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Isolation and Identification of fungal species from dried fruits in Sulaimani City, Kurdistan Region-Iraq.

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ABSTRACT

Dry fruits are the best source of minerals and vitamins and are essential for human health; they are exposed to microbial contamination at any stage of growth and processing. The current study aims to investigate and identify fungi associated with dried fruit available from local markets of Sulaimani City and also detect the aflatoxigenic strain of *Aspergillus* by various methods. Forty-eight samples of dried fruits were collected; the fungi were isolated by using the dilution plate method on dicloran rose bangle chloramphenicol agar medium. The isolated fungal species identified depend on morphological characters (macroscopic and microscopic features). Cultural (ammonium vapor and UV fluorescence); and molecular methods were used to detect the potential of aflatoxigenicity of *Aspergillus* spp. A total of (353 * 10^2) CFU/gm of dried fruit of fungal species were isolated, *Aspergillus niger* and *Penicillium* spp. are the predominant species isolated, and *Aspergillus parasiticus* was isolated on dry fig only; All of *A. parasiticus* isolates were toxigenic strains as indicated by cultural and molecular methods. In addition, some species of *Penicillium* and *Aspergillus niger* also produced ochratoxin. Therefore, the implementation of hygienic conditions is necessary during the process of harvesting, desiccating, and handling dry fruits.

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Keywords: Dried Fruits, Aspergillus Species, Toxigenic Aspergillus, Aflatoxin Detection Methods.

1. Introduction

Fruits contain significant substances beneficial for human well-being, like vitamins, minerals, fiber, and antioxidants[1, 2]. Fresh fruit preservation through drying provides an alternative method for maintaining the fruits for long periods and concentrating their nutritional value, reducing the cost of packaging, handling, and transportation[1, 2, 3]. Traditional dried fruit (i.e., without added sugar) found in the Mediterranean and other regional diets are important dietary groups[2]. Dried fruits are essential for human health, as they reduce the possibility of obesity, cardiovascular disease, and the chance of diabetes[4].

Dried fruits are exposed to microbial contamination at any stage of growth and processing. When the environmental condition is favorable, some of these organisms might start to grow before the fruits or nuts get to the processing facility; they might keep growing there until the products are dried, at which point workers and equipment might contaminate the final products[5], Mold has the potential to contaminate dry fruits and spread quickly as well as even when a small area of fruit peel is contaminated[6]. Microorganisms can cause food spoilage to a valuable food staff[3]; fungi are the most common organisms that spoil foods worldwide[3, 7]. The USDA-Economic Research Service reported that "18.9 billion pounds of fruits and vegetables are lost annually due to spoilage by fungi"[8]. The existence of some fungi in food, *Alternaria, Aspergillus, Candida, Fusarium, Mucor, Rhizopus, Penicillium,* etc. cause food deterioration, as well as the consumption of this food might lead to the emergence of a variety of health problems, and fungal diseases, ranging from moderate to severe clinical situations, especially in individuals with weakened immune systems[9]**.** Food contamination by fungi can cause a noticeable decline in the quality and even destruction of food. Deterioration of food and mycotoxin formation relies on several factors, such as the type of food and its constitution and handling and storage conditions[10]. The formation of mycotoxin by some species of fungi is compelling and more subtle to control their growth[11]. Mycotoxin is a toxic secondary metabolite produced naturally by some mold[12], having detrimental consequences that might be sudden or persisting, such as those that are oestrogenic, carcinogenic, mutagenic, teratogenic, and atherogenic in both people and animals[13]. Few molds produce mycotoxins, including *Aspergillus, Fusarium, Penicillium*, and *Alternaria*; however, chief mycotoxin-producer fungi belonging to *Aspergillus* spp. (produces aflatoxin), *Penicillium* spp. (makes ochratoxin) and *Fusarium* spp. (which forms T-2, HT-2 toxin, deoxynivalenol, nivalenol, zearalenone, and fumonisins)[14].

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Aspergillus flavus and *Aspergillus parasiticus* are particularly important for community health because they produce aflatoxin, a toxic mycotoxin that adversely affects human health[15]. The effects of aflatoxin on human health include impairing children's development, weakening the immune system, increasing the risk of cancer, and causing death in cases of severe acute exposure[16].

The dried fruit might be contaminated from the fields or spoiled during the drying and storage with some of the toxigenic fungal species in optimum environmental conditions (favorable temperature and moisture) these species produced mycotoxins such as aflatoxin and ochratoxin; ingestion of mycotoxin by humans cause mycotoxicosis[9, 17], continuous screening of food staffs for the possible occurrence of fungal contamination is necessary. The current study aims to investigate and identify fungi associated with dried fruits and screening mycotoxin production of A*spergillus* spp. isolates from the dried fruit of local markets in Sulaimani city.

2. Materials and methods

2.1 Collection and processing of the samples

Forty-eight samples of eight dried fruits with six replicates from each, as shown in Table (1), were purchased from different places in the Sulaimani market. Two hundred grams of each sample were placed in clean polyethylene bags; the bags were tightly closed, labeled, and stored in the refrigerator (4 ºC) until use.

Table 1: Number of samples of dried fruits and their scientific names.

No.	Dried Fruit	Scientific Name
1	Mulberry	*Morus alba*
2	Apricot	*Prunus armeniaca*
3	Dried grape	*Vitis vinifera*
4	Barberry	*Berberis vulgaris*
5	Raisin	*Visit vinifera*
6	Prune	*Prunus domestica*
7	Dried lime	*Citrus aurantiifolia*
8	Fig	*Ficus carica*

2.2 Isolation of fungi

The dilution plate method was used to isolate fungal species. Two hundred grams of dried fruit were crushed with an electric blender (previously sterilized with alcohol) to powder. The homogenized suspension was prepared by mixing dried fruit powder with sterile distilled water with a ratio of (1:10) respectively[9, 18]. Triplicate plates containing Dichloran-Rose Bengal Chloramphenicol Agar (DRBCA) were inoculated with 1 ml of homogenized sample from appropriate dilution for each dried fruit sample and then incubated at 28°C for one week. The fungal colony on dichloran rose bengal chloramphenicol agar was enumerated and presented as a colony forming unit per gram (cfu/g)[19, 20].

2.3 Identification of isolated fungi

Identification of fungal isolates was performed depending on macroscopic characteristics of fungal colony morphology (topography, pigmentation, and texture) and microscopic features of fungal strains, using the taxonomic keys[21-25]. A wet mount technique was used to prepare a slide[3]; a drop of lacto phenol cotton blue (LPCB) was deposited on the center of the slide and mixed well with a small amount of fungal growth with the help of a needle. A cover slip was positioned and inspected under a microscope[23, 26].

2.4 Identification of Aspergillus species

The genus *Aspergillus* was identified at the species level according to the standardized procedure described by Pitt and Hocking, 2009; depending on the color and diameter of the colony and the microscopic features of the asexual reproduction structure. A pure colony was transferred to Czapek yeast extract agar, malt extract agar, and 25% glycerol nitrate agar supplemented with chloramphenicol to inhibit bacterial growth and incubated at three different temperatures (25 ºC, 5 ºC and 37 ºC) for one week[23, 26].

2.5 Screening for aflatoxin producer strain of Aspergillus

Various methods are available for screening and detecting toxigenic strains of *Aspergillus favus* and *A. parasiticus*, such as analytical methods, cultural methods, and molecular techniques. In the current study, both (cultural and molecular) were applied to screen the potential of toxigenicity[4, 27].

2.5.1 Cultural techniques

2.5.1.1 Ammonium vapor:

In 1999, Saito and Machida introduced a sensitive and rapid method for detecting toxin-producing strains of *A. flavus* and *A. parasiticus*. Isolates of *A. flavus* and *A. parasiticus* were grown on yeast extract sucrose agar and one or two drops of ammonium hydroxide were put on the edge of the inverted cover of the plate. The reverse side of the colony of the aflatoxin-producing strain rapidly turns to plum red, while the non-aflatoxin isolates retained the original color of the colonies[28].

2.5.1.2 Ultraviolet (UV) photography:

This technique was used to detect toxin-producer strains of A. *flavus* and *A. parasiticus.* A fungus was grown on yeast sucrose agar, stored in a dark incubator at 28 ºC for one week then exposed to Ultraviolet light at a wavelength of 365 nm. Under UV light, aflatoxigenic strains produced a fluorescence ring around the colony, and the absence of a fluorescence ring in non-aflatoxin producer strains[28-30].

2.5.2 Molecular method

2.5.2.1 DNA Extraction of Aspergillus

The DNA was extracted from all isolated species of *Aspergillus parasiticus*. Isolates were grown on PDA media, and the mycelia were collected and ground in liquid nitrogen to powder[31]. DNA extraction was performed with the help of the Easy Pure Plant Genomic DNA Kit (South Korea) described in the manufacturing kit.

2.5.2.2 Amplification of aflatoxin genes by PCR

Two genes (*nor1*(*aflD*) and *omtA* (*aflP*) of aflatoxin cluster genes were amplified by PCR to confirm the aflatoxin production by *A. parasiticus* strain primer pairs for each gene presented in Table (2) were used to amplify the particular region of the selected gene. The amplification mixture of 50 µl reaction contains 25 µl PCR master mix (add Taq Master (2X conc.), 2.5 µl of 10 mM of each gene-specific primer, 5 µl DNA template, and 15 µl nuclease-free water. The following cycling PCR programs were applied; initial temperature 95 ºC for 1 min, 35 cycles, 95 ºC for 30 sec, annealing temperature for nor1 of (67 ºC), and omtA (61 ºC) was used for 1 min, 72 ºC, 1 min; then a final extension at 72 ºC for 5 min hold at 10 ºC. The PCR products were visualized by gel electrophoresis.

Table 2: Primers for amplification of nor1 and omtA aflatoxin genes.

Target Gens	Primer codes	Primer Sequences (5' to 3')	Expected product Size (bp)	References
aflP (omtA)	omt1	5'-GGC CCG GTT CCT TGG CTC CTA AGC-3'	1024	32
	omt2	5'-CGC CCC AGT GAG ACC CTT CCT CG-3'		
aflD (nor-1)	nor1	5'C GCT ACG CCG GCA CTC TCG GCA C-3'	400	33
	nor2	5'-GTT GGC CGC CAG CTT CGA CAC TCC G-3'		

2.5.2.3 Gel Electrophoresis

The PCR products run on an agarose gel. The agarose gels were prepared by dissolving (1.5 - 2%) agarose in Tris Borate EDTA (TBE) buffer. The ethidium bromide with a concentration of (0.2 µg/ml) was added to the prepared agarose gel for visualizing DNA. PCR amplicons and DNA ladder were loaded into the wells, and electrophoresis was run at 100 V for 1 hour to separate PCR products. The UV transilluminator was used to visualize the DNA fragments.

3. Results

3.1 Isolation and Identification of Fungi

Forty-eight dried fruit samples were collected from the Sulaimani city markets, and fungi were isolated using dilution plate methods. The data presented in Table (3) revealed that 353 * 10^2 CFU/gm of dried fruit fungal isolates recovered; they belonged to seven genera. The predominant fungi associated with dried fruit are *Aspergillus niger,* followed by *Penicillium* spp. The most contaminated dried fruit is mulberry, while dried lime and raisin contain the fewest numbers of fungi. The *Aspergillus parasiticus* was isolated in the fig fruit only.

Table 3: Occurrence and number of fungal species isolated in dried fruits.

Isolated fungal species	Mulberry	Apricot	Dried grape	Barberry	Raisin	Prune	Dried lime	Fig	Total CFU/gm *10^2
Aspergillus parasiticus	-	-	-	-	-	-	-	25	25
Aspergillus niger	152	1	58	5	5	6	3	-	230
Cladosporium spp.	-	-	-	-	-	2	-	-	2
Fusarium spp.	1	-	-	-	-	-	-	1	2
Mucor spp.	-	5	-	-	-	-	-	-	5
Penicillium spp.	4	12	23	33	2	3	-	-	77
Rhizoctonia spp.	5	-	-	-	-	3	1	-	9
Stemphylium spp.	2	-	-	-	-	1	-	-	3
Total CFU/gm *10^2	164	18	81	38	7	15	4	26	353

Note: - six replicate samples per each dried fruits were collected and analyzed.

3.2 Screening of aflatoxin producer strain of Aspergillus spp.

Toxigenic strains of Aspergillus spp. were identified using cultural and molecular techniques (conventional PCR).

3.2.1. Cultural techniques

3.2.1.1 Ammonium vapor test

Twenty- five strains of *A. parasiticus* were tested to detect the potential of their toxigenicity. The color of the reverse side of the colony of all of the tested isolates changed to plum red after exposure to ammonium hydroxide, as shown in Fig. (1).

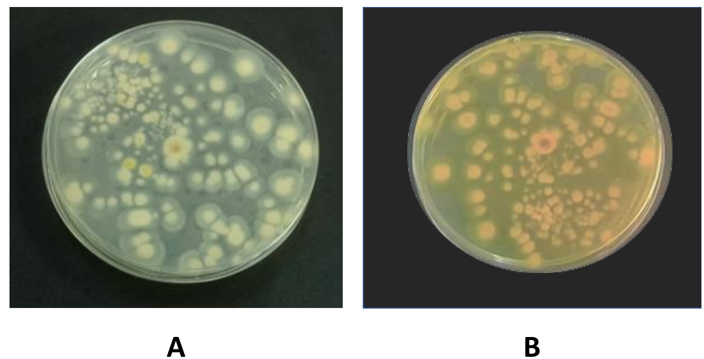

Figure 1: Representative picture of the detection of aflatoxin by using ammonium vapor.
A. Reverse of the colony of A. parasiticus on Yeast extract sucrose agar before exposure to ammonium hydroxide. **B.** Reverse of the colony of A. parasiticus on Yeast extract sucrose agar after exposure to ammonium hydroxide, the color of the colony turned to plum red or pink due to aflatoxin detection.

3.2.1.2 Ultraviolet (UV) photography

All isolated strains of *A. parasiticus* that grow on Yeast extract sucrose agar are exposed to UV translaminar at the wavelength of 365 nm to detect the aflatoxin production. As shown in Fig. (2 B), the toxigenic strain of *A. parasiticus* forms a fluorescence ring around the colony after exposure to UV light; in the present study, all Twenty- five isolates of *A. parasiticus* are toxigenic.

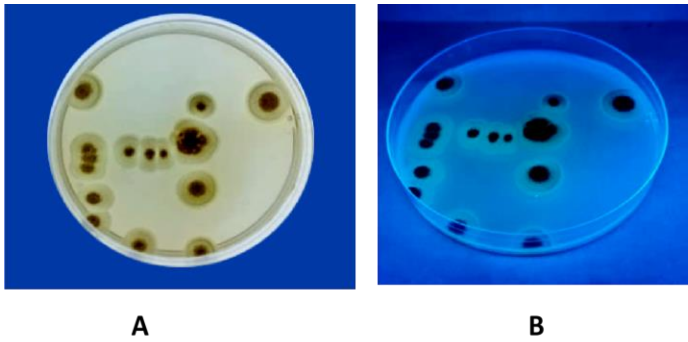

Figure 2: Representative picture of aflatoxin detection using ultraviolet (UV) photography. **A.** Colony of A. parasiticus on YES agar before exposure to UV light. **B.** Colony of A. parasiticus on YES agar after exposure to UV light, formation of fluorescence ring around the colony, indicate toxin production.

3.2.2 Molecular method

Two aflatoxin genes (*aflP* (*omtA*) and *aflaD* (*nor1*)) are selected to use as the markers to distinguish toxigenic *A. parasiticus* from non-toxigenic strains. The expected size of *omtA* is 1024 bp, and *nor1* is 400 bp Fig. (3, 4) all isolates of *A. parasiticus*.

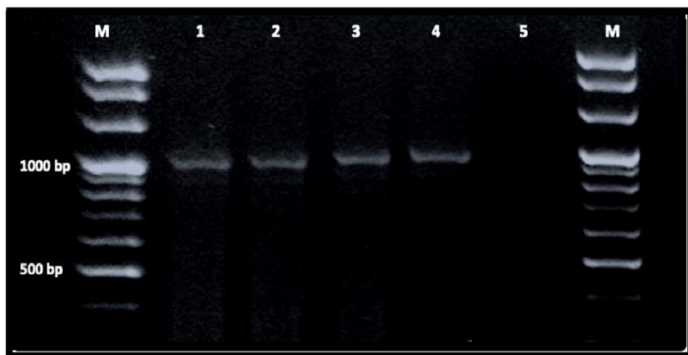

Figure 3: Agarose gel of PCR amplification test of aflatoxin gene (omtA).
M is 100 bp DNA ladder, (1, 2, 3 and 4) are the representative example of A. parasiticus isolates showing DNA fragments of omtA with 1024 bp, and 5 is negative control.

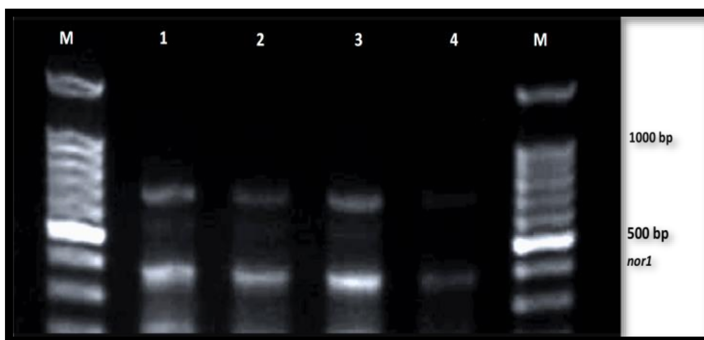

Figure 4: Agarose gel of PCR amplification test of aflatoxin gene (nor1).
M is 100 bp DNA ladder, (1, 2, 3, and 4) are the representative example of A. parasiticus isolates showing DNA fragments of nor1 with 400 bp.

4. Discussion

Desiccation of food is one of the earliest techniques for preserving food[34, 35]. The shelf-life of fruits increases through drying, which decreases water contents and prevents enzymatic change and microbial growth. Dried fruits contain high amounts of sugar because of the reduced water activity of the drying process and become more acidic; all of these make dried fruit more resistant to microbial contamination[34]. Fungal organisms can survive in environments with low water activities; the water content of dry fruits is unstable and affected by environmental humidity during storage, which might create a suitable condition for fungal growth and deteriorate it[36]. The current study aims to screen some dry fruits available in the Sulaimani markets and identify the types of fungal species associated with dried fruits. As well as distinguishing the toxigenic strain of *Aspergillus* species using cultural and molecular methods.

A standard dilution plate was used to isolate the fungi using dichloran rose bengal chloramphenicol agar (DRBC). It is a selective medium that supports the growth of fungi. The dichloran component of DRBC reduces the size of colony diameter; rose bengal restricts the overgrowth of fast-growing molds such as *Mucor* spp. and *Rhizopus* spp.[37]. The mycological study of dried fruits revealed a total of 358 isolates of fungi belonging to seven fungal genera, as shown in Table (3). *Aspergillus* spp. were the most prevalent fungi isolated from dried fruits, followed by *Penicillium* spp. A similar finding has been reported by Ramadan et al. (2021); they stated that the genus *Aspergillus* is the dominant fungi isolated in the dried fruit collected from Duhok and Mosul, Iraq[3]. Moreover, a study of fungal contamination of dried fruit in Hila-Iraq provided that *Aspergillus* spp. are the common fungi that contaminated the dried fruits[38]. Also, Abbas et al. (2019) reported that the most common fungal species associated with dried fruit belong to *Aspergillus* spp. and *Penicillium* spp.[4]. The high frequency of *Aspergillus* spp. in dried fruits might be because the genus *Aspergillus* can withstand low water activity and unfavorable environmental conditions, in addition to its ability to produce and secrete various enzymes[39]. Among Aspergilli, *Aspergillus niger* was the most abundant fungus isolated, as reported by[4, 9, 36, 37].

In the current study, *A. parasiticus* was the only species isolated in dried figs, and it was found just in dried figs and not recovered in any other dry fruits Table (3).

Some species of fungi produce mycotoxins, particularly *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. are the leading mycotoxins producers[40, 41]. Various methods are available to differentiate the aflatoxin producer strain from the aflatoxin non-producer strain[25]. In the present study, twenty-five isolates of *A. parasiticus* were examined by cultural (ammonia vapor test and UV fluorescence) and molecular methods (amplification of both *omtA* and *nor1* genes) to determine the potential of aflatoxin-producing strain. Detection of toxin formation by cultural method was performed by culturing the isolates of *A. parasiticus* on (YES) agar and incubating for one week before exposure to UV light and ammonium hydroxide. The YES media was selected because Fente et al. (2001) and Ritter et al. (2011) reported that the YES agar is superior to Czapek's yeast extract gar (CYA) for aflatoxin production[42, 43]. Also, Reddy et al. stated that YES media support aflatoxin B1 (AFB1) production; they reported that only 65% and 53% of toxigenic *A. flavus* produce aflatoxin on CYA and potato dextrose agar compared to YES[44]. Ammonium Vapor and UV

fluorescence are rapid and easy methods, not necessitating special equipment, but are less sensitive to the detection of toxin production[4]; in the current study, all isolates of *A. parasiticus* are toxigenic Fig. (1, 2). The results presented in Fig. (1) showed that the color of the reverse side of the toxigenic strain of *A. parasiticus* colonies turned to plum red after exposure to ammonium hydroxide, and no color change in the non-toxigenic one. The color change is due to an intermediate product of aflatoxin production, yellow anthraquinone, which acts as a pH indicator turned to red in alkaline pH[28]. UV fluorescence is a qualitative method to detect aflatoxin production Fig. (2). The aflatoxin producer strain emits a fluorescence ring around the colony under UV light with a wavelength of 365 nm due to the absorption of UV light by aflatoxin B1 and G1[45].

The conventional molecular method is another method used to test the potential of toxigenicity; it is a rapid and reliable method to discriminate the toxigenic strain from the non-toxigenic *A. flavus* and *A. parasiticus*. A study by Sohrabi and Taghizadeh (2018) reported that there is a correlation between the detection of some aflatoxin genes (*aflP* (*omtA*), *aflD* (*nor1*), and *aflR*) by PCR amplification and quantification of aflatoxin by using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC)[46]. In the present study, two structural genes (*omtA* and *nor1*) of the aflatoxin biosynthesis pathway are selected as a marker for potential aflatoxingenicity. The *nor1* gene required at the early stage is responsible for converting norsolorinic acid to averantin, and *omtA* needed at the later stage of the aflatoxin biosynthesis pathway, converts sterigmatocystin to aflatoxin in the final steps[47, 48]. All isolates of *A. parasiticus* contained both genes, indicated by positive DNA fragments with sizes of 1024 bp and 400 bp for both genes *omtA* and *nor1* Fig (4, 5). In the current study, all isolates of *A. parasiticus* are toxigenic strains and produced aflatoxin, and there are no differences between the cultural and molecular methods for detecting the toxigenicity of the isolates. Considerable research provided that *A. parasiticus* is more toxigenic, and non-toxigenic strain was infrequent and occasionally reported[49-51].:

As mentioned previously, the most predominant fungal species isolated from dried fruits belonging to *Aspergillus niger* and *Penicillium* spp. Some strains of these fungi produce a mycotoxin called ochratoxin, which has adverse effects on human and animal lives, such as nephrotoxicity and renal tumors in animals upon consumption of contaminated foods[52, 53]. Therefore, extra care should be applied to protect dried fruits and other foodstuff from fungal contamination during harvesting, drying, marketing, and maintaining proper environmental moisture and temperature during storage to prevent post-harvest contamination.

Conclusion

In conclusion, our study highlights the significant issue of fungal contaminations in dried fruits; *Aspergillus* spp. and *Penicillium* spp. emerging as the dominant fungi. Some species of these fungi produce mycotoxins, including the highly concerning aflatoxin and ochratoxin A, which pose severe health risks upon consumption.

Our findings underscore the urgency of adopting strict hygienic practices during harvesting, drying, and handling of fruits, as well as maintaining proper storage conditions and monitoring environmental factors during storage is essential to prevent fungal growth and contaminations.

To further advance our understanding of this critical issue, future research should focus on quantifying the extent of contamination, identifying specific fruits most susceptible to fungal contamination, and evaluating the effectiveness of different preventive measures. Additionally, studies should explore innovative methods for fungal control and assess the impact of various drying techniques on mycotoxin formation.

The implications of our research extend beyond the laboratory, as they inform food safety regulations, consumer awareness, and international trade standards for dried fruits. By addressing this issue proactively, we can safeguard public health and ensure the quality and safety of dried fruit products in the market.

Conflict of interests

None

Author Contribution

The first author contributed to the practical works and writing of the first draft, and the second author contributed to the supervision and revision.

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References

1. M. Masood, S.Z. Iqbal, M.R. Asi, and N. Malik. Natural occurrence of aflatoxins in dry fruits and edible nuts. *Food Control*; 55:62-5, (2015).
2. M. Radojčin, I. Pavkov, D. Bursać Kovačević, P. Putnik, A. Wiktor, Z. Stamenković, K. Kešelj, A. Gere. Effect of selected drying methods and emerging drying intensification technologies on the quality of dried fruit: A review. *Processes*, vol. 9, no. 1, pp. 1–21, 2021, doi: 10.3390/pr9010132.
3. E. A. Ramadan, N. A. Ramadan, and A. A. H. Mohammed. Aflatoxigenic Fungi in Nuts and Dried Fruits in Mosul and Duhok City. Mil. Med. Sci. Lett. (Vojenske Zdr. List., vol. 91, no. 3, pp. 224–234 (2022).
4. M. Abbas, S. A. Naz, M. Shafique, N. Jabeen, and S. Abbas. Fungal contamination in dried fruits and nuts: A possible source of mycosis and mycotoxicosis. *Pakistan J. Bot.*, vol. 51, no. 4, pp. 1523–1529, 2019, doi: 10.30848/PJB2019-4(31).
5. F. C. Wehner and C. J. Rabie. The microorganisms in nuts and dried fruits. *Phytophlactica*, vol. 170, pp. 165–169, 1970,.
6. H. Ozer, H. I. O. Basegmez, and G. Ozay. Mycotoxin risks and toxigenic fungi in date, prune and dried apricot among Mediterranean crops. *Phytopathol. Mediterr.*, vol. 51, no. 1, pp. 148–157, 2012.
7. P. B. Njobeh, M. F. Dutton, S. H. Koch, A. Chuturgoon, S. Stoev, and K. Seifert. Contamination with storage fungi of human food from Cameroon. *Int. J. Food Microbiol.*, vol. 135, no. 3, pp. 193–198, 2009.
8. M. Barth, T.R. Hankinson, H. Zhuang, and **F**. Breidt**.** Microbiological spoilage of fruits and vegetables. In Compendium of the microbiological spoilage of foods and beverages (pp. 135-183). Springer, New York, NY, 2009. DOI: 10.1007/978-1-4419-0826-1_6
9. V.H.Tournas, N.S. Niazi and J.S. Kohn. 2015. Fungal presence in selected tree nuts and dried fruits, Microbiol. Insights, 8: pp. 1-6.

10. N. Misra. Influence of temperature and relative humidity on fungal flora of some spices in storage. Z. Lebensm. Unter. Forsch, 172(1): 30-31 (1981).
11. L. Sibanda, L. T. Marovatsanga, and J. J. Pestka, Review of mycotoxin. Food Contro; vol. 8, no. 1, pp. 21–29, 1997.
12. M.E. Zain. Impact of mycotoxins on humans and animals. J Saudi Chem Soc 15: 129–144, 2011.
13. H. S. Hussein and J. M. Brasel. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, vol. 167, no. 2, pp. 101–134, 2001.
14. M. F. Abdallah, R. Krska, and M. Sulyok. Occurrence of Ochratoxins, Fumonisin B2, Aflatoxins (B1 and B2), and Other Secondary Fungal Metabolites in Dried Date Palm Fruits from Egypt: A Mini-Survey. *J. Food Sci.*, vol. 83, no. 2, pp. 559–564, 2018, doi: 10.1111/1750-3841.14046.
15. L. Lewis, M. Onsongo, H. Njapau, H. Schurz-Rogers, G. Luber, S. Kieszak, J. Nyamongo, L. Backer, A. M. Dahiye, A. Misore, K. DeCock, C. Rubin, and Keya Aflatoxicosis Investigation Group. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environ. Health Perspect., vol. 113, no. 12, pp. 1763–1767, 2005, doi: 10.1289/ehp.7998.
16. P. J. Cotty and R. Jaime-Garcia. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *Int. J. Food Microbiol.*, vol. 119, no. 1–2, pp. 109–115, 2007, doi: 10.1016/j.ijfoodmicro.2007.07.060.
17. A. Elkenany and A. Awad, (2021). Types of Mycotoxins and different approaches used for their detection in foodstuffs. *Mansoura Veterinary Medical Journal*, 22(1), pp. 25-32. doi: 10.21608/mvmj.2021.161191.
18. K.M. Thalij., J.M., Hajeej and M.J. Mohammed. Study the Occurrence of Aflatoxins in some Crops and Dry Fruits in Iraqi Markets. *Journal of Natural Sciences Research*, 5, 22-26, 2015.
19. V. R. Patil, N. S. Kute, M. Phule, and K. Vidyapeeth. Combining ability studies in grain sorghum. Journal of Global Biosciences: 4 (1), pp. 1902–1909, 2015.
20. M. S. Alhussaini. Mycobiota and Mycotoxins of Nuts and Some Dried Fruits from Saudi Arabia. J. Am. Sci., vol. 8, no. 12, pp. 525–534, 2012.
21. G C. Ainsworth, F.K. Sparrow, and A.S. Sussman. The Fungi, an advanced treatise, volume IV A. A taxonomic review with keys: Ascomycetes and fungi imperfecti. Academic Press London. 620 pp. 1973.
22. M.B. Ellis. Dematiaceous Hyphomycetes. CAB. England. 608 pp. 1971.
23. D.H. Larone. Laboratory procedures. Medically important fungi: a guide to identification. 3rd ed. Washington DC ASM press 1995.
24. J.I. Pitt and A.D. Hocking. Fungi and Food Spoilage. 2nd.ed. Gaithersburg, Maryland, Chapman and Hall..593 pp. (1997).
25. T. Watanabe. Pictorial Atlas of Soil and Seed Fungi, Morphologies of Cultured Fungi and Key to Species, 2nd Ed., CRC Press LLC. 486pp. (2002).
26. J.I. Pitt and A.D. Hocking. Fungi and Food Spoilage. 3rd.ed. Springer Dordrecht Heidelberg London New York, 524 pp. (2009).
27. H. Sudini, P. Srilakshmi, K. Vijay Krishna Kumar, S.M.C. Njoroge, M. Osiru, S. Anitha and F. Waliyar. Detection of aflatoxigenic *Aspergillus* strains by cultural and molecular methods: A critical review. African Journal of Microbiology Research, 9 (8). pp. 484-491, 2015.
28. H.K. Abbas, W.T. Shier, B.W. Horn and M.A. Weaver. Cultural methods for aflatoxin detection. J. Tox. Toxin Rev., 23: 295-315, 2004.
29. N. Davis, S. Iyer, and U. Diener. Improved Method of Screening for Aflatoxin with a Coconut Agar Medium. Appl. and Environ. Microbiol 53(7):1593-1595, 1987.
30. S.C. Nair, R.K. Bhagobaty, K. Nampoothiri, V. Kalaigandhi and K.R. Menon. Detection of Aflatoxin production by fungi in spice samples using HPLC and direct visual cultural methods. Inn Romanian Food Biotech., 1: pp. 1-12, 2014.
31. Davari E, Mohsenzadeh M, Mohammadi G, Rezaeian-Doloei R. Characterization of aflatoxigenic Aspergillus flavus and A. parasiticus strain isolates from animal feedstuffs in northeastern Iran. Iran J Vet Res.; 16(2), pp. 150-5, 2015.
32. R. Shapira, N. Paster, O. Eyal, M. Menasherov, A. Mett and R. Salomon. Detection of aflatoxigenic molds in grains by PCR. Appl. Environ. Microbiol. 1996, 62, 3270‑3273, 1996.
33. G. Criseo, A. Bagnara, and G. Bisignano. Differentiation of aflatoxin-producing and non-producing strains of *Aspergillus favus* group. Lett. Appl. Microbiol, 33, 291‑295, 2001.
34. N. Magan, A. Dobson, F. L. Al Ghamdi, F. M. Bokhari, and M. M. Aly, “Toxigenic fungi associated with dried Fruits and fruit-based products collected from Jeddah province,” *IOSR J. Pharm. Biol. Sci. (IOSR-JPBS*, vol. 14, no. 1, p. 10, 2019.
35. I. Gientka, B. Grinke and M. Janowicz. Fungi contamination of dried fruits sold without packaging in the context of water activity, water content and pH. Zeszyty Problemowe Postępów Nauk Rolniczych., 603: pp. 29–39, 2020.
36. F.L. Ghamdi, F.M. Bokhari and M.M. Aly. Toxigenic fungi associated with dried Fruits and fruit-based products collected from Jeddah province. Journal of Pharmacy and Biological Sciences: 14 (1) p. 10-20, 2019.
37. A.D. Hocking, J.I. Pitt, R.A. Samson and U. Thrane. Advances in Food Mycology. Springer, New York, 2006.
38. M.J. Al-Jassani. Aflatoxins and Aflatoxigenic Fungi Contamination of Dried Fruits in Iraqi Market. Journal of Global Pharma Technology. 10(9): pp. 299-308, 2017.
39. J.I. Pitt. The current role of *Aspergillus* and *Penicillium i*n human and animal health. Journal of Medical and Veterinary Mycology 32(1): pp. 17-32, 1994.
40. B. Kabak. The fate of mycotoxins during thermal food processing. J Sci Food Agric 89: 549–554, 2009.
41. FDCO Freire, and MEB da Rocha. Impact of mycotoxins on human health. In: Mérillon JM, Ramawat K. (Eds), Fungal metabolites. Reference Series in Phytochemistry. Springer, Cham, 239–261, 2016.
42. C.A. Fente, J.J. Ordaz, B.I. Vazquez, C.M. Franco and A. cepeda. New additive for cultural media for rapid identification of aflatoxin producing *Aspergillus* strains. Appl. Environ. Microbiol. 67: 4858-4862, 2001.
43. A.C. Ritter, M. Hoeltz and I.B. Noll. Toxigenic potential of *Aspergillus flavus* tested in different culture conditions. Ciencia e Tecnologia de Alimentos Campinas 31(3): 623-628, 2011.
44. K. R.N. Reddy, P. Saritha, C.S. Reddy and K. Muralidharan. Aflatoxin B1 producing potential of *Aspergillus flavus* strains isolated from stored rice grains. Afr. J. Biotechnol. 8 (14): 3303-3308, 2009.
45. D. Yazdani, Z.A.M. Abidin and S. Kamaruzaman. Evaluation of the detection techniques of toxigenic *Aspergillus* isolates. *African J.Biotechnol*. 9(45):.7654-7659, 2010 .
46. N. Sohrabi, and M.Taghizadeh. Molecular identification of aflatoxigenic *Aspergillus* species in feedstuff samples. *Curr Med Mycol.* 2018 Jun;4(2):1-6. doi: 10.18502/cmm.4.2.66. PMID: 30324150; PMCID: PMC6181065.
47. A. Abdel-Hadi, D. Carter and N. Magan, Temporal monitoring of the nor-1 (aflD) gene of *Aspergillus flavus* in relation to aflatoxin B1 production during storage of peanuts under different water activity levels. J. Appl. Microbiol. 109, 1914–1922, 2010.
48. A.C. Baquião, A.G. Rodriges, E.L. Lopes, S.M. Tralamazza, P. Zorzete and B. Correa. Expression of genes by aflatoxigenic and nonaflatoxigenic strains of *Aspergillus flavus* isolated from Brazil Nuts. *Foodborne Pathog Dis.* 2016; 13(8):434-40.
49. W.B. Horn, R.L. Greene, V.S. Sobolev, J.W. Dorner, and J.H. Powell, Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus, A. parasiticus*, and *A. tamarii. Mycologia* 88, 574–587, 1996.
50. G. Vaamonde, A. Patriarca, V.F. Pinto, R. Comeria and C. Degrossi. Variability of aflatoxin and cyclopiazonic acid production by *Aspergillus* Section Flavi from different substrates in Argentina. *International Journal of Food Microbiology* 88, 79–84, 2003.
51. M. Razzaghi-Abyaneh, M. Shams-Ghahfarokhi, A. Allameh, A. Kazeroon-Shiri, S. Ranjbar- Bahadori, H. Mirzahoseini, and M.B. Rezaee. A survey on distribution of *Aspergillus* Section Flavi in corn field soils in Iran: population patterns based on aflatoxins, cyclopiazonic acid and sclerotia production. *Mycopathologia* 161, 183–192, 2006.
52. T.R. Bui-Klimke and F. Wu. Ochratoxin A and human health risk: a review of the evidence. Crit Rev Food Sci Nutr. 2015;55(13):1860-9. doi: 10.1080/10408398.2012.724480. PMID: 24874522; PMCID: PMC4247821.
53. A. Salasib. A Review of Ochratoxin A Occurrence, Condition for the Formation and Analytical Methods. Int J Agric Sc Food Technol 6(2): pp. 180-185. 2020.