

First report of leaf blight disease of *Livistona chinensis* caused by *Alternaria alternata* in Pakistan

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*Equal contribution; Hanyue Zhu and Tanvir Ahmad contributed equally.
Funding: The National Key Research and Development Program of China (2019YFC1604502).

Livistona chinensis (Jacq.) is also known as fan palm and is commonly grown in the subtropical region of the world. This plant is widely cultivated in Asia for ornamental purpose and also used in Chinese medicines (Li et al. 2019). In May 2021, severe leaf blight was observed on *L. chinensis* leaves in ornamental plant nurseries, located at Pattoki (30°59'41.5"N 73°48'43.8"E) District Kasur, Punjab province, Pakistan. The disease incidence was up to 50% and the initial symptoms appeared as chlorotic brown spots on the upper portion of leaves. Later, the spots expanded and changed into elliptical lesions on the leaves. The lesions with dark brown margins coalesced to cause extensive tissue necrosis of leaves and exhibited blight (Figure 1). Two to three leaves were taken from each infected plant. Infected leaves of each sample of *L. chinensis* were excised into small pieces (3-4 mm) with the help of sterilized scissor and surface disinfected with 1% NaClO for 20s and rinsed 3 times with sterilized distilled water. To isolate the potential causal organism, these pieces were plated on Potato Dextrose Agar (PDA) medium and incubated at 28 °C with 70 % relative humidity for 7 days. Purified cultures were obtained through single spore culture on PDA. All obtained isolates were preserved in 30% glycerol at -80°C. The fungal colony colour was olive to dark greenish and dark brown to black on the reverse side. The conidia (n=36 per isolate) were greenish to brown in colour, ellipsoid to obclavate, ovoid, irregular and measured an average range from 10.9 to 30.7 µm long x 6.3 to 12.5 µm wide with 2 to 5 transverse and 0 to 3 longitudinal septa (Figure 2). The genomic DNA was extracted from all isolates (n=40) and multi-locus sequence analysis approach was used for molecular identification. The Internal Transcribed Spacers (ITS) region, Alt a1 major allergen (ALT) gene, glyceraldehyde-3-phosphate dehydrogenase (GPD), actin (ACT) gene and histone 3 genes were amplified using ITS1/4 (White et al. 1990), Alt-4for/Alt-4rev

(Lawrence et al. 2013), GPD1/GPD2 (Guerber et al. 2003), ACT512F/ACT783R (Carbone and Kohn, 1999) and H3-1a/H3-1b (Luan et al. 2007). Based on morphological and molecular characteristics, all isolates were identified as *Alternaria alternata*. The sequences of the representative isolate APLB-3 were submitted in the GenBank with the accession numbers (ITS: MZ663802), (ALT: MZ666883), (ACT: MZ666885), (GPD: MZ666884), and (Histone3: MZ666886) showing 100% similarity with ITS accession MK968038, ALT accession MN702781, ACT accession MT318253, GPD accession MT524743 and histone 3 accession MH824369. For pathogenicity test, potted *L. chinensis* plants (n=9) leaves were pin-pricked with sterilized needle (Bajwa et al. 2010) and inoculated with spore suspensions (10^7 spore/ml) of APLB-3 (1ml/leaf) to confirm Koch's postulates. After 14 days, the inoculated leaves showed chlorotic brown spots and leaf blight symptoms similar to those observed on infected plants in nurseries. The plants grown as the control group (n=9) were sprayed with sterilized distilled water and had no symptoms (Figure 3). The experiment was performed three times. The fungal pathogen was re-isolated from the artificial inoculated leaf tissues and identified as *A. alternata* based on morphological and molecular characterization. To our knowledge, this is the first record of *A. alternata* causing leaf blight disease of *L. chinensis* in Pakistan. This disease may potentially decrease the value of ornamental plants in Pakistan under favourable conditions and proper management strategies should be applied.

Reference:

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Figure 1. Leaf blight disease symptoms on *L. chinensis* plant’s leaves collected from nurseries.

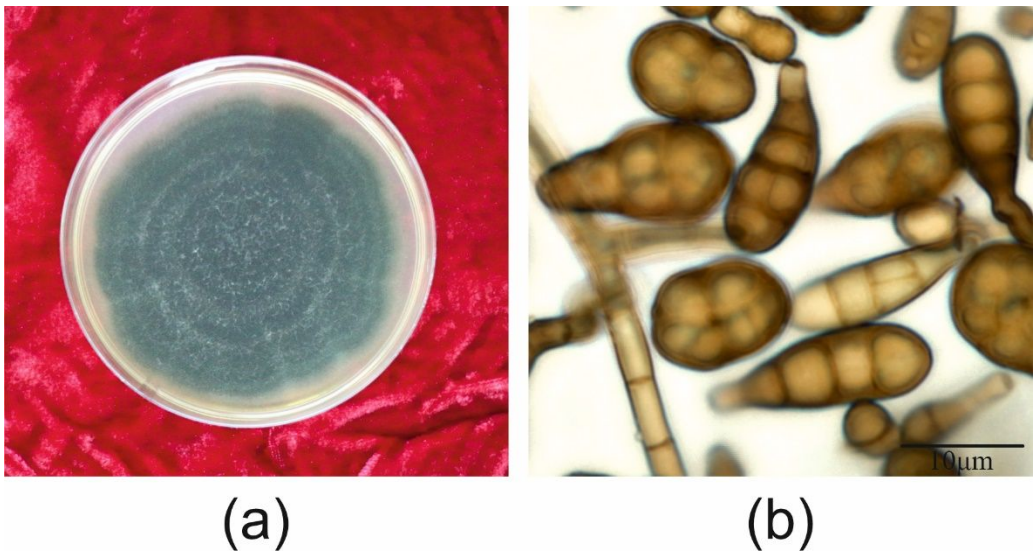


Figure 2. Morphological features of *A. alternata* isolated from *L. chinensis* (a) fungal culture on PDA medium at 28 °C (b) conidia of *A. alternata* (scale bar = 10 μm).

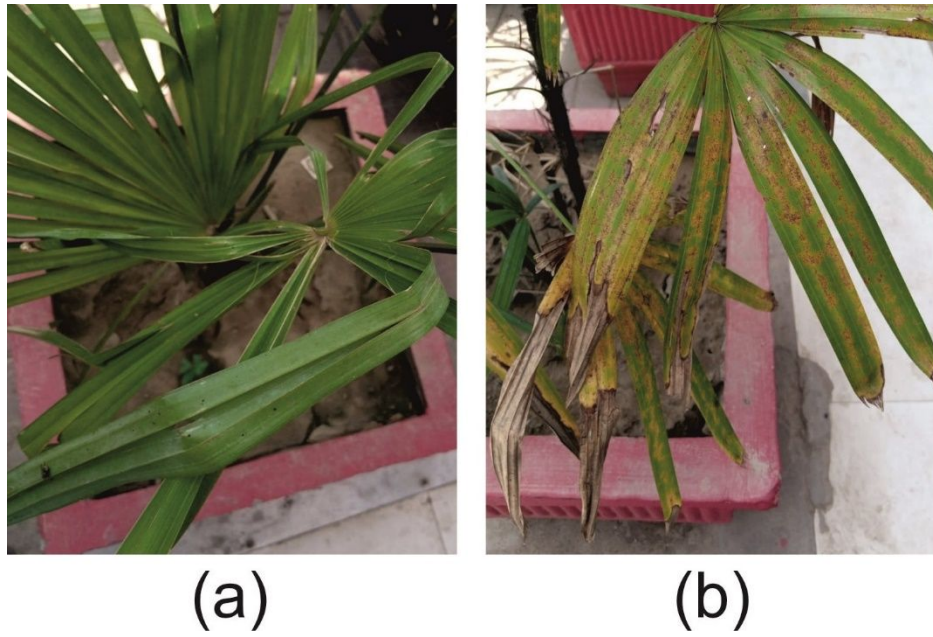


Figure 3. Pathogenicity of *A. alternata* on healthy *L. chinensis* plants (a) Control treatment (b) Inoculated *L. chinensis* with APLB-3 (10^7 spores/mL) suspension.