



## ***Alternaria alternata* associated with leaf spot disease on *Ipomoea purpurea* in Pakistan**

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### **Abstract**

During the 2024 growing season, severe leaf spot symptoms were observed on the ornamental plant *Ipomoea purpurea* in Swat, Pakistan. Affected leaves showed circular to irregular necrotic lesions, ranging from dark brown to black. The causal pathogen was isolated and identified through morphological examination and multilocus phylogenetic analysis using ITS, LSU, and RPB2 gene regions. Maximum likelihood phylogenetic analysis clustered the isolate within the *Alternaria alternata* clade with 98% bootstrap support. Pathogenicity was confirmed by the detached-leaf method, fulfilling Koch's postulates. To our knowledge, this study represents the first report of *A. alternata* causing leaf spot disease on *I. purpurea* in Pakistan.

**Key words** – Fungal pathogen – Morning glory – Phylogeny – Symptoms

### **Introduction**

Morning glory, *Ipomoea purpurea* (L.) Roth, belongs to the family Convolvulaceae. The species has a worldwide distribution across tropical, subtropical, and warm temperate regions (Sastry et al. 2019). The species is cultivated primarily as an ornamental plant but is also recognized as an agricultural and environmental weed. Additionally, it holds significance in traditional medicine (Srivastava & Rauniyar 2020). The flowers of *Ipomoea purpurea* contain antioxidant compounds such as pelargonidins and cyanidins (Srivastava et al. 2017). Similar to its related species *I. tricolor* and *I. violacea*, *I. purpurea* exhibits entheogenic properties due to the presence of ergoline alkaloids, including ergine, isoergine, and lysergol (Srivastava et al. 2017).

*Ipomoea purpurea* is a popular ornamental plant with attractive trumpet-shaped flowers. However, it is susceptible to various biotic stresses, particularly fungal infections. Several fungal pathogens have been reported in *I. purpurea*, including *Alternaria alternata* and *A. hydrangeae* cause leaf spot disease in China (Yang et al. 2018, Liu et al. 2023). Additionally, blight caused by *Phytophthora ipomoeae* has been documented in Mexico (Badillo-Ponce et al. 2004).

The aim of this study was to isolate and identify the causal pathogen responsible for the *Ipomoea purpurea* leaf spot through morphology and molecular analysis using LSU, ITS, and RPB2 genetic markers. This study provides the first report of *A. alternata* causing leaf spot disease on *I. purpurea* in Pakistan.

## Materials & Methods

### Isolates and morphology

During July–August 2024, leaf spot symptoms were observed on *Ipomoea purpurea* plants, which presented as dark brown to black necrotic spots with yellow halos or no halos (Fig. 1). Infected leaves were collected from Swat, Pakistan. The leaves were washed with water to remove dust and then cut into small pieces (4x4 mm) from the margins of the brown spots. These pieces were surface-sterilized by rinsing them in a 2% sodium hypochlorite solution for one minute, followed by three rinses in sterile distilled water for 45 seconds each. The sterilized fragments were blotted dry on sterile filter paper and transferred onto potato dextrose agar (PDA) plates. The plates were incubated at 25 °C and 70% relative humidity for two days. After fungal growth appeared, subcultures were performed on new PDA plates to obtain pure cultures.



**Fig. 1** – The leaf spot symptoms observed on *Ipomoea purpurea* plants.

Colony morphology of the isolates was recorded from 7 days old cultures grown on Potato Dextrose Agar (PDA) at 25°C. Colony characteristics such as color, texture, growth pattern, and pigmentation of the reverse side were documented. For microscopic examination, slides were prepared from 7-day-old cultures. Small portions of mycelium were mounted in 5% KOH and stained with 2% Congo red. Observations of hyphae, conidiophores, and conidia were made under a compound microscope at 40× and 100× magnifications. Morphological features, including septation, branching of conidiophores, and conidial shape and size, were recorded for species identification.

### DNA extraction and PCR amplification

For molecular identification, genomic DNA was extracted from a representative isolate, LMPP-2407, using the CTAB method described by Doyle and Doyle (1987). The internal transcribed spacer (ITS) region, the large subunit (LSU) rRNA gene, and the RNA polymerase II second largest subunit (RPB2) gene were amplified using the primer pairs ITS1/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), and RPB2-5F2/RPB2-7cR (Liu et al. 1999), respectively. Polymerase chain reaction (PCR) was performed following Lan et al. (2023). The resulting amplicons were sent for sequencing to Macrogen Inc. (Seoul, South Korea). The newly generated sequences were deposited in GenBank.

### Phylogenetic analysis

The generated sequences were BLAST-searched in the NCBI database, and the corresponding reference sequences were downloaded and are listed in Table 1. Sequence

alignments were performed using the MAFFT online server (Katoh & Standley 2013). The alignment files were manually edited using BioEdit (Hall et al. 2011). A combined dataset of ITS, LSU, and RPB2 was analyzed using the Maximum Likelihood method with 1000 bootstrap replicates in IQ-TREE v. 1.6.12 (Nguyen et al. 2015). The final phylogenetic tree was visualized using FigTree.

**Table 1** Strains of the *Alternaria* species and related GenBank accession numbers of taxa included in this study. Isolate obtained in this study is in bold.

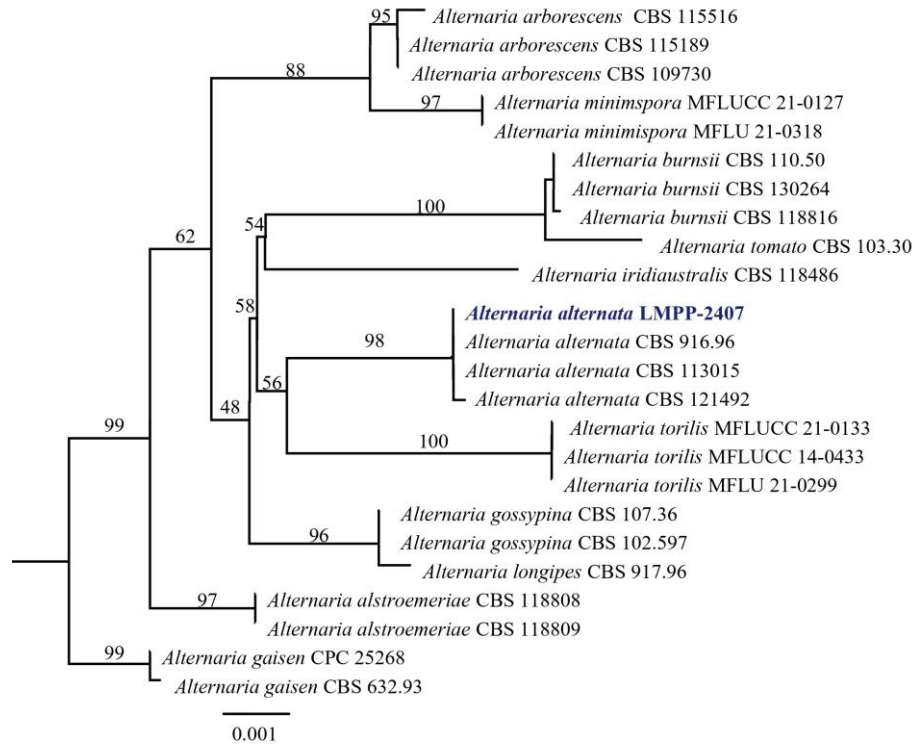
Taxa	Isolate no.	<i>rpb2</i>	ITS	LSU
<i>A. alstroemeriae</i>	CBS 118808	KP124764	KP124296	KP124447
<i>A. alstroemeriae</i>	CBS 118809	KP124765	NR_163686	NG_069882
<i>A. alternata</i>	CBS 113015	KP124811	KP124343	KP124495
<i>A. alternata</i>	CBS 121492	KP124840	KP124370	KP124524
<i>A. alternata</i>	CBS 916.96	KC584375	AF347031	DQ678082
<b><i>A.alternata</i></b>	<b>LMPP-2407</b>	<b>PV770579</b>	<b>PV768217</b>	<b>PV760618</b>
<i>A. arborescens</i>	CBS 115189	KP124872	KP124402	KP124555
<i>A. arborescens</i>	CBS 109730	KP124869	KP124399	KP124552
<i>A. burnsii</i>	CBS 110.50	KP124890	KP124421	KP124574
<i>A. burnsii</i>	CBS 118816	KP124892	KP124423	KP124576
<i>A. burnsii</i>	CBS 130264	KP124894	KP124425	KP124578
<i>A. gaisen</i>	CBS 632.93	KC584399	KC584197	KC584275
<i>A. gaisen</i>	CPC 25268	KP124898	KP124428	KP124582
<i>A. gossypina</i>	CBS 107.36	KP124901	KP124431	KP124585
<i>A. gossypina</i>	CBS 102597	KP124902	MH862797	MH874393
<i>A. iridialustralis</i>	CBS 118486	NG_069258	KP124905	NR_136120
<i>A. longipes</i>	CBS 917.96	KP124912	KP124442	KP124596
<i>A. muriformispora</i>	MFLUCC 22-0073	MZ621923	OK236630	MZ621976
<i>A. muriformispora</i>	MFLU 21-0309	MZ621924	OK236631	MZ621977
<i>A. tomato</i>	CBS 103.30	KP124915	KP124445	KP124599
<i>A. torilis</i>	MFLUCC 21-0133	OK236640	MZ621986	MZ621933
<i>A. torilis</i>	MFLU 21-0299	OK236642	MZ621987	MZ621934
<i>A. torilis</i>	MFLUCC 14-0433	OK236641	MZ621988	MZ621935
<i>A. arborescens</i>	CBS 115516	KP124873	KP124403	KP124556

## Results

### Phylogenetic analyses

Initial BLAST analysis of the ITS, LSU, and RPB2 sequences showed 100% similarity with *Alternaria alternata* strain CBS 916.96. To further confirm the phylogenetic placement, an analysis was performed using a combined dataset of ITS, LSU, and RPB2 sequences from 24 *Alternaria* species. The final alignment consisted of 2119 characters, which contained 68 distinct patterns, 39 parsimony-informative sites, 15 singleton sites, and 2065 constant sites. The maximum likelihood analysis was conducted under the GTR+I+G substitution model with the following estimated parameters: rate matrix (A–C = 1.74668, A–G = 2.28050, A–T = 1.94123, C–G = 0.55642, C–T =

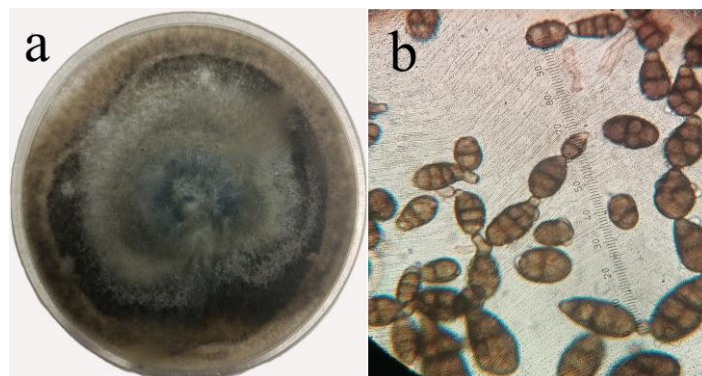
11.73791, G-T = 1.00000); base frequencies (A = 0.253, C = 0.231, G = 0.271, T = 0.244); a proportion of invariable sites (I) of 0.489; and a gamma shape parameter (G) of 0.020. In the resulting tree, rooted with *Alternaria gaisen* (CPC 25268 and CBS 632.93), our sequence grouped within the *Alternaria alternata* clade with 98% bootstrap support (Fig. 2) confirming its identification as *A. alternata*.



**Fig. 2** – Maximum-likelihood phylogeny inferred from the combined ITS, LSU and rpb2 sequence alignment of *Alternaria* species. The tree was rooted with *Alternaria gaisen* (CPC 25268 and CBS 632.93). Bootstrap support values from 1,000 replicates. Sequence obtained in the present study is indicated in **blue**.

### Morphology

The fungal colonies were dark green with white margins, produced black pigment, and showed abundant aerial hyphae. Microscopic examination Conidiophores were unbranched, bearing solitary conidia.

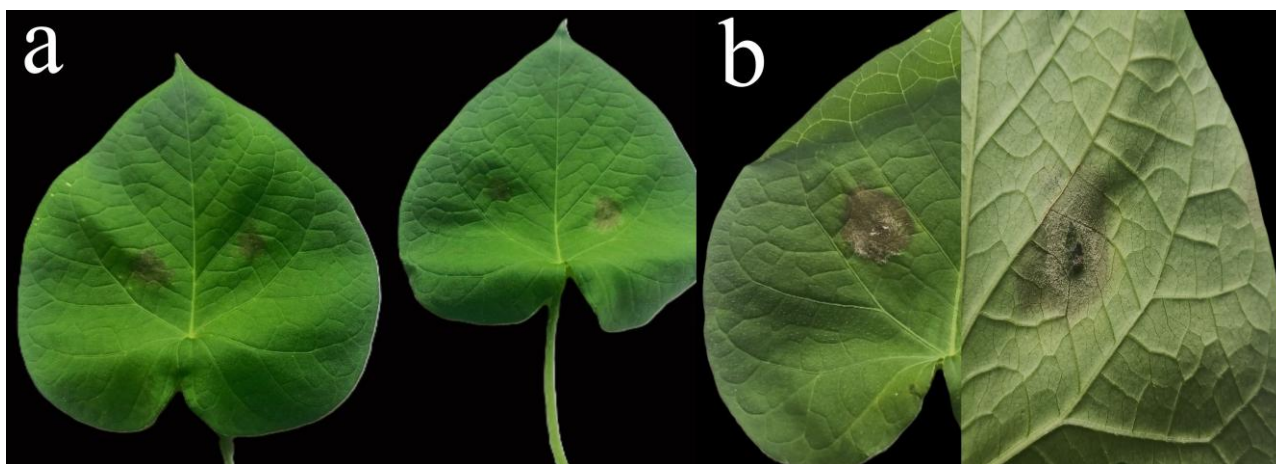


**Fig. 3** – *Alternaria alternata* isolate LMPP-2407. a Colony morphology on culture plate. b Microscopic view at 100× magnification.

The conidia were produced singly and were light brown to dark brown, short conical and obovate, and contained one to five transverse septa with ranged from 19.7–42.7  $\mu\text{m}$  long and 7.4–16.9  $\mu\text{m}$  wide  $n=25$  (Fig. 3).

### Pathogenicity

Pathogenicity tests were performed by placing 2 mm mycelial plugs from a 7 days old culture onto detached healthy leaves and inoculated at 25°C with 80% relative humidity. After 5 days, inoculated leaves developed spots identical to those observed in the field (Fig.4). The taxon was re-isolated and identified as *A. alternata* fulfilling Koch's postulates.



**Fig. 4** – Pathogenicity test showing disease symptoms on artificially inoculated host leaves. (a) Symptoms on day 4 post-inoculation, (b) symptoms on day 7 upper and lower leaf surfaces.

### Discussion

This study provides the first confirmed report of *Alternaria alternata* causing leaf spot disease on *Ipomoea purpurea* in Pakistan. The identification was based on both morphological characteristics and phylogenetic analysis, which consistently matched descriptions of *Alternaria alternata* (Simmons 2007). *Alternaria alternata* is a well-documented pathogen with a broad host range, infecting numerous economically important crops and ornamentals worldwide. Report of *A. alternata* on *Ipomoea purpurea* has previously been limited to China (Yang et al. 2018). The pathogen has also been associated with leaf spot on *Broussonetia papyrifera* in China (Ma et al. 2025) and potato in Korea (Park et al. 2024). Our findings therefore expand both the known geographic distribution and host range of *A. alternata* in South Asia.

In Pakistan, *Alternaria alternata* has been documented on other hosts, including strawberry, fig, and eggplant (Mehmood et al. 2017, Shafique et al. 2021, Anwaar et al. 2023). However, its association with leaf spot disease of *Ipomoea purpurea* has not been documented previously. The present study therefore, extends the known host range of *A. alternata* in Pakistan and provides basic data essential for future disease management and epidemiological studies in the country.

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