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Detection of *Aspergillus* Species in Dried Fruits Collected from Duhok Market and Study their Aflatoxiginic Properties

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ABSTRACT

Samples from four types of dried fruits (apricot, fig , grapes and plum) were collected from local markets at Duhok governorate and surveyed for their contamination with *Aspergillus* species. A total of 20 species of *Aspergillus* were isolated on DRBC medium. *A.awamori, A.carbonarius, A.flavus, A.fumigatus, A.japonicus, A.niger, A.ochraceus, A.parasiticus* and *A. tubingensis* were detected from all types of dried fruits. The most frequent species was *A.niger* followed by *A.flavus*. Aflatoxigenic potentials of selected strains from *Aspergillus* section *Flavi* were detected by ELISA technique. Aflatoxin potential was detected in cultures from all isolates of *A.flavus* and *A.parasiticus* .Aflatoxin was found at levels from 10-344 ppb for *A.flavus* isolates and from 71-355 ppb for *A.parasiticus*.

Keywords: *Aspergillus*, aflatoxin, dried fruits. Duhok, Iraq.

الكشف عن أنواع الاسبرجلس في الثمار الجافة من اسواق محافظة دهوك ودراسة قابليتها على انتاج الافلاتوكسينات

الملخص

اجري التحري عن تلوث عينات لأربعة أنواع من الثمار الجافة وهي المشمش و التـين و العنـب و الاجاص بانواع الفطر اسبرجلس والتي جمعت من أسواق محافظة دهوك. تـم تـشخيص 20 نوعـا مـن الاسبرجلس والتي عزلت على وسط DRBC. الأنواع التي وجدت على جميع أنواع الثمار هي *Aspergillus awamori* و *A.flavus* *A.carbonarius* و *A.fumigatus* و *A.japonicus* و *A.niger* و *A.ochraceus* و *A.parasiticus*, و *A .tubingensis*. النوعان الأكثر ترددا هما *A.niger* و *A.flavus* .

تم قياس قابلية عزلات *Aspergillus* section *Flavi* على إنتاج الافلاتوكسين باستخدام طريقة الاليـزا ELISA. تم الكشف عن تواجد السم في جميع المزارع الفطرية لعزلات *A.flavus, A.parasiticus*. انحصر

تركيز الافلاتوكسين بين 10-344 ppb في *A.flavus* في حـين انحـصر التركيـز فـي هـزارع *parasiticus*. بين 71-355 ppb.

INTRODUCTION

Species in the genus *Aspergillus* are among the most important fungi involved in food spoilage and deterioration and they occur more in subtropical than the temperate climate (Hocking, 1997) although some isolates in species such as *A.oryzae, A.sojae, A.tamarii* are used in oriental food fermentation processes (Campbell-Platt and Cook, 1989; Oxenboll,1994) and *A.nige*r in citric acid production (Brook,1994).

Most *Aspergillus* species that produce aflatoxins are members of *Aspergillus* section. *Flavi, A.flavus* and *A.parasiticus* which coexist with and grow on many agricultural commodities are the most significant aflatoxigenic fungi. *A. nomius* is also strongly aflatoxigenic but it has been rarely associated with foods other than Brazilian nuts (Olsen *et al*., 2008).

Two new aflatoxigenic species in section *Flavi* have been described namely *A.bombycis* (Peterson *et al*., 2001) and *A.arachidicola* (Pildain *et al*., 2008). More recently, two aflatoxin producing species namely *A.pseudocaelatus* and *A. pseudonomius* have been discovered and therefore,the section *Flavi* includes 22 species(Varga *et al.*, 2011). Despite the ability of aflatoxin production by nine of these species, *Aspergillus flavus* has been considered the most important for food products in general (Pitt and Hocking, 2009). However, isolates of several *Aspergillus* species outside section *Flavi* have been also found to produce aflatoxin such as *A.ochraceoroseus* in section *Circumdati* and *Emericella astellata* in section *Nidulans* (Cary *et al*., 2005).

Aflatoxins are toxic naturally occurring as secondary metabolites produced by filamentous fungi mostly by members of *Aspergillus* section *Flavi*. These toxic substances have a wide occurrence in different agricultural commodities including dried fruits. The contamination of fruits by these fungi can be a sign of inadequate drying processing and storage. Aflatoxins have been implicated in human diseases including liver cancer (Eaton and Groopman, 1994; Hedayati *et al.*, 2007).

The aim of the present work was to study the incidence of aspergilli species in dried fruits collected from Duhok market and to assess the ability of isolates of *Aspergillus* section *Flavi* to produce aflatoxins *in vitro* using ELISA technique.

MATERIALS AND METHODS

Dried fruit samples

Dried fruit samples (30 samples each from apricot, fig, plum and zibib (dried grape)) were purchased from local markets in Duhok governorate. The collected samples were put in polythene bags and were brought into laboratory for isolation of fungi.

Mycological analysis

Larger fruits (apricot, fig and plum) were cut aseptically into small pieces, whereas, fruits (zibib) were treated as the whole piece. The fruit pieces were surface disinfected with 2% sodium hypochlorite for 1 min., and then rinsed with sterile distilled water. Ten pieces were placed onto Dichloran Rose Bengal Chloramphenicol (DRBC) agar medium (Fluka-Germany) and examined daily for growth and sporulation of fungi for 7 days using a

stereomicroscope. After 7 days all fungi belonging to genus *Aspergillu*s were transferred to Petri dishes of malt extract agar (Pitt and Hockin, 2009).

Identification of *Aspergillus* species

Culture media used for identification of species in the genus *Aspergillus* include Czapek yeast extract agar incubated for 7 days and 25^{o}C (CYA25) and at 37^{o}C (CYA37), Czapek yeast Extract Agar with 20% sucrose incubated for 7 days at 25^{o}C (CY20S), malt extract agar (MEA) incubated for 7 days at 25^{o}C and Czapek Dox solution Agar (CZ) for 7d at 25^{o}C. Other confirmatory tests include Ehrlerch reagent test and growing on creatine medium were also applied (Samson *et al*., 2000, 2007; Klich 2002).

For each isolate, five plates were used, two of CYA and one each of CY20S, MEA and CZ. Each plate inoculated at the center and incubated in the dark for 7days. The media were prepared according to Pitt and Hocking (2009). Klich (2002), Samson *et al*., (2004)

Isolation frequency of *Aspergillus* species from samples was calculated by the following formula.

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

Species identifications were according to the keys and descriptions provided by Pitt and Hocking (2009), Klich (2002), Samson *et al*., (2000, 2004, 2007).

Aflatoxin extraction from fungal culture

Production of aflatoxin (AF) by randomly chosen isolates of *Aspergillus* section *Flavi* was screened according to the method of Bragulat *et al.*, (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 and the centre of the colony 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 millipore filter (0.22 µm) diameter (Millex GP Filter Unit Coringhwohill Co. Ireland).

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 part per billion (ppb).

RESULTS AND DISCUSSION

Data in Table (1) showed that aspergilli are very common fungi associated with dried fruits. A total of 20 *Aspergillus* species have been isolated and identified. The highest diversity of *Aspergillus* species (17) was detected from zibib, followed by 16 species on plum, 14 species on apricot and 13 species on fig. The majority of isolated species were members of *Aspergillus* section *Nigri* (8 species), followed by *Aspergillus* section *Flavi* (5 species). This is in line with data presented by Heperkan *et al*., (2012) and Ozer *et al.*, (2012). Nine *Aspergillus* species were found common to the four types of dried fruits surveyed in the present study. These include *A.awamori, A. carbonarius, A. flavus, A.fumigatus, A. japonicas, A. niger, A. ochraceus, A. parasiticus* and *A. tubingensis*.

The most frequently isolated species from Apricot was *A.niger*, followed by *A.carbonarius* and then *A. flavus* with percentage frequencies of 76.6%, 40.0% and 36.0% respectively, whereas on fig the most fungal species was *A.niger* followed by *A.flavus* and then *A. carbonarius* and *A.parasiticus* with a percentage frequencies of 76.65, 66.6%, 33.1%, 33.1% respectively. *A.flavus* and *A.niger* were reported as being the most common species on dried fig (Pitt and Hocking, 2009). *A.flavus* and *A.parasiticus* in general instances and in very rare cases *A.niger* and *A.fumigatus* were detected from dried fig in Turkey (Steiner *et al*., 1988). Embaby *et al*., (2012) recorded *A.niger*, *A.flavus* and *A.parasiticus* as the most frequent species on dried fig in Egypt. Similar results were reported by Doster *et al*., 1996 from fig in California. Javanmard (2010) reported that the most frequent species in Iranian dried fig was *A. niger* aggregate (90%) followed by *A.flavus* (63.76%). On plum, the most frequently isolated species was *A. niger* followed by *A. flavus* and *A.carbonarius* with a percentage frequency of 66.6%, 46.6% and 33.3% respectively. Among *Aspergillus* species, the ones belonging to section *Nigri* and *Flavi* are the most frequently identified species in dried apricot and prunes (Zohri and Abdel-Gawad,1993; Heperkan, 2006).

The two black aspergilli (*A.niger* and *A.carbonarius*) were the most fungal species detected from zibib and with a percentage frequency of 93.0% and 66.65 respectively. Predominant species in dried vine fruits in Argentina were members of *Aspergillus* section *Nigri* and were isolated with relatively high frequency. *Aspergillus niger* was the most common species (Romero *et al*., 2005). High incidence of black aspergilli in dried fruits can be explained due to their black spores that can provide protection from sunlight and ultraviolet light, giving them competitive advantages in this habitat. Moreover, these fungi are xerophilic thus can tolerate high sugar concentrations and low water activity (Iamanaka *et al.*, 2005).

In general, *A. niger, A. flavus* and *A.carbonarius* showed the highest percentage frequency of occurrence on the tested four types of dried fruits.

Table 1: Percentage occurrence of *Aspergillus* species on dried fruits as detected on DRBC medium

	% Occurrence			
Fungal species	**Apricot**	**Fig**	**Plum**	**Zibib**
Aspergillus aculeatinus	3.3	0.0	6.6	6.6
A.aculeatus	0.0	10.0	0.0	6.6
A.alliaceus	10.0	13.3	0.0	3.3
A.awamori	6.6	3.3	3.3	6.6
A.candidus	0.0	0.0	3.3	3.3
A.carbonarius	40.0	33.3	33.3	66.6
A.flavipes	3.3	3.3	0.0	0.0
A.flavus	36.6	66.6	46.6	33.3
A.fumigatus	10.0	10.0	6.6	6.6
A.japonicus	3.3	3.3	3.3	6.6
A.niger	76.6	76.6	66.6	93.3
A.niveus	3.3	0.0	0.0	3.3
A.ochraceus	3.3	10.0	10.0	6.6
A.oryzae	3.3	0.0	3.3	0.0
15. A.parasiticus	26.6	33.3	10.0	6.6
16. A.sclerotioniger	0.0	0.0	3.3	3.3
17. A.tamarii	0.0	3.3	3.3	0.0
18. A.terreus	0.0	0.0	3.3	3.3
19. A.tubingensis	6.6	3.3	6.6	10.0
20. A.wentii	0.0	0.0	3.3	3.3

Dried fruits are susceptible to fungal infection and mycotoxin formation because of their high level of sugars, possess ideal water activity, methods of harvest and drying conditions (Trucksess and Scot, 2008; Ozer *et al*., 2012).

Aspergillus flavus and *A.parasiticus* are the most important species contaminating food including dried fruits because of their potential to produce aflatoxins. These two aflatoxigenic species are cosmopolitan and may grow and form aflatoxins under many conditions (Hocking,1997).

Table (2) showed the results of screening *Aspergillus* section *Flavi* strains for aflatoxigenic production abilities in culture media as detected by ELISA technique. All strains tested from *A.flavus* and *A.parasiticus* showed aflatoxigenic potential. *A. flavus* isolates obtained from apricot and fig showed the highest abilities (334.0 ppb and 333.0 ppb) respectively compared with two isolates obtained from zibib 10.2 ppb and 79.4 ppb respectively and an isolate from plum (37.4 ppb). Two isolates of *A.parasiticus* from fig showed a marked variation in their aflatoxin potential (81.0 ppb and 344.0 ppb). However, in a recent study in Iraq, Mohammed *et al*., (2010) showed that 81.8% of *A.flavus* strain and 100% of *A.parasiticus* were positive. Abdullah and Al-Mousawy (2009) showed that out of 24 and 18 strains of *A.flavus* obtained from corn grains and sunflower seeds respectively, 15 strains (62.5%) from corn and 10 strains (55.5%) from sunflower seeds showed a positive aflatoxigenic activity. Several studies indicated that not all strains of *A.flavus* can produce aflatoxin and the ratio of the non-aflatoxigenic strains to aflatoxin producing strains varied according to the source and location of the isolates (Schroeder and Bolla, 1973; Abdel-Malek *et al*.,1993; Abdullah *et al*., 2009).

Table 2: Quantitative production of aflatoxin by *Aspergillus* section *Flavi in vitro* by ELISA technique

Fungal isolate	Source	Aflatoxin (ppb)
Aspergillus flavus (isolate 1)	Zibib	79.4
A.flavus (isolate 2)	Zibib	10.2
A.flavus	Fig	333.0
A.flavus	Plum	37.4
A.flavus	Apricot	334.0
Aspergillus parasiticus (isolate 1)	Fig	344.0
A.parasiticus (isolate 2)	Fig	81.0
A.parasiticus	Plum	355.0
A.parasiticus	Zibib	266.0

CONCLUSION

In conclusion, a survey for fungal contamination in dried fruits leading to detection of many toxigenic fungi such as *A.flavus*, *A.parasiticus* and others. Good manufacturing practice after harvest such as cleaning, drying and packaging will minimize fungal contamination and then prevent mycotoxin formation in order to ensure that dried fruits are safe for consumers.

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