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ORIGINAL ARTICLE

THE FIRST REPORT OF *PHOMA COSTARRICENSIS* AS A CAUSAL AGENT OF LEAF SPOT DISEASE OF DATE PALM IN IRAQ

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Abstract: Leaf spot disease is considered one of the most common diseases that affect the date palm. Many fungi have been recorded across the world causing this disease. *Phoma costarricensis* was isolated from date palm trees affected by leaf spot disease. Morphological and molecular characteristics confirmed the identification of *P. costarricensis*. The Nitrogen bases sequence was deposited in the National Center for Biotechnology Information (NCBI) GenBank with Accession No: OK255499.1. Koch's postulates were confirmed the pathogenicity of the fungus. Based on the best knowledge, this report is the first registration of *P. costarricensis* as a leaf spot pathogen on palm trees in Iraq.

***Keyword*:** Date palm, Leaf spot disease, *Phoma costarricensis,* Morphological.

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1. Introduction

Date palm (*Phoenix dactylifera* L.) has been cultivated in the desert oases of the Arab world for several centuries. Their fruits contain carbohydrates source, fiber, vitamins and minerals. Additionally, their fruits can be used in medicinal field because they contain different chemical compounds, having beneficial effect. such as procyanidins, sterols, carotenoids, phenolics and anthocyanins. Morever, some studies have shown that the date fruits have free radical scavenging activities, antimutagenic, antimicrobial, anti-inuammatory, antioxidant, anticancer, gastroprotective, hepatoprotective, nephroprotective and immunostimulant activities. The production of date Palm is influenced by many factors; one of the crucial factors is the fungal disease [Abdullah *et al.* (2010)]. In the literature references, Ammar and El-Naggar (2011) considered that leaf spot diseases are extremely prevalent one of the most important and widespread date palm disease in Najran region in Saudi Arabia. Other studies that carried out by Livingston *et al.* (2005) mentioned that leaf spot diseases have become one of the sources of threat to palm cultivation in recent years. Many researches indicated that the causes of spot disease are numerous, however, the fungal genus Phoma is one of the most important [El Hadrami and Al-Khayri (2012), Abd AL-Hseen and Manea (2020]. In Costa Rica, *P. costarricensis* was recorded as a cause of leaf, stem and fruit blight on coffee trees by Echandi (1957) and Barguil *et al.* (2005) repotted it in Brazil as a cause of coffee plant leaf spot. In India, this species was found by Patil *et al.* (2012) on delphinium leaves infected with leaf spot disease. In Iraq there are no references that mentioned this fungus as a pathogen on plants and because we noted this fungus as a cause of date palm leaf spot disease, for this reason, this study was conducted to define the significant role of this fungus as a pathogen on palm trees.

2. Materials and Methods

Isolation of *P. costarricensis*

Date palm leaves affected with leaf spot disease were cut into little pieces. These pieces were washed with tap water first, then superficially sterilized with

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10% sodium hypochlorite solution for 3 minutes. After that, those pieces were washed again with sterile distilled water several times in order to remove the sterile solution's traces. The pieces were dried by sterile filter papers. Then, a pieces were transferred into 9 cm Petri dishes containing PDA emended with chloramphenicol antibiotic. 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 using the morphological features such as, the colony texture, the color, size of pycnidia and conidial characteristic. The morphological identification was done according to Boerema *et al.* (2004).

Molecular Identification

Molecular Identification was performed using a 10 day-old culture of *P. costarricensis* that incubated on PDA medium at 25±2°C. The DNA was extracted using g-DNA extraction kit (Plant Genomic DNA Mini Kit (GP100), Taiwan) by following the manufacturer's instructions. The presence of DNA was confirmed through agarose gel electrophoresis [Sambrook *et al.* (1989)]. The molecular identification was depended on the 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 100bp Ladder. The PCR product proposed to sequencing in Macrogen Co. (Korea). The sequence was processed by 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 the registration.

Pathogenicity test

Pathogenicity experiment of *P. costarricensis* was conducted by using three-month old seedlings. Those seedlings resulted from Barhi seeds that were grown in the plastic pots. Each pot contains a mixture of peat moss and soil with ratio of 1:2. The leaves surface was sterilized by ethyl alcohol 70%, then left for 10 minutes. After that, leaves were washed by sterling distilled water to obtain the rid of ethyl alcohol and also to provide suitable moisture in order for inoculation by pathogenic fungus. Fungal suspension was prepared using an isolate grown on PDA for 14 days at 25°C ± 2°C. A 10 mL of sterile distilled water was added for each plate. The plates were left for 30 minutes on the table, then the surface layer of a colony scraped by metal scraper. The conidial suspension was placed in a glass beaker and stirred for 15 minutes by a magnetic stirrer. This step was performed to completely mix the conidia. The conidial suspension is adjusted by Haemocytometer to a final concentration of 106 conidia/mL [Davari and Hajieghrari (2008)].

Symptoms

The Symptoms have appeared in the form of irregular brown spots on the fronds (rachis). These spots enlarge during the time, causing color turns brown. Meanwhile, the center of this spot was converted into pale white. On the other hand, the spot surface might be sunken from the surface of the tissue (Fig. 1).

Description of the fungus

The results of the isolation and inoculation showed that *P. costarricensis* (Fig. 3) was the cause of the date palm leaf spot disease. The cultural characteristics revealed that the fungus colony grew healthy on the medium (PDA) at 23°C. The color of the colony ranged from olive to dark olive (Fig. 2A) and its growth was regular and sometimes with a white edge. The microscopic characteristics of pycnidia were globose to flask shaped, smooth with one ostiole, Often solitary, it is formed scattered on or partially in the agar, their dimensions ranged from 80-180 µm in length and 45-135 µm in width (Fig. 2B). Conidia assemble in pycnidia, then it emerges from the top of the pycnidia in the form of Conidial matrix whitish (Figs. 2C, 2D). Conidia were

Fig. 1: Symptoms of Leaf Spot disease caused by *P. costarricensis*

white ellipsoidal to sub cylindrical its dimensions range from 2.6-4.5 µm in length and 2-2.3 µm in width.

Molecular identification of *P. costarricensis* using PCR technique

The molecular identification revealed that the examined isolate was 95.34% compatible with NCBI reference isolate *P.costarricensis* (Accession No: OK255499.1)

Pathogenicity Test

The Pathogenicity on date palm seedlings using conidial suspensions confirmed that *P.costarricensis* resulted in the symptoms of leaf spot after 21 days of inoculation. It can be seen clear via appearance of small irregular brown spots, which rapidly converted into a large brown lesion, then also expanded to include most of the inoculated leaves. While, these symptoms did not appear at the control treatments, the pathogen then re-isolated from infected sites to confirm the pathogenicity test.

3. Discussion

In this study, a new pathogen was described on date palms, *P. costarricensis*, which was isolated from

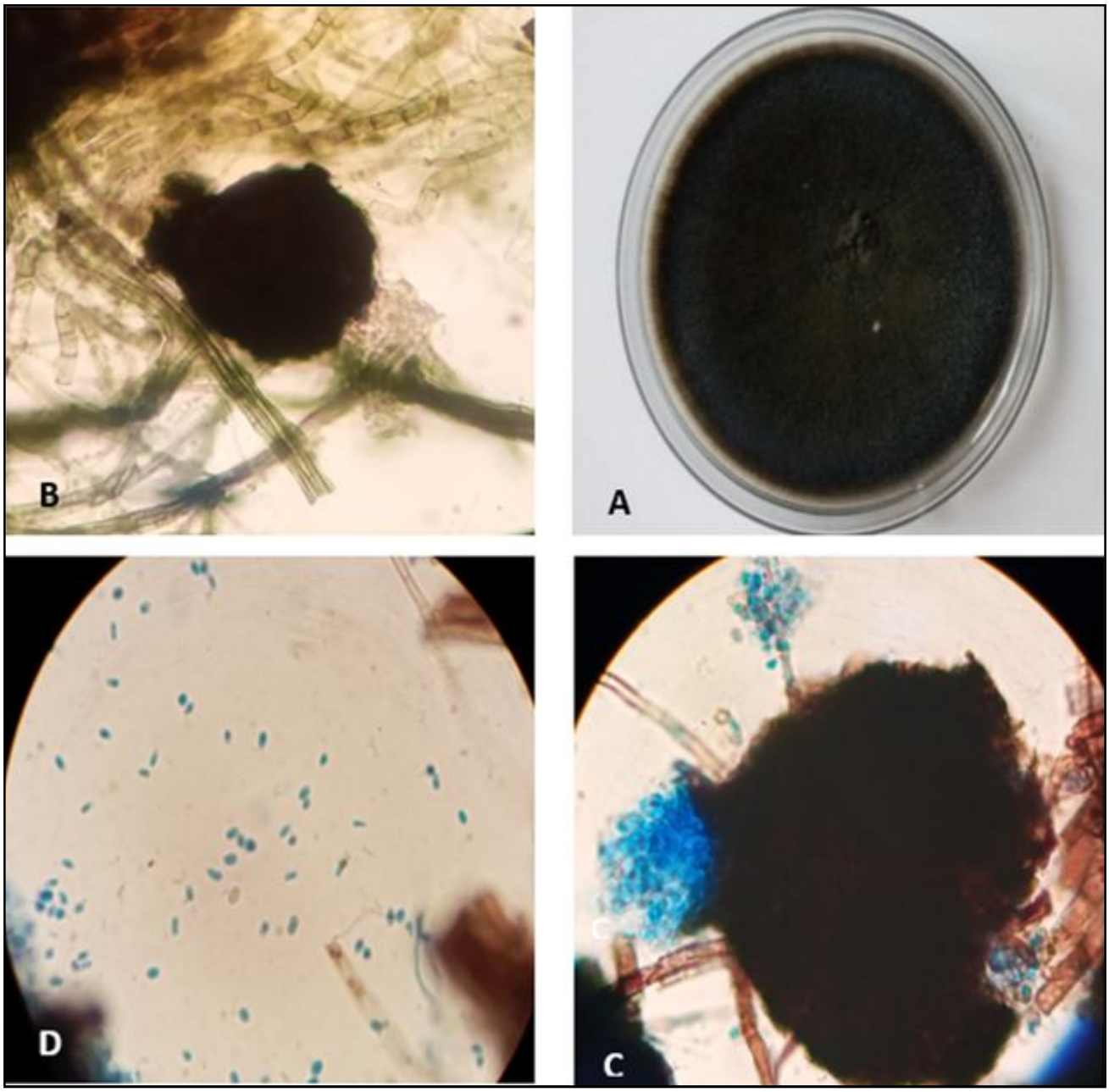

Fig. 2: (A) Petri dish of *P. costarricensis* colony, (B) Pycnidia of *P. costarricensis*, (C) Form of conidial matrix whitish (D) Conidia of *P. costarricensis*

Fig. 3: Pathogenicity test, right un inoculated seedling ,left inoculated seedling with a fungal suspension of *P. costarricensis*

the symptoms of leaf spot date palms in Basrah Governorate in Iraq. Morphological characteristics was closed to the study of [Boerema *et al.* (2004)]. They found that single cell ellipsoidal conidia1size is 5-3-4× 1-1.5-2 µm, pycnidia globose to flask shaped with one ostiole, its dimensions rate are 50-150 µm. And the DNA sequence data was applied by using internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA). Both the sequenced data and the Blast showed the identity (95.34%) of the fungus to be Phoma costarricensis (Gen Bank accession No. OK255499.1). This study gave a clear indication that the fungus Phoma costarricensis caused leaf spot and blight symptoms on date palm seedlings based on the current in pathogenicity experiment. These results are in agreement with several studies that showed that the fungus *P.costarricensis* causesd severe symptoms in different hosts after artificial inoculation. *P.costarricensis* is also known as a pathogen on several hosts worldwide, such as leaf spot and blight pathogen on Arabian coffee (*Coffea arabica* L.) in Brazil [Salgado *et al.* (2005), Mesquita *et al.* (2016)]. Although many authors did not mention this disease on the date Palm [El Bouhssini *et al*. (2018)]. This study is considered the first registration in Basrah-Iraq. Based on that, further studies are required to understand the relationship between this pathogen with the another leaf spot pathogens and its control on date Palm trees.

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