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OCCURRENCE OF AFLATOXINS AND AFLATOXIGENIC FUNGI IN SOME COMMON TYPES OF DRIED FRUITS IN EGYPT.

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Abstract: The mycobiota of dried fruits was investigated in 40 samples of apricots, plums, raisins and figs collected from different markets in Assiut city, Egypt. There was a remarkable variation in the fungal count and diverse among the studied types of dried fruits. Ten species appertaining to four genera were isolated from the four types of dried fruits on 20% sucrose-Czapek's agar medium at 28°C. Samples of figs and apricots were highly polluted than those of plums and raisins. The genera of the highest occurrence and their respective species were *Aspergillus (A. niger, A. flavus, A. sydowi, A. parasiticus* and *A. versicolor); Penicillium (P. oxalicum* and *P. chrysogenum*).

The different dried fruit samples were analyzed for the presence of aflatoxins. There was aflatoxin contamination in apricots and raisins (one sample out of 10 tested, 0.4-4.0 µg/kg) and figs (5 samples, 0.4->100 µg/kg). For the potential of contamination, spores of two highly toxic strains, *A. flavus* and *A. parasiticus* were applied to the surface of the four types of dried fruits as well as to the surface of their corresponding fresh fruits. All samples were incubated at 25°C for 2 weeks. Data revealed that, dried fruits proved to be unsuitable for fungal sporulation and aflatoxin production. Comparatively, the fresh fruits of apricots, grapes and figs stimulated mold growth and aflatoxins formation. Levels of the produced aflatoxins were fungal strain dependent.

Key words: Dried fruits, fungi, Aflatoxins.

Introduction

Fungi are of ubiquitous distribution and regarded more or less as a source of contamination of foods leading to spoilage and/or food-borne mycotoxins. Owing to the role played by fungi, whether from economic or public health point of view, advanced countries considered mold and yeast counts as a standard test for checking general sanitary conditions (Foster *et al.*, 1958). Mold growth on foods that are to be consumed directly can result in direct exposure to mycotoxins.

Mycotoxins exert a diverse range of toxic effects because their chemical structures are very heterogeneous. Apart from their

acute and chronic toxixity, mycotoxins may possess carcinogenic, mutagenic, and teratogenic properties. They may act primarily on the liver (hepatotoxicity), kidney (nephrotoxicity), nervous (neurotoxicity), and immune systems (immunotoxicity or immunosuppression), on the uterus (uterotropism), and on the skin (dermatotoxicity), or they may act as general cytotoxins (Weidenbörner, 2000). Aflatoxins are the most potent toxic, mutagenic, teratogenic and carcinogenic metabolities produced by some strains of *Aspergillus flavus, A. parasiticus* and *A. nomius* (Kurtzman *et al.*, 1987). Aflatoxins B_1, B_2, G_1 and G_2 are the most commonly encountered forms, with the former being the most potent (Eaten and Ramsdell, 1992).

Mycotoxin contamination of foods may cause considerable economic losses. On a global perspective, aflatoxins in tree nuts, dry fruits, and spices, *Fusarium* toxins in cereals (particularly maize, wheat, and barley), and ochratoxin A in cereals and coffee are of major importance (Bhat and Vasanthi, 1999). Regional problems also may arise from mycotoxins in fruits such as patulin in apples, ochratoxin A in grapes and dried vine fruits, or aflatoxins in different dried fruits.

Mycotoxins diffuse into the environment and can be found in food and feed areas, which do not show any sign of mycelial growth. Therefore, the absence of molds does not guarantee freedom from mycotoxins, and conversely, the presence of a toxin-producing mold does not automatically imply the presence of mycotoxins in foods and feeds. Generally, three causes for contamination of foods are distinguished: a primary contamination of agricultural commodities in the field and upon postharvest storage, a secondary contamination during processing as a consequence of poor hygienic processing conditions, and finally, a carryover effect may occur with residues in animal-derived food via mycotoxins-contaminated feed (Drusch and Aumann, 2005).

The most important dried fruits produced for human consumption are raisins, plums, figs, apricots, and dates. Because all these fruits are cultivated in warm climates, mycotoxins associated with these fruits are aflatoxins and ochratoxin A (Doster *et al.*, 1996).

Due to the significant health risks associated with aflatoxins in foods specially these which are normally consumed directly such as dried fruits, it is important to verify the quality of these products, mainly if they are imported. The objective of this study was to investigate the presence of toxigenic fungi and aflatoxins in four common types of dried fruits namely: raisins, apricots, plums and figs. The aflatoxigenic

potential of those dried fruits was also investigated.

Materials and Methods

Sampling:

Forty samples, (ten of each of dry apricots, plums, raisins and figs) were collected from different markets in Assiut city, Egypt. The samples were transferred to the laboratory and kept in a refrigerator (3-5°C) until analysis.

Approximately 500 g of each sample were employed. For mycological analysis, a sub sample of 150 g from a total of 500 g was used. Raisins were plated as a whole fruits, while dried apricots, plums and figs fruits were cut into small pieces. For aflatoxin determination the remaining weight (350 g) of each sample was homogenized thoroughly by blending in an electric blender.

Chemicals:

Standard aflatoxins B_1, B_2, G_1 and G_2 were obtained from the Southern Regional Research Center, New Orleans, Louisiana, USA. TLC aluminum plates 20x20 cm precoated with 0.25 mm silica gel G-25 HR as well as, silica gel for column chromatography were obtained from Sigma Chemicals Co. Other chemicals were analytical grade.

Mycological analysis:

The dilution-plate method (Johnson and Curl, 1972) was applied for isolation of fungi. 20% (w/v) sucrose-Czapek's agar medium (Raper and Fennell, 1977) was employed. Chloramphenicol (20 µg/ml) and rose bengal (30 ppm) were used as bacteriostatic agents. Four plates of each sample were prepared and incubated at 28°C for one week. The developing fungi were counted (per g dry fruit) and identified according to the following references: Booth (1971); Ellis (1976); Raper and Fennell (1977); Pitt (1979); Domsch *et al.* (1980); Kozakiewicz (1989); Moubasher (1993) and Samson *et al.* (1995).

Chromatographic analysis of aflatoxins:

Thin-layer chromatography was routinely used for qualitative and quantitative estimations of aflatoxins (if any) in the resulting chloroform extracts.

Extraction and purification:

Aflatoxins were extracted and purified according to the method of AOAC (1984). The extraction was performed using chloroform:water (10:1 v/v) mixture. The obtained crude extracts were purified by column chromatography containing anhydrous sodium sulphate (15 g) and silica gel (10 g).

Qualitative estimation of aflatoxins:

Rectangular glass jar (30x15x30 cm) was used for developing chromatoplates. A suitable volume of solvent mixture (chloroform:

methanol, 97:3 v/v) was placed in the bottom of the jar so that the starting spots on the plates would be one cm above the upper surface of the solvent mixture. Chromatographic plates (20x20 cm) were activated by heating one h at 120°C in a hot air oven and removed immediately to a desiccator to cool. Parallel starting spots, 2 cm from each side of the plate and 1.5 cm apart, were made with micropipets from chloroform extracts with authentic reference aflatoxins. Spots were left to dry in air. Prepared plates were then transferred to the chromatographic jar, developed to a suitable distance (10 cm), and removed. The solvent front was marked and the plates were dried in air. Spots were viewed under UV light (366 nm) and the outline of each fluorescent spots was marked by sharp pin. R_f values fluorescence, colors, and intensities of the unknown spots were compared with those of the authentic reference aflatoxins (El-Bazza *et al.*, 1982).

Quantitative determination of aflatoxins

The dilution-to-extinction (Coomes *et al.*, 1965) and comparison of standards (AOAC, 1984) techniques were used for the quantitative estimation. Aflatoxin concentrations in the tested samples were calculated from fluorescence intensities compared with those of known concentrations of standard commercial aflatoxins.

Preliminary detection of aflatoxin-producing fungi:

Thirty-nine isolates of *A. flavus* and 8 isolates of *A. parasiticus* recovered from the studied dry fruit samples were screened for their ability to produce aflatoxins on Sabouraud-yeast extract agar plates, using the fluorescent agar technique of Hara *et al.* (1974). Each of the isolated molds was inoculated as a single short streak at the center of the plate surface. Plates were then incubated at 25°C for 7 days and viewed under UV light (366 nm); the presence of any fluorescence in the medium surrounding the fungal growth was recorded. A plate of non-inoculated medium was similarly incubated and viewed under UV light as a control. This control was used to rule out any fluorescence that may be produced by the constituents of the medium.

Experimental production of aflatoxin:

To evaluate the ability of aflatoxigenic molds to growth and aflatoxin formation on the dried fruits, the highest aflatoxin-producing strain of each of *A. flavus* and *A. parasiticus* isolated during the present study was used to inoculate dried and fresh samples of apricots, plums, raisins and figs. 50g samples of dried fruits and 100 g samples of fresh fruits of each type under investigation were placed in separate autoclaved 500 ml flasks and sealed with sterile cotton plugs. All samples

were inoculated by 1 ml of heavy spore suspension ($1x10^6$ spores) of *A. flavus* and/or *A. parasiticus*. The infected samples were incubated at 25±1°C for two weeks. Control samples of each type were not inoculated but were otherwise carried through the experiment. At the end of incubation period, extraction and estimation of aflatoxins were made as previously mentioned. All treatments were carried out in triplicate.

Results and Discussion

Fungal flora of dried fruit samples:

The mycological analysis of forty samples of dried fruits (10 samples of each of apricots, plums, raisins and figs) revealed that, the total count of the isolated fungi ranged from 19 to 162 colonies/g (Table 1). Samples of dried figs and apricots were highly contaminated with fungi in comparison with plums and raisins samples. As shown in Table (1), ten species belonging to four genera were isolated from the four types of dried fruits.

Table(1): Fungal genera and species isolated from different types of dried fruits (colonies/g).

Genera and species	Apricots		Plums		Raisins		Figs	
	No. of isolates	Occurr-ence %	No. of isolates	Occurr-ence %	No. of isolates	Occurr-ence %	No. of isolates	Occurr-ence %
Aspergillus	53.0	48.6	16.0	84.2	30.0	88.2	120.0	74.0
A. flavus	15.0	13.8	4.0	21.0	0.0	0.0	20.0	12.3
A. parasiticus	0.0	0.0	0.0	0.0	0.0	0.0	8.0	4.9
A. versicolor	2.0	1.8	0.0	0.0	0.0	0.0	6.0	3.7
A. sydowi	0.0	0.0	0.0	0.0	0.0	0.0	14.0	8.6
A. niger	36.0	33.0	12.0	63.2	30.0	88.2	72.0	44.5
Penicillium	12.0	11.0	3.0	15.8	0.0	0.0	36.0	22.2
P. oxalicum	9.0	8.2	2.0	10.5	0.0	0.0	26.0	16.0
P. chrysogenum	3.0	2.8	1.0	5.3	0.0	0.0	10.0	6.2
Alternaria	26.0	23.9	0.0	0.0	0.0	0.0	6.0	3.7
A.chlamidospora	10.0	9.2	0.0	0.0	0.0	0.0	1.0	0.6
A. alternata	16.0	14.7	0.0	0.0	0.0	0.0	5.0	3.1
Paecilomyces variotii	18.0	16.5	0.0	0.0	4.0	11.8	0.0	0.0
	109.0		19.0		34.0		162.0	

Aspergillus was the most predominant genus encountering in 48.6-88.2% of the total fungi. Five species of *Aspergillus* were identified of which, *A. niger* was the most prevalent species in all the studied types of dried fruits (33.0-88.2% of the total fungi), followed by *A. flavus* (12.3-21.0%). *A. versicolor* was isolated only from apricots and figs samples whereas, the remaining *Aspergillus* species (*A. parasiticus* and *A. sydowi*) were encountered only from figs samples. Raisins samples were contaminated only with *A. niger* which represented 100% of the recovered aspergilli. These results are in agreement with those obtained by Abdel-Hafez and Saber (1993), Abd-Alla *et al.* (1999), Ragab *et al.* (2001), Rosa *et al.* (2002) and Magnoli *et al.* (2003). They found that *Aspergillus* was the predominant genus on many types of dried fruits and the most prevalent species were *A. niger, A. flavus* and *A. fumigatus*.

Penicillium occupied the second place with regard to the total count of the isolated fungi. It recovered from apricots, plums and figs samples at levels of 11.0, 15.8 and 22.2% of the total fungi, respectively. Among the two isolated *Penicillium* species, *P. oxalcium* was the most common whereas, *P. chrysogenum* was rarely occurred (Table 1). *Penicillium* species were also isolated at various levels of occurrence from different types of dry and fresh fruits as reported by Pitt (1985); Pruski and Ben-Arie (1985); Nassar (1986); Benkhemmar *et al.* (1993); Abdel-Sater *et al.* (1996) and Ragab *et al.* (2001).

Data in Table (1) also showed that the genus *Alternaria* ranked third in the number of cases of isolation, occurring at levels of 23.9 and 3.7% of the total fungi contaminated apricots and figs fruits, respectively. This genus was represented by two species, *A. chlamidospora* and *A. alternata*, the latter proved to be more frequent.

Paecilomyces variotii was detected as a single representative of the genus *Paecilomyces* and infrequently encountered. It was occurred only in apricots and raisins at levels of 16.5 and 11.8% of the total count of fungi, respectively. Species of the genera *Alternaria* and *Paecilomyces* were also isolated, but with various frequencies and occurrences from similar foodstuffs (Logrieco *et al.*, 1990; Delgado and Goméz-Cordovés, 1998; Scott, 2001; Tournas and Stack, 2001 and Magnoli *et al.*, 2003).

In the present study, the highest fungal count and diverse was mainly observed in figs samples. This observation was in consistent with the results of other investigators. According to Pitt and Hocking (1997), *A. flavus* and *A. niger* were reported as being the most common

species in dried figs which was explained by their high sugar content, making them more susceptible than other types of fruits.

Natural occurrence of aflatoxins in dried fruits:

Table (2) shows the results of aflatoxin determinations in dried fruits analyzed. Aflatoxins were not detected in any samples of dried plums. One sample of each of apricots and raisins presented detectable aflatoxin levels with a maximum of 4.0 µg/kg. On the other hand, data in Table (2) also indicated that dried figs are a highly-risk commodity among dried fruits. 5 samples out of 10 analyzed (50%) presented aflatoxins at different levels, one sample only contained more than 100 µg/kg.

Table(2): Aflatoxin contamination levels detected in various types of dried fruits.

Total aflatoxins (µg/kg)	No. of samples out of 10 samples analyzed			
	Apricots	Plums	Raisins	Figs
ND	9.0	10.0	9.0	5.0
0.4-4.0	1.0	0.0	1.0	2.0
5.0-20.0	0.0	0.0	0.0	2.0
20.0-100	0.0	0.0	0.0	0.0
>100	0.0	0.0	0.0	1.0

ND = Not detected.

These results are in agreement with that obtained by other investigators. Herry and Lemetayer (1992) detected aflatoxin B_1 at low levels in 1 out of 52 raisins samples. Youssef *et al*. (2000) examined 100 dried raisins samples for mycotoxin contamination. Aflatoxin B_1 was detected in two of these samples at concentrations of 300 and 220 µg/kg. The highly incidence of aflatoxins in dried figs showed in the present study has been also reported by other investigators. In Turkey, Özay and Alperden (1991) analyzed 103 samples collected from various orchards and at various stages of figs processing, including samples of dried figs and figs paste produced from these dried figs. Overall, aflatoxins B_1, B_2, G_1 and G_2 were present in 29% of the examined samples at levels ranged from 0.5 to 78.3 µg/kg.

In an attempt to explain conditions leading to the occurrence

of mycotoxin in dried figs, Özay and Alperden (1991) described the traditional method applied in Turkey for the harvesting and drying of figs as follows.

The ripe figs are left on the trees until they shrivel. The figs fall to the ground and are gathered up and dried further in the sunlight on a drying device. This sun-dryign process takes almost 5 days. After drying, the sound fruits are sorted out from the damaged ones and stored in farm storehouse. Obviously, the environmental conditions associated with these procedures seem favorable for mycotoxin production in infected fruits. Anton (1988) reported that the temperature in the figs cultivation regions during the ripening, harvesting and drying of the fruits ranged from 27 to 30°C, which is close to the optimum temperature for mold growth and mycotoxin formation. Bullerman *et al*. (1984) demonstrated that if the moisture content of the stored crop exceeds 13 to 18%, mycotoxin formation can occur.

Accordingly, it could be concluded that the contamination of dried figs with aflatoxins can start with fungal contamination on the trees, increase during harvesting and sun drying, and continue to accumulate during storage as a result of poor drying and of improper conditions such as high temperature and relative humidity, as well as the rewetting of dried fruits.

Aflatoxigenic potential of dried fruits:

Data in Table (3) indicated that, no mold growth or aflatoxin production was observed in all the dried samples of apricots, plums, raisins and figs, in addition to the fresh plums fruits when infected by the isolated aflatoxigenic fungi. In contrary, samples of fresh apricots, raisins and figs inhanced mold growth and aflatoxin formation. *A. parasiticus* grown well and produced aflatoxins B_1 and G_1 at levels of 4.0-20.0, 0.4-4.0 and 4.0-20.0 μg/g of fresh apricots, raisins and figs, respectively. Meanwhile, *A. flavus* produced only aflatoxins B_1 + B_2 and the amount of toxins were lower than that produced by *A. parasiticus* when grown on fresh apricots and figs, but not on plums and raisins. Mycelial growth of the tested fungi on grapes fruits was slight and observed only on the area of the removed fruit neck. This implies that the intact outer skin may protect the fruit from sporulation and penetration of fungal spores, and thus may help to explain the lower susceptibility of plums and grapes fruits for fungal infestation and aflatoxin formation.

Table(3): Mycelial growth (M.G., visual) and aflatoxins production on infected samples of dried and fresh fruits of apricots, plums, raisins and figs.

Substrate	*Aspergillus flavus*			*Aspergillus parasiticus*		
	M.G	Aflatoxins		M.G.	Aflatoxins	
		Type	Concentra-tion (μg/g)		Type	Concentra- tion (μg/g)
Dried apricots	-	Non	ND*	-	Non	ND
Fresh apricots	++	B_1	0.4-4.0	++	B_1+G_1	4.0-20.0
Dried plums	-	Non	ND	-	Non	ND
Fresh plums	-	Non	ND	-	Non	ND
Dried raisins	-	Non	ND	-	Non	ND
Fresh grapes	+	Non	ND	+	B_1+G_1	0.4-4.0
Dried figs	-	Non	ND	-	Non	ND
Fresh figs	++	B_1+B_2	0.4-4.0	++	B_1+G_1	4.0-20.0

* ND = Not detected. (++) = Strongly growth.

(+) = Slightly growth (-) = Negatively growth.

The obtained results (Table 3) consistent with the hypothesis that contamination of the dried fruits with aflatoxin can start on the trees and continue during harvesting and sun drying.

To avoid or at least to decrease the incidence of aflatoxins in dried fruits, Splitstoesser (1987) summarized some important control practices at harvesting, grading, and packing stages as follows: (1) harvest fruit when at optimum maturity, (2) handle the fruit gently to prevent bruises and punctures that would permit the entry of saprophytic fungi, (3) maintain good sanitation to minimize the buildup of mold on fruit-contact surfaces. Mechanical harvesters, lug boxes, and packinghouse equipment should be cleaned and sanitized regularly. Live steam, formaldehyde, and fumigation with chlorine gas were some of the treatments used to destroy fungal spores. (4) moldy fruits and those showing skin breaks and bruises should be culled out during the sorting and grading operations.

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تواجد الأفلاتوكسينات والفطريات المفرزة لها فى بعض أنواع الفواكه المجففة الشائعة فى مصر

وفيق سند رجب

قسم علوم وتكنولوجيا الأغذية ، كلية الزراعة ، جامعة أسيوط ، أسيوط ، ج.م.ع.

استهدف البحث دراسة مدى تلوث بعض أنواع الفواكه المجففة الشائعة فى مصر بالأفلاتوكسينات والفطريات المفرزة لها . استخدمت في البحث عدد أربعون عينة من الفواكه المجففة تم شراؤها من المحلات الكبيرة بمدينة أسيوط بواقع عشر عينات من كل من المشمش المجفف ، البرقوق المجفف (القراصيا) والزبيب والتين المجفف . وقد أظهرت النتائج وجود تفاوتا واضحا فى أعداد وأنواع الفطريات الملوثة لأنواع الفواكه المختلفة ، حيث تم عزل وتعريف عشرة أنواع فطرية تتبع أربعة أجناس وذلك على بيئة تشابك آجار ٢٠% سكروز . كانت عينات التين والمشمش أكثر تلوثا بالفطريات مقارنة بعينات القراصيا والزبيب وكانت الأجناس الفطرية الأكثر تواجداً والأنواع التابعة لها هى :

Aspergillus (A. niger, A. flavus, A. sydowi, A. parasiticus and *A. versicolor); Penicillium (P. oxalicum* and *P. chrysogenum*).

أظهرت نتائج تحليل الأفلاتوكسينات للفواكه المجففة المختبرة بواسطة كروماتوجرافيا الطبقة الرقيقة وجود تلوث فى عينة واحدة من عشر عينات لكل من المشمش والبرقوق بتركيز يتراوح بين ٠,٤ – ٤ ميكروجرام / كجم وكذلك فى خمس عينات من العينات العشر للتين المجفف بتركيز يتراوح بين ٠,٤ ميكروجرام إلى أكثر من ١٠٠ ميكروجرام / كجم . كذلك تناول البحث دراسة مدى ملائمة الثمار المجففة لإنبات جراثيم الفطريات المعزولة وإنتاج سموم الأفلاتوكسينات وذلك عن طريق تلقيح أسطح عينات الثمار الجافة وكذلك أسطح عينات ثمار طازجة لنفس الأنواع (كمقارنة) بجراثيم نوعين من الفطريات المعزولة من نفس الثمار والتى أثبتت الدراسة قدرتهما العالية على إنتاج الأفلاتوكسينات وهما *Aspergillus flavus* and *A. parasiticus* .

وأظهرت النتائج أن ثمار الفواكه المجففة لا تلائم ولا تفى بالاحتياجات اللازمة لإنبات جراثيم الفطر وبالتالى عدم إنتاج الأفلاتوكسين على عكس الثمار الطازجة المستخدمة للمقارنة والتى أظهرت تحفيزاً بدرجات متفاوتة لنمو جراثيم الفطر وإنتاج الأفلاتوكسينات ، وكانت مستويات السموم المفرزة تختلف باختلاف نوع الفطر المستخدم .

تشير نتائج هذه الدراسة إلى ضرورة توجيه عناية خاصة للتقليل قدر الإمكان من تلوث الثمار الطازجة بالفطريات خلال مراحل نضجها وحصادها وإتباع تقنيات حديثة للإسراع من عملية تجفيفها وتقليل الفترة المنقضية بين نضج الثمار وإتمام تجفيفها إلى حدود الرطوبة الآمنة .