Al-Farouk journal of sciences
Volume (2), Issue (3) ( JUNE, 2026)
ISSN: 3135-2359

مجلة الفاروق للعلوم
المجلد (2) العدد 3 يونيو 2026
ردمد: 3135-2359

# Isolation and Identification of Some Fungal Genera Associated with Faba Bean (*Vicia faba* L.) Seeds

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تاريخ الاستلام: 2026/04/21 تاريخ المراجعة 2026 /05/23 تاريخ القبول:2026/06/07- تاريخ النشر: 2026 /06/30

**Abstract**

The study was carried out to isolate and identify fungal genera associated with faba bean (*Vicia faba* L.) seeds. This study was conducted in the botany department of Life Sciences, Faculty of Arts and Sciences, Qasr Al-Akhyar.

The results indicated that, five fungal genera: *Aspergillus*, *Alternaria*, *Penicillium*, *Fusarium*, and *Rhizoctonia* were detected among the non-surface-sterilized seeds; *Aspergillus* was the most commonly detected fungus in the samples (100%), followed by *Alternaria* (70%), *Penicillium* (60%), *Fusarium* (40%), and *Rhizoctonia* (30%). *Aspergillus* also displayed the greatest growth density, with an average of 3.9 CFU/plate, followed by *Penicillium* (2.4 CFU/plate), *Alternaria* (1.8 CFU/plate), *Fusarium* (1.1 CFU/plate), and *Rhizoctonia* (0.6 CFU/plate).

Additionally, in the surface sterilization samples, the occurrence of all isolated fungi declined. However, *Aspergillus* remained the dominant genus, with a frequency of 50% and an average growth density of 1.3 CFU/plate. *Alternaria* revealed a higher frequency of occurrence than *Penicillium*. Whereas, *Penicillium* produced a greater growth density, displayed by a higher average colony count. In an other hand, *Fusarium* shows the lowest frequency (10%) and growth density (0.2 CFU/plate).

The isolation of *Aspergillus* and *Penicillium* from both treatments suggests their strong association with faba bean seeds and their ability to persist on or within seed tissues. This finding is of particular importance because some species within these genera are known producers of mycotoxins, which may pose risks to seed quality as well as to human and animal health.

**Keywords**: Faba bean, Seeds-borni fungi, Fungal, isolation.

## 1.Introduction:

Faba bean (*Vicia faba* L.) is one of the most important leguminous crops belonging to Fabaceae family cultivated worldwide. It is extensively grown for both human and animal consumption because of its high nutritional value, mainly its rich content of proteins, carbohydrates, and vital minerals. Also faba bean plays a major role in improving soil fertility through its ability to fix atmospheric nitrogen in cooperation with root nodule bacteria, thus enhancing its agricultural and economic importance in many countries (El-dawy et al, 2021).

Seed-borne fungi are among the most significant plant pathogens, it can be present either on the seed surface or within seed tissues. These fungi can cause many detrimental effects,

including seed decay, reduced germination, and seedling mortality. Moreover, seed-borne fungal diseases be able to persist from one growing season to another through infected seeds (Neme et al, 2023). Many studies have reported that, seed-borne fungi are usually associated with faba bean seeds and play a significant role in dropping seed quality and causing seedling diseases.

A study by Neme et al reported that seed-borne fungi in *Vicia faba* were mainly represented by *Fusarium*, *Aspergillus*, and *Penicillium*, with evidence of their transmission from seeds to seedlings, which causing diseases such as root rot and seedling death (Neme et al. 2023). Likewise, Firdu et al found high levels of fungal infection in faba bean seeds (up to 87%) under storage conditions, with *Aspergillus* and *Fusarium* as the dominant genera (Firdu et al. 2025). The study also highlighted that storage conditions, particularly moisture and duration, significantly influence fungal proliferation (Firdu et al. 2025).

These studies reveal that the variety and frequency of seed-borne fungi in faba bean vary according to environmental and storage conditions. But, information on fungal genera associated with faba bean seeds under local conditions remains limited.

Therefore, the present study aims to isolate and identify the fungal genera associated with faba bean (*Vicia faba* L.) seeds. As well as, to determine their frequency of occurrence and identify the most dominant fungal taxa using morphological and microscopic characteristics based on agar plate techniques.

## 2.Materials and Methods:

Seed samples of broad bean (faba bean) were collected from five commercial stores in the Qasr Al-Akhyar area and transported to the laboratory. Approximately 250 g of seeds were obtained from each store and placed in paper bags. The collected samples were then thoroughly mixed to represent a single sampling site within the study area.

Sterile distilled water was used as a control treatment on Czapek Dox Agar (CDA) medium to ensure that the culture medium, water, and laboratory equipment were free from any microbial contamination. The seeds were washed with sterile distilled water for three minutes, and a few drops of the washing water from each sample were inoculated onto the same medium to detect surface-associated fungi present on the seeds. Ten replicates were used for each treatment.

### 2.1. Chemicals and medium:

Sterile distilled water, 96% ethanol, Sodium hypochlorite (Clorox), Czapek Dox Agar (CDA), Lactic acid, Petri dishes, Autoclave, Conical flasks (1000 mL and 1500 mL capacities), Incubator, Microscope slides, Light microscope, Bunsen burner, Water bath, Filter paper, Scalpel.

### 2.2. Without surface sterilization:

The seeds were directly placed onto Petri dishes containing (CDA) medium, with three seeds per dish and ten replicates for each treatment. Data were recorded separately for each treatment and each Petri dish. All dishes were then incubated at 25°C.

### 2.3. Surface Sterilization:

The seeds were surface-sterilized for one minute in a disinfectant solution consisting of sodium hypochlorite (Clorox), ethanol, and sterile distilled water in a volumetric ratio of 1:1:8, following the seed surface sterilization method described by (Davoudpour et al. 2020). The seeds were then rinsed three consecutive times with sterile distilled water and placed on sterile filter paper to absorb excess moisture before being transferred onto the culture medium. Fungal growth was examined after three days of incubation.

### 2.4. Purification and Identification of Fungi:

Fungal colonies were purified by transferring a 0.5 cm diameter agar disc from each fungal culture onto fresh Petri dishes containing (CDA) medium. The fungi were identified based on the morphological characteristics of the colonies growing on the culture medium, including colony size, shape, and color. Microscopic slides were prepared to examine the morphological

features of each fungus under a light microscope. The fungal isolates were identified using the comparative identification method by comparing their characteristics with standard taxonomic descriptions (pitt et al,2009).

**2.5. Frequency Percentage Calculation:**

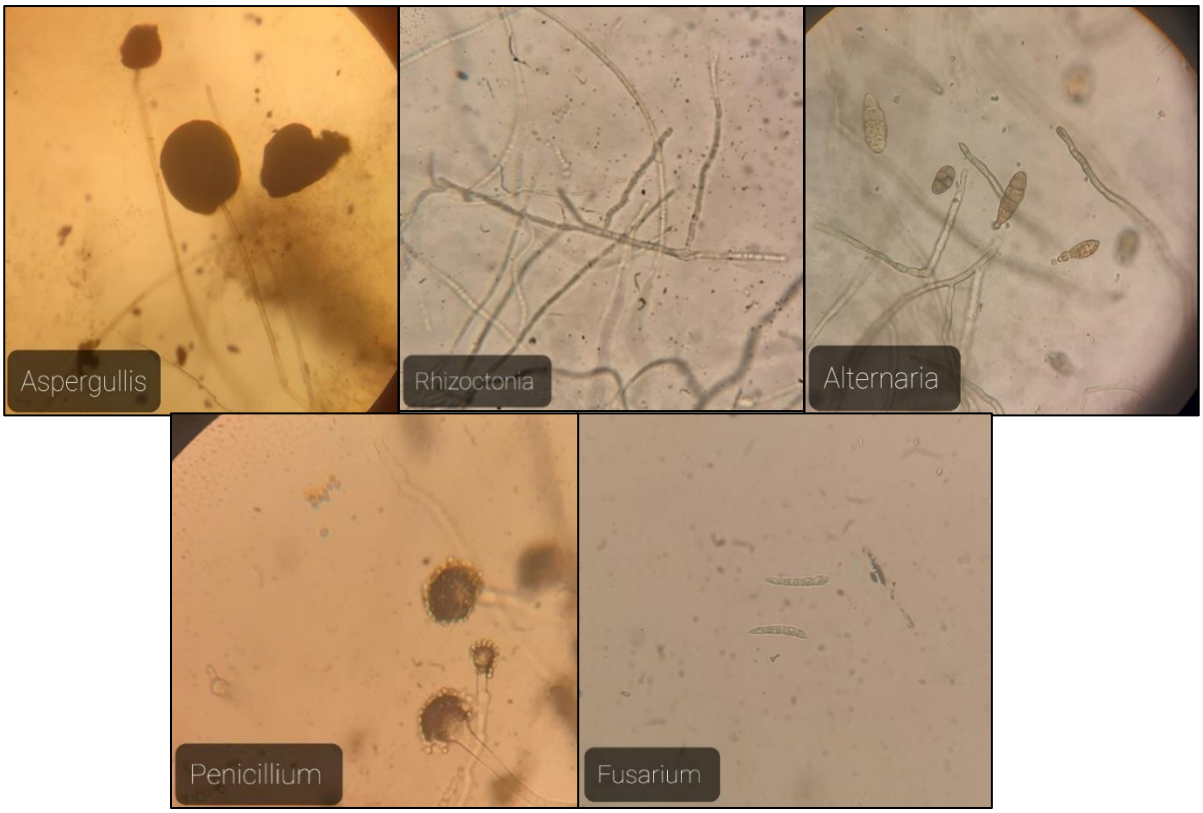
The frequency percentage of fungal occurrence was calculated using the following equation: Frequency percentage(%)= (Number of plates in which the fungus appeared / Total number of plates) × 100

**3. Results and Discussion:**

The results indicated that the water used to wash the seed samples contained high frequencies of microorganisms that may contribute to seed contamination during storage. These microorganisms included bacteria and fungi belonging to the genera *Aspergillus* and *Alternaria*. The presence of these contaminants suggests that improper storage conditions were a major source of contamination, which can adversely affect seed quality and nutritional value. Furthermore, some of these fungi are capable of producing toxins that may pose a risk to human health (Awuchi et al,2022).

**3.1. Seeds Without Surface Sterilization:**

The results revealed the presence of five different fungal genera isolated from the seeds. Some of these fungi were pathogenic, while others were classified as storage fungi. The identified




fungal genera were *Aspergillus*, *Alternaria*, *Penicillium*, *Rhizoctonia*, and *Fusarium* (Figure 1).

**Figure 1. Fungal genera isolated from the seed samples**

The isolated fungal genera differed in both Frequency Percentage (Frequency %) and Mean Colony Forming Units per Plate (Mean CFU/plate).

The results of the present study showed that *Aspergillus* was the most frequently occurring and predominant fungus associated with the examined faba bean seeds, indicating its high ability to survive and persist during storage. These findings are in agreement with those reported by (Kesho et al.2020), who identified several seed-borne fungal genera associated with cereal and legume crops, including faba bean (*Vicia faba*), with *Aspergillus* being among the most

important isolated fungi and some faba bean samples exhibiting infection rates of up to 100%. Similarly, (Nuwara et al. 2013) reported the occurrence of several seed-borne fungi in legume crops, including *Penicillium*, *Fusarium*, *Aspergillus*, *Rhizopus*, and *Alternaria*. Their study also detected the presence of aflatoxins in seeds. The consistency of these findings with the present study further emphasizes the importance of Aspergillus as a common storage-associated fungus and highlights its potential threat to seed quality, nutritional value, and human health due to its ability to produce mycotoxins*, Alternaria* ranked second in frequency of occurrence, followed by *Penicillium*. In contrast, *Fusarium* and *Rhizoctonia* were detected at lower frequencies compared with the other isolated fungal genera (Tables 1 and 2).

**Table 1. Frequency Percentage (%) of Fungi Isolated from Faba Bean Seeds Without Surface Sterilization:**

| Isolated Fungus | No. of Positive Plates | Total No. of Plates | Frequency (%) |
|---|---|---|---|
| *Aspergullis* | 10 | 10 | 100% |
| *Alternaria* | 7 | 10 | 70% |
| *Penicellium* | 6 | 10 | 60% |
| *Fusarium* | 4 | 10 | 40% |
| *Rhizoctonia* | 3 | 10 | 30% |

**Table 2. Mean Colony Forming Units per Plate (CFU/Plate) of Fungi Isolated from Faba Bean Seeds Without Surface Sterilization:**

| Isolated Funges | Total CFU | Mean CFU/Plate |
|---|---|---|
| *Aspergullis* | 39 | 3.9 |
| *Alternaria* | 18 | 1.8 |
| *Penicellium* | 24 | 2.4 |
| *Fusarium* | 11 | 1.1 |
| *Rhizoctonia* | 6 | 0.6 |

### 3.2. Surface Sterilization:

The results of surface sterilization of faba bean seeds showed a marked reduction in both Frequency Percentage (Frequency %) and Mean Colony Forming Units per Plate (Mean CFU/plate) compared with the non-surface-sterilized treatment. This finding indicates the effectiveness of the sterilization procedure in reducing the fungal load present on the seed surface.

*Aspergillus* recorded the highest frequency of occurrence, followed by *Alternaria* and *Penicillium*. *Fusarium* was detected at a relatively low frequency, whereas no growth of *Rhizoctonia* was observed in the surface-sterilized seed samples. These results suggest that *Rhizoctonia* was primarily associated with the seed surface and was effectively eliminated by the sterilization treatment (Tables 3 and 4).

**Table 3. Frequency Percentage (%) of Fungi Isolated from Surface-Sterilized Faba Bean Seeds:**

| Isolated Fungus | No. of Positive Plates | Total No. of Plates | Frequency (%) |
|---|---|---|---|
| *Aspergullis* | 5 | 10 | 50% |
| *Alternaria* | 3 | 10 | 30% |
| *Penicellium* | 2 | 10 | 20% |
| *Fusarium* | 1 | 10 | 10% |
| *Rhizoctonia* | 0 | 10 | 0% |

**Table 4. Mean Colony Forming Units per Plate (CFU/Plate) of Fungi Isolated from Surface-Sterilized Faba Bean Seeds:**

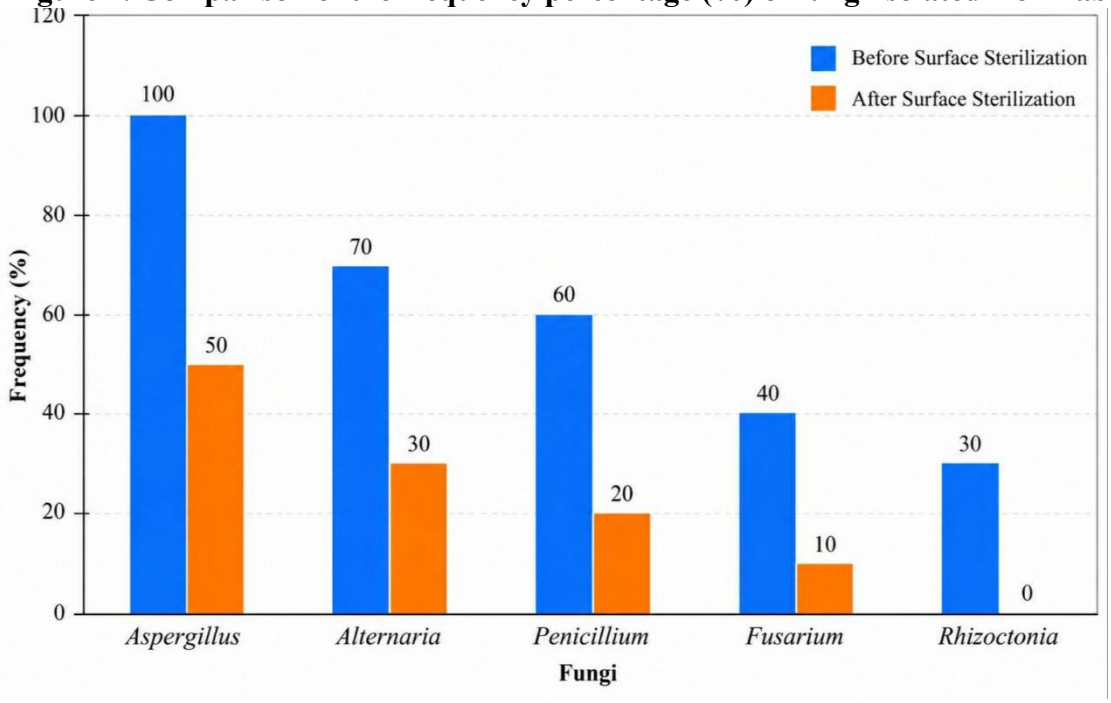
| Isolated Funges | Total CFU | Mean CFU/Plate |
|---|---|---|
| *Aspergullis* | 13 | 1.3 |
| *Alternaria* | 5 | 0.5 |
| *Penicellium* | 6 | 0.6 |
| *Fusarium* | 2 | 0.2 |
| *Rhizoctonia* | 0 | 0 |

These results indicate that storage fungi such as Aspergillus, *Alternaria*, and *Penicillium* were still able to appear after the sterilization treatment, although at lower frequencies. This may reflect their relative resistance to the sterilization process or the possibility that they are located within seed tissues rather than only on the surface.

In contrast, the complete absence of *Rhizoctonia* suggests that this fungus is highly sensitive to surface sterilization.

Overall, the comparison between the two treatments (before and after surface sterilization), as shown in Figures 2 and 3,

**Figure 2. Comparison of the frequency percentage (%) of fungi isolated from faba bean seeds before and after surface sterilization.**



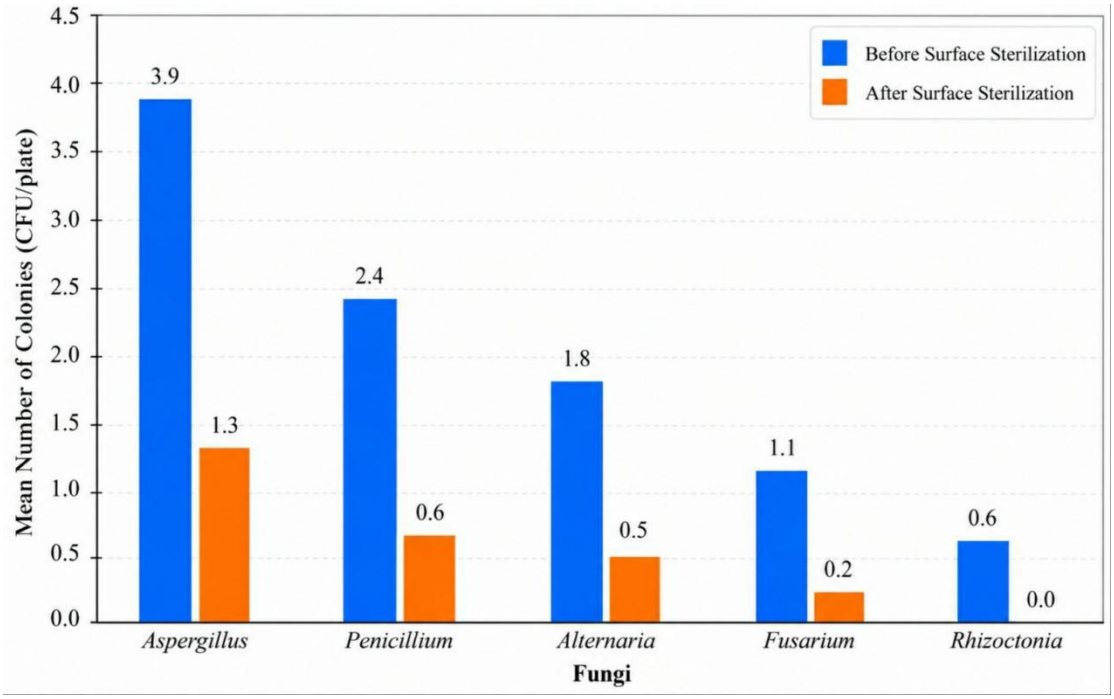



**Figure 3. Comparison of the mean colony forming units per plate (CFU/plate) of fungi isolated from faba bean seeds before and after surface sterilization**.

The results clearly demonstrated that surface sterilization of faba bean seeds resulted in a marked reduction in both the frequency and number of fungal colonies compared with non-sterilized seeds. The occurrence of *Aspergillus*, *Alternaria*, *Penicillium*, and *Fusarium* decreased after sterilization, whereas *Rhizoctonia* was completely eliminated. This reduction can be attributed to the effectiveness of the sterilizing agent in destroying fungal spores and propagules present on the external surface of the seeds, indicating that fungal contamination was predominantly superficial. In contrast, the persistence of some fungi after sterilization suggests their ability to survive within seed tissues or between seed coats, which protects them from the effects of surface disinfectants.

These findings are consistent with those reported by (Mathuret et al, 2003), who stated that surface sterilization significantly reduces fungi present on the seed surface but does not completely eliminate internal seed-borne fungi located within seed tissues. The present results also agree with those of (Rashid et al. 2021), who reported that *Aspergillus* and *Penicillium* are among the most common fungi associated with legume seeds and possess a high capacity to tolerate various environmental and storage conditions. This may explain their continued occurrence after sterilization, albeit at lower frequencies. The complete disappearance of *Rhizoctonia*, however, indicates its high sensitivity to surface sterilization treatments and suggests that it relies predominantly on external colonization of the seed surface compared with other fungi. These findings emphasize the importance of surface sterilization in studies of seed-borne fungi, as it enables differentiation between superficial and internal fungal contaminants. Furthermore, they indicate that faba bean seeds may serve as an effective means for the transmission of several fungal pathogens from one growing season to another if appropriate management and seed treatment measures are not implemented before planting.

**4. Conclusion:**

The presence of certain fungi was recorded on faba bean seeds both before and after surface sterilization, with *Aspergillus* and *Penicillium* being the most dominant genera. These fungi are considered highly significant due to their ability to produce mycotoxins that pose serious public

health risks, particularly affecting the respiratory system (Mannaa et al., 2017). In addition, these fungi contribute to the reduction of the nutritional value of legume seeds, which are an important dietary staple for humans. Consequently, their presence may be associated with potential health problems when contaminated seeds are consumed without proper safety measures.

**5. Recommendations:**

- Conduct further studies on the mycotoxins produced by the isolated fungi and their effects on the nutritional value of legume crops.
- Apply alternative and more efficient sterilization methods to reduce surface-associated fungal contamination on seeds. Improve seed storage and preservation conditions in order to minimize fungal contamination.

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