African Journal of Advanced Pure and Applied Sciences (AJAPAS)
Online ISSN: 2957-644X
Volume 5, Issue 2, 2026
Page No: 192-199
Website: https://aaasjournals.com/index.php/ajapas/index
ISI 2025: 1.126 **SJIFactor 2024: 6.752** **معامل التأثير العربي: 1.62**

Isolation and identification of fungi associated with strawberry fruits collected from three local markets in Al-Bayda City, Libya

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عزل وتوصيف الفطريات المرتبطة بثمار الفراولة المجمعة من ثلاثة أسواق محلية في مدينة البيضاء، ليبيا

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Received: March 10, 2026	Accepted: May 16, 2026	Published: May 27, 2026

Abstract:
Strawberries (*Fragaria x ananassa*) are a fruit crop of high nutritional value and significant economic importance, cultivated worldwide. However, they are susceptible to fruit rot, leading to substantial crop and economic losses. This study was conducted to isolate the fungi associated with strawberries. Strawberry samples were collected from local markets in the city of Al-Bayda. The isolation was carried out by the agar plate method on PDA medium at 28 ºC following a period of incubation. Strawberries sterilized with sodium hypochlorite and non-sterilized strawberries were used. The isolated fungi were identified depending on some characteristic morphologically such as colony characters include shape, size, color, surface texture, size and reverse of the colony/bottom of the colony. and microscopically such as the shape of conidia, conidiophores and septate hyphae (septate) and aseptate hyphae (non-septate). In this study, the results indicated that the fungi identified from the tested strawberries were as follows: *Alternaria alternata*, *Aspergillus niger*, *Fusarium chlamydosporium*, *Penicillium crustosum* and *Rhizopus* sp. The strawberries contained a higher percentage of contaminated fungi due to their high moisture content, which reached 85.7%. Therefore, it is essential to monitor moisture levels to prevent fungal growth.

Keywords: Isolation, Strawberry, Fruit rot, Spoilage.

الملخص

تُعد الفراولة (*Fragaria x ananassa*) من محاصيل الفاكهة ذات قيمة غذائية عالية وأهمية اقتصادية كبيرة تُزرع في جميع أنحاء العالم إلا أنها عرضة لتعفن الثمار مما يؤدي إلى خسائر كبيرة في الإنتاج والعائد الاقتصادي. أُجريت هذه الدراسة لعزل الفطريات المرتبطة بثمار الفراولة، حيث جُمعت عينات الثمار من الأسواق المحلية في مدينة البيضاء. تمت عملية العزل باستخدام طريقة أطباق الأجار على وسط (PDA) وحُضنت عند درجة حرارة 28 درجة مئوية .شملت الدراسة استخدام ثمار فراولة معقمة سطحياً بمحلول هيبوكلوريت الصوديوم وأخرى غير معقمة. اعتُمد في تعريف الفطريات المعزولة على الخصائص المورفولوجية الظاهرية، مثل: (شكل المستعمرة، حجمها، لونها، ملمس السطح، والمظهر السفلي للمستعمرة)، والخصائص المجهرية، مثل: (شكل الكونيديا، الحوامل الكونيدية، ووجود الحواجز في الخيوط الفطرية "المقسمة وغير المقسمة). أظهرت نتائج الدراسة أن الفطريات التي تم تشخيصها من ثمار الفراولة المختبرة هي: *Alternaria alternata*، *Aspergillus niger*، *Fusarium chlamydosporium*، *Penicillium crustosum*، *Rhizopus* sp. تحتوي الفراولة على نسبة أعلى من الفطريات الملوثة بسبب محتواها العالي من الرطوبة، والذي بلغ 85.7%. لذا، من الضروري مراقبة مستويات الرطوبة لمنع نمو الفطريات.

الكلمات المفتاحية: العزل، الفراولة، تعفن الفاكهة، التلف.

Introduction

Among all the fruits available in international markets, strawberries have gained high demand and earned substantial profits in the regions where they are produced [1]. Strawberries are invaluable due to their delicious taste and numerous health benefits, both nutritional and medicinal. They are particularly rich in sugars, organic acids, pectin, vitamins (C, B, B1, B2, E, K), and minerals (calcium, magnesium, sodium, phosphorus, potassium, iron, and iodine) [2]. The economic and nutritional importance of strawberries is high, and their distinctive flavor results from a complex mixture of several volatile and sensory compounds, in addition to their unique characteristics such as texture and taste [3].

Fungi belong to the kingdom fungi and are eukaryotic organisms. Their name is derived from the Latin word "mycus" meaning mushroom. Some fungi live naturally in water, soil, and decaying organic matter, but other types colonize on living organisms such as plants and animals, taking their food from them and causing diseases; therefore, they are called parasitic fungi. Fungi are known to have a rigid cell wall containing substances such as chitin, mannan, and other sugars. These substances provide the fungi with strength and protection. Some fungi are beneficial to humans, such as edible mushrooms [4].

The presence of certain types of fungi in food, such as *Alternaria, Aspergillus, Candida, Fusarium, Mucor, Rhizopus, Penicillium,* etc. causes spoilage and contamination. consuming food contaminated with fungi can lead to various health problems and fungal infections, ranging from mild to serious. Individuals with weakened immune systems are at increased risk of developing this type of infection, as their bodies are less able to resist fungal infections [5]. Food contamination with fungi can lead to a significant decrease in food quality, such as changes in taste, smell, or appearance, and may even result in complete spoilage and rendering the food inedible [6]. Food spoilage and mycotoxin formation depend on several factors, including the type of food, its ingredients, how it is handled, and food storage conditions such as humidity and temperature [7]. Some types of fungi can easily and dangerously produce mycotoxins, and controlling their growth and preventing their spread is a difficult and delicate task [8]. Mycotoxins are toxic secondary metabolites produced by some types of mold [9], these toxins can cause serious harm to humans and animals, and this harm may be sudden or long-term. They may affect hormones, cause cancer, induce genetic mutations, cause birth defects, and lead to problems with blood vessels and hardening of the arteries [10]. Microbial food safety is currently a very important issue for public health and the economy [11]. The World Health Organization [12] has recorded that one in ten people become ill each year due to eating contaminated food, resulting in approximately 420,000 deaths annually worldwide.

In study [13] conducted on rotten strawberries and were able to isolate five fungi, including *A. niger, A. fumigatus, M. fragalis* and *M. genevensis* as well as other species and an unknown fungus. Several fungi such as *Alternaria alternata, Aspergillus niger, Botrytis cinerea, Cladosporium cladosporioides, Fusarium oxysporum* f. sp. *fragariae, Nigrospora sphaerica, Penicillium raistrickii, Penicillium griseofulvum* and *Rhizopus stolonifer* others were found in a study carried out by [14] and various types of fungi were examined and studied. [15] mentioned that there are pathogenic fungi that attack strawberry fruits, and among the most important of these are *Penicillium spp., Colletotrichum spp.,* and *Botrytis cinerea*. [2] demonstrated that the fungi isolated from strawberry fruits included several types, such as *Botrytis cinerea, Alternaria spp, Penicillium spp, Podosphaera aphanis,* and *Fusarium spp.* According to the study [2], *Alternaria* and *Fusarium* were found to be the most widespread fungi among the 27 fungal genera associated with strawberry plants. Researchers in the study [16] found fifteen species of fungi in infected strawberry fruits. *Botrytis cineraria* was the most prevalent, causing damage to approximately 64.6% of the fruit. Another study [17] identified nine genera: *Aspergillus, Alternaria, Cercospora, Cladosporium, Cylindrocladium, Cunninghamella, Geotricum, Penicillium,* and *Phytophthora*. This study aimed to isolate and identify fungi associated with strawberry fruits collected from markets in Al Bayda, Libya.

Material and methods

Samples Collection

Strawberry samples were randomly collected from markets in the city of Al-Bayda, Libya. The samples were placed separately in clean plastic bags, labeled with information such as (date and market name), and then transported to the microbiology laboratory in the Department of Botany, Faculty of Science, Omar Al-Mukhtar University, and were ready for analysis.

Isolation of fungi from strawberry fruits

To isolate fungi from strawberry fruits, the following steps were followed: The fruits were first washed with water, then exposed for two minutes to a 1% sodium hypochlorite solution to sterilize their surface, and then washed three times with sterile distilled water. Any remaining water was dried with filter paper. Also, non-sterilized strawberry were used. Using a sterile scalpel, the strawberries were cut into small pieces, placed in Petri dishes on a pre-prepared potato and glucose (PDA) medium, and incubated at 28 °C for 7 days. Three duplicate samples were used for each sample.

Purification and Identification of Fungi associated with Strawberry Fruits

The obtained fungi were purified then identified by observing macroscopic morphological characters (colony characters include shape, size, color, surface texture, size and reverse of the colony/bottom of the colony). Microscopic morphology includes the shape of conidia, conidiophores and septate and aseptate hyphae. The microscopic detection was conducted for them after staining the slides with Lactophenol cotton blue [18]. Identification to the genus level using fungal identification key books, namely [19, 20, 21].

Determination of Moisture Content

The moisture content was determined using the method [22]. 30 grams were placed in a pre-weighed dish and then dried at 130°C for two hours in a hot air oven.

Results and Discussion

In this study, the percentage of moisture in the strawberry samples was determined to be 85.7%, which represents a critical physiological parameter, as measuring the moisture content of fruits is essential to ensure their quality, safety, and proper storage, given that both high and low humidity significantly affect strawberries by potentially leading to spoilage or loss of quality. High humidity inherently elevates the microclimatic moisture, increasing the probability of mold growth and subsequent fruit rot, whereas low humidity causes severe water loss and desiccation; these physiological outcomes align consistently with the findings of [23]. Mycological evaluation led to the identification of five genera of fungi isolated from post-harvest strawberry fruits, namely *Alternaria alternata*, *Aspergillus niger*, *Fusarium chlamydosporium*, *Penicillium crustosum*, and *Rhizopus* sp., among which *Rhizopus* sp. was found to be more prevalent than any other isolated species (Table 1, Fig. 1). More specifically, as illustrated in Figure 2, statistical evaluation of the Isolation Frequency Index (IF%) demonstrated the absolute dominance of *Rhizopus* sp. (100%) across all market treatments, followed by *Fusarium chlamydosporium* (83.3%), whereas *Aspergillus niger*, *Alternaria alternata*, and *Penicillium crustosum* displayed a moderate overall prevalence of 50%. This statistical analysis confirmed that surface sterilization significantly suppressed the external mold load—particularly that of *P. crustosum*—while internal, deep-seated infections by *Rhizopus* sp. and *F. chlamydosporium* remained unaffected, a resilience highly driven and aggravated by the elevated internal moisture level (85.7%) of the strawberry fruits which establishes an optimal microenvironment for deep tissue fungal proliferation.

Table 1: Species of fungi isolated from sterile and non-sterile tested strawberries

Strawberries	Market A	Market B	Market C
Unsterilized	*A.alternata* *A.niger* *F.chlamydosporium* *P. crustosum* *Rhizopus* sp.	*A.niger* *F. chlamydosporium P. crustosum* *Rhizopus* sp.	*A.alternata* *F.chlamydosporium P. crustosum* *Rhizopus* sp.
Sterilized	*A.alternata* *F.chlamydosporium* *Rhizopus* sp.	*F.chlamydosporium* *Rhizopus* sp.	*A.niger* *Rhizopus* sp.

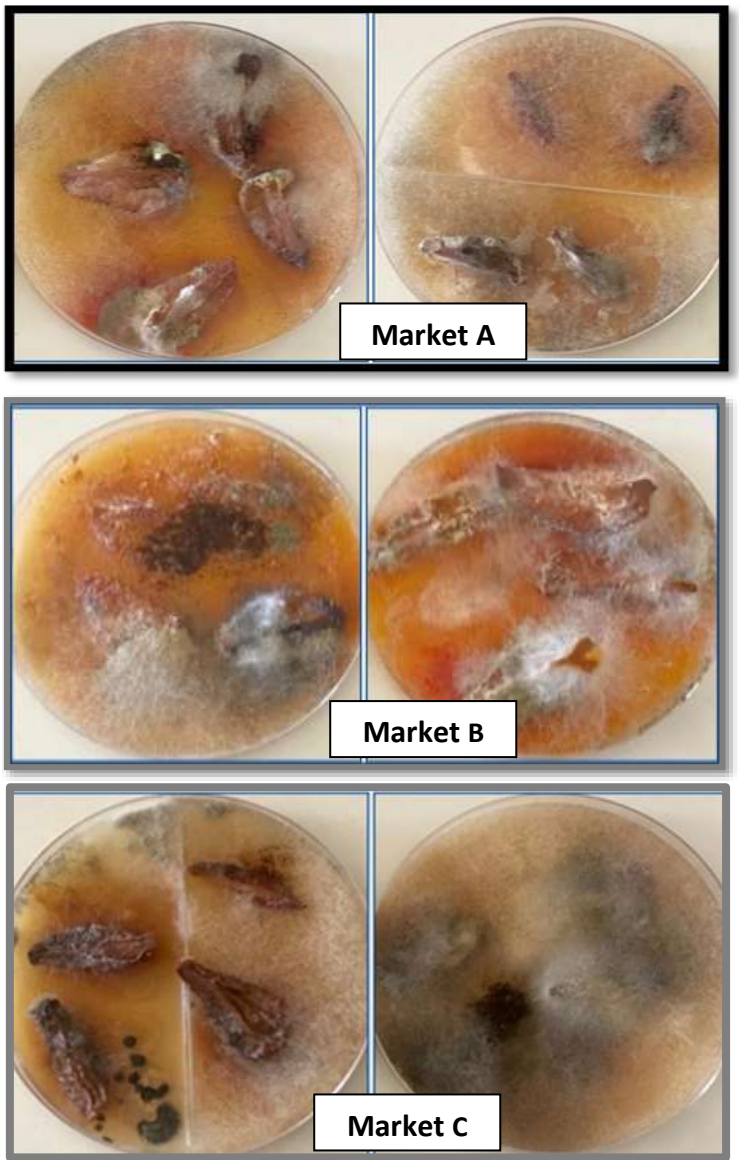

Figure 1 : Fungal contamination of sterilized and unsterilized strawberries (Market A, B, C).

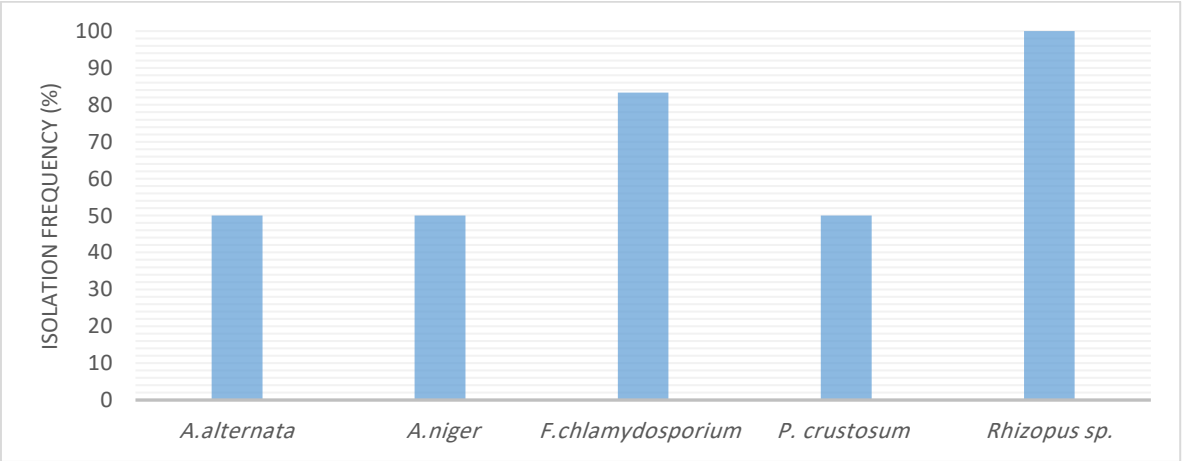

Figure 2: Isolation frequency (%) of the five fungal species isolated from strawberry fruits across three local markets (A, B, and C) in Al-Bayda City.

Alternaria alternata

The *A. alternata* colony first exhibited a white and fluffy appearance, accompanied by abundant aerial mycelium that progressively changed to a gray color. The conidiophores varied in length, ranging from short to long. They were either simple or branching, and they grew individually. The conidiophores ranged in color from golden to brown, with septa arranged both horizontally and vertically. Conidia were born, beaked, with thick walls and brown. Fig 3. The findings were consistent with those [24].

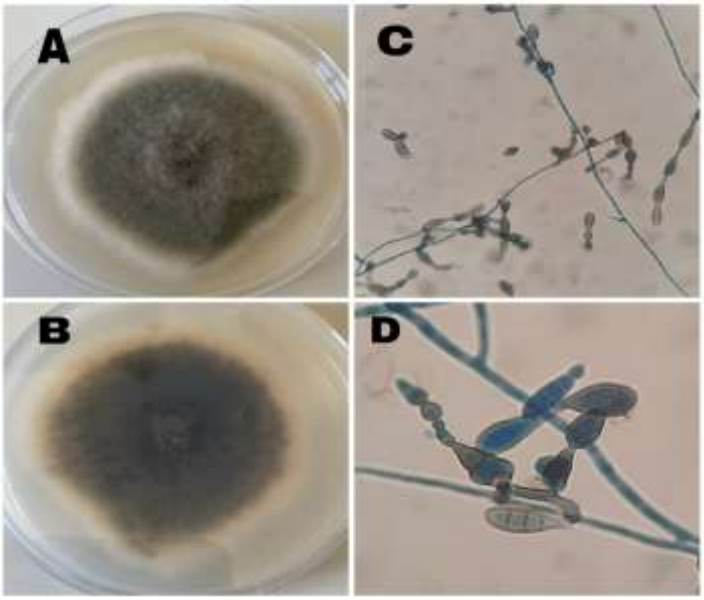

Figure 3 *Alternaria alternata*: A- Growth colonies on PDA at 28ºC, B- Reverse, C- Septate mycelium and conidiophores, D- Conidia in chains.

Aspergillus niger

This genus is characterized by large, spherical, dark brown, and biseriate. The conidia themselves are spherical with rough walls, while the smooth walls in conidiophores. The colonial features are Black Colonies with white edges. as seen in Fig 4. The results were in agreement with [25].

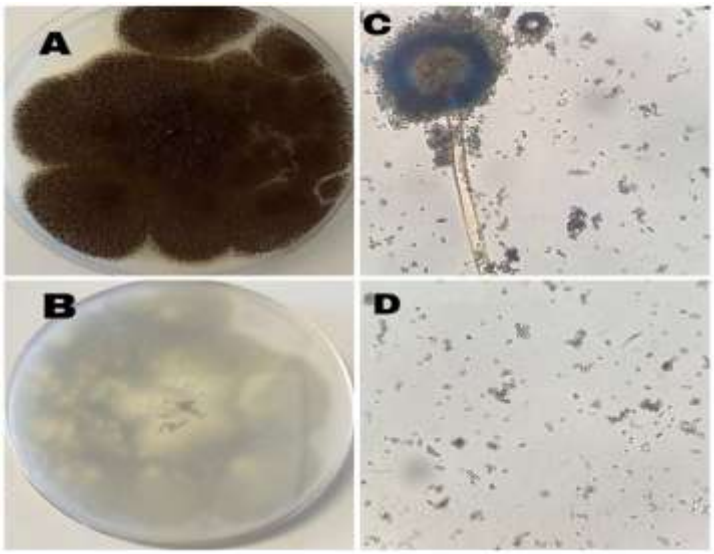

Figure 4 ***Aspergillus niger*:** A- Growth colonies on PDA at 28ºC, B- Reverse, C-conidiophores and radial conidial head and sterigmata typically in two series, thickly covering the vesicle, D- conidia.

Fusarium chlamydosporium

Septate hyphae, morphological features of Microconidia are oval. Macroconidia are slightly curved and pointed at both ends, borne on phialides which are on branched conidiophores. Colonial features are varying in color from white to Congo pink. As shown in Fig 5. The results were in line with the findings reported by [25].

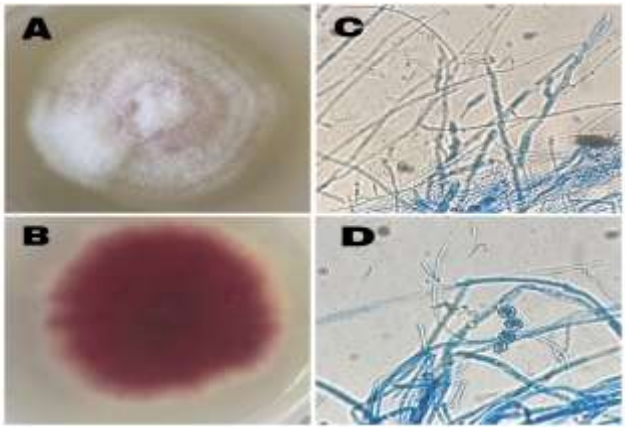

Figure 5 ***Fusarium chlamydosporum*****:** A- Growth colony on PDA at 28ºC, B- Reverse, C- Conidiophores are short branched and polyphialides and conidia, D- Septate mycelium and chlamydospores.

Penicillium crustosum

The morphological characteristics of this genus are as follows: the conidia are small and smooth, and the fungal hyphae are arranged irregularly on branches of varying lengths. Colonial features are Green and velvety. Fig 6. The results align with the studies carried out by [25].

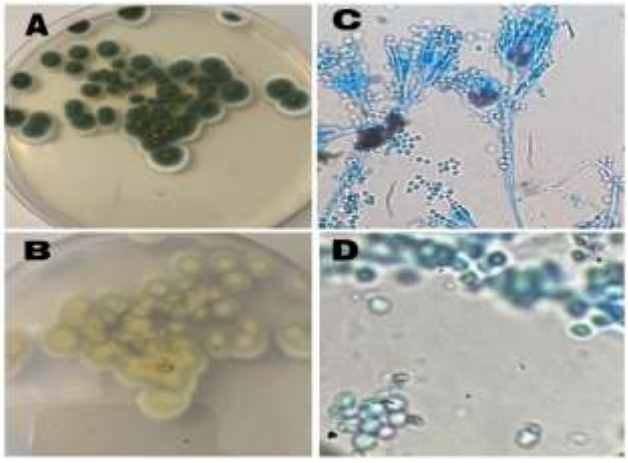

Figure 6 ***Penicillium crustosum*****:** A- Growth colonies on PDA at 28ºC, B- Reverse, C- terverticillate conidiophores and Metulae and phialides (sterigmata), D- conidia are globose to subglobose.

Rhizopus sp.

These fungi are characterized by aseptate hyphae, smooth-walled sporangiophores, sub globose columella, and oval-shaped sporangiospore. The colonies are initially white, growing rapidly and filling a Petri dish with dense, cotton-like hyphae, which then turn dark brown over time as seen in Fig 7. The results were in agreement with [26].

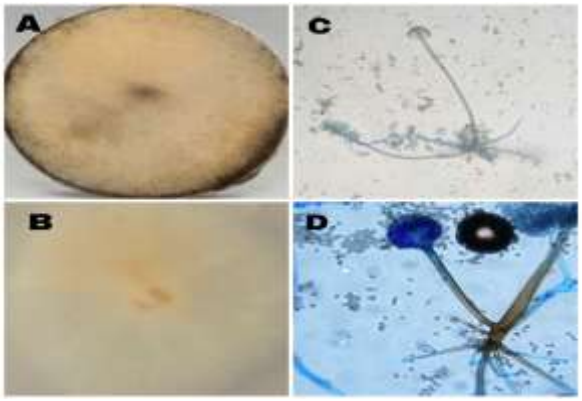

Figure 7 ***Rhizopus sp.*****:** A- Growth colonies on PDA at 28ºC, B- Reverse, C- Sporangium and sporangiophores, D- Rhizoids and Sporangiospores.

Conclusion

The study isolated five species of fungi from strawberries: *Alternaria alternata, Aspergillus niger., Penicillium crustosum, Fusarium chlamydosporium.* and *Rhizopus* sp., with *Rhizopus* sp. being the most prevalent. Based on these findings, it can be inferred that strawberries are heavily contaminated with fungi, This may be due to handling methods such as washing, harvesting, and sorting, as well as the fruit's exposure to environmental contamination. The study also indicates that some fungi found on spoiled strawberries may produce mycotoxins harmful to consumers. Therefore, all fruits, including strawberries, require more careful handling to be edible and safe for human health.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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