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Original article

Isolation and Identification of Fungi Associated with Certain Imported Nuts in Al-Qubbah City, Libya

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Abstract
Although numerous bioactive substances with established health advantages, including vitamins, phenolic compounds, and tocopherols, can be found in nuts, they can also be a source of various aflatoxins due to fungal contamination. This investigation's goal was to identify and classify the fungi in various nuts imported into local markets in Libya; the nuts included walnuts, almonds, peanuts, and cashews. The study's experimental analyses were conducted in the Botany Department's lab, Faculty of Science at Derna University (Al-Qubbah), during January 2025. Isolation and identification results showed widespread fungal contamination in the oilseed samples (four types of nuts) under study. The results included the presence of six pathogenic fungi in all samples: *Rhizopus sp.*, *Penicillium chrysogenum*, *Penicillium glabrum*, *Alternaria alternata*, *Aspergillus parasiticus*, and *Aspergillus niger*, but in varying proportions. The maximum number of fungal isolates of contamination recorded by *Rhizopus spp.* was the most frequently occurring fungus, accounting for 95% of the samples, while *A. alternata* was the least frequent occurring, representing only 10% of the samples.
Keywords. Isolation, Identification, Fungi, Oilseeds, Nuts.

Introduction

Nuts are a great method to get fiber, and eating more fiber has been linked to a lower risk of heart disease and obesity. As well as adding rich vitamins and minerals to the diet, nuts also include phytochemicals that have anti-inflammatory, antioxidant, phytoestrogen, and other protective properties [1]. Making a varied and well-balanced diet is a worry for consumers nowadays. Consequently, as more people become aware of nuts' special nutritional value, unique flavor, taste, and nutraceutical qualities, the inclusion of these components in the diet has significantly increased, along with their beneficial bioactive elements, such as minerals, fiber, high-quality proteins, tocopherols, phytosterols, and phenolic compounds [2]. Also [3] reported that nuts are known to contain a variety of nutrients, such as monounsaturated and polyunsaturated fatty acid profiles, vitamins E and K, carotenoids, and many other components have an antioxidant effect, like, proteins, squalene, fibers, vitamins, as well as specific minerals including potassium, magnesium, copper, and selenium eating them is frequently linked to lowering risk factors for chronic diseases [4]. Nuts are popular and widely consumed in the Mediterranean region due to their many health benefits, which include their high protein, fiber, vitamin E and K, magnesium and potassium, and beneficial fats like monounsaturated and polyunsaturated [5].

Nuts are dry fruits with a hard shell and an edible seed. The most produced nuts in the world are cashews, pistachios, almonds, walnuts, and hazelnuts [6]. A lot of national and international organizations have taken notice of mycotoxins due to their widespread presence and significant toxicity. To deal with the issue of mycotoxins in food, including nuts and feed, the US (FDA) and the European Food Safety Agency (EFSA) have developed several regulations and guidelines [5]. Nut eating has been shown in numerous studies to have positive health impacts, such as on antidiabetic, neuroprotective, cardioprotective, and other qualities [7].

According to the USDA, nuts are regarded as an excellent source of essential amino acids, among other nutrients [8]. Additionally, more nutrients help people stay healthy, enhance the function of the brain, and maintain good skin [9]. Nuts are also a major source of minerals like K and Mg. In recent years, eating more nuts has been associated with increased consumption of certain minerals, which have been suggested to promote human health. It is also believed that nuts are heart-healthy snacks when eaten in moderation [10]. Also, these are an important dietary source of copper and magnesium, which can play a good role in preventing coronary heart disease. Additionally, these contain a considerable amount of iron, zinc, and potassium. According to studies, eating almonds enhances the high-density lipoprotein profile [11]. According to [12], the presence of several chemicals, particularly phenolics, is primarily responsible for these health impacts. Every nut species exhibits a unique phenolic content and character.

Fungi are among the most diverse types of life on Earth. These fungi have a vast range of favored environments and are vital to ecosystems. The versatility of fungi to thrive in ecological niches may be attributed to their efficient extracellular enzyme machinery, which allows them to adapt to environmental conditions and utilize their trophic habitats [13]. Many filamentous fungi, including Aspergillus species, create aflatoxins, which are secondary metabolites that are frequently discovered as pollutants in many agricultural goods, including oilseeds [14]. Aflatoxin-producing molds are known to proliferate more easily

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in warm, humid areas, which is a serious risk. However, poor harvesting and storing methods, environmental factors, and weather patterns frequently combine to cause contamination [15]. At every stage of a person's life, aflatoxins pose serious health concerns. Aflatoxins can cross the placenta and harm a fetus's growth and development, resulting in serious issues for the fetus. Aflatoxin exposure in early childhood has also been strongly associated with immunosuppression, growth, and development problems in early adulthood [16]. Moreover, the cytotoxic effects of aflatoxins on kidney and liver cells include chromosomal segregation, delayed progression during mitosis, and genotoxicity in bone marrow and spermatocyte cells [17]. The current study aims to investigate the extent of fungal growth on some oilseeds and to classify those fungi associated with four of the most common types of nuts in Libyan markets.

Materials and methods

Oilseeds (Nuts)

Almonds (*Prunus sp.*), Peanuts (*Arachis hypogea*), Walnuts (*Juglans regia*), and Cashews (*Anacardium occidentale*) were collected from various local markets in Derna city, located in eastern Libya, for the purpose of isolating and identifying growing fungi present.

Isolation fungi from seeds

Twenty-five (25) seeds from each type were collected and plated in five replicates for the purpose of isolating fungi. For one minute, each seed was submerged in a solution of NaOCl (1%) to surface sterilize it, as outlined by [18]. After that, the seeds were washed with distilled water and then dried on filter paper before being put onto the plates. The seeds were then placed at equal intervals in a Petri dish after being prepared by using PDA. The plates were incubated at 25°C for 7 days, following the methodology outlined by [19]. After the Petri dishes were routinely checked, the fungi growing from the nut's seeds were separated and preserved as pure cultures in preparation for identification and classification.

Identifying growing fungi

Fungi isolated from various nuts and seeds were identified based on mycelium and spore characteristics and the definition of fungal species with the aid of standard mycology [20, 21, 22, 23].

Results

At the end of inoculation and incubation, six distinct fungi were found in all nut-seeds following the separation of the fungal colonies and their microscopic and cultural identifications; these fungi have been categorized into four genera: *Rhizopus*, *Penicillium*, *Alternaria*, and *Aspergillus* (Figure 1, 2). *Rhizopus sp., Penicillium chrysogenum, Penicillium glabrum, Alternaria alternata, Aspergillus parasiticus*, and *Aspergillus niger*. The findings indicated that the six isolated fungi's frequency of occurrence (%) ranged between 10 to 95%. *Rhizopus sp.* had the highest frequency of occurrence, recorded 95%, while *Alternaria alternata* had the least, recorded 10%. Additionally, the frequency of *Penicillium chrysogenum* and *Aspergillus parasiticus* fungal infections was equal, reaching 20%. However, *Penicillium glabrum* recorded a frequency 25%, and *Aspergillus niger* 15%.

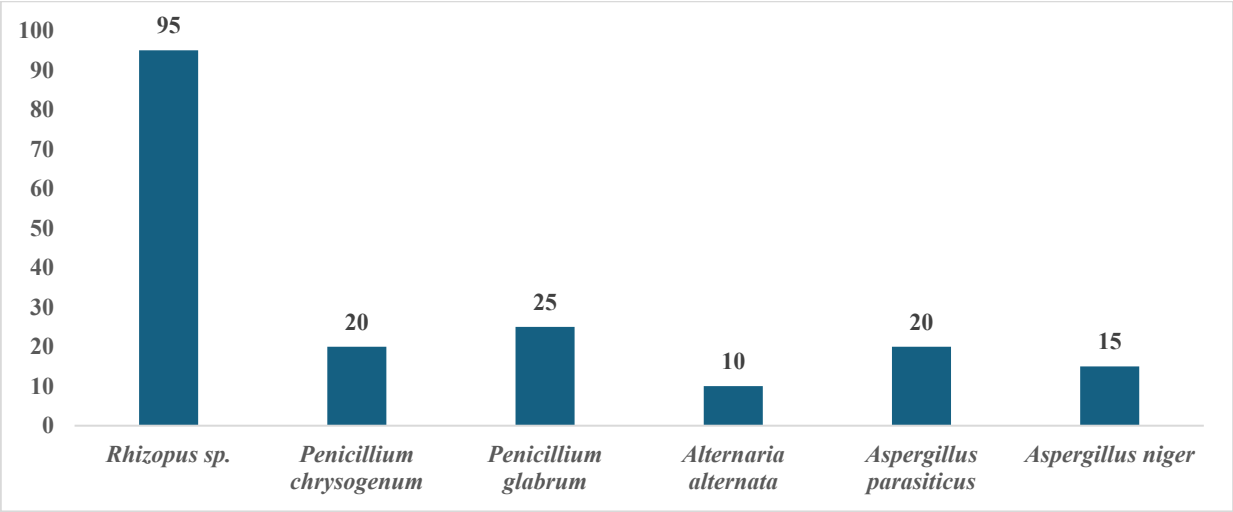

Figure 1. Frequency of occurrence of isolated fungi (%) in 4 types of nut seeds

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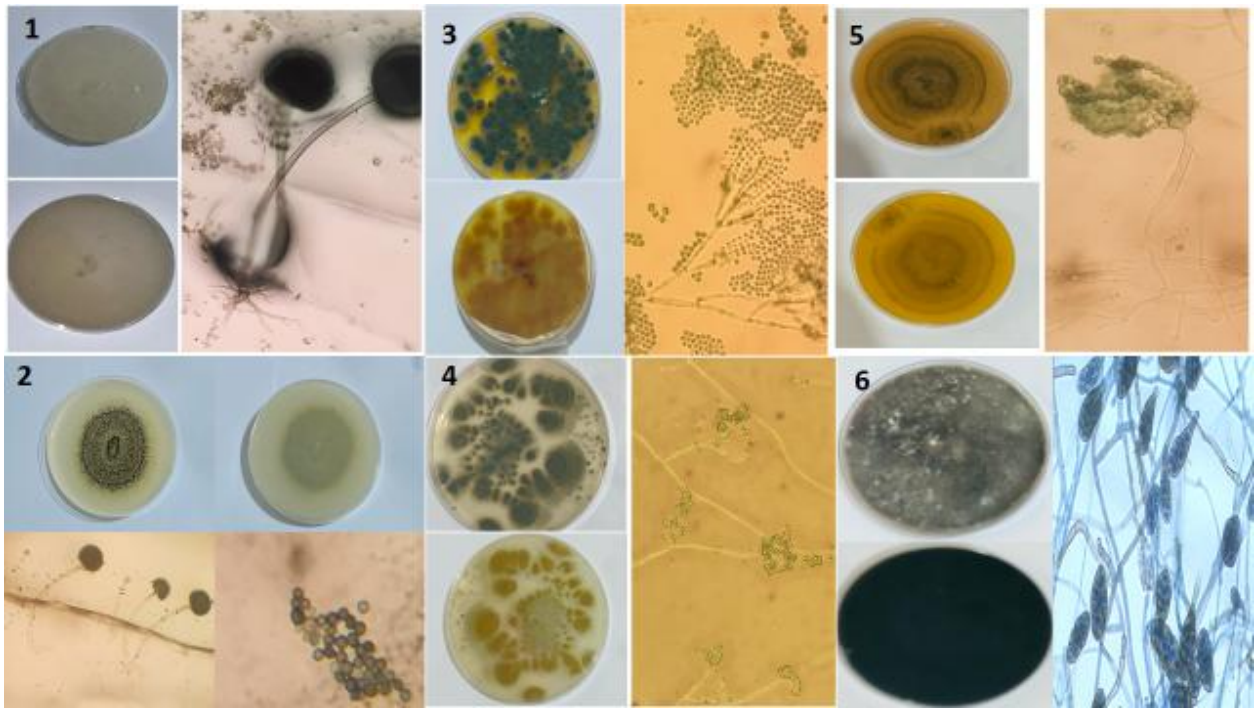

Figure 2. Fungi isolated from varied seed nuts. 1. Rhizopus sp., 2. Aspergillus niger, 3. Penicillium chrysogenum, 4. Penicillium glabrum, 5. Aspergillus parasiticus, 6. Alternaria alternata)

Data in Table 1 showed the percentage of isolated fungi in each of the various nut seeds under study. The findings indicate that the four grains differed in terms of the fungi that grew and were isolated. However, all contained five fungi; three of these are isolated from all varied nut seeds: *Rhizopus sp., Penicillium chrysogenum*, and *Penicillium glabrum*. Almonds and cashews were similar in that all five fungi were isolated. In addition to the three mentioned, *Aspergillus parasiticus* and *Aspergillus niger* were also isolated, but at varying rates of frequency.

The fungus *Rhizopus sp.* was found to be the most prevalent and frequent in all types of grains studied, ranging from 33.3% to 42.8%. Cashew nuts recorded the highest percentage of this fungus at 42.8%, while almond nuts had the lowest percentage of 33.3%. On the other hand, *Alternaria alternata* wasn't isolated from either walnuts or cashews, but it was isolated at the same rate of 12.5% from peanuts and almonds. While *Aspergillus n*iger wasn't isolated on almonds, it reached its highest rate of 25% on peanuts, and *Aspergillus parasiticus* did not grow on peanuts.

Table 1. The percentage of isolated fungi in each type of various nut seeds under study

Taxon	Peanuts	Walnuts	Almonds	Cashews
Rhizopus sp.	37.5	33.33	37.5	42.8
Penicillium chrysogenum	12.5	16.67	12.5	14.3
Penicillium glabrum	12.5	16.67	25	14.3
Alternaria alternata	12.5	0	12.5	0
Aspergillus parasiticus	0	16.67	12.5	14.3
Aspergillus niger	25	16.66	0	14.3

The data in Figure 3 shows the frequency of occurrence of the isolated fungi in peanut seeds (*Arachis* hypogaea). The results show that *Rhizopus sp.* recorded the highest frequency on peanut seeds when isolating the group of fungi that grew on the seeds, reaching 37.5%, while *Aspergillus niger* came in second most frequent fungus at 25%. On the other hand, *Penicillium chrysogenum, Penicillium glabrum,* and *Alternaria alternata* had an equal frequency in isolated fungi, with each having a frequency of 12.5%.

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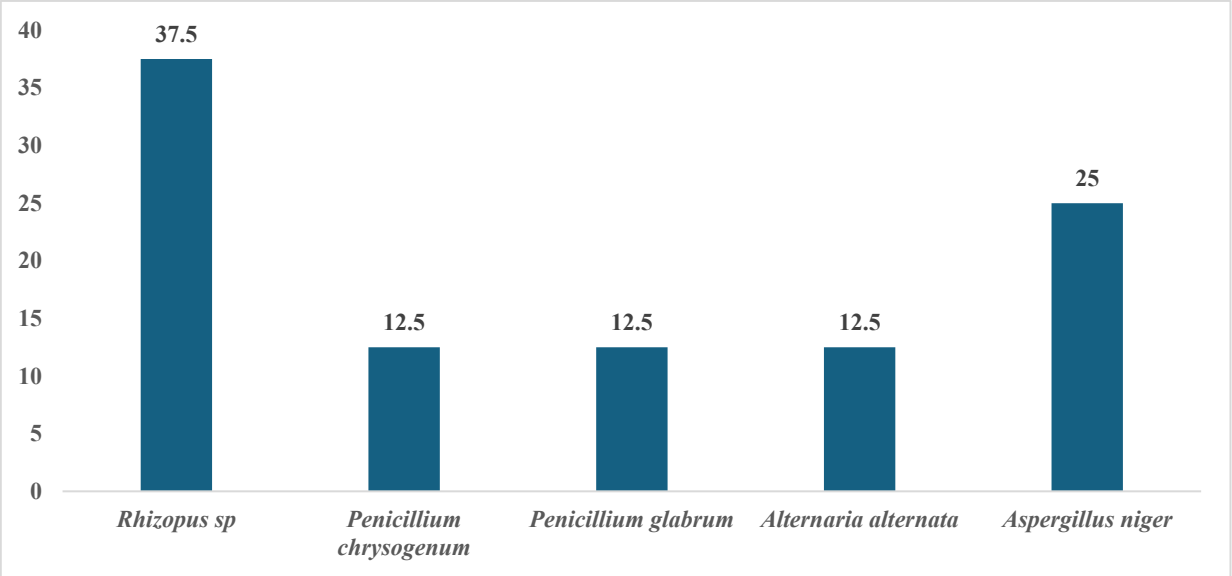

Figure 3. Frequency of occurrence of isolated fungi (%) in peanuts

The results in the following Figure 4 show the percentage of fungi present in the isolates taken from almonds. By identifying these fungi, it became clear that *Rhizopus sp.* recorded the highest percentage of presence when isolating the group of fungi growing on them, reaching 37.5%. On the other hand, *Penicillium chrysogenum, Alternaria alternata*, and *Aspergillus parasiticus* recorded the lowest percentage, which reached 12.5%. However, *Penicillium glabrum* recorded 25% of the group of fungi isolated from almonds.

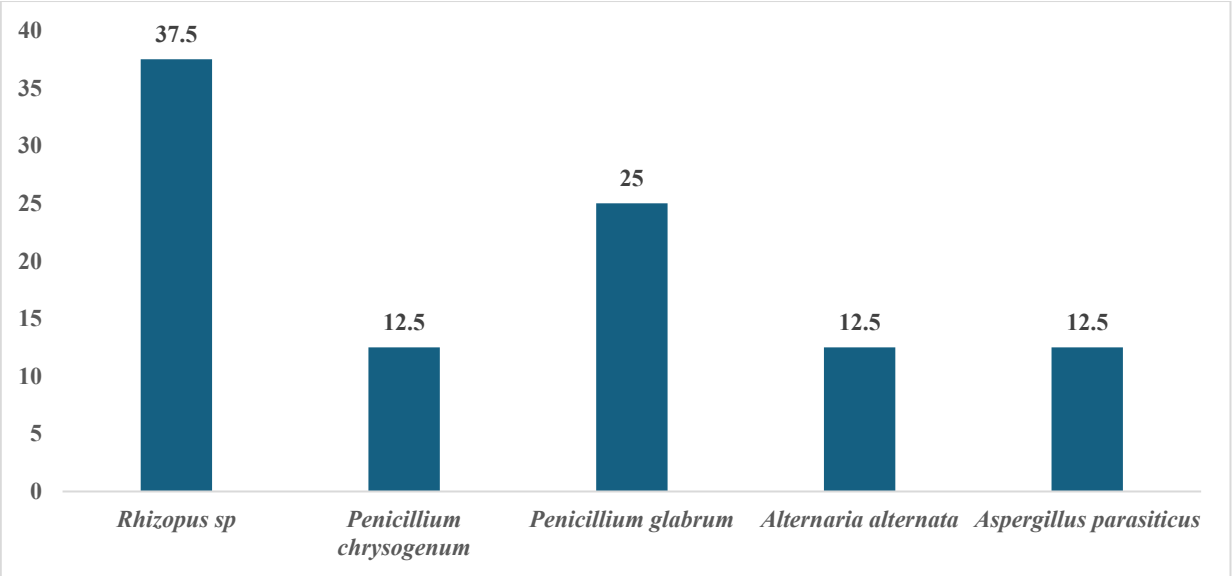

Figure 4. Frequency of occurrence of isolated fungi (%) in almond (Prunus sp.)

(Figure 5) presents the results of mycological analyses of walnut and cashew seeds; five fungi were isolated and identified according to morphologically were found in cashews and walnuts, following three various genera. *Rhizopus sp.* isolate was the most present in all seeds of both nuts. Frequencies of 33.3% and 42.8% of fungal isolates were detected in walnuts and cashews, respectively. However, the frequency of the remaining four fungi, namely *Penicillium chrysogenum, Penicillium glabrum, Aspergillus parasiticus*, and *Aspergillus niger*, was similar, as their presence rate reached 16.67% in walnut isolates and 14.3% in cashew nut isolates.

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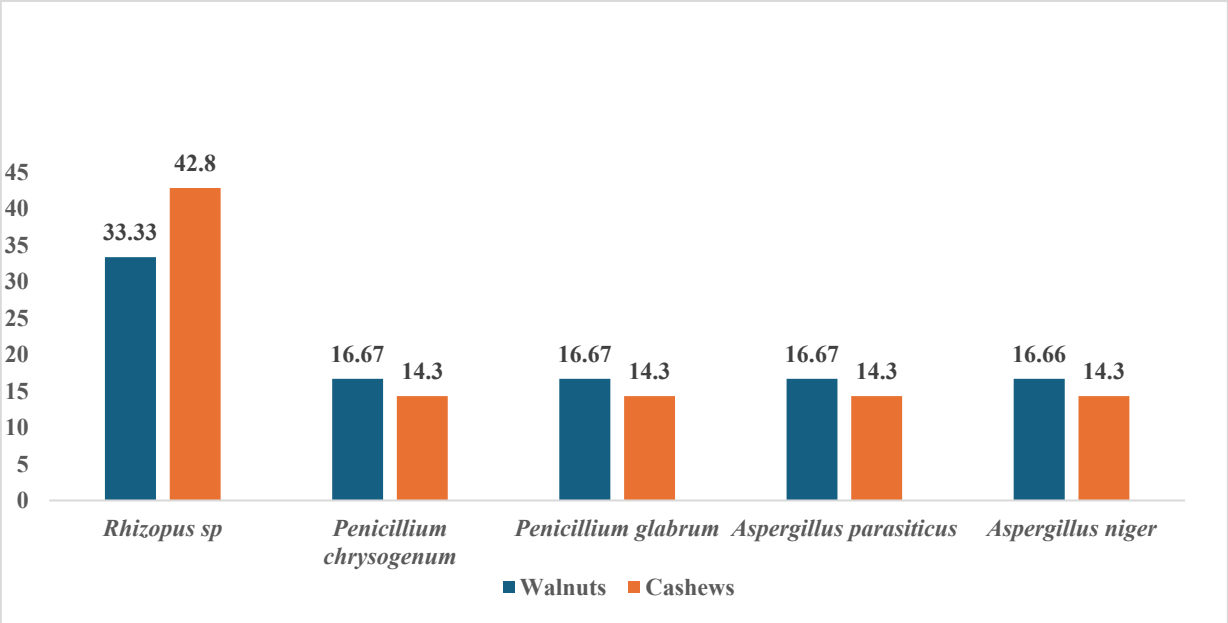

Figure 5. Frequency of occurrence of isolated fungi (%) in walnuts and cashews

Discussion

The results analysis investigation based on the microscopic features of the fungi, six fungal isolates were highlighted i.e., (*Rhizopus sp., Penicillium chrysogenum, Penicillium glabrum, Alternaria alternata, Aspergillus parasiticus*, and *Aspergillus niger*), following 4 genera (*Rhizopus, Penicillium, Alternaria,* and *Aspergillus*) in 4 imported nut-seeds i.e., almonds, walnuts, peanuts, and cashews, were collected from some Libyan markets in Derna city at the eastern part. The variation in storage facilities offered in these markets could be the cause of the discrepancy in the fungi found in this investigation; the findings concur with those of [24, 25]. The findings are consistent with [25] finding that the primary fungus affecting the nutritional value of peanut seeds is *Aspergillus spp.*, which aligns with the findings of [26, 27].

Several studies have shown comparable results regarding the contamination of oilseeds with various fungi. This is due to attributed to practices during and after harvesting that may contribute to fungi growth in oilseeds, grains, and their derivatives [28, 29, 30]. Traditional oilseed processing has been demonstrated to produce an environment that is favorable to contamination by various fungi and to make these and their products more susceptible to contamination compared to mechanized seed processing [31]. Additionally, oilseeds might become more contaminated during storage due to storage methods and relative humidity [32, 33]. Numerous investigations have demonstrated that differences in climatic conditions, particularly high humidity, which contributes to elevated microbial counts, may be the cause of variances in microbial load in samples. Inadequate wrapping or the use of subpar packing materials may be the cause of high humidity levels in samples. Because they are colloidal, nuts have a propensity to absorb moisture from the air around them. The results of this study may also be supported by the possibility that poor drying, inappropriate handling, insufficient processing methods, and inappropriate storage strategies are all connected to this [34, 35, 36].

Conclusion

According to this investigation, nuts were tainted with harmful fungi, which might cause the items to become infected with aflatoxin and put their consumers' health at risk. Enforcing rules, improving hygienic practices, and routinely monitoring quality criteria for various food markets are all necessary to reduce the public's risk of contracting mycotoxicosis and food-borne illnesses. In addition, proper packing materials and storage methods must be used.

Conflicts Of Interest

The authors declare no conflicts of interest.

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