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Surveillance of Mycotoxigenic Fungi in Legume Seeds and Their Potential Implications for Consumer Health in Libyan Markets

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Abstract
Legume products are highly susceptible to fungal contamination throughout various stages of the supply chain, including cultivation, processing, transport, and storage. Under favorable environmental conditions, such contamination may lead to the biosynthesis of mycotoxins, posing substantial risks to food safety and public health. This study aimed to isolate and identify fungal species colonizing legume seeds available in Libyan markets. A total of eight legume types—beans, chickpeas, faba beans, peas, lentils, fenugreek, reeds, and corn—were collected from retail outlets in the city of Al Khoms. In total, 95 fungal isolates were recovered, revealing significant variation in contamination levels among legume types. The highest fungal incidences were observed in reeds (36.8%) and lentils (35.8%), while bean samples exhibited no detectable fungal growth. Faba beans (11.6%), fenugreek (6.3%), corn (4.2%), peas (3.2%), and chickpeas (2.1%) showed varying levels of contamination. Taxonomic analysis revealed the dominance of *Aspergillus* spp. (including *A. flavus*, *A. niger*, and *Aspergillus* sp.), particularly in faba beans, fenugreek, and reeds (53.8%). *Penicillium* species were primarily identified in lentils and peas (38.5%), while *Fusarium* (3.3%), *Rhizopus* (2.2%), *Paecilomyces* (1.1%), and *Pythium* (1.1%) were detected at lower frequencies. The absence of fungal contamination in beans and the variable presence in other legumes suggest that post-harvest handling, environmental factors, and intrinsic seed properties contribute to fungal colonization. These findings underscore the urgent need for improved quality control measures in agricultural processing and storage practices to mitigate the risk of fungal proliferation and mycotoxin exposure in staple food commodities.
Keywords: Legumes, Mycotoxigenic fungi, *Aspergillus*, *Penicillium*, Food safety, Libya

Introduction

Legumes, members of the Fabaceae family, are globally cultivated for a variety of applications, including human consumption, livestock forage, silage, and as nitrogen-fixing green manure for sustainable agriculture (1). They exhibit a broad spectrum of morphological and nutritional characteristics, offering a rich source of plant-based proteins, dietary fiber, and bioavailable micronutrients such as iron, zinc, and magnesium. Additionally, legumes are low in fat and caloric content, making them an essential component of health-promoting diets (2). Their bioactive compounds—including antioxidants, hypoglycemic, hypolipidemic, and anticarcinogenic agents—confer significant protective effects against non-communicable diseases such as cardiovascular disorders, diabetes, and various cancers (3). As a result, legumes are considered among the most nutritionally dense food sources globally, particularly in low-resource settings where access to animal-based proteins may be limited (1).

Despite their agronomic and nutritional value, legume seeds are prone to microbial deterioration, particularly from spore-forming fungi that compromise seed quality and reduce crop yields (4, 5). Cereals and oilseeds, in particular, serve as primary reservoirs for fungal proliferation in the human and animal food chain. The contamination of agricultural commodities with toxigenic fungi has become a major concern due to its direct association with chronic disease incidence and global food insecurity (7, 6). Legumes, like other grains, are susceptible to colonization by mycotoxigenic molds capable of producing hazardous secondary metabolites such as aflatoxin B_1 and ochratoxin A. These mycotoxins are frequently synthesized under high-moisture and temperature conditions, particularly during post-harvest handling, transport, and storage (8).

Among the most notorious fungal genera implicated in mycotoxin contamination are *Aspergillus*, *Fusarium*, and *Penicillium*, all of which have been frequently isolated from economically important crops such as corn, sorghum, and legumes (9,7). Aspergillus flavus, in particular, is known for its high aflatoxin production and its resilience under desiccation stress, contributing to its persistence in storage environments. The aggressive nature and widespread distribution of these fungi necessitate preventive strategies, including the use of resistant crop varieties, good agricultural practices (GAP), and rigorous storage protocols (10). The current study seeks to contribute to this growing field by documenting the diversity and prevalence of fungal contaminants in legume seeds distributed in Libyan markets, with the aim of informing food safety frameworks and public health interventions.

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Methods

Study Site

This study was conducted at the Food Inspection Center in Al Khoms, Libya.

Sample Collection

Eight legume types—beans, chickpeas, faba beans, peas, lentils, fenugreek, reeds, and corn—were purchased from local markets in the city of Al Khoms, west of Tripoli, Libya. Representative samples of each legume were aseptically transferred into sterile, sealed polyethylene bags, transported to the mycology laboratory, and stored at a refrigerated temperature of 3–5 °C until further processing for fungal isolation and identification.

Enumeration of Fungi

The enumeration and isolation of fungal species from legume seeds were conducted in accordance with the International Organization for Standardization (ISO) method ISO 21527-1:2008. Fungal colony counts were performed using the spread plate technique on appropriate culture media, and incubation was carried out at 28 ± 2 °C for a period of 7 to 15 days. Following incubation, colony-forming units (CFUs) were visually examined, and morphologically distinct colonies, including slow-growing isolates and filamentous mycelia, were subcultured for further taxonomic characterization.

Fungal Identification

Fungal isolates were identified based on macroscopic and microscopic morphological features using standard mycological keys and taxonomic references. The identification was conducted following the protocols described by Samson et al. (11), Pitt and Hocking (12), Ellis et al. (13), Pitt (14), Raper and Fennell (15), and Moubasher (16).

Results

This study yielded a total of 95 fungal isolates, which were taxonomically assigned to six genera: *Aspergillus*, *Penicillium*, *Fusarium*, *Paecilomyces*, *Rhizopus*, and *Pythium*.

Table 1. Fungal Incidence and Predominant Genera in Legume Samples

Legume	Fungal Presence (%)	Number of Isolates	Primary Fungal Isolates
Reeds	36.8%	35	*Aspergillus*
Lentils	35.8%	34	*Penicillium, Paecilomyces*
Faba Beans	11.6%	11	*Aspergillus*
Fenugreek	6.3%	6	*Aspergillus*
Corn	4.2%	4	*Fusarium*
Peas	3.2%	3	*Penicillium, Rhizopus*
Chickpeas	2.1%	2	*Rhizopus, Pythium*
Beans	0%	0	---

The highest fungal contamination was observed in reeds (36.8%), with *Aspergillus* species predominating, followed closely by lentils (35.8%), where *Penicillium* and *Paecilomyces* were the primary genera isolated. Faba beans exhibited moderate susceptibility, with 11.6% contamination mainly attributed to *Aspergillus*. Fenugreek samples demonstrated a lower contamination rate of 6.3%, also predominantly colonized by Aspergillus. Corn presented a contamination frequency of 4.2%, largely due to Fusarium species. Minimal fungal presence was recorded in peas (3.2%) and chickpeas (2.1%), with isolates belonging primarily to *Penicillium*, *Rhizopus*, and *Pythium*. Remarkably, bean samples were free from detectable fungal contamination, suggesting potential inherent resistance or optimal storage and handling conditions.

Table 2. Fungal Isolates and Their Presence in Different Legumes

Fungal Isolate	Presence (%)	Type of Legumes
Aspergillus	53.8%	Faba Beans, Fenugreek, Reeds
Penicillium	38.5%	Lentils, Peas
Fusarium	3.3%	Corn
Rhizopus	2.2%	Chickpeas, Peas
Paecilomyces	1.1%	Lentils
Pythium	1.1%	Chickpeas
---	0%	Beans

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The predominance of *Aspergillus* (53.8%) in this study, particularly within faba beans, fenugreek, and reeds, highlights its notable adaptability and prevalence in these legumes. *Penicillium* was the second most abundant genus (38.5%), primarily isolated from lentils and peas. The presence of *Fusarium* (3.3%) was limited to corn samples, while *Rhizopus* (2.2%) and *Pythium* (1.1%) were found mainly in chickpeas and peas. *Paecilomyces* isolates (1.1%) were confined to lentils. The absence of fungal isolates in beans points toward potential biological resistance mechanisms or superior post-harvest management practices that inhibit fungal colonization.

Discussion

Fungal Species Associated with Faba Beans

In this study, *Aspergillus* demonstrated a marked prevalence in faba bean samples, constituting 53.8% of isolates and underscoring the crop's pronounced susceptibility to fungal colonization (Table 2). This finding corroborates previous reports by Anderson and Kumar (5), who identified *Aspergillus* as a pervasive contaminant in grains and legumes, attributable to its resilience under low-moisture environments and post-harvest storage conditions. Similarly, Martínez and Vasquez (4) emphasized that *Aspergillus* incidence is frequently exacerbated by inadequate drying and storage in humid atmospheres. The moderate contamination rate observed in faba beans (11.6%, Table 1) aligns with these insights. Additionally, Stukenbrock and Gurr (7) highlighted the adaptive capacity of *Aspergillus* to fluctuating climatic factors, which may elevate contamination risks for faba beans and reeds under variable environmental stressors. This adaptive versatility accentuates the imperative for stringent storage controls to mitigate fungal proliferation (Table 2).

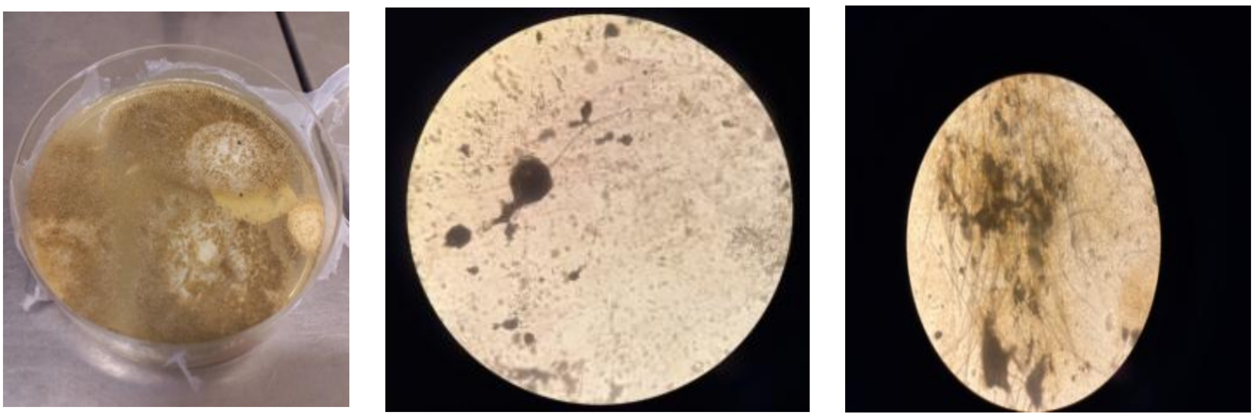

Figure 1. Fungi Associated with Faba Beans.(Right = Aspergillussp; left= Aspergillus.sp

Fungal Species Associated withLentils

Lentils exhibited significant fungal colonization, with *Penicillium* representing 38.5% of isolates and reflecting the legume's heightened vulnerability (Table 2). This concurs with Martínez and Vasquez (4), who described Penicillium as a prevalent storage fungus in legumes, often linked to suboptimal moisture management. The contamination rate of 35.8% in lentils (Table 1) further substantiates this susceptibility. Harris and Patel (9) reinforced these findings by demonstrating the necessity of meticulous moisture and temperature regulation to curtail *Penicillium* growth during storage.

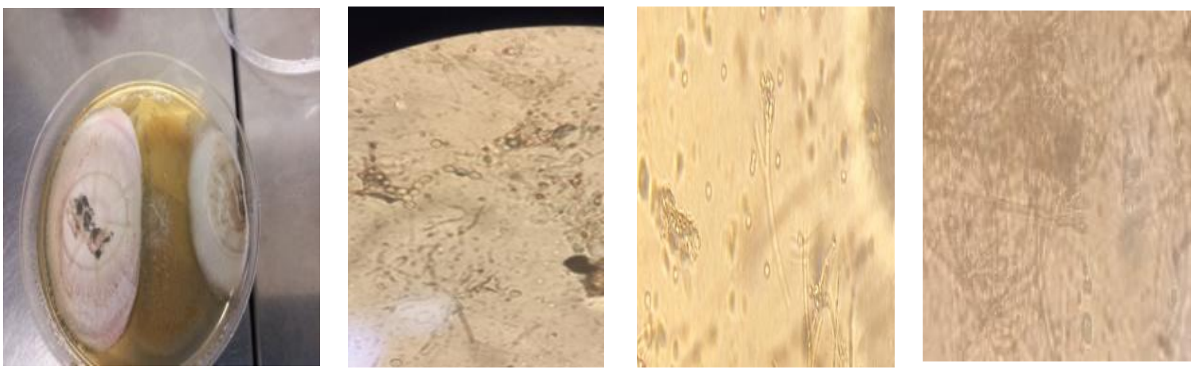

Figure 2. Fungi Associated With Lentils; Right = Penicillium sp.(midum = Penicilliumsp; left= Penicilliumsp)

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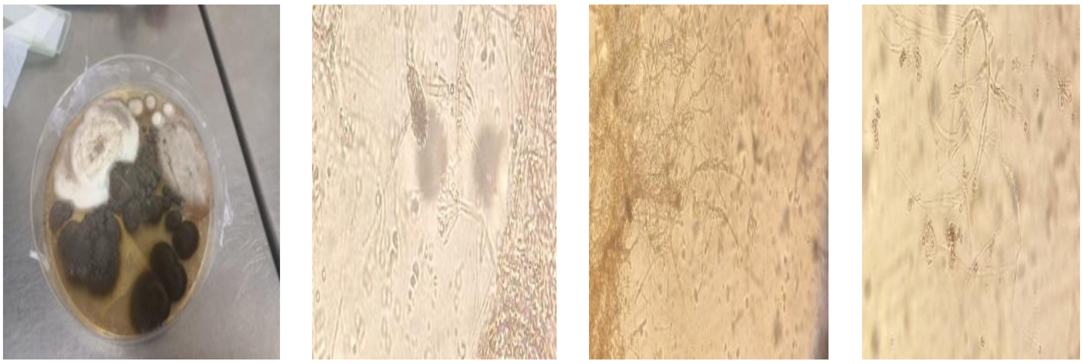

Figure 3. Fungi Associated with Lentils; Right = Penicillium sp. (midum = Penicilliumsp; left= Penicilliumsp)

Fungal Species Associated with Chickpeas

Chickpeas exhibited minimal fungal contamination (2.1%), predominantly involving *Rhizopus* and *Pythium* species (Table 1). These results are consistent with Harris and Patel (9), who observed chickpeas' general resilience to fungal colonization but noted susceptibility to soil-borne fungi like Rhizopus under excessive moisture conditions. Effective moisture control during storage was underscored as critical to minimizing fungal proliferation. Jones and Bhat (17) similarly reported that optimized drying and low-humidity storage protocols effectively reduce *Rhizopus* and *Pythium* contamination in chickpeas.

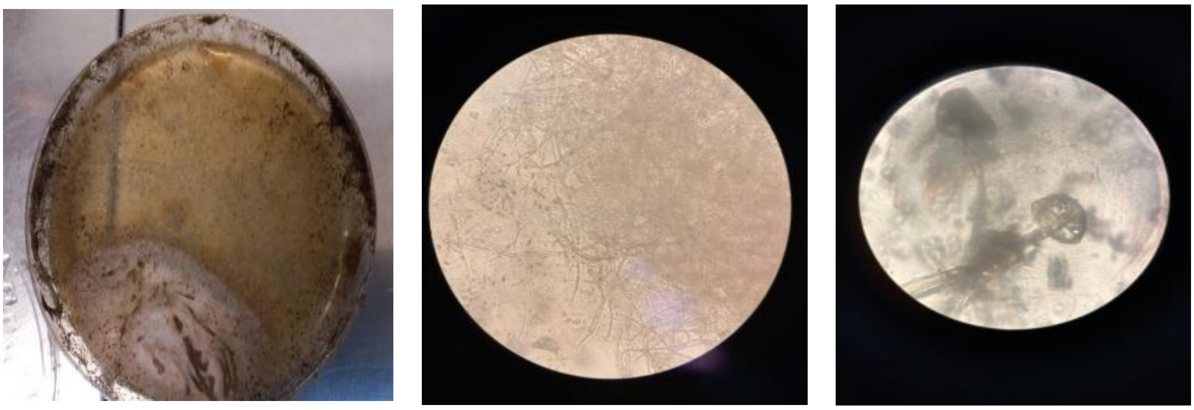

Figure 4. Fungi Associated with Chickpeas. (Right = Rhizopus, left= Pythium

Fungal Species Associated with Corn

Corn samples displayed a low incidence of Fusarium contamination (4.2%), accounting for 3.3% of fungal isolates (Tables 1 and 2). This aligns with Harris and Patel (9), who described Fusarium as a common contaminant in corn, especially under humid conditions conducive to spore germination and growth. Martínez and Vasquez (4) further elucidated that Fusarium contamination in corn, particularly under warm and moist storage environments, elevates the risk of mycotoxin production, posing significant health hazards

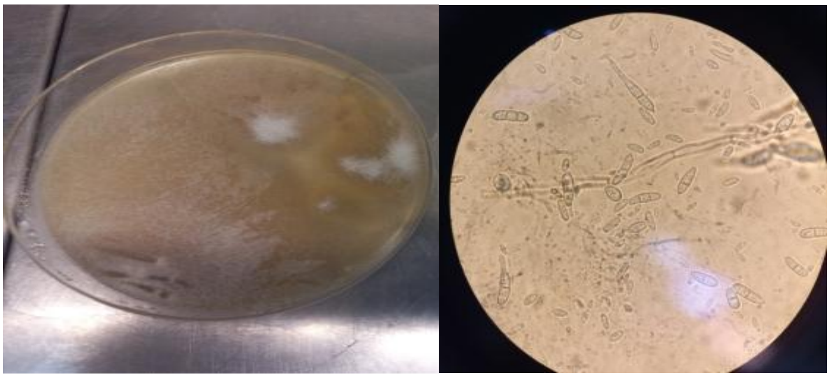

Figure 5. Fungi Associated with Corn. (Right = Fusarium sp)

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Fungal Species Associated withFenugreek and Reeds
Reeds exhibited the highest contamination level (36.8%), predominantly by *Aspergillus*, while fenugreek showed moderate contamination (6.3%) by the same genus (Table 1). These findings are consistent with Dufrenne and Mambou (8), who reported the susceptibility of medicinal legumes such as fenugreek to *Aspergillus* colonization under humid storage. Table 2 data reinforce that *Aspergillus* constitutes a majority (53.8%) of isolates, primarily affecting faba beans, fenugreek, and reeds. Both *Aspergillus* and *Penicillium* are recognized for their potential to compromise legume quality and pose significant health risks through mycotoxin production under unfavorable storage conditions (9, 17). These observations highlight the critical need for climate-controlled storage solutions to inhibit fungal growth and preserve legume integrity.

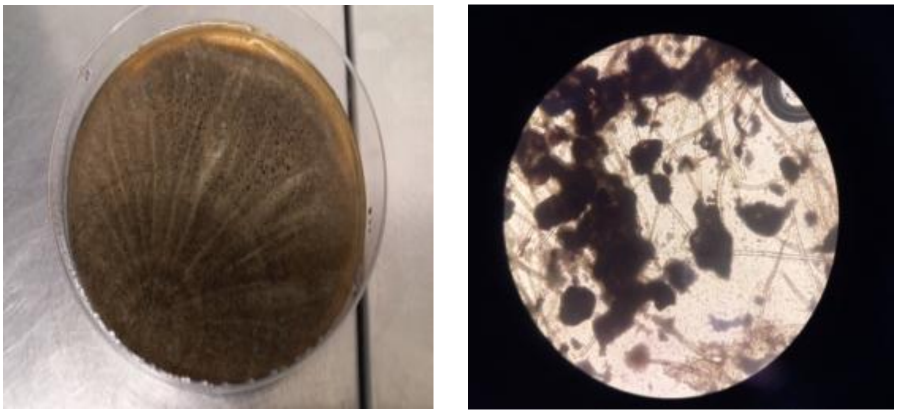

Figure 6. Fungi associated with Fenugreek and Reeds. (Right = Aspergillussp)

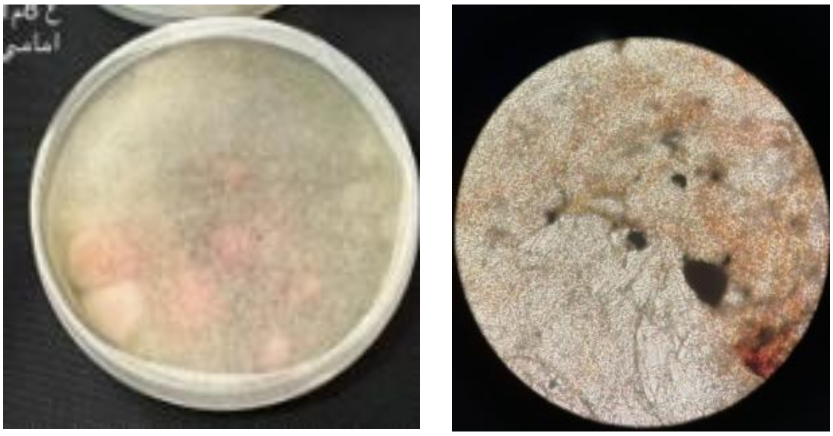

Figure 7. Fungi associated with Fenugreek and Reeds. (Right = Aspergillussp).

Fungal Species Associated with Beans
Beans were uniquely devoid of detectable fungal contamination in this study (Table 1), indicating possible inherent resistance or the benefit of optimal post-harvest storage practices. This absence of fungal colonization aligns with Jones and Bhat (17), who documented the relative resilience of certain legumes, including beans, to fungal infestation when stored under controlled environmental parameters. The complete lack of fungal isolates in beans (Table 2) further supports their potential as a less vulnerable legume species with regard to mycological contamination.

Conclusion
The current study provides a comprehensive mycological assessment of legume seeds sourced from Libyan markets, revealing a diverse and taxonomically significant array of fungal contaminants with potential implications for food safety. A total of 95 fungal isolates spanning six genera were recovered from eight distinct legume types—beans, chickpeas, faba beans, peas, lentils, fenugreek, reeds, and corn. Notably, the genus *Aspergillus* was predominant, represented by three species (*A. flavus*, *A. niger*, and *Aspergillus* spp.), while *Penicillium* encompassed six morphologically distinct species. Other genera identified included *Fusarium* (4 species), *Rhizopus* (2 species), *Paecilomyces* (1 species), and *Pythium* (1 species), indicating a wide fungal spectrum potentially conducive to mycotoxin biosynthesis. The prevalence of *Aspergillus* and

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Penicillium, both known for their prolific production of aflatoxins and ochratoxins under favorable environmental conditions, underscores the latent risk posed to consumers via chronic dietary exposure. Furthermore, the distribution of fungal species varied markedly among legume types, with reeds and lentils exhibiting the highest rates of contamination (36.8% and 35.8%, respectively), while beans exhibited no detectable fungal presence, suggesting either intrinsic resistance or favorable post-harvest handling.These findings highlight a critical need for systematic surveillance and the implementation of preventive frameworks within the food supply chain. Emphasis should be placed on post-harvest hygiene, controlled storage environments, and regular microbial testing to mitigate fungal proliferation and subsequent mycotoxin contamination. Collectively, this research contributes valuable baseline data to the regional food safety literature and serves as a call to action for policymakers and public health stakeholders to adopt integrative strategies that enhance both microbial food safety and consumer protection in Libya.

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