First Report of *Neodeightonia phoenicum* As the Causal Agent of Date Palm Inflorescence Rot in Iraq

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Abstract

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The inflorescence rot disease is considered an important disease, caused by various pathogens, including *Mauginiella scaettae*, *Fusarium* spp. and *Thielaviopsis paradoxa*. In this study, the fungal pathogen *Neodeightonia phoenicum* was isolated from different date palm fields located in Basra Province, Iraq, as the causal agent of the inflorescence rot disease. The disease symptoms included brownish to grey inflorescences, yellowing, and withering, which under severe attack causes inflorescence death. The majority of the symptoms manifested were close to the inflorescence's apex. Molecular identification was performed using the polymerase chain reaction (PCR) technique, and the identified isolate was registered in the National Centre for Biotechnology Information (NCBI) under the name *N. phoenicum* BYM-6 (Accession No. LC831993.1). In healthy palm pollen, *N. phoenicum's* pathogenicity test clearly revealed rot symptoms of inoculated inflorescences, indicating a high pathogenic potential for causing inflorescence rot disease. To the best of our knowledge, this work represents the first identification of *N. phoenicum* as the causal agent of date palm tree inflorescence rot disease (Khamedj). Results obtained showed that *Trichoderma longibrachiatum* is capable to restrict the growth of *N. phoenicum* in vitro. Thus, it can potentially be used in biological control to reduce the incidence of inflorescence rot disease on date palms, but require further investigations.

Keywords: Inflorescence rot, Iraq, date palm, khamedj, *Neodeightonia phoenicum*.

Introduction

The date palm, *Phoenix dactylifera* L. is one of the most significant fruit trees in the Arabian region, and it is known to be attacked by several pests (Fayyadh *et al*., 2022). Currently, inflorescence rot disease of date palm (also called Khamedj in North Africa) is a common disease that affects palm trees and is found in most palm-growing regions, including Egypt, Saudi Arabia, Iraq, and North African countries, causing yield losses of 30-40% in some regions (Abdullah *et al*., 2005; Bouhlali *et al*., 2021; Djerbi, 1998; Sana *et al*., 2022).

According to Riaz *et al*. (2009), *Serratia marcescens* is the causal agent of pink rot disease in Kuwaiti date palms, which is characterized by pinkish lesions encircled by pink bacterial secretions and dark brown patches on the inflorescence. In addition, Mohammed & Alfahad (2022) reported *Alternaria radicina* in different areas in central and northern Iraq.

Mauginiella scaettae Cav. is the main fungal pathogen of inflorescence rot disease. Cavara was the first to report it in Libya in 1925. Nevertheless, some fungal species, such as *Fusarium oxysporum*, *F. solani*, *F. moniliforme* and *Thielaviopsis paradoxa* (Abdullah *et al*., 2005; Hameed, 2012) were found associated with rotted date palm inflorescences. Additionally, *Neodeightonia phoenicum* is a significant pathogen that affects a variety of palm species. It causes root rot of date palm in Qatar (Nishad & Ahmed, 2020), leaf blight and stem rot on date palm P. dactylifera and Canary palm *P. canariensis* in Greece (Ligoxigakis *et al*., 2013). Likewise, Manea *et al*. (2022) reported *N. phoenicum* to cause black scorch disease in tissue culture date palm fields in Basra province, Iraq. The fungus was first recorded as a causal agent of black scorch disease in Arizona, USA (Hu & Wright, 2024).

On the other hand, Hu & Wright (2024) indicated that *N. phoenicum* has a high infection potential with economic impact on palm trees, which requires immediate attention. Consequently, the objective of the current study was to identify the causal agent causing inflorescence rot disease based on morphological and molecular characteristics, sequence analysis, and pathogenicity tests.

Materials and Methods

Isolation of *Neodeightonia phoenicum*

The infected inflorescence samples were collected during March and April 2024 from Safwan and Al-Nashwa locations in Basra province from Barhi and Sayer varieties. All samples were washed with sterile water, and then cut into 1-2 cm pieces, sterilized with 1% sodium hypochlorite for two minutes, and then dried on sterile filter paper and washed with sterile distilled water. The pieces were then placed in Petri plates containing potato-dextrose- agar (PDA) (39 g/L) medium, supplemented with chloramphenicol (125 mg/L). Plates were incubated at 25±2˚C for five days. Colonies obtained were transferred into new plates and incubated at 25±2˚C for 7 days.

Morphological identification of *N. phoenicum*

Morphological identification of *N. phoenicum* included colony shape, and color, in addition to conidia characteristics such as shape, size, and color (Phillips *et al*., 2013).

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Molecular identification of *N. phoenicum*

The molecular identification was performed using the polymerase chain reaction (PCR). Mycelium of *N. phoenicum* was scraped directly from the plate surface, and the DNA was extracted by using the gSYNC DNA extraction kit. The ribosomal RNA (rRNA) gene was amplified using universal fungal primers: internal transcribed spacer 1 (ITS1: 5'-TCC GTA GGT GAA CCT GCG G-3'), and internal transcribed spacer 4 (ITS4: 5'-TCC TCC GCT TAT TGA TAT GC-3'). The DNA products were transferred to agarose gel to confirm the DNA extraction. The amplicons were sent to Macrogen Company in South Korea for sequencing. The ITS sequences were analyzed using BLAST software and comparisons were made with standard sequences available in the GenBank (NCBI).

Pathogenicity test of *N. phoenicum*

The pathogenicity test for *N. phoenicum* was conducted in vitro using the detached plant parts (Manea *et al*., 2023; Soytong, 2005). Non-infected palm pollen was rinsed with sterile water to remove dust and any other contamination, and dried with sterile wipes. The samples were then surface sterilized with 70% ethyl alcohol, rinsed with sterile water to remove the remaining trace of alcohol, and dried again. With a cork borer, 0.5 cm diameter holes, 15 cm apart, were made in the pollen sheath using the crock borer. Each hole was inoculated with a 0.5 cm *N. phoenicum* mycelium disc grown for 7 days. The treatment samples and the control (PDA medium without pathogen) were placed in the sterile plastic bags and incubated at 25±2˚C for two weeks. The completely randomized design (CRD) was used in this experiment, with three replicates. Rot development in the inflorescences was monitored 7, 10, and 14 days after inoculation. Development of brown rot was considered an indicator of pathogenicity (Manea *et al*., 2022).

Antagonistic activity of *Trichoderma longibrachiatum* against *N. phoenicum*

The double-culture technique was applied to evaluate the antagonistic efficiency. *T. longibrachiatum* FBY1 (Accession No.: LC499793.1) was used against *N. phoenicum*. The method involved the preparation of PDA plates inoculated with two opposite discs of 0.5 cm diameter, at 1 cm from the edge of the culture. The experiment followed the CRD design with four replicates. Each dish was centrally inoculated by the pathogen only and then incubated at 25±2 °C. The antagonism level was calculated after the pathogen growth reached the edge's plate in the control plate. The scale of Bell *et al*. (1982) 1-5 was applied, and a score of 1 or 2 represents the highly biologically effective fungi.

Results and Discussion

Symptoms of infection

N. phoenicum infection symptoms were expressed as yellowing and brown discoloration and lead later to severe drying and death of the inflorescences. Symptoms started from the tip of the panicle towards the cluster, followed by a mass of grey mouldy growth appeared in the infected area as fungal hyphae and conidia (Figure 1). Furthermore, the pollen sheath appeared dry as reddish-brown or in blackish discoloration. These symptoms were similar to the symptoms of black scorch that resulted from the infection with the same pathogen on palm leaves (Figure 2) (Manea *et al*., 2022).

Figure 1. Symptoms of the inflorescence rot infection on date palm pollen.

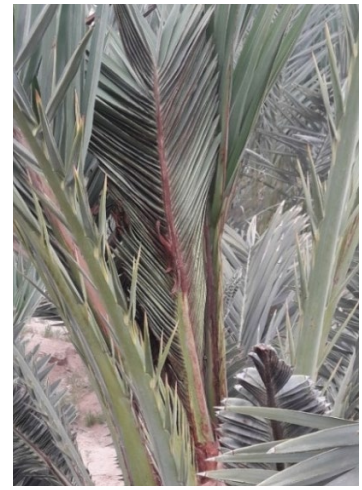

Figure 2. Symptoms of the black scorch infection accompanying with inflorescences rot in the Barhi date palm variety.

Morphological identification of *N. phoenicum*

Culture characteristics of *N. phoenicum* showed that the fungus had a rapid growth with dense-hyphae raised above the medium, with the color gradually changed to olive or gray, then became black in the one-week-old culture. The fungal colony's back side was dark, hyphae were septate and the conidia were either ovoid or ellipsoid. The conidia apex and base were broadly rounded, widest in the middle, initially hyaline and aseptate, becoming later dark brown and sometime with one septum, with melanin deposits on the inner surface of the wall. The species *N. phoenicum* belongs to the family Botryosphaeriaceae and order

Pseudosphaeriales (Manea *et al*., 2022; Phillips *et al*., 2013; Zhang & Song, 2022).

Molecular identification of *N. phoenicum*

The results of polymerase chain reaction (PCR) using ITS2-ITS4 primers produced an amplicon of 540 bp in size. Sequence analysis showed high similarity of the ITS region with high similarity (99-100%) with isolates available in the GenBank. The investigated fungal isolate was 100% identical with *N. phoenicum* isolate AMN (Accession No. OL589157.1) (Figure 4). This isolate is implicated to cause date palm tissue culture contamination in Iraq. This isolate is 100% identical to *N. phoenicum* strain CBS 122528 (Accession No. KF766198.1). The Iraqi isolate investigated in this study was registered in the NCBI with the name *N. phoenicum* BYM-6 (Accession No. LC831993.1).

Pathogenicity test of *N. phoenicum*

The pathogenicity test conducted on *N. phoenicum* revealed its capability to cause rotting symptoms after the date palm was inoculated with the pathogenic fungus (Figure 3). The rotting symptoms produced were extended to a distance of 8 cm, seven days after the pollen sheath was inoculated, and to 13 cm 10 days later. Meanwhile, the rotting symptoms overlapped between the pollen areas to include the entire pollen treated with the pathogen, 14 days after inoculation. In contrast, no rotting symptoms appeared in the palm inflorescences in the control treatment. Results obtained showed that the fungus *N. phoenicum* was highly pathogenic, as confirmed by the symptoms produced on the palm inflorescences (Manea *et al*., 2022).

Several reports have shown that the pathogen *N. phoenicum* has a high ability to infect the dwarf date palm *P. roebelenii*, resulting in the formation of spots on palm leaves and buds (Zhang & Song, 2022). Those spots can be rapidly extended to become a large area, which appears with dark brown edges, eventually leading to wilting and leaf drop. As *N. phoenicum* known to be severely pathogenic on palm trees, the potential spread of the fungal pathogen may cause a significant economic loss on date palm trees, which requires taking appropriate measures to manage the disease (Hu & Wright, 2024).

Figure 3. Symptoms of inflorescence rot on the pollen of Sayer palm trees, one week after inoculation with *N. phoenicum*.

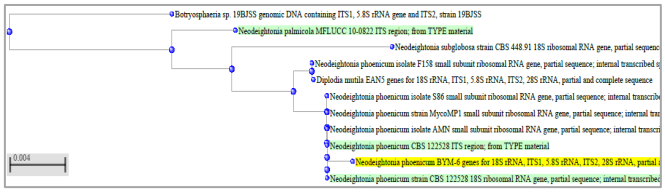

Figure 4. Phylogenetic tree of *N. phoenicum* BYM-6.

The antagonistic efficiency of the biological agent *T. longibrachiatum* against *N. phoenicum*

The results obtained showed that the antagonistic fungus *T. longibrachiatum* against *N. phoenicum* hyphae had a strong inhibitory and parasitic effect (scale 1) based on a 1-5 scale (Bell *et al*., 1982). *T. longibrachiatum*bhad had visible antagonistic effects, as it caused cell death and decline of the fungal pathogen colony (Figure 5). These findings are consistent with that of previous workers (Mahdi *et al*., 2019; Mahdi & Al-Waiely, 2024; Manea *et al*., 2022).

It can be concluded from this investigation that *N. phoenicum* is a pathogenic fungus responsible for causing inflorescence rot of date palm of different palm varieties, such as Barhi, and can cause serious damage to date palm trees. To the best of our knowledge, this is the first report on *N. phoenicum* that causes inflorescence rot disease of date palm in Iraq.

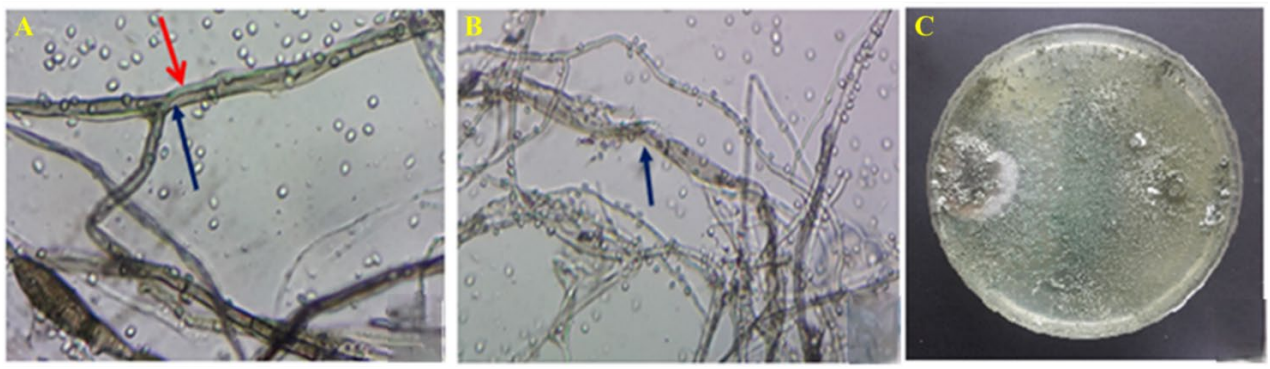

Figure 5. Antagonism between the fungus *N. phoenicum* and the biological agent *T. longibrachiatum*. A= adhesion of T. *longibrachiatum* mycelium to pathogenic fungi, B= mycelium decomposition of *N. phoenicum*, C= antagonism between *T. longibrachiatum* and *N. phoenicum* at five days on PDA medium.

الملخص

مهدي، باسل يوسف وضياء سالم علي الوائلي. 2026. التسجيل الأول للفطر *Neodeightonia phoenicum* كمسبب لمرض تعفن النورات الزهرية في نخيل التمر في العراق. مجلة وقاية النبات العربية، 44(2):168−172. https://doi.org/10.22268/AJPP-001398

يعدّ مرض تعفن النورات الزهرية في نخيل التمر من الأمراض المهمة، والذي تسببه فطور مختلفة، بما في ذلك *Mauginiella scaettae*، *Fusarium* spp. و *Thielaviopsis paradoxa*. في هذه الدراسة، تم عزل الفطر *Neodeightonia phoenicum* من بعض حقول نخيل التمر الواقعة في محافظة البصرة، العراق، كمسبب لمرض تعفن النورات الزهرية. تضمنت أعراض الإصابة بالمرض تلون النورات الزهرية بلون بني إلى رمادي واصفرارها وذبولها، كما تسببت حالة الاصابة الشديدة بموت النورات، وظهرت معظم الأعراض بالقرب من قمة النورة. تمّ إجراء التشخيص الجزيئي للفطر باستخدام تقنية التفاعل التسلسلي للبوليميريز (PCR)، مع تسجيل عزلة الفطر في المركز الوطني لمعلومات التقانة الحيوية (NCBI) باسم *N. phoenicum* BYM-6 (رمز التعريف: LC831993.1). أظهر اختبار المقدرة الإمراضية للفطر *N. phoenicum* ظهور أعراض واضحة لتعفن النورات الملقحة، مما يشير إلى مقدرة إمراضية عالية للفطر في التسبب بمرض تعفن النورات الزهرية. على حدّ علمنا، يمثل هذا العمل أول تسجيل للفطر *N. phoenicum* كمسبب لمرض تعفن النورات في أشجار النخيل (الخامج) في العراق. كما بينت النتائج أن للفطر *Trichoderma longibrachiatum* مقدرة تضادية عالية في الحدّ من نمو الفطر *N. phoenicum* مختبرياً. وبالتالي، ثمّة احتمال لاستخدامه في المكافحة الحيوية لتقليل حدوث الاصابة بمرض تعفن النورات الزهرية في أشجار النخيل، إلا أن ذلك يتطلب المزيد من الاختبارات، وبخاصةٍ الحقلية منها.

كلمات مفتاحية: تعفن النورات، الخامج، نخيل التمر، العراق، *Neodeightonia phoenicum*.

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