Journal of Kerbala for Agricultural Sciences Issue (4), Volume (9), (2022)

First Report of *Alternaria alternata* causing leaf spot on fig (*Ficus carica* L.) in Karbala Province, Iraq

Zainab L. Hameed[1*], Adnan A. Lahuf[2]

[1] **Department of Field Crop, Agriculture College, University of Kerbala, Karbala, Iraq**
[2]**Department of Plant Protection, Agriculture College, University of Kerbala, Karbala, Iraq**
***Corresponding author email:** zainab.l@uokerbala.edu.iq

Received: Oct. 10, 2022

Accepted: Nov.15, 2022

Published: Dec. 5, 2022

Abstract

Fig is an important tree that is cultivated worldwide including Iraq. During a survey held in 2018 in orchards of Alhusaynia territory, Karbala Province, Iraq, an epidemic leaf spot disease affecting fig trees was observed. Based on morphological appearances and rDNA-ITS sequence analyses, the fungi caused this disease was recognized as *Alternaria alternata*. To our knowledge, this is the first record of *A. alternata* causing leaf spot disease on fig trees in Karbala, Iraq.

Keywords: *Ficus carica*, Foliage fungal disease, rDNA-ITS sequence

Introduction

Fig (*Ficus carica* L., family *Moraceae*) is a deciduous tree native to the Mediterranean and western Asia regions [1]. Presently, this tree is cultivated globally for dry and fresh consumption of its fruit due to the high content of minerals, vitamins and dietary fibres [2]. For instance, it has higher iron and copper content fourfold compared to other fresh and dry vegetables and fruits [3]. It is also rich in vitamins E and C [4]. Additionally, it is one of those medicinally imperative trees in terms of its various parts, such as fruits, leaves, shoots, tender, bark, and seeds, with medicinal appliances. For example, its phenolic compounds have a vital role in its antioxidant capacity leading to positive consequences to human health [5] .

Fig commercial production is accomplished in different countries, particularly those in the Mediterranean region, such as Turkey, Egypt and Morocco which lead fig production in 2020 with 665458 tons representing more than 50% of international production [6]. However, in Iraq, the production was 9322 tons, representing only 0.72 % of global production. This low percentage of production is due to the influence of numerous stresses to which the fig tree in Iraq is exposed including various pathogens. These pathogens have caused diseases such as roots rot, wilt and others [7].

Approximately 300 species of Alternaria genus exist in a wide range of environments [8, 9]. These species were either saprotrophic or parasitic, infecting a diversity of plant hosts and causing various diseases [10,11, 12]. For example, various species of Alternaria, particularly the *A. alternata* have caused leaf spot disease in different plant hosts, including fig [13]. On the other hand, the profuse number of recorded Alternaria species has led to a complicated accurate diagnosis of them [14, 15]. However, identification of them based on their morphological and molecular characteristics has offered a practical and accurate description [16, 17] .

In Iraq, the causative pathogen of this disease on Fig trees has not described yet. Thus, the object of this investigation was to identify the pathogen causing leaf spot of fig trees in Karbala Provinces of Iraq.

Materials and Methods

During a survey held in 2018 in orchards of Alhusaynia territory, Karbala Province, Iraq, an epidemic leaf spot disease affecting fig trees was observed. These leaf spots initially appeared as small in a circular shape and light brown colour, which developed to be mostly an irregular shape in blackish brown to grey colour. However, a minority of them persisted in circular shapes with concentric zones. Some of these spots, subsequently merged to create sizeable necrotic expanses ending with the yellowing and drying of the infected leaves (Fig. 1).

Figure (1): Leaf spot symptoms on fig leaves.

The symptomatic leaves of fig trees were collected, cut into small sections (1-2 cm), disinfected with sodium hypochlorite solution (2%) for a couple of minutes, and washed thoroughly with distilled water. They were then placed aseptically on water agar media for a couple of days at 25±2 °C. Afterwards, a hyphal tip technique was applied to purify emerged fungal colonies on potato dextrose agar that was amended with the antibiotics ampicillin and kanamycin monosulphate (50 µg/mL). The inoculated plates were subsequently incubated for a week at 25±2°C [18, 19].

The genomic DNA of a representative isolate was extracted [20]. Then, the sequence of the internal transcribed spacer region of rDNA was gained using the primer set ITS1 and ITS4 described previously [21]. The phylogenetic tree was built based on the alignment of the ITS-rDNA nucleotide sequences using MEGA version 10.1.5.

The pathogenicity of the isolated fungus was examined via the detached leaf assay [22]. Healthy fig leaves were surface sterilized using 70% of Ethanol for half minute, dry, and each inoculated with 500 µl of conidia suspension (1x10^6 conidia/mL). However, the leaves of control were treated with sterile distilled water. All leaves were subsequently incubated at 25±2°C in a growth chamber.

Results and Discussion

Several fungal isolates were obtained from the diseased fig leaves, and their morphological characteristics were examined. Initially, the mycelial growth of fungal colonies raised on PDA was a radial, airy, condensed and cottony structure in a greyish-white colour that converted latterly to blackish olivaceous (Fig. 2). The conidiophores were short, simple and mostly curved, carrying olive to dark brown, oval to pyriform like-shape conidia with transverse (1–3) and longitudinal (0–2) septate, and a short apical beak-shaped. The average length and width of these conidia (n=50) ranged from 16 to 36 µm and 7 to 11 µm, respectively. They were commonly lonely and rarely noticed in short chains (Fig. 3). These morphological characteristics proposed that the fungal species isolated in this study was likely *Alternaria alternata* as described previously [23, 11, 12, 17]. A BLAST and phylogenetic analyses of the isolate sequence obtained (GenBank Accession No. MK070016.1) showed that it was almost identical (99%) to several global strains of *A. alternata* such as MK371774.1, MN872494.1 and MN615420.1 (Fig. 4).

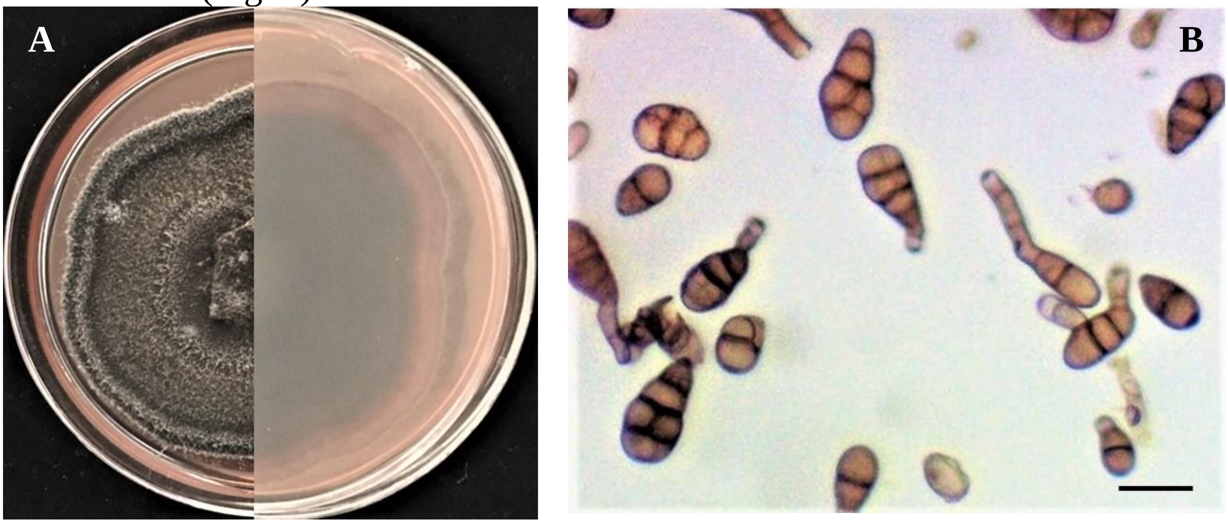

Figure (2): Cultural and microscopic features of *A. alternata*; (A) The up (Left) and down (Right) surfaces of *A. alternata* culture on PDA media; (B) Various forms and sizes of *A. alternata* conidia. The scale bar is 20 µm.

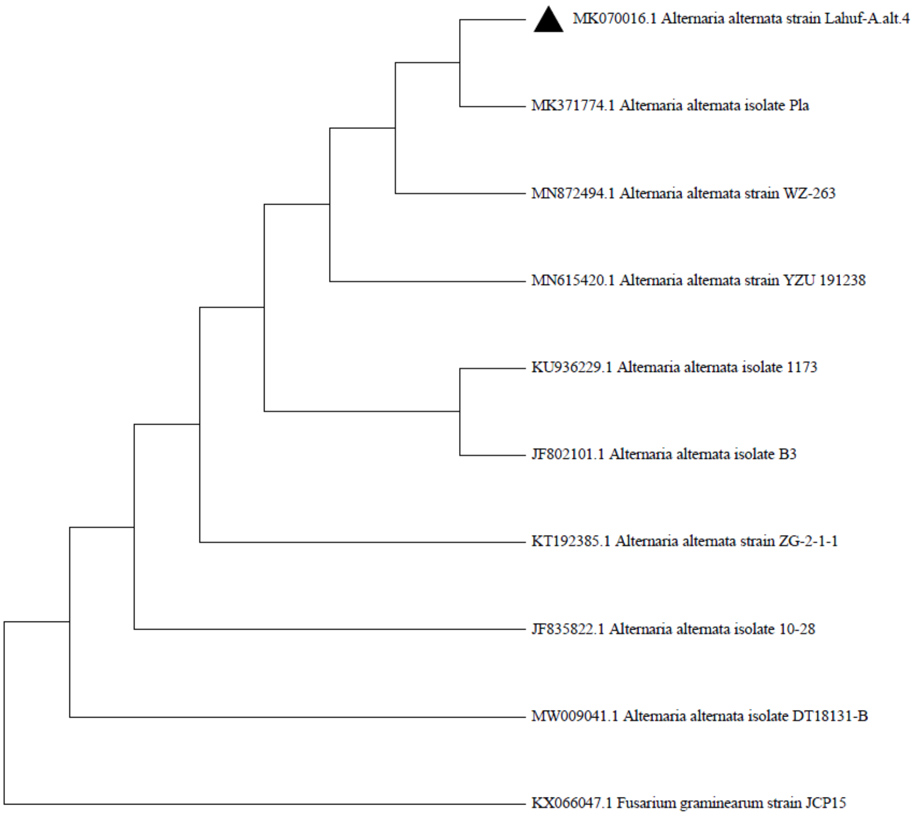

Figure (4): Phylogenetic tree displaying the relationship of the *A. alternata* isolated in this study (indicated with a black triangle) with other global *A. alternata* strains deposited in GenBank database.

After one week of inoculation, small circular spots of light brown colour emerged on the inoculated leaves. Conversely, leaves of control were symptomless. The pathogen (*A. alternata*) associated with these diseased leaves was re-isolated and re-identified. To our knowledge, this is the first record of fig leaf spot incited by *A. alternata* in Karbala, Iraq. Thus, additional investigations and surveys which should be accomplished in the rest of Iraqi fig orchards are demanded to reveal the incidence and severity of this disease besides detailing a comprehensive characterization of the fungal pathogen.

Acknowledgements

We would like to thank the undergraduate students B.J. Kadim, H.M. Mohsen, A.F. Mohamed and S.R. Saleh for their assistance in conducting the survey and collecting the diseased leaves samples.

References

1) Ersoy, N.; Gozlekci, S.; Gok, V. and Yilmaz, S. (2017). Fig (*Ficus carica* L.) fruit: some physical and chemical properties. Acta Horticulturae, 1173, 329-334.

2) Oskouei, T.E.; Allahyari, S.A.; Atashkhosrow, A.A.; Delazar, A.; Pashaii, M., Gan, S.H.; and Najafi, M. (2015). Methanolic extract of Ficus carica Linn. leaves exerts antiangiogenetic effects based on the rat air pouch model of inflammation. Evidence-Based Complementary and Alternative Medicine 2015 Article ID 760405.

3) Sadia, H. M.; Ahmad, S.; Sultana, A.Z.; Abdullah, T. L.; Zafar, L. M. and Bano, A. (2014). Nutrient and mineral assessment of edible wild fig and mulberry fruits. Fruits, 69(2): 159-166.

4) Javed, G.; Majid, S.A ; Khatoon, S. and Ulfat, A. (2017). Biochemical and nutritional evaluation of *Ficus palmata* Forssk. fruits from different ecotype population. Bangladesh Journal of Botany, 46(4): 1277 1283.

5) Joseph, B. and Raj, S.J. (2011). A comparative study on various properties of five medicinally important plants. International Journal of Pharmacology, 7 (2): 206-211.

6) FAOSTAT (2020). FAOSTAT (Food and Agriculture Organization of the United Nations statistics). Retrieved October 6, 2022, from http://www.fao.org/faostat/en /#data/QC.

7) Kadhim, W. R. and Matloob, A. A. H. (2022). Biological control of fig root rot disease. Journal of Kerbala for Agricultural Sciences, 9 (3): 139-149.

8) Lourenco, V. J.; Moya, A.; Gonzalez-Candelas, F.; Carbone, I.; Maffia, L.A. and Mizubuti, E. S. G. (2009). Molecular diversity and evolutionary processes of Alternaria solani in Brazil inferred using genealogical and coalescent approaches. Phytopathology, 99:765–774.

9) Lahuf, A. A.; Abdalmoohsin, R. A. G. and Alhusani, A. H.; Al-Asadi, A. (2018). First report of leaf blight disease in lily (*Lilium candidum*) caused by *Alternaria alternata* in Iraq. Biopesticides International, 14 (2): 123-126.

10) Konstantinova, P.; Bonants, P.J.M.; Gent-Pelzer, M.P.E.; Zouwen, P. and Bulk, R. (2002). Development of specific primers for detection and identification of Alternaria Spp. In carrot material by PCR comparison with blotter and plating assays. Mycological Research, 106: 23–32.

11) Lahuf, A. A. (2019). *Alternaria alternata* causes leaf blight of rosy periwinkle (Catharanthus roseus) in Iraq. Australasian Plant Disease Notes, 14(4):1-4.

12) Hameed, Z. L.; Lahuf, A. A. ; Jasim, M. T.; Mohsen, H. M. ; Kadim, B. J. ; Saleh, S. A. ; Mohamed, A.F. (2021). First Report of *Alternaria Alternata* causing brown leaf spot on apricot (*Prunus Armeniaca*) in Karbala Province of Iraq. Earth Environ. Sci. 910: 012080.

13) Anwaar, H. A. ; Perveen, R.; Chohan, S.; Saeed, A.; Cheema, M.T.; Qadeer,A.; Aslam, U.A.; Ahmed, M.Z.; Alam, M.; Shafique, M.S. and Ali, A. (2022). First Report of Alternaria alternata causing Alternaria leaf spot of fig in Pakistan. Plant Disease, 106(2): 759- 759.

14) Pryor, B.M. and Michailides, T.J. (2002) Morphological, pathogenic, and molecular characterization of Alternaria isolates associated with Alternaria late blight of pistachio. Phytopathology, 92: 406–416.

15) Woudenberg, J.H.C., Groenewald, J.Z. and Crous, P.W. (2013) . Alternaria redefined. Studies in Mycology, 75:171–212.

16) Andrew, M., Peever, T.L. and Pryor, B.M. (2009). An extended multilocus phylogeny does not resolve morphological species with the small-spored Alternaria species complex. Mycologia, 101: 95–109.

17) Lahuf, A. A., Kareem, A.A., Mohammadali, M.T., Mohsen, H.M., Li, J. (2020). First report of *Alternaria alternata* causing leaf spot on quince (*Cydonia oblonga*) in Iraq. Plant Disease,104 (5): 1538-1538.

18) Lahuf, A. A.; Jaafar, O. H.; Al-mosoy, M.; Hameed ,Z. L. (2018). First record of the crown rot fungus *Fusarium equiseti* affecting *Triticum aestivum* L. and *Aptenia cordifolia* in Iraq .Asian Journal of Agriculture and Biology, 6 (4): 543-548.

19) Lahuf, A. A.; Kadhim, A. Q.; Hameed, Z. L. and Almulla, A. M. N. (2022). First report of *Paecilomyces* rot on rose (*Rosa* sp.) in Iraq. Journal of Plant Pathology https://doi.org/ 10.1007/s42161-022-01162-7.

20) Lahuf, A. A.; Jaafar, O. H. and Hameed, Z. L. (2019). A simple, rapid, safe and low-cost method to extract DNA from phytopathogenic fungi. Asian Journal of Agriculture and Biology, 7(2):197-203.

21) White, T.J.; Bruns, T.; Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. San Diego, CA, USA: Academic Press, 315-322.

22) Akhtar, K.P.; Sarwar, N.; Saleem, M.Y. and Asghar, M. (2011). Convolvulus arvensis, a new host for Alternaria solani causing early blight of Solanum lycopersicum in Pakistan. Australasian Plant Disease Notes, 6 (1):84–86.

23) Simmons, E.G. (2007). Alternaria: an identification manual. Utrecht, Netherland: CBS Fungal biodiversity center.