25	0	0	0	0	0	0	0	0
Talaromyces thermophilus	0	0	0	0	0	0	0	0	8	3.6	1	33.3	0	0	0	0
sterile mycelium	0	0	0	0	0	0	0	0	8	3.6	1	33.3	0	0	0	0
Yeast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total count	56				136				224				104			
	Hazelnut (1 sample)				Walnut (1 sample)				Cashew (1 sample)				Karela (2 samples)			
Genera and species	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 1	%I	TC	%TC	I out of 2	%I
Absidia corymbifera	0	0	0	0	0	0	0	0	8	9	1	100	8	100	1	50
Aspergillus flavus	0	0	0	0	0	0	0	0	8	9	1	100	0	0	0	0
A. fumigatus	0	0	0	0	8	16.7	1	100	0	0	0	0	0	0	0	0
A. niger	0	0	0	0	32	66.7	1	100	72	82	1	100	0	0	0	0
A. terreus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Emericella nidulans	0	0	0	0	8	16.7	1	100	0	0	0	0	0	0	0	0

Table 5: Continued

Genera and species	Hazelnut (1 sample) TC	%TC	I out of 1	%I	Walnut (1 sample) TC	%TC	I out of 1	%I	Cashew (1 sample) TC	%TC	I out of 1	%I	Karela (2 samples) TC	%TC	I out of 2	%I
Rhizomucor pusillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Talaromyces thermophilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sterile mycelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yeast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total count	0				48				88				8			

Genera and species	Pumpkin seeds (11 samples) TC	%TC	I out of 11	%I	Almond (3 samples) TC	%TC	I out of 3	%I	Pistachio (5 samples) TC	%TC	I out of 5	%I
Absidia corymbifera	32	10	3	27.3	0	0	0	0	8	2.6	1	20
Aspergillus flavus	16	5	1	9.1	0	0	0	0	8	2.6	1	20
A. fumigatus	24	75	1	9.1	8	11.1	1	33.3	0	0	0	0
A. niger	232	72.5	8	72.7	32	44.4	1	33.3	248	79.5	4	80
A. terreus	8	2.5	1	9.1	0	0	0	0	40	12.8	2	40
Emericella nidulans	0	0	0	0	0	0	0	0	0	0	0	0
Rhizomucor pusillus	0	0	0	0	32	44.4	1	33.3	8	2.6	1	20
Talaromyces thermophilus	0	0	0	0	0	0	0	0	0	0	0	0
sterile mycelium	8	2.5	1	9.1	0	0	0	0	0	0	0	0
Yeast	0	0	0	0	0	0	0	0	0	0	0	0
Total count	320				72				312			

Table 6: Collective total counts (CTC) and incidences (I) of fungal species isolated from the 40 samples of nuts and dried seeds on different medium types

Fungal species	Mesophilic fungi (on glucose Cz) CTC	%CTC	I	%I	Cellulose decomposing fungi (on cellulose-Cz) CTC	%CTC	I	%I	Osmophilic fungi sucrose (on 40% sucrose-Cz) CTC	%CTC	I	%I	Thermophilic and thermotolerant fungi (on YPSS) CTC	%CTC	I	%I
Absidia corymbifera	0	0	-	-	40	1.7	3.0	7.5	0	0	-	-	56	4	6	15
Acremonium strictum	0	0	0	0	24	1.0	2.0	5.0	0	0	-	-	0	0	-	-
Alternaria alternata	0	0	0	0	8	0.3	1.0	2.5	8	0.3	1.0	2.5	0	0	-	-
Aspergillus flavus	600	23.9	26	65	768	33.0	29	72.5	640	22.5	23	58	56	4	6	15
A. fumigatus	0	0	0	0	32	1.4	4	10	0	0	-	-	296	21.3	7	17.5
A. niger	1136	43	28	70	896	38.5	32	80	1048	36.9	28	70	832	59.8	21	52.5
A. proliferans	0	0	-	-	0	0	-	-	8	0.3	1.0	2.5	0	0	-	-
A. tamarii	16	0.6	2	5	0	0	-	-	0	0	-	-	0	0	-	-
A. terreus	32	1.2	3	7.5	0	0	-	-	32	1.1	2.0	5.0	48	3.4	3	7.5
A. sclerotioniger	64	2.1	2	5	0	0	-	-	0	0	-	-	0	0	-	-
A. ustus	0	0	0	0	16	0.9	1.0	2.5	0	0	-	-	0	0	-	-
A. versicolor	8	0.3	1	2.5	0	0	-	-	0	0	-	-	0	0	-	-
Cephaliophora irregularis	0	0	0	0	8	0.3	1.0	2.5	0	0	-	-	0	0	-	-
Cladosporium cladosporioides	0	0	0	0	56	2.4	4.0	10	40	1.4	3.0	7.5	0	0	-	-
C. sphaerospermum	16	0.6	2	5	0	0	-	-	0	0	-	-	0	0	-	-
Colletotrichum coccodes	16	0.6	1	2.5	0	0	-	-	0	0	-	-	0	0	-	-
Corynascus sepedonium	0	0	-	-	8	03	1.0	2.5	0	0	-	-	0	0	-	-
Curvularia ovoidea	8	0.3	1	2.5	0	0	-	-	0	0	-	-	0	0	-	-

Table 6: Continued

	Mesophilic fungi (on glucose Cz)				Cellulose decomposing fungi (on cellulose-Cz)				Osmophilic fungi sucrose (on 40% sucrose-Cz)				Thermophilic and thermotolerant fungi (on YPSS)			
Fungal species	CTC	%CTC	I	%I	CTC	%CTC	I	%I	CTC	%CTC	I	%I	CTC	%CTC	I	%I
Emericella acrestata	8	0.3	1	2.5	0	0	-	-	0	0	-	-	0	0	-	-
E. nidulans	24	0.9	2	5	16	0.9	2.0	5.0	0	0	-	-	24	1.7	3	7.5
Eurotium amstelodami	32	1.2	2	5	16	0.9	2.0	5.0	264	9.3	11	27.5	0	0	-	-
E. montevidensis	0	0	-	-	0	0	-	-	368	13	10	25	0	0	-	-
E. repens	0	0	-	-	0	0	-	-	8	0.3	1.0	2.5	0	0	-	-
E. rubrum	0	0	-	-	0	0	-	-	208	7.3	9	22.5	0	0	-	-
E. umbrosum	0	0	-	-	0	0	-	-	40	1.4	3	7.5				
Fusarium sambucinum	0	0	0	0	16	0.9	2.0	5.0	0	0	-	-	0	0	-	-
F. verticillioides	0	0	0	0	8	0.3	1.0	2.5	0	0	-	-	0	0	-	-
Microascus cinereus	8	0.3	1	2.5	0	0	-	-	0	0	-	-	0	0	-	-
Mucor hiemalis	24	0.9	2	5	0	0	-	-	16	0.6	1.0	2.5	0	0	-	-
Neurospora crassa	8	0.3	1	2.5	8	0.3	1.0	2.5	0	0	-	-	0	0	-	-
Paecilomyces variotii	48	1.8	3	7.5	16	0.9	2.0	5.0	8	0.3	1.0	2.5	0	0	-	-
Penicillium aurantiogriseum	32	1.2	1	2.5	0	0	-	-	0	0	-	-	0	0	-	-
P. brevicompactum	16	0.6	2	5	0	0	-	-	0	0	-	-	0	0	-	-
P. chrysogenum	24	0.9	4	10	0	0	-	-	0	0	-	-	0	0	-	-
P. citrinum	8	0.3	1	2.5	0	0	-	-	0	0	-	-	0	0	-	-
P. glabrum	16	0.6	2	5	0	0	-	-	0	0	-	-	0	0	-	-
P. oxalicum	0	0	-	-	40	1.7	5.0	12.5	8	0.3	1.0	2.5	0	0	-	-
P. purpurogenum	16	0.6	2	5	0	0	-	-	0	0	-	-	0	0	-	-
Rhizomucor pussilus	0	0	-	-	0	0	-	-	0	0	-	-	48	3.4	3	7.5
Rhizopus oryzae	288	10.9	10	25	248	10.7	12	30	136	4.8	5	12.5	0	0	-	-
Scopulariopsis brevicaulis	0	0	-	-	24	1.0	2.0	5.0	0	0	-	-	0	0	-	-
Sporotrichum roseolum	0	0	-	-	8	0.3	1.0	2.5	0	0	-	-	0	0	-	-
Stachybotrys chartarum	0	0	-	-	8	0.3	1.0	2.5	0	0	-	-	0	0	-	-
Syncephalastrum racemosum	8	0.3	1	2.5	0	0	-	-	0	0	-	-	0	0	-	-
Talaromyces thermophilus	0	0	-	-	0	0	-	-	0	0	-	-	8	0.6	1	2.5
Trichoderma harzianum	0	0	-	-	16	0.9	1.0	2.5	0	0	-	-	0	0	-	-
Trichothecium roseum	8	0.3	1	2.5	8	0.3	1.0	2.5	0	0	-	-	0	0	-	-
Ulocladium consortiale	16	0.6	2.0	5.0	0	0	-	-	0	0	-	-	0	0	-	-
Unidentified yeasts	160	6.1	8	20	0	0	-	-	0	0	-	-	8	0.6	1	2.5
Sterile mycelia	0	0	-	-	40	1.7	4.0	10.0	8	0.3	1.0	2.5	16	1.1	2	5
Gross total count	2640				2328					2840				1392		

as 59.8% TC on YPSS). *A. flavus* was also isolated in all medium types (range: 4% TC on YPSS to as much as 33% on Cellulose-Cz agar). Six other species of *Aspergillus* were isolated from different medium types. A complete detailed fungal isolates collective total count is presented in Table 6.

Aflatoxin B_1 (8.5 μg mL^{-1}) was detected in a salted peanut sample containing *A. flavus*. Aflatoxin B_1 (1.7 μg mL^{-1}) and B_2 (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*. DAS (2.4 μg mL^{-1}) was detected in a salted peanut sample containing *Trichthecium roseum*. No mycotoxins were detected in the chloroform extracts of the different samples analyzed (Table 7).

Table 7: Mycotoxins (μg mL^{-1}) produced by some fungal species isolated from nut samples

Isolate No.	Fungal species	Source and sample No.	Aflatoxin B_1	Aflatoxin B_2	T_2 Toxin	DAS
89	*Acremonium strictum*	Afghani Roasted pumpkin seeds No. 28	-	-	-	-
28	*Aspergillus flavus*	Salted peanut No. 4	8.5	-	-	-
25	*A. flavus*	Peanut seeds (not salted) No. 3	-	-	-	-
70	*A. terreus*	Sunflower seeds No. 15	1.7	1.7	-	-
44	*A. tamarii*	Salted Peanut in kernels No. 7	-	-	-	-
98	*Curvularia ovoidae*	Roasted pumpkin seeds No. 22	-	-	-	-
95	*Paecilomyces variotii*	American Almond No. 35	-	-	-	-
71	*Penicillium chrysogenum*	Salted sunflower seeds No. 16	-	-	-	-
93	*P. purpurogenum*	Syrian Roasted pumpkin seeds No. 32	-	-	-	-
49	*P. purpurogenum*	Salted Turkish Chick-pea No. 9	-	-	-	-
86	*Stachybotrys chartarum*	Pumpkin seeds No. 27	-	-	2.8	-
68	*Trichoderma harzianum*	Chinese Pine seeds No. 14	-	-	-	-
27	*Trichthecium roseum*	Salted peanut No. 4	-	-	-	2.4

Table 8: Positive results of microbiological evaluation of nuts and seed samples in Riyadh, Saudi Arabia

Samples	TPC	TCC	FCC	B.C	Salmonella	Listeria
Turkish Pine seeds	12×10	16×10	-	-	-	-
Pakistani Pine seeds	3×10	4×10	5×10	-	-	-
American Walnut	12×10	-	-	-	-	18×10
Iranian salted Pistachio	3×0	2×0	4×10	-	-	-

TPC = Total Plate count, TCC = Total Coliform count, FCC = Faecal coliform count, B.C = *Bacillus cereus*. Note: cells indicated as (-) means no bacteria isolated from the sample/s

Four nut samples showed contamination with bacteria. Turkish pine seeds and American walnut had total plate counts of 12×10. Pakistani pine seeds and Iranian salted pistachio had TPC of 3×10. Listeria monocytogenes was isolated from American walnut samples (Table 8).

DISCUSSION

Present study confirmed the capability of *Aspergillus* species in producing mycotoxins which can be harmful for human consumption. This is in agreement with previous studies on this subject matter (Hedayati *et al*., 2007). In contrast to the findings made by Wang and Liu (2007), present results showed only 8.5 μg mL^{-1} of mycotoxins from salted peanut contaminated with *Aspergillus niger*. Wang and Liu (2007) reported upto 28.4 μg kg^{-1} of mycotoxins from peanuts. Furthermore, three strains of Aspergillus are identified from peanut samples, namely: *A. flavus*, *A. niger* and *A. fumigatus*. These have been mentioned by Hedayati *et al*. (2007).

In this study, we were able to demonstrate the diverse strains and species of fungi that can be isolated from nuts and edible seeds. Considering the fact that amongst these isolated fungi are strains or species that are capable of producing mycotoxins, to name a few, *A. flavus*, *A. terreus* and *S. chartarum* (Table 7). Unfortunately, these fungi are ubiquitous and widespread at all levels of the food chain. Their presence is considered unavoidable and it is not possible to predict or prevent entirely their occurrence during cultivation, harvest, storage and processing operations by current good agronomic and good manufacturing practices. As mentioned by Dorner (2008), measures to control levels of mycotoxins may not totally be a success story since a variety of environmental factors can affect storage and consumption thus making it very difficult to minimize mycotoxin porduction. Under favourable conditions of temperature and humidity, these fungi grow on certain foods especially on edible nuts and seeds resulting in the production of toxins. Much more, mycotoxins can also be metabolized by animals fed contaminated grains and pass into milk, eggs and other organs entering the food chain once again as previously reported by several researchers (Soubra *et al.*, 2009; Wang and Liu, 2007; Pacheco and Scussel, 2007; Kenjo *et al.*, 2007; Molyneux *et al.*, 2007; Abdulkadar *et al.*, 2002).

Mycotoxins may cause various adverse health effects from immediate toxic response and immune-suppression to the potential long-term carcinogenic effects. *S. chartarum* has been found to cause pulmonary diseases including pulmonary arterial hypertension (Shariat and Collard, 2007; Ochial *et al.*, 2008; Al-Ahmad *et al.*, 2010). The variety of symptoms also include dermatitis, recurring cold and flu-like symptoms, burning sore throat, headaches and excessive fatigue, diarrhea and impaired or altered immune function. *Cladosporium* and *Aspergillus* are commonly found fungi in ventilation systems and indoor environments making up to 75% of the particulates, also found in present study (Reboux *et al.*, 2009; Hedayati *et al.*, 2009; Bundy *et al.*, 2009). These organisms can occur naturally in the exterior environment and enter as spores or active fungi attached to dust particles. These families of molds have been implicated in being causative agents in asthma, hypersensitivity pneumonitis and pulmonary mycosis, including toxic pneumonitis, tremors, chronic fatigue syndrome, kidney failure and cancer. Exposure to molds has become a significant health risk to an increasing number of workers in various occupations throughout the nations. Fungal antigens are able to cause occupational asthma, rhinoconjunctivitis, hypersensitivity pneumonitis and organic dust toxic syndrome.

Growth of commonly occurring filamentous fungi result in production of mycotoxins, the most dangerous aflatoxins, ochratoxin A, fumonisins, trichothecenes and zearalenone, a toxin known for causing infertility and endometriosis in animals (Meyer *et al.*, 2000). Aflatoxins are potent carcinogens and in association with hepatitis B virus are responsible for many thousands of human deaths per annum, mostly in non-industrialized tropical countries. Ochratoxin A is a carcinogen and has caused urinary tract cancer and kidney damage in people from northern and eastern Europe. Fumonisins appear to be the cause of oesophageal cancer in southern Africa, parts of China and elsewhere. Trichothecenes are highly immunosuppressive and zearalenone causes oestrogenic effects in animals and man.

Surprisingly, only 4 samples of this study showed bacterial contamination with only 3 samples showing positivity for coliforms and 1 for listerial contamination. These levels of microbial contamination of food are influenced by harvesting/slaughtering technologies and by the processes applied during food manufacture. With current technologies it is impossible to guarantee the absence of pathogenic microorganisms on raw foods, both of plant and animal origin thus, increasing incidence of foodborne diseases and the resultant social and economic impact on the human population have brought food safety to the forefront of public health concerns. Many outbreaks are the consequence of a failed process, or inappropriate storage conditions (usually temperature abuse) during distribution, food service or by the consumer. Besides, mycotoxin-producing molds such as *A. ochraceus* and *P. varidicatum* can produce ochratoxin A 4-5 days after inoculation at 25°C to 46 $\mu g\ g^{-1}$ of any grain after 28 days.

Hope and Simon (2007) has reported the association between exposure to dampness and excess growth mold and the development of aeroirritant symptoms. Changes in temperature, relative humidity and moisture content of food products are important indicators of fungal mycotoxin production. For example, Ochratoxin A. production occur in products stored at the top of containers and in wet bags (Palacios-Cabrera *et al.*, 2007). In present study, fungal growth occurred in all types of media, osmophiles with 2840 gross total count, followed by mesophiles (2,640 total count) and the thermopiles (1,392 total count) (Table 6). These findings state that fungi whether they are mycotoxin or non-mycotoxin producers grow in environmental conditions with adequate temperature, relative humidity and moisture.

CONCLUSION

The microbiological safety of food can never be achieved by end-product testing, which only detects that a failure has occurred and can only contribute indirectly to identification and control of the cause of the problem. Furthermore, the isolation of a bacterial pathogen from a food does not mean that the food necessarily is dangerous, e.g., the food may be cooked before consumption. Hence preventive approaches are required, often as simple as control of storage time and temperature. Nevertheless, microbiological testing, used appropriately, is one of the measures that can be used to achieve microbiological safety.

There is a continuous need to protect the health of humans and susceptible animals by limiting their exposure to mycotoxins because of their toxicological manifestations and agricultural products contaminated with harmful microorganisms. Long-term sequelae including the development of cancer and other fatal conditions should prompt health and safety authorities to regulate for or suggest permitted levels of mycotoxins in foods and feed because of the public health significance and commercial consequences. This should be carefully accounted for since monitoring such contamination and health hazard, this can have profound economic implications resulting in losses of foodstuff due to mycotoxins and bacterial contamination. In conclusion, 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.

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