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Survey and Identification of Date Palm Pathogens and Indigenous Biocontrol Agents

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Abstract

Fungal diseases are considered a major threat to plant growth and productivity. However, some beneficial fungi growing in the same environment protect plants from various pathogens, either by secreting antifungal metabolites or by stimulating the host immune defense mechanism. Date palms are susceptible to several fungal pathogens. Nevertheless, information on the pathogenic fungal distribution in date palm fields across different seasons is limited, especially that from Qatar. Therefore, the current study's aim was to evaluate the pathogenic and beneficial fungal diversity and distribution, including the endophytic fungi from the date palm tissues and root-associated soil fungi, during different seasons, for the identification of indigenous biocontrol agents. Our results showed that the highest number of fungal species was isolated in fall and spring, and pathogenic fungi were isolated mainly in spring. This is the first report that in Qatar, *Neodeightonia phoenicum* and *Thielaviopsis punctulata* cause date palm root rot disease, *Fusarium brachygibbosum* and *Fusarium equiseti* cause date palm wilting, and *N. phoenicum* causes diplodia disease in date palm offshoots. The combinations of the fungi that did not frequently occur together in date palm rhizosphere soil were investigated to identify indigenous biocontrol agents. Based on the results, we determined that *Trichoderma harzianum* and *Trichoderma longibrachiatum* are effective antagonistic fungi against *T. punctulata*, *N. phoenicum*, *F. brachygibbosum*, and *Fusarium solani*, qualifying them as potential biocontrol agents. Antagonistic activity of endophytic fungi against the pathogens was tested; except for *Ulocladium chartarum*, no endophytic fungi showed antagonistic activity against the tested pathogens.

Keywords: biocontrol agent, date palm phytopathogen, endophyte, *Fusarium brachygibbosum*, *Neodeightonia phoenicum*, rhizosphere soil fungi, *Thielaviopsis punctulata*

Date palm (*Phoenix dactylifera* L.) is an important tree commonly grown in arid regions for its edible fruit. Currently, more than 5,000 date palm cultivars have been recognized, but only a few are used for agronomic purposes (Ibrahim 2008). Date palm is used as a source of fruit and for its herbal medicinal values, and its parts have been traditionally used as medicine to treat various conditions (Amal et al. 2015). As a dominant plant in a harsh ecosystem, date palms constantly interact with heterotrophic microbes present in their niche. Date palms are susceptible to various pathogens, such as *Fusarium oxysporum* f. sp. *albedinis*, which causes Bayoud disease and Fusarium wilt; *Thielaviopsis punctulata*, which causes black scorch, basal trunk disease, trunk rot, root rot, wilt, and rhizosis; and other fungal pathogens such as *Omphalia pigmentata*, *Omphalia tralucida*, *Thielaviopsis paradoxa*, *Mycosphaerella tassiana*, and *Graphiola phoenicis*, which cause foliar diseases, root diseases, and false smut (Abbas and Abdulla 2004; Abdullah et al. 2009; Al-Naemi et al. 2014; Al-Raisi et al. 2011; El-Deeb et al. 2007, El Modafar 2010; Suleman et al. 2002; Zaid et al. 2002).

To survive in such harsh and competitive environments, constant interactions between the plant and other organisms, particularly microorganisms, are essential. These interactions can be either beneficial or deleterious to the host plant. The microorganisms associated with the plants may play an important role in controlling plant growth and in disease resistance. Therefore, studying microorganisms associated with the date palm is necessary to identify the pathogens and any beneficial organisms that help in plant growth or play important antagonistic roles in preventing infections by pathogenic microbes. To establish high-quality date palm cultivars that are free of disease, research on disease-suppressive surrounding soil is important because diseases can be controlled by antagonistic microorganisms inhabiting the soil. Rhizosphere microorganisms are capable of activating the plant defense pathway, that is, they induce systemic resistance, which protects the plant from biotic attacks (Bakker et al. 2013). Certain microbes, such as actinomycetes spp., *Aspergillus* spp., *Bacillus* spp., *Penicillium* spp., and *Pseudomonas* spp., isolated from the soil surrounding the roots of the date palm, have been found to be antagonistic against *Fusarium oxysporum* f. sp. *albedinis*, thus offering protection to date palms from the disease (Chakroune et al. 2008; El Modafar 2010).

To the best of our knowledge, no comprehensive study has been conducted in Qatar on microbial associations with date palms that include the microbes present in soil and on the tree. The current study was designed for the following purposes: to evaluate the fungal species diversity in both healthy and naturally infected date palm tissues and the surrounding soil at different times of the year; and to identify pathogenic and endophytic fungi and the rhizosphere-associated fungi for their potential antagonistic activity against date palm pathogens. The ultimate objective of the study was to assess the potential of these beneficial fungi as biocontrol agents to control date palm diseases.

Materials and Methods

Isolation of fungal pathogens from diseased plants and pathogenicity test. Naturally infected and healthy tissues of date palm were collected from four farms located in northern Qatar, including three private farms (26°03′21.8″N 51°08′35.4″E) and one government farm (25°48′43.8″N 51°21′09.6″E) and two farms located in the southern part of Qatar (24°50′09.4″N 50°53′08.0″E). Infected plant tissues, such as those from the leaf, trunk, and root, were collected for the isolation of pathogenic fungi, and healthy asymptomatic leaf samples were collected for the isolation of endophytic fungi.

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Plant materials collected from the diseased date palm trees were washed thoroughly under running tap water, followed by a round of washing under sterile distilled water. Infected tissues were surface sterilized by soaking them in 1.0% NaOCl for 60 s, followed by rinsing with distilled water. Sterilized tissues were plated on potato dextrose agar (PDA) for pathogen isolation. After 2 days of incubation, the hyphal tip of each fungus was transferred onto the PDA medium. Single isolates were transferred onto the following different media, namely, malt extract agar, Czapek-Dox agar (30 g of sucrose, 2 g of $NaNO_3$, 1 g of KH_2PO_4, 0.5 g of KCl, 0.5 g of $MgSO_4 20.7H_2O$, 0.1 g of $FeSO_4 0.7H_2O$, 1 g of $ZnSO_4$, 0.5 g of $CuSO_4 0.5H_2O$, and 15 g of agar per liter), and PDA. Each fungal isolate was identified on the basis of morphological (color of the fungal colony in different media, pattern of growth, sporulation, presence of secretion or exudates, and the number of days needed for growth on different media) and microscopic (evaluation of hyphal and spore structures) characteristics. Microscopic analysis was performed by the examination of fungal slides stained with lactophenol cotton blue examined under a compound light microscope (Aina et al. 2011). Sporangia and spores of each fungus were examined carefully under 10× and 40× magnifications. Additional structure and surface patterns of each spore were recorded carefully. Fungus identification and confirmation were based on relevant reference illustrations (Barnett and Hunter 1998; Leslie and Summerell 2006; Moubasher 1993; Nagamani et al. 2006).

All isolated fungi were subjected to the pathogenicity test conducted by the detached leaf assay. Detached leaf assays were performed to evaluate the pathogenicity of fungi isolated from the rhizosphere of date palms. Leaves from 5-year-old date palm cultivars of Barhi, Khalas, and Khneezi were collected and surface sterilized by soaking in 1% NaOCl for 5 min, followed by rinsing in sterile distilled water three times. To facilitate pathogen infection, a small incision was made in the midvein of the leaves with a sterile scalpel. An actively growing fungal mycelial disk (diameter of 4 mm) was cut out from the medium and inoculated onto the incision made in the midvein of the leaf. An agar disk without fungal mycelium was used to inoculate the control samples. The inoculated area was covered by moist cotton wool. Ten leaves from each cultivar were inoculated with each fungal isolate. Leaves were placed in a 150-mm × 30-mm sterile Petri dish containing sterile filter paper layered on moist cotton wool. Then the Petri dish was then sealed with semitransparent film and incubated in the light under a 12-h photoperiod at 25°C. The frequency and size of necrotic lesions on the infected leaves were recorded after 72 h of infection.

The fungi that caused higher necrotic intensity in leaves as assessed by the detached leaf assay were selected for further pathogenicity tests on date palms in vivo by inoculation of the rachis and leaves of 7-year-old date palms grown in large pots under greenhouse conditions (27°C ± 2°C and 12-h/12-h light/dark cycle). The pathogenicity test was conducted by inoculating a disk, with a diameter of 8 mm, of actively growing fungal mycelium from the PDA plate onto a 10-mm incision created on the rachis region; the disk was also placed on the leaves, wherein small incisions were made to facilitate pathogen entry. The inoculated area was covered with wet cotton wool and sealed with transparent adhesive tape. Then, the whole plant was covered with a polythene bag to maintain humidity and was maintained under greenhouse conditions until the appearance of necrosis. Mock-inoculated trees, with 8-mm PDA disks, were used as controls.

Isolation of fungi from soil. Soil samples needed for this study were collected from the same farms where date palm tissues were collected. Rhizosphere soil samples from healthy and naturally infected date palms were collected. Soil samples were collected carefully by removing 1 cm of soil around the root of a date palm with a sterile spatula to avoid contamination with surface organic matter in the collected samples. Then, the soil was collected from a depth of 15 cm with a steel borer. The soil samples were placed in individual sterile polythene bags to avoid contamination and were stored at 4°C under aseptic conditions until use. Soil samples were collected during different seasons as follows: winter (December to February, temperature of 9 to 29°C and humidity of 17 to 100%), spring (March to May, 16 to 41°C, 7 to 94%), summer (August to September, 28 to 45°C, 8 to 89%), and fall (November, 19 to 34°C, 17 to 89%).

In total, 15 rhizosphere soil samples were collected in each season, five samples were collected from the rhizosphere of naturally infected date palms, and 10 samples were collected from the rhizosphere of healthy date palms. Because the number of diseased date palms was lower than that of the healthy date palms and a high disease incidence was observed in northern Qatar, we opted to collect the samples in a 2:1 ratio of rhizosphere soil under the healthy date palms and under the naturally infected date palms.

Direct inoculation method and soil dilution plate method were used for fungal isolation from the soil. The direct inoculation method was conducted by adding 0.1 g of finely ground soil to 25 ml of melted PDA at 40°C that was spread evenly in a Petri dish. Once the medium solidified, it was incubated at 25°C for 2 days. For the soil dilution method, 1 g of soil was added to 10 ml of sterile distilled water, and the mixture was shaken for 1 h in a shaker at room temperature to ensure thorough mixing. Dilutions of 10^{-1} to 10^{-5} were prepared; of the prepared dilutions, dilutions of 10^{-4} and 10^{-5} were inoculated on PDA by the pour plate method by adding 1 ml of diluted sample to 24 ml of melted PDA in a Petri dish and incubated at 25°C for 2 days. Two technical replicate plates were prepared for all samples. Fungi were categorized as pathogenic and nonpathogenic based on the results of the pathogenicity test.

All the isolated fungi from rhizosphere soil samples were subjected to the detached leaf assay to detect their pathogenicity. The pathogenicity of fungi isolated from the rhizosphere soil, where diseased offshoot plants exhibited more symptoms, were analyzed with offshoot plants under greenhouse conditions. The test was conducted by inoculating a mycelial disk (diameter of 5 mm) onto the small incision made with a sterile needle. The inoculated area was covered with wet cotton wool, and the plant was covered with a polythene bag to maintain humidity. Mock-inoculated offshoot plants, inoculated with 5-mm PDA disks, served as the controls.

Isolation of endophytic fungi and yeast from healthy plants. Endophytic fungus isolation was performed according to the method of Arnold et al. (2000). Healthy plant tissues were cut into small square pieces. These pieces were surface sterilized by soaking them in 70% ethanol for 30 s, after which they were soaked in 1.0% NaOCl for 60 s. The plant tissues were then rinsed in sterile distilled water and blot dried. The inner tissues of the surface-sterilized samples were plated on water agar and incubated at 25°C until the appearance of fungal growth. After the hyphal tip of each fungus were removed under the microscope, the tips were transferred onto fresh PDA medium. Subculturing and incubating at 25°C were performed subsequently until pure cultures were obtained.

The collected leaf samples were kept in a moist chamber after surface sterilization to allow endophytic fungi to grow and induce sporulation (Shurtleff and Averre 1997; Waller et al. 1998). Any fruiting body that was generated after 7 days of incubation in the moist chamber at room temperature was examined under the microscope. Characteristic features observed under the microscope were similar to those of yeasts. For confirmation, the fruiting bodies were grown on malt extract agar at 25°C until the appearance of growth. Pinkish-orange yeast-like colonies appearing on the medium were examined under a compound microscope. Yeast cells were further grown in 3 ml of malt extract broth at 25°C overnight for DNA extraction. The overnight culture was centrifuged for 5 min at 3,000 rpm for collecting the cell pellet. Then, 0.5 ml of lysis buffer (50 mM of EDTA, 1% sodium dodecyl sulfate, and 0.1 M of Tris-Cl pH 8.0) was added to the pellet along with sterile glass beads. The mixture was vortexed at high speed for 1 min and then kept on ice for 1 min. This process was repeated six times. Then, the tubes were incubated in a water bath at 70°C for 10 min. An aliquot (200 µl) of 3 M of potassium acetate and 150 µl of 4 M of NaCl were added to the tubes, which were then maintained on ice for 10 min. After incubation, the samples were centrifuged at 13,000 rpm for 10 min. The supernatants were transferred into fresh tubes, after which 2.5 times of the volume of absolute alcohol was added to each,

followed by incubation at −20°C for 1 h. After incubation, the samples were centrifuged at 13,000 rpm for 5 min, after which the supernatants were discarded. The pellet was air dried under sterile conditions and was dissolved in Tris-EDTA buffer. The extracted DNA was stored at −20°C until use. Species identity was confirmed via rDNA internal transcribed spacer (ITS) region sequence as described below.

DNA isolation and identification of fungi. The pathogens that caused the necrotic lesions of the highest intensity, as assessed by the detached leaf test, were selected for ITS sequencing to confirm the species. DNA extraction from the fungal samples performed as follows. Fungal mycelium was scraped from the 7-day-old fungal culture grown on PDA and ground into powder with liquid nitrogen. Qiagen Mini kit (Qiagen, Redwood City, CA) was used for DNA extraction. Then, the ribosomal DNA (rDNA) ITS region of the extracted DNA was amplified with the universal primers ITS1 (5′-TCCGTAGGT GAACCTGCGG-3′) and ITS4 (5′-CCTCCGCTTATTGATATGC -3′) (White et al. 1990). For the 25-µl PCR, 2 µl of DNA (25 ng/µl) was taken as the template, and 10 µM dNTP, 5 µM of ITS1 and ITS4 primer, 1.5 mM of $MgCl_2$, 1× buffer, and 1 unit of *Taq* polymerase (Applied Biosystem Thermal Cycler, Foster City, CA) was added. PCR was carried out with a Veriti thermal cycler (Applied Biosystems) under the following conditions: 94°C for 4 min for initial denaturation followed by 35 cycles of 1 min at 94°C for denaturation, annealing at 55.5°C for 2 min, extension at 72°C for 2 min, and final extension at 72°C for 10 min. QIAQuick PCR Purification Kit (Qiagen, Hilden Germany) was used for PCR product purification according to the manufacturer's procedure. DNA sequencing was performed using the BigDye Terminator Ready Reaction Kit (Life Technologies, Foster City, CA). The sequences obtained with an average length of 550 bp were analyzed in the NCBI database by BLASTn. ITS rDNA sequences showing >98% identity with the sequence in NCBI database were considered the same species. The e-value cutoff was 0.0 for BLASTn analysis. DNA extraction and rDNA ITS sequencing and BLASTn analysis were performed to confirm the antagonistic fungi species, as described above.

In vitro antagonism assay. After detailed analysis of fungal combination present in rhizosphere soil and plant tissues from infected and healthy date palm trees, some fungal species were characterized based on their antagonistic activity against other fungal species. To study the antagonistic activity of such isolated fungi, in vitro analysis was conducted by the coinoculation assay. Mycelial disks (diameter of 5 mm) were cut out from the edge of the actively growing antagonistic fungal and pathogenic fungal cultures; then, one fungal disk from both species was placed 4 cm apart at two ends of the PDA plate of diameter 8 cm. This experiment was performed in triplicate. The control plates were incubated without the antagonistic fungus, and they served as negative controls. The plates were incubated at 25°C for 7 days. The growth of the pathogenic fungus was measured from 3 days after inoculation (dpi). Percentage inhibition was calculated according to the following formula:

$$I = [(C - T)/C] \times 100$$

where I is the percentage of inhibition of the pathogen, C is the radial growth of the pathogen in the control, and T is the radial growth of pathogen in the presence of antagonistic fungus.

Different types of fungal interactions between antagonistic fungi and pathogenic fungi were assessed to determine whether any clear inhibition zone was present. Six types of interactions have been reported and categorized as types A to F. "(1) mutual intermingling growth, where both fungi grew into each other without any macroscopic signs of interaction: Type A; (2) mutual inhibition on contact or space between colonies small (<2 mm): Type B; (3) inhibition of one species on contact, the inhibited species continued to grow at a significantly reduced rate, while the inhibitor species grew at a slightly reduced rate or unchanged: Type C; (4) mutual inhibition at a distance (>2 mm): Type D; (5) inhibition of one species on contact, the inhibitor species continued to grow at a reduced rate through the inhibited colony: Type E; and (6) inhibition of one species on contact or at a distance, the inhibitor species then continuing to grow at an unchanged rate through or over the inhibited colony: Type F" (Wheeler and Hocking 1993).

Statistical analysis. Significant differences in the antagonistic activities of *Trichoderma harzianum* and *Trichoderma longibrachiatum* against the fungal pathogens were statistically analyzed by one-way analysis of variance (ANOVA) with $P < 0.05$ as the level of significance.

Results

Fungal isolation from date palm tissue samples. Date palms with various disease symptoms were surveyed during different seasons as follows: winter (December to February), spring (March to May), summer (August to September), and fall (November). Infected plant tissues from the leaf, root, and trunk of diseased date palm trees were collected from three private farms located in northern Qatar for the isolation of pathogens, whereas healthy leaves, trunk, inflorescence, and roots were collected from six farms located in northern and southern Qatar (four in northern and two in southern Qatar) for the isolation of nonpathogenic endophytic fungi. Because a high disease incidence was observed in northern Qatar, the farm located in northern Qatar was selected for sample collection from diseased date palms.

Date palm pathogenic fungi isolation. Date palms with symptoms resembling those of palm diseases such as black leaf scorch, trunk rot, inflorescence blight wilt, offshoot diplodia disease, and root rot were surveyed during different seasons. Black scorch, trunk rot, and root rot symptoms were observed mainly from November to April. This survey on the diseases was conducted in six farms (five private farms and one government farm) located in northern and southern Qatar. All diseased trees recorded were from the three private farms located in northern Qatar. Therefore, the samples were collected only from the farms located in northern Qatar. The pathogenic fungi *Neodeightonia phoenicum*, *T. punctulata*, *Fusarium brachygibbosum*, *Fusarium equiseti*, and *Fusarium solani* were isolated from the diseased trees tissues obtained during different seasons. The BLASTn analysis of ITS sequences identified as *N. phoenicum*, *F. brachygibbosum*, *F. solani*, *T. punctulata* and *F. equiseti* from date palm were deposited in GenBank (Table 1).

N. phoenicum was isolated from the samples obtained from the parts affected by root rot in fall and winter along with other nonpathogenic fungi. The isolation frequency of *N. phoenicum* was 28 and 27% in fall and winter, respectively. *T. punctulata* was isolated from the samples obtained from parts affected by trunk rot and root rot in spring (isolation frequency of 27 and 31%, respectively). *F. brachygibbosum* was isolated from wilted date palms leaf samples in spring and summer (isolation frequency of 28 and 25%, respectively). *F. equiseti* was isolated from the wilted plant samples in fall (isolation frequency of 29%). *F. brachygibbosum* and *F. solani* (isolation frequency of 19 and 26%, respectively) were isolated from the samples obtained from the parts affected by root rot in spring (Table 2).

Date palm endophytic fungi isolation. Of the 26 endophytic fungal isolates identified, 17 belonged to five species: *Cladosporium sphaerospermum*, *Verticillium theobromae*, *Macrophomina* spp. (all were isolated from the leaf and trunk), *Penicillium* spp. (leaf, trunk, and inflorescence), and *Ulocladium chartarum* (leaf); the remaining isolates were categorized as cryptic fungi with immersed mycelia (leaf, trunk, inflorescence, and root). *U. chartarum* and *Penicillium* spp. were isolated consistently from leaf samples, and unknown cryptic fungal isolates were purified from trunk and inflorescence tissue samples. Some cryptic fungi were also isolated from leaf and root tissue samples. The isolated fungi were confirmed to be nonpathogenic by the detached leaf test. An endophytic yeast isolated from date palm leaf samples was confirmed to be the red yeast *Rhodotorula marina* based on rDNA ITS region sequencing, and its sequence was deposited in GenBank; 490 bp of *R. marina* (MN203096) had 98% sequence identity with KY744117 in the NCBI database. *R. marina* did not show any antagonistic activity against the pathogenic fungi but showed antagonistic activity against

Penicillium expansum, which is known to be nonpathogenic to date palms. Thus, the other potentially beneficial roles of endophytes, such as growth promotion and biotic stress tolerance with respect to date palm, must be studied further.

Pathogenicity assay. The pathogenicity of fungi isolated from the diseased tree samples was assessed by the detached leaf assay, and the pathogenicity of fungi that induced high necrosis in leaves was confirmed by a pathogenicity test conducted by inoculating date palm seeds under greenhouse conditions. A black rusty infection appeared at 5 days dpi in *T. punctulata*-inoculated rachis, and black scorch disease symptoms appeared at 20 dpi. However, necrosis in *F. brachygibbosum*-inoculated rachis appeared at 7 dpi, and wilting was observed in rachis and the leaf base at 20 dpi. Although necrosis in *N. phoenicum*-inoculated rachis appeared at 7 dpi, no further changes or disease symptoms were observed after the appearance of necrosis. Mock-inoculated control date palm showed no symptoms (Fig. 1). Necrosis was observed in leaves on the third day of *T. punctulata* inoculation, and black scorch disease symptoms and wilting were observed at 20 dpi. On inoculation of *F. brachygibbosum*, leaf necrosis was found at 4 dpi, and wilting was observed at 18 dpi. In *N. phoenicum*-inoculated leaves no further changes or disease symptoms were observed after necrosis was observed at 4 dpi. No symptoms were observed in mock-inoculated date palm leaves (Fig. 2). *F. equiseti*- and *F. solani*-infected tissues showed necrosis in rachis at 10 dpi and in leaf at 7 dpi.

Seasonal distribution of date palm-associated fungi isolated from the rhizosphere. In total, 182 soil fungal isolates were successfully obtained during different seasons, comprising 26 species belonging to 12 genera; of these isolates, 53 were isolated in fall, 34 were isolated in winter, 62 were isolated in spring, and 26 were isolated in summer. The most common genera of fungi in the rhizosphere of date palms were *Rhizopus* and *Aspergillus*, followed by *Fusarium* (Fig. 3). *Aspergillus niger* was found throughout all the seasons in the rhizosphere of both healthy and diseased date palms, along with *Rhizopus* spp., although *Trichoderma* spp were found in fall, winter, and spring, the highest number of isolates obtained in fall and winter. *Trichoderma* spp. were isolated mainly from healthy date palm rhizosphere soil, whereas *Aspergillus* spp., *Rhizopus* spp., and *Fusarium* spp. were isolated from in soils under both healthy and diseased date palms. The frequently isolated fungus in summer was *Fusarium* spp (Fig. 3). The highest number of fungal species was isolated in fall and spring, with the lowest being isolated in summer.

Isolation of date palm pathogenic fungi from the rhizosphere. The pathogenicity of all fungi isolated from date palm rhizosphere soil samples was determined by the detached leaves test, with the virulence of the isolated fungi on date palm being determined by detecting the intensity of necrosis under ultraviolet light and by measuring the lesion size (Table 3). Based on the appearance of the necrotic lesions, fungi were categorized as pathogenic or nonpathogenic. *Pythium* spp., *Fusarium moniliforme*, and *Chaetomium globosum* were lost during subculturing, and thus no pathogenicity tests were carried out for these species.

Date palm pathogenic fungi were isolated mainly in spring. Wilt-causing *F. brachygibbosum* and *F. equiseti*, root rot-causing *T. punctulata* and *F. solani*, and diplodia disease- and root rot-causing *N. phoenicum* were isolated from the rhizosphere soil mainly in spring. *T. punctulata* was also isolated from the soil in early summer. *N. phoenicum* was found in the rhizosphere soil of date palm and its offshoot plants affected by diplodia disease in winter apart from spring. The pathogenicity test conducted on the offshoots of the plant under greenhouse conditions revealed the presence of diplodia disease in the tested plant and confirmed the pathogenicity of *N. phoenicum*. *N. phoenicum*-inoculated offshoot plants showed typical symptoms

Table 1. Summary of BLASTn results of the ribosomal DNA internal transcribed sequence of date palm-associated fungi isolated in this study, showing base pair and GenBank accession numbers of deposited sequence, coverage, and sequence identity with target sequence (e-value 0.0)

Fungal isolate	Base pair	GenBank accession number	Query coverage	Percentage of sequence identity	GenBank accession number of organism with the highest sequence identity
Neodeightonia phoenicum	517	MN201622	97	98	KF766198
Thielaviopsis punctulata	542	MN202794	94	99	KU365774
Fusarium brachygibbosum	523	MN201962	100	99	KR071881
Fusarium solani	598	MN202790	94	99	MG836251
Fusarium equiseti	587	MN202016	86	99	MK290391
Trichoderma harzianum	565	MN263160	100	100	KT285888
Trichoderma longibrachiatum	631	MN203130	100	99	KY859792
Rhodotorula marina (*Symmetrospora marina*)	490	MN203096	100	98	KY744117

Table 2. Percentage of fungal isolates from naturally infected date palm tissues in different seasons

	% Isolation (season)[b]		
Isolated fungi[a]	Root rot	Trunk rot	Wilted leaf
Aspergillus niger	18 (fall), 23 (winter), 8 (spring)	12 (spring)	6 (fall), 10 (spring), 13 (summer)
Cryptic fungal species	25 (fall), 30 (winter), 12 (spring)	23 (spring)	21 (spring), 25 (fall) 20 (summer)
Fusarium brachygibbosum	18 (spring)	–	28 (spring), 25 (summer)
Fusarium equiseti	–	–	29 (fall)
Fusarium solani	21 (spring)	–	–
Macrophomina spp.	5 (spring)	–	7 (spring)
Neodeightonia phoenicum	28 (fall), 27 (winter)	–	–
Penicillum spp.	14 (fall), 20 (winter), 3 (spring)	14 (spring)	16 (spring), 7 (fall), 15 (summer)
Pythium spp.	5 (spring)	–	–
Thielaviopsis punctulata	30 (spring)	27 (spring)	5 (spring)
Ulocladium chartarum	10 (fall)	13 (spring)	13 (spring), 20 (fall), 12 (summer)
Verticillium theobromae	5 (fall)	–	–
Cladosporium sphaerospermum	–	11 (spring)	–

[a] Fungi in boldface are pathogenic fungi.
[b] Sign (–) indicates absence of fungi.

of diplodia disease at 15 dpi (Fig. 4). The obtained *F. oxysporum* isolate did not cause leaf necrosis, as assessed by the pathogenicity test on leaf tissue samples. *F. brachygibbosum* was isolated from the rhizosphere in spring and summer. *Fusarium* spp., such as *F. brachygibbosum* and *F. equiseti*, capable of causing date palm wilt, were isolated from the soil under date palms in spring to summer and winter to spring, respectively.

Antagonistic fungal isolates from rhizosphere soil. From the rhizosphere of healthy date palms, 10 isolates of *Trichoderma* were purified. After the isolates showing similar morphological characters were excluded, two isolates were selected for rDNA ITS region sequencing for species confirmation. rDNA ITS regions from the *Trichoderma* isolates were sequenced and deposited in GenBank. Isolated *Trichoderma* spp. were confirmed to be *T. harzianum* and *T. longibrachiatum* via BLASTn analysis of the ITS rDNA sequences against the NCBI database. The length of ITS rDNA sequence of *T. harzianum* (MN263160) and *T. longibrachiatum* (MN203130) was 565 bp and 631 bp, respectively, and had 100 and 99% sequence identity with ITS rDNA region from *T. harzianum* (KT285888) and *T. longibrachiatum* (KY859792), respectively. Species identification was further performed in TrichOKEY 2 (Druzhinina et al. 2005), and the species were confirmed to be *T. harzianum* and *T. longibrachiatum*. TrichOKEY 2 is a program for quick molecular identification of *Hypocrea* and *Trichoderma* on the genus and species levels based on an oligonucleotide DNA BarCode, a diagnostic combination of several oligonucleotides (hallmarks) specifically allocated within the ITS1 and ITS2 sequences of a ribosomal RNA gene cluster.

Coinoculation assay. The antagonistic activity of *Trichoderma* spp. isolated from the rhizosphere soil samples was tested against the date palm pathogenic fungi, namely, *N. phoenicum*, *F. brachygibbosum*, *F. solani*, and *T. punctulata* by the coinoculation assay.

Fig. 1. Pathogenicity test of *Thielaviopsis punctulata*, *Fusarium brachygibbosum*, and *Neodeightonia phoenicum* inoculated on the rachis of date palms. **A,** A rusty black infection observed in *T. punctulata*-inoculated rachis at 5 dpi. **B,** Necrosis was observed around *F. brachygibbosum*-inoculated rachis at 7 dpi. **C,** Necrosis was observed around *N. phoenicum*-inoculated rachis at 7 dpi, after which no more changes were observed. **D,** No necrosis was observed in mock-inoculated rachis at 7 dpi. **E,** Symptoms of black scorch disease were observed at 20 dpi in *T. punctulata*-inoculated rachis. **F,** Symptoms of wilting were observed in *F. brachygibbosum*-inoculated rachis at 20 dpi. **G,** No symptoms were observed in mock-inoculated rachis at 20 dpi.

Type E interactions were observed between the antagonistic fungi (*T. harzianum* and *T. longibrachiatum*) and pathogenic fungi (*T. punctulata*, *N. phoenicum*, *F. brachygibbosum*, and *F. solani*), with the inhibitory activity of *T. longibrachiatum* being lesser than that of *T. harzianum* (Table 4). Type E interactions do not affect the growth of antagonistic fungi but reduce pathogen growth. We analyzed the antagonistic activities of *T. harzianum* and *T. longibrachiatum* against the date fruit pathogen *A. niger*; type D and type B interactions were observed between *T. harzianum* and *A. niger* and between *T. longibrachiatum* and *A. niger*. *T. harzianum* and *A. niger* showed mutual inhibition and displayed a >2-mm clear inhibition zone in between, whereas *T. longibrachiatum* and *A. niger* mutually inhibited one another without a clear inhibition zone. Because *A. niger* is not a date palm pathogen, data are not included in Table 4.

To investigate the role of endophytic fungi in the potential biocontrol activity against pathogenic fungi, the antagonistic activities of the isolated endophytic fungi were analyzed by coinoculation assay. None of the endophytic fungal isolates, with the exception of *U. chartarum* against *T. punctulata* and *N. phoenicum*, were effective against the pathogens tested. *U. chartarum* showed a type D interaction against *T. punctulata* and *N. phoenicum*. Because both organisms were mutually inhibitory, *U. chartarum* cannot be considered a potential biocontrol agent.

Discussion

This is the first study to examine the distribution of fungi in date palm tissues and rhizosphere soil to identify date palm pathogens and indigenous biocontrol agents in Qatar. In the present study, we

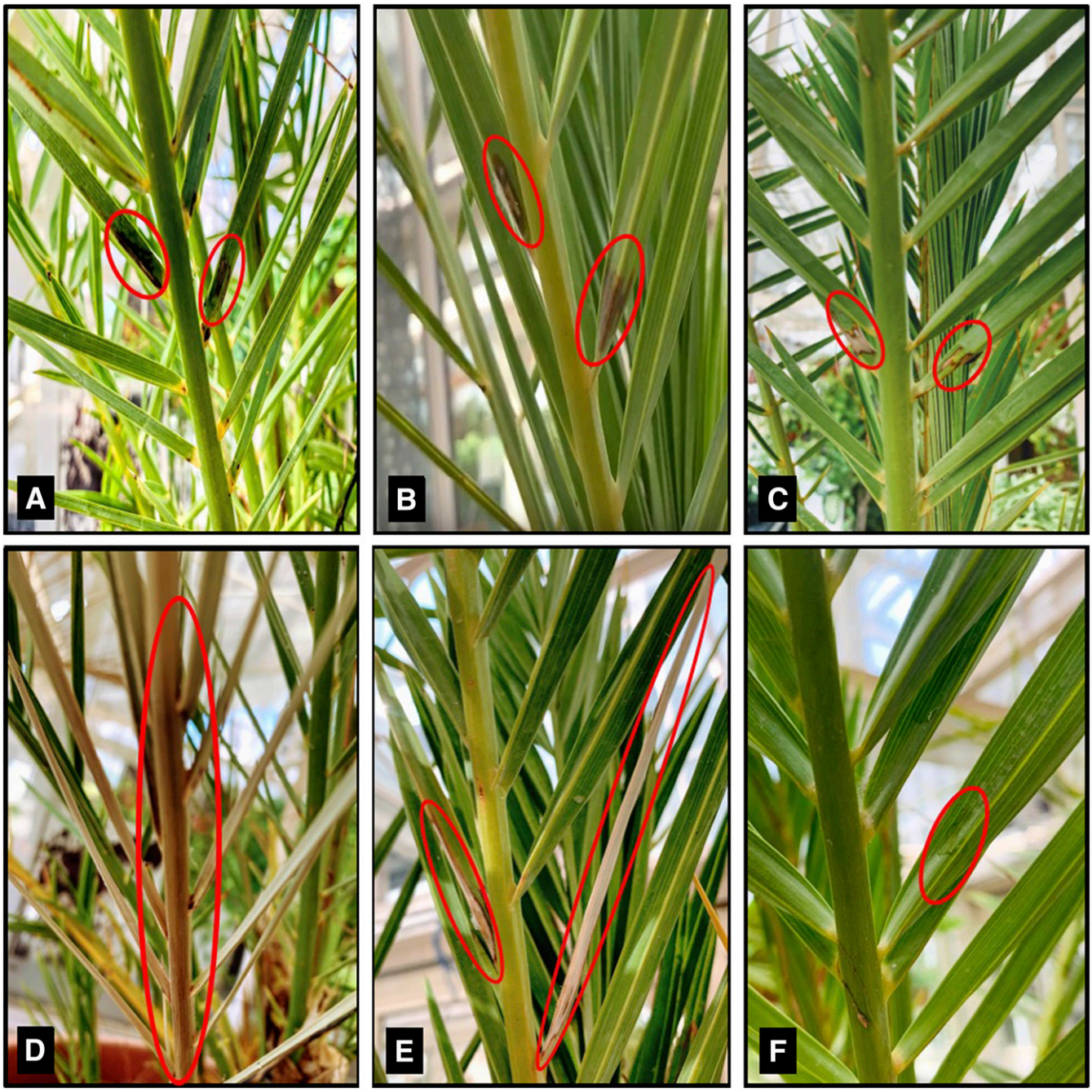

Fig. 2. Pathogenicity test of *Thielaviopsis punctulata*, *Fusarium brachygibbosum*, and *Neodeightonia phoenicum* inoculated on the leaves of date palms. **A to C,** Necrosis was observed in *T. punctulata*-inoculated leaves at 3 dpi and in *F. brachygibbosum*- and *N. phoenicum*-inoculated leaves at 4 dpi. No more changes were observed in *N. phoenicum*-inoculated leaves. **D,** Symptoms of black scorch disease and wilting were observed in *T. punctulata*-inoculated leaves at 20 dpi. **E,** Leaf wilting was observed in *F. brachygibbosum*-inoculated leaves at 18 dpi. **F,** No necrosis was observed in mock-inoculated leaves at 4 dpi, and no more changes were observed.

analyzed the pathogen distribution over four seasons. To the best of our knowledge, this is the first report of root rot and diplodia disease caused by *N. phoenicum* in Qatar. Although root rot caused by *N. phoenicum* has been infrequently reported, the incidence and severity of diplodia disease caused by *N. phoenicum* have been reported worldwide (Zaid et al. 2002). *N. phoenicum* associated with severe rot diseases was reported in Greece, with the symptoms including leaf decay and premature death, terminal bud necrosis, stalk rot, and shoot blight (Ligoxigakis et al. 2013). To evaluate the pathogenicity of *N. phoenicum* in date palms, a pathogenicity test was conducted on leaves and rachis. The pathogenicity test was also conducted on offshoot plants. Although inoculated leaves and rachis did not exhibit any symptoms, the offshoot plants were adversely affected by inoculation, and yellowing was predominant at 20 dpi, indicating symptoms of diplodia disease.

The association of *F. brachygibbosum* with date palm wilting in the present study is the first such report in Qatar. Isolation of *F. brachygibbosum* from wilted date palm indicated a new host for the pathogen. *F. brachygibbosum* is the causal agent of canker in cold-stored and bare root-propagated almond, leaf spot on the ornamental plant oleander, and dieback disease on *Euphorbia larica* (Al-Mahmooli et al. 2013; Mirhosseini et al. 2014; Stack et al. 2017). *F. brachygibbosum* had previously been reported to be a weakly pathogenic fungus isolated from root rot lesions of date palm in Oman (Al-Sadi et al. 2012). We isolated *T. punctulata* from diseased date palms exhibiting trunk rot and root rot symptoms. In Qatar, *T. punctulata* infection in date palm is more commonly shown as black scorch of leaves and trunk rot (Al-Naemi et al. 2014); consistent isolation of *T. punctulata* from diseased date palms exhibiting trunk rot in the present study confirmed this finding. Severe *T. punctulata* infection in date palms has also been reported in the United States, Spain, and Africa (Abdullah et al. 2009; Linde and Smit 1999). *T. punctulata* has a wide host range, causing root rot in pineapple and fruit rot in lemon (Kile 1993; Mirzaee and Mohammadi 2005).

The other two pathogens isolated from tissues of date palm affected by wilt and root rot were *F. equiseti* and *F. solani*, respectively. The pathogen *F. oxysporum* f. sp. *albedinis*, causing a major date palm disease, Bayoud disease, was not found in soils in Qatar during this survey. Date palms infected with *F. equiseti* were reported in Iraq (Abbas et al. 1991), but no other studies have reported on the association of *F. equiseti* with date palm diseases. *F. equiseti* causes vascular wilt on cumin plants, damping-off disease on Aleppo pine, severe wilt in cauliflower plants, and foliar necrosis and wilt on pecan (Lazarotto et al. 2014; Lazreg et al. 2014; Li et al. 2017; Ramchandra and Bhatt 2012). Although *F. solani* is a virulent pathogen present on several plant species, our findings indicate that it is less virulent for date palms (Han et al. 2017; Pérez-Hernández et al. 2017).The association of *F. solani* with date palms has been reported in Iran and Oman. It causes yellow death of date palms in Iran, whereas it was found to be a weak pathogen causing root rot in Oman (Al-Sadi et al. 2012; Mansoori and Kord 2006). Although *A. niger*, *Penicillium* sp., and some cryptic fungi were isolated from diseased date palm samples, they were categorized as nonpathogenic fungi after detached leaf pathogenicity tests were conducted. However, Zaid et al. (2002) reported that *A. niger* is a common fungus associated with date palm fruit spoilage. The presence of this fungus may severely affect date fruit production during the fruiting time.

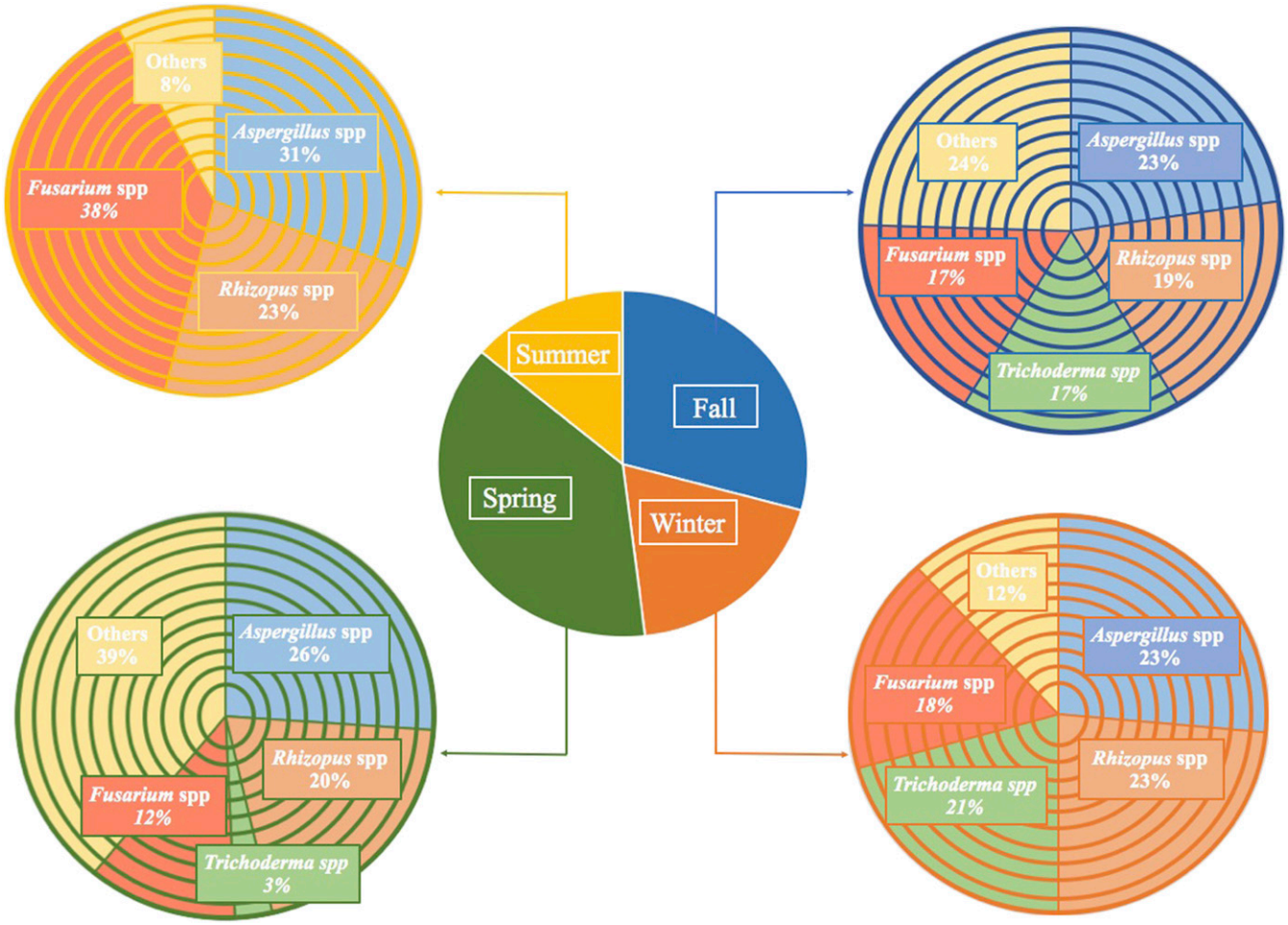

Fig. 3. Seasonal distribution of fungal isolates obtained from the rhizosphere soil of date palms. In total, 182 fungal isolates were obtained over the four seasons; of these, 53 were isolated in fall, 34 were isolated in winter, 62 were isolated in spring, and 26 were isolated in summer. *Aspergillus* spp., *Rhizopus* spp., *Trichoderma* spp., and *Fusarium* spp. were the most common genera. Isolated date palm pathogenic fungi were categorized as other fungal species. The isolation frequency of pathogenic fungi was highest in spring (39%) and lowest in summer (8%).

Identification of the seasonal patterns of the distribution of date palm rhizosphere fungi suggests that environmental conditions have a great influence on pathogen growth. Of a total of 182 fungal isolates obtained in all four seasons, 69 isolates, including pathogenic and nonpathogenic fungi, were isolated in spring. Our study shows that the seasons with the greatest fungal diversity in the rhizosphere are spring and fall. Because pathogenic fungi were isolated mainly in spring, precautionary steps taken before spring could prevent date palm diseases. Fewer fungal isolates were obtained in summer, at which time *Aspergillus* spp. and *Rhizopus* spp. were found to be the major fungi in the date palm rhizosphere soil. Pathogens were also isolated in summer and winter but in lesser numbers than those obtained in spring and fall; thus, our study indicates that date palms are at risk of developing diseases throughout the year.

Because the identified date palm diseases in Qatar can lead to serious losses in date fruit production, proper disease preventive measures must be taken. *T. punctulata* is a pathogen causing the most severe disease in date palms; date palm death caused by *T. punctulata* root infection has been reported (Al-Raisi et al. 2011). *N. phoenicum* causes diplodia disease on palm offshoots, resulting in death when the offshoot is still attached to the mother tree or when it is detached and planted separately (Zaid et al. 2002). The newly identified date palm pathogens *F. brachygibbosum*, *F. equiseti*, and *F. solani* were found to cause wilt and root rot.

Different disease control strategies, including chemical control and the use of biocontrol agents, have been applied to prevent diseases caused by *T. punctulata*. Successful in vivo chemical control of *T. punctulata* with difenoconazole has recently been reported (Saeed et al. 2016). Application of chemical pesticides is the common practice adopted for disease control in date palm plantations. Despite the negative impacts of plant protection chemicals on the environment and human health, this strategy is considered to be a major way of controlling crop diseases, although there is the risk of fungicide-resistant isolates of the pathogen evolving as a result of widespread use of a single active ingredient. The recent increase in interest in the use of biocontrol agents has reduced the use of hazardous chemicals to some extent. Al-Naemi et al. (2016) isolated and identified a biocontrol fungus, *T. harzianum*, that inhibited *T. punctulata* in vitro and in vivo. The biocontrol agent *Streptomyces globosus* UAE1 has also been successfully tested against *T. punctulata* (Saeed et al. 2017). Biological control of *N. phoenicum* has not been reported, but the use of copper-based fungicides is recommended to control diplodia disease (Zaid et al. 2002). Concern about the use of copper-based fungicides has been raised because of excessive copper

Table 3. Pathogenicity of isolated fungi recovered from the rhizosphere of date palm trees in different seasons and from the date palm tissues

					Pathogenicity[b]		
Fungal isolate	**Summer[a]**	**Fall**	**Winter**	**Spring**	**Necrosis**	**Lesion length (cm)**	**Virulence**
Aspergillus awamori	+	+	+	+	–	0.0	NP
Aspergillus carbonarius	+	+	+	+	–	0.0	NP
Aspergillus flavus	+	–	+	+	–	0.0	NP
Aspergillus nidulans	–	–	+	–	–	0.0	NP
Aspergillus niger	+	+	+	+	–	0.0	NP
Aspergillus terreus	–	–	+	–	–	0.0	NP
Aspergillus ustus	–	–	+	–	–	0.0	NP
Chaetomium globosum	–	–	–	+	ND	NA	ND
Cunninghamella echinulata	–	+	–	–	–	0.0	NP
Fusarium brachygibbosum	+	–	–	+	+	1.7 ± 2	MV
Fusarium chlamydosporum	–	+	–	–	–	0.0	NP
Fusarium equiseti	–	–	+	+	+	1.0 ± 2	LV
Fusarium graminearum	–	+	–	–	–	0.0	NP
Fusarium moniliforme	+	+	+	+	ND	NA	ND
Fusarium oxysporum	+	+	+	+	–	0.0	NP
Fusarium solani	+	+	+	+	+	0.8 ± 1	LV
Gliocladium virens	–	+	–	–	–	0.0	NP
Mucor spp.	–	+	–	–	–	0.0	NP
Neodeightonia phoenicum	–	–	+	+	+	1.4 ± 3	MV
Penicillium expansum	+	–	+	+	–	0.0	NP
Penicillium funiculosum	–	+	–	–	–	0.0	NP
Pythium spp.	–	–	–	+	ND	NA	ND
Thielaviopsis punctulata	+	–	–	+	+	2.6 ± 4	HV
Trichoderma harzianum	–	+	+	+	–	0.0	NP
Trichoderma longibrachiatum	–	+	+	+	–	0.0	NP
Rhizopus arrhizus	+	+	+	+	–	0.0	NP
Rhizopus oryzae	+	+	+	+	–	0.0	NP
Rhizopus stolonifer	+	+	+	+	–	0.0	NP

	Pathogenicity		
Endophytic fungal isolate	**Necrosis**	**Lesion length (cm)**	**Virulence**
Cladosporium sphaerospermum	–	0.0	NP
Cryptic fungal species	–	0.0	NP
Macrophomina sp.	–	0.0	NP
Rhodotorula marina	–	0.0	NP
Ulocladium chartarum	–	0.0	NP
Verticillium theobromae	–	0.0	NP

[a] The + sign indicates the presence of the fungal species and – indicates absence of the fungal species in different seasons. The + and – signs in the pathogenicity section indicate symptoms and no symptoms, respectively, developing on the leaves.

[b] Detached leaf assays were conducted for pathogenicity test. Lesion length was measured at 4 dpi. Virulence was determined by analyzing the intensity of necrosis. HV, highly virulent; LV, low virulent; MV, moderately virulent; NA, not applicable; ND, not determined, fungi were lost during subculturing, so no pathogenicity tests were carried out; NP, nonpathogenic.

accumulation in the soil, resulting in losses of soil biodiversity and fertility (Komárek et al. 2010; Wightwick et al. 2008); therefore, alternative disease control strategies have become necessary. The use of biocontrol agents to replace chemical fungicides, in part or in whole, will solve many of these problems. In most cases, a single biocontrol agent produces different types of antimicrobial components, minimizing the risk that the use of biocontrol agents would inadvertently select for resistant pathogen populations.

Although biocontrol agents are considered safe, there is a concern regarding potential invasiveness if a new nonnative biocontrol organism is introduced into the environment. Therefore, the use of indigenous biocontrol fungi would be preferable to prevent this problem. To screen for indigenous biocontrol agents from the agriculture soil in Qatar to protect date palms, the combinations of fungi that do not occur together frequently were investigated. We conducted a screening process in which we studied different combinations of fungi present in healthy and diseased date palms. Certain combinations of fungi, such as *Rhizopus arrhizus* and *Cunninghamella echinulata*, *N. phoenicum* and *R. arrhizus*, *T. harzianum* and *R. arrhizus*, *T. harzianum* and *N. phoenicum*, *T. harzianum* and *Thielaviopsis punctulata*, and *T. longibrachiatum* and *Thielaviopsis punctulata*, did not occur. In vitro antagonistic studies showed that *R. arrhizus* inhibited the growth of *C. echinulata*, whereas *N. phoenicum* inhibited the growth of *R. arrhizus*. Considering that *C. echinulata* and *R. arrhizus* are not pathogenic to date palms, they were excluded from further experimental study. Because *Trichoderma* spp. have been extensively and successfully studied as biocontrol agents, antagonistic studies were continued with them. It was observed that *Trichoderma* spp. were absent from rhizosphere soils from which *T. punctulata* and *N. phoenicum* were isolated.

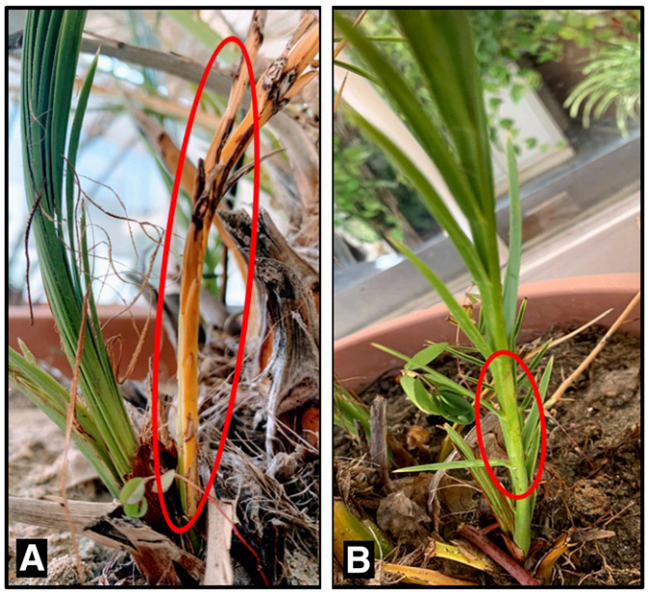

Fig. 4. Pathogenicity test of *Neodeightonia phoenicum*-inoculated offshoot plants. **A,** *N. phoenicum*-inoculated date palm offshoot plant showed typical symptoms of diplodia disease at 15 dpi. **B,** No symptoms were observed in the mock-inoculated offshoot plant.

Trichoderma spp. were more frequently isolated from the rhizosphere of healthy date palms than from that of diseased date palms. Therefore, it was postulated that *Trichoderma* spp. could be biocontrol agents capable of protecting date palms in Qatar from the most important pathogens. This survey resulted in the identification of two *Trichoderma* species, namely, *T. harzianum* and *T. longibrachiatum.* In vitro antagonistic studies on *T. harzianum* and *T. longibrachiatum* against the date palm pathogens *T. punctulata*, *N. phoenicum*, *F. brachygibbosum*, and *F. solani* resulted in the identification of *T. harzianum* and *T. longibrachiatum* as potential biocontrol agents to manage date palm trunk rot and root rot.

The role of endophytic fungi in plant defense activity has been reported widely, including their ability to produce bioactive compounds that protect hosts from adverse conditions, such as abiotic and biotic stresses, and their ability to live within host plants without causing any disease (Aly et al. 2011; Amin 2016; Dutta et al. 2014). In accordance with previous studies (Mahmoud et al. 2017; Mefteh et al. 2017), we isolated endophytic fungi from the internal tissues of date palm to investigate their antagonistic activity toward date palm pathogens. The following fungi were isolated from date palm tissues: *C. sphaerospermum*, *U. chartarum*, *V. theobromae*, and *Penicillium* sp. Although the pathogenicity of *U. chartarum* on downy oak (*Quercus pubescens*) has been reported (Vannini and Vettrain 2000), our study on its pathogenicity confirmed it to be nonpathogenic for date palms. *U. chartarum* showed antagonistic activity against *T. punctulata*, but none of the other endophytes showed antagonistic activity. Antimicrobial activity of endophytic *U. chartarum* isolated from a different host plant was reported earlier (Selim 2011). The endophytic fungus *C. sphaerospermum* isolated from soybean plants showed host plant growth-promoting characteristics (Hamayun et al. 2009). Moreover, in vitro growth-promoting activity of *C. sphaerospermum* applied to tobacco has also been reported recently (Li et al. 2019). Because *C. sphaerospermum* did not exhibit antifungal activity against the date palm pathogens in the current study, it would be interesting to study its growth-promoting role in date palms.

The red yeast *R. marina* was successfully isolated from date palm leaf samples, and its identity was confirmed by rDNA ITS region sequencing. The biocontrol activity of different endophytic *Rhodotorula* spp. has been reported previously. *Rhodotorula mucilaginosa* strain S-33 is a biological agent used to control *Botrytis cinerea*, causing stem canker of tomato (Utkhede et al. 2001). Moreover, *R. mucilaginosa* has been reported to be antagonistic against bacterial and fungal pathogens (Akhtyamova and Sattarova 2013) and to play a growth-promoting role for *Typha angustifolia* (narrowleaf cattail) (Sen et al. 2019). Antagonistic activity of *Rhodotorula glutinis* against *B. cinerea* has also been reported (Li et al. 2016). The antagonistic study we conducted with *R. marina* showed that it was ineffective against the pathogens against which it was tested but that it showed antagonistic activity against *P. expansum* isolated from the date palm rhizosphere, although the pathogenicity test we conducted with *P. expansum* confirmed that it is nonpathogenic for date palms. *P. expansum* has been previously reported as an etiological agent for postharvest diseases on date fruits (Palou et al. 2013); hence, it would be interesting to study *R. marina* as a possible biocontrol agent to manage postharvest diseases. *Rhodotorula kratochvilovae* strain LS11, isolated from olives, is an efficient biocontrol agent, exhibiting antagonistic activity against pathogens *P. expansum* and *B. cinerea* that affect olives (Castoria et al. 1997).

Table 4. Growth inhibition (%, type E interaction) of pathogenic fungi by *Trichoderma harzianum* and *Trichoderma longibrachiatum*[a]

	Growth inhibition rate in relation to pathogenic fungi (%)			
Antagonistic fungus	***Thielaviopsis punctulata***	***Neodeightonia phoenicum***	***Fusarium brachygibbosum***	***Fusarium solani***
Trichoderma harzianum	54.40 ± 0.87	51.5 ± 1.78	56.6 ± 1.54	56.3 ± 1.18
Trichoderma longibrachiatum	46.5 ± 1.29	46.4 ± 0.53	44.2 ± 1.33	47.2 ± 1.82

[a] Data are presented as mean ± SE. Differences between the antagonistic activity of *T. harzianum* and *T. longibrachiatum* against the fungal pathogens were found to be statistically significant for $P < 0.05$ via ANOVA.

Rhodotorula graminis, isolated from the *Populus trichocarpa*, was identified as a major producer of the plant hormones indole acetic acid and gibberellic acid; it also showed a significant plant growth-promoting role (Khan et al. 2012, 2016; Knoth et al. 2014; Xin et al. 2009). Genome sequencing of *R. graminis* WP1 showed presence of three putative proteins, aromatic-L-amino-acid decarboxylase, monoamine oxidase, and indol-3-acetaldehyde dehydrogenase; these are involved in the synthesis of indole acetic acid, a phytohormone that promotes plant growth, from L-tryptophan via tryptamine (Firrincieli et al. 2015). Plant growth-promoting *Rhodotorula* spp. (strain CAH2) isolated from the rhizosphere soil of *Beta vulgaris* was found to produce exopolysaccharide that helps microbes survive under abiotic stress conditions (Silambarasan et al. 2019). Extracellular polymeric substances produced from such plant growth-promoting microbes bind Na^+ cations and promote plant growth under salt stress in saline environmental conditions (Geddie and Sutherland 1993). Because the soil in Qatar has a high salt content, it would be interesting to study the role of *R. marina* in conferring salt tolerance to date palm. A siderophore known as rhodotorulic acid has been isolated from some *Rhodotorula* spp. It is an efficient iron-chelating agent capable of binding iron from soil and providing it to the plant in a soluble form. Rhodotorulic acid has been found to be helpful in preventing chlorosis in plants and to help plants tolerate an iron-deficient environment. A high concentration of the hydroxamate siderophore has been isolated from cultures of *Rhodotorula pilimana* grown under iron-deficient conditions. The identification of genes involved in trehalose biosynthesis and those involved in the synthesis of volatile organic compounds indicated that *R. graminis* may be able to confer drought tolerance ability on associated plants (Khan et al. 2016). Therefore, the presence of *R. marina* in date palms suggests possible roles in growth promotion and abiotic stress tolerance. It would be interesting to test the role of *R. marina* in date palms because other *Rhodotorula* species have been shown to have plant growth-promoting and abiotic stress tolerance effects. However, there is a lot of research to be conducted in identifying any role of *R. marina* in date palm growth promotion and abiotic stress tolerance.

Conclusions

This article documents the findings of the first year-round Qatari survey of pathogenic fungi, rhizosphere fungi, and endophytic fungi associated with date palms. It also presents details on the in vitro assessment of endophytic and rhizosphere fungi for their biocontrol traits against the pathogenic fungi. This survey has resulted in identifying new fungal pathogens for date palm in Qatar, such as date palm wilt-causing *F. brachyglossum* and *F. equiseti*, diplodia-causing *N. phoenicum*, and trunk rot-causing *T. punctulata*, which was identified as the cause of root rot. In the current study we were particularly interested in fungal pathogen distribution in Qatar. However, considering the similar climatic conditions in nearby countries, the pathogen outbreak may affect the other countries too. Of the rhizosphere fungi, *T. harzianum* and *T. longibrachiatum* proved to be effective antagonists of date palm pathogens, and the endophyte *U. chartarum* was an effective antagonist against *T. punctulata*. The endophytic red yeast *R. marina* acted as an antagonist against *P. expansum*, which causes postharvest spoilage of fruits. Because the application of biocontrol agents in date palm fields is not reported, the extensive climate-based study is needed to elucidate the time and amount of application of biocontrol agents in date palm fields. It would be interesting to test these biocontrol agents in date palm plantations in different seasons for controlling date palm diseases.

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