ألمجلة ألليبية لوقاية النبات
Libyan Journal of Plant Protection
http://www.ljpp.org.ly
ISSN : 2709-0329

Fungal Diseases of Strawberry in Tripoli Area of Libya with Emphasis on Gray Mold Disease.

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Received –April 29, 2025; Revision – March 15, 2025; Accepted – June 28, 2025; Available Online – August 10, 2025.
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Abstract \

This study was conducted on strawberry plant fruits, crown, leaves and the soil surrounding the roots. Samples were collected from Tripoli and its vicinity. Fourteen genera of fungi were isolated including B. *cinerea, Alternaria* spp., *Colletotrichum* spp., *Fusarium* spp., *Rhizoctonia* spp. and other genera. Based on the morphological characteristic and the pathogenicity degree of the fungus; *Botrytis cinerea* was proved to be the causal agent of the gray mold disease in Tripoli area. This study showed strong evidence that covering of strawberry fruits with polyethylene will indeed increase the rate of spreading *B. cinerea* on the fruits during marketing and storage. This study is the first to document the isolation and identification of strawberry fungal diseases in Tripoli and its suburbs.

Key words: Strawberry, gray mold disease, grape, *Botrytis cinerea*, Libya

Introduction \

Strawberry is a perennial, stoloniferous herbaceous belongs to the family Rosaceae, genus *Fragaria* , There are several varieties of strawberries, only one species, *F. vesca* is cultivated in Libya [17], It is one of the most popular fruits growing in the Northern hemisphere in temperate and sub temperate environment zone [5]. Strawberry is highly perishaple fruit due to its extreme tenderness, its vulnerability to mechanical damage, high level of respiration and its susceptibility to fungal spoilage [8,20]. For these reasons the strawberry has a very limited postharvest life and cannot be stored except briefly [8]. There are many pests and diseases affect strawberry crop, which are caused by fungi, bacteria, viruses and nematodes, whether in the field or during transportation, storage or marketing facilities. Strawberry diseases caused by fungi lead to significant economic losses throughout the world [24]. Strawberry is affected by several fungal diseases such as: coronary mold disease caused by *Phytophthora cactorum* [29], Black root disease caused by *Rhizoctonia* sp. [6], leaf spots caused by *Alternaria* sp. [3], Charcoal rot of strawberry, which is caused by *Macrophomina phaseolina* [20]. Verticillium wilt disease caused by *Veticillium albo-atrum* [24]. Strawberry is also infected with anthracnose caused by *colletotrichum* spp. [12,15] and frequently infected with both leaf scorch *Diplocarpon earlianawere* [23] and rhizobic mold caused by *Rhizopus stolonifera*. Moreover [27] reported that the most Common strawberry phytopathogenic fungi are *Botrytis cinerea*, *Colletotrichum acutatum*, *Phytophthora cactorum*, *Mycosphaerella fragariae*, *Verticillium* and *Rhizopus* sp.

Botrytis fruit rot, also known as gray mold, caused by *Botrytis cinerea* and is one of the most important diseases of strawberry worldwide. The disease affects fruit in the field resulting in severe pre-harvest losses. It also affects fruit after harvest, since infections that begin in the field continue to develop during storage [11]. The name *Botrytis cinerea* is derived directly from the fungus' morphology: "*Botrytis*" is named after the Greek word for “bunch of grape berries”, describing the grape-like morphology of conidiophores, and "cinerea" refers to the gray color of sporulation, *Botrytis cinerea* was first described by Persoon and the name was accepted by the Swedish botanist Magnus Fries [13,14]. The gray mold disease caused by *Botrytis cinerea* is considered one of the most important diseases which affect strawberry production world wide, the infection start from the petals, stamens and pistils of the flowers and then the fruit. The infections are not developing until the weather

becomes favourable for the disease [7,18,22,31], It was found that the primary source of the flowers infection was from old senescent leaves [22,26]. *Botrytis cinerea* is an opportunistic fungus with a wide range of hosts, which can colonize dead tissue and from abundant germs. The fungus favors daily temperatures ranging from 27-28 ◦C when plants are wet for long periods, and can cause crop losses of up to 50%, and is dangerous for the strawberry crop when the period of moisture extends during the stages of flowering and holding fruits [2]. *Botrytis cinerea* the causal agent of grey mold or botrytis bunch rot in grapes is responsible for significant economic damage in vineyards worldwide [10]. The grape crop is one of the most economically important fruit crops with 8 million hectares in the world [30] and the estimated crop losses due to *B. cinerea* were 2 billion US per annum [9]. The aim of this study is to survey and identify some of the strawberry diseases caused by fungi.

Materials and methods \

The experiments of this work were carried out in at the laboratory of the Botany Department - Faculty of Science / University of Tripoli.

Sample collection and fungal isoltion: Samples were collected from various fields and markets in the city of Tripoli, specifically from the area of Tajoura, Al-Madal, Al-Nasheea, Qaraboolly and Ein- Zara. Fourty samples from leaves, crown and soil were randomly collected from strawberry fields. However to determine the effect of humidity on the spread of *Botrytis cinerea* infection on strawberry fruits two hundred samples of fruits were collected randomly from different markets, classified to 100 samples covered with transparent polycethylen and 100 samples not covered by transparent polyethylene during spring and summer 2016-2017-2018 [22]. Strawberry infected tissues were cut with sterilized scalpel, superficially sterilized in 10% sodium hypochlorite solution for two min., then washed three times with sterilized distilled water, and dried on sterilized filter paper, then the specimens were plated on PDA-medium and incubated at (25± 2˚C) for 5-7 days. Transfer each fungus with the same characteristics and morphology into separate petridishes [22]. To isolate pathogenic fungi from soil of strawberry fields, serial dilution was performed on PDA-medium.one gram of each soil sample was suspended in 10 ml of sterile distilled water. Serial dilution was performed by adding 1 ml of soil suspension into 9 ml of sterile distilled water to get dilution of 10 -1. Transfer 1 ml of each dillution to PDA-medium, incubated at (25± 2˚C) for 5-7 [1] Small fragment of each infected were transferred onto potato dextrose gar (PDA) plates

and icubated at 25°C for 5-7 days. All isolates from leaves, fruits and soil were purified by transferring hyphal tips from the edges of developing colonies to fresh PDA plates. All isolated cultures were kept in PDA slants and stored at a temperature of 4°C [1].

Morphological characterization: All isolates were incubated on PDA plates to detect color, density, growth, texture, and aerial hyphae of the colonies. These include the shape, color, texture of the colony, as well as the presence of spores, it is shape and the number of cell in each spore. Microscopic examination of fungal isolates were recorded to measure the size and the shape of spores and then the morphological characteristics were compared with those available in the literature [28].

Counting the percentage for the presence of fungi: The percentage for the presence of each genus of the isolated fungi from the fruits, crown, leave, and soil was colculated then were compared to each other the following equation was used for this purpose:

% of the fungus presence = No. of samples which containe one genus / No. of total sample*100

Pathogenisty test: To determine the pathogenicity of the *Botrytis* isolates, Healthy strawberry leaves and fruits were washed by tap water and sterilized with SDW. Disinfected leaves and fruits were distributed on petridishes containing moistened filterpaper. A small piece of *Botrytis spp* growth was transferred to the surface of the wounded leaves and fruits. Control fruits and leaves were treated the same way, but a sterile PDA disk was placed in each wound instead of fungal growth. All inoculated leaves and fruits of strawberry were kept at room temperature for one week, then results were evaluated.

Host range of *Botrytis* isolates: A Grape plant was used for this experiment.

The same procedure used for the pathogenicity test of *Botytis* on strawberry leaves and fruits was used for the host rang of *Botrytis* on grape leaves and fruits.

Results and discussion \

Identification of fungi isolated from different parts of strawberry plants and from the soil: Figure 1 shows the fungi isolated in this study. Fourteen genera of fungi were identified. The most prominent fungus isolated from the leaves was *Alternaria* spp. (87%), followed by *Fusarium* spp. (42%) and *Botrytis* spp. (32%) (Figure1.A). These fungi can cause foliar and wilt diseases on stawberry as reported by [3,27]. Figure 1.B Shows the fungi isolated from the crown. The highest percentage was recorded for, *Fusarium* spp. (65%), *Colletotrichum* spp.

(47%), *Alternariaspp and mucor* spp. (40%) *and Rhizoctonia* spp. (22%) respectively. These fungi can cause different diseases on strawberry plant as reported by [6,16,18]. *Botrytis* spp, which was reported as *B. cinerea* was the most prominant fungus isolated from the fruits, (Figure 2.B). *B. cinerea* is considered the most important fungus whic can cause heavy loses on strawberry fruits. Regarding density, the fungi isolated from the soil showed different percentages (Figure 2.A). Figures 3 showed the morphogical characters of fungi isolated from strawberry plant and from the soil. The results obtained considered the first record in Tripoli area and its visinity**.** It is recomended that other studies showd be done in different areas of strawberry production in Libya including the effects of *Fusarium* spp., *Colletotrichum* spp*., Alternaria* spp. *and Rhizoctonia* spp. on strawberry plants in the field.

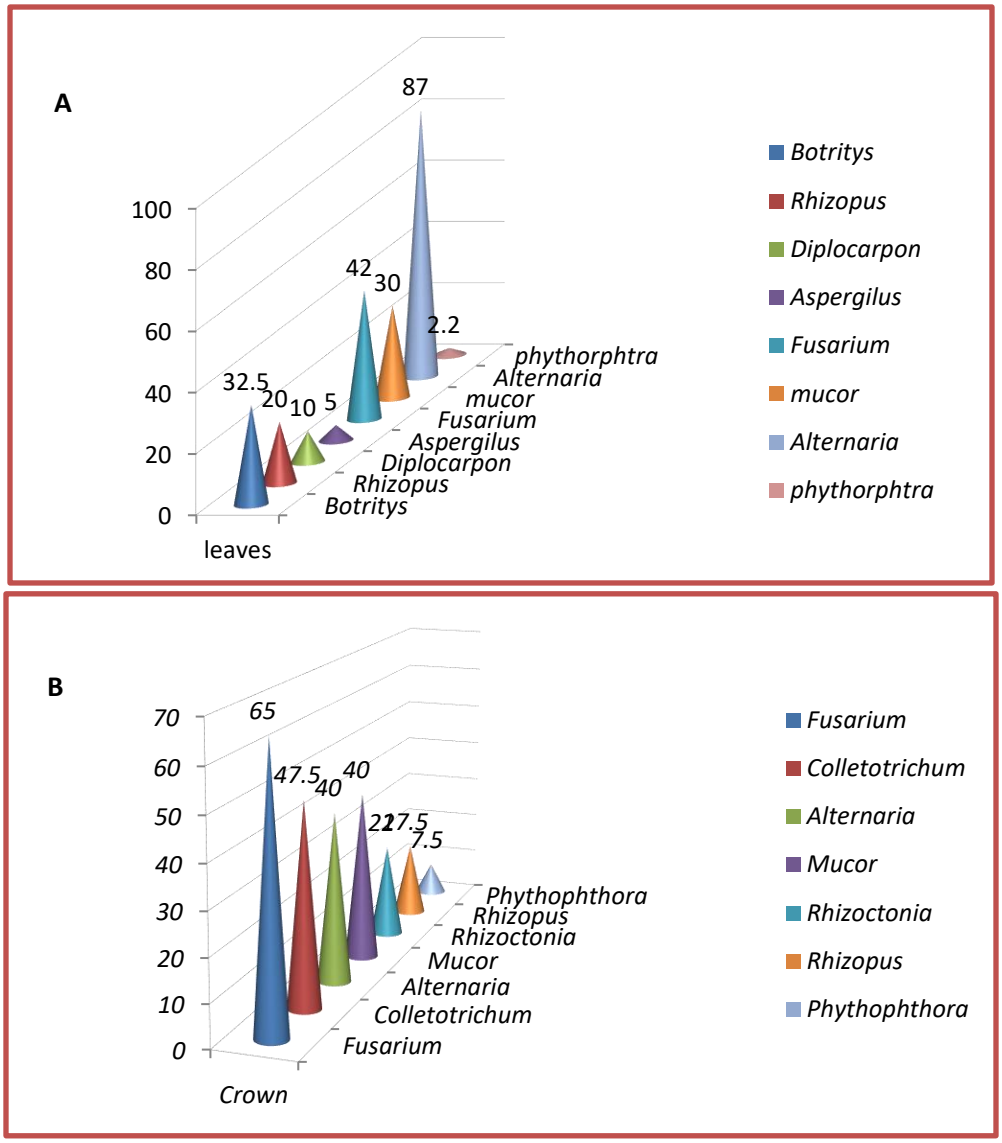


Figure 1. The Percentage of fungi isolated from strawberry leaves and crown.

A - The Percentage of fungi isolated from strawberry leaves.

B - The Percentage of fungi isolated from strawberry crown.

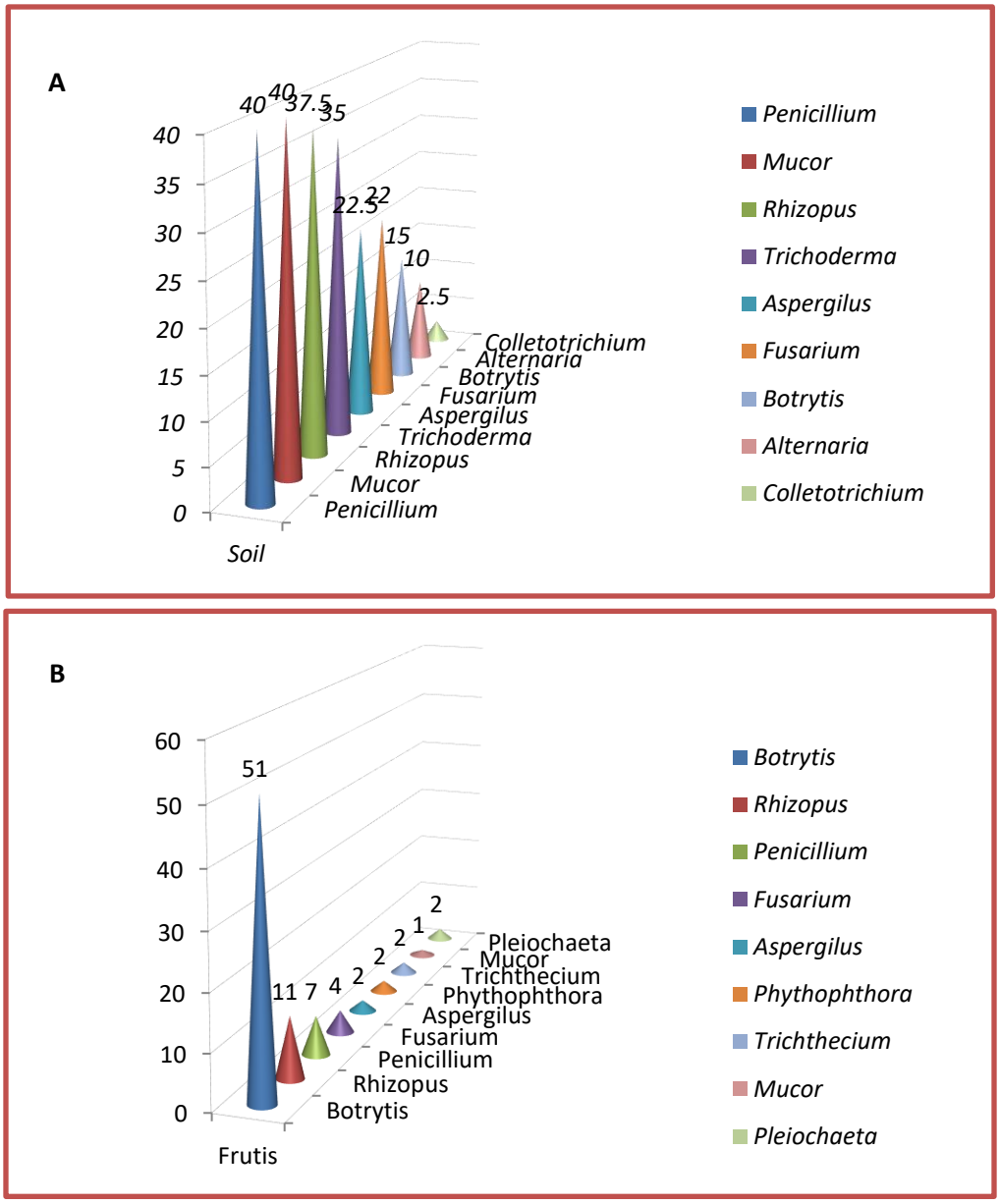
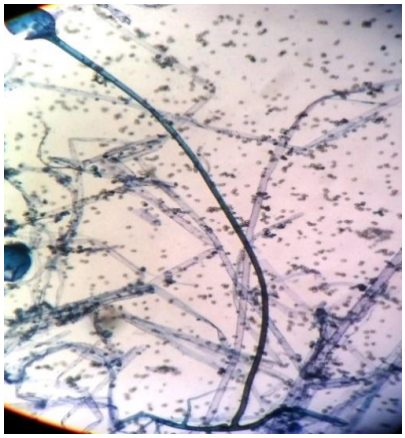


Figure 2. The Percentage of fungi isolated from strawberry fruits and soil.

A - The Percentage of fungi isolated from soil.

B - The Percentage of fungi isolated from strawberry fruits.

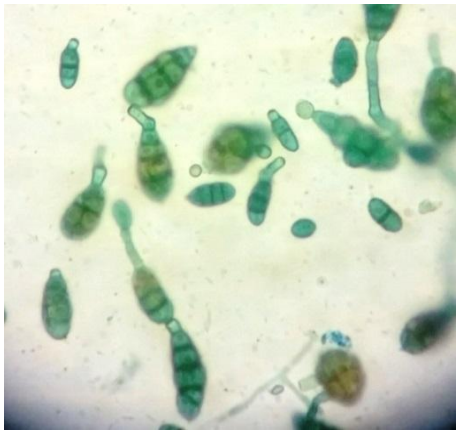
Botrytis cinerea



Mucor spp.

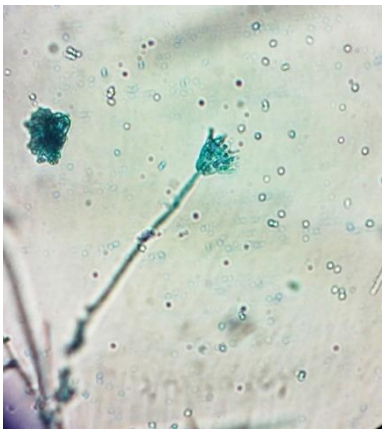
Fusarium spp.

Asperaillus spp.

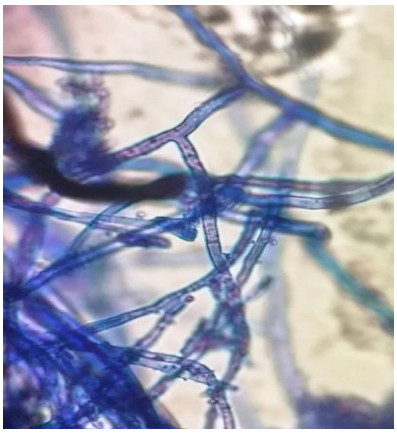


Alternaria

Pleiochaeta spp.

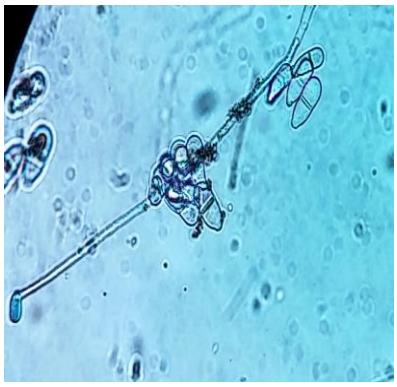


***Penicillium* spp.**

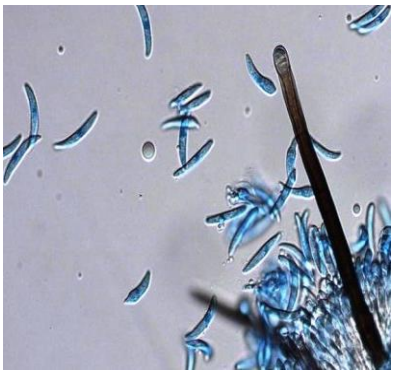


***Rhizoctonia* spp.**

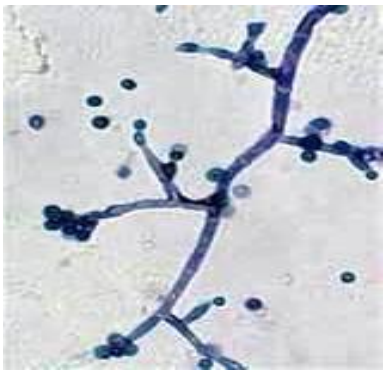
***Rhizopus* spp.**



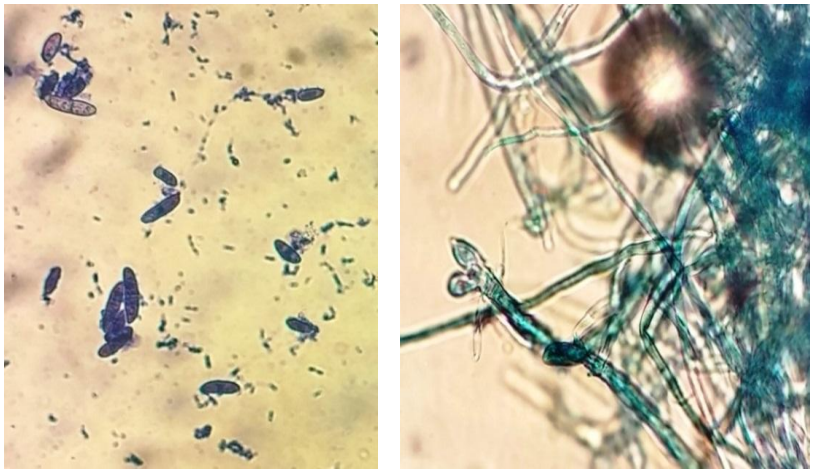
***Tricothecium* sp.**



***Colletotrichum* spp.**



***Trichoderma* spp.**



***Aschocyta* spp.** ***Phythophthora* spp.**

***Diplocarpon* spp.**

Figure 3. Morphogical of fungi isolated from strawberry plant and from the soil.

Identification of *Botrytis* spp: *Botrytis* was considered the most important fungus infected strawberry plant as indicated in previous studies [7,18,22,31]. *Botrytis* was identified to the species level. Results indicated the culturs of the fungus were gray or greyish brown. Sclerotia black in color produced on PDA medium, vary in size,with black colour. Conidiophores, dark, tall, errect, branching irregularly or dictomously, septate 2-3 mm in length. Terminnal cells swell conidia arising simulataneously on each ampula, globose to avoid, 10^{-12} x 6-9 mM. The most characteristic feature of the genus is the dark, branching conidiophores which bear clusters of gray conidia on denticles from apical ampulae [4].

Figure 4 shows the morpholigical of the fungus. The results were confirmed when compared to the characteristis of *Botrytis cinerea* in diffrent identification keys. The identification of *Botrytis cinerea* on strawberry plant is considered the first report in Libya.

To confirm that *Botrytis cinerea* is the causal agent of gray mold on strawberry plants. A pathogenecity test was carried on the fruits and leaves of strawberyy. The results as showed in figure 5 that *Botrytis cinerea* is the causal agent of the disease on strawberry.

On the other hand a host range study was conducted on grape plants collected from Tripoli area. The results showed that isolates of the fungus from strawberry plants can cause infection on grap plants (Figure 6). It is recomended that further studies should be conducted to isolate *Botrytis* spp from grape and compare it with the isolate of *Botrytis* from strawberry plant using PCR method.

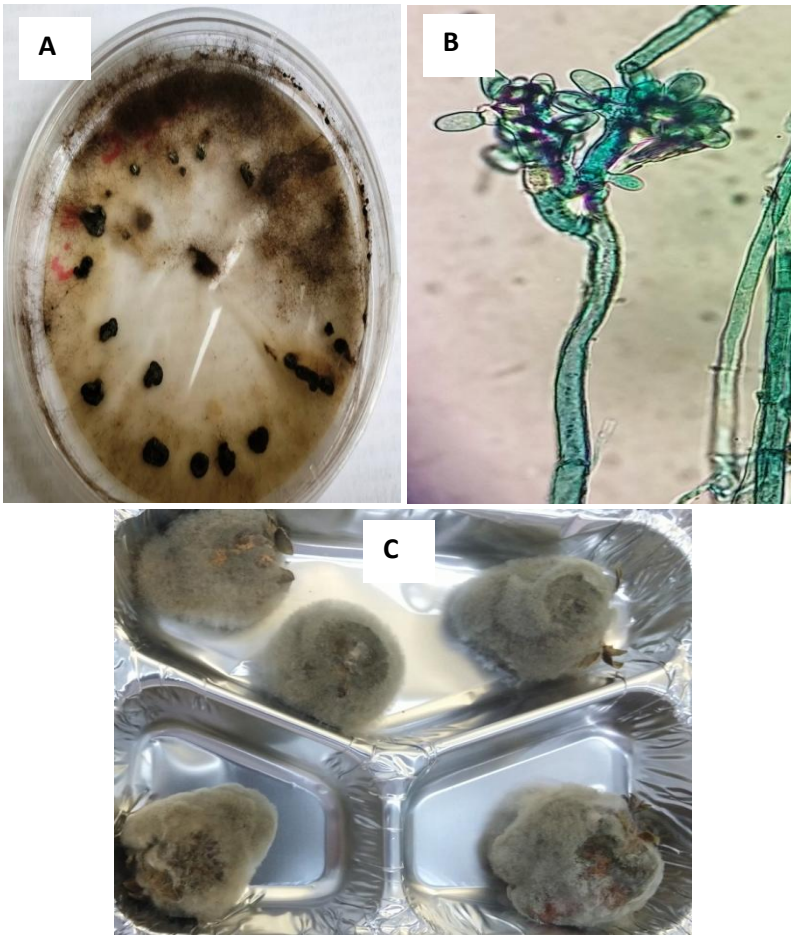


Figure 4. A conidiophore, spore, gray mycelia and sclerotia of *B. cinerea*

A – conidiophore and spore of B.cinerea.

B – Sclerotia of *B.cinerea*.

C – Gray mycelia of *B.cinerea.*

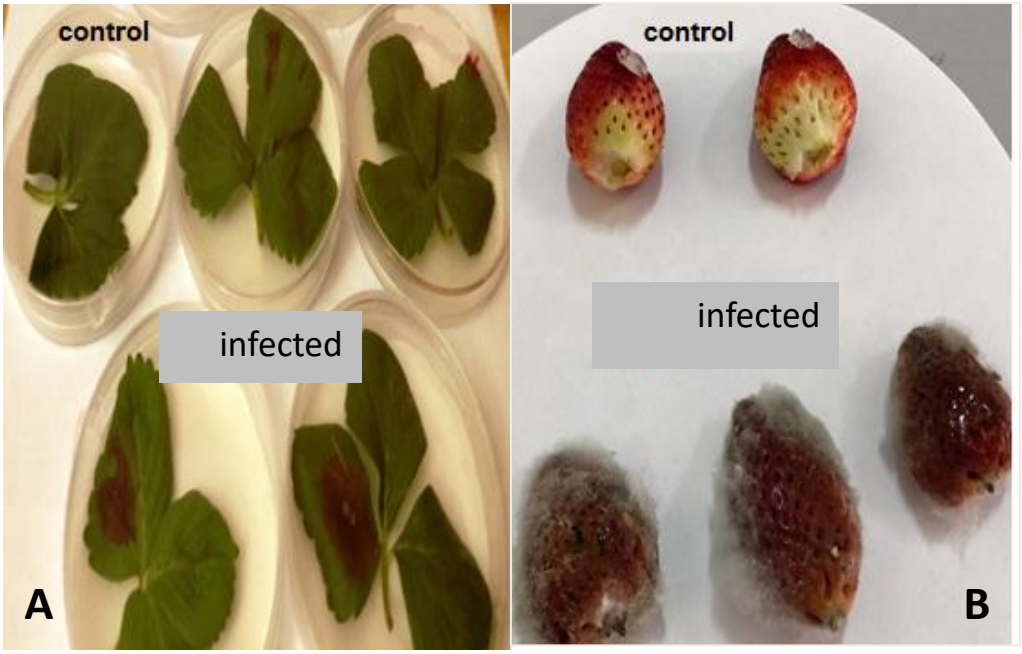


Figure 5. Pathogenicity test on strawberry leaves and fruits.

A - Pathogenicity test on strawberry leaves.

B - Pathogenicity test on strawberry fruits.

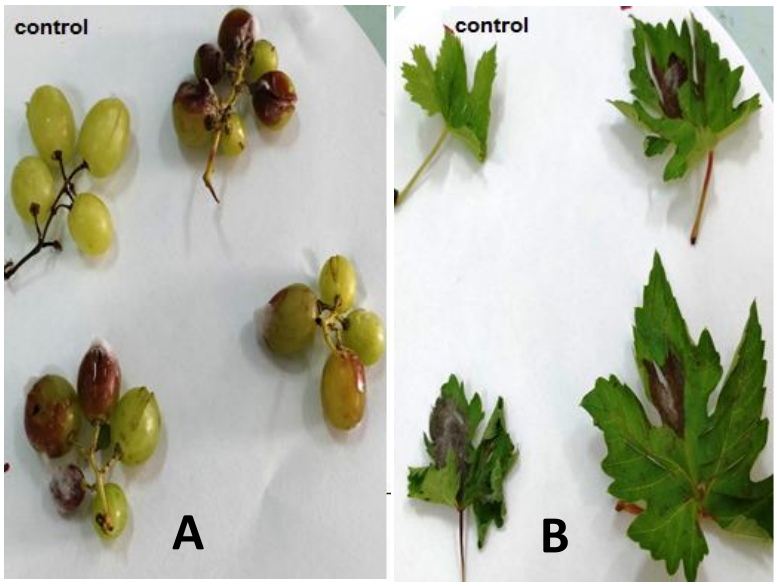


Figure 6. Pathogenicity test of *B. cinerea* isolated from strawberry on grape fruits and leaves.

A - Pathogenicity test of *B. cinerea* isolated from strawberry on grape fruits.

B - Pathogenicity test of *B. cinerea* isolated from strawberry on grape leave.

Effect of humidity on the spread of *Botrytis cinerea* infection on strawbery fruits: Strawbery fruits covered by polyethylene showed faster rate of infection compared to the non covered fruits. The comparison of treatment between the covared and non covered fruits. Among isolated fungi, *B. cinerea* was the most prominent fungus in infected strawberry fruits. No significant differences between covared and non covered fruits in regard to rate of infection with *B. cinerea* (Figure 7) . Therefore strawberry fruits should not be covered after harvest to avoid the accumulation of humity which may increase the spread of the fungi.

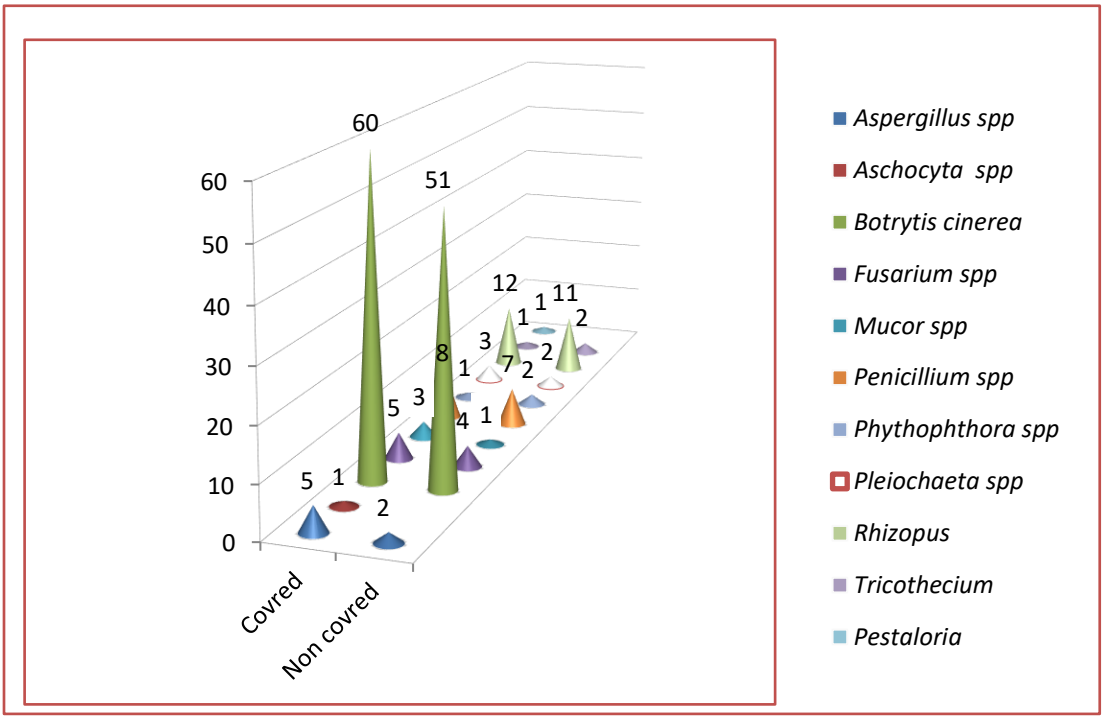


Figure 7. Percentage of fungi isolated from strawberry fruits covred and non covred with polyethylene

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أمراض الفراولة المتسببة عن الفطريات في طرابلس ليبيا وضواحيها مع التركيز على مرض العفن الرمادي

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أجريت هذه الدراسة خلال الموسم الزراعي 2016-2018 على نبات الفراولة والتي تشمل الأوراق, التاج, الثمار والتربة المحيطة بالجذور. جُمعت العينات من منطقة طرابلس والمناطق المحيطة بها. تم عزل أربعة عشر جنسا من الفطريات من أهمها *Botrytis cinerea,* *Alternaria* spp, *Colletotrichum* spp, *Fusarium* spp, *Rhizoctonia* spp وأجناس أخرى من الفطريات. برهنت الدراسات على أن فطر *Botrytis cinerea* هو المسبب لمرض العفن الرمادي في منطقة طرابلس طبقا للموصفات المورفولوجية والقدرة الأمراضية لهذا الفطر. أظهرت الدراسة أن تغطية ثمار الفراولة بغشاء من البولي إيثلين الشفاف يساعد على انتشار فطر *Botrytis cinerea* على ثمار الفراولة أثناء فترة التسويق والتخزين. تعتبر هذه الدراسة هي الأولى في ليبيا التي تم فيها عزل وتعريف الفطريات الممرضة لمحصول الفراولة في منطقة طرابلس وضواحيها.

الكلمات الدالة: العفن الرمادي، الفراولة، العنب، ليبيا.