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Novel Fungal Pathogens Associated with Date Palm Leaf Spot in Egypt

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Abstract

Date palms in Egypt are susceptible to leaf spot diseases caused by various fungal pathogens. The aim of this study was to identify and characterize these pathogens to understand their distribution and impact on date palm health. During 2019–2021 Leaf samples were collected at five locations for each of the five districts with infected date palms in New Valley Governorate, Egypt. The occurrence incidence and severity of disease were recorded at each location as a natural infection. Fungal isolates were identified using morphological characters and internal transcribed spacer (ITS) sequencing, yielding five genera: *Alternaria.*, *Aspergillus*, *Curvularia*, *Neoscytalidium* and *Nigrospora*. Pathogenicity assays were performed in both wounded and unwounded states to evaluate the virulence of each isolate. *Asp. terreus* showed the highest virulence using the unwounded method, while *A. terreus* and *Cur. siddiquii* were the most virulent using the wounded method. A comprehensive analysis identified 22 different fungal species, including several novel reports of leaf spot pathogens on date palms: *A. angustiovoidea*, *A. botrytis*, *Asp. Terreus*, *Cur. clavata*, *Cur. lunata*, *Cur. mebaldsii*, *Cur. siddiquii*, *Cur. specifera*, *Neo. novaehollandiae* and *Nig. lacticolonia.* These findings provide valuable insights into the diversity and virulence of fungal pathogens threatening date palm health in Egypt.

Keywords: Date palm, leaf spot diseases, fungal pathogens, disease severity, ITS

Introduction

The date palm (*Phoenix dactylifera* L.) is a multi-purpose tree cultivated for its nutrient-rich fruits as well as other components such as trunk, leaves, pulp, and seeds. The wood of the trunk is used as fuel and for various types of furniture, while the leaves are used for handicrafts, roofing, and walls. Alcohol and antibacterial gel can be made from the pulp, and high-quality oil can be obtained from the date seed for cosmetic and pharmaceutical applications. The date palm is also used as an ornamental plant in gardens and resorts. Dates are a source of carbohydrates, fiber and micronutrients and contain bioactive compounds with potential therapeutic benefits against diseases such as cancer and heart disease. The palm tree has a dioecious habit with separate male and female flowers on different trees. It is the highest species of the *Phoenix* genus, with large leaves that serve as a defense against grazing animals (Bhatt et al., 2023; Obón et al., 2023; Salomón-Torres, 2023). Leaf spot diseases in date palms pose a significant challenge to plant health and can lead to blight, spotting, and burns that negatively impact plant vigor. The diversity of leaf spot diseases complicates control and management efforts. This study aims to address these challenges in date palms in the New Valley Governorate. The specific objectives include studying date palm leaf spot pathogens, isolating and identifying causative fungi using traditional methods, mapping the distribution and severity of leaf spot in the New Valley Governorate, conducting pathogenicity tests, and using molecular techniques for precise identification of pathogenic fungi. Date palms (*Phoenix dactylifera* L.) are highly valued in Egypt for their economic and cultural importance, as they are extensively cultivated for their edible fruit and used as ornamental and landscape trees (García-Díaz et al., 2023). The date palm is a vital crop cultivated in arid and semi-arid regions worldwide, including Egypt's New Valley Governorate. Egypt leads global date production, with a cultivated area of 48,031 hectares and a yield of 1,306,762 tons in 2021, accounting for 18% of the world's date supply. However, date palms are susceptible to fungal diseases, which can significantly reduce fruit production and even lead to tree death. Leaf spot diseases, caused by various fungi such as *A.* sp., *Fusarium* sp., and *Phytophthora* sp., can cause significant yield reductions and economic hardship. Despite their impact, research on these diseases is limited. Annual yield losses due to leaf spot diseases are estimated at 50%. It is crucial to address these diseases and their associated fungal pathogens to ensure the sustainability and productivity of date palm cultivation (Aribi, 2023; Khan et al., 2023). Extensive surveys of date palm orchards worldwide have revealed the widespread nature of leaf spot diseases, with over 10 causal agents identified. These diseases manifest in various symptoms, including gray to brown powdery spots (Khudhair et al., 2015), yellow to brown semicircular or irregular spots (Alam et al., 2020), and small, dispersed brown to black irregular spots (Farrag & Abo-Elyousr, 2011). Studies in diverse regions have successfully isolated and identified numerous fungal pathogens responsible for these symptoms. In Egypt, symptomatic date palm samples yielded *A. alternata*, *Drechslera halodes*, *D. spicifera*, *Mycosphaerella* sp., *Phoma* sp., *Fusarium moniliforme*, and *F. equiseti* (El-Morsy, 1999). *Nig.* sp. has been isolated from diseased leaves exhibiting severe black spots (Khudhair et al., 2015). Similarly, research in Iran identified a pathogen responsible for circular leaf spots with concentric rings (Mirhosseini et al., 2017). Chinese investigations revealed a brown leaf spot disease with yellow margins (Tao et al., 2021). In Egypt, Diplodia leaf spot occurs on various date palm cultivars, including Zaghloul and Hayany (Abdel-Megid & Gafar, 1966). Leaf spot diseases caused by *Helminthosporium* spp. and *A.* spp. have been documented in the Al-Qassim region of Saudi Arabia (Al-Rokibah, 1991). Leaf spot diseases represent a significant threat to date palm cultivation across the Middle East and

North Africa (Hassan, 2018; Matrood et al., 2021; Russomanno et al., 2010). While *A. alternata*, *Helminthosporium* sp., and *Thieloviopsis* sp. have been identified as primary causal agents, numerous other fungi including species of *Pestalotia*, *Mycosphaerella*, and *Phoma* are also responsible (Abass et al., 2013). In Iraq, research within date palm orchards of the Shatt Al-Arab region has documented a wide range of fungal pathogens associated with leaf spots (Hassan, 2018; Mansoori, 2012; Sattar et al., 2021). Diverse phytopathogenic fungi cause leaf spot diseases in date palms, characterized by circular or elongated brown or black spots, sometimes with an oily texture. Visual identification of specific fungal pathogens is challenging (Holliday, 1995). These diseases negatively impact the yield and quality of date palm fruit (Nishad & Ahmed, 2020). Research on date palm leaf spot diseases has identified a diverse array of fungal pathogens across multiple regions. In Iraq, common culprits include *Biopolaris australiensis*, *Nig.* species, *A.* species, and many others (Al-Asad, 2010; Al-Nadabi et al., 2021). Studies from Pakistan highlight *Nig. sphaerica* (Alam et al., 2020), while *Cur.* sp. is a primary concern in Tunisia (Ben Chobba et al., 2013). Investigations in Oman identified *Mycosphaerella tassiana*, *A.* spp., and *Dreshcleri* sp. (Sam et al., 2002), and *Pseudopestalotiopsis theae* has been reported in China (Tao et al., 2021). Studies from the Middle East and North Africa further expand the list of pathogens: Qatar: *A.* sp., *Asp.* sp., and *Helmenthosorium* sp. (Manzelat, 2019). Iran: *Neopestalotiopsis clavispora* (Basavand et al., 2020). Saudi Arabia: *A. alternata* and *Xylohypha nigrescens* (Sheir et al., 1982). Egypt: *A.*, *Botryodiplodia*, *Chaetosphaeropsis*, *Diplodia*, *Fusarium*, *Graphiola*, *Gliocladium*, *Mycosphaerella*, *Phoma*, *Phomopsis* and *Thielaviopsis* (Atallah, et al., 2008; El-Deeb et al., 2006; Farrag & Abo-Elyousr, 2011). Standard protocols for identification of pathogenic fungi at the genus level include macroscopic and microscopic examinations. Macroscopic analysis focuses on colony characteristics such as color and shape, while microscopic examination includes observation of characteristics such as hyphae, conidia, conidiophores, and spore arrangement. These protocols are described in detail in various articles. The work of Rahman et al. discusses the use of deep convolutional neural networks to classify pathogenic fungi from microscopic images (Rahman et al., 2023). The work of Kowalski and Cramer's discusses the variation in the macroscopic morphology of pathogenic microbes and their possible relationship to virulence (Jayawardena et al., 2021). Finally, the paper by Kowalski and Cramer focuses on the identification and classification of plant pathogenic fungi and highlights the use of morpho-taxonomy and molecular tools (Kowalski & Cramer, 2020). The swift and accurate identification of plant pathogens, including fungi, is crucial for effective crop disease management. Traditional methods for identification, such as isolation and microscopic analysis, are time-consuming and labor-intensive (Dayarathne et al., 2023). However, molecular techniques, particularly those based on polymerase chain reaction (PCR), have revolutionized fungal pathogen detection (Mourou et al., 2023). These methods offer enhanced precision, sensitivity, speed, and reliability, enabling the identification of pathogens in both symptomatic and asymptomatic plant materials (Jeon et al., 2023). PCR and sequencing have been successfully applied to identify pathogenic fungi responsible for specific diseases (Kumar et al., 2023). Molecular techniques also provide valuable insights into fungal taxonomy and epidemiology, allowing for the study of phylogenetic relationships and differentiation between pathogenic fungal species (Feau et al., 2023; Jayalakshmi et al., 2023). The ITS region of ribosomal DNA is widely targeted for specific fungal identification. Universal primers such as ITS1 and ITS4 can be used to amplify this region.

Table (1): Date palm fungal diseases

Disease	Causal organisms	References
Bayoud disease	*Fusarium oxysporum f. sp. albedinis*	(El Modafar, 2010; Khayi et al., 2021)
Black scorch	*Thielaviopsis paradoxa, T. punctulata*	(Abdullah et al., 2009; Al-Naemi et al., 2014; Saeed et al., 2016)
Inflorescence rot	*Mauginiella scaettae*	(Bouhlali et al., 2021)
Pollen rot	*Fusarium fujikuroi*	(Abedalred et al., 2019)
Date palm root rot and decline	*Fusarium oxysporum, F. proliferatum, F. solani, Neodeightonia phoenicum, Thielaviopsis punctulata*	(Alwahshi et al., 2019; Baraka, et al., 2011; Haq & Khan, 2020; Mahmoud et al., 2016; Metlo et al., 2021; Nishad & Ahmed, 2020)
Botryodiplodia theobromae rot	*Botryodiplodia theobromae*	(Arafat et al., 2013)
Pestalotia leaf spot	*Pestalotia spp.*	(Tao et al., 2021)
Bending head disease	*Ceratocystis paradoxa, Thielaviopsis paradoxa*	(Abdullah et al., 2010)
Heart and trunk Rot disease	*Botryodiplodia theobromae, Fusarium spp., Gliocladium spp., Thielaviopsis paradoxa*	(Baraka, et al., 2011; Haq & Khan, 2020; Polizzi et al., 2006)
Belaat disease	*Phytophthora spp.*	(Abdelmonem & Rasmy, 2007; Russomanno et al., 2010)
Drying of apical leaves	*A. sp., F. solani, Phoma sp.,*	(Hassan, 2018; Matrood et al., 2021)
Bunch fading	*F. proliferatum*	(Mansoori, 2012)
Graphiola spot	*Graphiola phoenicis*	(Abbas & Abdulla, 2004; Sattar et al., 2021)
Omphalia root rot	*Omphalia pigmentata, O. tralucida*	(Abdullah et al., 2010)
Fruit rot	*A. alternata, Asp. flavus, A. fumigatus, A. japonicus, A. niger, A. ochraceus, Botryodiplodia sp., Fusarium spp., Ceratostomella sp., Cladosporium sp., Penicillium sp., Thielaviopsis paradoxa*	(Matrood et al., 2021)

Materials and Methods

Survey the leaf spot fungal diseases of date palm

A survey area of date palm leaf spot diseases were carried out in five districts in New Valley Governorate, *viz*.; Kharga, Baris, Balat, Dakhla and Frafra, with five locations for each one, and GPS were recorded for each location.

Symptoms

Leaf spots disease symptoms were photos as natural infections in all locations studied.

Evaluation of natural disease incidence and severity %.

Disease incidence % (DI%)

The percentage of DI was estimate in each location and calculate as followed (Cooke, 2006):

% DI%) = B / A x 100

whereas

A = total number of trees (healthy and infected) of units assessed.

B = number of infected trees with leaf spots disease.

Disease severity % (DS%)

The severity% of the disease is assessed in each field as natural infection. The disease severity index (DSI) was assessed according to (Ilias, 2000) with modifications. For measurements on the leaf scale, the percentage of diseased leaf area of the measured leaf in relation to the healthy leaf tissue was estimated visually. The range of observed external disease symptoms of trees with natural infection was assessed using the disease index using a scale of 0 to 4 (Table 2).

Table (2): Disease numerical scale and their corresponding disease symptoms

Scale	Disease symptoms
0	No spots = Healthy
1	1-3 spots = Gradual spot occurred on 1-25 % of date palm leaves
2	4-6 spots = Gradual spot occurred on 26-50 % of date palm leaves
3	7-9 spots = Gradual spot occurred on 51-75 % of date palm leaves
4	<10 spots = Gradual spot occurred on 76-100 % of date palm leaves

Severity data were processed using the Townsend–Heuberger formula (Townsend, 1943), DS (%) = $\Sigma(v0n0 + v1n1 + v2n2 + v3n3 + v4n4)/(\Sigma N * V) \times 100$, whereas V represents the numeric value of the disease index scale, n is the number of plants assigned to the disease index scale, N is the total number of plants and V is the numerical value of the highest disease index scale. The DSI was calculated from four leaves of each date palm seedling (Rakib et al., 2019).

Samples and isolation of microorganisms associated with date palm leaf spot

Sample collection

Diseased leaves were sampled from naturally infected date palm trees and offshoots across various districts in New Valley Governorate, Egypt. The symptoms were documented and photographed. Samples were placed in sterile plastic bags, stored at 4 °C, and transported to the Plant Pathology Laboratory at the Faculty of Agriculture, New Valley University, Egypt. A total of 125 samples were collected.

Sample preparation

Date palm leaves were cleaned of dust and debris. Infected leaf and midrib sections were cut into 0.5 cm^2 pieces, washed with tap water, surface-sterilized with 75% ethyl alcohol for five minutes, rinsed with sterile distilled water, and dried on sterile filter paper.

Isolation and identification

Five sterilized leaf pieces were placed on potato dextrose agar (PDA) amended with chloramphenicol (250 mg/L) and lactic acid. The plates were incubated at 25 ± 2 °C for 4-5 days. Fungi were purified on PDA and identified using published keys. Five replicates were used per leaf sample. Fungal stocks were maintained on PDA slants with monthly subculturing.

Fungal frequency calculation

The frequency of each isolated fungus was calculated as follows:

% Fungal frequency = (Number of isolates of the individual fungus / Total number of all isolates) x 100

Identification of the pathogenic fungi

Morphological identification of fungi causes leaf spot diseases in date palm

Fungal isolates causing leaf spot diseases in date palm were identified based on their morphological characteristics. Isolates were cultured on PDA and incubated at 25±2 °C for 10 days. Colony characteristics such as texture, color, and size of pycnidia were recorded. Microscopic examination was performed using a Zeiss Axiolab compound light microscope to observe hyphal structures and conidial characteristics under both low (10x) and high (40x) magnification. Identification was carried out in accordance with established mycological references (Agrios, 2005; Barnett & Hunter, 1998; Campbell & Johnson, 2013; Dhingra & Sinclair, 2017; Matsushima, 1975).

Pathogenicity tests

Unwounded method

Twenty separated seeds of the Saidy date palm cultivar were surface-sterilized and sown in plastic pots containing a mixture of sand, peat moss, and vermiculite. The seeds were germinated in a nursery and maintained there for three months until seedling emerged. Fungal cultures were grown on PDA medium and spore suspensions were prepared. The spore concentration was adjusted to 10^6 spores/mL using a hemocytometer and used to spray the

detached leaflets of the seedlings. The seedlings were then incubated separately at 25 ± 2 °C for 15, 30 and 45 days, respectively. Entire seedlings arranged in a randomized complete block design (RCBD). Symptom development on the inoculated leaves was recorded by determining the DS% with leaf spot within the DS index of the inoculation site and at 15, 30 and 45 days, after inoculation, respectively. To fulfill Koch's postulates re-isolations were performed from leaves that developed leaf spot symptoms (Al-Nadabi et al., 2020; Alemayehu, 2023; El Badawy et al.; Osman et al., 2012).

Wounded method

This method is the same as above, but the date palm seedlings had injuries on the leaves. The leaves were injured with a sterile toothbrush.

Molecular identification of fungi causing leaf spot diseases in date palm

The pathogens causing the leaf spot with the highest intensity were selected for ITS sequencing to confirm the species (Yaser & Abass, 2022). DNA extraction from the fungal samples was performed using the genomic DNA Prep kit and the SDS/CTAB lysis and phenol/chloroform extraction method (Choi et al., 2021). The ITS region, including ITS1, 5.8S, and ITS4, 28S rRNA, was amplified via PCR using specific primers (Wang et al., 2022). The obtained ITS sequences were compared with known homologous sequences of pathogenic fungi in the NCBI and EMBL databases using the BLAST search program (Fan et al., 2023). The sequences were aligned and analyzed using the CLUSTALW program, and phylogenetic analysis was performed using the neighbor-joining method with Kimura 2-parameter distances (Chitrakani et al., 2019). Bootstrap replicates were performed to assess the statistical support for each tree (Al-Nadabi et al., 2020; Matrood et al., 2021).

RESULT

Survey the leaf spot fungal diseases of date palm

A survey conducted in Egypt's New Valley Governorate from 2019 to 2021 revealed the presence of date palm leaf spot diseases occurs in all districts, including Kharga, Baris, Balat, Dakhla, and Frafra. Typical symptoms of date palm leaf spot were observed in these districts. The study also identified several fungal species associated with leaf spot symptoms. Some of these fungal pathogens were first described as causative agents of leaf spot disease in date palms.

Symptoms

Symptoms of date palm leaf spot disease, caused by various pathogenic fungi, vary in color depending on the severity of the infection. The spots initially appear yellow and turn brown, black, or gray as the infection progresses. The size of the spots ranges from 0.2 to 5 cm, and in severe cases, multiple spots may combine to form larger lesions. Figure (1) shows the natural symptoms of date palm leaf spot disease caused by various pathogenic fungi.

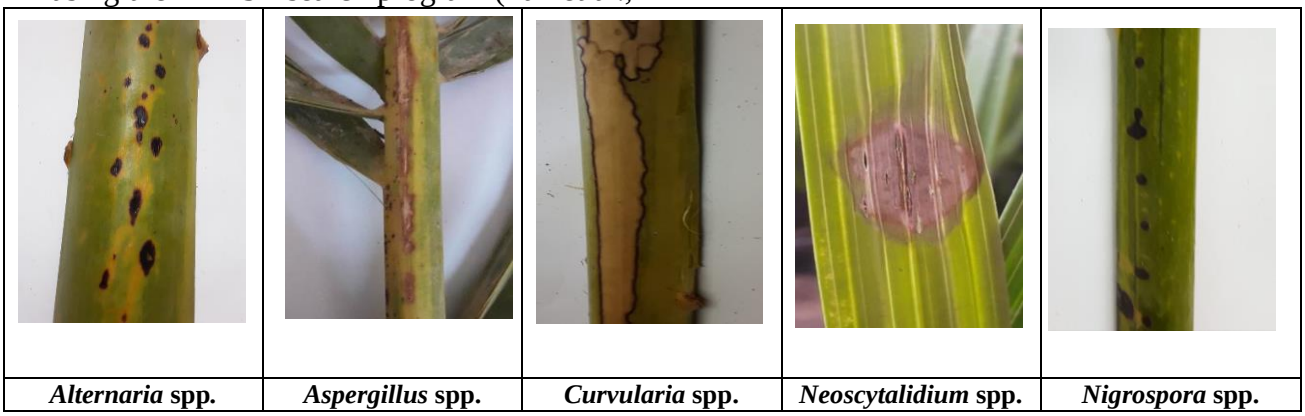

Figure (1): Symptoms of natural leaf spot infection on date palms caused by various pathogenic fungi, assessment of the frequency and severity of natural diseases %

The data in Table (3), provides an overview of the frequency and severity of natural diseases which vary across districts. In Kharga district, DI was 41.55% and DS was 15.26%. In the Baris district, the DI was higher at 53.06% and the DS was 13.26%. Balat district had a DI of 37.10% and a DS of 12.20%. Dakhla district had a DI of 31.00% and a DS of 13.40%. Frafra district had the lowest DI at 28.86% but the highest DS at 17.20%. These results suggest that disease frequency and severity varied between districts, with Baris district having the highest DI and Frafra district having the highest DS.

Table (3): DI and DS% in twenty-five locations in five district of date palm leaf spot diseases

District	Location		Total Area (Fadden)	Total No. of date palm trees	No of trees observed	Diseased tree	DI%	DS%	Mean DI%	Mean DS%
	Latitude (N)	Longitude (E)								
Kharga	25°24'24.61"	30°34'38.96"	522	52,149	76.00	35.00	46.05	12.74	41.55	15.26
	25°26'1.39"	30°34'43.66"			75.00	30.00	40.00	19.00		
	25°28'13.14"	30°32'12.38"			51.00	20.00	39.22	14.95		
	25°24'47.96"	30°32'39.71"			49.00	20.00	40.82	13.14		
	25°23'34.76"	30°33'15.43"			36.00	15.00	41.67	16.49		
Mean					57.00	24.00	41.55	15.26		
Baris	24°41'18.23"	30°35'4.28"	6825	261,826	74.00	30.00	40.54	10.14	53.06	13.26
	24°40'37.20"	30°36'16.78"			73.00	25.00	34.25	8.56		
	24°40'58.19"	30°37'2.49"			51.00	30.00	58.82	14.71		
	24°39'15.63"	30°35'58.13"			86.00	50.00	58.14	14.53		
	25°30'23.41"	29°20'7.29"			68.00	50.00	73.53	18.38		
Mean					70.40	37.00	53.06	13.26		
Balat	25°30'23.41"	29°20'7.29"	1774	141,921	40.00	10.00	25.00	10.00	7.10	2.20
	25°30'32.17"	29°20'21.76"			30.00	12.00	40.00	13.00		
	25°30'43.61"	29°20'15.36"			35.00	20.00	57.14	15.00		
	25°31'9.92"	29°20'6.41"			50.00	15.00	30.00	10.00		
	25°31'36.34"	29°16'34.49"			60.00	20.00	33.33	13.00		
Mean					43.00	15.40	37.10	12.20		
Dakhla	25°29'50.911"	29°0'34.141"	3336	219,361	50.00	10.00	20.00	10.00	1.00	3.40
	25°30'7.933"	29°0'31.457"			30.00	5.00	16.67	12.00		
	25°29'28.051"	28°59'25.0613"			40.00	10.00	25.00	15.00		
	25°30'26.476"	28°58'34.591"			25.00	15.00	60.00	14.00		
	25°30'17.444"	29°3'33.117"			30.00	10.00	33.33	16.00		
Mean					35.00	10.00	31.00	13.40		
Frafra	27° 3'41.31"	27°57'51.76"	12,092	1,207,212	100	30.00	30.00	17.00	8.86	7.20
	27° 3'33.19"	27°57'37.14"			70.00	25.00	35.71	20.00		
	27° 3'29.46"	27°57'46.19"			60.00	10.00	16.67	19.00		
	27° 3'28.63"	27°57'25.77"			70.00	20.00	28.57	15.00		
	27° 0'55.47"	27°58'14.60"			60.00	20.00	33.33	15.00		
Mean					72.00	21.00	28.86	17.20		

Pathogenicity test of the fungal genera most commonly isolated from date palm leaf spot diseases

The most frequently isolated fungi, namely *Alternaria* spp. (*A*), *Aspergillus* spp. (*Asp*.), *Curvularia* spp. (*Cur*.), *Neoscytalidium* spp. (*Neo*.) and *Nigrospora* spp. (*Nig*.) from various districts and locations in New Valley Governorate, which were used to study the pathogenic abilities on leaf seedlings of young date palms grown from seeds of cv. Saidy.

Pathogenicity testing was performed for 22 identified fungal species *viz., Cur. siddiquii* OK340657, *A. alternata* OM281844, *Cur. spicifera* OM283786, *Asp. terreus* OK346632, *Cur. siddiquii* OM283787, *A. alternata* OM281779, *Nig. lacticolonia* OM281785, *Cur. siddiquii* OM281805, *Nig. lacticolonia* OK340130, *Cur. lunata* OM180001, *Cur. lunata* OK338697, *A. angustiovoidea* OM202461, *Asp. terreus* OK094927, *Neo. novaehollandiae* OM280142, *A. botrytis* OK346254, *Neo. novaehollandiae* OM283736, *A. alternata* OM280071, *Cur. clavata* OM280074, *A. alternata* ON113023, *Cur. mebaldsii* OK349683, *A. alternata* OK345332 and *Cur. lunata* MW048511.

Unwounded method

Pathogenicity test on date palm seedlings using the unwounded method after

three months. Data were recorded for 22 fungi at 15, 30 and 45 days as DI% and DS%.

DI%

The data in Table (5) evidence that all the tested fungi were able to induce leaf spot diseases reaction, except four fungi *viz.*, *A. alternata* OM281844 (Kharga district), *Cur. siddiquii* OM281805 (Baris district), *Neo. novaehollandiae* OM283736 (Dakhla district) and *Neo. novaehollandiae* OM280142 (Dakhla district) were nonpathogenic of date palm leaf spot diseases. Results of the pathogenicity test of inoculation in the unwounded leaf showed that, the fungi highest percentage of mean DI% was *A. alternata* OM281779 (Baris district) ranged (20.00%), followed by *Asp. terreus* OK346632 (Kharga district) ranged (16.67%), *Asp. terreus* OK094927 (Dakhla district) ranged (16.67%), *Nig. lacticolonia* OK340130 (Baris district) ranged (13.33%), *Cur. siddiquii* OM283787 (Kharga district) ranged (13.33%), *Cur. lunata* MW048511 (Frafra district) ranged (13.33%), *A. angustiovoidea* OM202461 (Balat district) ranged (13.33%), *A. alternata* ON113023 (Frafra district) ranged (11.67%) and *A. botrytis* OK346254 (Dakhla district) ranged (11.67%). While the fungi moderate percentage of mean DI% were *Cur. mebaldsii* OK349683 (Frafra district) ranged (8.33%), *Nig. lacticolonia* OM281785 (Baris district) ranged (6.67%), *Cur. clavata* OM280074 (Frafra district) ranged (6.67%), *A. alternata* OK345332 (Frafra district) ranged (6.67%) and *Cur. lunata* OK338697 (Balat district) ranged (6.67%). Moreover, the fungi latest percentage of mean DI% were *Cur. spicifera* OM283786 (Kharga district) ranged (5.00%), *Cur. lunata* OM180001 (Balat district) ranged (5.00%), *A. alternata* OM280071 (Frafra district) ranged (5.00%) and *Cur. siddiquii* OK340657 (Kharga district) ranged (3.33%). Data also show that increased time led to increasing the DI% for fungi evaluated. Furthermore, DI% at 45 days was (13.26%), followed by DI% at 30 days (10.65%). While DI% at 15 days was (0.00%).

DS%

The next section of the pathogenicity test was concerned with DS%. The results obtained from the preliminary analysis of DS% are presented in Table 5. The highest mean score for DS% were *Asp. terreus* OK094927 (Dakhla district) ranged (5.83%) and *Asp. terreus* OK346632 (Kharga district) ranged (5.83%), followed by *Cur. lunata* MW048511 (Frafra district) ranged (4.17%), *Cur. siddiquii* OM283787 (Kharga district) ranged (4.16%), *A. botrytis* OK346254 (Dakhla district) ranged (3.75%), *Nig. lacticolonia* OK340130 (Baris district) ranged (3.33%) and *A. alternata* ON113023 (Frafra district) ranged (2.92%). While the fungi moderate percentage of mean DS% were *A. alternata* OM281779 (Baris district) ranged (2.50%), *Cur. mebaldsii* OK349683 (Frafra district) ranged (2.08%), *A. angustiovoidea* OM202461 (Balat district) ranged (1.67%), *A. alternata* OK345332 (Frafra district) ranged (1.67%), *Cur. lunata* OK338697 (Balat district) ranged (1.67%), *Cur. lunata* OM180001 (Balat district) ranged (1.67%), *Cur. clavata* OM280074 (Frafra district) ranged (1.42%), *Cur. spicifera* OM283786 (Kharga district) ranged (1.25%) and *A. alternata* OM280071 (Frafra district) ranged (1.25%). Moreover, the fungi latest percentage of mean DS% were *Nig. lacticolonia* OM281785 (Baris district) ranged (0.92%) and *Cur. siddiquii* OK340657 (Kharga district) ranged (0.83%). By contrast, *A. alternata* OM281844 (Kharga district), *Cur. siddiquii* OM281805 (Baris district), *Neo. novaehollandiae* OM283736 (Dakhla district) and *Neo. novaehollandiae* OM280142 (Dakhla district) were nonpathogenic fungi through artificial unwounded method. Data also show that increased time led to increasing the DS% for fungi evaluated. Furthermore, DS% at 45 days was (3.48%), followed by DI% at 30 days was (2.65%). While DI% at 15 days was (0.00%).

Wounded method

Pathogenicity test on date palm seedlings by the wounded method after three months, data recorded for twenty-two fungi

after 15, 30 and 45 days as DIs%) and severity (DS%).

DI%:

The data in Table (6) and Figure (3) demonstrate that all fungi tested were able to incidence a leaf spot response. The results of pathogenicity tests of inoculation in the wounded leaf showed that the fungi with the highest percentage of mean DI% were *Cur. lunata* OK338697 (Balat district) (56.67%), followed by *A. alternata* OM280071 (Frafra district) (53, 33%)., *Nig. lacticolonia* OM281785 (Baris district) in the distribution area (53.33%), *A. alternata* OK345332 (Frafra district) in the distribution area (51.67%), *Asp. terreus* OK094927 (Dakhla district) in the distribution area (50.00%), *Cur. lunata* MW048511 (Frafra district) in the distribution area (46.67%). %) and *Asp. terreus* OK346632 (Kharga district) ranged (45.00%). While the mean of median DI% of fungi *A. alternata* OM281779 (Baris district) was in the ranged (41.67%), *Neo. novaehollandiae* OM280142 (Dakhla district) ranged (41.67%), *Cur. lunata* OM180001 (Balat district) ranged (40.00%), and *Cur. clavata* OM280074 (Frafra district) ranged (40.00%), *A. botrytis* OK346254 (Dakhla district) ranged (40.00%) followed by *Neo. novaehollandiae* OM283736 (Dakhla district) ranged (38.33%), *Nig. lacticolonia* OK340130 (Baris district) ranged (36.67%), *A. angustiovoidea* OM202461 (Balat district) ranged (36.67%) and *Cur. siddiquii* OM281805 (Baris district) ranged (36.67%). Furthermore, the latest final percentage of fungi in mean DI% were *A. alternata* ON113023 (Frafra district) ranged (33.33%), *A. alternata* OM281844 (Kharga district) ranged (33.33%), and *Cur. siddiquii* OM283787 (Kharga district) ranged (33.33%) followed by *Cur. siddiquii* OK340657 (Kharga district) ranged (26.67%), *Cur. spicifera* OM283786 (Kharga district) ranged (25.00%) and *Cur. mebaldsii* OK349683 (Frafra district) ranged (25.00%). The data in Figure (4) also shows that longer time led to an increase in DI% in the fungi studied. Furthermore, the DI% at 45 days was (58.26%), followed by DI% at 30 days (55.43%). While DI% at 15 days was (1.74%).

DS%

The next section of the pathogenicity test was concerned with DS%. The results obtained from the preliminary analysis of DS% presented in (Table 6 and Figure 5). The highest mean score for DS% was *Asp. terreus* OK094927 (Dakhla district) ranged (8.67%) followed by *Cur. siddiquii* OK340657 (Kharga district) ranged (7.25%), *Cur. lunata* MW048511 (Frafra district) ranged (6.33%), *A. botrytis* OK346254 (Dakhla district) ranged (6.25%), *A. alternata* ON113023 (Frafra district) ranged (6.17%), *Asp. terreus* OK346632 (Kharga district) ranged (6.17%), *Nig. lacticolonia* OK340130 (Baris district) ranged (6.00%), *Cur. mebaldsii* OK349683 (Frafra district) ranged (5.67%), *Cur. spicifera* OM283786 (Kharga district) ranged (5.33%), *Cur. lunata* OM180001 (Balat district) ranged (5.00%), *A. alternata* OK345332 (Frafra district) ranged (4.75%) and *Neo. novaehollandiae* OM283736 (Dakhla district) ranged (4.75%). While the fungi moderate percentage of mean DS% were *A. alternata* OM281779 (Baris district) ranged (4.58%), *Cur. siddiquii* OM283787 (Kharga district) ranged (4.25%), *Cur. lunata* OK338697 (Balat district) ranged (4.08%), *Neo. novaehollandiae* OM280142 (Dakhla district) ranged (3.83%), *Cur. siddiquii* OM281805 (Baris district) ranged (3.50%) and A. *alternata* OM280071 (Frafra district) ranged (3.42%). Moreover, the fungi latest percentage of mean DS% were *Nig. lacticolonia* OM281785 (Baris district) ranged (2.5%), *Cur. clavata* OM280074 (Frafra district) ranged (2.50%), *A. angustiovoidea* OM202461 (Balat district) ranged (2.50%) and *A. alternata* OM281844 (Kharga district) ranged (1.54%). Data also show that in (Figure 6), increased time led to increasing the DS% for fungi evaluated. Furthermore, DS% at 45 days was (7.09%), followed by DI% at 30 days was (6.18%). While DI% at 15 days was (0.43%).

Molecular identification of fungi cause leaf spot diseases in date palm

The presence of five different genera, namely *A.* spp., *Asp.* spp., *Cur.* spp., *Neo.* spp., and *Nig.* spp., was confirmed through molecular methods and comparison with sequences in public gene bank databases.

The genera *A. alternata* (OM281844, OM281779, OM280071, OK345332, and ON113023) had 100% sequence identity with *A. alternata* (MN615420, MT420637, MN615420, MN481948, and MN481948, respectively). The genera *A. angustiovoidea* (OM202461) had 99.82% sequence identity with *A. angustiovoidea* (MN242398). The genera *A. botrytis* (OK346254) had 100% sequence identity with *A. botrytis* (LC440625).

The genera *Asp. terreus* isolates with accession numbers OK346632 and OK094927 showed 100% sequence identity with *Asp. terreus* sequences OP268179 and OW985952, respectively.

The genera *Cur. lunata* isolates of the genera with accession numbers (OM180001, OK338697, and MW048511) had 100% sequence identity with the *Cur. lunata* sequence (MK690419, OK138910, and MN213745, respectively). *Cur. siddiquii* accession numbers OK340657, OM283787, and OM281805 had sequence identities of 99.82%, 99.82%, and 99.64% with the *Cur. siddiquii* sequences MN688823, NR_170009, and NR_170009, respectively. *Cur. spicifera*, accession number OM283786 had 99.82% sequence identity with *Cur. spicifera* (MK956807). *Cur. clavata* (accession number OM280074) showed 100% sequence identity with *Cur. clavata* (MN718986). *Cur. mebaldsii* (accession number OK349683) also showed 100% sequence identity with *Cur. mebaldsii* (MN759651)

The genera *Neo. novaehollandiae*, accession number OM280142 and OM283736 had (99.82% and 99.82%, respectively) sequence identity with *Neo. novaehollandiae* (MT195553 and MT195552, respectively).

The genera *Nig. lacticolonia* accession numbers OM281785 and OK340130 exhibited 100% sequence identity with *Nig. lacticolonia* sequences MT043787 and MN173122, respectively.

The most dominant genus was *Cur.* spp. (9 isolates), followed by *A.* spp. (7 isolates), *Asp.* spp. (2 isolates) *Neo. spp*. (2 isolates) and *Nig.* spp. (2 isolates) that were detected in all the surveyed districts (Table 7). The data in Figure (11) allows a comparison between the different pathogenic fungi.

DISCUSSION

Date palm leaf spot is common in hot and humid regions, with the lower and older leaves being more affected than the upper young leaves and becoming worse as the leaves age. Pathogenic fungi persist on infected tree parts in various forms such as spores, mycelium or perfect forms and survive on dead tissue and other substrates. In addition, phylloplane fungal pathogens are present in dust and air. In favorable environments, spores germinate and attack pinnae, spines and leaf veins. The parasites then sporulate and release new spores that multiply, contaminating and infecting other parts of the leaf (El Bouhssini, 2018).

The aims of this study were to survey the pathogens causing date palm leaf spot diseases in the New Valley Governorate, Egypt isolate and identify the pathogenic fungi using traditional methods, conduct pathogenicity tests, and perform molecular identification of the fungi.

Date palms are susceptible to leaf spot diseases caused by phylloplane fungal pathogens. This is consistent with the findings of previous researchers (Al-Naemi et al., 2014; Al-Raisi et al., 2011; El Modafar, 2010).

The study on mapping leaf spot diseases of date palms in New Valley Governorate, Egypt. It records the DI% and DS% in different districts. The research shows that counties with similar climates had similar disease rates, with the isolated Frafra county having a lower DI, likely due to its

unique climate. Both location and plant age had a significant impact on disease frequency and severity. Baris district had the highest DI and Frafra the highest DS. These results are consistent with previous studies demonstrating the global prevalence of leaf spot diseases in date palm plantations (Abdullah et al., 2010; Al-Nadabi et al., 2020b; Bokhary, 2010; Manzelat, 2019).

The observed differences in the occurrence of fungal diseases on date palms or new offshoots of cv. Saidy in different nurseries and orchards in New Valley Governorate areas may be due to differences between environmental factors and management practices that were also used in the studied areas (El-Morsi et al., 2012).

One hundred and forty different genera of fungi that are the causative agents of leaf spot have been isolated from the phylloplane of the date palm. The main fungi isolated during this study were purified and identified to 22 species belonging to five genera, namely (*Alternaria.*, *Aspergillus*, *Curvularia*, *Neoscytalidium* and *Nigrospora*). The abundance of isolated fungi varied greatly, with *Cur.* spp. being the predominant genus isolated at high frequency with nine isolates, followed by *A.* spp. with seven isolates. This is consistent with previous studies that indicated the prevalence of this genus and its pathogenicity on date palm leaves (Al-Nadabi et al., 2021; Alam et al., 2020; Manea et al., 2021; Tao et al., 2021).

The results showed that there were significant differences in the virulence of the tested isolates of 22 fungi. The pathogenicity test of the isolated fungi was carried out in the greenhouse. All fungi tested cause leaf spotting using the wounding method and some fungi cause leaf spotting using the unwounding method, but with varying degrees of susceptibility. In this regard, *Cur. spicifera* was the most virulent fungus using unwounded methods, followed by *A. alternata*. In addition, *Cur. lunata* and *Cur. siddiquii* were the most infectious fungi. While the other fungi had moderate to low virulence. These results are consistent with the findings of previous studies (Atallah, Aly, et al., 2008; El-Deeb et al., 2006) that found *Curvularia* and *Alternaria* were highly pathogenic fungi for date palm leaves.

Characterization based on morphological characteristics and molecular techniques helped identify fungal isolates from leaf spot disease of date palm to the species level. The study showed the association of *Cur. siddiquii, A. alternata, Cur. spicifera, Asp. terreus, Cur. siddiquii, A. alternata, Nig. lacticolonia, Cur. siddiquii, Nig. lactolonia, Cur. lunata, Cur. lunata, A. angustiovoidea, Asp. terreus, Neo. novaehollandiae, A. botrytis, Neo. novaehollandiae, A. alternata, Cur. clavata, A. alternata, Cur. mebaldsii, A. alternata* and *Cur. lunata* with leaf spot on date palm.

It is the first study to report *A. angustiovoidea*, *A. botrytis*, *Asp. terreus*, *Cur. lunata*, *Cur. mebaldsii*, *Cur. siddiquii*, *Neo. novaehollandiae* and *Nig. lacticolonia* as leaf spot pathogens of date palm.

Additionally, date palms are indeed susceptible to leaf spot diseases caused by phylloplane fungal pathogens, as various studies show. Research in Mexico identified *Neopestalotiopsis* sp. and *Colletotricum karstii* as pathogens causing leaf spot and anthracnose in camedor palms (Khan et al., 2023). Similarly, in Mexico City, fungi such as *Nalanthamala vermoesenii*, *Lasiodiplodia* sp. and *A. alternata* were found to be associated with the decline and death of *Phoenix canariensis* palms (Sarmiento-Chacón et al., 2023). Furthermore, in Tunisia, *A.* and *Cur.* species have been identified as pathogens causing leaf spot on date palms, with *A.* strains producing various mycotoxins (Rabaaoui et al., 2022). These results highlight the importance of understanding and controlling fungal pathogens to alleviate leaf spot diseases in date palms.

The variability in the pathogenic copying abilities of the tested pathogenic fungi is very logical since these genera and isolates were isolated from different locations and may be genetically different. These results are critical to understanding the population structure and evolutionary

relationships within this fungal species, which may have implications for diverse applications ranging from biotechnology to disease management.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: 'Not applicable'

Availability of data and materials: “The datasets generated and/or analyzed during the current study are available in the Biosample.

Competing interests: "The authors declare that they have no competing interests"

Authors' Contributions: Not Applicable

List of abbreviations

BLAST	Basic Local Alignment Search Tool
bp	Bootstrap
DI	Disease incidence
DS	Disease severity
DSI	Disease severity index
EMBL	European Molecular Biology Laboratory
ICARDA	International Center for Agricultural Research in the Dry Areas
ITS	Internal transcribed spacer
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
RCBD	Randomized complete block design

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Table (4): Pathogenicity test for pathogenic fungi causing leaf spot diseases in date palm

No.	Fungi / Code	GB Accession no.	Unwounded Method							
			DI %			DI% MEAN	DS %			DS% MEAN
			15 DAYS	30 DAYS	45 DAYS		15 DAYS	30 DAYS	45 DAYS	
0	Control	----------	0.00	0.00	0.00	0.00d	0.00	0.00	0.00	0.00d
1	*Cur. lunata*	OM180001	0.00	5.00±5.00	10.0±6.88	5.00±2.84cd	0.00	1.25±1.25	3.75±2.73	1.67±1.00bcd
2	*A. botrytis*	OK346254	0.00	15.0±8.20	20.0±9.18	11.67±4.18abc	0.00	5.00±2.92	6.25±3.07	3.75±1.43abc
3	*Neo. novaehollandiae*	OM283736	0.00	0.00	0.00	0.00d	0.00	0.00	0.00	0.00d
4	*Cur. lunata*	OK338697	0.00	10.0±6.88	10.0±6.88	6.67±3.25bcd	0.00	2.50±1.72	2.50±1.72	1.67±0.81bcd
5	*A. alternata*	OM281779	0.00	30.0±10.5	30.0±10.5	20.0±5.21a	0.00	3.75±1.31	3.75±1.31	2.500.65bcd
6	*A. angustiovoidea*	OM202461	0.00	20.0±9.18	20.0±9.18	13.33±6.67abc	0.00	2.00±0.92	3.00±1.38	1.67±0.56bcd
7	*Nig. lacticolonia*	OM281785	0.00	10.0±6.88	10.0±6.88	6.67±3.25bcd	0.00	1.00±0.69	1.75±1.23	0.92±0.50cd
8	*A. alternata*	OM280071	0.00	0.00	15.0±8.19	5.00±2.84cd	0.00	0.00	3.75±2.05	1.25±0.71bcd
9	*Cur. clavata*	OM280074	0.00	10.0±6.88	10.0±6.88	6.67±3.25bcd	0.00	1.75±1.32	2.50±1.72	1.42±0.72bcd
10	*Cur. siddiquii*	OM281805	0.00	0.00	0.00	0.00d	0.00	0.00	0.00	0.00d
11	*A. alternata*	ON113023	0.00	15.0±8.19	20.0±9.18	11.67±4.18abc	0.00	3.75±2.05	5.00±2.29	2.921.04abcd
12	*Nig. lacticolonia*	OK340130	0.00	20.0±9.18	20.0±9.18	13.33±4.42abc	0.00	5.00±2.29	5.00±2.29	3.33±1.11abc
13	*Cur. mebaldsii*	OK349683	0.00	10.0±6.88	15.0±8.19	8.33±3.60bcd	0.00	2.50±1.72	3.75±2.05	2.08±0.90bcd
14	*Cur. siddiquii*	OK340657	0.00	0.00	10.0±6.88	3.33±2.34cd	0.00	0.00	2.50	0.83±0.59cd
15	*A. alternata*	OK345332	0.00	10.0±6.88	10.0±6.88	6.67±3.25bcd	0.00	2.50±1.72	2.50±1.72	1.67±0.81bcd
16	*A. alternata*	OM281844	0.00	0.00	0.00	0.00d	0.00	0.00	0.00	0.00d
17	*Cur. spicifera*	OM283786	0.00	0.00	15.0±8.19	5.00±2.84cd	0.00	0.00	3.75±2.05	1.25±0.71bcd
18	*Asp. terreus*	OK094927	0.00	25.0±9.93	25.0±9.93	16.67±4.85ab	0.00	8.75±3.75	8.75±3.75	5.83±1.82a
19	*Neo novaehollandiae*	OM280142	0.00	0.00	0.00	0.00d	0.00	0.00	0.00	0.00d
20	*Asp. terreus*	OK346632	0.00	25.0±9.93	25.0±9.93	16.67±4.85ab	0.00	8.75±3.75	8.75±3.75	5.83±1.82a
21	*Cur. siddiquii*	OM283787	0.00	20.0±9.18	20.0±9.18	13.33±4.42abc	0.00	6.25±3.07	6.25±3.07	4.17±1.47ab
22	*Cur. lunata*	MW048511	0.00	20.0±9.18	20.0±9.18	13.33±4.42abc	0.00	6.25±3.07	6.25±3.07	4.17±1.47ab
Mean			0.00b	10.6±1.44a	13.3±1.58a	8.00±0.73	0.00b	2.65±0.40a	3.470.45a	2.04±0.21

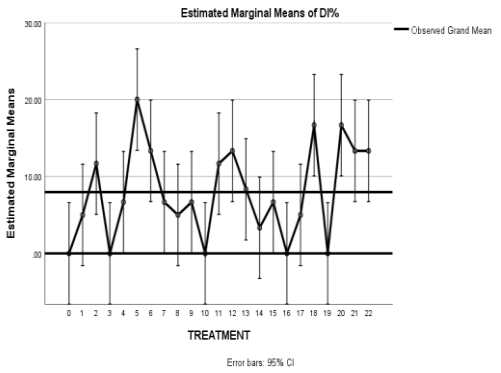

Figure (2): Relationship between fungi (Treatment) and DI % under unwounded method

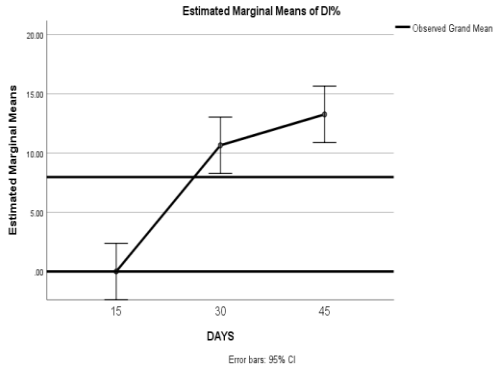

Figure (3): Relationship between Days and DI % under unwounded method

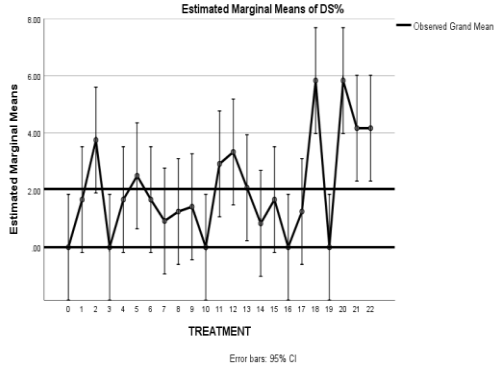

Figure (4): Relationship between fungi (Treatment) and DS% under unwounded method

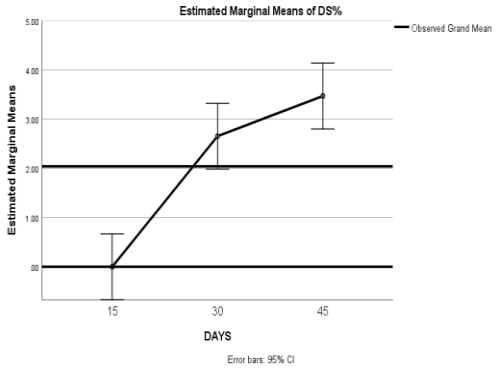

Figure (5): Relationship between Days and DS% under unwounded method

Table (5): Pathogenicity test for pathogenic fungi causing leaf spot diseases in date palm

No.	Fungi	GB Accession no.	Wounded method							
			DI %			DI% MEAN	DS %			DS% MEAN
			15 DAYS	30 DAYS	45 DAYS		15 DAYS	30 DAYS	45 DAYS	
0	Control	----------	0.00	0.00	0.00	0.00e	0.00	0.00	0.00	0.00g
1	*Cur. lunata*	OM180001	0.00	60.0±11.2	60.0±11.2	40.00±5.01abcd	0.00	6.25±1.2	8.75±1.7	5.00±0.8bcde
2	*A. botrytis*	OK346254	5.00±5.0	55.0±11.4	60.0±11.2	40.00±5.01abcd	1.25±1.2	7.50±1.7	10.0±2.1	6.25±0.8bc
3	*Neo. novaehollandiae*	OM283736	5.00±5.0	50.0±11.5	60.0±11.2	38.33±5.01bcd	1.25±1.2	6.25±1.5	6.75±1.6	4.75±0.8bcde
4	*Cur. lunata*	OK338697	0.00	85.0±8.2	85.0±8.2	56.67±5.01a	0.00	5.50±0.7	6.75±0.8	4.08±0.8cdef
5	*A. alternata*	OM281779	5.00±5.0	60.0±11.2	60.0±11.2	41.67±5.01abcd	1.25±1.2	6.25±1.2	6.25±1.2	4.58±0.8cde
6	*A. angustiovoidea*	OM202461	0.00	55.0±11.4	55.0±11.4	36.67±5.01bcd	0.00	3.75±0.9	3.75±0.9	2.50±0.8ef
7	*Nig. lacticolonia*	OM281785	0.00	80.0±9.2	80.0±9.2	53.33±5.01ab	0.00	3.75±0.5	3.75±0.5	2.50±0.8ef
8	*A. alternata*	OM280071	0.00	60.0±11.2	100.0±0.0	53.33±5.01ab	0.00	3.50±0.7	6.75±0.5	3.42±0.8def
9	*Cur. clavata*	OM280074	0.00	60.0±11.2	60.0±11.2	40.00±5.01abcd	0.00	3.75±0.8	3.75±0.8	2.50±0.8ef
10	*Cur. siddiquii*	OM281805	0.00	55.0±11.4	55.0±11.4	36.67±5.01bcd	0.00	5.25±1.4	5.25±1.4	3.50±0.8def
11	*A. alternata*	ON113023	0.00	50.0±11.5	50.0±11.5	33.33±5.01cd	0.00	9.00±2.2	9.50±2.2	6.17±0.8bc
12	*Nig. lacticolonia*	OK340130	0.00	55.0±11.4	55.0±11.4	36.67±5.01bcd	0.00	7.75±2.2	10.25±2.3	6.00±0.8bcd
13	*Cur. mebaldsii*	OK349683	5.00±5.0	35.0±10.9	35.0±10.9	25.00±5.01d	1.25±1.2	7.75±2.5	8.00±2.5	5.67±0.8bcd
14	*Cur. siddiquii*	OK340657	0.00	40.0±11.2	40.0±11.2	26.67±5.01d	0.00	10.25±3.0	11.50±3.3	7.25±0.8ab
15	*A. alternata*	OK345332	5.00±5.0	75.0±9.9	75.0±9.9	51.67±5.01ab	1.25±1.2	6.25±1.0	6.75±1.0	4.75±0.8bcde
16	*A. alternata*	OM281844	0.00	50.0±11.5	50.0±11.5	33.33±5.01cd	0.00	2.13±0.5	2.50±0.6	1.54±0.8fg
17	*Cur. spicifera*	OM283786	5.00±5.0	35.0±10.9	35.0±10.9	25.00±5.01d	1.25±1.2	7.25±2.3	7.50±2.4	5.33±0.8bcd
18	*Asp. terreus*	OK094927	0.00	75.0±9.9	75.0±9.9	50.00±5.01abc	0.00	12.75±2.0	13.25±1.9	8.67±0.8a
19	*Neo novaehollandiae*	OM280142	5.00±5.0	55.0±11.4	65.0±10.9	41.67±5.01abcd	1.25±1.2	3.75±0.9	6.50±1.1	3.83±0.8cdef
20	*Asp. terreus*	OK346632	5.00±5.0	65.0±10.9	65.0±10.9	45.00±5.01abc	1.25±1.2	8.50±1.5	8.75±1.6	6.17±0.8bc
21	*Cur. siddiquii*	OM283787	0.00	50.0±11.5	50.0±11.5	33.33±5.01cd	0.00	6.25±1.5	6.50±1.5	4.25±0.8cde
22	*Cur. lunata*	MW048511	0.00	70.0±10.5	70.0±10.5	46.67±5.01abc	0.00	8.75±1.4	10.25±1.6	6.33±0.8bc
Mean			1.74b	55.44a	58.26a	38.48	0.43c	6.18b	7.09a	4.57

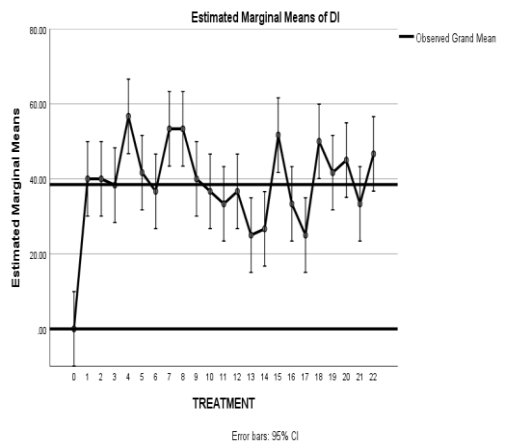

Figure (6): Relationship between fungi (Treatment) and DI % under wounded method

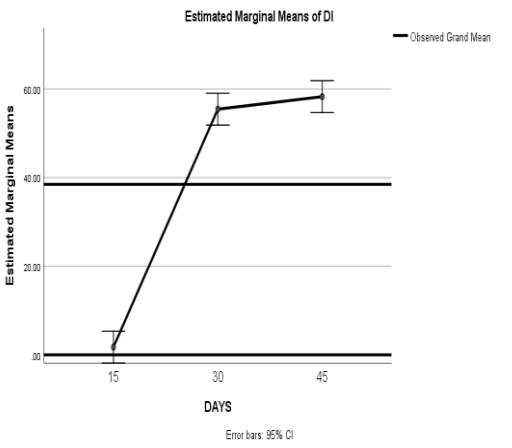

Figure (7): Relationship between Days and DI % under wounded method

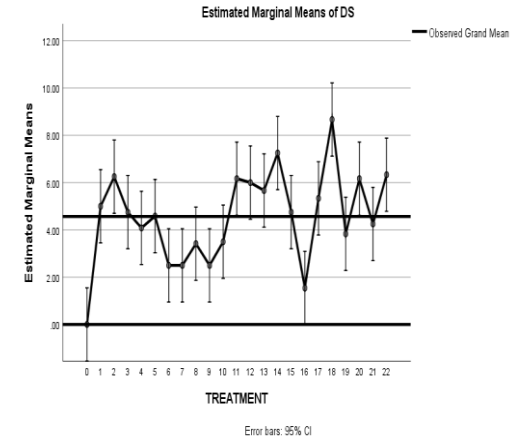

Figure (8): Relationship between fungi (Treatment) and DS% under wounded method

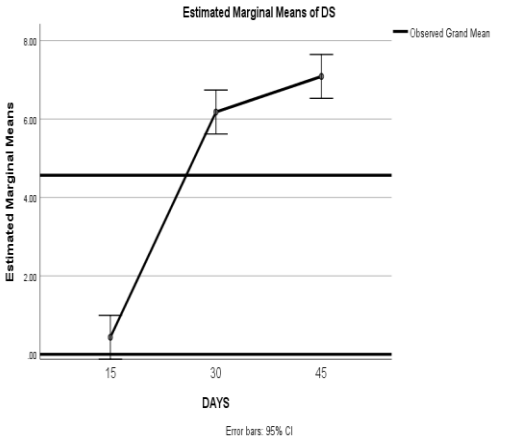

Figure (9): Relationship between Days and DS% under wounded method

Table (6): Molecular identification of fungi cause leaf spot diseases in date palm

Districts	Identification	Pb	Gb Accession no
KHARGA	*Cur. siddiquii*	576	OK340657
	A. alternata	556	OM281844
	Cur. spicifera	555	OM283786
	Asp. terreus	604	OK346632
	Cur. siddiquii	565	OM283787
BARIS	*A. alternata*	566	OM281779
	Nig. lacticolonia	548	OM281785
	Cur. siddiquii	553	OM281805
	Nig. lacticolonia	547	OK340130
BALAT	*Cur. lunata*	570	OM180001
	Cur. lunata	585	OK338697
	A. angustiovoidea	558	OM202461
DAKHLA	*Asp. terreus*	606	OK094927
	Neo. novaehollandiae	565	OM280142
	A. botrytis	576	OK346254
	Neo. novaehollandiae	566	OM283736
FARAFRA	*A. alternata*	557	OM280071
	Cur. clavata	575	OM280074
	A. alternata	541	ON113023
	Cur. mebaldsii	544	OK349683
	A. alternata	541	OK345332
	Cur. lunata	586	MW048511

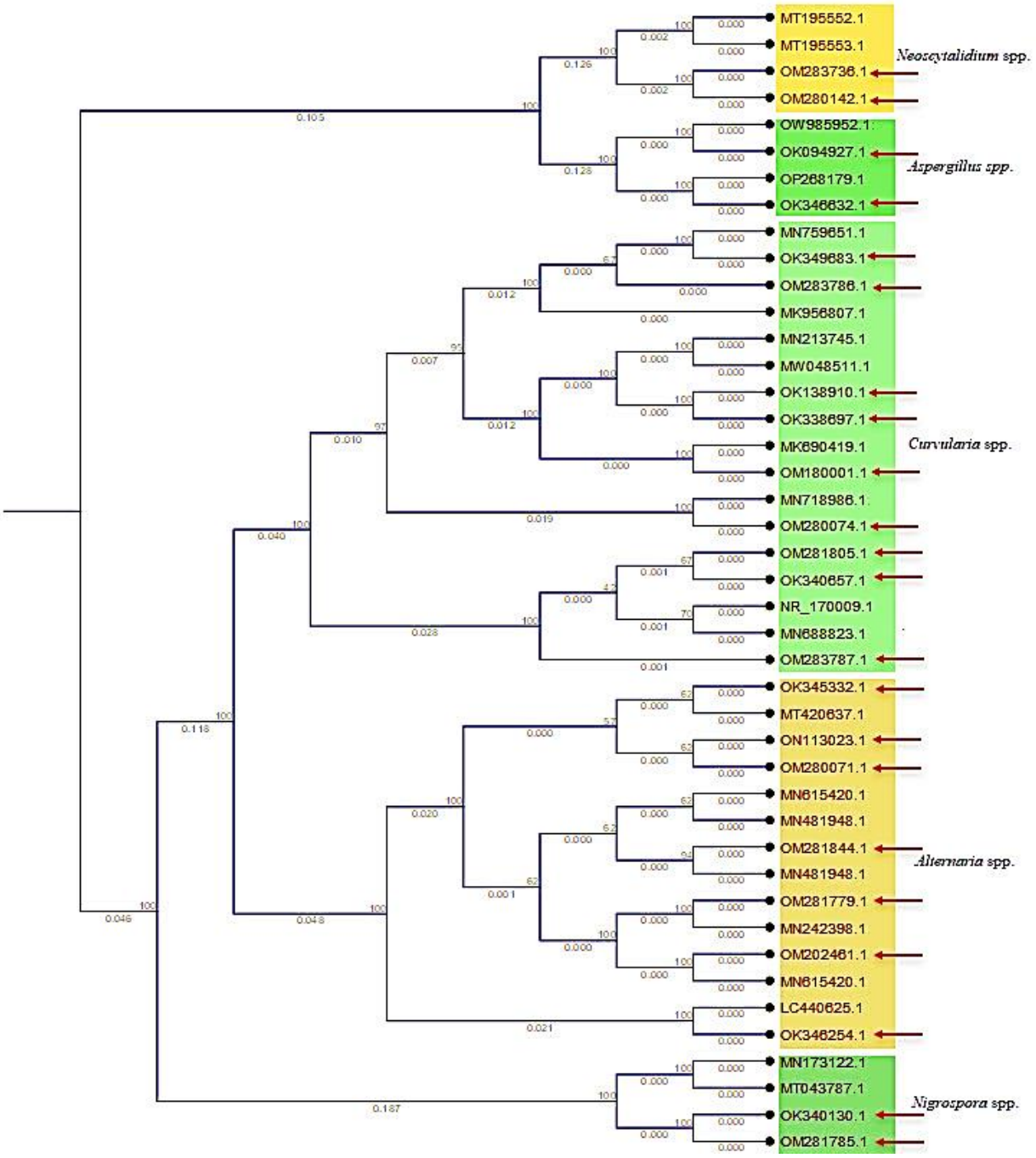

Figure (10): Phylogenetic tree of the pathogenic fungi caused leaf spot diseases of date palm

➡ These fungi were used in this study.