J. Agr. Sci. Tech. (2010) Vol. 12: 331-338

Occurrence of Mould Counts and *Aspergillus* Species in Iranian Dried Figs at Different Stages of Production and Processing

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ABSTRACT

The present study was carried out to investigate the occurrence of total mycobiota and *Aspergillus* species at different stages of fig production and processing in Iran. In the meantime, the potential of different culture media for isolation of fungal species from figs was also investigated. The mycobiota of 389 samples of dried fig were studied. Total fungal counts ranged from <2 to 6.74 $\log_{10}$ CFU g^{-1}. In general, the predominant species were *Aspergillus niger* aggregate, *Aspergillus flavus*, *Acremonium* spp. and *Mucor* spp. in percentages of 90.9%, 63.7%, 54.6% and 36.4% infection, respectively. Other *Aspergillus* spp. and *Mucor* spp. Corresponded to 36.7% and 28.3% infection, respectively. The lowest contamination was observed in dried figs. The predominant fungi were *Alternaria* spp. and *Penicillium* spp. (9.1% infection). On average, *Aspergillus* spp. comprised 34.4% of the total fungal population. *Aspergillus niger* aggregate was detected in 99%, and *A. terreus* was present in 11.3% of total samples. The results revealed that poor hygienic conditions in fig harvesting, drying procedures, collecting sites, sorting and packaging plants caused higher mould contamination and risk of the *A. flavus* growth in dried fig production in Iran. As the result obtained from this study, using more than one culture media for isolation of *A. flavus* is recommended.

Keywords: *Aspergillus flavus*, Culture media, Dried figs, Iran, Mycobiota.

INTRODUCTION

Fig (*Ficus carica*, Moraceae) probably originated in western Asia, and spread to the Mediterranean, is a very nourishing food. It is an important source of carbohydrates, essential amino acids, vitamins A, B_1, B_2 and C as well as minerals that are used in industrial food products as well. Figs occupy a special place of importance in nutrition due to being usually consumed either locally and in fresh form or in various dried, canned, and preserved forms. Countries import figs in dried form or fig paste. The main exporters of dried figs and paste are Turkey and the USA (Sadhu, 1990). Fig is a moderately important world fruit with an estimated annual production of 1,077,211 tons (FAO, 2003). Iran is the third producer of fig in the world. It produced more than 87,520 tones in 2005 and exported about 1,610 tones to different countries in the same year (FAO, 2007). Most fig in Iran is produced in Estahban region (Fars Province, central Iran). The general sun-drying process, employed, i.e. picking partially dried ripe fruits from the ground and exposing them to direct sunlight until further dehydration, dramatically increases the chance and potential for toxigenic fungal contamination, resulting in greater chances of aflatoxin development (Tosun and Delen, 1998). They are usually dried under sun on cement floors and therefore subjected to

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[DOR: 20.1001.1.16807073.2010.12.3.6.6]

microbiological contamination during harvesting, drying, storage, transportation as well as handling. In the case of pistachio economic impacts as well as health risk potential posed by aflatoxin-contaminated nuts prompted researchers to initiate studies to monitor the aflatoxin contamination in pistachio nuts. Rahimi *et al*. (2008) isolated *Aspergillus* species from the hull, kernel and shell of pistachio nuts of samples collected from Iran. There has not been any research carried on the fungal contamination in Iranian dried figs. Several researchers have reported toxigenic fungi and aflatoxin contamination in figs in Brazil (Iamanaka *et al*., 2007), Syria (Haydar *et al*., 1990), Turkey (Steiner *et al*., 1988; Boyacioglu and Gonul, 1990; Özay *et al*., 1995; Karaca and Nas, 2006; Zorlugenç *et al*., 2008), Morocco (Juan *et al*., 2008), the USA (Buchanan *et al*., 1975; Doster *et al*., 1996; Bayman *et al*., 2002), and UK (Sharman *et al*., 1991). Species belonging to *Aspergillus* section *Flavi* are among the most intensively studied of all fungi, due largely to the formation of potent carcinogenic metabolites (as aflatoxins in agricultural commodities) that impact animal and human health thus causing severe human and economic losses (Brul and Klis, 1999; Peraica *et al*., 1999; Hussein and Brasel, 2001).

In this study, dried figs have been screened to determine the occurrence of total mycobiota and *Aspergillus* species at different stages of fig production in Iran. In the meantimes, the potential of different culture media for isolation of fungal species from figs was also put into investigation.

MATERIALS AND METHODS

Materials

Dichloran 18% Glycerol Agar (DG18), Dichloran Rose Bengal Chloramphenicol Agar (DRBC), Acidified Potato Dextrose Agar (PDA) and universal peptone M66 were procured from Merck (Darmstadt, Germany). Rose Bengal Chloramphenicol Agar (RBC) was supplied through Difco (Detroit, MI).

Sampling

Three hundred eighty nine fruit samples from Estahban region were collected from different farms, sorting and processing plants, and from local markets during the following phases: 122 from collecting sites, where figs are bought from small scale farmers and then sold to fig sorting plants (A and E), 106 from sun drying places or Eshfangs (B) (Figure 1c), 52 collected from the ground under the trees (C), 63 samples from sorting and packaging plants (D and F) and finally 46 manually harvested from the trees (G) (Figure 1b) in August 2007 in Estahban region (Table 1). The collected samples were kept at refrigeration temperature (4°C) until analysis. Towards

Table 1. Sample size and different places of sampling (Estahban region during the period of harvesting (August 2007).

Sample	Number of samples	Sampling site
A	82	Collecting site 1
B	106	Sun drying place (Eshfang)
C	52	Collected from the ground under tree
D	10	Sorting and processing plant 1
E	40	Collecting site 2
F	53	Sorting and processing plant 2
G	46	Manually harvested

[DOR: 20.1001.1.16807073.2010.12.3.6.6]

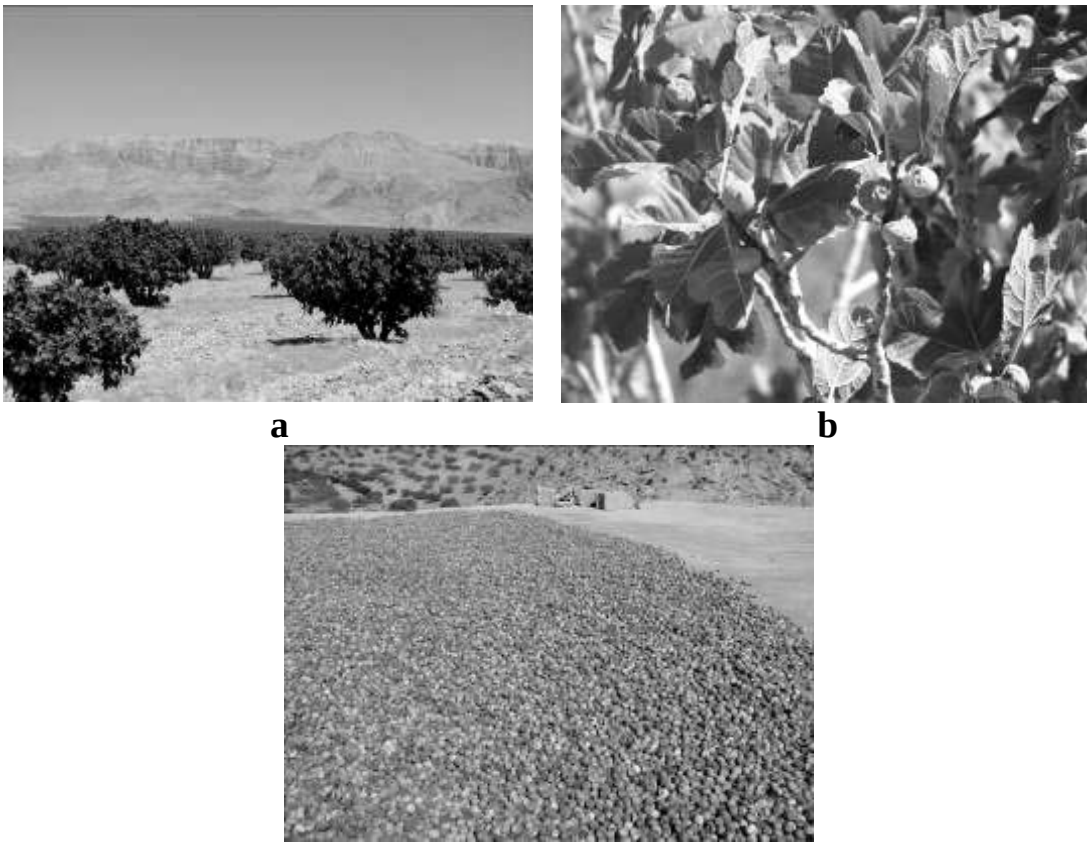

Figure 1. A fig fruit farm(a), Dried figs on the tree(b), and Eshfang, a sun drying place(c).

this and, approximately 1,000 g of each sample were collected.

Mycobiota Determination

For mycological analysis, figs (whole or halves) were plated aseptically in direct plating or indirect plating (serial dilution plating) according to Pitt and Hocking procedure (1999). In direct plating, small figs were applied whole and big ones cut into halves directly on solid culture media. Whole figs had incisions in opposite sites and were plated to determine the percentage of figs infected by fungi. A sample of 150 g from a total of 500 g of figs was disinfected with sodium hypochloride solution 0.4% for 2 minutes and stirred for 2 minutes (Iamanaka *et al*., 2007). The figs disinfection was done to specifically eliminate bacterial flora and facilitate identification of mould colonies. Samples were then rinsed twice by 500 ml of sterile water. Ten whole figs or eighteen pieces of disinfected figs were transferred to DG18, DRBC, PDA and RB plates (9 cm).

In indirect plating, quantitative determination of fungal propagules was performed on solid media and the results expressed as percentage of fig samples contaminated with fungal genera or species. The dilution plating was performed as fallows: The samples were homogenized by thoroughly blending and serial dilutions made in peptone water (40g 360 ml^{-1}) and then shacken up with shaker for 1 hour in order to facilitate microorganisms' recovery. Further decimal dilutions were prepared in peptone water solution and aliquots of each dilution (100 µl) inoculated by duplicate onto four plates of DG18, DRBC, PDA and

[DOR: 20.1001.1.16807073.2010.12.3.6.6]

RBC agar. Plates with 10–100 CFU were used for enumeration and the results expressed as $\log_{10}$ CFU g^{-1} of the sample. Plates were incubated at 30ºC in darkness for 7 days. Molds were identified by examination of colony and their macroscopic charactersistics. For fungal isolation, isolates were cultured on DG18, DRBC, PDA and RB plates and for fungal identification PDA was used for subculture. Plates were incubated at 25°C for 7 days (Samson *et al*., 2004).

Statistical Analysis

Data were subjected to one way analysis of variance (ANOVA) using the SPSS (2007) package. Means were separated, when required, by multiple Duncan's range test at $P< 0.05$ level of significance.

RESULTS AND DISCUSSION

Total mould counts varied from 2 to 6.74 $\log_{10}$ CFU g^{-1} in dried figs. The highest total mould counts in dried figs were obtained in the samples obtained from colleting sites (A and E) and the samples that had been dried in low hygienic conditions (B). The lowest mould counts occurred in manually harvested samples (Table 2). Results indicate that highest mould contamination occurred in samples collected from sun drying places and from collecting sites, respectively. Öztekin *et al.* (2006) showed that the initial yeast/mould count was 1.46 $\log_{10}$ CFU g^{-1} in dried figs.

The data obtained from the total of samples in different culture media revealed a high diversity of fungal species (a total of 389 isolates were obtained) examples being: *Aspergillus flavus*, *A. niger* aggregate, *A. terreus*, other *Aspergillus* spp., *Acremonium* spp., *Penicillium* spp., *Alternaria* spp., and *Mucor* spp. These fungi can be classified into three groups according to their presence and abundance. In the first group *A. niger* aggregate, *A. flavus*, *Acremonium* spp. and *Mucor* spp. were the most frequent corresponding to 90.9%, 63.7%, 54.6% and 36.4% of infections, respectively. The second group with moderate infection included: other *Aspergillus* spp. and *Mucor* spp. corresponding to 36.7% and 28.3% of infections, respectively. The third group, composed of those fungi that are less frequent such as: *Alternaria* spp. and *Penicillium* spp. (9.1% of infections). *Aspergillus parasiticus* was not isolated from the dried figs.

According to Pitt and Hocking (1997), *A. flavus* and *A. niger* aggregate were reported as being the most common species in dried figs, explained by their high sugar content,

Table 2. Total mould counts in the dried fig samples through indirect plating.

Sampling site	N [a]	Mould count (Log_{10} CFU g^{-1})			
		PDA [b]	DG18 [c]	DRCB [d]	RBC [e]
A [f]	82	5.77 ± 0.44^{a}	5.81 ± 0.07a	5.92 ± 0.16a	5.84 ± 0.34a
B [g]	106	6.65 ± 0.02^{b}	6.74 ± 0.08^{b}	6.74 ± 0.12^{b}	6.65 ± 0.26^{b}
C [h]	52	4.00 ± 0.24^{c}	4.07 ± 0.84^{c}	4.07 ± 0.44^{c}	4.17 ± 0.02^{c}
D [i]	10	4.87 ± 0.82^{a}	5.07 ± 0.28^{a}	5.07 ± 0.06^{a}	5.14 ± 0.18^{a}
E [f]	40	3.47 ± 0.66^{c}	3.51 ± 0.42^{c}	3.60 ± 0.04^{c}	3.38 ± 0.16^{c}
F [j]	53	3.60 ± 0.24^{c}	3.51 ± 0.08^{c}	3.60 ± 0.22^{c}	3.54 ± 0.12^{c}
G [k]	46	< 2	< 2	< 2	< 2

[a] Number of samples; [b] Potato dextrose agar; [c] Dichloran glycerol agar; [d] Dichloran rose bengal chloramphenicol agar; [e] Rose bengal chloramphenicol agar; [f] Collecting sites; [g] Sun drying place; [h] Collected from the ground under the tree on the ground; [i, j] Sorting and packaging plants, [k] Manually harvested from the tree.

Values quoted are mean values ± SDs of results for three experiments. Means bearing different superscripts are significantly different ($P< 0.05$).

[DOR: 20.1001.1.16807073.2010.12.3.6.6]

making them more susceptible than apricots and sultanas. Steiner *et al*. (1988) investigated aflatoxin distribution in a naturally contaminated batch of dried figs in Turkey. *Aspergillus flavus* and *A. parasiticus* identified in several instances, and in very rare cases *A. fumigatus* and *A. niger* were isolated. Zorlugenç *et al*. (2008) isolated *A. flavus, A. niger, A. parasiticus, Byssochlamyys fulva, Cladosporium clodosporiodes, Mucor hiemalis, Mucor plumbeus Bon. Mucor racemosus Fres and Scopulariopsis bain* from Dried Sarılop (Calimyrna) figs in Turkey. Bayman *et al*. (2002) reported that *Aspergillus alliaceus* (now in section *Flavi*) rather than *Aspergillus ochraceus* could be responsible for the ochratoxin, a contamination observed in Californian figs. Piga *et al*. (2004) tested milder hot air dehydration processing to produce better quality figs. Their findings showed that dehydrated figs no longer had any microbial growth, whereas yeast counts did occur to an extent of 3.30 logs $_{10}$ CFU g^{-1} in fresh figs.

Occurrence (percent contamination in each sample) of *A. flavus* strains isolated with different media are summarized in Table 3. The highest contamination with *A. flavus* was found in sorting and processing plant 1 (sample D). There was no presence of *A. flavus* in samples collected manually feom the trees. The findings revealed that poor hygienic conditions in traditional harvesting, collecting sites, sorting and packaging plants as well as in drying procedures in fig production process caused higher mould contamination and consequently the risk of the *A. flavus* growth (Table 2). According to Özay *et al*. (1995) findings, in which it was supposed that manual harvesting can reduce fungal contamination, there was significant difference observed in mycobiota among different harvesting procedures. The results of contamination with fungi and *A. flavus* through different media are shown in Table 3. *Aspergillus flavus* occurred in 8.6%, 4.2% and 4.1% PDA, DG18 and DRCB, respectively. *Aspergillus flavus* was not detected in RBC medium. Potato dextrose agar was not able to isolate *A. flavus* in any one of the samples.

The occurrence of contamination with spoilage and toxigenic fungi in dried figs could be avoided or at least diminished if good agricultural (harvesting and handling) and manufacturing (sorting and packaging) practices from harvesting to processing were applied. Harvesting is a probable incurring stage in the production of aflatoxins in figs. Figs should be allowed to dry on the tree until over-ripe. After they lose enough moisture, and are partially dry and shriveled, an abscission layer forms and the fruits naturally fall from the trees onto the ground (Codex Alimentarius Commission, 2007), a phenomenon which must be taken advantage of in the prevention of any incurring damage.

Table 3. Contamination (%) of the dried figs with total mould and *Aspergillus flavus* through direct plating**.**

Sampling site	n [a]	Contamination (%)							
		Total mould				*Aspergillus flavus*			
		PDA	DG18	DRCB	RBC	PDA	DG18	DRCB	RBC
A [b]	82	62	50	90	ND	ND	15	ND	ND
B [c]	106	100	86	100	100	14	14	ND	ND
C [d]	52	8	67	67	86	ND	ND	8.5	ND
D [e]	10	36	67	67	45	36	ND	ND	ND
E [b]	40	100	40	100	100	10	ND	10	ND
F [f]	53	25	55	60	60	ND	ND	10	ND
G [g]	46	20	35	45	45	ND	ND	ND	ND

[a] Number of samples; [b] Collecting sites; [d] Sun drying place; [e] Collected from the ground under the tree; [e, f] Sorting and packaging plants, [g] Manually harvested from the trees, [h] Not detected.

[DOR: 20.1001.1.16807073.2010.12.3.6.6]

CONCLUSIONS

In order to report on the contamination state of dried figs and to allow elaborating strategies to control pathogens, information on the fungal contamination during the production process is indispensable. Three hundred eighty nine samples of dried figs were studied to investigate the total mould counts and occurrence of *Aspergillus* spp. during the production process. On average, *Aspergillus* spp. comprised 34.4% of the total fungal population.

High mould counts in collecting sites and sun drying places indicated that hygienic practices, implementing good storage and transportation procedures and post harvesting conditions played important roles in contamination of dried figs. Therefore, the authorities should take the lead in the efforts to establish mandatory regulations in fig farming to decrease contamination risk to toxigenic fungi. These would lead to enhanced food safety, enhanced international trade efforts and improved public health. Development of efficient pre- and post-harvest hygienic practices must be considered as components to be integrated into fig production processing. Nowadays, research and practical directions for establishment of such good agricultural practices as hygienic harvesting (using nets under trees for fig collection), drying (mesh apparatus with about 1 meter height instead of drying on the cement flooring) and processing plants are being provided by authorities. A survey of occurrence of aflatoxins in dried figs in Iran will be needed to further and to completely assess the situation.

ACKNOWLEDGEMENT

Funding of this research was provided by a grant from Iranian Research Organization for Science and Technology (IROST) for which the author is grateful to.

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شمارش کپک‌ها و رخ‌دهی گونه‌های آسپرژیلوس در انجیر خشک ایرانی در مراحل مختلف تولید

م. جوانمرد

چکیده

این بررسی جهت تعیین میزان وقوع فلور قارچی انجیر خشک ایران انجام گرفت. در این بررسی فلور قارچی ۳۸۹ نمونه از انجیر خشک از نظر نوع فلور قارچی و میزان وقوع قارچ آسپرژیلوس فلاوس با کمک محیط های کشت مختلف مورد آزمون قرار گرفتند. میزان کل آلودگی قارچی از کمتر از $۱۰^{۲}$ تا $۵/۵\times۱۰^{۶}$ cfu/gr بود. گونه های غالب قارچی عبارت بودند از: *Aspergillus flavus* *Aspergillus niger*, *Aspergillus* spp., *Acremonium* spp. and *Mucor* spp.

[DOR: 20.1001.1.16807073.2010.12.3.6.6]

گونه های *Aspergillus* به ترتیب به میزان ۵۷/۸ ، ۱۴/۴، ۷/۴ و ۰ درصد با کمک محیط های PDA ، DG18، DRCB و RB جدا گردیدند. به طور میانگین ۳۴/۴ درصد از کل فلور قارچی را گونه های آسپرژیلوس تشکیل می دادند .آسپرژیلوس نایجر در ۹۹ درصد از نمونه ها جدا گردید. آسپرژیلوس ترئوس در ۱۱/۳ درصد از نمونه ها موجود بود. آسپرژیلوس پارازیتیکوس در هیچ نمونه ای جدا نشد. عملیات کشاورزی و تولید مناسب از زمان برداشت تا فرآیند باعث کاهش معنی دار در میزان کل شمارش قارچی می گردد. این بررسی همچنین نشان داد که برای جدا سازی قارچ آسپرژیلوس فلاووس به جای یک محیط بایستی از چند محیط کشت قارچی بهره گرفت.

[DOR: 20.1001.1.16807073.2010.12.3.6.6]