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FIRST REPORT OF LEAF BLIGHT OF *JASMINUM SAMBAC* CAUSED BY *BIPOLARIS AUSTRALIENSIS* IN PAKISTAN

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ABSTRACT

During disease surveys of plants in 2021, leaf blight was observed on *Jasminum sambac* plants in City Housing Society, Gujranwala, Punjab, Pakistan. Infection spots occurred on the margins of leaves in the form of large patches on both abaxial and adaxial surfaces of *J. sambac*. These spots began as small necrotic spots and developed into irregular pale brown patches with black powder covering the surfaces. The causative organism was consistently isolated from infected leaves on potato dextrose agar. Based on morphological analysis, the species isolated from *Jasminum sambac* during this research belongs to the genus *Bipolaris* and has been identified as *Bipolaris australiensis*. This fungal species has not previously been reported on this host plant worldwide, and this fungal taxon has been characterized for the first time in detail from Pakistan. To the best of our knowledge, *J. sambac* is a new host record for this fungal species in Pakistan and worldwide. A key to the identification of Pakistani species of the genus *Bipolaris* is presented in this paper.

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INTRODUCTION

Over 19,000 fungal species are considered to be the cause of diseases in economically important plants worldwide. Fungi might stay dormant on leaf surfaces, and can survive both on dead and living plant tissues until conditions are favorable for their growth (Lazarovits et al., 2014; Ahmed et al., 2018). Leaf spot, which is a widespread foliar disease in plants, is caused by a number of pathogens. Pathogens that cause leaf spot vary in severity according to the geographic location. Currently, leaf spot is acknowledged as one of the major plant diseases that limits yields globally (Boka et al., 2018; Iqbal et al., 2022; Gorea et al., 2023).

Broadleaf evergreen shrub, *Jasminum sambac* (L.) Aiton, sometimes known as Arabian jasmine, is most likely native to tropical Asia. It grows as a twining shrubby vine on a support. It develops into a spreading shrub when unsupported. Its ovate leaves measure 4.0–12.5 cm in length and 2.0–7.5 cm in width. *J. sambac* produces salver-shaped, white, tiny, waxy blooms that are typically one inch in diameter. This species can reach a height of 25 cm in its natural habitat and blooms all year long. It grows best in humus-rich, evenly moist, well drained loose soil, in partial shade to full sun and its cuttings can be propagated in the summers (Flora of China Editorial Committee, 2016).

Since the very beginning, *J. sambac* has been grown for a variety of purposes. Jasmine flowers have been used for ceremonial purposes, as religious offerings, and to scent hair oils in many Asian countries like China, India and Pakistan. Buds and flowers are used to make bouquets, garlands and veni for religious prayers. Moreover, they are employed in the manufacturing of attar and fragrant

hair oils. In China, dried flowers of Arabian jasmine are used for aroma in the tea. They are used as a saffron alternative since they also contain yellow pigments. Medicines also contain flowers and other components of this plant. It is raised in landscapes, gardens, and by companies that make lei flower necklace. It is one of the three national flowers in Indonesia. It has long been characterized as a sacred flower as it is a symbol of love, purity, and simplicity. This plant also carry the legacy of being national flower of Pakistan as well (Leonhardt and Teves, 2002; Singh, 2006; Fernando et al., 2010). *J. sambac* gives economic yield only from the third year and up to 12-15 years. A number of studies have been taken to identify the fungal pathogens affecting this host plant. But there is still a need for the precise and more accurate identification of variant fungal pathogens, day by day, affecting host plant and help breeders in evaluating potential ways for plant's security in more efficient ways.

It is estimated that significant numbers of new fungal-host associations are still unidentified and unreported. Species-level identification of fungi is essential for both fundamental (ecology, taxonomy) and practical (scientific research) purposes. Since the scientific name of a species connects knowledge about that species' biology, host range, distribution, and potential risk, accurate identification of fungal pathogens is crucial in developing effective control strategies.

To explore the prevalence of fungal diseases on *J. sambac* plants during winters, in 2021, a survey was conducted. Severe spotting and drying were noticed on *J. sambac* leaves growing in Gujranwala. The current study was conducted to analyze the leaf spot symptoms in *J. sambac* and to pinpoint the causal agent of the disease. Previously reported fungal taxa on this host are *Aithaloderma setosum* (Zimm.) Boedijn, *Aschersonia philippinensis* Petch, *Asterina spissa* Syd. & P. Syd., *Bartalinia robillardoides* Tassi, *Botryodiplodia theobromae* Pat., *Capnodium* Mont. sp., *Colletotrichum* Corda sp., *Curvularia* Boedijn sp., *Neocapnodium tanakae* (Shirai & Hara) W. Yamam., *Phyllosticta* Pers. sp., *Rhizoctonia* DC. sp., and *Uromyces hobsoni* Vize (USDA fungal-host database). From Pakistan, *Hendersonia obtusa* Cooke, *Microdiplodia jasmini* Syd. & P. Syd., and *Pleospora coronata* Niessl, are the fungal species reported on this host (Ahmad et al., 1997).

This time a new fungal species, *Bipolaris australiensis*, belonging the genus *Bipolaris*, is reported as a new fungal record for this host. The genus *Bipolaris* includes a number of significant plant pathogens with worldwide distribution. These species are commonly associated with leaf spots, leaf blights, melting outs, root rots and other disease symptoms mainly in high value field crops, including rice, maize, wheat and sorghum and on various other host plants (Ellis, 1971; Sivanesan, 1987; Berbee et al., 1999; Boka et al., 2018). Devastating diseases caused by species of *Bipolaris* on staple crops such as rice and wheat have been the cause of historical famines resulting in the starvation of large human populations in several regions in the world. The new host report of *Bipolaris* species on this economic plant is a serious threat to the plants health. It is the need of hour to save *J. sambac* from the pathogenic potential of *B. australiensis*.

Current paper also aims to provide modern description and illustration of fungal species resulting in a modern monographic treatment for the pathogen. Additionally, this research will be useful for plant taxonomists, plant quarantine officials and pathologists for identification of species together with the access to information on biology and geographic distribution. This article will led to a series of researchers interest to control the pathogenic impact of the fungal species on this economic plant by introducing new and effective fungicides in the market.

MATERIALS AND METHODS

Collection of infected plant samples

In December 2021, during a disease survey of plants in Gujranwala, Pakistan, leaf spot disease of *J. sambac* was observed. About 15-20 infected leaves were plucked from the plant, temporarily stored in sterile paper bags, labeled, and brought to the laboratory.

Isolation of the pathogen

Potato Dextrose Agar (PDA) media was used for the isolation and growth of the fungus causing leaf spot on the plant. For the preparation of media, a small amount of distilled water was used to dissolve 39 g of powdered PDA, and the final volume was raised up to 1000 ml. The mixture was then autoclaved for 15 minutes at 121ºC and 15 psi. After cooling, PDA media was poured in 3 Petri plates (25 ml in each) until 1/3 of the plates base got covered and were allowed to solidify. Surface sterilization of infected leaves was

done with 0.1 % $HgCl_2$ for 45 seconds, followed by a rinse using 70% ethanol for 30 seconds and then washing with autoclaved double distilled water for 2-3 times. Afterwards, three small pieces of the infected leaves were placed on the PDA plates. The inoculum was given at three different points on the PDA plates (at right angle to each other). The plates were then labeled, wrapped tightly with the parafilm, and incubated for 6-7 days in the dark. The incubation temperature for plates was 25°C.

Morphological and microscopic identification of the fungal pathogen

On PDA media, there was a noticeable mycelial growth after 8 days of incubation. To examine the morphology of mycelia, petri plates were carefully examined from the dorsal and ventral views of the colony. Color, shape, size, margins and structure of the colonies were noted.

For microscopic analysis, slide preparation was done using 5% KOH as mounting medium. For this purpose, 1-2 drops of 5% KOH were placed on a clean glass slide. A little amount of material from the fungal cultures was taken and dissolved well in the mounted glass slide. Glass cover-slip was placed on the material in such a way to avoid trapping of air-bubble. The slide was then observed under light microscope (Labomed) at different magnification powers of 5×, 10× and 40×. Microscopic characters i.e., appearance, color, shape, size, branching and septal behavior of conidia and conidiophores were noted down. Measurement of microscopic characters was done using ocular micrometer at 40× and length along with width of conidiophores and conidia was measured and calculated. Illustrations were made with the help of a camera Lucida. Light micrographs of microscopic characters were also captured.

The identification of the isolated fungal species was later confirmed by comparing with published literature and keys (Shamsi and Yasmin, 2009; Arvind et al., 2011; Manamgoda et al., 2014). The species identification line was retrieved from the database of "Index Fungorum" (https://www.indexfungorum.org/names/names.asp).

RESULTS

Taxonomy

***Bipolaris australiensis* (Bugnic. ex M.B. Ellis) Tsuda & Ueyama,** *Mycologia* **73**(1): 90 1981) (Figures 1-4).

Colonies show dark brownish to black surface growth on PDA, margins irregular, convex, effusive, velvety appearance from the top (obverse view), colonies are black (reverse view). **Conidiophores** septate, thin-walled, brown, branched, simple, flexuous, and geniculate, long, bears conidia sympodially, 60– 120 × 3.5–6.0 µm.

Conidia thick-walled, smooth, ellipsoidal, or oblong, fusoid to cylindrical, brown to chocolate brown, mostly 3 septate but 4 or 5 septa also present, bipolar, hilum present, 15–40 × 7–10.5 µm.

Material examined (Infected plant: *J. sambac*): Pakistan: Province Punjab, district Gujranwala, City Housing Society, Gujranwala, growing as a shrub in the garden, 20th January 2021 (Collector: Maryam Nawaz).

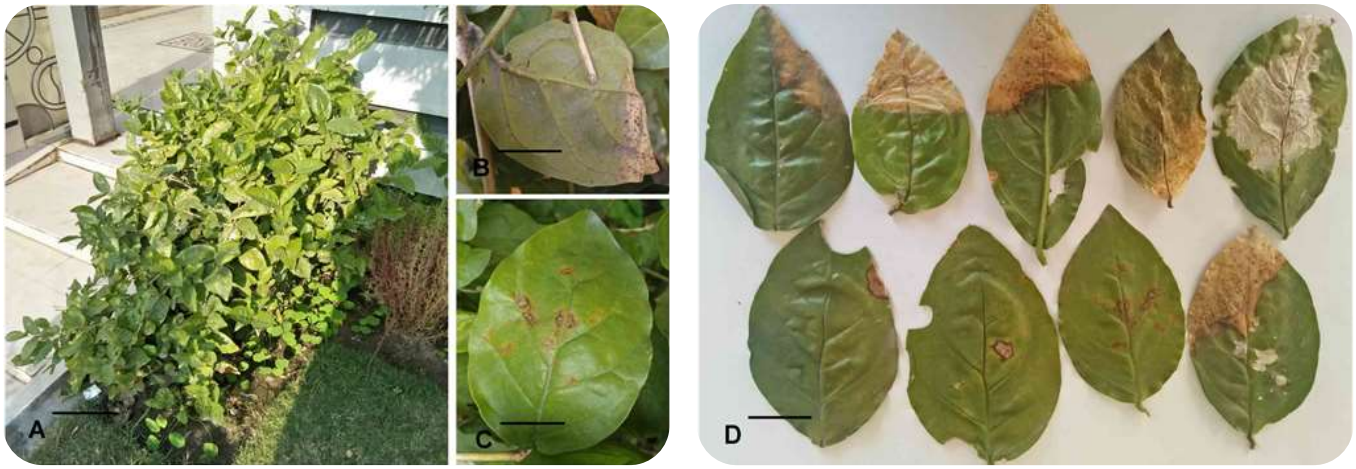

Figure 1 (A-D): Fungal infection on host plant, *Jasminum sambac.* (A). Infected host plant. (B-D). Leaves of *Jasminum sambac* showing fungal infection. Scale bars for A = 14 cm, B and C = 2.5 cm, D = 3 cm.

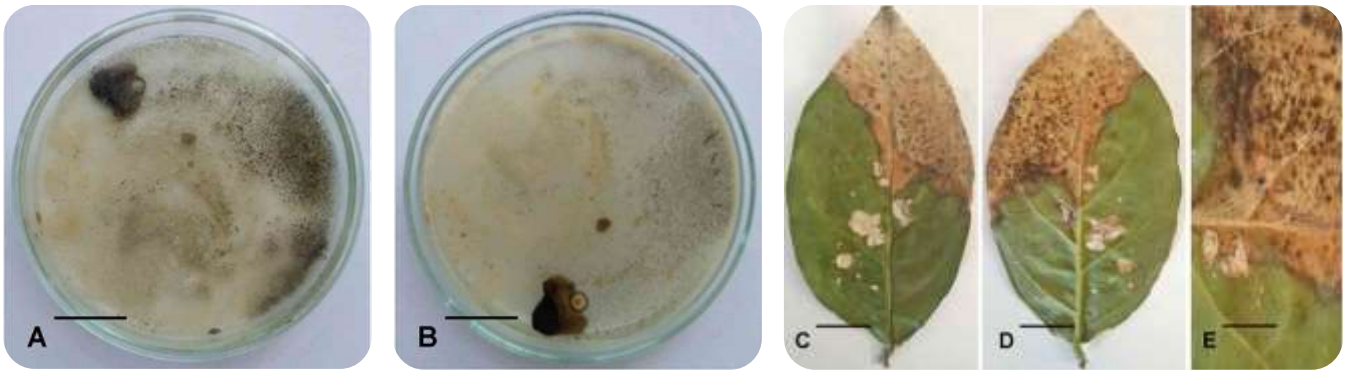

Figure 2 (A-E): *Bipolaris australiensis* colony morphology and leaf blight symptoms on *Jasminum sambac* (A and B) Pure cultures; (C-E) Largely infected leaf patches of *Jasminum sambac.* Scale bars for A and B = 2.5 cm, C and D = 1.5 cm, E = 0.7 cm.

Figure 3 (A-K): Light micrographs of microscopic features of *Bipolaris australiensis* (A-D) Conidiophores and conidia; (E-K) Multi-celled conidia. Scale bars for A and D = 0.4 µm, B and C = 0.8 µm, E = 1.2 µm, F = 1.7 µm, G and J = 2 µm, H = 2.3 µm, I and K = 2.5 µm.

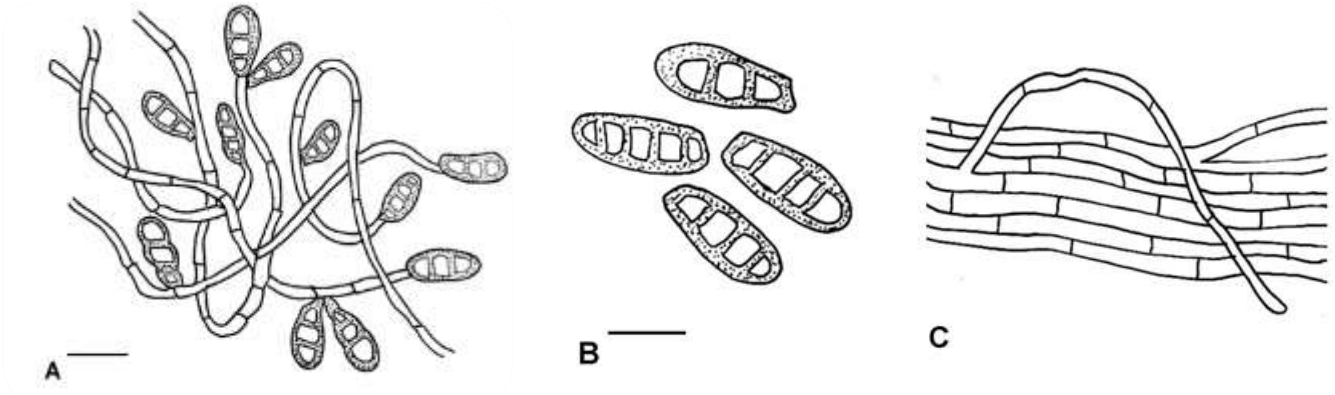

Figure 4 (A-C): Illustrations of microscopic features of *Bipolaris australiensis* (A) Conidiophores and conidia; (B) Conidia; (C) Septate hyphae. Scale bars for A = 0.3 µm, B = 1.2 µm, C = 0.7 µm.

A key to identification of Pakistani species of genus *Bipolaris*

1 Conidiophores branched ……2

1* Conidiophores unbranched or rarely branched……..3

2 Conidia 15–40 × 7–10.5 µm, brown to chocolate brown, disto-septa 3– 4 (– 5); conidiophores reddish brown, 60–120 × 3.5– 6 µm; PDA colony velvety, effusive, dark brownish to black; leaf spots black……***B. australiensis***

2* Conidia (50–)68–108(–155) × (10–)14–20(–22) µm, slightly brown, disto-septa (6–)10(–12); conidiophores brown to black, (150–)405–625 × 6–8 µm; PDA colony cottony, fluffy, white at young, slight grey at mature; leaf spots brown……..***B. oryzae***

3 Disto-septa less than or equal to 4……..***B. tetramera***

3* Disto-septa usually more than 4……4

4 Conidial maximal length not exceedingly more than 110 µm, straight or curved……5

4* Conidia maximal length not exceedingly more than 243 µm, strongly rostrate….***B. rostratum***

5 Conidiophores mid brown to olivaceous brown……..6

5* Conidiophores greyish brown…….. ***B. cookie***

6 Hilum conspicuous; conidia (59–)66–102 × (12–)14–18 (–20) µm, slightly curved, fusiform, pale to mid brown; conidiophores 51–198 × 5–9 µm causing; southern corn leaf blight, leaf lesions with purplish to brown tinge…….. ***B. maydis***

6* Hilum inconspicuous; conidia (50–)65–100(–108) × (10–)13–16 µm, straight or curved, fusoid or navicular, pale brown to mid golden brown; conidiophores (96–)145–207(–218) × 4–6 µm; causing leaf spots with white or pale grey centre…….. ***B. setariae***

DISCUSSION

Bipolaris contains significant plant pathogens with worldwide distribution. It belongs to Pleosporales of Dothideomycetes class in division Ascomycota (Rossman and Hernandez, 2008). These species are often associated with various plant diseases infecting leaves in high important crops of the Poaceae family as mentioned in introduction section (Ellis, 1971; Ou, 1985; Sivanesan, 1987; Scheffer, 1997; Berbee et al., 1999; Khan et al., 2023).

B. australiensis isolated from the leaf spot of *J. sambac*, is characterized by its dark colored, 3 or 4 celled septa, oblong spores that generally arises from basal pores along a gradually elongating hypha. The cells of the spores often appear to be very thick-walled. Its hyphae are frequently septate, long, intermingled, and brown. *B. australiensis* is a plant pathogen and known to cause various diseases in plants and humans as well (Fajolu, 2012).

World-wide host records for *B. australiensis*

B. australiensis has previously been reported on *Chloris gayana* Kunth, from Australia (Sivanesan, 1987) and Kenya (Caretta et al., 1999), on *Cymbopogon winterianus* Jowitt, *Pennisetum americanum* (L.) Leeke, *P. typhoides* (Burm.) Stapf & Hubb., *Senna angustifolia* Vahl., *Sorghum* Moench, Methodus sp. and *Zea mays* L. from India (Sivanesan, 1987; Saroj et al., 2011), on *Cynodon dactylon* (L.) Pers., *Dactylis* L. sp., *Pandanus* L. f., Suppl. sp., *Paspalum* Linn. sp., *Setaria italica* (L.) P. Beauv. and *Sorghum halepense* (L.) Pers. from Iran (Mirzaee et al., 2010; Ahmadpour et al., 2012), on *Cynodon transvaalensis* Burtt Daly, and *Oryza sativa* Linn., from Australia (Sivanesan, 1987; Matsushima, 1989), on

Cynodon dactylon-transvaalensis from China (Fang et al., 2007), on *Hordeum vulgare* L., from Libya (Sivanesan, 1987), on *Medicago laciniata* (Linn.) Mill., *Senecio mesogrammoides* O. Hoffm. and *Setaria pumila* (Poir.) Roem. & Schult. from Kenya (Caretta et al., 1999), on *Passiflora edulis* Sims. from Japan (Kobayashi, 2007), on *Quercus xalapensis* Bonpl. and in Mexico (Mena-Portales et al., 1995).

Host records for *B. australiensis* from Pakistan

From Pakistan, *B. australiensis* has been reported on *Abelmoschus esculentus* (L.) Moench, *Allium cepa* L., *Arachis hypogaea* L., *Avena sativa* L., *Beta vulgaris* L., *Brassica rapa* L., *Cannabis sativa* L., *Capsicum annuum* L., *Carica papya* L., *Cicer arietinum* L., *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cocos nucifera* L., *Coriandrum sativum* L., *Cucumis melo* L., *Cucurbita foetdissima* H.B.K., *C. pepo* L., *Cynodon dactylon*, *Gossypium arboretum* L., *Helianthus annuus* L., *Ixora* sp., *Lactuca sativa* L., *Lagenaria siceraria* (Mol.) Standl., *Luffa aegyptica* Mill., *Lycopersicon esculentum* Mill., *Mangifera indica* L., *Medicago sativa* L., *Mentha piperita* L., *Momordica charantia* L., *Musa paradisiaca* L., *Pennisetum americanum* (L.) Leve, *Petunia violacea* Lindl., *Piper betel* L., *Pisum sativum* L., *Portulaca oleracea* L., *Praecitrullus fistulosus* (Stocks) Pangal, *Raphanus sativus* L., *Saccharum officinarium* L., *Sesamum indicum* L., *Solanum melongena* L., *S. nigrum* L., *Spinacea oleracea* L., *Triticum aestivum* L., *Vigna mungo* (L.) Hepper, *V. radiata* (L.) Wilczek, from different areas of Abbotabad, Hyderabad, Islamabad, Jacobabad, Karachi, Khairpur, Lahore, Mirpurkhas, Nawabshah, Quetta, Rawalpindi, Sakrand, Sukkur, Tandojam, Thatta (Shahzad and Ghaffar, 1995; Ahmad et al., 1997; Shahzad and Zareen, 1999).

Based on the above-mentioned information that stated previous records, *J. sambac* is reported as a new host for this fungal taxon in current study.

Previous reports of *Bipolaris* species from Pakistan

Other reported species of genus *Bipolaris* includes *B. cookei* (Sacc.) Shoemaker (Manamgoda et al., 2014), *B. maydis* (Y. Nisik. & C. Miyake) Shoemaker on maize crop from Khyber Pakhtunkhwa (KPK) (Manamgoda et al., 2014; Ullah et al., 2022), *B. oryzae* (Breda de Haan) Shoemaker (Manamgoda et al., 2014), *B. rostrata* Raghv. Singh & Shambhu Kumar from Faisalabad, Islamabad, Jacobabad, Karachi, Khairpur, Lahore, Risalwala and Thatta (Shahzad and Ghaffar, 1995; Ahmad et al., 1997; Manamgoda et al., 2014; Shafique et al., 2020), *B. setariae* Shoemaker (Manamgoda et al., 2014) and *B. tetramera* (McKinney) Shoemaker from Faisalabad and Risale Wala, Punjab, Pakistan (Ahmad et al., 1997; Manamgoda et al., 2014). In the current study, Gujranwala is added as a new locality for *B. australiensis.*

CONCLUSION

In this investigation, leaf blight disease of *Jasminum sambac* was studied which was found affecting almost 25% of the leaves of an individual plant. Fungal pathogen was isolated using PDA medium and identified based on macroscopic and microscopic characters. Identification confirmed that *B. australiensis* is responsible for causing leaf blight symptoms on the host. As it was mentioned earlier that *Bipolaris* species are potential pathogenic destructors of plants and have caused major famines and crop losses in the history. Therefore, report of any plant disease being caused by *Bipolaris* species is a red flag to the health of such economic and commercially important plant that could lead to massive loss of crop if left untreated. Accurate identification of this fungal species is essential before taking into account possible control measures for disease. This study can be useful for the selection of new and efficient fungicides or introduction of new strategies to treat jasmine plants against this disease.

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AUTHORS' CONTRIBUTION

MN collected samples, isolated and identified the pathogen, wrote the manuscript, NY designed the study, helped in conducting the research work, guided and supervised all the lab work and proofread the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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