

Isolation and Identification of pathogenic fungi causing Apple disease in Baghdad

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ABSTRACT:

Fungi are the main cause of most plant diseases, including those that grow and produce mycotoxins that are dangerous to humans and their health. This study was conducted to isolate and identifying types of fungi that infect fruit-bearing apple trees, which were collected from three areas in Baghdad (Abu Ghraib, Al-Fahhama, and Al-Rashidiya) over a period of three months (August, September, and October). Nine types of fungi were isolated (*Schizophyllum commune*), *Fusarium solani*, *Alternaria solani*, *Fusarium oxysporum*, *Curvularia hawaiiensis*, *Penicillium chrysogenum*, *Aspergillus niger* and *Aspergillus flavus*), and the most prevalent isolate was *Alternaria solani*, with a frequency of (26.53%), followed by *A. niger*, (21.52%) while *Fusarium solani* (19.68), *Aspergillus flavus* (17.48%), *Scisophyllum commune* (5.5%), *Curvularia hawaiiensis* (4.4%) *Fusarium oxysporum* (3.06%) and *Penicillium chrysogenum* (1.83%). The study includes several issues, including educating farmers and selecting tree varieties that are resistant to fungi.

Keywords : Fungi, apple tree , mycotoxins.

عزل وتشخيص الفطريات

المسببة للأمراض التي تصيب التفاح في بغداد

حنين علاء عبد الرزاق ، عادل حمدان علوان ، نضال عبد الامير علي

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مستخلص

تعد الفطريات المسبب الرئيسي لمعظم أمراض النباتات، خاصة تلك المنتجة للسموم الفطرية والتي تشكل خطراً على صحة الإنسان. أجريت هذه الدراسة لعزل وتشخيص أنواع الفطريات التي تصيب أشجار التفاح المثمرة والتي جمعت من ثلاث مناطق في بغداد (أبو غريب، الفحامة، الراشدية) ولمدة ثلاثة أشهر (آب، أيلول، تشرين الأول). عزلت 9 أنواع فطرية (*Schizophyllum commune*)، *Fusarium solani*، *Alternaria solani*، *Fusarium oxysporum*، *Curvularia hawaiiensis*، *Penicillium chrysogenum*، *Aspergillus niger* و *Aspergillus flavus*، وكانت العزلة الأكثر انتشاراً هي *Alternaria solani* بنسبة تكرار (26.53%)، تليها *A. niger* (21.52%)، بينما سجلت *Fusarium solani* نسبة (19.68%) و *Aspergillus flavus* (17.48%) و *Scisophyllum commune* (5.5%) و *Curvularia hawaiiensis* (4.4%) و *Fusarium oxysporum* (3.06%) و *Penicillium chrysogenum* (1.83%). اقترحت هذه الدراسة عدة قضايا منها تثقيف المزارعين واختيار أصناف لأشجار مقاومة للفطريات.

الكلمات المفتاحية: الفطريات، شجرة التفاح، السموم الفطرية.

Introduction

Fruits are very important in human nutrition, as they provide the human body with many necessary growth factors, such as essential minerals and vitamins, which help maintain human health[1] [2].

Apples are considered one of the excellent table fruits loved by many people due to their distinctive taste and many health benefits because they contain nutrients for humans, especially fats, carbohydrates, calcium, iron, and antioxidants. Because it contains nutrients and sugars [3].

The phrase “an apple a day, keeps the doctor away” is commonly used to highlight the many health advantages that are commonly linked to consuming this fruit. Nevertheless, the efficacy of these advantages has primarily been demonstrated through in vitro and in vivo experiments, along with a limited number of clinical trials. Several observational studies have demonstrated positive associations between consuming apples and a reduced likelihood of developing various chronic diseases or experiencing mortality due to such diseases [4].

it helps Easily spoiled by fungi , And the growth of fungi and yeasts. Recent research has found that more than twenty-five percent of the fruits and vegetables produced are exposed to spoilage, especially during the harvest period and relatively long storage periods[5].

While winter prunings are crucial, it has been observed that postharvest pathogens, specifically *Alternaria* and *Fusarium*, can lead to heart rot, potentially as a result of early flower infection. Furthermore, certain pathogens can exist on the surface of wood tissues, while others have already established colonies and are currently present within those tissues[6].

The primary factors contributing to apple decay are the fungal species *Penicillium expansum* and *Monilinia fructigena* [7]. Additional fungal genera that were obtained from apples include *Colletotrichum*, *Xylaria*, *Botryosphaeria*, and *Rhizopus oryzae* [8]. *Aspergillus* spp. has been identified and recognized as a source of infections or allergies [9].

This study was conducted to identify and isolate the types of fungi that grow on apple fruit trees in the city of Baghdad.

Materials and methods

Study area

The study was conducted on the city of Baghdad, which is the capital of Iraq, with an area of 657 square kilometers according to the report of the Central Statistical Organization for the year 2018.

The coordinates are located at length 33.3128057 and width 44.3614875. Its climate is characterized by a desert climate, characterized by little rain in the winter and hot, dry summers in which average temperatures reach 45c .

Sample collection

Collection operations were carried out from several apple orchards located in three areas of Baghdad (Abu Ghraib, Al-Fakhama, Al-Rashidiya) over two periods.

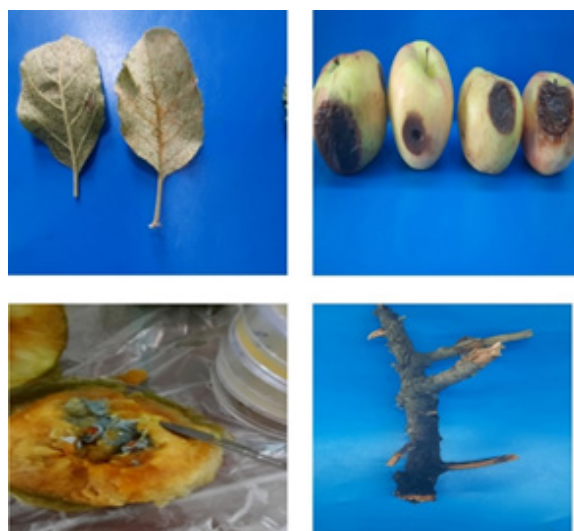


Figure1: Some of fungal infection on different parts of the apple plant

The first period was before the winter pruning (dormancy period) in August, and the second period was during the vegetative growth period in the months of September and October. The samples contained all parts of plants of varying severity of infection, and they were transported in plastic bags and in refrigerated containers to the mycology laboratory. In the Department of Life Sciences at Al-Mustansiriya University for the purpose of isolating fungi from them.

Surface sterilization of roots

Collected roots from apple trees were soaked in sterile water for 5 hours at room temperature before processing for rhizobial isolation purpose. Then roots were washed with 70% ethanol for 1 minute followed by 3min washing with 2% NaOCl. Then, roots were washed 7 times with sterile distilled water (dH₂O) to remove extra surface disinfectant[10].

Isolation and identification of fungi

The sterilized root, stem leaves, and fruits samples were cut into pieces (0.5 × 0.5) cm and placed in Petri dishes containing potato dextrose agar(PDA) after wash plant parts under tap water [11]. medium is giving positive result. . The identification of the pathogens

on the twigs up to the level of genera was accomplished by studying the reproductive structures of the pathogens (such as conidia, ascospores, pycnidia, or perithecia) using an Olympus light microscope after the incubation period [12]. The morphological characteristics of the pathogens were cross-referenced with the existing literature

to verify their genera. The presence of pathogens in each plant was reported.

Then, a number was allocated to each group, and on the basis of that, a representative isolate of the group was randomly selected for molecular analysis, and then the fungal frequency was calculated according to the equation The following :

$$\text{percentage of species frequency} = \frac{\text{colonies number of species}}{\text{total number of species colonies}} \times 100$$

Identification of Endophytic Fungi by molecular method:

The DNA extraction was performed using the Qiaamp Mini Kit (Qiagen) with some modifications. Small sub-unit ribosomal RNA was then amplified by PCR using primer pairs ITS1/ITS4b For the PCR analysis, specific

sterile tubes were made, and the reaction mixture's constituent parts were Primer 1 µl Forward, Reverse primer 1 pl. DNA 5 µl and 13 µl of deionized free nuclease water to PCR tube contain master mix (bioneer), then put them in PCR device[11].



Figure 2 Verification of fungal DNA by gel electrophoresis of PCR.

Result and Discussion

The results showed that they were infected with eight different isolates of fungi, which are as follows (*Schizophyllum commune*, *Fusarium solani*, *Alternaria solani*, *Fusarium oxysporum*, *Curvularia hawaiiensis*, *Penicillium chrysogenum*, *Aspergillus niger*, *Aspergillus flavus*), figure (3) and the percentages of their appearance and the number of colonies were as shown in the table (1).



Figure 3 growth of fungi in PDA agar

They are more or less responsible for damage to fruits and some of these isolated fungi, such as *Aspergillus flavus* are producers of mycotoxins that have a number of harmful effects on health[13].

Table 1 The total count and frequency of occurrence of different fungal strains, and also their percentage of frequency, were determined in 30 samples on Potato Dextrose Agar (PDA) supplemented with chloramphenicol (30 mg/l).

Isolates	Total count	Occurrence %	Frequency
Alternaria solani	217	26.53	11
Aspergillus flavus	143	17.48	9
Aspergillus niger	176	21.52	11
Curvularia hawaiiensis	36	4.40	2
Fusarium oxysporum	25	3.06	1
Fusarium solani	161	19.68	12
Penicillium chrysogenum	15	1.83	2
Schizophyllum commune	45	5.50	2
Total	818	100.00	

The majority of the fungal organisms identified in this research are crucial in causing food spoilage and contaminating feed systems. Additionally, some of these organisms have the ability to produce harmful substances

that can be toxic to both humans and animals. The fungi mentioned can produce mycotoxins that have various harmful effects on health, such as being carcinogenic, immunotoxic, neurotoxic, and hepatotoxic [14].

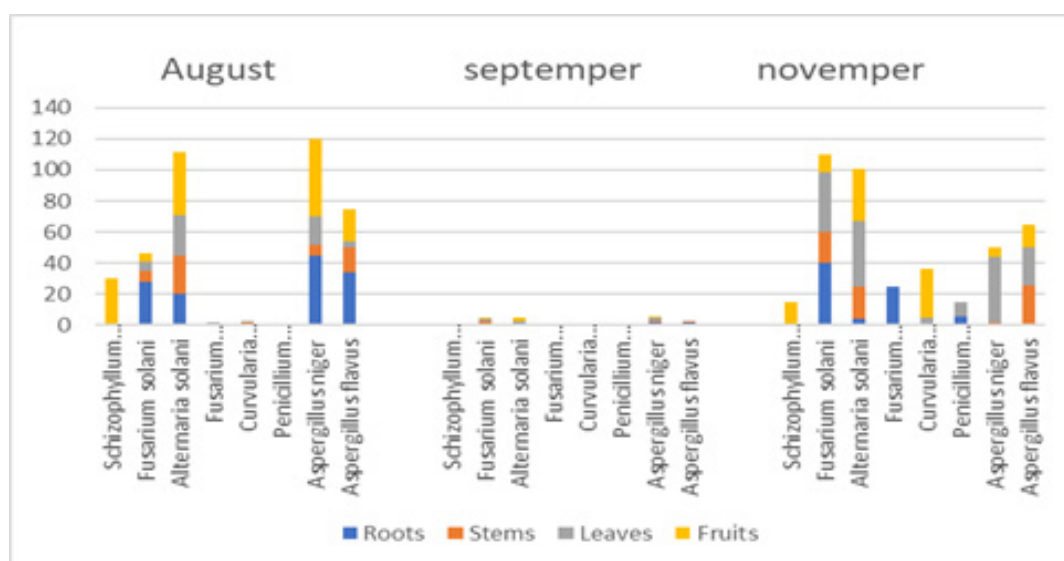


Figure 4 Number of fungi present in the stems, roots, fruits and leaves of the apple tree in the months of August, September and October.

Among the fungi isolated in this study, the most prevalent one was *Alternaria solani*, which is an airborne fungus. It was also found that relative humidity is a significant factor that strongly affects the infection of *Alternaria* spp. [15]. and the occurrence of *Alternaria* leaf spot [16]. Consequently, the increase in temperature during the vegetative growth phase, combined with the presence of rainfall, may have created optimal circumstances for the

pathogen to produce spores in the twigs [17].

There are no fungicides currently available that are both effective and environmentally friendly for controlling this fungus. The most prevalent fungi causing diseases on certain fruits and vegetables are *A. niger* (26.53%), which causes black mold and produces harmful mycotoxins called ochratoxins, followed by *fusarium solani* (19.68%), *aspergillus flavus* (17.48%),

scizophyllum commune (5.5%), curvularia hawaiiensis (4.4%), fusarium oxysporum (3.06%), and penicillium chrysogenum (1.83%). These frequencies of occurrence are shown in Table 1. However, Tafinta et al. [18] reported a frequency of occurrence of 36%, 25%, 22%, and 17% for *R. stolonifer*, *A. flavus*, *A. fumigatus*, and *A. niger*, respectively, from some fruit. The variations can be ascribed to the types of fruits, their quantities, and the disparity between Iraq and Niger, which were analyzed in both studies.

Penicillium species are a group of fungi. The majority of fungi found in the environment are generally recorded as non-pathogenic to humans[19][20].

Most fungal infections occurred in August and October, whereas on winter pruning no fungi were found in the evaluated root stems, leaves and fruit (Figure 4).

Most report indicated that *Schizophyllum commune* it is a type of fungi found in the wild on decaying trees, The species was recorded as nonpoisonous by [21]. The first report indicate infected of apple wood rot with *Schizophyllum commune* was in Tunisia on apple stem[22].

While the other isolated fungi are present in varying proportion after the end of winter pruning as mentioned in (Figure 4). *Fusarium*, *Curvularia* and *Pencilinium sp.*, are clearly absent in dormant period. Fungal species causing apple tree infested were significantly higher in November especially for leaf as mentioned for *Fusarium solani*, *Alternaria solani* and *Aspergillus niger* (Figure 4).The reasons for multiple species causing the same disease on one host are not understood and further research needed to determine the role of genetic recombination affecting fruit and trees.

However in this study *Alternaria solani* showed that the widespread prevalence during August and November (Figure 4).respect to the other isolated species and this may not due to its pathogenicity or greater virulence over the other, *Alternaria sp.*, has other competitive or ecological fitness advantages over the other species, which could explain the high prevalence of the species. Biological or ecological fitness of pathogen is a combination of pathogenic traits, such as aggressiveness and infection efficiency [23], with other fitness traits, such as sporulation and growth rate [24][25].

Pencillium chrysogenum isolated only from infected roots in the vegetation growth[26], demonstrated several *Pencillium* species which are common apple rot-causing pathogens.

Alternaria solani and *Aspergillus niger* caused fruit spot alternating as shown in (Figure 3). No infested symptoms for *Fusarium* sp., contrary to *Fusarium solani* for both collection during dormancy and vegetative growth, the incidence of the genus *Fusarium* on vegetative growth did not differ (Figure 3).

The increase in the incidence of the majority of fungi can be explained by the lower affecting of fungicide during winter pruning, which is one of the basis controls when the removal of infested symptom [27].

CONCLUSIONS

Apple and pear trees grown in Baghdad are exposed to various fungal diseases, which are one of the main factors responsible for the decline in crop quality. Among the diseases, gum, fire blight, and apple scab were the most serious diseases that occurred in most of the areas included in the study. Powdery mildew has been observed on apples in a few fields in some locations.

Therefore, we can recommend that management strategies require certain practices, including:

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