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Evaluation of Fungal Flora in Some Important Nut Products (Pistachio, Peanut, Hazelnut and Almond) in Tehran, Iran

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Abstract: The natural occurrence of fungal contamination was evaluated in stored nuts including pistachio, peanut, hazelnut and almond selected randomly from different regions of Tehran. After initial preparation, the samples were cultured on Sabouraud glucose agar, Dichloran rose-bengal agar and Czapek-dox agar. A total of 60 samples were analyzed and 158 fungal isolates were identified. 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%). Significant difference was observed between the frequency of fungal isolates of pistachio, peanut and hazelnut with almond ($p<0.05$). The results demonstrated that nut samples with a relative high incidence of diverse species of fungi to need for proper surveillance and monitoring exclusively for the prevention of fungal and mycotoxin contaminations before it reaches the consumer.

Key words: Fungal contamination, peanut, pistachio, *Aspergillus*, *Penicillium*

Introduction

Fungi can grow on simple and complex food products and produce various metabolites. These microorganisms exist in the environment and distribute by wind, insects and raining (Brus *et al*., 2005). Up to now, more than 100000 fungal species are considered as natural contaminants of agricultural and food products (Kacaniova, 2003). The majority of the toxic species belongs to the genera *Aspergillus, Penicillium* and *Fusarium* (Kaushal and Sinha, 1993). A major problem related to fungal attack in nuts is the production of toxic secondary metabolites, particularly fumonisin, zearalenone and aflatoxin, produced by *F. verticillioides, F. graminearum* and *A. flavus*, respectively (Scott, 1993). Aflatoxin has powerful teratogenic, mutagenic and hepato-carcinogenic effects (Wang *et al*., 2001), while fumonisins are reported to have cancer-promoting activity, in addition to causing several diseases in human (Bullerman and Draughon, 1994). The co-occurrence of fumonisin with aflatoxin B1 (AFB1) is presumed to play an important role in the promotion of carcinogenesis (Ueno, 2000). Previous studies showed that 30.97 million tones of greasy seed products, mainly pistachio and peanut, of different Asian and African countries were contaminated by *Aspergillus flavus* (*A. flavus*) and *A. parasiticus* (Christensen and Meronuck, 1986; Dekoe *et al*., 2000; D'Mello and Macdonald, 1998). They had been infected during or after harvesting, storage and transition (Bilgrami and Choudhary, 1990; Bruce *et al*., 2003). The storage temperature, moisture content, presence of oxygen and gaseous composition are the most important factors influencing the development of fungi during storage (Kubatova, 2000). Both fungal and mycotoxin contamination are currently regarded as the primary concern in the effort to reduce problems in the global trade of agricultural commodities. Hence, there is a great need for more extensive investigations, where nuts production and consumption are predominant. Since, the naturally fungal contamination of nuts from Iran had not been studied to date, the aim of the present study, was to determine mycoflora distribution of Iranian nuts such as pistachio, peanut, hazelnut and almond.

Materials and Methods

Sampling: Sampling was done randomly on some human food products such as pistachio, peanut, hazelnut and almond. A total of 50-100 gr of each sample were taken in sterile pockets coincidentally among three geographic regions in Tehran (south, east and west) under standard conditions. The date and place of the sampling were labeled on the pockets and immediately sent to the Mycology Research Center, Tehran.

Mycological analyses: One hundred grams from each sample were sterilized in a 0.4% sodium hypochlorite solution for 2 minutes. Subsequently, the supernatant solution was discarded and the sample was rinsed once in distilled water and followed to dry. Each sample was grinded into powder by vortex and then 1 gr was poured into 100 mL of sterile distilled water and stirred. Subsequently, 1 mL of supernatant was inoculated into Dichloran Rose-Bengal Chloramphenicol (DRBC, *Sigma, USA*) agar. The cultures were then incubated for 5 to 14 days at 25°C in a dark chamber. The colonies were exactly isolated and sub-cultured on slant Potato Dextrose agar (PDA, *Merck, Germany*) and Sabouraud

Table 1: Frequency distribution (count,%) of fungi isolated from different nut products

Sample	Mean Fungal	Aspergillus	Penicillium	Mucor	Fusarium	Paecilomyces	Yeast	Total Contamination (%)
Pistachio	1.6	10 (20.4)	13 (26.5)	12 (24.4)	2 (4.1)	8 (16.3)	4 (8.2)	49
Peanut	2.1	26 (45.6)	17 (29.8)	8 (14)	5 (8.8)	1 (1.8)	0	57
Almond	1	1 (20)	1 (20)	0 (0)	0 (10)	0 (0)	3 (60)	5
Hazelnut	1.5	14 (29.7)	17 (36.1)	7 (14.8)	6 (12.7)	2 (4.3)	1 (2.1)	47
Total	-	51 (32.2)	48 (30.3)	27 (17.1)	13 (8.2)	11 (6.9)	8 (5.1)	158

Glucose agar (SGA, *Merck*, *Germany*) media. *Fusarium* species were isolated, transferred onto Spezieller Nahrstoffarmer agar (SNA, *Difco*, *Germany*) and incubated at 25°C for 7 days. Final identification of *Fusarium* species was conducted according to method of Nelson *et al.* (1983). Also, *Aspergillus* species were identified using PDA and Czapek-dox agar (CZA, *Merck*, *Germany*) media and according to the method of Raper and Fennell (1965). The other genera were identified using PDA and SGA media.

Statistical analyses: Unpaired Student' s t test was performed using SPSS software (Version 13.0) and differences were considered significant at $p<0.05$.

Results

As shown in Table 1, 6 genera were isolated from different nuts including *Aspergillus*, *Penicillium*, *Mucor*, *Fusarium*, *Paecilomyces* and *yeast*. The most predominant isolated fungus was *Aspergillus* (51, 32.2%), followed by *Penicillium* (48, 30.3%), *Mucor* (27, 17.1%), *Fusarium* (13, 18.2%), *Paecilomyces* (11, 6.9%) and *yeast* (8, 5.1%). *Penicillium* in pistachio (26.5%) and hazelnut (36.1%), *Aspergillus* in peanut (45.6%) and *Yeast* in almond (60%) were the highest contaminated fungi. The most common contaminated nuts were peanuts (57, 36.1%), pistachio (49, 30), hazelnut (47, 29.7%) and almond (5, 3.2%). The contamination mean values of peanut and pistachio were 2.1% and 1.6%, respectively. It is worth to mention that in this study there were significant differences between fungal contaminations in the peanuts, pistachio and hazelnut with almond.

Discussion

Fungi accidentally contaminate food products and decaying them (Pitt and Hocking, 1985). Fungal contamination of edible greasy seeds, mostly pistachio and almond, were reported in different countries. Most of the peanut products of Indonesia, Philippine and Thailand are unusable due to fungal contaminations (Kendrick, 1992; Francisco *et al*., 1999; Hocking and Coventry, 2003). Those studies showed that *Aspergillus*, *Penicillium* and *Fusarium* were the most important toxigenic fungi that decay food products. In addition, fungal contaminations due to *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma* and *Cladosporium* in nuts especially almond and pistachio and other greasy edible seeds were reported in America, Brazil and western African countries (JEFCA, 2000; Weidonborner, 2001). In this regard, 97.3% and 94.5% of American pistachio products were contaminated by *Aspergillus* in 1995 and 2001, respectively (Schatzki, 1995; Doster *et al*., 2001). *Aspergillus* and other fungal contaminations such as *Penicillium* (with lower incidence) were also reported in the seeds in Netherlands, Denmark, Australia and India (Ghosh and Desai, 1997; D'Mello and Macdonald, 1998). Research on Iranian pistachio in Kerman gardens showed that 25% of the pistachio products that have gapped or lost their shells during early period of growth and 12.5% of the immature splitted ones were contaminated by *A. flavus* and other *Aspergillus* species (Shahidi Bojar, 2004). Early splitting of the pistachio and other edible greasy seeds, immature nuts and the shell splitting in early growth period causes fungal contamination. Wind and insects can be other factors of fungal contamination in nuts products (FAO, 1997). Previous study revealed that the presence of *Penicillium* and *Aspergillus* in soil may be the main causes of the contaminations in Peanuts. Regarding to direct contact of the soil with the peanuts in growth phases, fungi can penetrate through the peanut's shell and grow there (Pitt *et al*., 1991). Considering a relative high incidence of fungal contamination of nuts, it seems that difference in climate conditions of different regions of Iran and also, the traditional methods of handling grains during harvesting in the field, drying process and transferring lead to mechanical damages of grains. In this condition, broken and ground grains are more vulnerable to fungal attack than whole grains. 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.

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