Original article

Mycoflora of pistachio and peanut kernels from Sari, Iran

Mohammad Taghi Hedayati, Saied Kaboli, Sabah Mayahi

Department of Medical Parasitology and Mycology, School of Medicine, Mazandaran University of Medical Sciences, Km 18 Khazarabad Road, P.O. Box: 48175-1665, Sari, Iran

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Abstract

Introduction and objective: Fungal contamination of various foodstuffs and agricultural commodities is a major problem in the developing countries; therefore the aim of this study was to evaluate the mycoflora of pistachio and peanuts from retailers and dried fruit retail shops of Sari, Iran.

Materials and methods: A total number of 100 peanut and pistachio kernel samples in two consumption forms (dry roasted and raw) were collected from retailers and dried fruit retail shops in Sari city. Samples were analyzed for the presence of fungi by culture on Sabouraud dextrose agar media.

Results: Fungi were detected in almost 70% of the samples. The genus *Aspergillus* was the most predominant isolate from peanut (70.5%) and pistachio (62.7%) kernel samples. Among the species of *Aspergillus*, *A. flavus* was the most frequently isolated species in the collected samples. *A. flavus* also had the highest contamination mean value in dry roasted and raw form of peanut and pistachio kernels.

Conclusion: Because of the isolation of high percentage of *A. flavus* as the main aflatoxins producer in nature we recommend also the need of good storage practices in order to prevent the occurrence of aflatoxins in peanuts and pistachio.

Keywords: Mycoflora, *Aspergillus*, *Aspergillus flavus*, Kernels

Introduction

Pistachio and peanut kernels are nuts the most consumed in Iran. In addition, according to FAO statistics (2004), Iran is the number one exporter of pistachios in recent years and Iranian pistachios have been exported to many countries [1]. Peanuts are used in the fabrication of sweets, candies and pastes and mainly as a raw material in oil production. Peanut kernels have also human consumption in dry roasted form in Iran, especially by children. These kernels are usually contaminated by a range of different fungi during growth, harvesting and storage. Contamination of nut seeds by fungi and their toxic metabolites is a serious problem as it has adverse effects on human health

and causes economic problems for international trade in particular that of developing countries [2].

Some 300 to 400 compounds are now recognized as mycotoxins, of which approximately a dozen groups regularly receive attention as threat to human and animal health. The most important groups of mycotoxins that occur quite often in grains are: aflatoxins, ochratoxins, trichothecenes (deoxynivalenol, nivalenol), zearalenone, T-2 toxin and fumonisins. The fungi that produce these mycotoxins are various species of *Aspergillus*, *Penicillium*, and *Fusarium*, but mycotoxin production is restricted to only a few species within these genera.

The toxicity of the mycotoxins varies considerably with the toxin, the animal species exposed to it, and the extent of exposure, age and nutritional status. Most of the toxic effects of mycotoxins are limited to specific organs, but several mycotoxins affect many organs. Mycotoxins have four basic kinds of toxicity: acute, chronic, mutagenic and teratogenic. However, induction of cancer by some mycotoxins is a major concern as a chronic effect of these toxins [3]. However drying of fruit is a usual procedure to preserve them against mould contamination, mould attack still may be seen on these types of fruits.

Different studies from developing countries showed that the threat of fungi and mycotoxin contamination of foods and feeds resulting in human and livestock poisoning is really a major problem [4,5]. It has also been estimated that 25% of the world's crops are affected by mould or fungal growth [6]. The economic loss resulting from fungal and mycotoxin contamination of nuts is difficult to estimate. However, judging from the widespread occurrence of fungal and mycotoxin contamination and the large number of nuts affected, one can assume that such losses must be large [7].

Fungal contamination of various foodstuffs and agricultural commodities is a major problem in the tropics and sub-tropics, where climatic conditions and agricultural and storage practices are conducive to fungal growth and toxin production [8-12]. In view of these facts, together with the lack of studies regarding fungal contamination of dried fruit in Sari the capital of Mazandaran, a northern province of Iran with high humidity and temperate climate conditions. The aim of the present investigation was to evaluate the mycoflora of pistachio and peanuts from retailers and dried fruit retail shops of Sari.

Materials and methods

Sampling

The study was conducted in, Iran. A total number of 100 peanut (n=50) and pistachio (n=50) kernel samples in two consumption forms (dry roasted and raw) were collected from retailers and dried fruit retail shops between July and September 2008 among five geographic regions in Sari (south, north, east, west and center) and transferred to the laboratory in plastic bags of 100g. These nuts usually are sold without packaging and kept in normal room conditions.

Mycoflora identification

Approximately 100g from each sample were disinfected with 0.4% sodium hypochlorite solution for two minutes, followed by washing with sterile distilled water for elimination of external contaminants and then dried. Each sample was grinded into powder by vortex and then 1g was poured into 100ml of sterile distilled water and stirred. Aliquots of 1ml of each dilution were plated on Petri dishes containing Sabouraud dextrose agar (Scharlau, Spain) supplemented with 100g/ml chloramphenicol, and the plates were incubated at 25°C for five days. The grown fungi were identified by standard

mycological techniques based upon gross cultural and microscopic morphology. The fungi that could not be identified by this manner were sub cultured on potato dextrose agar, water agar and /or slide cultures for further study. The *Aspergillus* species were identified by subculture onto Czapek Dox agar (HIMEDIA, Mumbai, India) medium and described according to macroscopic and microscopic characteristics of each colony. These were then identified up to the species level using keys of Raper and Fennel [13]. The yeast was identified with subculture onto corn meal agar + Tween 80 and CHROMagar Candida (Becton Dickinson, Spark, MD).

Results

Of 50 pistachio samples, 17 raw form and 33 dry roasted form produced, 13(76.5%) and 24(72.7%) fungal growth, respectively. Fungi were also detected in 68% (11/16) and 70.6% (24/34) of raw and dry roasted form of peanut samples, respectively. In total, 862 fungal colonies were isolated from the peanut and pistachio kernel samples (Table 1).

Table 1: Frequency of fungi isolated from peanut and pistachio kernel samples

Isolated fungi	Peanut no (%)	Pistachio no (%)	Total no (%)
A. flavus	236(58.6)	235(51.2)	471(54.6)
Aspergillus spp.	48(11.9)	53(11.5)	101(11.7)
Penicillium	29(7.2)	23(5.0)	52(6.0)
Cladosporium	44(10.9)	60(13.1)	104(12.1)
Alternaria	0(0)	1(0.2)	1(0.1)
Fusarium	0(0)	1(0.2)	1(0.1)
Yeast	45(11.2)	71(15.5)	116(13.5)
Rhodotorula	1(0.2)	15(3.3)	16(1.9)
Total	403(100.0)	459(100.0)	862(100.0)

[a]Mean values for positive samples

The isolates belonged to seven fungal genera. The genera were *Aspergillus, Yeast, Cladosporium, Penicillium, Rhodotorula, Fusarium* and *Alternaria*. The genus *Aspergillus* was the most predominant isolate which covers 70.5% and 62.7% of the total isolates from peanut and pistachio kernel samples, respectively. The genus *Fusarium* and *Alternaria* had the least frequency (0.1%).

Out of 572 grown colonies of *Aspergillus* species from peanut and pistachio samples, *A. flavus* (82.3%) had most frequency, followed by other *Aspergillus* species (9.6%), *A. niger* and *A. fumigatus* (Table 2). *A. flavus* was also the most commonly isolated species by covering 58.6% and 51.2% of the total isolates from peanut and pistachio kernel samples, respectively. Tables 3 and 4 show the mean fungal contamination and frequency of fungi isolated from two consumption forms of peanut and pistachio kernel samples.

Aspergillus flavus had most frequency in dry roasted (63.7%) and raw (48.9%)

form of peanut kernels. The contamination mean values for positive samples of dry roasted and raw form were 1.09 and 1.28×10^3 CFU/g, respectively. *A. flavus* also had the highest contamination mean value in dry roasted ($0.70\ 10^3$ CFU/g) and raw (0.63×10^3 CFU/g) form of peanut kernels. The total viable count of *A. flavus* varied from 0 to 3.8×10^3 CFU/g in dry roasted form of peanut kernels. This value was $0\text{-}5.0\times10^3$ CFU/g for raw form materials.

Aspergillus flavus had most frequency in dry roasted (54.7%) and raw (45.3%) form of pistachio kernels. The contamination mean values for positive samples of dry roasted and raw form were 1.20 and 1.31×10^3 CFU/g, respectively. *A. flavus* also had the highest contamination mean value in dry roasted (0.66×10^3 CFU/g) and raw (0.59×10^3 CFU/g) form of pistachio kernels. The total viable count of *A. flavus* varied from 0 to 3.7×10^3 CFU/g in dry roasted form of pistachio kernels. This value was $0\text{-}4.1\times10^3$ CFU/g for raw form.

Table 2: Frequency of different species of *Aspergillus* isolated from peanut and pistachio kernel samples

Isolated fungi	Peanut	Pistachio	Total
A. flavus	236(83.1%)	235(81.6%)	471(82.3%)
A. niger	7(2.5%)	30(10.4%)	37(6.5%)
A. fumigatus	0(0%)	9(3.1%)	9(1.6%)
Aspergillus spp.	41(14.4%)	14(4.9%)	55(9.6%)
Total	284(100%)	288(100%)	572(100%)

Table 3: Mean fungal contamination (viable count, $\times10^3$CFU/g) and the frequency of fungi isolated from two consumption forms of peanut kernel samples

Isolated fungi	Dry roasted (n=34)		Raw (n=16)	
	Mean fungal[a]	No (%)	Mean Fungal[a]	No (%)
A. flavus	0.70	167(63.7)	0.63	69(48.9)
Aspergillus spp.	0.04	10(3.8)	0.34	38(26.9)
Penicillium	0.09	21(8.0)	0.07	8(5.7)
Cladosporium	0.08	19(7.2)	0.23	25(17.7)
Yeast	0.19	45(17.2)	0.0	0.0
Rhodotorula	0.0	0.0	0.01	1(0.7)
Total	1.09	262(100.0)	1.28	141(100.0)

[a]Mean values for positive samples

Table 4: Mean fungal contamination (viable count, × 10^3CFU/g) and the frequency of fungi isolated from two consumption forms of pistachio kernel samples

Isolated fungi	Dry roasted (n=34)		Raw (n=16)	
	Mean Fungal[a]	No (%)	Mean Fungal[a]	No (%)
A. flavus	0.66	158(54.7)	0.59	77(45.3)
Aspergillus spp.	0.11	27(9.3)	0.20	26(15.3)
Penicillium	0.09	21(7.3)	0.01	2(1.2)
Cladosporium	0.17	41(14.2)	0.15	19(11.2)
Alternaria	0.004	1(0.3)	0	0
Fusarium	0.004	1(0.3)	0	0
Yeast	0.10	25(8.6)	0.35	46(27.0)
Rhodotorula	0.06	15(5.2)	0	0
Total	1.20	289(100.0)	1.31	170(100.0)

[a]Mean values for positive samples

Discussion

Nut seeds including peanut and pistachio are very susceptible to contamination with fungi. Mycotoxins may occur in contaminated nut seeds with these fungi. This situation causes both potential health hazards for humans and economic losses. In the present study we analyzed fungal contamination of two important kinds of nut seed in Iran. In our study, fungal contamination was observed approximately in 70% of sampled kernels. This data is almost similar to Mikaeili [14] in another part of Iran on stored pistachio. In a study in Turkey [15], out of 18 collected peanut kernel samples, only nine (38.9%) samples showed fungal growth.

In Nakai *et al*. study [16], fungi were detected in all peanut samples tested. The report of different fungal contamination rate by different investigators may be a result of adverse pre-harvest conditions of temperature and humidity in the field and improper post-harvest handling and storage. In the present study, the genus *Aspergillus* and *Fusarium* were the most predominant and the least isolates, respectively. The predominance of *Aspergillus*, together with the reduced presence of *Fusarium* and *Alternaria* observed for all samples studied may be due to great adaptation of this fungus to these substrates, especially during storage [17].

The increase in the frequency of *Aspergillus* can also be explained by the fact that this genus is considered to be storage one unlike *Fusarium* which is exist in field fungi. In addition, *Aspergillus* as a storage fungus also grows well at lower moisture content in contrast to *Fusarium* and *Alternaria* as major field fungi which they require very high moisture content in the substrate for growth and mycotoxin synthesis. In Gonçalez *et al*. study [18], *Fusarium* was the most prevalent fungus in Brazilian peanut kernels from sowing to harvest. The isolation of *Aspergillus* spp. agrees with the findings of other investigators studying peanut and pistachio kernels in tropical countries [8-12]. This may be due to the climate condition of these countries, which can be favorable for moulds like *Aspergillus* to grow. These are the reasons, why *Aspergillus* is a prevalent fungus in as stored pistachio and peanut kernel samples in this study.

Among the species of *Aspergillus*, *A. flavus* was the most frequently isolated species found in both consumption forms of peanut and pistachio kernel samples in our study. These findings are also similar to those of other studies [11,15,19]. Nakai *et al*. [16] showed the susceptibility of peanuts to colonization to *A. flavus*, especially during storage. Smith and Ross [20] have also suggested that the contamination of peanuts with *A. flavus, A. parasiticus* and *A. nomius* (main aflatoxin producers) is one of the major problems in tropical and subtropical regions during the storage period. In our previous study [21] on stored wheat samples, *A. flavus* was also the most prevalent isolated. In Mikaeili study [14] in the cold region of Iran, 14.2% of stored pistachio samples were contaminated by *A. flavus*. This could also indicate thermophilic characteristic of *A. flavus*.

The result of our study showed no significant difference in the contamination mean values for positive samples between dry roasted and raw form of peanut and pistachio kernels. However *A. flavus* had the highest contamination mean value in dry roasted and raw form of peanut and pistachio kernels. Although the mean of all fungal contaminations varies in different studies, it is emphasized the importance of fungal type which they infested various foodstuffs and agricultural commodities [11,15,19]. In this view *Aspergillus* species, especially *A. flavus* was considered as an important mycotoxins producer [22]. *A. flavus* is the predominant species responsible for aflatoxin contamination of crops prior to harvest or during storage [23].

Conclusion

Because the isolation of high percentage of *A. flavus* from two consumption forms of pistachio and peanut kernel (dry roasted and raw) samples in the present study we recommend good storage practices in order to prevent the occurrence of aflatoxins in peanuts and pistachio.

Acknowledgement

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Address for correspondence:
Mohammad Taghi Hedayati, Department of Medical Mycology and Parasitology, School of Medicine, Km 18 Khazarabad Road, PO. Box: 48175-1665, Sari, Iran
Tel: +98151 3543088; Fax: +98151 3543087
Email: hedayaty2001@yahoo.co.uk