

The Preliminary Study on The Contamination of Warehouse-Stored Pistachios with Aflatoxinogenic Fungi in Kermanshah, Iran: The Tip of The Iceberg

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Received (Geliş Tarihi): 12.04.2010

Accepted (Kabul Tarihi): 23.07.2010

ABSTRACT: The aim of this study was to determine the amount and type of aflatoxinogenic fungi on samples of pistachios collected from warehouses in Kermanshah, Iran. Overall, a total of 632 samples were randomly collected and based on their physical properties the samples were categorized into following groups: (i) raw pistachio with intact shells, (ii) raw pistachio with broken and damaged shells, (iii) salted pistachio with intact shell, and (iv) salted pistachio with broken and damaged shell. Then 158 subsamples were chosen from each category. The overall contamination rate was 66%. The fungus contamination rates were significantly lower in salted in compared to raw samples ($p<0.05$). The percent contaminated samples recorded 45.6% and 33.3% in salted pistachio with intact shell and salted pistachio with damaged shell, respectively. The most fungal contamination rate was recorded for *Aspergillus niger* followed by *Aspergillus flavus* and *Aspergillus terreus* in all categories. The percent contamination of samples by *Aspergillus niger* was significantly higher in raw samples in comparison to salted samples. The salted pistachio with damaged shell was significantly lesser contaminated to *Aspergillus niger* when compared with the salted pistachio with intact shell ($p<0.05$). The *Penicillium* spp. contamination rates were significantly higher in damaged shell samples compared to intact shell samples and apparently this type of contamination did not decrease in salt-treated samples while, the salted, intact shell samples have shown significantly more *Aspergillus flavus* contamination rates than salted, damaged shell ($p<0.05$). The *Aspergillus terreus* contamination rates were significantly higher in intact shell samples compared to damaged shell samples. These toxigenic fungi isolated raises concerns on the potential of pistachio products as a natural substrate prone to the mycotoxin formation.

Key words: *Aspergillus* species, *Penicillium* species, pistachio, Iran.

İran, Kermanshah Bölgesinde Depolanmış Antep Fıstıklarında Aflatoxinogenic Fungus Kontaminasyonu Hakkında Ön Çalışma: Buzdağının Görünen Kısmı

ÖZET: Bu çalışmanın amacı, İran, Kermanshah bölgesinde bulunan depolardaki Antep fıstığının aflatoxinogenic fungus tipi ve miktarını belirlemektir. Yaklaşık 632 örnek fiziksel örnekleri baz alınarak toplanmış ve i) Sağlam kabuklu Antep fıstığı, ii) Kabuğu kırılmış Antep fıstığı, iii) Sağlam kabuklu tuzlanmış Antep fıstığı, ve iv) Kabuğu kırılmış tuzlanmış Antep fıstığı şeklinde dört gruba ayrılmıştır. Her gruptan 158 örnek seçilmiştir. Genel kontaminasyon oranı % 66 olarak bulunmuştur. Tuzlanmış fıstıkların fungus kontaminasyon oranı daha düşük bulunmuştur ($p<0.05$). Sağlam kabuklu tuzlanmış Antep fıstığı ve kabuğu kırılmış tuzlanmış Antep fıstığında kontaminasyon oranı sırasıyla % 45.6 ile % 33.3 bulunmuştur. Bütün kategorilerde en fazla kontaminasyon sırasıyla *Aspergillus niger*, *Aspergillus flavus* ve *Aspergillus terreus* bulunmuştur. *Aspergillus niger* ile kontaminasyon sağlam kabuklu Antep fıstıklarında tuzlanmış Antep fıstıklarına göre daha yüksek bulunmuştur. Kabuğu kırık tuzlanmış Antep fıstıklarında *Aspergillus niger* kontaminasyonu kabuğu sağlam tuzlanmış fıstıklara oranla daha az bulunmuştur ($p<0.05$). Kabuğu kırık Antep fıstıklarında *Penicillium* spp kontaminasyon oranı kabuğu sağlam olan fıstıklara oranla daha yüksek bulunmuştur. Bu tip kontaminasyon tuzlama ile azaltılamamıştır. Bununla birlikte sağlam kabuklu tuzlanmış Antep fıstığının *Aspergillus flavus* ile kontaminasyonu, kırılmış ve tuzlanmış Antep fıstığından daha fazla bulunmuştur ($p<0.05$). Kabuğu sağlam Antep fıstıklarının *Aspergillus terreus* ile kontaminasyon oranı kabuğu kırılmış Antep fıstıklarının kontaminasyon oranından daha yüksek bulunmuştur. Toksik özelliğe sahip fungusların Antep fıstıklarında izole edilmesi Antep fıstıklarından elde edilen ürünlerin, mikotoksin oluşumuna meyilli doğal ürünler olabileceğini gündeme getirmektedir.

Anahtar Kelimeler: *Aspergillus* türleri, *Penicillium* türleri, Antep fıstığı, İran

INTRODUCTION

Pistachio (*Pistacia vera* Anacardiaceae) is a broad, bushy, deciduous tree which grows slowly to a height and spread of 25 to 30 feet, with one or several trunks. All of Iranian, Pakistani, Tajikistani, Afghani and

Turkish varieties are landrace (pure) varieties that belong to three species *Pistacia vera*, *Pistacia mutica*, and *Pistacia khinjuk*. The most important pistachio products in the food and drug trade are whole nut, kernels, roasted and salted pistachio. In Iran, Pakistan

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and Turkey roasted and salted pistachio (Per: Pesteh) still sold by street vender and herbal stores and it is very popular especially among children as an edible nut, along with walnuts, almonds, macadamias and cashews. Whole pistachio seeds contain approximately 17% protein, 31% fat (in the form of nutritious oil), and 51% carbohydrate, in addition to an interesting array of vitamins and minerals (Sheridan et al. 2007)

The pistachio is a fragile nut susceptible to fungus infection in the orchard and during improper storage conditions. Damaged nuts caused by either pistachio weevil (*Amyetois transiella*) or mechanically during harvest and transportation are particularly subjected to fungal invasion and decay (Nawar, 2008). The saprophytic fungi including species of *Alternaria*, *Aspergillus*, *Chladosporium*, *Eurotium*, *Fusarium*, *Penicillium*, *Trichoderma*, *Ulocladium*, *Epicoccum* and *Rhizopus* decay kernels of pistachio (Michailides, 2006). Denizel et al. (2006) reported that the *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* spp. as dominant external mycoflora of the immature pistachio in Turkey. *Aspergillus niger* and *Aspergillus flavus* were found common in all pistachio samples collected from the three locations in Saudi Arabia (Nawar, 2008). Aflatoxinogenic fungi and their related mycotoxins e.g., aflatoxins are one of the major health issues in humans and animals. Main producers of these mycotoxins are fungi of genus *Aspergillus*, although some other species of fungi from genera *Penicillium* and *Rhizopus* produce those (Peraica and Domijan, 2008). Aflatoxins as unavoidable contaminants of food are involved in pathogenicity of several diseases (Mahoney and Rodriguez, 1996). Aflotoxins (B1, B2, G1, and G2) are highly toxic hepatotoxins produced by various strains of *Aspergillus* in tropical and subtropical regions which naturally contaminate maize, sorghum, nuts, cotton seed, sunflower seed, coffee, pistachios, and other energy-rich products (Peraica and Domijan, 2008; Mahoney and Rodriguez, 1996). The high level of aflatoxins in several lots of imported pistachios from Iran, as dominant producer and the exporter of pistachios, led the European Union (EU) implemented restrictive measures on the import of pistachios in 1997 (Germain, 2003). In spite of the improvements of agricultural practices, and hygiene practices in handling, processing, storage and distribution of the product in Iranian pistachio industry, the fungal contamination is the most public health concern. Pistachio as a strategic crop has various features of storage which differ from

place to place. Since there have not been any published reports on pistachio stored in Kermanshah warehouses, the aim of this research was to determine the amount and type of aflatoxinogenic fungal contamination in pistachio warehouses of Kermanshah, Iran.

MATERIAL and METHODS

In this study 632 samples were randomly collected from different warehouses in Kermanshah. Based on physical properties the samples were categorized into following groups: (i) raw pistachio with intact shell, (ii) raw pistachio with broken and damaged shell, (iii) salted pistachio with intact shell, and (iv) salted pistachio with broken and damaged shell.

Mycological analyses were done according to the standard procedures that reported elsewhere (Raper and Fennel, 1973; Gilman, 1975; Barnett and Hunter, 1984). Briefly, 100 g from each sample were sterilized in a 0.4% sodium hypochlorite solution for 2 min, followed by rinsing with three washes of sterile distilled water. Then, 1 g of homogenized sample was added into 100 ml of sterilized distilled water and stirred. Finally, 1 ml of the resulting suspension was inoculated into Sabouraud dextrose agar (SGA, Merck, Germany) media containing antibiotics. The media were incubated at 30°C for 5 days and the fungal colonies exerted were evaluated daily. The fungal colonies were identified using the most documented keys in fungal identifications (Raper and Fennel, 1973; Gilman, 1975; Barnett and Hunter, 1984).

Statistics

The mean percentage of recovery of each fungus was calculated as the ratio of the number of samples contaminated by each fungus over the total number of samples submitted to analysis. The results were expressed as mean, differences between means analyzed using paired sample-t-test. All data were analyzed using the software SPSS ver.16. Statements of significance were based on $p < 0.05$.

RESULTS and DISCUSSION

The results of fungus culture in four groups were presented in Table.1. The fungus contamination rates were significantly ($p < 0.05$) lower in salted in comparison to raw samples. The percentage contaminated samples recorded 45.6% and 33.3% in salted pistachio with intact shell and salted pistachio with damaged shell, respectively.

Table 1. Percentage of fungal contamination associated with pistachio nuts obtained from different warehouses in Kermanshah

Pistachio group	<i>Pen.spp</i>	<i>A.terreus</i>	<i>A.flavus</i>	<i>A.niger</i>	Negative	Total no.
Raw, intact shell	0.00 ^a	1.3 ^a	25.9 ^a	67.1 ^a	5.7 ^a	158
Raw, damaged shell	0.07 ^b	0.0 ^b	26.1 ^a	64.3 ^a	8.9 ^a	157
Salted, intact shell	0.00 ^a	1.2 ^a	3.2 ^b	41.0 ^b	54.4 ^b	158
Salted, damaged shell	0.06 ^b	0.0 ^b	1.9 ^b	38.0 ^c	66.7 ^b	159

Note: In columns, values with different superscripts are significantly different ($p < 0.05$)

The most fungal contamination rate was recorded for *Aspergillus niger* followed by *Aspergillus flavus* and *Aspergillus terreus* in all categories. The percentage contamination of samples by *Aspergillus niger* was significantly ($p < 0.05$) higher in raw samples in comparison to salted samples. The salted pistachio with damaged shell was significantly lesser contaminated to *Aspergillus niger* compared with the salted pistachio with intact shell.

The *Penicillium* spp. contamination rates were significantly ($p < 0.05$) higher in damaged shell samples in comparison to intact shell samples and apparently this type of contamination did not decrease in salt-treated samples while, the salted, intact shell samples have shown significantly more *Aspergillus flavus* contamination rates than salted, damaged shell. The *Aspergillus terreus* contamination rates were significantly ($p < 0.05$) higher in intact shell samples in comparison to damaged shell samples.

The 3 species of *Aspergillus* isolated in our study have been reported in other food commodities elsewhere. *Aspergillus niger* and *Aspergillus flavus* from pistachio nuts in Saudi Arabia (Nawar, 2008), these species also detected in our samples. Their recovery from the samples examined in this study suggests that stored pistachio products are suitable substrates for the growth and development of *Aspergillus* spp. While, the highest recovery of *Aspergillus niger* from raw, intact shell pistachio samples in this study implies that contamination may occur during harvesting procedures and is partially independent to the availability of suitable substrates for fungal growth. *Aspergillus niger* is involved in the pathogenicity of various diseases such as mold onychomycosis (Hilmioglu et al. 2005), and pulmonary diseases (Severo et al., 1997). *Aspergillus* blights as a fungal disease of pistachio trees and nuts caused by *Aspergillus niger* and other *Aspergillus* spp. (Eskalen et al., 2001). Therefore, high contamination of *Aspergillus* spp. in our study may be secondary to the contamination throughout storage in warehouses and may result in contamination in orchard. In the present study, the amount of recovery of *Aspergillus flavus* as an organism that causes aspergilloma, and chronic pulmonary aspergillosis (Pasqualotto and Denning, 2007; Hedayati et al. 2007), endophthalmitis (Aydin et al. 2007), and keratolytic malignant glaucoma (Jain et al. 2007) significantly decreased in salted samples in comparison to raw samples. Also the lowest recovery of *Aspergillus terreus* from intact shell pistachios samples in comparison to other *Aspergillus* spp. suggests that pistachios are not suitable media for this organism. *Aspergillus terreus*, a less common pathogen causes aspergillosis with severe neutropenia (Tokimatsu et al. 2007).

Application of pistachio nuts with sodium chloride was found to increase the resistance of pistachio nut to invasion and colonization by the fungi during storage (Nawar, 2008). Also, in the present study, the salt

treated pistachio samples showed lower contamination of *Aspergillus niger* and *Aspergillus flavus* spp. than raw pistachio samples but the former were more contaminated *Penicillium* spp. Also, *Penicillium* spp. were found to occur sporadically and to constitute small numbers of the fungal isolates. Khosravi et al. (2007) reported the contamination rates of pistachios that collected from different regions of Tehran, Iran were 26.5% and 20.4% for *Penicillium* and *Aspergillus* spp., respectively. Nawar (2008) has been reported low *Penicillium* spp. contamination in pistachio nuts in Saudi Arabia. Research on Iranian pistachio in Kerman orchards 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 split ones were contaminated by *Aspergillus flavus* and other *Aspergillus* spp. (Shahidi, 2004). Apparently, salt treatment did not change the contamination rate of pistachio to *Aspergillus terreus* and the lack of recovery of this species in damaged samples suggest that pistachio is not suitable media for growing of this fungus. In general, all *Aspergillus* spp. referred to the present study are common and distributed in nature worldwide, and have been isolated in a wide array of substrates (Kozakiewicz, 1994).

CONCLUSION

The growing interest attached to aflatoxinogenic fungi on pistachio as an Iranian indigenous nut is of special concern mainly because of the increasing roles of this nut as nutraceutical in the confectionary and its usage as a remedy in orthodox and traditional medicine for treatment of various maladies functional food. These three *Aspergillus* species which were recovered from the samples examined in this study, share with *Penicillium* spp. a common potential capability to produce the aflatoxins. This would indicate that further research on mycotoxicological aspects of derived pistachio products is required.

ACKNOWLEDGEMENT

We would like to appreciate all members of research council of Kermanshah University of Medical Sciences for their cooperation.

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