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Molecular Identification of Fungal Pathogens of Date Palm Root Rot in Egypt

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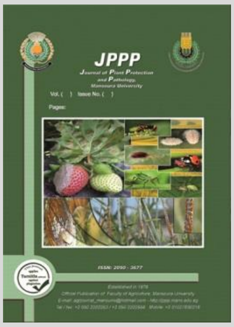

ABSTRACT

In tropical and subtropical regions, date palm is a highly significant fruit tree, known for its more than 3,000 different varieties found around the world. However, in Egypt, date palms are susceptible to infection by root rot disease (RRD) caused by various harmful soil borne fungi. This leads to significant damage to both trees and their offshoots. The most harmful fungi found in Egypt included: *Fusarium oxysporum*, which causes deterioration of palm roots, as well as *F. moniliforme*, *F. solani*, *Thielaviopsis paradoxa*, *Botryodiplodia theobromae* and *Rhizoctonia solani*. A survey and collection of date palm samples was carried out in five districts (Kharga, Baris, Balat, Dakhla and Frafra) within New Valley Governorate, Egypt were carried out. Pathogenicity tests and disease severity confirmed the presence of 15 fungal species belonging to six genera, which identified as *Acrocalymma vagum*, *Curvularia australiensis*, *C. lunata*, *C. spicifera*, *Exserohilum rostratum*, *F. oxysporum*, *F. solani*, *Marasmius palmivorus* and *Talaromyces pinophilus*. Through molecular identification using internal transcribed spacers (ITS), it has been determined that these eight new pathogenic fungal isolates, were not previously identified in date palms in Egypt, belong to the species: *A. vagum*, *A. vagum*, *C. australiensis*, *C. lunata*, *C. spicifera*, *E. rostratum*, *M. palmivorus*, *T. pinophilus*. This finding represents the first documented association of these eight fungi with date palm RRD in Egypt.

Keywords*:* Date palm; root rot diseases; soilborne pathogenic fungi; ITS.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is a perennial, diploid, monocot, dioecious, large, evergreen plant grown for its edible fruits in semiarid and arid regions (Salomón-Torres, 2023). It is considered one of the oldest tree fruits and is known as the "tree of life" (Alemayehu, 2023). Date palm has multiple uses and products beyond its fruit. The trunk of the tree is used for fuel and to make furniture, artwork, and shoe soles (Obón et al., 2023). Leaves can be used for handicrafts, roof covers, and in the production of biofertilizers (Bhatt et al., 2023). The pulp of the fruit can be used to produce alcohol and antibacterial gel (Mahomoodally et al., 2023). Date seed can produce high-quality oil for cosmetic and pharmaceutical applications (Alahyane et al., 2022). The date palm is also used as an ornamental plant in gardens and resorts (Halabi et al., 2023). Furthermore, researches have shown that date palms require specific climatic conditions and well-drained soils for successful fruit production (Jiddeine et al., 2022). The nutritional and medicinal properties of different parts of the date palm have been extensively studied, highlighting their potential as functional foods and nutraceuticals (Achour et al., 2022). Palm trees are planted for fruit; they are also grown as ornamental or landscape trees in many countries (Biglari et al., 2009).

The cultivation of date palms has been extended to novel regions such as India, Pakistan, Australia, South America, Mexico, North Africa, and the United States. The worldwide output of date palms has escalated by 18% in the previous ten years, attaining a quantity of 8.53 million metric tons across a combined expanse of 1.11 million hectares (Alotaibi et al., 2023). Date palm plantations are found in 37 countries on five continents, making them one of the most widespread plantations in the world (Ibrahim, 2022). Date palm production requires temperatures ranging from 18 to 22 °C during flowering and from 25 to 29 °C during fruiting, as well as sufficient irrigation water or a shallow water table (Alemayehu, 2023). Date palm fruits are of a high value commercial crop with numerous health benefits, including antioxidant, antimicrobial, and anti-inflammatory properties (Musthafa and Sandhu, 2022). In Egypt, date palms can develop RRD from a variety of soilborne pathogenic fungi, such as *Fusarium oxysproum Schlecht*, *F. solani (Mort.) Sacc*, *F. moniliforme Sheldon*, *F. semitectum Berk and Ravenel*, *Botryodiplodia theobrome Pat*, *Thielaviopsis thielavioides Peyr. and Rhizoctonia solani Kuhn.* (Baraka et al., 2011; Arafat, 2013).

Palm trees are indeed grown in Egypt, with a total area of 134,100 feddan dedicated to their cultivation. Palm tree production in Egypt is expected to reach 1,710,000 tons, accounting for approximately 18% of the world's production. The average yield per hectare is projected to be 33,390.1 kg ha^{-1} in 2021. These figures indicate that Egypt is a significant player in the global palm tree industry (Ahmed, 2019, Mobilization and Statistics, 2022).

The etiological agents responsible for root rot in date palms have been determined to be pathogenic fungi such as *Alternaria* sp., *Chaetomium* sp., *Cladosporium* sp., *Diplodia phoenicum*, *F.* spp., *Macrophomina phaseolina*, *Phoma* sp., *Phomopsis phoenicola*, *R. solani* and *Thielaviopsis paradoxa*. These fungal pathogens induce various diseases in date palms, resulting in decreased fruit quality and quantity, as well as the death of the main tree and its offshoots. This research investigated the prevalence of root rot disease (RRD) in date palm cultivation within the New

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Valley Governorate of Egypt. The study aimed to determine the specific fungal pathogens responsible for RRD across various sites in the region. Previous research (Abdelmonem and Rasmy, 2007, Abdullah et al., 2009, Baraka et al., 2011, Khan et al., 2023, Quezada-Salinas et al., 2023) has also focused on this issue.

MATERIALS AND METHODS

The research was carried out between 2020 and 2021 in five different districts and 25 locations in the New Valley Governorate, Egypt.

Isolation of Pathogenic Fungi

The isolation of pathogenic date palm fungi with natural infections and their outgoings from different locations have been used to collect root samples (cv. Saidy) from commercial farms. The five districts of the New Valley Governorate, Kharga, Baris, Balat, Dakhla and Frafra, each with five locations, were the sites of the RRD study. The percentage of disease incidence (DI) was determined using the following formula (Cooke et al., 2006):

$$DI= B/A \times 100$$

Where;

A is the total number of trees or offshoots (both healthy and infected) of the units evaluated, and B= the quantity of affected trees or offshoots.

The formula for calculating disease severity (DS) was as follows:

$$DS= (\Sigma (A \times B/ \Sigma n x4) \times 100$$

Where,

A denotes the disease class (0, 1, 2, 3 or 4); B is the number of plants exhibiting this disease class per treatment; n is the total number of replicas (constant) and an (n). Class 4= has the highest score. The infected trees were scored as follows: 0 represented no symptoms and healthy plants; one represented grade wilting in 1 to 25% of date palm leaves; 2 represented grade wilting in 26 to 50% of date palm leaves; 3 represented grade wilting in 51 to 75% of date palm leaves; and four represented grade wilting in 76% to 100% of date palm leaves (Abdalla et al., 2000).

The disease scale was used to calculate the percentage of DS. After carefully cleaning large soil particles, date palm roots (trees or offshoots) are removed from the infested areas of the orchard. To reduce the growth of saprophytic microorganisms, root samples were collected at a minimum distance of 5 cm and then stored in a cool and dry environment during transport to the laboratory. The samples were surface sterilized with a 0.5% sodium hypochlorite solution for three minutes, rinsed in sterile distilled water (SDW) and dried between two folds of sterilized filter paper after thorough cleaning with tap water to remove adhered dust particles. Sections of each tree or seedling, a total of 20 randomly selected 1 cm root sections per plant, were cut into 1 cm lengths and placed on potato dextrose agar (PDA) and incubated at 25 ± 2°C. In preparation for further studies, the stock cultures were stored in a slanted PDA cabinet and cooled to 4 °C. Based on this color, type of spores, presence of sporodochia or appressoria, structure, colony structure and other growth characteristics of the fungi, the fungal colonies growing in the culture plates were morphologically identified. Microorganisms and purified fungi were identified according to (Alexopoulos et al., 1979, Nelson et al., 1981, Barnett and Hunter, 1998, Leslie and Summerell, 2006). The following formula is used to calculate the frequency of isolated root rot separately: Percent FR = (N / T) × 100, where percent FR indicates fungal frequency, N is the number of isolates for each fungus and T is the total number of isolates.

Pathogenicity test of the Isolated Fungi

To fulfill Koch's postulates, a pathogenicity test of all isolated fungi from diseased roots was carried out in the greenhouse of the Faculty of Agriculture at New Valley University in Egypt. A for-profit producer provided *P. dactylifera* (cv. Saidy). Following a 2-minute surface disinfection with sodium hypochlorite and 1% available chlorine solution available, washed in sterile distilled water (SDW), and then dried between folds of sterilized filter papers. Soil, sand, and vermiculite were mixed in equal amounts and seeded (v / v) in 15 cm black plastic bags. The seedlings were allowed to be developed at six months of age in date palms with two to three leaves were used as duplicates for each fungus that was studied. To perform the experiment on diseased seedlings, two methods were used: (a) Seedling injection: Six-month-old plants were inoculated according to instructions, injecting 1 ml of spore suspension and measuring the concentration with a hemocytometer at 1 × 10^6 conidia/ml. (Baraka et al., 2011). Spore suspensions are injected into the corolla using a hypodermic needle and a syringe designed specifically for each type of fungus. To maintain high humidity, each plant was individually covered with black plastic bags for 48 hours after inoculation. 25 seedlings of the cv. Saidy were injected with each isolate, and the appropriate controls received an injection of SDW (????). The way the pots are arranged makes no sense at all. (b) Soil infestation: 100 ml of spore suspensions obtained from each pathogenic fungus, at a density of 4 × 10^6/ml were placed inside each black plastic bag. For plants to remain healthy and the soil to spread, watering was done every three to four days. Pathogenicity assays were assessed as DS percent 90 days after inoculation, as previously mentioned. The isolated fungi were compared to the original cultures following the re-isolation of the infected tissues. Every experiment was repeated twice and the results were consistent.

Molecular Identification of Pathogenic Fungi

In culture medium, the fungi were grown and incubated for 15 days at 25 °C. Subsequently, the fungal growth was scraped and suspended in 100 µL of distilled water. Then they were boiled for 15 minutes at 100 °C and kept in storage at 80 °C. Based on SDS/CTAB lysis and phenol/chloroform extraction techniques, DNA was extracted from fungal cultures using a genomic DNA preparation kit (SolGent, Daejeon, Korea), (White et al., 1990). ITS sequence acquired through Macrogen's commercial service (Macrogen Comp. Korea (South). Comparisons between the sequences and known homologous sequences of pathogenic fungi are made using databases from the European Nucleotide Archive (ENA) (https://www.ena.org) and the National Center for Biotechnology Information (NCBI) (http://www.ncbi.gov/Genbank).

Phylogenetic Analysis

DNA sequences were first aligned using Clustal Omega Multiple Sequence Alignment (MSA) (https://www.ebi.ac.uk/Tools/msa/clustalo/). CLC Genomics Workbench version 22.0.2 was used to construct a neighboring tree.

RESULTS AND DISCUSSION

Results

Isolation of Pathogenic Fungi

A survey was conducted in the New Valley Governorate, consisting of five districts and five locations in each district, to assess the occurrence and severity of date palm RRD. Frequency and severity were taken, and root rot samples were collected at each location. The data in Table (1) indicate that all surveyed date palm locations surveyed in each district were affected by RRD, with varying proportions. The district with the highest average proportion of observations affected by RRD (DI%) was Dakhla (42.67%), while the district with the lowest proportion was Frafra (19.37%). Furthermore, Dakhla had the highest average severity of RRD (DS%) at 14.27%, and Kharga had the highest overall average severity at 6.54%.

Table 1. Incidence and severity of date palm disease root rot in different districts and locations in the New Valley Governorate

District	Latitude (N)	Longitude (E)	DI%	Mean DI%	DS%	Mean DS%
	25°24'24.61"	30°34'38.96"	28.57		8.93	
	25°26'1.39"	30°34'43.66"	16.67		5.42	
Kharga	25°28'13.14"	30°32'12.38"	25.00	20.05	8.33	6.54
	25°24'47.96"	30°32'39.71"	10.00		2.50	
	25°23'34.76"	30°33'15.43"	20.00		7.50	
	24°42'0.16"	30°36'48.63"	45.00		13.75	
	24°41'18.23"	30°35'4.28"	13.33		4.17	
Baris	24°40'37.20"	30°36'16.78"	20.00	21.67	6.25	6.77
	24°40'58.19"	30°37'2.49"	10.00		2.17	
	24°39'15.63"	30°35'58.13"	20.00		7.50	
	25°30'23.41"	29°20'7.29"	37.50		12.50	
	25°30'32.17"	29°20'21.76"	26.67		7.92	
Balat	25°30'43.61"	29°20'15.36"	34.29	26.69	11.07	8.61
	25°31'9.92"	29°20'6.41"	10.00		2.17	
	25°31'36.34"	29°16'34.49"	25.00		9.38	
	25°29'50.911"	29°0'34.141"	40.00		13.75	
	25°30'7.933"	29°0'31.457"	33.33		11.67	
Dakhla (Mut)	25°29'28.051"	28°59'25.0613"	50.00	42.67	17.19	14.27
	25°30'26.476"	28°58'34.591"	40.00		10.00	
	25°30'17.444"	29°3'33.117"	50.00		18.75	
	27° 3'41.31"	27°57'51.76"	16.00		4.75	
	27° 3'33.19"	27°57'37.14"	12.50		4.06	
Frafra	27° 3'29.46"	27°57'46.19"	15.00	19.37	5.31	6.62
	27° 3'28.63"	27°57'25.77"	8.33		2.08	
	27° 0'55.47"	27°58'14.60"	45.00		16.88	

All fungal isolation was conducted in the Department of Plant Pathology of the Faculty of Agriculture at New Valley University. A total of 125 diseased root samples were collected from five districts in the New Valley Governorate, namely Kharga, Baris, Balat, Frafra and Dakhla (Mut). Each district had five different locations from which samples were collected. In these samples of diseased date palm roots, 56 species belonging to 28 fungal genera were isolated. Fungal identification was performed after conducting pathogenicity tests. Abundance and newly discovered fungi can be utilized in pathogenicity tests. The data displayed in Table (2) reveal that Dakhla (Mut) district had the highest number of fungi isolated from the diseased samples, with Kharga district following closely. On the contrary, the lowest count of isolated fungi was observed in the Frafra district.

Pathogenicity Assays of Commonly Isolated Fungi

The pathogenic potential of date palm seedlings grown from the seeds of cv. Saidy was examined using 28 isolated fungi. Wounded and unwounded date palm seedlings were subjected to pathogenicity testing. The data in Figure (1) show that all fungi can cause a root rot response, except for the isolates that were found to be non-pathogenic (0, NP) fungi that caused RRD in date palm seedlings and they were obtained using the unwounded method (K103 - K105 - K106 - BR201 - BR204 - BR205 - B305 - F502 -F504 - M403 - M406 - M407 and M488). Furthermore, the most aggressive isolates were B304 (*F. oxysporum*) and M402 (*F. oxysporum*), which originated from Mut and Balat districts, respectively. Subsequently, B301 (*A. vagum*), M405 (*F. oxysporum*), and F501 (*F. solani*) were all moderately aggressive. On the other hand, isolated B302 (*A. vagum*) and BR202 (*F. solani*) were both less aggressive. More specifically, the least aggressive isolates were K101 (*E. rostratum*), K102 (*T. pinophilus*), K104 (*C. australiensis*), BR203 (*C. spicifera*), B303 (*F. oxysporum*), M401 (*F. oxysporum*) and M404 (*M. palmivorus*) and F503 (*C. lunata*).

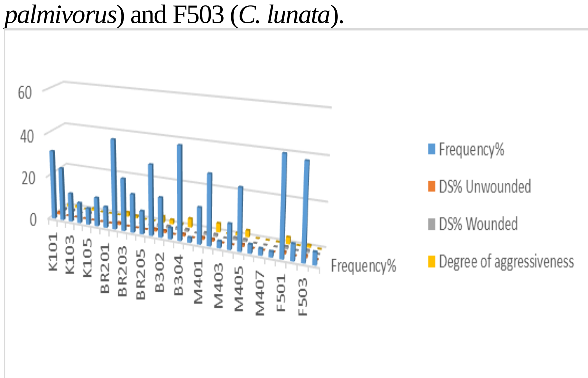

Figure 1. Percent frequency, pathogenicity tests and degree of aggressiveness of all isolated fungi.

Molecular Identification of Pathogenic Fungi

The study classified 15 pathogenic fungal isolates that were isolated from date palm root rot. The findings provide compelling evidence for further research into the prevalence and severity of root rot diseases in date palms in districts and locations of the New Valley Governorate. Furthermore, certain pathogenic fungi in date palms (8 genera) that were initially identified in Egypt. Table (2) provides an overview of these results, which indicates that there are three pathogenic fungi in the Kharga district, namely E. rostratum (first detection in Egypt), T. pinophilus (first detection in Egypt), and C. australiensis (first recorded in Egypt). The Baris district harbors two pathogenic fungi, F. solani and C. spicifera, the latter of which was initially identified in Egypt. The Balat district is home to four pathogenic fungi: two isolates of A. vagum (which was first identified in Egypt) and two isolates of F. oxysporum. Two pathogenic fungi have been identified in the Frafra district: F. solani and C. lunata (first recorded in Egypt). The Dakhla district (Mut) is home to four pathogenic fungi: M. palmivorus (first detected in Egypt) and three isolates of F. oxysporu

Table 2. DNA identification of pathogenic fungi, bootstrap, weight, and accession number in in GenBank

District	Isolate code	DNA identification	Bootstrap (bp)	Weight kDa	Gb Accession No.	% similarity to GenBank sequences	GenBank reference
Kharga	K101	*E. rostratum*	587	188.839	*MZ707080	100	MT322129
	K102	*T. pinophilus*	565	182.675	*OL985894	100	OL985894
	K104	*C. australiensis*	557	179.411	*OL984253	100	MN173134
Baris	BR202	*F. solani*	509	164.432	MZ702989	99.80	MK367716
	BR203	*C. spicifera*	545	175.406	*MZ675605	100	MN173128
Balat	B301	*A. vagum*	532	171.334	*ON926983	98.18	KF494164
	B302	*A. vagum*	522	168.047	*ON926985	98.38	KF494164
	B303	*F. oxysporum*	521	167.820	MZ703641	100	MT560381
	B304	*F. oxysporum*	540	174.094	MZ675609	100	MN153518
Dakhla (Mut)	M401	*F. oxysporum*	541	174.441	ON924754	100	MN153518
	M402	*F. oxysporum*	540	174.111	MZ675611	99.81	MN153518
	M404	*M. palmivorus*	583	187.534	*MZ675612	97.09	KF718233
	M405	*F. oxysporum*	531	171.057	MZ675610	100	MN153518
Frafra	F501	*F. solani*	565	182.510	MZ675606	100	MK418649
	F503	*C. lunata*	558	179.546	*ON926994	100	MH183192

***First record in Egypt**

Phylogenetic Analysis

According to sequence analysis, the ITS regions of the nuclear encoded rDNA shown in Figure 2 contain six genus identifications, such as: *F.* spp. *C.* spp. *A.* sp. *E.* sp. *P.* sp. and *T.* species. Genus *Fusarium* consisted of two species: *F. oxysporum*, an isolate of 99.81 percent of isolate MZ675611 (540 bp) and a significant alignment of 100% of the four isolates, MZ675609 (540 bp), MZ675610 (531 bp), MZ703641 (521 bp) and ON924754 (541 bp) were identified as 100%.

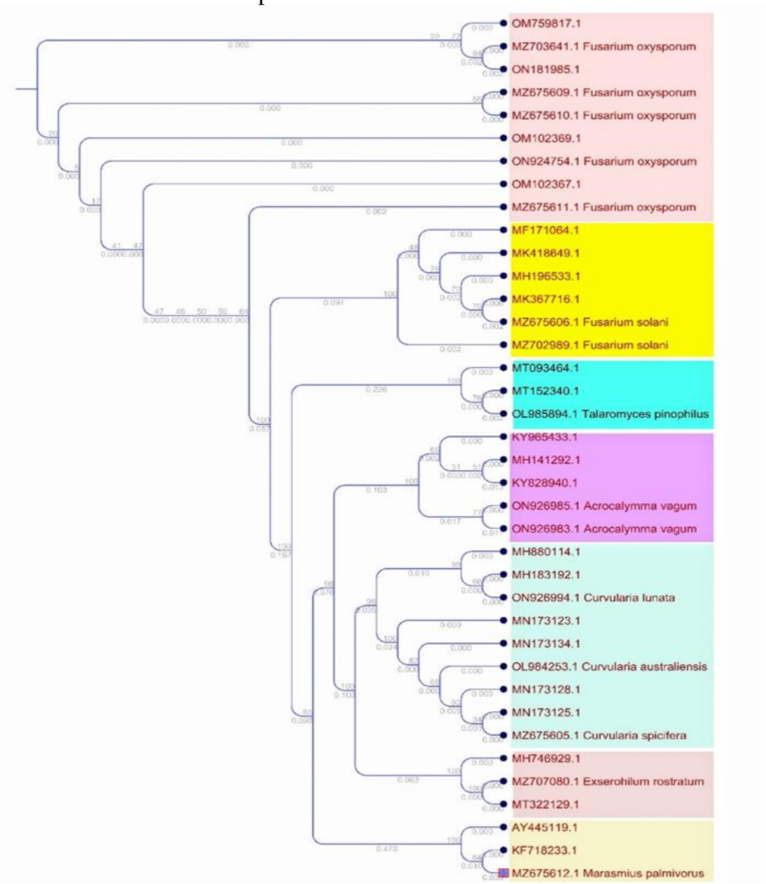

Figure 2. Phylogenetic relationship based on the ITS rDNA regions of the associated fungi isolated from date palm root rot. (Data supporting the results of this figure have been deposited in GenBank with accession numbers from the list of accession numbers with links in Table 2.)

Second, *F. Solani* has a significant alignment of 99.80% of the isolated MZ702989 (509 bp) and 100% of the isolate MZ675606 (565 bp). *C.* spp. represented a remarkable convergence of all three species. *C. australiensis* (557 bp) OL984253, *C. lunata* (558 bp) ON926994 and *C. spicifera* 100% of the samples, MZ675605 (545 bp), of the two isolates *A. vagum* showed a significant concordance of 100%; ON926983 (532 bp) was identified at 98.08% and ON926985 (522 bp) at 98.08%. *E. rostratum* was found to represent a significant alignment of 100% of the MZ707080 sequence (587 bp). A significant alignment of 100% of MZ675612 (583 bp) with a percentage of 97.09 was found in *M. palmivorus*. *T. pinophilus* was found to be a significant alignment of MZ707080 (565 bp), which was detected at 100%.

Discussion

Palms of date are a valuable and affordable tree crop that is widely grown in Egypt and other tropical and subtropical regions. In Egypt, date palms cover about 134,100 Fadden, and in 2021, production is expected to reach about 1,710,000 tons (18% of global production), with an average of 33390.1kg / ha^{-1} (Food). Date palms are affected by several soilborne and aerial pathogenic plant fungi.

Date palm diseases caused by pathogenic fungi, can sometimes result in the death of parent plants or offspring in addition to reducing fruit production and quality (Haq and Khan, 2020). The main factors influencing commercial ration and preventing date yield are diseases, including those caused by pathogenic fungi (Zaid et al., 1999). The dates in Egypt are attack by a number of pathogenic soil fungi that can lead to severe diseases such as root rot (Baraka et al., 2011).

Studies on date palm RRD were conducted in New Valley Governorate, Egypt, in five districts and five locations each. Natural infection rates for DI and DS were recorded for each site and district in cv. Saidy. The distribution of the mean DI and DS percentages was mapped in the New Valley Governorate. The Kharga, Baris, Balat, and Dakhla districts had similar climates, while the Frafra district, located in a different geographic region, had a different climate. The study also observed discrepancies in the incidence of fungal diseases among date palms or new offshoots of cv. Saidy. Differences in environmental factors and management practices were also observed in the areas under study, could account for the statements made in different nurseries and orchards in the New Valley. According to published research, there is a direct correlation between date palm root rot diseases and climatic conditions (Abdullah et al., 2010; Baraka et al., 2011;Shabani et al., 2012).

In Egypt's New Valley Governorate, date palm root rot has been detected in various nurseries and orchards. The severity and frequency of these diseases varied by area, suggesting that environmental factors and management practices may be play a role in their occurrence. However, a strong association between date palm root rot diseases and meteorological factors has been documented in the literature. Studies have identified several pathogenic fungi as causative agents of root rot and wilt diseases in date palm offshoots (Abdullah et al., 2010; Baraka et al., 2011; Shabani et al., 2012; Khairi, 2015). These fungi were differed in their pathogenic abilities, where *F. oxysporum*, *F. solani*, and *F. moniliforme* caused the highest disease incidence and severity (Saremi et al., 2007).

Pathogenicity tests of isolated fungi showed that *F. oxysporum* (isolates B304 and M402) from the Balat and Mut districts were highly pathogenic isolates, while the virulence of the other fungi was low to moderate. These results are consistent with those of Baraka et al. (2011); Ben Chobba et al. (2013); Saleh et al. (2016),who reported that *F. oxysporum* is highly pathogenic to date palms. The virulence of the fungal species tested was greatly differed significantly.

All studied fungi exhibit varying degrees of pathogenicity, which makes sense given that the isolates come from different locations and may have different genetic makeups. However, as noted by Al-Sadi et al. (2012), certain species of fungi on date palm roots have been found to be either nonpathogenic or mildly pathogenic. However, this does not prevent them from causing serious disease in other sections of old trees or their offspring. Date palm root rot samples were collected from the five districts of Egypt's New Valley Governorate. Dakhla, Kharga, Baris, Balat and Frafra. Various fungal isolates were produced by testing the roots collected.

The isolated fungi were purified and 15 species of six genera were identified. *F. oxysporum* (5 isolate), *F. solani* (2 isolates), *A. vagum* (2 isolates), *C. australiensis* (1 isolate), *C. lunata* (1 isolate), *C. spicifera* (1 isolate), *E. rostratum* (1 isolate), *M. palmivorus* (1 isolate) and *T. pinophilus* (1 isolate).

Understanding the genetic variability within and among phytopathogenic strains is crucial for enhancing date palm disease control strategies against pathogenic fungi (Ayaz, et al., 2023). Fungal diseases, like root rot, pose significant threats to agriculture, causing economic losses globally (Singh, et al., 2023). Biological control, utilizing biocontrol agents like fungi and bacteria, offers an environmentally friendly alternative to conventional pesticides for managing plant diseases effectively (Maral & Eltem, 2024).

The use of molecular diagnostic tools, such as DNA-based approaches and PCR assays, enables precise and rapid detection of plant-infecting fungi, aiding in disease diagnosis and management. Additionally, the complex interactions among microorganisms, NPK, soil types, organic fertilizers, and environmental factors influence the diversity and distribution of isolated fungi in different districts, highlighting the need for a comprehensive understanding of these interactions for tailored disease control strategies.

The current study's isolation results agree with those of Baraka et al. (2011), who found that root rot can be caused by soilborne pathogenic fungi. Previous research has also, shown that organic fertilizers and irrigation systems can spread fungal pathogens, including some of the sporadic pathogens that cause date palm root rot diseases. Since most organic fertilizers used on farms to grow date palms are not certified, these fertilizers have contributed to the spread of certain fungi that cause diseases in date palm plantations. Many of these infections may have spread within a single farm or among farms due to flood irrigation, a popular irrigation technique in date palm plantations (Al-Sadi et al., 2011; Al-Sadi et al., 2012).

According to the investigation's findings, eight fungal species are linked to diseased date palm roots for the first time in Egypt. These include *A. vagum* (B301 and B302). The enigmatic darkly septate endophyte occurs in a variety of environments, particularly semiarid and arid. In many plants it is called an endophyte (Deshmukh et al., 2022), The first known causes of strawberry root rot in India were *C. australiensis* (K104), *C. lunata* (F503) and *C. spicifera* (BR203) (Verma and Gupta, 2010). Furthermore, *C.* is a prominent genus, whose species cause widespread, opportunistic phytopathogenic infections in animals and humans (Manamgoda et al., 2015).

Alamri, et al. (Alamri et al., 2019) combined *E. rostratum* (Drechsler) (K101) containing *F. oxysporum* and were found to be the cause of the disease known as lettuce root rot.

According to Pham et al. (2020); Sridhar et al. (2022) the fungus *M. palmivorus* (M404) Sharples was initially identified as the cause of stem rot in the native Formosa palm *Arenga engleri* and white root rot in Arachis

The *T. pinophilus* (K102) (Hedgc.) is widely used as an efficient cellulose-degrading species (Pol et al., 2012).

According to the current study, eight species of fungi have been linked to diseased date palm roots in Egypt. These results have not been reported in any other studies and may have application in integrated disease control initiatives.

CONCLUSION

The New Valley Governorate in Egypt hosts approximately two million date palm trees yielding around 60,000 tons of fruit, establishing date palms as the most crucially cultivated plant in the region. Many soilborne pathogenic fungi infect date palms in Egypt, causing a variety of diseases that cause severe root rot in trees and their offshoots. The current investigation revealed that several pathogenic fungi, including eight newly identified isolates are the main causes of root rot in Egypt. The degree to which the environment influences the difference between the DI and the DS percent in different districts. In addition, pathogenic fungi containing ITS1 and ITS4 were verified to be identified molecularly. In general, the occurrence of date palm root rot diseases is influenced by both environmental factors and management practices, highlighting the need for integrated disease management strategies.

Availability of data and materials

"The datasets generated and/or analyzed during the current study are available in the Bioproject (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA956228)

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List of abbreviations:

ENA	European Nucleotide Archive
FAO	Food and Agriculture Organization
ITS	Internal transcribed spacers
MSA	Multiple Sequence Alignment
NCBI	National Center for Biotechnology Information
PDA	Potato dextrose agar
RRD	Root rot disease
SDW	Sterile distilled water
DI	Disease incidence
DS	Disease severity
RFLP	restriction fragment length polymorphism

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التعريف الجزيئي لمسببات الأمراض الفطرية لمرض عفن جذور نخيل التمر في مصر

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الملخص

تعتبر نخلة التمر من اشجار الفاكهة ذات أهمية كبيرة في المناطق الاستوائية وشبه الاستوائية، وتشتهر بأكثر من 3000 صنف مختلفة موجودة في جميع أنحاء العالم. وتتعرض اشجار نخيل التمر في مصر لمرض تعفن الجذور (RRD) الناتج عن مجموعة متنوعة من فطريات التربة الممرضة للجذور، ويؤدي ذلك إلى إحداث أضرار كبيرة بالأشجار وفسائلها على حد سواء. تشمل الفطريات الأكثر ضررًا في مصر كل من فيوزاريوم أوكسسبورام، والذي يسبب تدهور جذور النخيل، وكذلك فيوزاريوم مونيليفورم، و فيوزاريوم سولاني، ثيلافيوبسيس بارادوكسا، بوتريودبلوديا ثيوبروما، وريزوكتونيا سولاني. تم إجراء مسح شامل وجمع عينات من جذور نخيل التمر في خمس مراكز (الخارجة وباريس وبلاط والداخلة والفرافرة) داخل محافظة الوادي الجديد، مصر. وقد أكدت اختبارات العدوى الصناعية للفطريات ومؤشرات شدة المرض وجود 15 نوعا من ستة أجناس مسببة لمرض عفن الجذور تم تحديدها وهى: *Acrocalymma, Curvularia, Exserohilum, Fusarium, Marasmius, Talaromyces* ومن خلال التحديد الجزيئي باستخدام الفواصل النصية الداخلية (ITS)، تم تحديد أن هذه العزلات الفطرية الثمانية المسببة للأمراض، والتي لم يتم التعرف عليها من قبل في نخيل التمر في مصر، تنتمي إلى الأنواع: *A. vagum, A. vagum, C. australiensis, C. spicifera, C. lunata, E. rostratum, M. palmivorus, T. pinophilus.* ويمثل هذا الاكتشاف أول ارتباط موثق لهذه الفطريات الثمانية مع مرض عفن نخيل التمر في مصر.

الكلمات الدالة: نخيل التمر؛ مرض عفن الجذور؛ فطريات التربة الممرضة؛ (ITS).