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New Record of Neodeightonia Phoenicum as a Cause of Black Scorch Disease on Date Palms in Iraq

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Abstract

Black scorch is one of the most important diseases that affect date palm trees, *Thielaviopsisparadoxa* is the main cause of this disease all over the world **.**In this study, *Neodeightoniaphoenicum* isolated from palm trees infected with black scorch disease in tissue culture palm orchards in Basrah Governorate .The fungus identified morphologically and molecularly using the primers ITS1-ITS4. Molecular levels have been deposited in National Center for Biotechnology Information (NCBI)GenBankwith (Accession No: MZ675601.1)**.**The results of the morphological and molecularidentification were consistent**.** A pathogenicity test showed the ability of the fungus *N. phoenicum* to cause infection on the palm rachis inside the laboratory, and the infection was confirmed using Koch's postulates. This is the first report of *N. phoenicum* as a cause of black scorch disease in palm trees in Iraq

Key word : Date palm , Black scorch disease ,*Neodeightoniaphoenicum*

Introduction

Date palm *Phoenix dactylifera* L. is considered one of the most important plants in arid and semi-arid lands that tolerate harsh desert environmental conditions. Palm trees have great nutritional and economic value in the Mediterranean, Middle East, Australia, China, North Africa and warm regions of the USA (30). Their production is affected by several factors,

including infection with pathogens (26). The spread of plant diseases and intensity differs from one region to another and from one species to another. Environmental conditions, agricultural methods and tree ages play a significant role in determining higher diseases spread in the orchard. Leaf spot and black scorch diseases are most prevalent among date palms grown in heavy and saline soils (14). Black scorch disease is considered an important problem facing palm cultivation, where it may cause losses to reach 50% in newly planted offshoots (3).A first registration of *Thielaviopsisparadoxa* on date palm as the black scorch pathogen was performed by (15). After that, majority of researchersmentioned this fungus as a cause of black scorch disease on date palms. Previous studies classified *T. paradoxa* as a causative agent of neck bending disease on date palms in Kuwait (20). also (28) recorded *T. paradoxa* in Oman, in Iraq, it was mentioned by (2)Fayyad et al. (31) were also able to isolate *C. radicicola* from the symptoms associated with leaf blight on date palms in Basrah, while (25) recorded it in Egypt. In Qatar, it was documented by (1) and mentioned by (8) as a pathogen in Saudi Arabia. The genus *Neodeightonia* belongs to the family Botryosphaeriaceae that has the potential to cause important and common diseases on various plants (24). (18) reported the *N. palmicola* on palm trees in Thailand. (17) recorded*N. Phoenicum* on palms in Greece. (16) reported both types of *N. rattanica*and *N. rattanicola* on (rattan palm) trees, as well as *N. palmicola* on Fishtail palm trees in China (21). (12) mentioned*N. Phoenicum* on Queen palm *Syagrusromanzoffiana*in Brazil. In Qatar, *N. Phoenicum* mentioned by (22) on date palm trees.

Materials and Methods

Isolation of *N. phoenicum*

The small pieces of date palm leaves were taken from *Phoenix dactylifera* L. that are infected with black scorch disease. They washed with tap water first, then superficially sterilized with 10% sodium hypochlorite solution for 3 minutes. After that, those pieces were washed with sterile distilled water several times in order to remove the sterile solution's traces, and dried by sterile filter papers. All pieces were put in 9 cm Petri dishes containing Potato Dextrose Agar after sterilizing it by autoclave at 121°C and 15 pound / $inch^2$ a pressure. A 250 mg/L of Chloramphenicol was added to agar as an antibiotic agent. These dishes incubated at 25 ± 2ºC for 7 days. The dishes were examined to identify the fungal colonies.

Morphological Identification

The fungus was phenotypically identified by the characteristics of colony color, pycnidium color and the size of conidia as mentioned in (23).

Molecular Identification

Molecular Identification was performed using a 10 day-old culture of *N. phoenicum*that incubated on PDA medium at 25±2°CTheDNA was extracted using g-DNA extraction kit (Plant Genomic DNA Mini Kit (GP100), Taiwan) according to manufacturer's instructions. The presence of DNA was confirmed using agarose gel electrophoresis (Sambrook, et al., 1989). The molecular identification depended on ITS1-ITS4 marker using F:TCCGTAGGTGAACCTGCGG and R:TCCTCCGCTTATTGATATGC, primers (Bellemain et al., 2010).The PCR conditions included 95°C for initiation step followed by 35 cycle of: denaturation at 95°C for 1 min, annealing at 58°C for 30s, extension at 72°C for 1 min and the reaction ended with 10 min of final extension at 72°C. The PCR product was visualized on agarose gel using 100bpLadder (Abd-Elsalam et al., 2003), the PCR product proposed to sequencing in Macrogen Co. (Korea). The sequence was processed using Chromas 2.6.5 software (Al-Saad et al 2018) then multiple alignment was performed with BLAST software using NCBI database. The identified isolates were submitted to the NCBI for registration.

Pathogenicity test

The detached leaves method was used in (29, 26). A 20 cm long piece (rachis) was taken from Sayer cultivar from the third basement. These leaves were removed , and washed using tap water,then superficially sterilized with 70% ethanol alcohol for several times, after that this piece was washed with sterile distilled water several times to remove traces of the sterile substance. The pieces were left on the sterile filter paper until they dried. Three holes were then used in each piece of rachis by a sterile cork borer 0.5 cm. A disc with 0.5 cm of a diameter was taken from the edge of the colony 7-day-old of *N. phoenicum*, which grew on PDA media and put in the hole that was made in rachis of leaves. Each hole was wrapped by wrapfilm, which was removed after two days from inoculation with the pathogen. Then the leaves were placed in sterile (1 L) glass bottles containing sterile distilled water with a height of 2 cm. The nozzle of the glass bottles was sealed by sterile aluminum foil. The glass bottles were incubated in the

incubator at 25 ± 2º C for a month. The control treatment put only a 0.5 cm diameter PDA disc inside the leaf pieces.

It was considered the appearance of the brown pathological spot indicator of pathogenicity of the fungus according to (10).

Results

Symptoms

Symptoms appeared in the form of brown to black scorch hard charcoal-like appearance of the leaves, in particular in the rachis region with the appearances of clear curly leaflets. These symptoms have been extended alongside the infected region (Fig. 1).

Fig 1: Symptoms of Black scorch disease caused by *N.phoenicum*

Description of the fungus

The results of the isolation are shown in Figure (2-A) that *N. phoenicum* causes the symptoms of black scorch. The characteristics of *N. phoenicum* cultivation demonstrated that the fungus colony is rapidly grown on the PDA at 25°C. Initially, the color of the colony changed from light gray to dark, then turned to black during one week. The Figure (2-B) illustrated the microscopic

characteristics of conidia. They are oval to elliptical with a base and rounded apex thick walls at the beginning, transparent and undivided. These conidia become dark brown and divided into two cells after emerging from the mature stage (pycnidiun). The average of conidia length ranged15 - 23 microns, and their width 9-12 microns. The fungus characteristics, and its morphology determined according to (23)

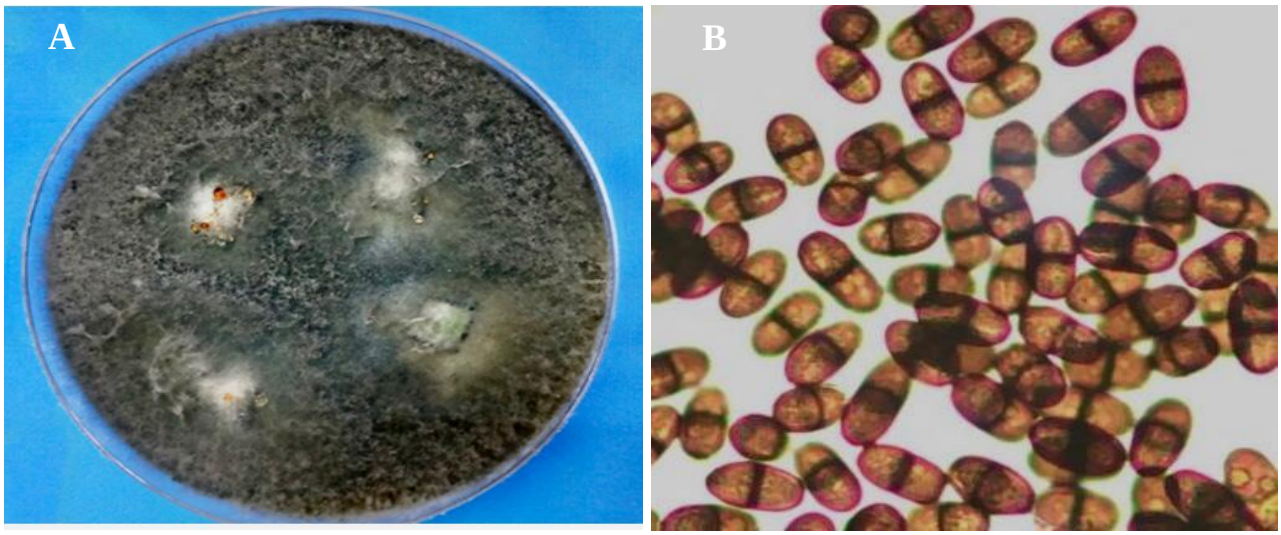

Fig 2: A.*N.phoenicum*colonies on PDA medium, which isolated from black scorch disease. B Conidia of*N.phoenicum*

Molecular identification of *N. phoenicum* using PCR technique

The bioinformatics results analysis using Basic Local Alignment Search Tool (Blast) showed that the nitrogenous base sequences for PCR product for both ITS 1 and ITS 4 primers the tested isolate *N. phoenicu*m with matching proportion 99%. The sequence of the nitrogenous bases of the fungus studied was registered with the (NCBI) under Accession No: MZ675601.1 The phenotypic description was strongly compatible with the molecular analysis.

Pathogenicity Test

The results of this experiment displayed that *N. phoenicum* caused a change in the color of the inoculated rachis tissue. This color changed into brown after 5 days of the inoculation. Then, the coloration area increased, and the *N. phoenicum* mycelium has speared throughout all parts of the inoculation rachis. Finally, the inoculation rachis color become black (Figure 3-A). After 30

days, the rachis was contained the aggregation of pycnidiun, in addition, the conidia was observed, which represents the asexual phase of the fungus (Fig. 3-B, C, DS). The pathogenic fungus was re-isolated from the leaf tissue on P.D.A. according to Koch's postulates to confirm the results of the research and prove the relationship of the pathogen *N.phoenicum* to causing symptoms of the disease.

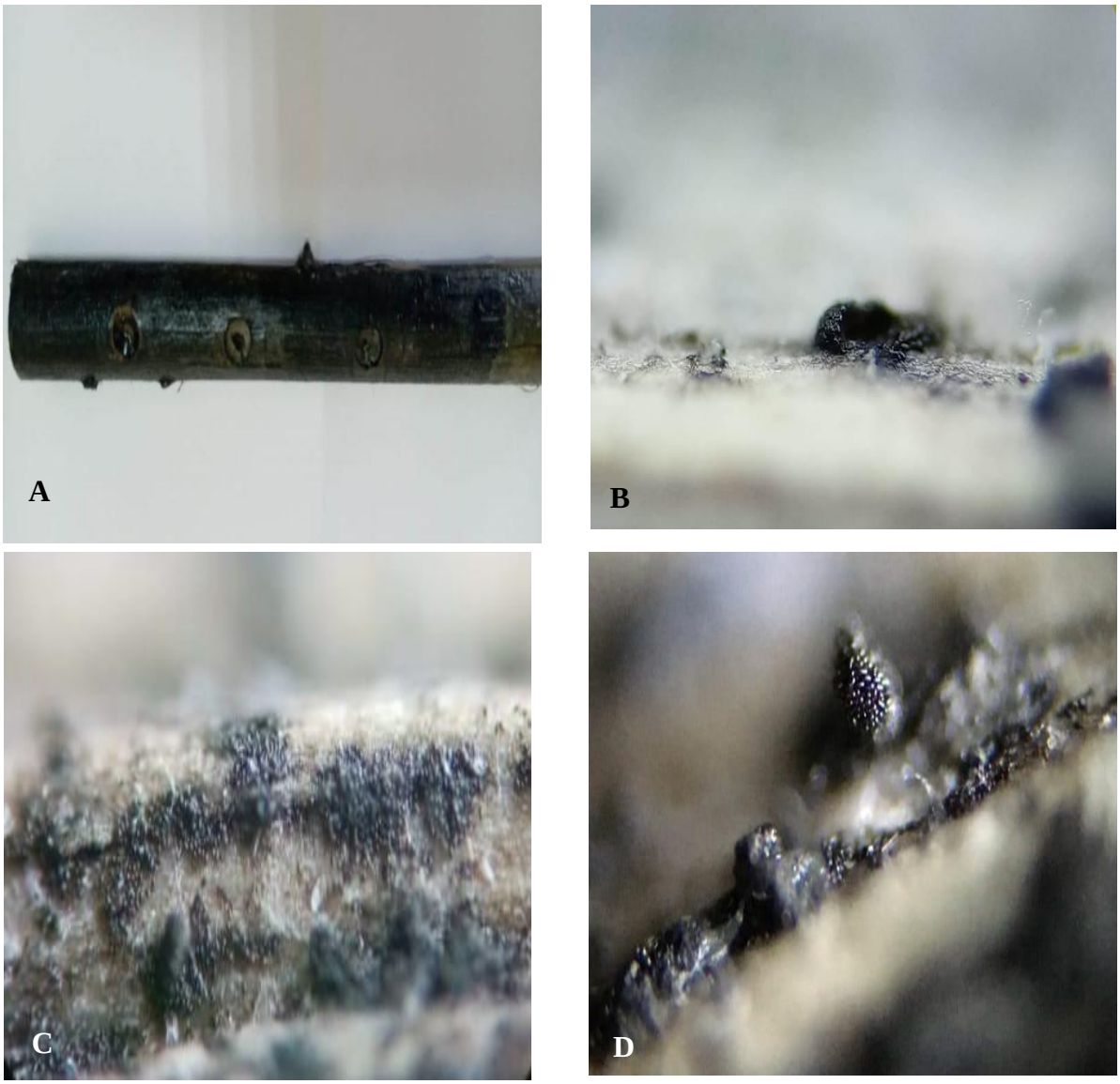

Figure 3: A.Turning of tissues inoculated by *N.phoenicum*into black due to formation of pycnidium bodies and the conidia on leaves surface. B-C . The formation of conidia on the

inoculated leaves surface by *N.phoenicum*. D .Aggregation of conidia afterreleasing it from pycnidium.

Discussion

In this study, a new pathogen was described on date palms. *N. phoenicum*, which was isolated from symptoms of black scorch on date palms that resulted from tissue culture in Basra Governorate in Iraq, involving Safwan and Hartha regions. This result is consistent with other studies that proved the possibility of *N. phoenicum* in causing different diseases on palms, for instance,(17) mentioned that *N. Phoenicum* is the cause of diseases, such as leaf death, stem rot and shoot blight on palm in Greece, also (22) revealed that the *N. phoenicum* results in root rot and diplodiadiseases on date palm in Qatar.

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