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Contamination of Dried Figs with Fungi and Aflatoxigenic Potential of Some Isolates of Aspergillus Section Flavi

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Abstract
Thirty samples of dried figs were collected from local shops at Duhok governorate, Kurdistan region of Iraq during 2012-2013 and surveyed for their fungal contamination. Thirty one species assigned to 14 genera were isolated on DRBC medium. *Aspergillus* was represented by 12 species and showed the widest diversity among all recorded genera. *Penicillium* was second in the number of species and was represented by five species. Three teleomorphic ascomycetes namely, *Emericella nidulans, E.qudrilineata* and *Eurotium amstelodami* were also detected. *A,niger, A.flavus, A.carbonarius* and *A.parasiticus* were the most frequent species. Aflatoxigenic potential of selected strains of *A.flavus* and *A.parasiticus* were secreened for their aflatoxigenic potential by ELISA technique. Aflatoxin potential detected in culture for *A.parasiticus* isolates was ranging between 81.0 to 355 ppb, whereas, the level of aflatoxin production for *A.flavus* isolates was from 193 to 333 ppb.
Keywords: Fungi*, Aspergillus,* aflatoxin, dried fig, Duhok, Iraq.

1.Introduction

Ficus carica L. (Moraceae) is a native to south west Asia and spread to Mediterranean by human (Tous and Ferguson, 1996). The plant occurs wild throughout Kurdistan region of Iraq in rocky mountain slopes, and valleys, hill sides, road sides, degraded Oak and Pine forests.Figs have also been cultivated in irrigated orchards in dry vine orchards and as house plant (Shahbaz, 2010).

Dried fruits of fig are good source of minerals, vitamins, carbohydrates, dietary fibers and polyphenols (Veberic *et al.,* 2008; Slatnar *et al.,* 2011).

Dried figs have been used in traditional medicine to treat gastrointestinal, respiratory and cardiovascular disorders and as anti-inflammatory and anti-spasmodic remedy (Duke *et al.,* 2002).

Fungal infections to figs may occur on the tree during ripening stages, after falling from the tree and during the drying process (Ozay *et al*., 1995; Heperkan et *al*., 2012a).Figs infection by toxigenic fungi has been reported in a number of studies and revealed a high risk due contamination with mycotoxins (Doster *et al*., 1996; Alghalibi and Shater, 2004; Bircan, 2009; Heperkan *et al* .,2012 b) .Moreover, fungi contaminated dry figs caused considerable changes of all the biochemical contents (total carbohydrates, sugars, proteins, fats and dietary fibers) as well as affecting quality (Embaby *et al*.,2012).

The aim of the present study was to determine the mycobiota contaminated dried fig samples collected from Duhok shops and to assess the aflatoxigenic potential of some isolates of *Aspergillus* section *Flavi*.

2. Materials and Methods

2.1. Dried fruit samples

Thirty samples of dried figs were purchased from local markets in Duhok governorate. The collected samples were put in paper bags and were brought into laboratory for isolation of fungi.

2.2. Mycological analysis

The dried fig pieces were surface disinfected with 2% sodium hypochlorite for 1 min., and then rinsed with sterile distilled water. Five pieces were placed in each Petri plate containing Dichloran Rose Bengal Chloramphenicol (DRBC) agar medium (Fluka-Germany) and examined daily for growth and sporulation of fungi using a stereomicroscope. Six Petri plates were used for each sample. Pure colonies were established on appropriate media for identification. Majority of detected species were identified to species level based on morphological and cultural characteristics. Fungi other than the genera *Aspergillus* and *Penicillium* were identified according to the manuals of Domsch *et al.,* 1980 and Pitt and Hocking, 1997.

For identification of species in the genera *Aspergillus* and *Penicillium*, pure colonies were grown on four media according to Klich (2002) and Samson *et al.,* (2000). The media are as follows: Czapeck Yeast Extract Agar incubated for seven days at 25C˚ (CYA25), Czapeck Yeast Extract Agar incubated for seven days at 37C˚ (CYA37), Czapeck Yeast Extract Agar with 20% Sucrose incubated for seven days at 25C˚ (CY20S), Malt Extract Agar (MEA) incubated for seven days at 25C˚.

Ingredients and preparation of the above five media were mentioned in Klich (2002), Pitt and Hocking (1997). Each medium was supplemented with 250 mg / L chloromphenicol (SDI) to suppress bacterial growth. For each culture four plates were used, two of CYA and one each of CY20S, MEA. Each plate is inoculated at

the center and incubated in the dark for seven days. One CYA is incubated at 37C°. The rest are incubated at 25C°.

All species identifications were according to the keys and descriptions provided by Klich (2002); Frisvad and Samson; (2004) ; Abarca *et al.,* (2004) ; Samson *et al.,* (2004) ; Frisvad *et al*.,(2004); Samson *et al.,* (2007) .

Isolation frequency of detected species from samples was calculated by applying the following formula.

$$\text{Isolation frequency \%} = \frac{\text{Number of samples on which a fungus appeared}}{\text{Total number of samples}} \times 100$$

2.3. Aflatoxin extraction from fungal cultures.

Production of aflatoxin (AF) by randomly chosen isolates of *Aspergillus* section *Flavi* was screened according to the method of Bragulat *et a*l.,(2001) by centrally inoculating yeast extract sucrose (YES) plates and then incubated in the dark at 25^{o} C for 7 days. Agar plug (0.5 cm) diameter was removed from the edges of the colony, from the centre and a midway between the edge and the centre of the growing colonies. The three plugs were mixed with 1 ml methanol in a small vial ,shaking vigorously and left at room temperature for 1h , mixed again and the extracts were filtered through milpoore filter (0.22 um) diameter (Millex GP Filter Unit Coringhwohill Co. Ireland).

2.4. Aflatoxin analysis

The quantitative analysis of AF was performed with the enzyme linked immunosorbent assay (ELISA). The aflatoxin assay was performed according to the instructions provided by the manufacture (Veratox Aflatoxin quantitative Test, Neogen Corporation,USA). Aflatoxin produced by isolates was calculated from the standard curve derived from aflatoxin standards and expressed in ppb.

3. Results and discussion

Fungal contamination of 30 samples of dried figs collected from Duhok shops was examined. Thirty one species representing 14 genera were isolated. Their frequency of occurrence is presented in Table 1. Most of the recovered fungi were previously reported from dried figs in many parts of the world (Zohri and Abdel-Gawd,1993 ; Bayman *et al* ., 2002; Alqhalibi and Shater, 2004; Iamanaka *et al.,*2007,Senyave *et al*., 2008; Embaby *et al.,*2012; Heperkan *et al*., 2012a).

Two species were isolated with high frequency namely: *Apergillus niger* (73.4%) and *A. flavus* (66.0%), followed by *A. carbonarius* and *A. parasiticus* (31.3% each). Our result is in line with the report of Herperkan *et al*., (2012b), on the fungi associated with figs in the Mediterranean area.

Aspergillus was represented by 12 species and showed the widest diversity among all recovered genera. Black aspergilli were represented by five species (*A . aculeatus , A . awamori , A . carbonarius , A . japonicus* and *A . niger*). These species were found common to soil and different agricultural commodities in Kurdistan region (Abdullah, and Abdullah, 2009; Abdullah and Muhammed, 2011; Saadullah and Abdullah, 2012a, b,c; Abdullah and Saadullah, 2013).

Isolates from *A. carbonarius* and to less extent *A. niger* were showed potential for ochratoxin A production and were isolated with high frequency from dried vine contaminated naturally with orchratoxin A in Kurdistan region (Saadullah and Abdullah ,2012a,c)

Apart from black aspergilli and aspergilli in section *Flavi , A .alliaceus* was also encountered from dried figs in this study . *A. alliaceus* was reported to be ochratoxigenic and commonly occurring in figs in California (Bayman *et al*., 2002).

Penicillium was second in the number of species isolated from dried figs and was represented by five species. *P.glabrum* was the most frequent species (11.33%), followed by *P. expansum* (7.0%) and *P . verrucosum* (6.0%). Alghalibi and Shater (2004) isolated P. *Chrysogenum* and *P. oxalicum* from dried figs in Yemen, whereas, P. *expansun* and *P. chrysogenum* were reported as the most frequent species on Turkish dried figs (Senyuva *et al*., 2008).

Three teleomorphic ascomycetes, namely, *Emericella nidulans ,E. qudrilineata and Eurotium amstelodami* were detected with percentage frequencies 3.3% ,3.3% and 6.0 % respectively .The later species has been detected from dried figs in several surveys from different countries (Zohri and Abdel-Gawad ,1993; Alghalibi and Shater ,2004; Senyuva *et al*., 2008). Furthermore, isolates of *E. amstelodami* from Turkish dried figs produced Ochratoxin A and Aflatoxin B1 when grown on potato dextrose broth (PDB) medium (Senyuva *et al*., 2008).

A. flavus , A . parasitius , A. carbonarius and to less extent some species in the genus *Fasarium* are the most important species contaminating dried figs because of their potential to produced mycotoxins (Moretti *et al*.,2005 , 2010 ;Heperkan *et al.* ,2012b).

Pitt and Hocking (2009) reported that *A. flavus* and *A. niger* were the most common species on dried figs. Steiner *et al*., (1988) showed that *A. flavus* and *A. parasiticus* in general instance and in very rare cases *A. niger* and *A. fumigatus* were frequently encountered on figs in Turkey , whereas , *A . niger* aggregate followed by *A . flavus* were the most frequent species in Iranian dried figs (Javanmard , 2010) .

Table 2 showed the results of screening three isolates for each of *A. flavus* and *A. parasiticus* isolated from dried figs for their aflatoxigenic production abilities in culture media as detected by ELISA technique. Two strains of *A. flavus* showed positive abilities as aflatoxin producers, whereas all isolates from *A. parasitius* were positive .The tested isolates showed marked variation in their aflatoxin potential. This is in line with several other studies indicate variability in aflatoxin production abilities for *Aspergillius* section *Flavi* isolates (Vuamonde *et al* ., 2003; Senyuva *et al.,* 2008 ,Embaby *et al.,* 2012) . Several studies indicated that not all strains of A. *flavus* have aflatoxigenic potential and the ratio of the non-aflatoxigenic isolates to the aflatoxin producing strains varied depending on the source and location of the isolates (Schroeder and Bolla 1973; Abdel-Malik *et al*., 1993; Abdullah and Al-Mousawi, 2009; Abdullah *et al*., 2009).

4. Conclusion, the present study revealed that dried figs are highly contaminated with several mycotoxigenic fungi such as *A.flavus ,A.parasiticus* and others. Therefore, strict hygiene mycological measured should be done during harvest, storage and drying to minimize contamination with such fungi.

Table 1. Percentage occurrence of fungi on dried fig fruit as detected on DRBC medium.

No.	Fungi	Frequency %
1	*Alternaria alternata* (Fr.) Keissl	6.0
2	*Aspergillus aculeatus* Iizuka	10.12
3	*A.alliaceus* Thom & Church	13.22
4	*A.awamori* Nakaz	10.00
5	*A.carbonarius* (Bainier) Thom	31.3
6	*A.flavus* Link	66.0
7	*A.flavipes* Bainier & Sartory	1.5
8	*A.fumigatus* Fresen	10.0
9	*A.japonicus* Saito	5.50
10	*A.niger* Tiegh.*nom.cons*.	73.4
11	*A.ochraceus* K.Wilh	10.0
12	*A.parasiticus* Speare	31.5
13	*A.tamarii* Kita	3.33
14	*Penicillium expansum* Link	7.0
15	*P.glabrum* (Wehmer) Westling	11.33
16	*P.oxalicum* Currie & Thom	2.66
17	*P.spinulosum* Thom	3.33
18	*P.verrocosum* Dierckx	3.67
19	*Cladosporium cladosporoides* (Fresen.) G.A. de Vries	3.33
20	*Cladosporium herborum* (Pers.) Link	1 .0
21	*Drechslera* sp. .	1 .0
22	*Emericella nidulans* (Eidam) Vuill	3.33
23	*E.quadrilineata* (Thom &Raper)CR.Benj.	3.33
24	*Eurotium amestelodami* Mangin	6.0
25	*Fusarium oxysoprum* Schlecht	1.0
26	*Geotrichum candidum* Link ex Fr.	1.0
27	*Gliocladium* sp.	2.0
28	*Mucor circinelloides* Tiegh	4.0
29	*Rhizopus stolonifer* (Ehrenb.) Vuill.	1.0
30	*Stachybotrys* sp.	3.0
31	*Trichoderma* sp.	3.33

Table 2. *In vitro* quantitative production of aflatoxin by isolates of *Aspergillus* section *Flavi* from dried figs as detected by ELISA technique.

Fungal isolate	Aflatoxin (ppb)
Aspergillus flavus (isolate 1)	N.D
A.flavus (isolate 2)	333.0
A.flavus (isolate 3)	193.0
Aspergillus parasiticus (isolate 1)	355.0
A.parasiticus (isolate 2)	344.0
A.parasiticus (isolate 3)	81.0

N .D ... Not detected (negative)

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