



Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)
<http://esciencepress.net/journals/PP>

Research Article

Molecular and Pathological Characterization of *Alternaria burnsii* as an Emerging Foliar Pathogen of Eggplant (*Solanum melongena* L.)

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ARTICLE INFO

Article history

Received: 22nd October, 2025Revised: 14th December, 2025Accepted: 18th December, 2025

Keywords

Alternaria burnsii

Eggplant leaf spot

Molecular identification

Fungal pathogenicity

Plant-pathogen interaction

ABSTRACT

The present study reports *Alternaria burnsii* as the causal agent of leaf spot disease on eggplant (*Solanum melongena* L.) for the first time. Symptomatic leaves exhibiting dark necrotic lesions with yellow margins were collected from multiple locations in Basrah province, southern Iraq. The fungus was isolated on potato dextrose agar which produced creamy-orange to white colonies with a cottony to velvety texture. Microscopic examination revealed oval to rectangular conidia with 3-6 transverse septa, formed in chains of 4-8 conidia, consistent with characteristics of the genus *Alternaria*. Molecular identification based on ITS sequencing confirmed the isolate as *A. burnsii*, forming a distinct subclade within the species complex and the sequence was deposited in GenBank under accession number PV961372.1. Pathogenicity assays under greenhouse conditions confirmed its virulence on eggplant, with necrotic lesions appearing five days after inoculation and progressively expanding thereafter. These findings demonstrate that *A. burnsii* is a previously unreported foliar pathogen of eggplant and highlight its potential threat to economically important vegetable crops.

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Introduction

Eggplant (*Solanum melongena* L.) also known as brinjal and aubergine, belonging to the Solanaceae family, is one of the main vegetables cultivated and consumed worldwide. Due to rich in high nutraceutical contents, extensive culinary uses, and being economically vital, eggplant is cultivated widely throughout the subtropical, tropical, and warm temperate regions of the globe. This vegetable can significantly adapt to broad climatic and edaphic environments and is being integrated into varied cropping patterns by small and large farmers. However, in spite of its wide adaptableness, the yield and production of eggplant are often adversely threatened by a large number of biotic and abiotic

stresses. Amongst the biotic stresses, plant pathogens pose a significant and continuing threat to lucrative and sustainable production of eggplant resulting in substantial reduction in income of the farmers (Kaniyassery et al., 2023; Mishra et al., 2024). Amongst the biotic restraints limiting eggplant production, fungal diseases are of significant importance, cause substantial damage and reduce yield significantly. The prominent symptoms caused by these foliar pathogens include leaf spots and blights. These diseases usually develop on the hosts when the environmental conditions are conducive and spread rapidly within fields, resulting in heavy tissue necrosis, premature falling of leaves, and a considerable reduction in photosynthetic

potential. Moreover, these severe infections also cause reductions in plant vigor, poor flowers and fruit development and significant yield losses.

In this regard, pathogenic species belonging to the genus *Alternaria* are generally recognized as main causes of leaf spot and blight diseases in a large numbers of agricultural and horticultural crops. Diseased plants usually show typical necrotic lesions with concentric rings, which are responsible for physiological dysfunction and premature senescence (Meena et al., 2025; Yang et al., 2025).

The genus *Alternaria* comprises a huge and a phylogenetically complex assemblage of phytopathogenic fungi showing extensive cultural and morphological variations and genomic diversity. Even though numerous *Alternaria* species have been widely investigated and extensively studied, a considerable number of species are still insufficiently characterized in terms of their pathogenic potential, host specificity, and molecular identity. Among these poorly understood species, *Alternaria burnsii*, is one. This species has been reported to infect only a limited number of economically significant crops, such as onion, tomato, wheat, cumin, date palm, and faba bean (Shekhawat et al., 2013; Woudenberg et al., 2015; Htun et al., 2022; Ofi et al., 2025). These studies show that *A. burnsii* has a wide host range than previously recognized; however, the information on its incidence, prevalence, epidemiology, pathogenicity and virulence is lacking on eggplant.

The current study was, therefore, carried out to isolate and characterize *A. burnsii* causing leaf spot symptoms on eggplant by employing comprehensive morphometric and molecular methods. Furthermore, pathogenicity tests were performed to fulfill Koch's postulates and to affirm its roles as the causal organism of leaf spot disease of *eggplant*. The outcomes of the resent investigation aim to advance the understanding of *Alternaria*-associated diseases in eggplant, develop accurate diagnostic tools and design control strategies for its management.

Materials and Methods

Sample collection

A comprehensive field survey was conducted during the months of January and February 2025 at various greenhouse locations cultivating eggplant in Basrah province, southern Iraq. Leaves exhibiting prominent symptoms of leaf spot infection were collected from

eggplant plants during the 2024-2025 growing season. The samples thus collected were transported to the laboratory and maintained at 4°C for further analysis.

Isolation of fungal pathogens

Fungal isolates were obtained from symptomatic eggplant leaves showing typical leaf spot symptoms. The infected leaves of eggplant plants were surface-sterilized with 70% ethanol for 1 min, subsequently rinsed three times with sterile distilled water, and dried on sterile Whatman No. 1 filter paper. Small sections, about 2 mm in diameter, were cut from the margins of diseased leaf patches and inoculated into Potato Dextrose Agar (PDA) plates supplemented with chloramphenicol (250 µg/L). The petri plates were incubated for 7 days at 25°C ± 1 to allow the fungus to proliferate. A representative isolate was selected for further study based on consistent colony morphology, sporulation capacity, and symptom association across multiple samples.

Characterization of *A. burnsii*

The purification of *A. burnsii* isolate was done on PDA plates using the single-spore isolation technique, followed by incubation for 7 days at 25 ± 1°C. The morphometric and microscopic features of the fungus such as colony morphology, growth pattern, mycelial pigmentation, shape and size of conidia were examined under the light microscope (Olympus BX51, Tokyo, Japan). The size of conidia was determined as an average of 100 conidia measurements (Ahmed and Abass, 2022).

Molecular diagnosis *A. burnsii*

Genomic DNA extraction of *A. burnsii*

The gDNA of *A. burnsii* was extracted from a 5-day old purified culture using the Fungal MinKit supplied by Favorgen Biotechnology Crop (Korean Company). The instructions of the manufacturer of the Kit were followed. Subsequently, the purity of DNA and the concentration were measured using the Nanodrop technique (Thermo Fisher Spectrophotometer).

Amplification and sequencing of fungal DNA

The gDNA of *A. burnsii* was amplified using the Internal Transcribed Spacer (ITS) primers according to White et al. (1990). The forward primer was: 5' TCCGTAGGTGAACCTGCGG-3' and the reverse ITS4 primer was: 5'-TCCTCCGCTTATTGATATGC-3'. The composition of a 25 µL of the PCR reaction mixture used was: 9 µL double distilled H₂O; 1 µL of both forward and reverse primers, 1.5 µL fungal gDNA and 12.5 µL master mix. The conditions of PCR were: initial denaturation at 95°C for 5 min in one cycle; denaturation at 95°C for 30

sec; annealing at 53°C for 2 min; extension at 72°C for 30 sec for 35 cycles; and a final extension at 72°C for 7 min. The efficiency of PCR reaction was evaluated by running of the PCR product on 1.5% agarose gel (Ofi et al., 2025). The PCR fragments were sequenced by Macrogen Company (South Korea), the ITS sequences of *A. burnsii* were deposited in the GenBank with the gene accession number PV961372.1.

Phylogenetic analysis

ITS sequence of *A. burnsii* PV961372.1 was searched with the help of the BLAST search tool on the NCBI site (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The results of the BLAST search were downloaded, and similar sequences were aligned using the Clustal Omega program. A phylogenetic tree was then built with MEGA 11, employing the maximum likelihood method as described by Kumar et al. (2016) and Tamura et al. (2021). The percent replicate trees (in which the corresponding taxa were grouped) were compared with the bootstrap test (1000 replicates), and the distances between the evolutions were calculated with the help of the Poisson correction method and expressed in the number of amino acid substitutions per site (Jones et al., 1992).

Pathogenicity test of *A. burnsii*

A pathogenicity test was conducted in a controlled greenhouse with a temperature of $25 \pm 2^\circ\text{C}$ and a relative humidity of 85 percent. The seeds were surface sterilized with 75 percent ethanol followed by three washings with distilled water. A 1 Kg-capacity pot was filled with a mixture of peat moss and soil in 3:1 (v/v) and 10 surface sterilized seeds were sown. The pot was irrigated daily and monitored regularly.

The inoculum of *A. burnsii* with a concentration of 1×10^6 cfu/ml was prepared using the procedure described by Rashid et al. (2016). The plants were inoculated at the 4-5 leaf stage with the fungal suspension. Ten plants were used per treatment with three replicates while the control group (without the fungus) was sprayed with distilled water only following the protocol of Ofi et al. (2025).

The plants were arranged in a completely randomized design to minimize environmental variations. Macroscopic and microscopic evaluation of disease symptoms was done at 14, 28, and 42 days after inoculation. The severity of infections was assessed with the help of a 0-9 scale described by Simko and Piepho (2012) as follows: 0 = no symptoms (no visible damage); 1 = 1-3 spots per plant; 2 = 4-6 spots per plant; 3 = up to 10% of the plant surface covered with spots; 4 = 11-

25%; 5 = 26-50%; 6 = 51-75%; 7 = >75% of the plant surface covered with spots; 8 = complete plant surface covered with spots; 9 = dead plant. To confirm *A. burnsii* as the causal organism of the disease, Koch's postulates were fulfilled. A standard disease severity scale was used to measure the severity of the disease 10 days after inoculation and the DSI values were determined according to the equation of McKinney (1923). Data were analyzed statistically to determine significant differences between inoculated and control plants.

Statistical analysis

The experiments were carried out following completely randomized design. The data were analyzed using a One-Way ANOVA, adopting a significance level of 0.01. The least significant difference (LSD) test was employed to assess the significance of differences between means. Statistical analysis was conducted using the statistical software SPSS version 24.

Results and Discussion

Field survey and disease symptom of eggplant leaf spot

The incidence of the leaf-spot disease in the above-ground parts of the eggplant was observed at different regions surrounding the Basrah province during the growing season of 2024 to 2025. Under cool and moist conditions, the disease followed a certain persistence and then spread on the leaves. Symptoms began as small, necrotic, circular spots on leaves with white centers and brown to dark-brown margins; which later on expanded, coalesced, and became dense, dark-brown to black areas (Figure 1).



Figure 1. Field symptoms of eggplant leaf spot disease in Basrah province, Iraq.

Under favorable conditions, whole leaves were covered with necrotic lesions, which often resulted in the death of the plants. The present findings have broadened the existing host list of *A. burnsii* which has previously been limited to cumin, tomato, onion, wheat, date palm, and faba bean (Shekhawat et al., 2013; Dominique et al., 2022; Htun et al., 2022; Ofi et al., 2025). The successful infection and symptom development observed on eggplant suggested that *A. burnsii* possessed a broader pathogenic adaptability than it has been previously considered. Similar host expansion has been documented in other *Alternaria* species, where environmental conditions and host availability contribute to cross-host infection potential. The present report therefore supports the emerging view that *A. burnsii* should be considered as a potentially adaptable foliar pathogen rather than a host-restricted species.

Morphometric and microscopic characteristics of *A. burnsii*

A. burnsii showed optimal growth (90 mm) on PDA plates at seven days after incubation at $25\pm 1^\circ\text{C}$. The colonies of *A. burnsii* growing on PDA media were white with white margins forming a circular and entire border with a flossy to smooth velvety texture. Moreover, a yellowish-white or yellowish-brown hue was observed at the growth center at the bottom of the plate (Figure 2 A-B). The conidiophores of *A. burnsii* were branching, upright, straight, irregularly curved, and geniculate, producing a cluster of 4-8 conidia. Conidia were oval to oblong in morphology and contained 3-6 transverse septa and measured $10\ \mu\text{m} \times 45.5\ \mu\text{m}$ (Figure 2 C). The morphometric and microscopic properties of *A. burnsii* are in line with the description of Simmons (2007).

Molecular diagnosis of the pathogenic isolate of *A. burnsii*

The amplification and sequencing of the ITS region of the pathogenic isolate of *A. burnsii*, collected from the infected leaves of eggplant in Basrah province, Iraq was done to confirm the morphometric identification. The amplified product was deposited in GenBank under the accession number of PV961375.1 (Gene length 505 bp). The BLAST analysis of the obtained sequences revealed the highest level of similarity ($\geq 99\%$) with the other sequences of *A. burnsii* collected from different regions of the world, especially from India, Pakistan and Saudi Arabia.

Further analysis was done to confirm the taxonomic position based on a phylogenetic tree, constructed using

the *A. burnsii* sequences of ITS retrieved from GenBank in addition to *Rhizoctonia solani* (PV419863.1) as an outgroup to root the phylogenetic tree (Figure 3). Cluster analysis clearly revealed that the *A. burnsii* Basrah isolate was clustered within *Alternaria burnsii* group, forming a relationship with many Indian isolates (MN795639.1, PB855652.1, KR604843.1, and PQ285439.1). Generally, the phylogenetic analysis provides conclusive molecular evidence that the Basrah isolate belongs to *Alternaria burnsii*. The strong bootstrap support and minimal genetic variation within the *A. burnsii* cluster further indicate a stable population structure and high sequence conservation of the ITS region within this species.

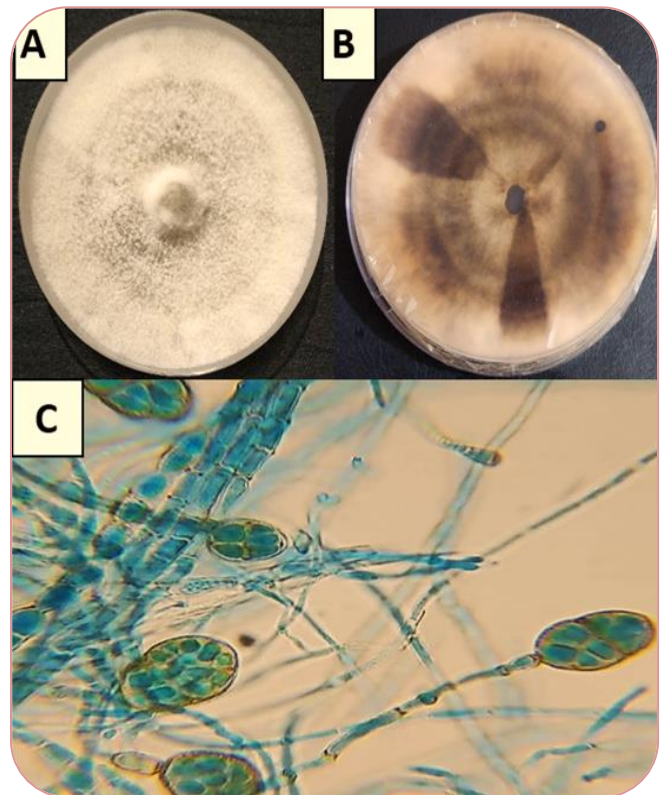


Figure 2. Colony morphology of *A. burnsii* on PDA plates: (A) upper surface of the colony, (B) lower surface of the colony, and (C) conidia observed at $40\times$ magnification.

Our results of the ITS primers efficiency in confirming plant pathogenic fungal identity are in agreement with several studies that showed this efficiency such as *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Fusarium*, *Stemphylium*, and many other pathogens infecting important plant hosts (Ahmed and Abass, 2022; Razaq and Abass, 2022; Lan et al., 2023; Ofi et al., 2025).

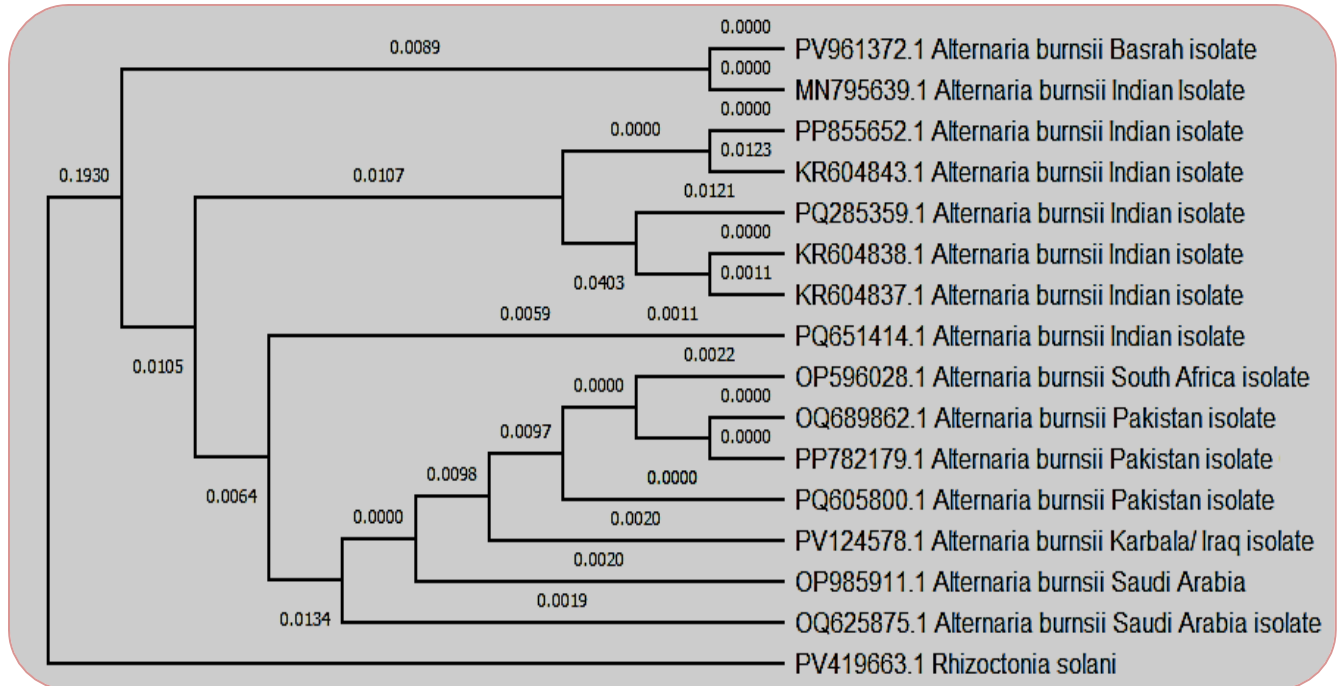


Figure 3. Maximum likelihood phylogenetic tree based on ITS sequences of the fungal pathogen *A. burnsii* (Basrah, Iraq isolate; PV961372.1) and closely related *A. burnsii* isolates retrieved from GenBank. *Rhizoctonia solani* (PV419663.1) was used as an outgroup. Numbers on the branches indicate genetic distances.

Pathogenicity of *A. burnsii*

A conidial suspension of *A. burnsii* at a concentration of 1×10^6 Cfu/ml was used to inoculate 45-day-old eggplant plants. Symptoms of the disease manifested five days post-inoculation as small, round to oval, pale-centered necrotic lesions (0.2-0.5 cm in diameter) on young leaves.

As the disease progressed, the lesions grew larger and formed dark brown to black margins, eventually becoming irregular necrotic patches on leaves, strongly mirroring the symptoms seen in naturally infected eggplant fields in Basrah (Figure 4).



Figure 4. Symptoms of eggplant leaf spot disease caused by *A. burnsii* in a pathogenicity trial.

The “Barcelona” cultivar showed disease severity of about 25, 33 and 46% at 14, 28, and 42 days post-inoculation, respectively (Table 1). On the other hand, control plants treated with sterile distilled water remained asymptomatic throughout the experiment. *A. burnsii* was regularly re-isolated from the necrotic tissues, agreeing with the initial morphological and molecular characteristics of the injected isolate and thus confirmed Koch’s postulates.

Table 1. Disease severity of *A. burnsii* on the eggplant cultivar ‘Barcelona’

Treatment	Days post inoculation	(%) Disease severity
Control*	-	00.00
<i>A. burnsii</i> treatment	14	25.50 ^c
<i>A. burnsii</i> treatment	28	33.25 ^b
<i>A. burnsii</i> treatment	42	46.50 ^a
LSD (0.01)	5.25	

*Each treatment represents the mean of three replicates.

Although various recent reports have indicated the pathogenic potential of *A. burnsii* against an extensive array of economically significant hosts such as date

palm, a number of ornamental plants like *Impatiens hawkeri*, medicinal plants like *Polygonatum odoratum*, and forest and landscape trees like *Conocarpus lancifolius*, among others, in addition to its confirmation of being a pathogenic agent of faba bean (*Vicia faba*) in Iraq, where it has been linked to both leaf spot and blight symptoms (Al-Nadabi et al., 2018; Chang et al., 2023; Ahmed et al., 2025; Li et al., 2025; Ofi et al., 2025), no previous study has documented its pathogenicity on eggplant. This highlights the novelty of the study and its relevance in increasing the scope of knowledge regarding the possible hosts of this emerging fungus.

Conclusions

The present research was conducted in the greenhouse and laboratories of the Faculty of Agriculture, University of Basrah, in order to isolate and identify the pathogen that causes leaf spot disease in eggplant plants. The findings revealed for the first time in the world that *Alternaria burnsii* is a true foliar pathogen of eggplant. The identification of the pathogen was confirmed through a comprehensive morphometric and molecular procedures followed by amplification of ITS region, its sequencing and submission to the NCBI GenBank database (access number: PV961372.1). In pathogenicity tests on the "Barcelona" cultivar, *A. burnsii* was shown to produce clear necrotic lesions. Disease severity increased greatly over 42 days after artificial inoculation in greenhouse conditions. This information offers *A. burnsii* as a possible novel pathogen of the eggplant production systems and provides the broadest host range record for this fungus. Further studies are required to analyze its association with other *Alternaria* species that could influence the leaf spot complex to influence eggplant cultivation.

Authors' Contributions

AAM conceptualized and designed the study, performed the data analysis, and drafted the original manuscript. MAH provided critical guidance, constructive feedback, and valuable suggestions to improve the content and clarity of the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work, ensuring accuracy and integrity in reporting.

Research Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

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