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Toxigenic fungi associated with dried Fruits and fruit-based products collected from Jeddah province

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Abstract: *Dried fruits may be contaminated with mycotoxins which are dangerous health problems. Forty samples fruits and fruit-based products were purchased from markets in Jeddah governorate, Saudi Arabia and were mycologically analyzed. The mycoflora were isolated from the samples by direct plating method on Potato Dextrose Agar (PDA) medium. The total counts of the isolated fugal colonies were recovered. Seven different fungal genera and 13 species were isolated and identified. The most dominant genera were Aspergillus (80.60%), Rhizopus (13.58%) and Penicillium (3.30%). About 83 fungal isolate were tested for toxin productions using thin layer chromatography (TLC). It was found that only A. flavus and A. parasiticus were toxigenic fungi and could produce mycotoxins. The factors affecting A. flavus and A. parasiticus growth and Aflatoxins production, including temperatures and types of growth media, were also studied. The optimum temperatures for the growth and Aflatoxins production of the two tested fungi were 25°C using PDA medium. It was also found that PDA and Sabouraud Dextrose Agar (SDA) media were the best media for fungal growth and Aflatoxins production.*

Key words: *Dried fruits, mycotoxins, fungi, aflatoxins, Potato Dextrose Agar, Aspergillus, Penicillium*

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I. Introduction

Mould growth in agricultural products may cause an important hazard to human health by producing toxic metabolites called ‘‘mycotoxin” (Set and Erkmen, 2010) which are secondary metabolites of filamentous fungi, occur naturally in food. Mycotoxins represent a very large group of different substances including aflatoxins, ochratoxin A, patulin and toxins of *Alternaria* which were produced by different mycotoxigenic species (Fernández-Cruz *et al*., 2010, Gonçalves *et al*., 2018). Moulds can infect agricultural crops during crop growth, harvest, storage or processing. The major affected food commodities are cereals, nuts, dried fruit, coffee, cocoa, spices, oil seeds, dried peas, beans and fruit, particularly apples. Dried fruits are susceptible to mould growth and mycotoxin formation because of their high sugar content, method of harvest and drying conditions (Trucksess and Scott, 2008). Factors contributing to the presence or production of mycotoxins in foods or feeds include storage, environmental, and ecological conditions (Luttfullah and Hussain, 2011; Salem and Ahmad, 2010; Zain, 2011). Decreases of moisture content led to a favorable medium for xerotolerant moulds such as *Aspergillus* section *nigri* (Iamanaka *et al*., 2005).

The most important dried fruits produced for human consumption are raisins, sultanas, figs, apricots, and dates ad their mycotoxin contamination may start with on the trees, increase during harvesting and sun drying, and continue to accumulate during storage. Because all these fruits are cultivated in warm climates, mycotoxins associated with these fruits are aflatoxins and ochratoxin A. Aflatoxins in figs are mainly produced by *Aspergillus flavus* or *Aspergillus parasiticus* (Doster and Michailides, 1998).There are many studies on microflora of dried fruits which indicate growth of microorganisms occurs mostly on the outer surfaces with a load of a few hundreds to thousands per gram of fruits. Even if a small part of surface is infected by mold, they may grow quickly in a short time. Furthermore, the numbers of infected fruit may increase rapidly if the drying process is not performed properly (Montville and Matthews, 2008). Species belonging to the genera *Aspergillus*, *Penicillium* and *Alternaria* are major causative agents of fruit spoilage; in addition, these fungi can produce mycotoxins and, in this way, can cause significant economic losses for any process of the food industry, including drying (Jackson and Al-Taher, 2008). In Egypt, Abdel-Sater and Saber, (1999) found that *Aspergillus* was the most frequently isolated genus with contamination of 100% of the date samples while *Penicillium* was less frequently isolated with contamination of only 30% of the date samples. On the other hand, Zohri and Abdel-Gawad, (1993) found that *Penicillium* was the most predominant genus isolated from dried apricots and prunes in a survey performed also in Egypt. In Morocco, the most abundant species found contaminated dates was *A. niger* (Hasnaoui *et al*., 2010). Morton *et al*., (1979) studied the aflatoxin risk for dried figs, apricots, pineapples and raisins and demonstrated that dried apricots have highest potential for aflatoxin, along with dried figs. Shenasi *et al*., (2002) investigated the microflora of date fruits and production of aflatoxins at three stages

of maturation: inedible green fruit (Kimri), edible soft and brown colored fruit (Rutab) and dried, dark brown colored fruit (Tamr). The levels of OTA and AFs in dried prunes produced in Iran were analyzed by Janati *et al.* (2012) who showed that 13.33% of the prune samples had aflatoxin. Several reports have shown that fig fruits are a high risk commodity with respect to toxigenic fungi and their mycotoxins. The most common toxigenic fungi reported are *Aspergillus* section Nigri, *Fusarium* spp., *Aspergillus* section flavi and *Penicillium* species (Heperkan, 2006). Other genera of moulds were also found in Turkish dried figs such as *Acremonium*, *Byssochlamys*, *Cladosporium*, *Trichoderma*, *Mucor* and *Scopulariopsis* (Isman and Bıyık, 2009).

Dried raisins are susceptible to fungal growth and mycotoxins production because of their favourable moisture and sugar contents (Alghalibi and Shater, 2004). El Khoury *et al*. (2006) conducted a study to determine the fungal species responsible for aflatoxin production in grapes cultivated in Lebanon. Their findings show that aflatoxin is primarily generated by *A. flavus.* Abdel-Sater and Saber, (1999) determined the concentrations of aflatoxin B1 in raisins grown in Egypt to be 300 and 550 µg/kg respectively. Intrinsic factors include moisture content or water activity, Hydrogen ion concentration, redox potential, nutrient content (substrate), inhibitors and osmotic pressure. Factors promoting mycotoxin production can differ from mold to mold. Recently, effects of environmental factors, especially temperature, on aflatoxin biosynthetic genes were intensively studied. Light, nitrogen, carbon source, temperature and pH influence the regulation of aflatoxin biosynthesis (Ehrlich *et al*., 2010). Therefore, the aim of this study was to isolated toxigenic and non-toxigenic fungi, from different dried fruits. Moreover, factors affecting fungal growth, and mycotoxins production were also studied.

II. Material and methods

Collection of dried fruits

Forty samples which include 20 samples of dried fruits (raisins, dates, dried plums, figs and apricot, 10 samples of mix of nuts (almonds, pistachios, walnuts, cashews, hazelnuts and peanuts and 10 samples of mix of dried fruits and nuts were collected from different markets of Jeddah governorate, Saudi Arabia in August 2015. Samples were collected in sterilized polyethylene bags, transferred into laboratory and preserved in refrigerator until the study time.

Moisture content of dried fruits

The moisture content of the samples was calculated by a dry weight basis (Christensen, 1957). Moisture content was calculated as the following: $\text{Moisture content}=\frac{W1-W2}{W1}\times 100$

W1 : Weight before drying, W2: Weight after drying

Mycological analysis of dried fruits

Fungal isolation and counting from dried fruits

3.3.1.1 Direct plating method

Each fruit sample was cut aseptically into small pieces, whereas, fruits (raisins) were treated as the whole piece. Three pieces were transferred to the surface of agar plate containing Potato Dextrose Agar medium (PDA) from OXOID, UK. The plates were incubated at 25°C for 7 days (Pitt *et al*., 1992). PDA medium was used as a subculture medium to identify the isolates.

Identification of fungal isolates

Culture and morphological characteristics of the isolated fungi of 5-10 days, grown on PDA were assessed by examination under a light microscope. Subsequently, slide preparation of fungi taken from the culture were stained with drop of Lacto phenol cotton blue and examined for spores and mycelia features using light microscope (x 400).

Identification of fungi was based on comparison of morphology, color, shapes of spores and colony characters as descript by Coony and Emerson, (1964), Raper and Fennell (1965), Samson and Van Reenen-Hoekstra (1988), Moubasher (1993) and Samson *et al.* (1995).

Maintenance of fungal isolates

All isolates of fungi were identified and maintained on PDA slopes held at 4°C. At 4-month intervals, fresh cultures were prepared by streaking out from stock slopes onto PDA plates to check for purity and then sub-culturing onto fresh PDA slopes, both plates and slopes being incubated at 25°C (Smith and Onions, 1994).

Screening of fungi for mycotoxins production

Growth of fungal isolates

All fungal isolated were plated in duplicate on PDA to test their ability to produce mycotoxins according to Samson and Hoekstra (1988). The centre of each plate was inoculated with a plug cut from a plate culture using a flame-sterilized cork-borer (4 mm internal diameter) and the plates were incubated at 25°C for 10 days. The diameter of the fungal colony (mm) was determined.

Preparation and screening of the fungal extracts

All fungi isolated were tested by a rapid screening method for mycotoxin production (Filternborg and Frisvad, 1980). From 10-day old plate culture, 5 agar discs were cut out near the center of the colony with a flame- sterilized stainless steel cork-borer (4 mm) and removed using a flame- sterilized scalpel. Five discs of growth were removed and extracted by mixture of chloroform: methanol (2/1 V/V). The organic layer were collected in small begoes and preserved at -20°C until used.

Thin layer chromatography methods for detection of mycotoxins

Toxigenic fungal isolates used as standards

Five toxic strains of fungal species, *Aspergillus parasiticus* (ATCC 15517), *Aspergillus flavus* (ATCC 16875), *Aspergillus flavus* (ATCC 11498), *Aspergillus ochraceus* (ATCC 22947) and *Aspergillus niger* (ATCC16404) were used as standards. The toxic fungi produce aflatoxin B1, B2, G1, G2 and ochratoxin A. These toxic strains of fungi were obtained from Cairo Microbiological Resources Centre, Faculty of Agriculture, ASU, Egypt.

Preparation and development of TLC plates

For routine examination of extracts, aluminum-backed, DC-Alufolien- Kieselgel 25 plates, silica gel matrix, with fluorescent indictor at 254 nm, were cut to 10 x 10 cm and spotted along a line 1.5 cm from the bottom with 10 µl aliquots of extracts or standards. The plates were developed in the solvent system TEF :Toluene, Ethyl acetate 90% and Formic acid (5:4:1 v/v) at the room temperature until the solvent front reached a line marked 2 cm from the top of the plate(Roberts and Patterson, 1975, Bokhari, 1993). After development, the plates were removed from the solvent, and air-dried in a fume cabinet. The plates are sprayed and dried at 100 °C in a hot-air oven, then examined in a Chromato-Vue cabinet (Model UVP Upland, CA USA) under visible light or short wavelength UV light (254 nm) and long wavelength (366nm) as described by Samson *et al*., (2000).

Detection and identification of mycotoxin on TLC plates

Development chromatograms were examined under visible light for colored substances, under UV light for fluorescent substance with and without the use of spray reagent. The detection system for particular toxin is shown Table 1. The aflatoxins could be detected under UV light without any additional treatment; aflatoxin B1 and B2 fluorescent blue and aflatoxin G1 and G2 gave a green fluorescent color. Ochratoxin gives a fluoresced blue color after exposing the Chromatography plate for Ammonia vapor for 10 min and heating at 110°C for 5 min.

Table 1. Detection and identification of mycotoxin by (TLC)

Toxin	Treatment	Visualization under UV
Aflatoxin B1, B2	NO	Blue fluorescence
Aflatoxin G1, G1	NO	Green fluorescence

Fungal growth by linear extension method

The fungal growth of *A. flavus and A. parasiticus* was measured using the liner method after 7 days of incubation, as described by Korzets *et al*., (2001).The average growth was calculated and this method reflects the daily growth rate increase.

Effect of different media on toxigenic fungal growth and mycotoxin production

At this experiment five different media were used to determine fungal growth and mycotoxin production by *A. flavus and A. parasiticus*. The media used were PDA, Czapek Dox Agar medium (HIMEDIA, India), Sabouraud Dextrose Agar medium (OXOID, UK), Malt Extract Agar medium (DIFCO, US), and Yeast Extract Agar medium (HIMEDIA, India).Hydrogen ion concentration (pH) was adjusted for all the used media to6.5. A disc of fungal growth of 7 day old culture was transferred to the center of a Petri dish containing the tested medium. The plates were incubated at 25°C for 7 days. Three replicates were including and fungal growth was measured as the mean diameter of the three colonies. Mycotoxins were extracted and detected.

Effect of different temperatures on the growth and production of mycotoxins

Five different temperatures 18°C, 25°C, 30°C, 37°C and 40°C were used to study the effect of temperatures on fungal growth and toxin production. Plates with PDA media were inoculated with a 4 mm disc of *A. flavus* and *A. parasiticus*. The plates were incubated for 7 days at different

temperatures. The liner examination method was used to estimate rate of fungal growth (Korzets *et al*., 2001). Mycotoxins were extracted and examined according to Filternborg and Frisvad, (1980).

III. Results

Forty samples including 20 dried fruits (7 samples from raisins, 2 from dried prunes, 6 from dates, 2 from fig and 3 from apricot), 10 samples from mix nuts (almonds, pistachios, walnuts, cashews, hazelnuts and peanuts) and 10 samples from mix of dried fruits and nuts were collected. The percentage of moisture content of each sample was determined and they were ranged from 17.7 to 18.0% (Table 2).

Table 2. Moisture content of forty collected dried fruit and nut samples

Dried fruit	No. of sample	% of moisture content
Prunes	2	18.0
Raisins	7	14.3
Dates	6	10.7
Dried Figs	2	18.0
Apricot	3	12.7
Mixed nuts	10	13.2
Mixed fruit	10	14.9

The total number of isolated fungi from the collected dried fruit and nuts samples were determined and identified. They were belonging to 7 genera and the most important was *Aspergillus* which contained 5 species (*A. flavus*, *A. niger*, *A. parasiticus*, *A. terreus* and *Aspergillus* sp.). Three species of genus *Penicillium* (*P. citrinum*, *P. italicum* and *P. digitatum*), one species of the genus *Rhizopus (R. stolonifer*), one species of the genus *Mucor* (*M. racemosus*), one species of the genus *Alternaria (A. alternata*), one species of the genus *Paecilomyces* (*Paecilomyces variotii*) were obtained other isolated fungi include: Sterile mycelium isolated in lower incidence rate. Table 3 showed the identified and the total number of fungi isolated on PDA medium by direct plate method.

Fungal occurrence was ranged from highly, moderate and low. Other isolated fungi belong to sterile fungi was in lower incidence rate. *Aspergillus* was the most common isolated genus. *Rhizopus stolonifer* was the second most common fungus isolated from dried fruits. The percentage of occurrence was estimated for different types of fungal isolates of dried fruits. The results indicated that, among the isolated genera, *Aspergillus* was the most common. It was found by 80.6% on PDA, of the total number of fungi isolated on PDA medium. The most dominant species was *A. niger* which appear by 75.62% on PDA. Moreover, the second common isolated fungus was *Rhizopus stolonifer*. It was found by 13.58% on PDA, of the total number of fungi isolated from PDA medium (Table 4).On the other hand, *Penicillium* was the third isolated fungal genera. It was found by 3.30% on PDA, of the total number of fungi isolated from PDA medium.

Table 3. Fungi isolated from 40 samples of dried fruits and nuts on PDA medium at 25 ˚C using direct plating method

Sample (number) / Isolated fungi	**Prunes (2)**	**Raisin (7)**	**Dates (6)**	**Figs (2)**	**Apricot (3)**	**Nuts (10)**	**Mix (10)**
1- *Aspergillus*	20	**558**	**437**	**-**	**-**	**192**	**330**
A. flavus	**2**	**16**	**9**	**-**	**-**	**10**	**36**
A. niger	**14**	**542**	**426**	**-**	**-**	**175**	**285**
A. parasiticus	**-**	**-**	**-**	**-**	**-**	**-**	**4**
A. terreus	**-**	**-**	**2**	**-**	**-**	**7**	**5**
***Aspergillus* sp.**	**4**	**-**	**-**	**-**	**-**	**-**	**-**
2- *Penicillium*	**5**	**15**	**2**	**-**	**-**	**7**	**34**
P. citrinum	**-**	**13**	**-**	**-**	**-**	**6**	**22**
P. italicum	**5**	**2**	**2**	**-**	**-**	**1**	**10**
P.digitatum	**-**	**-**	**-**	**-**	**-**	**-**	**2**
3- *Rhizopus*	**12**	**71**	**35**	**-**	**-**	**58**	**83**
R. stolonifer	**12**	**71**	**35**	**-**	**-**	**58**	**83**
4-*Mucor*	**-**	**4**	**2**	**-**	**-**	**-**	**1**
M. racemosus	**-**	**4**	**2**	**-**	**-**	**-**	**1**
5- Alternaria	**-**	**-**	**-**	**-**	**-**	**3**	**10**
A. alternata	**-**	**-**	**-**	**-**	**-**	**3**	**10**
6- Paecilomyces	**-**	**-**	**-**	**-**	**-**	**5**	**6**
Paecilomycesvariotii	**-**	**-**	**-**	**-**	**-**	**5**	**6**
7- Sterile mycelium	**4**	**2**	**-**	**-**	**-**	**5**	**6**

(-) = Not detected

Table 4 .Total colony (counts per g), number of appearance out of 40 samples and occurrence remarks of fungal species recovered from samples at 25°C after 7 days on PDA medium

Fungal species	Total count	Occurrence (%)	Frequency of occurrence in 40 samples	Frequency (%)	Occurrence remark
Aspergillus	1537	80.60	69	48.25	
A. flavus	73	3.83	25	17.48	H
A. niger	1442	75.62	35	24.48	H
A. parasiticus	4	0.21	2	1.40	R
A. terreus	14	0.73	6	4.20	L
Aspergillus sp.	4	0.21	1	0.70	R
Penicillium	63	3.30	20	13.99	
P. citrinum	41	2.15	11	7.69	L
P. italicum	20	1.05	8	5.59	L
P. digitatum	2	0.10	1	0.70	R
Rhizopus	259	13.58	32	22.38	
R. stolonifer	259	13.58	32	22.38	H
Mucor	7	0.37	3	2.10	R
M. racemosus	7	0.37	3	2.10	R
Alternaria	13	0.68	7	4.90	L
A. alternata	13	0.68	7	4.90	L
Paecilomyces	11	0.58	5	3.50	L
Paecilomyces variotii	11	0.58	5	3.50	L
Sterile mycelium	17	0.89	7	4.90	L
Total count in all samples	1907	100.00	143	100.00	

H: High occurrence (25-40 time), M: Moderate occurrence (15-24 time), L: Low occurrence (5-14 time), R: Rare occurrence (1-4 time)

Fungi were grown on agar plates and five discs were taken, extracted using organic solvents, and mycotoxins were separated. Detection was carried out chromatographically using TLC which was examined under long- wavelength (366 nm) and short- wavelength UV light (254nm). Fungal species has been tested for the production of different mycotoxins, 8 fungal isolates was chosen for testing the production of mycotoxins (Aflatoxins B1, B2, G1, G2 and Ochratoxin A). The tested fungal species were *A. flavus*, *A. parasiticus*, *A. niger*, *A. terreus*, *Aspergillus* sp., *P. citrinum*, *A. alternate* and *Paecilomyces variotii*. Two samples from 40 samples of dried fruits were contaminated with two toxigenic fungal isolates. The toxigenic fungi, recorded in the examined sample, were *A. flavus*, *A. parasiticus*, were (Table 4, Figure 1). The results showed that two toxigenic fungi were producer of aflatoxin B1 and one isolate was producer of aflatoxin B1 and G1.One isolate of *A. flavus* was found to produce aflatoxin B1 and one isolate of *A. parasiticus* produced both aflatoxin B1 and G1. No mycotoxins were recorded for the other fungal species under the condition of this study. The current study focused on two species that secrete mycotoxin, they are *A. flavus* and *A. parasiticus*.

Table 5. Screening of some selected isolates for the production of mycotoxins by TLC

Toxin	Tested species	No. of tested isolates	No. of toxigenic isolates	Characteristics
Aflatoxin B1	*A. flavus*	25	1	Blue fluorescence without treatment
	A. niger	30	-	
	A. parasiticus	1	1	
	A. parasiticus	3	-	
	A. terreus	6	-	
	Aspergillus sp.	4	-	
	P. citrinum	5	-	
	A. alternate	4	-	
	Paecilomyces variotii	5	-	
Aflatoxin G1	*A. flavus*	25	-	Green fluorescence without treatment
	A. niger	30	-	
	A. parasiticus	1	1	
	A. parasiticus	3	-	
	A. terreus	6	-	
	Aspergillus sp.	4	-	
	P. citrinum	5	-	
	A. alternate	4	-	
	Paecilomyces variotii	5	-	

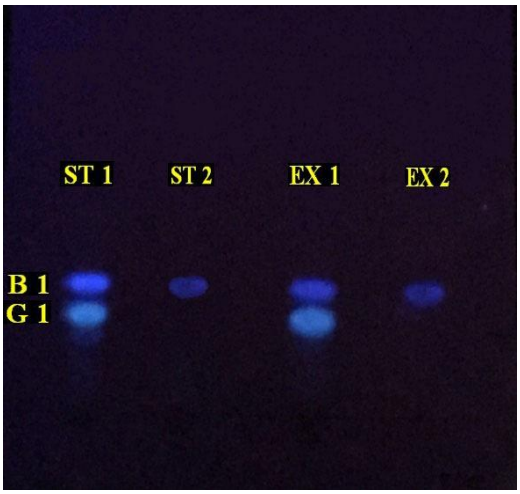

Figure 1: TLC showed aflatoxins(B1and G1) extacted from *A. flavus* (EX1) and *A. parasiticus*

Two different toxigenic isolates, *A. flavus* and *A. parasiticus* were selected to be examined for their ability to grow and produce toxin on different agar media (PDA, Cz, SDA, MEA and YES) and growth rate were taken after 7 days at 25°C.The growth was either good or moderate using SDA or PDA as shows in Table 5. It was found that PDA medium was the best for both fungal growth and the toxin production, followed by SDA medium for both *A. flavus* and *A. parasiticus* while only PDA, SDA and YES media supported the toxin production by *A. flavus* (Table 6).In *A. parasiticus*, PDA, SDA and YES media supported both Aflatoxin B1 and G1 production while MEA media supported only the Aflatoxin B1 production. Moreover, five different temperatures 18°C, 25°C, 30°C,37°C and 40°C were used to study the growth rate of toxigenic fungi and toxin production after 7 days. The result revealed that the best temperature for grow and toxin production was 25°C after 7 days for both *A. flavus* and *A. parasiticus* (Table 6). The mean diameter of the fungal growth after 7 days at 25°C was 82.50 and 71.67mm for *A. flavus* and *A. parasiticus*, respectively. Production of aflatoxin B1 to aflatoxin G1 varied with temperature. Aflatoxin production was not related to growth rate of *A. flavus* where at 37°C, at almost maximal growth, no aflatoxin was produced. No aflatoxins were produced at 37-40°C by the two isolates.

Table 6. Effect of different media on growth (diameter of fungal colony) and aflatoxin production by *Aspergillius flavus* and *Aspergillius parasiticus*, grown for 7 days at 25°C

Media used	*Aspergillius flavus*		*Aspergillius parasiticus*		
	Fungal growth (mm)	Aflatoxin B1 production	Fungal growth (mm)	Aflatoxin G1 production	Aflatoxin B1 production
PDA	80±0.316	+++	81.33±0.378	+++	+++
SDA	85±0.000	++	84.83±0.041	+++	+++
Czapek	67.5±0.689	-	76.67±0.753	-	-
Malt agar	64.17±0.204	-	72.50±0.274	-	+
Yeast agar	61.67±0.516	+	61.67±0.816	+	+

+ : weak production, ++ : moderate production, +++ : high production

Table 7. Effect of different temperature on growth (diameter of fungal colony) and Aflatoxin production by *Aspergillius flavus*and *Aspergillius parasiticus*, grown on PDA for 7 days

Temperature (◦C)	*Aspergillius flavus*		*Aspergillius parasiticus*		
	Fungal growth(mm)	Aflatoxin B1 production	Fungal growth (mg/ml)	Aflatoxin G1 production	Aflatoxin B1 production
18	35.83±0.801	+	55.00±0.775	++	+
25	82.50±0.274	+++	71.67±0.408	+++	+++
30	51.67±1.538	++	67.50±0.689	+	+++
37	46.67±1.125	-	67.50±0.274	-	+
40	34.17±0.376	-	31.67±0.258	-	-

+ : weak production, ++ : moderate production, +++ : high production

IV. Discussion

Food drying is one of the oldest methods for preserving foods for centuries. Fruit may be dried as whole fruit e.g., grapes, in sliced form e.g., banana, mango, papaya, kiwi, etc.), in puree form e.g., mango, apricot (Ratti and Mujumdar, 2004). The drying of fruits allows for their better preservation by reducing water content, thus inhibiting microbial growth and enzymatic modifications. These products are thought to be resistant to microbial spoilage because of their low water activity, high acidity and sugar content, as a consequence of drying process.

Mycotoxins affect food quality, resulting in huge economic losses in addition to being hazardous to consumer health for producing countries (Richard, 2007, Gonçalves *et al*., 2018). Therefore, mycotoxins are considered an important problem throughout the world in terms of public health, agriculture and economics. The contamination of dried fruits and nuts with mycotoxins has not only caused health hazards but also resulted in economic losses, especially for exporting countries. The numbers of microorganisms on most dried fruits vary from a few hundreds per gram of fruits to thousands and they are mostly on the outer surfaces. When part of the fruit has supported growth and sporulation of mold before or after drying, mold spores may be present in large numbers. If drying trays are not clean and improperly loaded, a marked increase in the numbers of bacteria and fungi may take place during the drying process. Spoilage of most dry fruits usually occurs during storage, handling and transport (Frazier and Westhoff, 1988). In this study, 7 genera and 13 species of different fungi including 5 species of *Aspergillus*, three of Penicillium, one species of *Rhizopus* , one species of *Mucor*, one species of *Alternaria* ,one species of *Paecilomyces* and other isolated fungi include: Sterile mycelium, were isolated from 40 dried fruit and nut samples. Examination of mycotoxin positive dried Fruits by Toma and Rajab,2014 in Iraq yielded 15 genera and 16 species of different fungi including 16 species of *Aspergillus*, five species of *Penicillium*, two species of *Alternari*, two species of the *Gliocladium*, four species of *Mucor*,five species of *Rhizopus*, other isolated fungi include: *Emericella nidulans, Geotrichum* sp., *Helminthosporium australians, Mortierella* sp., *Neurospora* sp., *Paecilomyces victoria, Rhizoctonia* sp., and *Scopularisopsis* sp. while *Tetracocosporium sp. sporium Australians* were isolated in lower incidence rate (1 colony).

On PDA medium, Aspergillus was the most dominant genus. It was contaminating all nut and dried fruit samples contributing 80.60% of the total fungal counts on PDA .Our result agree with Alhussaini (2012).Differences in the fungal counts and composition of genera and species among samples are expected due to variations in geographical localities from which dried fruit and nut samples were collected. Types of isolation media, techniques for mycological analysis and the number of samples tested are all factors leading to variations in the final reports on these fungi.

Date fruits analyzed in the present study produced only three species of *Aspergillus (A. flavus, A. niger* and *A. terreus*). Gherbawy *et al*. (2012) studied the mycological profile of the retail date fruits distributed in different markets at Taif, Saudi Arabia in addition to the presence of aflatoxins and ochratoxin A. They isolated 22 fungal species belonging to 12 genera from 50 different datesamples. *Aspergillus flavus, A. niger, Penicillium chrysogenum,* and *Rhizopus stolonifer* were the most prevalent species among isolated fungi. Toxicity test using *Artimia* larvae indicated that seven out of 18 isolates of *A. flavus* had aflatoxins production potentials, while nine out of 36 isolates of *A. niger* were ochratoxigenic fungi.

Analysis of raisins showed contamination with *A. flavus, A. niger P. italicum, R. stolonifer, M. racemosus* and Sterile mycelium. Reports from Yemen Republic (Al-Ghalibi *et al*., 2008) revealed the prevalence of *A. niger* on dry raisins. A. flavus was less frequently isolated. *Penicillium* was isolated in moderate frequency on 1 and 20% sucrose Czapek's agar media, but in low frequency on Sabouraud dextrose agar medium. Their results revealed also that 3 out of 7 samples of raisins were contaminated with aflatoxins at levels ranging from 2.68 to 11.56 µg K/g). In Armenia, Hakobyan *et al*.(2010) were able to make mycological analysis of forty-one samples of Armenian made and eleven samples of imported raisins collected in several markets in Yerevan. They isolated and identified thirty two species of filamentous fungi from *Aspergillus, Penicillium, Alternaria, Trichoderm*a and *Syncephalastrum* genera. Species belonging to Aspergillus have a very high frequency of occurrence, 65.2% with species from Nigri section showing the highest occurrence (66.7% of all isolated fungi belonging to Aspergillus). *Aspergillus carbonarius* and *A. niger* were the dominant among fungi from section Nigri. Both Armenian and imported samples of raisin had a high contamination level by these fungi which are potential producers of ochratoxin A.

In the present work figs and apricot were samples were analyzed for isolation fungi and no fungi contamination was found. Iamanaka *et al*. (2005) analyzed 14 samples of dried apricots and none of them was contaminated with OTA. These studies imply that low incidence of fungi and mycotoxin contamination in apricot could be due to the sulfur dioxide treatment (Karaca *et al*., 2010).According to our findings, figs and apricot samples free of live fungi. Iamanaka *et al*., (2005) also reported the absence of live molds from some dried fruits including apricots. Scarcity or absence of live fungi from these commodities perhaps was due to a postharvest microbe-destructing treatment.

In the present work nuts were contaminated with nine fungal species of which *A. flavus, A. niger, A. terreus, P. citrinum, P. italicum, A. alternata, Paecilomyces variotii, R. stolonifer* and occurred in relatively high counts to low. Also similar findings were reported from Saudi Arabia by Alhussaini (2012). Nearly similar findings were reported from Brazil by Freire and Kozakiewicz (2005) who investigated the mycobiota of cashew kernels and found that members of *Aspergillus* and *Penicillium* were the dominant. Species potentially toxigenic such as *Alternaria alternata, Aspergillus clavatus, A. flavus, A. parasiticus, A. ochraceus, A. ustus, Penicillium citrinum* and *P. oxalicum* were frequently isolated. There are other comprehensive studies on the occurrence of filamentous fungi in cashews traded in different countries. In Thailand the study of Pitt *et al*., (1993) showed that the major fungi isolated were *A. flavus* (60%), *Nigrospora oryzae* (58%), *A. niger* (53%), *Chaetomium globosum* (47%) and *Eurotium chevalieri* (40%) A wide range of fungi, representing several genera and species, has been reported associated with deterioration of cashew kernels in Saudi Arabia and Nigeria (Adebajo and Diyaolu, 2003). Zohri and Abdel-Gawad (1993) recorded a wide range of moulds representing several genera and species from 5 seed samples of each almond, cashew nut, chestnut, hazelnut, pistachio nut and walnut collected from different markets. The total counts of fungi were widely fluctuated between 1960- 7704 and 1948-7434 colonies/g dry seeds on glucose- Czapek's and glycerol agar media respectively. During that study 20 genera, 53 species and 2 varieties of fungi were isolated. The prevalent fungi on the 2 agar media were *Aspergillus flavus, A. niger* and *Penicillium chrysogenum.* In this study, the mycotoxins are found to be produced by two toxigenic molds including *Aspergillus flavus* and *A. parasiticus*. Many of studied have proven that *Aspergillus* spp. secreted aflatoxins (Othman and Al-Delamiy, 2012). It was found that AFB1 production by the aflatoxigenic isolates of A. flavus and *A. parasiticus* isolated from peanuts, wheat flour and corn using synthetic media of potato dextrose agar. Mycotoxins produced by different genera of the isolated fungi were determined using Thin Layer Chromatography (TLC) which is the most common and inexpensive chromatographic technique, used to identify different mycotoxins. TLC, a technique used for the separation, purification assessment, and identification of organic compounds, is one of the most widely used separation techniques for aflatoxin, ochratoxin analysis and other toxins (Rizzo *et al*., 2004) and several TLC methods were developed for mycotoxin quantification and qualification determinations (Rizzo *et al*., 2004 ;Caldas and Silva, 2007).

In this research, TLC was used to assess aflatoxin B1, B2, G1, G2 and ochratoxin contamination in dried fruits and nuts samples by thin layer chromatographic (TLC) method. The mycotoxins that have been extracted from the selected fungal isolates were one isolates was producer of aflatoxin B1 from *Aspergillus flavus* and one isolate of *Aspergillus parasiticus* produced both aflatoxin B1 and G2. The only solvent system used was the best solvent system recommended to separate the toxins (Bokhari, 1993). On the other hand, some mycotoxins like aflatoxins are easy to detect under UV light, unfortunately, others mycotoxins cannot can be detected by such a simple method. According to the results obtained in this study and other countries, the presence and high levels of aflatoxins in dried fruits and nuts has become a serious concern for human health and cause huge economic losses.

The most important factor for microbial growth is availability of water. Fungi are better competitors for the free water then bacteria. Water activity (aw) is the measure of water available for use by microorganisms in their metabolic processes. In the present study, % of moisture content of each sample was ranged from 2-18.4%, these results show appropriate moisture content of the samples which allow the growth of xerophilic fungi. The concentration of high sugar and low water activity in dried fruits helps develop the species of *Aspergillus*, especially *A. niger, A. carbonaris* and *A. ochraceus* because they are Zerophilic, Because *A. niger* was the most common fungus in most dried fruit samples like (Toma and Rajab,2014).Increasing water content and high sugar of dried fruit samples compared with that of compound food enhanced fungal growth and/or mycotoxin accumulation. Growth of fungi can be prevented by drying and keeping the commodity at aw below 0.65 (Northolt *et al*. 1995). Averkieva (2009) reported that grains and other dry feedstuffs should be stored at a moisture level of less than 14% and/or with the use of chemical mould inhibitors to prevent mould growth.

There are factors affecting growth of *A. flavus* and *A. parasiticus* and toxin production. In this study two different toxic fungi isolated were tested different, factor such as temperature and growth media. Temperature and different media represent two key environmental factors influencing both the rate of fungal spoilage of food commodities and production of mycotoxins such as aflatoxins. This study has confirmed the significant influence of physical factors such as temperature and chemical factors such as different media on growth and mycotoxin production of both *A. parasiticus* and *A. flavus*. However, the conditions assayed affect, often, differently the two tested species. From this study, *A. flavus* and *A. parasiticus* showed good growth on SDA and PDA medium at temperature (25°C). Furthermore, higher values of temperatures corresponded to faster appearance of mycelial growth.

A really wide range of temperature permitting growth of *A. flavus* has been reported in the literature (Marín *et al*., 2009; Pitt and Hocking, 2009). This fact seems to depend from the availability of nutrients influenced by structure and composition of substrates, which define the minimum conditions for proliferation of

fungi but do not have effect under optimal environmental conditions (Pardo *et al*., 2004). The results reported herein add new information about the optimal conditions necessary for growth or inhibition of *A. flavus* and *A. parasiticus.* With regard to aflatoxin production, several reports have established the optimal conditions for A. flavus at 0.99 aw and 25–30 °C (Giorni *et al*., 2007). Also in this case the differences found in literature about the optimal conditions supporting aflatoxin production are likely due to the differences in media used and in fungal strain studied (Klich, 2007). In the system analyzed in our study, we found that 25 °C was the optimal conditions for the highest production of AFB1 on PDA for *A. flavus* while the optimal conditions for the highest production of AFB1 on PDA for *A. parasiticus* was 25 and 30°C. At 18°C, 37°C and 40°C and a marked decrease or total suppression of toxin production was observed, although good fungal growth occurred at 30 °C and 37°C for both *A. flavus* and *A. parasiticus*.

The production of various mycotoxins in response to abiotic and other stress conditions has been well documented in different mycotoxigenic fungal species. Temperature plays an important role in influencing the growth of fungi. Normally, the growth temperature for the majority of fungi is between 25˚C to 30˚C and above 40˚C the growth is poor (Cooney and Emerson, 1964). Similarly, Sharma and Sharma (2009) obtained maximum growth of *Chrysosporium tropicum* and *Trichophyton metagrophytes* at 28˚C and 30˚C temperatures. In this study 25˚C to 30˚C were observed to support maximum growth of *A. flavus* and *A. parasiticus*. The Aflatoxins production were also highly influenced by temperature .No correlation was found between mycelial growth of *A. parasiticus* and *A. flavus* and mycotoxins synthesis after 10 days of incubation. Indeed, different authors suggested that the production of mycotoxins is not associated with rapid growth of the fungi, rather higher growth rates seem to restrict mycotoxin synthesis. The maximum AFB1 production by A. flavus was detected at 25C after 10 days of incubation; whereas, the optimal temperature for AFB1 and AFG1 productions by *A. parasiticus* were 25 C and 30 C after 10 days of incubation.

These results indicated that *A. parasiticus* and *A. flavus* has a higher optimum temperature for mycotoxin synthesis and possibly also takes greater advantage of drier conditions for maximum Aflatoxins production. Those findings are supported by the fact that semi-arid to arid and drought conditions in tropical countries are more associated with aflatoxins contamination (Russell *et al*., 2010), Paterson and Lima (2010) considered *Aspergillus flavus* as thermotolerant species adapted to warmer climate. With regard to aflatoxin production, several reports have established the optimal conditions for *A. flavus* at 25 - 30◦C (Giorni *et al*., 2007). Differently, Gallo *et al.* (2016) found the 28◦C was the optimal temperature for the highest production of AFB1 by *A. flavus* on almond based medium.

The optimal conditions supporting aflatoxin production depend on the differences in media used and in fungal strain studied. It is worth mentioning that some interspecies differences emerge about fungal growth and mycotoxins biosynthesis as affected by environmental factors, probably, in relation to the substrates from which the various strains were isolated and to the natural genetic variation inside toxigenic strains. The optimal conditions for biosynthesis of AFB1 and AFG1 of *A. parasiticus* were similar. In addition, studies by Schmidt-Heydt *et al.* (2010) suggested that differences between AFB1 and AFG1 production by a strain of *A. parasiticus* were because of temperature changes. Temperature and different media has individually marked effects on growth and toxin production although each often responded differently to changes in environmental conditions.

In conclusion, the tested fruits and fruit-based products may be contaminated by toxigenic fungi but at low percentage. The studied fungi are isolated on specific culture medium for cultivation, preservation, microscopically examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture..The media components are important criteria for fungal culture and study, along with important physiological parameters that lead to maximum sporulation in fungi. In the present investigation, the different type of culture media and their chemical compositions significantly affected the mycelia growth rate and conidial production in *A. flavus* and *A. parasiticus*. All the five culture media used supported the growth of the two fungi i.e. *A. flavus* and *A. parasiticus* to the same degree as optimal pH and temperature.

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