2026,Volume 3 , Issue 9 , PSIJK (88-91)

Pure Sciences International Journal of Kerbala

First Report of Alternaria alternata Causing Leaf Spot on Orange (*Citrus sinensis* (L.) Osbeck) in Karbala Province, Iraq

Zainab L. Hameed [a*]

a Field Crop Department, Agriculture College, University of Kerbala, Karbala, Iraq

P A P E R I N F O

Received: 25.11.2025
Accepted: 12.03.2026
Published: 31.03.2026

***Keywords*:**
Citrus sinensis; citrus leaf spot; Alternaria alternata; ITS-rDNA; fungal foliar disease

A b s t r a c t

During a field survey conducted in 2022 in citrus groves in Alhusaynia territory, Karbala Province, Iraq, leaf spot symptoms were observed on orange foliage. A fungal pathogen was regularly isolated from diseased leaves and identified as Alternaria alternata based on colony and conidial morphology and internal transcribed spacer (ITS-rDNA; PX763615.1) sequence analysis. Pathogenicity was evaluated using a detached-leaf assay, and typical symptoms were reproduced on inoculated leaves, followed by re-isolation of the same fungus. This investigation is the first documented occurrence of A. alternata associated with leaf spot on orange in Karbala Province, Iraq

DOI: 10.53851/psijk.v3.i9. 88-91

1. INTRODUCTION

Sweet orange (Citrus sinensis (L.) Osbeck; family Rutaceae) is an evergreen fruit tree and one of the most widely grown citrus crops (Xu et al.,2012). Globally, it is valued for its nutritional and industrial importance and its central role in fresh fruit supply chains and juice production (Favela-Hernández et al.,2016). Global citrus production and trade are regularly summarized in FAO statistical reporting, underscoring the continuing economic relevance of citrus crops worldwide (FAO, 2022)

Foliar and fruit diseases can constrain citrus productivity and market quality. Among these, diseases caused by Alternaria species are well recognized in citrus-growing regions. Citrus can be affected by several Alternaria-associated disease syndromes, and host susceptibility often depends on citrus genotype and pathogen pathotype (Timmer et al., 2003). In particular, Alternaria brown spot—frequently discussed in mandarins, tangelos, and susceptible hybrids—has been linked to A. alternata (often described as the tangerine pathotype or A. alternata pv. citri), where infections may lead to necrotic lesions on young tissues and can contribute to defoliation and fruit losses in susceptible cultivars (Cuenca et al.,2016) .

Because Alternaria is a species-rich genus with complex taxonomy, accurate identification benefits from integrating morphological assessment with molecular tools such as ITS-based sequencing and phylogenetic analysis. The present study aimed to determine the pathogen associated with leaf spot symptoms seen on orange foliage in Karbala Province, Iraq.This is due to the best of our knowledge, there are no published reports documenting Alternaria alternata causing leaf spot on sweet orange in Iraq, particularly in Karbala Province.

2. MATERIALS AND METHODS

2.1. Symptomatic sample collection

A survey was accomplished in 2022 in orange groves located in Alhusaynia orchards, Karbala Province, Iraq. A leaf spot disease influencing orange trees was noted. The disease symptoms primarily seemed as small in a circular shape and light brown colour, developing to be irregular form in dark brown colour. Nevertheless, a smaller zones stayed in circular shapes with concentric spotes. Some of them, afterward united to generate considerable necrotic spreads ending with the yellowing and drying the leaves on susceptible citrus tissues (Figure 1). Leaves showing suspected fungal leaf spot symptoms were

*Corresponding Author Institutional Email:
zainab.l @uokerbala.edu.iq (Zainab L. Hameed)

collected and transported to the laboratory for pathogen isolation. This is because alternaria leaf spot is a serious fungal disease that significantly reduces citrus yield and fruit quality by causing premature leaf drop and fruit blemishes. (Riyahi et al.,2021).

Figure 1. Typical Leaf spot symptoms on orange leafcollected in this study.

2.2. Isolation and Purification

Diseased orange leaves were cut into small sections (approximately 2 cm), surface-disinfested for two min in sodium hypochlorite (2%), washed thoroughly with sterile distilled water, and plated on water agar. After initial growth at 25 ± 2°C, hyphal tips were transferred to potato dextrose agar (PDA) attuned with the antibiotics ampicillin and kanamycin monosulfate (50 µg/mL each) to obtain pure cultures. (Lahuf et al.,2022) .

2.3. Molecular Identification And Phylogenetic Placement

The fungal DNA was extracted from the isolated fungus. The ITS region of rDNA was amplified with ITS1 and ITS4 primers (White et al.,1990), followed by sequencing. ITS sequences were aligned with reference sequences, and a phylogenetic tree was built via MEGA (version 11,0,13) (Tamura et al.,2021).

2.4.Pathogenicity Test

Pathogenicity was evaluated by means of a detached-leaf assay. Healthy orange leaves (young, fully expanded leaves) were surface-sterilized (70% ethanol, ~30 s), dried, and inoculated with 500 µL of a conidial suspension (1×10^6 conidia/mL). Control leaves were treated with distilled water only. Inoculated and control leaves were incubated at 25 ± 2°C under high humidity in a growth chamber. Symptom development was recorded, and the pathogen was re-isolated from lesions for confirmation. (Akhtar et al.,2011).

3. RESULTS AND DISCUSSION

3.1. Morphological Characterization

Fungal colonies consistently recovered from symptomatic orange leaves showed morphological features compatible with Alternaria spp. On PDA, colonies typically developed a radial growth pattern with initially pale to gray mycelium that darkened with age, becoming olivaceous to blackish. Conidiophores were short and simple, and conidia were pigmented (olive to dark brown), with variable shapes (often ovoid to pyriform) and transverse and occasional longitudinal septation; short beaks may be present depending on isolate and conditions. These characters are consistent with descriptions commonly used to recognize A. alternata in the small-spored Alternaria complex (Simmons, 2007).

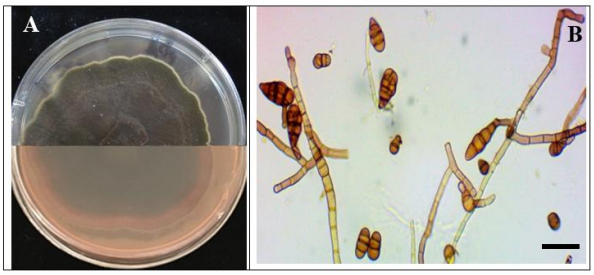

Figure 2: Features of the Alternaria sp. isolated from orange; (A) The upward and downward sides of A. sp. culture on PDA media; (B) Several shapes of A. sp. conidia.

3.2. Molecular Identification And Phylogenetic Placement

ITS-rDNA sequencing and database comparison (BLAST) supported identification of the representative isolate as Alternaria alternata. It showed >99% sequence similarity to several international strains of *A. alternata* such as OP959991.1, MZ047483.1 and OP955785.1. Thus, the sequence was submitted to GenBank database to receive a specific accession number (Alternaria alternata isolate Aa-orange-1; PX763615.1).

The phylogenetic tree constructed based on ITS-rDNA sequences revealed that the Iraqi isolate A. alternata Aa-orange-1 clustered firmly within the Alternaria alternata clade (Figure 3). The isolate grouped closely with reference A. alternata sequences retrieved from GenBank originating from Saudi Arabia (OP959991.1; OP955785.1), Egypt (OR358925.1), China (MZ047483.1; OK316967.1; MZ047506.1), Bangladesh (MH368103.1), and Turkey (KJ739872.1), indicating a high level of genetic similarity among these isolates.

The close clustering of Aa-orange-1 with previously authenticated A. alternata isolates from different geographical regions confirms its taxonomic placement within the A. alternata species complex. The short branch lengths separating Aa-orange-1 from other reference isolates further suggest limited sequence divergence in the ITS region, which is consistent with the conserved nature of ITS sequences within the *A. alternata* group.

An unrelated fungal species, Erysiphe lonicerae (GenBank accession ON248222.1), was used as an outgroup and was clearly separated from the Alternaria clade, supporting the robustness and correct rooting of the phylogenetic tree.

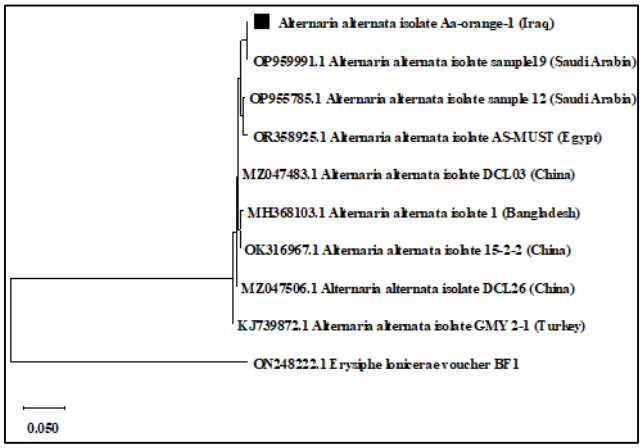

Figure 3: Phylogenetic tree based on ITS-rDNA sequences showing the relationship between A. alternata isolate Aa-orange-1 recovered from orange (Citrus sinensis) in Iraq and reference A. alternata isolates retrieved from GenBank. Erysiphe lonicerae (GenBank accession ON248222.1) was used as an outgroup.

Overall, the phylogenetic analysis, together with morphological characteristics, provides strong molecular evidence that the isolate recovered from orange leaves in Iraq belongs to *Alternaria alternata*.

3.3. Pathogenicity Test

Following seven days of inoculation, the disease symptoms were as slight spherical spots of light-dark brown colour appeared on the inoculated leaves (Figure 4). In contrast, the non-inoculated leaves were with no disease symptoms. The inoculated fungus was re-isolated from affected spotes tissues and re- recognized, implementation Koch's postulates at the assay scale.

Figure 4: Development of typical leaf spot symptoms on orange (Citrus sinensis) leaves one week after artificial inoculation with *A. alternata* isolate Aa-orange-1.

Alternaria diseases are widely recognized in citrus production systems, with important reports describing necrotic lesions and potential defoliation/fruit impacts in susceptible citrus genotypes (Peres et al.,2003; European and Mediterranean Plant Protection Organization, 2021). This report strengthens regional plant health knowledge for Karbala Province and motivate wider surveys across Iraqi citrus-producing areas. Future work should quantify incidence/severity, evaluate cultivar susceptibility.

4.ACKNOWLEDGEMENTS

The author gratefully acknowledges the Agriculture College, University of Kerbala, Iraq, for their support and for providing the necessary facilities to conduct this research.

REFERENCES

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.

Cuenca, J., Aleza, P., Juárez, J., Navarro, L., & Ollitrault, P. (2016). Fine mapping for identification of citrus alternaria brown spot candidate resistance genes and development of new SNP markers for marker-assisted selection. Frontiers in Plant Science, 7, 1948 .

European and Mediterranean Plant Protection Organization. (2021). Alternaria alternata pv. citri (Alternaria brown spot). EPPO Global Database. https://gd.eppo.int

Favela-Hernández, J. M., González-Santiago, O., Ramírez-Cabrera, M. A., Esquivel-Ferriño, P. C., & Camacho-Corona, M.delR. (2016). Chemistry and Pharmacology of Citrus sinensis. Molecules (Basel, Switzerland), 21(2), 247.

Food and Agriculture Organization of the United Nations. (2022). Citrus fruit: Fresh and processed – Statistical bulletin. FAO.https://www.fao.org/markets-and-trade/ commodities /citrus.

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.

Peres, N. A. R., Agostini, J. P., & Timmer, L. W. (2003). Outbreaks of Alternaria brown spot of citrus in Brazil and Argentina. Plant Disease, 87(6), 750–755.

Riyahi, M., Taheri, P., & Mamarabadi, M. (2018). Identification of Alternaria spp. in orange trees of Golestan and Mazandaran provinces and investigating their virulence on orange cultivars. *Iranian Journal of Plant Protection Science*, 48(2), 183–196.

Simmons, E. G. (2007). Alternaria: An identification manual. CBS Fungal Biodiversity Centre.

Tamura, K., Stecher, G., & Kumar, S. (2021). Molecular Biology and Evolution, 38(7), 3022–3027 .

Timmer, L. W., Pever, T. L., Solel, Z., & Akimitsu, K. (2003). Alternaria diseases of citrus—Novel pathosystems. Phytopathologia Mediterranea, 42, 99–112.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), PCR protocols: A guide to methods and applications (pp. 315–322). Academic Press.

Xu, Q., Chen, LL., Ruan, X. et al. (2013). The draft genome of sweet orange (Citrus sinensis). Nature Genetics 45, 59–66.

Potts, C. (1995). Using schematic scenarios to understand user needs. *Proceedings of the 1st Conference on Designing Interactive Systems: Processes, Practices, Methods, & Techniques*, 247–256.

Wang, L.-X., & Mendel, J. M. (1992). Generating fuzzy rules by learning from examples. *Systems, Man and Cybernetics, IEEE Transactions On*, *22*(6), 1414–1427.

Weiser, M. (1991). The computer for the 21st century. *Scientific American*, *265*(3), 94–104.