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Biodiversity of some Species of *Alternaria* Fungi Causing Spotting in Ornamental Plants

Noor A. Al-healy
Medical Laboratory Technique/ Al Noor University Collage/ Mosul/ Iraq
Warka S. Al-Taee
Department of Biology/ College of Science/ University of Mosul

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corresponding author:
Warka S. Al-Taee
warsbio22@uomosul.edu.iq

ABSTRACT

The pathogenic fungi causing leaf spotting of several species of ornamental plants were isolates, diagnosed and purified. a (229) fungal isolates belonging to several fungal genera was obtained and the species belonging to the genus *Alternaria* were selected to conduct experiments on them. Where (89) isolates of the fungal type *Alternaria dianthi* and (68) isolates of the type *A.alternata* were obtained in addition to three isolates of the type *A.longipes* and one isolate of the type *A.radicine*. The phenotypic qualities represented by the forms of colonies, which are considered evidence in classification and innate diagnosis, have also been studied, and the colors of colonies belonging to this genus ranged from dark olive to pale in addition to some other phenotypic qualities such as the emergence of the phenomenon of sectors characterized by some fungal isolates.

Keywords: *Alternaria dianthi*, spotting, *Alternaria Alternata*, Ornamental plants, leaf spotting, pathogen.

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INTRODUCTION

The fungal kingdom is interest in the study, which 100,000 species are described and 15,000 cause diseases to plants (Maharshi and Thaker, 2012). The complexity of pathogenic mechanisms in fungi at the morphological level is increased due to Increased fungal and plant diversity, as fungi form highly specialized structures of infection (Hawkswort, 1991; Horbach et al., 2011). The genus Alternaria species have been described based on the form (Kordalewskal et al., 2015). It is dedicated to the breadwinner and includes pathogenic species, (Woudenberg et al., 2015). As well as the ability of this genus to widely distributed and thus cause agricultural problems (Espiritu, 2018; Meena1 et al., 2020; Nishikawa and Nakashima, 2020). For example, Alternaria alternata alone is known for its ability to infect more than 100 species of plant, causing leaf spotting and other diseases (Rotem, 1994; Thomma, 2003; Shoaib et al., 2021). And that the occurrence of plant disease is the result of a complex interaction of the rapidly susceptible host plant and the presence of the pathogenic organism and the environment. Infected flowers are usually not exportable and are not recommended in local markets (Singh et al., 2016). Chase (1998) showed that species of the genus Alternaria cause diseases of ornamental plants, infecting their leaves and flowers, and when severe injuries occur, they cause the leaves to fall out quickly. In particular, type A. dianthi and A. dinthicola, which cause infection to the dianthus plant, the infection appears on the corolla leaves and is purple in color and then black lumps are spores inside (Pryor and Michailides, 2002) showing symptoms of infection with the fungus Alternaria in the form of spots on leaf spots (Gao et al., 2020; Kgatle et al., 2018; Li et al., 2022) or in the form of leaf blights (Laemmlen, 2001; Kokaeva et al., 2017).

Leaf Spots diseases is a term given to several diseases that have an impact on the leaves of trees for shade and ornamental plants that are usually caused by the presence of fungi or may sometimes occur due to bacteria as damage can occur in plant leaves and these spots vary in size and color depending on the affected plant and the stage of development of the disease and the affected organ for example, the symptoms of spotting on the leaves of the Leaf blight disease of jasminum appear as a result of infection with the fungus Alternaria alternata in the form of irregular brown areas surrounded by dark colored beams that appear on the surface of the leaf that sometimes cause "fire necrosis symptoms" (Matic et al., 2020). When the plant itself is infected with the fungus Alternaria jasmine, it appears in the form of circular spots of reddish-brown color with a diameter of (8-2) mm on the upper surface of the leaf, and the edges of the leaves appear affected internal wrinkles become hard and brittle, and in acute infection the terminal bouton and small branches are dry out, the infected leaves must be collected and burned to get rid of them (Singh et al., 2016). When the rose of ornamental plants are infected with the fungus Alternaria it causes them great losses, and the symptoms appear in the form of small to irregular oval spots, light brown to black scattered initially observed on the edge of the leaves which later grow and merge to cover the entire surface of the leaf, and the pathogens remain on the affected leaves and other parts of the plant, the fungal type Alternaria dianthi cause Rot diseases of flower bud to the plant and the symptoms of the disease appear mainly on young floral buds leading to their rotting, necrosis and discoloration of the stem, hood and small flowers also become brown. At the last stage of the disease the bud become wilt, dark brown and dry. Symptoms are less pronounced in mature buds but these buds do not bloom. As well as brown necrotic spots that appear on the edges and tops of old leaves (Singh et al., 2016; Matic et al., 2020).

Ornamental plants include all plants used for decorative purposes, with floral sales ranging from \$40-60 billion (Lecomte *et al.*, 2016). Many fungal pathogens cause leaf spotting and falling into their incubating plants and arguably the most harmful and widespread are caused by species of the genus Alternaria (Matić *et al.*, 2020). the most common fungal species found in ornamental plants are *Alternaria dianthi*, *Alternaria alternata* and *Alternaria dinthicola* which possess a wide range of plant hosts such as Ficus, Dahlia and Hedera. A single plant can also be attacked by more than one species of the genus *Altenaria*, as well as the presence of other fungal species that cause spotting to ornamental plants and include *Bipolaris*, *Corynespora*, *Drechslera*, *Helminthosporium*,

Stemphylium and Ulocladium. This fungus is closely associated with the genus Alternaria. Home gardens are a unique ecosystem, and the ornamental plants in them are exposed to many pathogens such as rusts that form fungi of virulence that cause damage to the plant but do not survive in the absence of plant host tissues, other species such as Botrytis that are opportunistic and found everywhere and attack only physiologically weak plants. The use of chemical control to control these pathogens is based on each group, in initially It is important to determine the nature of the disease to choose the right substance in control (Matić et al., 2019). and to use different fungicides because repeated use of the same fungicide causes the target pathogen to be resistant (Chung Hong, 2018).

MATERIAL AND METHODS

Sample Collection:

Samples of the leaves of ornamental plants infected with leaf spotting disease were collected from the nursery areas in Mosul including: Al-Faisaliah, Al-Darqzliyah, Mohandessin and Gardens of the University of Mosul for the period from the beginning of November 2017 to May 2018 and the rate of a visit every 20 days and these samples were represented by taking infected leaves for each of the plants (*Aralia elegantissima, Schefflera arboricola, Dracaena Fragrans, Thuja, Ficus, Nephrolepis exaltata, Syngonium Podophllum, Hedera, Lablab, Yucca, Gardenia Jasmineoides, Rosa, Gerbera Jasmesonii, Iris*). They were packed in clean Nylon bags labeled, and transported to the laboratory for study.

Potato-Sucrose Agar (PSA) Medium

This medium was used to isolate and preserve the used isolates and activate them and save the medium until use (Pitt and Hocking, 2009; Sibounnavonng *et al.*, 2009).

Isolation of fungus Alternaria from the leaves of infected Ornamental plants

Plant leaves that showed symptoms of spotting were selected and distributed to dishes containing potato extract, sucrose and agar, incubated in the incubator for 7 days at temperature (2±25) °C with daily observation during the incubation period (Naik *et al.*, 2010). After the end of the incubation period, the fungal isolates of the genus *Alternaria* were identified and preserved.

Conditions of preservation of fungal isolates

The various fungal isolates were kept in an inclined shape inside the Slants test tubes in the refrigerator at a temperature of 4°C, and the plantations were renovated every two weeks. (Aggarwal *et al.*, 2014).

Unification of fungal isolates of the genus Alternaria

The Single spore technique was used to purify fungal isolates (Simmons, 2007). For pure fungal cultures originating from a single fungal spore (Pryor and Michailides, 2002).

Diagnosis of fungal isolates

Lactofenol stain (phenol 20g, glycerol 40ml, lactic acid 20ml, distilled water 20ml) was used. To diagnose fungal isolates of the genus Alternaria using the implantation method on the slide which was described by (Deshmukh, 1998; Benson, 2002; Koneman *et al.*, 1997) and using the diagnostic keys described by (Ellis, 1971, 1976).

Morphological characteristics of the colony

The medium of potatoes, sucrose and agar was used to observe the formal qualities of colonies of all isolates of the genus *Alternaria* after their development on the center of the PSA where (7) qualities were selected based on taxonomic keys (Ellis, 1971; Moubasher, 1993; Pitt and Hocking, 2009) to describe the fungal colony.

Table 1: Morphological Characteristics of the fungal Colonies Approved for the Classification of Isolates of the genus *Alternaria* (Altaee, 2007)

Adjective	Extent					
The color of the colony	dark olive	pale olive	Gradient from pale to dark olive			
The symfort of the colony	Smooth	Warts	Cotton			
The surface of the colony	Un rough	Rough				
The center of the colony	Different	Not different				
Colonial edges	wide white edge	narrow white edge				
The perimeter of the colony	Plane	An uneven plane				
The phenomenon of sectors	Present	Non present				
The beek of the colony	dark olive		pale olive gradient to			
The back of the colony	Dark olive to black	gray to olive	bleach			

RESULT AND DISCUSSION

Fungi isolated from infected ornamental plants

The fungus was isolated from the leaf spots of various types of infected ornamental plants, and (229) fungal isolation of species belonging to the genus *Alternaria* as well as other fungal genera was obtained from the beginning of November 2017 until May 2018 during visits every 20 days as shown in (Table 2). Ornamental plants that are most susceptible to fungal infections during this period were selected, and cause economic losses to nursery owners as a result of their high prices and other plant species, despite their low price, have been selected but are among the most sought-after native plants. These plants fall into seven botanical groups, Some of them within the group of woody plants include thuja, aralia, shefflera, dracaena, ficus, and other plants within ferns including nephrolepis, group of climbers and purls including hedera, Syngonium and lablab, group of succulent plants including yucca plant, group of perennial herbaceous plants including gerbera, group of ornamental shrubs including cardenia and rosa and the group of ornamental bulbs represented by the Iris plant, and these plants belong to different plant families, namely:

Asparagaceae, Araliaceae, Agaraceae, Moraceae, Cupressaceace, Popilionaceae, Araceae, Lomariopsidaceae, Asteraceae, Rosaceae, Rubiaceae, Iridaceae.

The lowest incidence of fungi was recorded during the month of July and August due to the high temperature leading to the cessation of the vital activities of most fungi, which does not encourage any fungal infection, while fungal infections increase during the months of February and March to raise the temperature to the ideal degree of infection (30-25)°C. Some of these selected plants were flowering ornamental plants of aesthetic importance to gardens and nurseries and others of climbers that are considered to be vegetation that remains green throughout the year Fern and woody plant species were also selected. The fungal species of the genus Alternaria that appeared during isolation have varied, and this fungal genus is known to cause diseases of ornamental plants and in cases of severe infection may cause their leaves to fall (Chase, 1998). Also, the difference of the fungal species shown during isolation is due to the difference of the plant species isolated from it and four fungal species belonging to the genus Alternaria were obtained, namely A. dianthi, A. longipies, A. radicine, A. alternata and the fungal species A. dianthi was the most frequent as (15) isolation was obtained With a percentage of 100% isolation in Nephrolepis and (13) isolation of the same species and an isolation rate of 81.3% in the Ficus plant, while the number of isolates in the Dracaena plant was (15) isolation and with an isolation rate of 68.2%, and in the Shefflera plant the number of isolates (12) isolation and a percentage of isolation of 66.6%, while the Gerbera plant had a percentage of isolation of 65.5% and the number of isolates (19) isolation. It was followed by the fungal type A. alternata with (68) fungal isolates in all plants, while the type A. longipies was the least frequent with three isolates in lablab plant and a percentage of isolation of 17%. The fungal species A. radicine also has one isolation and a 12% isolation in the Thuja plant. Other fungal species also appeared during isolation, namely Stemphylium.sp, particularly in the yucca plant, where the number of isolates was (14) isolates and a percentage of isolation of 100% and

Ulocladium.sp, and in the Syngonium plant with an isolation rate of 6.25% and one isolation, which are considered to be taxonomic brothers of the genus Alternaria (Pryor and Gilbretson, 2000). as well as Cladiosporum sp. whose number of isolates in lablab plant was (14) isolates and with an isolation rate of 93%. Thus, it turns out that the most contagious and frequent fungal species in ornamental plants is A. dianthi because of its possession of this peculiarity in infecting ornamental plants (Thomma, 2003). In practice, the genus Alternaria depends in its pathogenesis on the resistance and sensitivity of the host (Meena et al., 2017a). The increase in disease is also influenced by various physical factors such as heat, light and pH (Jadhav and Wadikar, 2018). Therefore, we note a high incidence of fungal infection in plant species such as the rosa and the syngonium with thin leaves, while the species A. dianthi, A. alternata is absent in the yucca plant due to the thickness of its leaves and its resistance. And explain the emergence of one isolation for each of the species A. radicine in the thuja plant and three isolates A. longpies in lablab, although they specialize in infecting plant families as the type A. longipies are known to be infected with the tobacco plant (Meena et al., 2017). Type A. radicine infects the carrot plant, causing its black rot (Laemmlen, 2001), but this does not prevent them from infecting other plant families as pathogens when positive conditions are available to cause infection in the same season and thus the disease becomes a problem for these plant species, as stated (Thomma, 2003). Some species of the genus Alternaria can penetrate the tissues of the plant and remain static until the appropriate surrounding conditions are available and the infection appears.

Table 2: Fungi isolated from ornamental plants for the period from the beginning of November 2017 to May 2018

November 2017 to May 2018								
Percenta ge of Insulatio n	Numbe r of Isolate s	Fungal Species	Scientific Name	Common Name of Type	Botanical Family	Classi	nnical ficatio n	
21 31.5 47.4	4 6 9	A. alternate Cladiosporiu m sp. Stemphylium sp.	Finger Aralia plant paper	Aralia elegantissi ma	Araliaceae			
100	19		To	otal				
66.6	12 6	A. dianthi A. dianthi isolation A. dianthi Stemphylium sp.	Umberlla tree	Schefflera arboricola	Araliaceae			
100	18		Total					
22.7 68.2 9.09	5 15 2	Cladiosporiu m sp. Alternaria dianthi Alternaria alternate	Madagascar Dragontree	Dracaena Fragrans	Asparagaceae	Wooden plants	Leafy plants	
100	22	Total						
12 50 38	1 4 3	Alternaeia radicine Alternaria alternata Cladiosporiu m sp.	Thuja Orientalis (Biot Orientalis)	Thuja	Cupressaceace			
100	8	Total						
18.8	3	A. alternate A. dianthi	Ficus Hawaii	Ficus	Moraceae			

Percenta ge of Insulatio n	Numbe r of Isolate s	Fungal Species	Scientific Name of Type		Botanical Family	Botanical Classificatio n			
81.3	13	A. dianthi							
		isolation							
100	16	A. dianthi	To	l otal		1			
100				Nephrolepis	Lomariopsidace				
100	15	A. dianthi	dianthi Ladder Fern exaltata		ae	Ferns			
100	15		To	otal	1	Й			
6.25 62.5 31.3	1 10 5	Ulcladium sp. Stamphilum sp. A. alternate	Goose foot plant,Syngoniu m	Syngonium Podophllum	Araceae				
100	16		To	otal		and ers			
100	13	A. alternate	Canary island IVY	Hedera	Araliaceae	Purls and Climbers			
100	13		Total						
82 17.6	14 3	Cladiosporu m sp. A. longipies	Lablab Dolichos	Lablab	Popilionaceae				
100	17	3.7.00	1						
100	14	Stamphilum Spineless Yucca Asparagaceae sp.				Succule nt plants			
100	14	Total							
55 45	11 9	A. alternate A. dianthi	Gardenia Cape,Jasmine	Gardenia Jasmineoid es	Rubiaceae	Ornamental bushes			
100	20	Total							
69.2 30.8	9 4	A. alternata A. dianthi	Rose damascene	Rose mascene Rosa Rosaceae		Orname	Ornamental Bushis		
100	13			enta					
34.5 65.5	10 19	A. alternata A. dianthi	Gerbera Diasy,Barbeto n Daisy	Gerbera Jasmesonii	Asteraceae	Perennial herb	Orname		
100	29								
77.8 22.2	7 2	A. alternata A. dianthi	Iris Cristata	Iris	Iridaceae	Ornam ental bulbs			
100	9	Total							
229)	Total number of isolates							

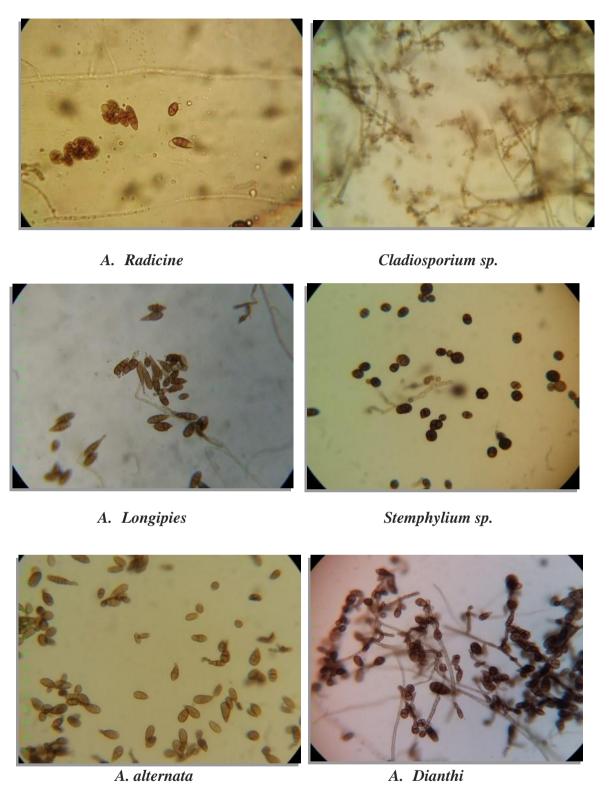


Fig. 1: Microscopic image of fungal species isolated under 40X zoom force, image resolution 8 MP.

Formal qualities of the colony

The variation in the traits of fungal cultures of all species visible during isolation belonging to the genus *Alternaria* and based on the taxonomic keys of the (Table 3) within the materials and methods, as the colors of the fungal colonies of the genus *Alternaria* ranged from dark olive to light as well as the color of the back of the colony between dark olive and gray to olive and pale olive to

white. The surface of the colony is characterized as smooth or contains cotton-shaped fluffs. The study showed that all colonies are not producing pigments that cause the medium color change and this is in line with their findings (Chethana et al., 2018). The innate isolation of A. dianthi4a isolated from the schefflera plant was characterized the surface color of the colony was pale olive with fluffs with wide edges and the center of the colony in some of them showed a difference, while the back of the colony was of a dark olive color to black with the perimeter of a zigzag colony as shown in Fig. (2). As for the fungal isolates of the type A. alternata, the isolation A. alternata 5 taken from the hedera plant was characterized the surface of the colony is smooth with wide and flat edges, and in some isolates the edges were narrow, as the color of the back of the colony ranged from brown to olive, and in other isolates belonging to the same species and was of a dark olive color with a different colony center, as well as the phenomenon of sectors in a distinctive way in it, which can express that the fungal filaments includes inside the nuclei and is a cellular integrated Some may vary heterokaryotic spores produce spores of two different colors and the growth of homokaryon produces a different color sector as the appearance of this phenomenon can be considered as a result of the pyramid of the colony due to the repeated transplantation of fungal filaments at the edge of the colony (Burnett, 1976). These sectors usually appear at or within the edge of the colony and differ in color from the rest of the colony. As for the fungal isolation of the type A. radicine shown in the thuja plant, the color of the colony was dark olive, a smooth surface with narrow edges, the color of the back of the colony gray to olive, the absence of the phenomenon of sectors in it, and the perimeter of the colony was flat, and this is confirmed (Gilbertson and Pryor, 2000) about the characteristics of the fungal colony. As for the fungal isolates of the type A. longipies isolated from the lablab plant, the colony had a smooth surface and a dark olive color, and the back of the colony was gray to olive in color and the phenomenon of sectors in it did not appear, and these qualities are similar to what he mentioned (Andersen et al., 2001).

Table 3 Formal qualities of the fungal colonies of some species of the genus Alternaria

Isolation	Color of the colony	Color of the back of the colony	Center of the colony	Edges of the colony	Phenomenon of sectors	Surface of the colony	Perimeter of the colony
A. dianthi ₅	Pale olive to bleach	olive to dark	No differ	Narrow	Present	Cotton	Plane
A. dianthi ₃	pale olive	Gray to Olive	Differ	Wide	No present	Rough	Plane
A. dianthi ₇	dark olive	Pale olive to bleach	No differ	wide	No present	Smooth	Plane
A. alternata ₄	dark olive	Dark olive gradient to pale	No differ	Wide	No present	Rough	Plane
A. dianthi _{6a}	dark olive	Dark olive to pale	No differ	Wide	No present	Smooth	Zigzag
A. dianthi ₂	dark olive	Pale olive to bleach	Differ	Narrow	No present	Smooth	Plane
A. radicine	dark olive	Gray to Olive	No differ	Narrow	No present	Smooth	Plane
A. dianthi _{4a}	Dark olive to black	Pale olive	Differ	Wide	Present	Rough	Plane\ zigzag
A. alternata ₅	Dark olive	Gray to olive	Differ	Wide	Present	Smooth	Plane
A. dianthi $_1$	Dark olive	Dark olive	Differ	Narrow	No present	smooth	Plane
A. alternata $_{I}$	Gray to black	dark olive	Differ	Narrow	No present	Rough	Plane
A. longipies	Dark olive	Gray to olive	Differ	Narrow	No present	Smooth	Plane
A. alternata ₁	Gray to black	dark olive	Differ	Narrow	No present	Rough	Plane
A. longipies	Dark olive	Gray to olive	Differ	Narrow	No present	Smooth	Plane

Isolation	Color of the colony	Color of the back of the colony	Center of the colony	Edges of the colony	Phenomenon of sectors	Surface of the colony	Perimeter of the colony
A. alternata ₁	Gray to black	dark olive	Differ	Narrow	No present	Rough	Plane
A. longipies	Dark olive	Gray to olive	Differ	Narrow	No present	Smooth	Plane
A. alternata ₁	Gray to black	dark olive	Differ	Narrow	No present	Rough	Plane
A. longipies	Dark olive	Gray to olive	Differ	Narrow	No present	Smooth	Plane

Pathogenesis and symptoms of infection with the fungus Alternaria in various types of ornamental plants

The development of signs of plant disease is associated with the accumulation of mycotoxins produced by fungi that colonize the living tissues of the plant and are responsible for the production and accumulation of these toxins within the plant tissue over time, as well as have a biological effect on the functions of plant organs and this effect is not noticeable as a result of the presence of mycotoxin, despite the absence of signs of the disease (Ismaiel and Papenbrock, 2015). Through the collected samples of the leaves of ornamental plants with spotting, it was found that the symptoms of the disease of the genus Alternaria were in the form of dark brown to black, multi-wide, centered in the form of rings and surrounded by a different color, as in the Fig. (2). Chester (1950) explained that the symptoms of spotting in nursery plants caused by infection with Alternaria fungus formed on the leaves are in the form of light brown spots and then turn dark brown over time as a result of the death of plant tissues, and may be irregular or irregular in shape (Woudenberg et al., 2015). If the spotting is caused by infection with type A. dianthi, the spots are luminous brown with purplebrown edges on the stems and leaves, the lower leaves attack first and then the disease progresses upwards to the upper leaves. As harm increases it leads to corruption and early death of the leaves (Kumar et al., 2012). Since the species of the genus Alternaria spp. have the ability to produce specialized and non-specialized toxins, they have the ability to spread to neighboring tissues from the site of injury and thus cause their death, as well as the species of this genus are characterized by symptoms of infection and the fact that a small number of Fungi have the ability to form such spots (Laemmlen, 2001; Aggarwal et al., 2014). Because of the necrotic nature of the Alternaria species, they lead to extensive damage to the plant (Mamgain et al., 2013).





Fig. 2: Symptoms of leaf spotting disease in various types of ornamental plants.

CONCLUSIONS

The fungi belonging to the genus *Alternaria* are among the important pathogens of Ornamental plants, especially the fungi belonging to the two species *A. dianthi* and *A. alternata*, and they are widespread in the nurseries and gardens of the city of Mosul.

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التنوع الحيوى لبعض أنواع الفطر Alternaria المسبب لتبقع أوراق نباتات الزينة

ورقاء سعيد قاسم الطائى

نور احمد شهاب احمد الحيالي

قسم علوم الحياة / كلية العلوم / جامعة الموصل

قسم تقنية المختبرات الطبية / كلية النور الجامعة

الملخص

تم عزل وتشخيص وتنقية الفطريات المُمرضة المسببة لتبقع الاوراق لعدة أنواع من نباتات الزينة، إذ تم الحصول على (229) عزلة فطرية عائدة لعدة أجناس فطرية وانتخبت الأنواع التابعة لجنس Alternaria لإجراء التجارب عليها. حيث تم الحصول على (89) عزلة للنوع الفطري A. alternata و (68) عزلة للنوع على (89) عزلة للنوع الفطري المستعمرات والتي للنوع المستعمرات والتي المستعمرات والتي المستعمرات والتي تعتبر من الادلة في التصنيف والتشخيص الفطري وقد تراوحت ألوان المستعمرات التابعة لهذا الجنس بين الزيتوني الغامق إلى الشاحب بالإضافة إلى بعض الصفات المظهرية الأخرى كظهور ظاهرة القطاعات التي تميزت بها بعض العزلات الفطرية.

الكلمات الدالة: Alternaria dianthi، فطريات، Alternaria Alternata، نباتات الزينة، تبقع الأوراق، امراضية.