Military Medical Science Letters
Since 1925

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2022, 91(3), 224-234
ISSN 0372-7025 (Print)
ISSN 2571-113X (Online)
DOI: 10.31482/mmsl.2021.049

ORIGINAL ARTICLE

AFLATOXIGENIC FUNGI IN NUTS AND DRIED FRUITS IN MOSUL AND DUHOK CITY

Eman A. Ramadan [1]✉, Nadeem A. Ramadan [2], Amjad Abdul Hadi Mohammed [1]

[1] College of Science, University of Mosul, Mosul, Iraq
[2] Hadbaa University College, Mosul, Iraq

Received 17th October 2021.
Accepted 21st December 2021.
Published 2nd September 2022.

Summary

Introduction: The present study aims to determine the contamination of eighteen samples from different nuts and dried fruits from the markets of Mosul and Duhok city.

Materials and Methods: This study included samples of local and imported nuts and dried fruits.

Results: The study showed different genera of contaminant fungi such as *Alternaria*, *Penicillium*, and *Aspergillus* species which are very common fungi associated with samples. The most fungal contamination was found in pistachio and black raisins, followed by balahsisi, taffy raisins, yellow raisins, dried apricot and peanut with shell. A total of nine species of *Aspergillus* were isolated on (potato dextrose agar, malt extract agar and dichloran rose bengal agar) media *A. niger*, *A. flavus*, *A. fumigatus*, *A. tamarii*, *A. aculeatus*, *A. parasiticus*, *A. oryzae*, *A. japonicus*, *A. ochraceus* were detected from all types of nuts and dried fruits samples. The most frequent species were *A. niger* followed by *A. flavus* and *A. fumigates* with percentage frequencies of 45.2%, 20.3% and 13.9% respectively. The ability of toxicogenic fungi to produce AflatoxinB1 by ELISA test of Sunlong corporation in nuts and dried fruits samples ranged from 94.8 to 136.4 µg/kg, with the highest levels of ability to produce AflatoxinB1 in nuts samples (peanut with shell, sunflower seeds and walnut) at levels 135.8, 129.0, and 128.9 µg/kg respectively, while in dried fruits samples the highest ability to produce AflatoxinB1in dried apricot, taffy raisins and yellow raisins was at levels136.4,123.1 and 118.0 µg/kg, respectively.

Conclusion: we conducted the fungal contamination and quantitative content of aflatoxin B1 in nuts and dried fruits notably those sold openly in food shops.

Key words: aflatoxigenic fungi; Nuts; Dried fruits

Introduction

Fungi are an exceptional group of organisms, different from all others in their behaviour and cellular organization. The exceptionality of fungi is reflected in the fact that they have the status of a kingdom, like a plant and animal kingdom. Hence, fungi represent one of the three major evolutionary branches of multicellular organisms (1).

✉ University of Mosul, College of Science, Department of Biology, Mosul, Iraq
eman9028@gmail.com
☎ +9647512035146

Fungi can grow on both simple and complex food products and produce different metabolites. These microorganisms are found in the environment and can spread by the wind, insects, and rain (2). More than 100000 fungal species have been identified as natural contaminants of agricultural and food products (3). The common poisonous species are found in the genera *Aspergillus*, *Penicillium*, and *Fusarium* (4). Nuts and dried fruits are measured as the best source of "essential nutrients" as they provide "protein, fatty acids, potassium, dietary fibres, and bioactive compounds". They also promote human health by lowering the risk of "obesity, cardiovascular disease, and diabetes" (5).

Nuts and dried fruits have a longer shelf life than "fresh fruits"; they are the best alternative to fresh fruits for long-term storage (6). The production of toxic secondary metabolites is the main problem associated with fungal attacks in nuts and dried fruits, particularly aflatoxin. Fumonisin and Zearalenone are produced by *A. flavus*, *F. verticillioides*, and *F. graminearum* respectively (7). They had been contaminated through or after harvesting, storage and transition (8). "The International Agency for Research on Cancer (IARC) classifies aflatoxins (AFs) as Group I carcinogens" produced by *Aspergillus flavus* and *A. parasiticus*, Aflatoxin is the most commonly known mycotoxin associated with storage development, however, a variety of other mycotoxins can also cause problems (9). the storage temperature, presence of oxygen, humidity, and gaseous composition is the major important factors influencing the development of fungi during storage (10).

Unfortunately, microorganisms can contaminate and spoiled valuable foodstuffs. As reported by "USDA-Economic Research Service, 18.9 billion pounds of fruits and vegetables are lost annually due to spoilage by fungi" (11), such as *Alternaria*, *Aspergillus*, *Candida*, *Fusarium*, *Mucor*, *Rhizopus* and *Penicilliumetc*. Ingestion of food-contaminated with fungi may contribute to the formation of many health-related concerns, such as mycoses, which can vary from moderate to life-threatening clinical diseases, notably in immunocompromised people (12). Among "1.5 million species of fungi" that may cause disease in humans and animals, roughly 300 are fungal. These illnesses can vary from minor allergic responses to life-threatening invasive infections (13). With the dangers of fungal development in products in mind, notably those sold openly in food shops, this study was conducted to assess the fungal content in nuts and dried fruits distributed by local suppliers in various regions of Mosul and Duhok city.

Materials and methods

Sampling: A total sample of nuts and dried fruits (150g) were collected randomly in clean paper bags from local markets in Mosul and Duhok city, from 28/1/2021 to 21/3/2021 (As shown in table 1). The bags were properly labelled, sealed and kept in the laboratory for isolation of fungi in the Ararat Private Technical Institute / Duhok city.

Table 1. Source and amount of samples of nuts and dried fruits.

Samples	Source	Amount
Cashew	Mosul/ imported	150g
Hazelnut	Mosul/ imported	150g
walnut	Duhok/ local	150g
Almond	Duhok/ local	150g
Pistachio	Mosul/ imported	150g
Peanut	Mosul/ imported	150g
Peanut with shell	Mosul/ imported	150g
Sunflower seeds	Mosul/ imported	150g
Pumpkin seeds	Mosul/ imported	150g
Melon seeds	Mosul/ imported	150g
Chickpeas nuts	Mosul/ imported	150g
Balahsisi	Mosul/ imported	150g
Yellow raisins	Duhok/ local	150g
Black raisins	Duhok/ local	150g
Taffy raisins	Duhok/ local	150g
Fig	Duhok/ local	150g
Dried apricot	Mosul/ imported	150g
Dried apples	Duhok/ local	150g

Culture Media

Preparation and ingredients for all below media were according to Pitt and Hocking, 1997 (14):

Potato Dextrose Agar PDA Medium

This medium was prepared as following steps:

First of all, 39 g of PDA powder was added to 1 litre of distilled water at pH 5.6. Then, it was sterilized by using autoclave at 121 °C, 15 psi for 20 minutes. After finishing the sterilization process, an antibiotic (Chloramphenicol at a concentration of 50 mg/litre) was added to the liquid of PDA at 45 °C. The steps mentioned above were followed as per manufacturer instructions. This medium was used to process the isolation and diagnose of fungi.

Malt Extract Agar MEA Medium

This medium was prepared as following steps:

To begin with, 50 g of medium powder was added to 1 litre of distilled water at pH 5.6. After that, it was sterilized by using an autoclave at 121 °C, 15 psi for 20 minutes. After finishing the sterilization process, an antibiotic (chloramphenicol a concentration of 50 mg/l) was added to a liquid medium at 45 °C. The steps mentioned above were followed as per manufacturer instructions. This medium was also used to process isolation and diagnose fungi.

Dichloran Rose Bengal Agar DRBC Medium

This medium was prepared as following steps:

To begin with, 15.75 g of medium powder was added to 500 ml distilled water at pH 5.6. After that, it was sterilized by using autoclave at 121 °C, 15 psi for 20 minutes. After finishing the sterilization process, an antibiotic (chloramphenicol at a concentration of 50 mg/l) was added to a liquid medium at 45 °C. The steps mentioned above were followed as per manufacturer instructions. This medium is for selective isolation and enumeration of fungi.

Isolation of fungi from samples:

Isolation of fungi associated with nuts and dried fruits samples according to previously described method (15) on PDA, MEA and DRBC media, used for isolation and enumeration of fungi from nuts and dried fruits (16). The media were supplemented with chloramphenicol (50 mg/L), Thirty specimens were taken randomly from each sample, were treated with 2 % sodium hypochlorite for 2 min, then rinsed in sterile water and dried on filter papers. Next, raisins were aseptically placed on plates (10 per plate), all plates were incubated in darkness for 7 days at 25 °C (17).

Identification of isolated fungi:

The isolated fungal genera were identified by macroscopic based on morphological and cultural characteristics (Color, texture, pigmentation and reverse of the colony) and microscopic characteristics and microscopic Characteristics, the wet mount technique was used to examine fungal strains under the microscope. In this technique, a drop of "lactophenol cotton blue" (LPCB) was placed on a "clean grease-free slide", then with the help of a sterile fungus needle, a tiny piece of fungal growth was transferred into the LPCB drop on the slide. A coverslip was placed and finally examined under the microscope at 10x and 40x magnification (18). Developing fungi were purified and maintained in a slanted PDA medium.

Frequency Proportion %:

The isolation frequency of genera from samples was calculated by the following formula.

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

Species identifications were according to the keys and descriptions provided by Pitt and Hocking (19, 20).

Detection of aflatoxin B1:

An enzyme-linked immunosorbent assay (ELISA) test was used to determine the quantitative level of AFB1. The aflatoxin B1 assay was carried out according to the manufacturer's instructions (Sunlong corporation kit).

The amount of AFB1 generated by isolates was determined using a standard curve constructed from aflatoxin B1 standards and expressed in parts per billion (21).

Results and discussion

Different genera of contaminant fungi were detected in our samples (see Tables 2, 3, 4), these mainly include *Aspergillus*, *Alternaria* and *Penicillium* species. The *Aspergillus* species are very common fungi associated with nuts and dried fruits. About nine *Aspergillus* species have been isolated and identified such as *A. niger*, *A. flavus*, *A. fumigatus*, *A. tamarii*, *A. aculeatus*, *A. parasiticus*, *A. oryzae*, *A. japonicus*, *A. ochraceus* as shown in (Figure1).

The highest percentage of *Aspergillus* appeared in pistachio and black raisins, followed by balahsisi, taffy raisins, yellow raisins, dried apricot and peanut with shell.

The most predominant of the isolated species were members of *Aspergillus* section *Nigri*, followed by *Aspergillus* section *Flavi*. This is consistent with the findings of a study conducted by Ozer *et al*. (22) and Heperkan *et al*. (23). In the study of the four types of dried fruits, nine *Aspergillus* species were found to be frequent. These include *A. niger*, *A. flavus*, *A. fumigatus*, *A. awamori*, *A. carbonarius*, *A. japonicas*, *A. ochraceus*, *A. parasiticus* and *A. tubingensis*. These results are similar to the findings of a study published by Khosravi *et al*. (24). The most frequent isolated fungi from different nuts were *Aspergillus* and *Penicillium*.

Among all isolated fungal strains from eighty-four samples of dried fruits and nuts, Abbas *et al*. (25) has confirmed that *Aspergillus niger* was isolated and reported as the most predominant species followed by *Aspergillus flavus*. Many fungal contaminants that belong to different genera were obtained in this survey with the predominance of *A. niger*. The presence of such fungi could be attributed to the high nutrition value of nuts and dried fruits as well as inappropriate marketing and storage conditions (26).

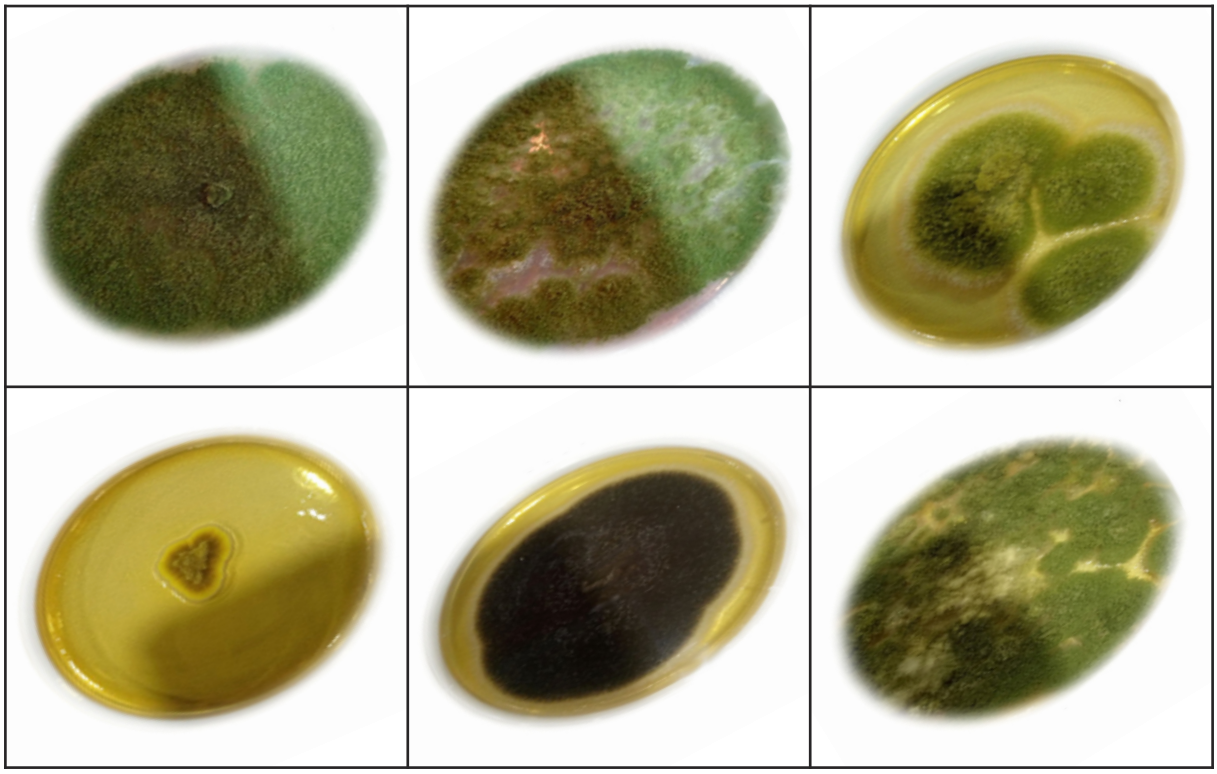

Figure 1. Some fungal isolation from nuts and dried fruits samples.

Table 2. Total counts of isolated fungal from samples of nuts and dried fruits on PDA medium.

No. of Frequency																		
Samples / Fungi	Cashew	Hazelnut	Walnut	Almond	Pistachio	peanut	Peanut with shell	Sunflower seeds	Pumpkin seeds	Melon seeds	Chickpeas nuts	Balahasisi	Yellow raisins	Black raisins	Taffy raisins	Fig	Dried apricot	Dried apples
Aspergillus niger	5		4	4	17		4			9		35	30	34	32	3	17	17
A. flavus	8	2	3	4	12	3	15	8	5	5	2	2	3	2	2	5	9	5
A. fumigatus	3	7	13	13	16		3							2		8		
A. tamarii	3				8	4			3		2					2		
A. aculeatus			8											4				
A. parasiticus	4			4						3								
A. oryzae							3			3								3
A. japonicus		4														2		
A. ochraceus						2											2	
Alternaria sp.			3					12										
Rhizopus sp.				3				3	7									
Penicillium							3											

Table 3. Total counts of isolated fungal from samples of nuts and dried fruits on MEA medium.

No. of Frequency																		
Samples / Fungi	Cashew	Hazelnut	Walnut	Almond	Pistachio	peanut	Peanut with shell	Sunflower seeds	Pumpkin seeds	Melon seeds	Chickpeas nuts	Balahasisi	Yellow raisins	Black raisins	Taffy raisins	Fig	Dried apricot	Dried apples
Aspergillus niger	3		8	6	25		4			10		25	10	38	37		19	25
A. flavus	10	3	4	6	18	3	6	10	6	7	4	2	4	3	2	8	12	3
A. fumigatus	8	3	23	17	20		3					1		2		8		
A. tamarii	2				10	2			7		3					5		
A. aculeatus			6											4				
A. parasiticus	2			3						3								
A. oryzae							2			2								6
A. japonicus		4														4		
A. ochraceus						2											4	
Alternaria sp.								8										
Rhizopus sp.					5			3	5	3								
Penicillium							2											

The frequency occurrence of fungal species from nuts and dried fruits samples:

The highest frequently occurrence of isolated genera of *Aspergillus* in tables (5, 6, 7) was *Aspergillus niger* followed by *A. flavus* and then *A. fumigatus* with percentage frequencies of 45.2 %, 20.3 %, and 13.9 % respectively (table 5). While the lowest percentage of occurrence was found in genera of *Alternaria* followed by *Rhizopus* and then *Penicillium* with percentage frequencies of 3.2 %, 2.7 %, and 0.6 % respectively (table 5).

Table 4. Total counts of isolated fungal from samples of nuts and dried fruits on DRBC medium.

No. of Frequency																		
Samples / Fungi	Cashew	Hazelnut	Walnut	Almond	Pistachio	peanut	Peanut with shell	Sunflower seeds	Pumpkin seeds	Melon seeds	Chickpeas nuts	Balahasisi	Yellow raisins	Black raisins	Taffy raisins	Fig	Dried apricot	Dried apples
Aspergillus niger	2		2	3	9		2			7		20	24	23	26	2	13	9
A. flavus	3	1	1	1	5	1	6	5	3	4	2	2	1	5	2	5	4	3
A. fumigatus	1	3	7	8	9		1							3		5		
A. tamarii	1				5	3			2		1					2		
A. aculeatus			4											4				
A. parasiticus	2			3						3								
A. oryzae							1			2								2
A. japonicus		2														1		
A. ochraceus						2											2	
Alternaria sp.			1					7										
Rhizopus sp.				1				2	4									
Penicillium							1											

Aspergillus flavus and *Aspergillus* niger were reported as being the most common species on dried fruits (27), in another study, which included 60 samples of dried fruits such as raisins, fig and dates, showed the genus *Aspergillus* as the higher rate of frequency than other genera (28), also Sultan and Magan (29) showed that *Aspergillus* was consistently the most frequent genus in seed and in-shell peanuts similarly (30) on mycobiota of almonds and chestnuts make it clear that *Aspergillus* species are frequent, sections *flavi* and *niger* being the predominant aspergilla.

In Argentina, by (31) six species of *Aspergillus* section *Nigri* were found in dried vine fruits samples, while the chief species were *A. niger*, *A. awamori*, and *A. carbonarius*. In general *Aspergillus niger*, *A. flavus* and *A. fumigatus* showed the highest percentage frequency of occurrence on the tested 18 types of "nuts and dried fruits" samples.

Table 5. Frequency of isolated fungi on PDA medium.

Fungi	Total Frequency	% Frequency
Aspergillus niger	211	45.2
A. flavus	95	20.3
A.fumigatus	65	13.9
A. tamarii	22	4.7
A. aculeatus	12	2.5
A. parasiticus	11	2.3
A. oryzae	9	1.9
A. japonicus	6	1.2
A. ochraceus	4	0.8
Alternaria sp.	15	3.2
Rhizopus sp.	13	2.7
Penicillium	3	0.6
Total	466	-

Table 6. Frequency of Isolated fungi on MEA medium.

Fungi	Total Frequency	% Frequency
Aspergillus niger	210	41.7
A. flavus	111	22.0
A. fumigatus	85	16.8
A. tamarii	29	5.7
A. aculeatus	10	1.9
A. parasiticus	8	1.5
A. oryzae	10	1.9
A. japonicus	8	1.5
A. ochraceus	6	1.1
Alternaria sp.	8	1.5
Rhizopus sp.	16	3.1
Penicillium	2	0.3
Total	503	-

Table 7. Frequency of Isolated fungi on DRBC medium.

Fungi	Total Frequency	% Frequency
Aspergillus niger	142	48.7
A. flavus	54	18.5
A. fumigatus	37	12.7
A. tamarii	14	4.8
A. aculeatus	8	2.7
A. parasiticus	8	2.7
A. oryzae	5	1.7
A. japonicus	3	1.0
A. ochraceus	4	1.3
Alternaria sp.	8	2.7
Rhizopus sp.	7	2.4
Penicillium	1	0.3
Total	291	-

Detection of AFB1 naturally contaminated nuts and dried fruits by ELISA:

Aflatoxigenic potentiate of samples were detected by Enzyme-linked immunosorbent assay (ELISA) technique as in the table 8, showed that AFB1 potentiate was found at levels from 94.8 µg/kg - 136.4 µg/kg for nuts and dried fruits samples as shown in Figure 2. Peanut with shell, Sunflower seeds and Walnut showed the highest abilities to produce AFB1 at levels 135.8, 129.0 and 128.9 µg/kg respectively in nuts samples, while in dried fruits samples the highest ability to produce AFB1in dried apricot, taffy raisins and yellow raisins was at levels of 136.4, 123.1 and 118.0 µg/kg respectively.

This is due to nuts and dried fruits containing rich nutrients and is thus highly vulnerable to contamination with toxigenic fungi and aflatoxins because of poor weather, processing and storage conditions (32). This is has been linked to climate changes in Iraq, including high humidity and low temperatures, particularly in the winter, which increase toxigenic fungal infection. The lowest abilities to produce AFB1 in dried apples, figs and almonds showed levels of 94.8, 99.1 and 99.4 µg/kg respectively in nuts and dried fruits samples. In a study conducted in Pakistan (33),

total aflatoxins were measured in 180 dried fruits and nuts samples. The highest aflatoxins contamination levels were found in peanut samples 14.5 g/kg and pistachio samples 14 g/kg, while the lowest contamination levels were established in dried apricot, dried fig, raisins, walnuts with shell, walnuts without shell, and dried apricot, dried fig, raisins, walnuts with shell, walnuts without shell, all of which were the recommended limit for the full amount of aflatoxins 4 g/kg set by EU regulations.

A study by Asia and Samir (34) showed apricot and fig as the highest abilities to produce aflatoxin. Similarly, another study (35) found the highest concentration of AFB1 in apricot and fig (138.28 µg/kg, 46.18 µg/kg) respectively. Another study showed 18 strains of *A. flavus* obtained from sunflower seeds of positive aflatoxigenic activity (36). In a recent study in Iraq, Alhamadani (37) had reported the results of ELISA examination for 60 samples chosen from grains and nuts which showed 49 samples were contaminated with aflatoxin in a range (0.5 µg/kg - 41.2 µg/kg). According to some studies, not all *A. flavus* strains can produce aflatoxins and the ratio of non-aflatoxigenic strains to aflatoxin produce different strains according to the source and location of the isolates (38).

Table 8. Aflatoxin B1 potentiate in nuts and dried fruits samples of Mosul and Duhok city.

Samples	Aflatoxin B1(µg/kg)
Dried apricot	136.4
Peanut with shell	135.8
Sunflower seeds	129.0
Walnut	128.9
Taffy raisins	123.1
Yellow raisins	118.0
Black raisins	114.8
Balahsisi	111.6
Chickpeas nuts	109.5
Cashew	108.9
Melon seeds	107.6
Peanut	105.7
Pistachio	105.4
Hazelnut	105.2
Pumpkin seeds	100.1
Almond	99.4
Fig	99.1
Dried apples	94.8

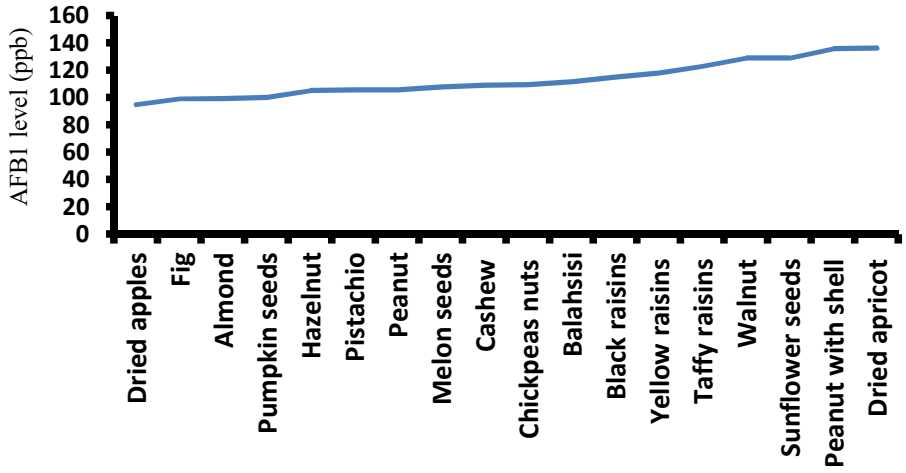

Figure 2. The concentration of AFB1in nuts and dried fruits samples was collected from Mosul and Duhok city.

The limitation of the study

In order to estimate fungal contamination as well as determine the level of aflatoxin in samples, a survey research was done for nuts and dried fruits available in local markets and widely traded in these areas. We do suggest to conduct a study on how to control the amount of fungal contamination, and thus the amount of mycotoxins.

Conclusion

Contamination with fungal and aflatoxin B1 in various nuts and dried fruits are a major problem and is mainly related to an improper condition of storage after harvest. Good post-harvest manufacturing practice such as drying, cleaning and packing reduces fungal infection and prevents the formation of mycotoxins to ensure that the nuts and the dried fruits are safe for consumers.

Acknowledgments

The authors would like to thanks Ararat Private Technical Institute in Duhok and College of Science/University of Mosul for their kindly assistance in carrying out this research.

Adherence to ethical standards

Not applicable. This article does not contain any studies involving animals performed by any of the authors. This article does not contain any studies involving human participants performed by any of the authors.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

References

1. Hawksworth D.L. The magnitude of fungal diversity: The 1.5 million species estimate revisited. Myco. Res. 2001;105(12):1422-1432. Doi:10.1017/S0953756201004725.
2. Horn BW. Colonization of wounded peanut seeds by soil fungi: selectivity for species from Aspergillus section Flavi. Mycologia. 2005 Mar 1;97(1):202-17. DOI: 10.1080/15572536.2006.11832854.
3. Kacaniova M. Feeding soybean colonization by microscopic fungi. Trakya Univ. J. Sci. 2003 Dec;4(2):165-8.
4. Khosravi AR, Shokri H, Ziglari T. Evaluation of fungal flora in some important nut products (pistachio, peanut, hazelnut and almond) in Tehran, Iran. Pakistan Journal of Nutrition. 2007;6(5):460-2. DOI:10.1.1.576.9456.
5. Carughi A, Feeney MJ, Kris-Etherton P, et al. Pairing nuts and dried fruit for cardiometabolic health. Nutrition journal. 2015;15(1):1-3. DOI: 10.1186/s12937-016-0142-4.
6. Masood M, Iqbal SZ, Asi MR, et al. Natural occurrence of aflatoxins in dry fruits and edible nuts. Food Control. 2015;55:62-5. DOI:10.1016/j.foodcont.2015.02.041.
7. Scott PM. Fumonisins. International Journal of Food Microbiology. 1993 Jun 1;18(4):257-70. DOI:10.1016/0168-1605(93)90149-B
8. Campbell BC, Molyneux RJ, Schatzki TF. Current research on reducing pre-and post-harvest aflatoxin contamination of US almond, pistachio, and walnut. Journal of Toxicology: Toxin Reviews. 2003 Jan 1;22(2-3):225-66. DOI:10.1081/TXR-120024093.
9. Bibani NM, Khidhir ZK, Shaker AS, et al. Analyses of mycotoxins in broiler's local and imported feeds. Iraqi Journal of Veterinary Sciences. 2019 Sep 1;33(2):267-71. DOI: 10.33899/ijvs.2019.162994
10. Kubatova A. New species of toxinogenic Penicillia found in foods and their identification. In[Current Problems of Food Microbiology-II], Liblice-Bysice (Czech Republic), 23-25 May 2000 2000. Dum Vedeckych Pracovniku, Akademie Ved.
11. Barth M, Hankinson TR, Zhuang H, et al. Microbiological spoilage of fruits and vegetables. InCompendium of the microbiological spoilage of foods and beverages 2009 (pp. 135-183). Springer, New York, NY. DOI: 10.1007/978-1-4419-0826-1_6

12. Tournas VH, Niazi NS, Kohn JS. Fungal presence in selected tree nuts and dried fruits. Microbiology insights. 2015;8:MBI-S24308. DOI: 10.4137/mbi.s24308.
13. Benedict K, Chiller TM, Mody RK. Invasive fungal infections acquired from contaminated food or nutritional supplements: a review of the literature. Foodborne pathogens and disease. 2016;13(7):343-9. DOI: 10.1089/fpd.2015.2108.
14. Pitt JI, and A D Hocking. "Fungi and food spoilage. Blackie Academic & Professional." New South Wales, Australia (1997).
15. Ichinoe M, Kurata H, Sugiura Y, et al. Chemotaxonomy of Gibberella zeae with special reference to production of trichothecenes and zearalenone. Applied and environmental microbiology. 1983 Dec;46(6):1364-9. DOI:10.1128/aem.46.6.1364-1369.1983.
16. Hocking AD, Pitt JI. Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. Applied and Environmental Microbiology. 1980 Mar;39(3):488-92. DOI: 10.1128/aem.39.3.488-492.1980.
17. Ronald MA, Alfard EB, and Lawrence CP. Laboratory manual of experimental microbiology. Mosby-Year Book.
18. Pitt JI, Hocking AD. Fungi and food spoilage Blackie Academic. London OpenURL. 1997.
19. Guchi E. Effect of storage time on occurrence of Aspergillus species in groundnut (Arachis hypogaea L.) in eastern Ethiopia. J Appl Environ Microbiol [Internet]. 2015;3(1):1-5. DOI: 10.12691/jaem-3-1-1.
20. Samson RA, Noonim P, Meijer M, et al. Diagnostic tools to identify black aspergilli. Studies in mycology. 2007;59:129-45. DOI: 10.3114/sim.2007.59.13.
21. Criseo G, Bagnara A, Bisignano G. Differentiation of aflatoxin-producing and non-producing strains of Aspergillus flavus group. Letters in Applied Microbiology. 2001 Oct 17;33(4):291-5. DOI:10.1046/j.1472-765X.2001.00998.x.
22. Ozer H, OKTAY BASEGMEZ HI, Ozay G. Mycotoxin risks and toxigenic fungi in date, prune and dried apricot among Mediterranean crops. Phytopathologia Mediterranea. 2012 Apr 1:148-57. DOI: 10.14601/Phytopathol_Mediterr-9806.
23. Heperkan D, Moretti A, Dikmen CD, et al. Toxigenic fungi and mycotoxin associated with figs in the Mediterranean area. Phytopathologia Mediterranea. 2012 Apr 1:119-30. DOI: 10.14601/Phytopathol_Mediterr-9467.
24. Khosravi AR, Shokri H, Ziglari T. Evaluation of fungal flora in some important nut products (pistachio, peanut, hazelnut and almond) in Tehran, Iran. Pakistan Journal of Nutrition. 2007;6(5):460-2. DOI: 10.3923/pjn.2007.460.462.
25. Abbas MU, Naz SA, Shafique M, et al. Fungal contamination in dried fruits and nuts: A possible source of mycosis and mycotoxicosis. Pak. J. Bot. 2019 Aug 1;51(4):1523-9. DOI: 10.30848/PJB2019-4(31).
26. Wu Q, Xie L & Xu H. "Determination of toxigenic fungi and aflatoxins in nuts and dried fruits using imaging and spectroscopic techniques." Food chemistry 252 (2018): 228-242. DOI.org/10.1016/j.foodchem.2018.01.076
27. Pitt JI, Hocking AD. Fungi and food spoilage. New York: Springer; 2009 Jul 25. DOI: org/10.1007/978-0-387-92207-2.
28. Alghalibi SM, Shater AR. Mycoflora and mycotoxin contamination of some dried fruits in Yemen Republic. Ass Univ Bull Environ Res. 2004 Oct;7(2):19-25. DOI: 10.21608/AUBER.2004.150709.
29. Sultan Y, Magan N. Mycotoxigenic fungi in peanuts from different geographic regions of Egypt. Mycotoxin Research. 2010 May;26(2):133-40. DOI: 10.1007/s12550-010-0048-5.
30. Rodrigues P, Venâncio A, Lima N. Mycobiota and mycotoxins of almonds and chestnuts with special reference to aflatoxins. Food Research International. 2012; 48(1):76-90. DOI:10.1016/j.foodres.2012.02.007.
31. Magnoli C, Violante M, Combina M, et al. Mycoflora and ochratoxin-producing strains of Aspergillus section Nigri in wine grapes in Argentina. Letters in Applied Microbiology. 2003 Aug;37(2):179-84. DOI:10.1046/j.1472-765X.2003.01376.x.
32. Gürses M. Mycoflora and aflatoxin content of hazelnuts, walnuts, peanuts, almonds and roasted chickpeas (LEBLEBI) sold in Turkey. International Journal of Food Properties. 2006 Sep 1;9(3):395-9. DOI: 10.1080/10942910600596597.
33. Luttfullah G, Hussain A. Studies on contamination level of aflatoxins in some dried fruits and nuts of Pakistan. Food Control. 2011 Mar 1;22(3-4):426-9. DOI: 10.1016/j.foodcont.2010.09.015.
34. A Saadullah A, K Abdullah S. Detection of Aspergillus species in dried fruits collected from Duhok market and study their aflatoxiginic properties. Rafidain journal of science. 2014 Feb 28;25(1):12-8. DOI: 10.33899/rjs.2014.86052.

35. Al-Haddad AA, HZ Hussain and MA Fayyadh."Evaluation of some physical means efficiency in degrading Aflatoxin B1 in some dry fruits." Arab J.Plant Prot. 34.2 (2016):148-155. DOI: 10.22268/ajpp-034.2.148155.
36. Abdullah SK, Al-Mosawi KA. Incidence of Aspergillus species in seeds of corn and sunflower cultivars grown in Iraq and aflatoxin-producing potential of Aspergillus section Flavi. InProc. 1st Sci. Conf. Biol. Sci. Sect. Bot. Mosul Univ. Iraq 2009 Apr (pp. 299-307).
37. Alhamadani AH. Qualitative and quantitative detection of aflatoxins produced by Aspergillus flavus isolated from clinical and food resources. Al-Qadisiyah Journal Of Pure Science. 2017;22(3):465-85.
38. Abdullah SK, Miryani MS, Al-Saadoon AH. Mycobiota and incidence of aflatoxigenic Aspergillus section Flavi in three medicinal plants in Iraq. J. Duhok Univ. 2009;12(1):262-7.