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Research Article

New record of anthracnose disease in leaves of *Plumeria pudica* in Sri Lanka

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Abstract: *Plumeria pudica* L. is a deciduous ornamental shrub that belongs to the family Apocynaceae and is widely cultivated in Sri Lanka as a cosmopolitan ornamental plant. It has been reported that the anthracnose disease affects many tropical and subtropical fruits, vegetables, and foliage plants. *Colletotrichum gloeosporioides* or C. *acutatum* were reported as pathogens presently accepted as species complexes. In January 2021, a new fungal disease was observed on *P. pudica*, within the premises of the Eastern University, Sri Lanka, Vantharumoolai, Sri Lanka. Symptoms initiated as irregular necrotic spots that enlarged with pale brown borders. Later, the spots turned grayish-black, nearly rounded without lesions and haloes on the adaxial side of the matured leaves. Diseased leaf samples (10) were collected from three different trees inside the premises. Small pieces of infected leaves were used to isolate the pathogen on Potato dextrose Agar (PDA) following the standard protocols. Forty colonies with similar morphology were obtained from diseased leaves. The colonies were white to gray and olivaceous in reverse after seven days of incubation under 27 °C. The morphological characters of fungal colonies, conidia, and appressoria were consistent with those of *Colletotrichum* sp., isolated from several other ornamental plants. Thus, based on these morphological characteristics, the pathogen was identified as *Colletotrichum* sp. Pathogenicity of the fungus was confirmed by performing Koch's postulates using healthy detached leaves of *P. pudica*. This is the first report of *Colletotrichum* sp. causing anthracnose in *P. pudica* in Sri Lanka. This finding warrants the species-level identification of the pathogen using molecular data. The present work also lays the foundation for future studies on managing the disease in *P. pudica* under nursery or marketing conditions.

Keywords: *Plumeria pudica*, Anthracnose, *Colletotrichum*, Pathogenicity.

1. Introduction

Plumeria pudica L. is a deciduous ornamental shrub belonging to the family Apocynaceae and was typically termed as bridal bouquet, an ornamental flowering plant associated with the typical frangipani. *P. pudica* belongs to the native of Columbia, Venezuela, and Panama. It has striking white flowers and a long blooming period that has ended it as a prevalent landscape plant in south Florida and the Caribbean (Suarez et al., 2017).

The pharmacological activities of *P. Pudica* were reported in various *in vivo* and *in vitro* applications, including algicidal, antibacterial, and cytotoxic activities (Choudhary et al., 2014) due to the presence of various active constituents present in latex. *P. pudica* is currently widely cultivated as a cosmopolitan ornamental plant in Sri Lanka. Although foliar diseases such as anthracnose (Ismail et al, 2021) and rust (Yang et al, 2014) have been reported from other ornamental *Plumeria* species such as *P. alba* and *P. rubra*, respectively, reports on diseases in *P. pudica* are sparse. Powdery mildew caused by *Erysiphe* sp was reported in *P. pudica* leaves in United States in 2017. (Suarez et al., 2017).

In January 2021, an unknown foliar disease was observed on *P. pudica*, within the premises of the Eastern University, Sri Lanka, Vantharumoolai, Sri Lanka. The spots were grayish-black, nearly rounded without lesions and haloes on the adaxial surface of the matured leaves (see Figure 1). No symptoms were observed on either flowers or stems. The symptoms closely resembled foliar anthracnose on *P. alba* ((Ismail et al., 2021))The incidence of diseased leaves ranged from 10% to 30%, affecting landscape aesthetic qualities. Thus, this study aimed to determine the causal agent of leaf spot disease in *P. pudica*.

2. Methodology

2.1. Isolation of the pathogen

Diseased leaves were collected from the Eastern University premises in January 2021. Small segments (5x5 mm^2) of the lesions from ten diseased leaf samples were surface sterilized by 2% (v/v) NaOCl for 1 min and then rinsed three times in sterile distilled water (Khoo et al., 2022). Cut pieces were placed on the surface of PDA and incubated at 27 °C. Forty colonies with similar morphology were recovered from 10 diseased leaves from three trees.

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Figure 1: Anthracnose symptoms on *P. pudica* leaves

2.2. Preparation of mono-conidial cultures

A suspension of 1 x 106 conidia ml-1 was prepared from two weeks old cultures. A loopful of the suspension from each isolate was streaked and spread on tap water agar plates, and the plates were incubated at 26-28 ℃ for 18 h. A single germinated conidium was located, and a small piece of agar with the germinated conidia was cut and transferred to fresh PDA plates. The plates were incubated for seven days at 28-30 ℃, and the isolates were sub-cultured on PDA (Norman et al., 2018).

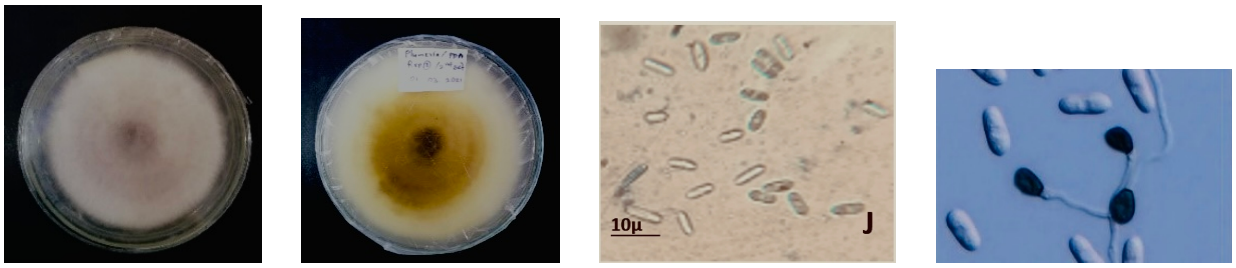
2.3. Morphological characterization of the pathogen

Two weeks old mono-conidial cultures were used to study the colony morphology, including color, texture, pigmentation underneath, the presence or absence of concentric rings, sectoring and reproductive morphology, acervuli, or the conidial masses.

Mycelial discs (4 mm diam.) were taken from actively sporulating areas near the growing edge of 7-day-old cultures and transferred to potato dextrose agar (PDA). After seven days, the size and shape of 25 conidia were recorded (Than et al., 2008). Colony diameter was measured daily for seven days, and growth rate was calculated as the 7-day average of mean daily growth (mm per day). After seven days, colony size, the color of the conidial masses, and zonation were recorded. Conidia were observed under the microscope for characteristics such as shape and color presence or absence of septa. Appressoria were produced using a slide culture technique, in which 10 mm^2 squares of PDA were placed in an empty Petri dish. The edge of the agar was inoculated with spores that were taken from a sporulating culture. A sterile cover slip was placed over the inoculated agar (Johnston and Jones, 1997). After 3-7 days, the shape and size of the appressoria formed across the underside of the coverslip were studied. Also, the lengths and widths of 50 conidia and appressoria were measured.

2.4. Pathogenicity test (Koch's postulate)

For artificial inoculation, uniform-sized, healthy leaves of *P. pudica* were used. A 1×10^6 conidia ml-1 suspension was prepared from the pure isolate cultures. Drops (20 µl) of conidial suspension were applied (6 drops per leaf) on wounded and unwounded sites of the adaxial sides of the leaves. Wounding was done superficially using a fine, sterile needle. Six replicate leaves were used for inoculation per isolate, and another three leaves treated with drops of sterile distilled water served as controls. Inoculated leaves were kept inside moisture chambers and incubated at 28 – 30 ℃, and the moisture chambers



(a) (b) (c) (d)

Figure 2: (a) Upper view of the seven days old monoconidial culture of the fungal pathogen, (b) Lower view of the seven days old monoconidial culture of the fungal pathogen, isolated from *P. pudica* on PDA, (c) Mature conidia of 7-days-old monoconidial culture, (d) Dark appressoria.

(a) (b)

Figure 3: (a) Anthracnose symptoms of *P. pudica* leaf, 7 days after inoculation with *Colletotricum* sp. (b) Upper surface views of the cultures of *Colletotrichum* sp.on PDA - re-isolated from artificially inoculated leaf of *P. pudica* (Right) and the initial monoconidial culture, isolated from naturally infected *P. pudica* leaves (Left).

were opened after 24 h. The symptoms that appeared on leaves were compared with those of the leaves used originally for isolations. The pathogens were re-isolated from the symptomatic leaves on PDA. The colonies of the fungal isolate were compared with the original isolate used for inoculation for the colony and asexual reproductive structure morphology (Adikaram and Yakandawala, 2020).

3. Results and discussions

3.1. Symptoms of Anthracnose disease

The first symptoms were small circular necrotic spots that enlarged into black lesions with pale brown borders. Later, the spots were grayish-black, nearly rounded without lesions and haloes on the adaxial surface of the matured leaves. No severe defoliation was observed due to this unknown leaf spot disease. The outer margin of the lesion was often shredded. Lesions then merged and enlarged along the leaf margin. Anthracnose lesions were not observed in younger leaves or any of the petioles.

Foliar anthracnose of ornamental plants has been recently reported from *Plumeria alba* L. (Ismail et al., 2021) and *Crinum asiaticum* (Khoo et al., 2022) in Malaysia, from *Heliconia rostrata* in Brazil (Chaves et al., 2020), and Persimmon (*Diospyros kaki* L. f.), grown in Korea (Chang et al., 2018). Anthracnose has also been reported on *Ocimum basilicum* leaves in Malaysia (Ismail et al, 2020) and on Begonia leaves in Sri Lanka (Wickramasinghe et al., 2020). Several other ornamental plants get affected by this destructive disease and put down the aesthetic values of these plants. *Sansevieria* (snake plant), a popular choice for the interior landscape, has also been reported to infect *Colletotrichum sansevieriae*, resulting in severe leaf blight (Palmateer et al., 2012). *Dracaena reflexa* L. has been observed with anthracnose in West Bengal of India (Banerjee et al., 2017). Thus, it clearly reveals that the foliage anthracnose caused by *Colletotrichum* sp. is becoming a widespread disease. Hence, there is a strong need to focus on the appropriate control measures to minimize the spread and occurrence of this disease at the initial stages of symptom development.

3.2. Morphological characteristics of the pathogen isolated

Seven days after incubation, colonies showed white-to-gray aerial mycelium with orange conidial masses in the center. The colony's lower surface was brown (see Figure 2(a) and Figure 2(b)). Conidia was falcate with a prominent area in the center with granulate cytoplasm

(see Figure 2(c)). The mycelium in the periphery was flat with dense conidia masses. Conidia were aseptate, single-celled, hyaline, smooth-walled, oblong, and measuring 13.3 to 16.2 × 3.2 to 6. 2 m, where the average of 15.3 × 4.8 m (n = 20). Appressoria ranged in size from 7.2 to 8.9 × 5.2 to 6.3 µm (n = 20) and were ovoid to clavate, spherical to irregular in shape, and dark brown (see Figure 2(d)). Morphological characteristics of the isolates were similar to those reported in the taxonomic description of *Colletotrichum* sp. (Prihastuti et al.2009).

Based on these morphological characteristics, the fungus was identified as *Colletotrichum* sp. The pathogenicity of isolates was also verified using five healthy detached leaves of *P. pudica*.

3.3. Pathogenicity test of Colletotrichum sp.

The pathogen with oblong conidia, subsequently identified as *Colletotrichum* sp., was re-isolated from diseased leaves of *P. pudica*, indicating its consistent presence in lesions and grown on fresh PDA as pure cultures. Wounded *P. pudica* leaves artificially inoculated with *Colletotrichum* sp. developed dark brown color and irregular, necrotic lesions typical to anthracnose seven days after inoculation, as observed in the naturally infected symptoms, collected from the field (Figure 3(a)). The leaves that were inoculated with the fungal conidia on unwounded tissue initiated anthracnose symptoms 3 - 4 days after inoculation and took a few more days for expansion than those in wound-inoculated leaves. The lesion development by *Colletotrichum* sp. was much slower. There were no symptoms observed in the control leaves. The culture plates prepared by re-isolation were morphologically similar to the cultures used for inoculation (Figure 3(b)).

Plant pathogens may highly affect a country's economy by reducing the yields and lowering the quality of plant products. Also, global plant and fresh produce trade can introduce new pathogens from one country to another (Hyde et al., 2018). An up-to-date understanding of these plant-pathogen interactions will ensure the safety of crops from prevailing diseases and exotic plant pathogens (Jayawardena et al., 2016).

4. Conclusion

Based on morphological features of the colony, conidia, and appresoria and also from the pathogenicity tests, the fungal pathogen isolated from anthracnose lesions in the leaves of *P. pudica* was identified as *Colletotrichum* sp. According to the literature, this is the first report of foliar anthracnose disease of *P. pudica* in Sri Lanka. This finding warrants the species-level identification of the pathogen using molecular data. The present work lays the foundation for future studies related to the management of this disease under nursery or marketing conditions.

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