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Fungal Flora Associated with Some Nuts in Three Different Regions in Saudi Arabia

Abeer R. M. Abd El-Aziz[1]*, Munirah F. Al-Gahtani[1], Mohamed A. Mahmoud[2] and Monira R. Al-Othman[1]

[1]Botany and Microbiology Department, College of Science, King Saud University, Riyadh 1145, Kingdom of Saudi Arabia.
[2]Plant Pathology Research Institute, Agricultural Research Center, Giza 12619, Egypt.

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The natural occurrence of fungal contamination was evaluated in stored nuts including Almond, cashew, peanuts and walnuts selected randomly from three different regions in Saudi Arabia. After initial preparation, the samples were cultured on potato dextrose agar. Sixty samples were analyzed and sixteen fungi belonging to eleven genera isolates were identified by morphological characteristics to genus level. The major genera of fungi isolated were *Aspergillus, Penicillium, Fusarium, Monilia, Chaetomium, Alternaria*, *Rhizopus* and *Mucor*. Mycological analyses revealed that the most frequent isolated fungi from different nuts were *Aspergillus spp.* Where it was *Aspergillus flavus* showed the highest prevalence in samples investigated followed by *Aspergillus niger* while *Aspergillus parasiticus* in the third rank.

Key words: Fungal flora, Nuts, Saudi Arabia.

Nuts are rich sources of protein, unsaturated fatty acids, minerals, vitamins, fiber, and polyphenols (Dreher *et al*., 1996) .So these are associated with several health benefits such as anticancer, anti-inflammatory, antioxidant and ant diabetic benefits (Vadivel *et al*., 2012). Nuts are subject to infection by a variety of microorganisms that can induce spoilage or produce metabolites that are toxic to humans, animals and birds. Although in many cases the sources of infections are not known, they are exacerbated by factors such as insect damage, drought and high temperatures. Fungi can grow on simple and complex food products and produce various metabolites. These microorganisms distribute in the environment by raining, wind and insects (Brus *et al*., 2005).

More than 100000 fungal species are natural contaminants of agricultural and food products (Kacaniova, 2003). A survey of incidence established that the most frequently found genera were Aspergillus, Rhizopus, and Penicillium (Bayman *et al*., 2002). Production of toxic secondary metabolites are Considered major problem related to fungal attack in nuts as zearalenone fumonisin, and aflatoxin which produced by *F. graminearum*, *F. verticillioides* and *A. flavus*, respectively (Scott, 1993). Fumonisins are reported cancer-promoting activity while Aflatoxin has powerful hepato-carcinogenic and mutagenic effects (Wang *et al*., 2001), in addition the co-occurrence of fumonisin with aflatoxin B1 (AFB1) lead to promotion of carcinogenesis (Ueno, 2000). The worldwide area harvested of nuts are

* To whom all correspondence should be addressed.

599368 hectare, production 835983 tons and crop yields 13948 tons/hectare (FAO, 2012).

The aim of the present study, was to determine mycoflora distribution of nuts such as Almond, cashew, peanuts and walnuts in three different location in Saudi Arabia.

MATERIALS AND METHODS

Collection of samples

Fifteen samples from four types of nuts (Almond, cashew, peanuts and walnuts) were collected randomly from different markets in three locations from Saudi Arabia (Riyadh, Al-Dammam and Abha) during 2012 for this experimental work. *The samples were* stored at 2 °C until used (Czerwiecki *et al*., 2002).

Isolation, purification and identification of pathogen

Samples were surface sterilized with 5% sodium hypochlorite solution for one minute, before they were rinsed three times with sterilized distilled water. Four pieces were placed on the surface of Petri dishes containing potato dextrose agar (PDA), and each entry replicated three times. Petri dishes were incubated at 25º ±2 C and observed daily for emergence of colonies, and then the colonies were counted. Isolates were purified either by hyphal tip methods and then transferred to PDA slants. The identification of isolates was confirmed by Regional Center of the Fungi and their Applications, Al-Azhar University, Cairo, Egypt.

The frequency of fungi of particu-lar species with in a genus of fungi was calculated using the formula of Ghiasian *et al.* (2004).

Frequency= Number of fungal species isolated X100

Total Number of fungi isolated

RESULTS

Isolation, purification and identification of fungi associated with Cashews, Walnuts, Almonds and Peanuts from Riyadh

Data presented in table (1) indicate that sixteen fungi belonging to eleven genera were isolated from five tested nuts. However, *Aspergillus* spp. were the most frequently from different nuts. *Aspergillus* spp. (45.8%), *Alternaria* spp. (12.5%), *Penicillium* (12.5%), *Eupenicillium crustaceum* (4.2%), *Rhizopus* spp (4.2%), *Fusarium* spp. (12.5%), and *Syncephalastrum spp.* (8.3%) were isolated from Cashews. *Aspergillus* spp. (46.9%), *Alternaria* (6.3%), *Penicillium* (9.4%), Eupenicillium crustaceum (3.1%), *Rhizopus* sp (3.1%), *Chaetomium murorum* (3.1), *Fusarium spp.*

Table 1. Frequency (%) of isolated fungi from Cashews, Walnuts, Almonds and Peanuts from Riyadh

Isolated fungi	Cashews	Walnuts	Almonds	Peanuts
Aspergillus flavus	25	25	19.2	20
Aspergillus niger	12.5	12.5	23.1	16
Aspergillus parasiticus	8.3	9.4	11.5	8
Onychocola canadensis	0	0.0	0	0
Syncephalastrum spp.	8.3	6.3	0	4
Penicillium citrinum	8.3	9.4	11.5	8
Penicillium islandicum	4.2	0.0	7.7	8
Eupenicillium crustaceum	4.2	3.1	3.8	4
Fusarium dimerum	8.3	6.2	7.7	8
fusarium equiseti	4.2	9.4	3.8	4
Monilia pruinosa	0	0.0	0	4
Chaetomium murorum	0	3.1	0	4
Alternaria phragmospora	4.2	6.3	3.8	0
Alternaria alternata	8.3	0.0	3.8	8
Rhizopus sp	4.2	3.1	3.8	4
Mucor	0	6.2	0	4
Total	100	100	100	100

(15.6%), *Syncephalastrum spp.* (6.3%) and *Mucor* (6.2%) were isolated from Walnuts. *Aspergillus* spp. (53.8%), *Penicillium* (19.2%), Eupenicillium crustaceum (3.8%), *Fusarium spp.* (11.5%), *Rhizopus* sp (3.8%), and *Alternaria* (7.6%), were isolated from Almonds. *Aspergillus* spp. (44%), *Syncephalastrum spp.* (4%), *Alternaria* (8%), *Penicillium* (16%), *Eupenicillium crustaceum* (4%), *Fusarium* spp. (12%), *Rhizopus* sp (4%), *Chaetomium murorum* (4%), and *Monilia pruinosa* (4%) were isolated from Peanuts.

In addition that, *Aspergillus flavus* showed the highest prevalence in samples investigated .The highest frequently of

Table 2. Frequency (%) of isolated fungi from Cashews, Walnuts, Almonds and Peanuts from Dammam

Isolated fungi	Cashews	Walnuts	Almonds	Peanuts
Aspergillus flavus	27.3	28.6	20	23.5
Aspergillus niger	18.2	14.3	25	17.6
Aspergillus parasiticus	9.1	10.7	10	11.8
Onychocola canadensis	0	0	0	0
Syncephalastrum spp.	4.5	3.6	0	0
Penicillium citrinum	4.5	0	0	11.8
Penicillium islandicum	9.1	7.1	10	17.6
Eupenicillium crustaceum	0	3.6	0	0
Fusarium dimerum	9.1	7.1	10	11.8
Fusarium equiseti	4.5	7.1	5	5.9
Monilia pruinosa	4.5	0	0	0
Chaetomium murorum	0	3.6	0	0
Alternaria phragmospora	4.5	3.6	5	0
Alternaria alternata	0	7.1	5	0
Rhizopus sp	4.5	3.6	5	0
Mucor	0.0	0	5	0
Total	100	100	100	100

Table 3. Frequency (%) of isolated fungi from Cashews, Walnuts, Almonds and Peanuts from Abha

Isolated fungi	Cashews	Walnuts	Almonds	Peanuts
Aspergillus flavus	16.7	21.0	15.4	17.4
Aspergillus niger	10	10.5	11.5	17.4
Aspergillus parasiticus	6.7	10.5	7.7	4.4
Onychocola canadensis	0	0.0	3.8	0
Syncephalastrum spp.	0	5.3	3.8	0
Penicillium citrinum	10	10.5	7.7	4.4
Penicillium islandicum	6.7	5.3	11.5	8.7
Eupenicillium crustaceum	6.7	5.3	3.8	0
Fusarium dimerum	13.3	5.3	7.7	8.7
Fusarium equiseti	0	10.5	7.7	13.0
Monilia pruinosa	0	0.0	3.8	8.7
Chaetomium murorum	10	5.3	0	8.7
Alternaria phragmospora	6.7	5.3	7.7	0
Alternaria alternata	3.3	0.0	0	4.4
Rhizopus sp	6.7	0.0	3.8	4.4
Mucor	3.3	5.3	3.8	0
Total	100	100	100	100

Aspergillus flavus was isolated from Cashews and Walnuts (25%) and the lowest frequently was isolated from Almonds (19.2%) followed by *Aspergillus niger* , The highest frequently were isolated from Almonds (23.1%) while *Aspergillus parasiticus* in the third rank.

Isolation, purification and identification of fungi associated with Cashews, Walnuts, Almonds and Peanuts from Dammam

Data presented in table (2) indicate that sixteen fungi belonging to eleven genera were isolated from five tested nuts. However, *Aspergillus* spp. was the most frequently from different nuts. *Aspergillus* spp. (54.6%), *Alternaria* (4.5%), *Penicillium* (13.6%), *Rhizopus* spp (4.5%), *Fusarium* spp. (13.6%), *Monilia pruinosa* (4.5%), and *Syncephalastrum spp.* (4.5%) were isolated from Cashews.

Aspergillus spp. (53.6%), *Alternaria* (10.7%), *Penicillium* (7.1%), *Eupenicillium crustaceum* (3.6%), *Rhizopus* spp. (3.6%), *Chaetomium murorum* (3.6), *Fusarium spp*. (14.2%), and *Syncephalastrum* spp. (3.6%) were isolated from Walnuts.

Aspergillus spp. (55%), *Alternaria* (10%), *Penicillium* (10%), *Fusarium spp.* (15%), *Rhizopus* sp (5%), and *Mucor (5%)* were isolated from Almonds.

Aspergillus spp. (52.9%), *Penicillium* (29.4%) and *Fusarium* spp. (17.7%), were isolated from Peanuts. In addition that, *Aspergillus flavus* showed the highest prevalence in samples investigated .The highest frequently *of Aspergillus flavus* was isolated from Walnuts (28.6%) and the lowest frequently was isolated from Almonds (20 %) followed by *Aspergillus niger* , The highest frequently were isolated from Almonds (25%) while *Aspergillus parasiticus* in the third rank.

Isolation, purification and identification of fungi associated with Cashews, Walnuts, Almonds and Peanuts from Abha

Data presented in table (3) indicate that sixteen fungi belonging to eleven genera were isolated from five tested nuts. However, *Aspergillus* spp. were the most frequently from different nuts. *Aspergillus* spp. (33.4%), *Alternaria* (10%), *Penicillium* (16.7%), *Eupenicillium crustaceum* (6.7%), *Rhizopus* sp (6.7%), *Fusarium* spp. (13.3%), *Chaetomium murorum* (10%) and *Mucor* spp (3.3%) were isolated from Cashews. *Aspergillus* spp. (42%), *Syncephalastrum* spp. (5.3%), *Alternaria* (5.3%), *Chaetomium murorum* (5.3%), *Penicillium* (15.8%), *Eupenicillium crustaceum* (5.3%), *Fusarium* spp. (15.8%), and *Mucor* spp. (5.3%) were isolated from Walnuts. *Aspergillus* spp. (34.6%), *Onychocola canadensis* (3.8%), *Syncephalastrum spp* (3.8%), *Alternaria* (7.7%), *Penicillium* (19.2%), Eupenicillium crustaceum (3.8%), *Fusarium spp.* (15.4%) *Monilia* (3.8%) *Mucor* spp (3.8%) *and Rhizopus* sp (3.8%) were isolated from Almonds. *Aspergillus* spp. (39.2%), *Penicillium* (13.1%), *Fusarium* spp. (21.7%) *Monilia pruinosa* (8.7), *Chaetomium murorum* (8.7%), *Alternaria* (4.4%), and *Rhizopus* spp. (4.4%) were isolated from Peanuts.

In addition that, *Aspergillus flavus* showed the highest prevalence in samples investigated. The highest frequently *of Aspergillus flavus* was isolated from Walnuts (21%) and the lowest frequently was isolated from Almonds (15.4%) followed by *Aspergillus niger*, the highest frequently were isolated from Peanuts (17.4%) and the lowest frequently was isolated from Cashews (10%) while *Aspergillus parasiticus* in the third rank.

DISCUSSION

Nuts rich in energy, protein, packed with antioxidants, vitamins, minerals and high lipid contents but with favorable profiles for promoting cardiovascular health, since they are low in saturated fatty acids and high in mono and polyunsaturated fatty acids (also known omega-3 fatty acids). (Sathe *et al*., 2008; Celik *et al*., 2010). Several fungi are capable of infecting growing nuts and causing damage to hulls and kernels (Denizel *et al*., 2006). Nuts are among the crops that can be contaminated by aflatoxins (AFs), mycotoxins mainly produced by *A. flavus* and *A. parasiticus*, peanuts (*Arachis hypogaea*) (Hedayati *et al*., 2007; Hedayati *et al*., 2010), Almond (*Prunus dulcis* M) (Deabes and Al- Habib, 2011), Walnut (*Juglans regia*) (Singh and Shukla, 2008), Cashew (*Anacardium occidentale*) (Deabes and Al- Habib, 2011). Extensive survey was conducted throughout three regions (Riyadh, Al-Dammam and Abha) in Saudi Arabia to determine the frequency of various fungi associated with Cashews,

Walnuts, Almonds and Peanuts. Sixteen fungi belonging to eleven genera were isolated from four tested nuts. However, *Aspergillus* spp. were the most frequently from different nuts in these findings are also similar to those of other studies (Shahidi, 2004; Yu *et al*., 2004; Hedayati *et al*., 2007; Hedayati *et al.*, 2010; Kabirian *et al.*, 2011; Khodavaisy e*t al*., 2012). Percentage of *Aspergillus* spp recorded the highest contamination in Dammam and the least value was in Abha in five tested nuts. *Aspergillus flavus* showed the highest prevalence in samples investigated followed by *Aspergillus niger*, while *Aspergillus parasiticus* in the third rank. Regional differences in aflatoxin contamination of crops may be attributable to climatic conditions and to agricultural practices that increase susceptibility of plants to invasion by *A. flavus* and relative humidity plays a vital role in the development and spread of fungal contaminations (Nawar, 2008) and pre-harvest conditions of temperature and humidity in the field and improper postharvest handling and storage (Nakai *et al.*, 2008; Khodavaisy *et al.*, 2012).

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