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Alternaria alternata causes leaf blight of rosy periwinkle (*Catharanthus roseus*) in Iraq

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Abstract
Nursery-grown rosy periwinkles (*Catharanthus roseus*) in Kerbala province, Iraq, showed severe symptoms of leaf blight. The causal agent associated with the diseased plants was identified as the fungus *Alternaria alternata*. This identification was based on the pathogen's morphological, molecular and pathogenic characteristics. To the best of the author's knowledge, leaf blight of rosy periwinkle caused by *A. alternata* is the first record in Iraq.

Keywords *Alternaria alternata* · Leaf blight · *Catharanthus roseus* · rDNA-ITS · Iraq

Catharanthus roseus, known as rosy periwinkle or Madagascar periwinkle, is an annual or perennial evergreen herbal plant that is native from Madagascar (Stearn 1975). Nowadays, it is cultivated globally, including in Iraq, for its therapeutic and ornamental attractive features. It has an ornamental aesthetic appearance related to its green variegation and prolific flowers in splendid colors (Nejat et al. 2015). The plant is also a source of pharmaceutical compounds such as terpenoid indole alkaloids including vincristine and vinblastine that have valuable antitumor properties (Aslam et al. 2010).

Several phytopathogens have been reported to cause a variety of diseases on periwinkle plants worldwide. The majority of the fungal diseases recorded were root rot and damping-off incited by numerous fungi such as *Rhizoctonia solani, Fusarium oxysporum, F. solani, Thielaviopsis* sp., *Sclerotinia sclerotiorum, Sclerotium rolfsii* and *Pythium aphanidermatum* and foliar blight caused by *Phytophthora parasitica, P. tropicalis, Colletotrichum dematium* and *Botrytis cinerea* (Nejat et al. 2015; Ou-Yang and Wu 1998). However, there are no published studies relating to pathogens infecting periwinkle plants in Iraq.

During the growing season 2015/ 2016, severe symptoms of foliar blight were observed on rosy periwinkle in one of the main ornamental nurseries in Kerbala province, Iraq. The symptoms initiated as small circular to irregular light brown spots that gradually enlarged and darkened to cover most of the leaf surface. The diseased leaves ultimately became withered and dry leading to the death of the plant.

Representative symptomatic leaves of rosy periwinkle plants were collected, dissected, surface sterilised (2% sodium hypochlorite solution) and placed on water agar (WA). After two days of incubation at 25 °C in darkness, a hyphal tip of each emerging colony was sub-cultured on potato dextrose agar (PDA). Nine single-hyphal-tipped fungal isolates were recovered from the diseased leaf samples. Morphological examinations of these isolates showed that the colonies grown on PDA were white in the beginning, converting to brownish olivaceous in the later stage of growing (Fig. 1a). The reverse surface of the colony was dark brown to black. Conidiophores were usually short being either ramified or simple, mostly curved measuring 13–15 μm long and 3–6 μm wide. Conidia (Fig. 1b) produced usually in long branched strings in diverse shapes; oval, obclavate, obpyriform or ellipsoidal with average size 20–30 μm long and 15–18 μm wide. They possessed two types of septa; transverse (1–5) and longitudinal (0–2), with a short apical beak shaped either conical or cylindrical and a prominent basal hole. The morphological features of all nine isolates were similar and agreed with those of *Alternaria alternata* described by Simmons (2007). One representative isolate of the fungus *A. alternata* was deposited in the microorganisms culture collection of phytopathogens and biological agents, University of Kerbala, Kerbala, Iraq with the accession No. IRAQAA 00025P.

To confirm this identification, one of the representative isolates was selected for amplifying and sequencing of the

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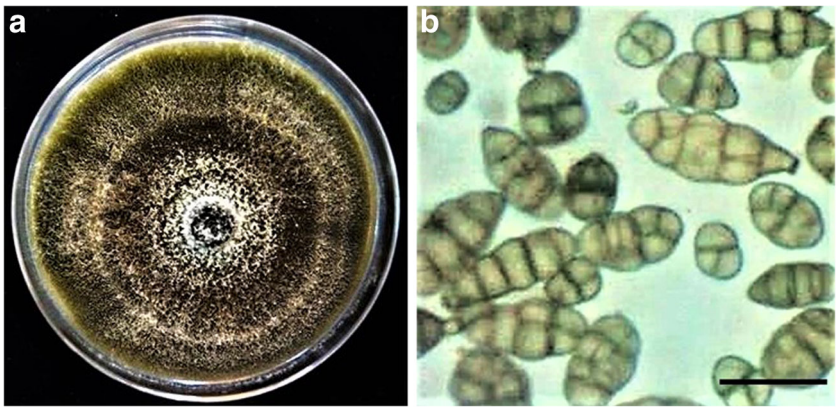

Fig. 1 Morphological characteristics of *Alternaria alternata* (**a**) pure colony (**b**) diverse shapes and sizes of conidia. Scale bar =20 μm

ribosomal DNA internal transcribed spacer region (rDNA-ITS) utilising the ITS1 and ITS4 primers (White et al. 1990). The sequence obtained (GenBank Accession No. MF099863) was highly similar to those of *A. alternata* isolates recorded in GenBank database. Accordingly, the phylogenetic analysis using MEGA7 with neighbour-joining method showed that it respectively assembled with numerous reference of *A. alternata* strains (Fig. 2).

Pathogenicity of all nine *A. alternata* isolates were assessed using the detached leaf assay (Akhtar et al. 2011). Healthy looking leaves of rosy periwinkle were surface sterilised using 70% ethanol and placed into transparent plastic boxes on moist tissues. A drop of conidial suspension (50-μl of 2000 conidia/ml concentration), harvested from 7 day old pure cultures of each isolate, were placed on the sterilised leaves while others were left without inoculation for control. All boxes were closed firmly, incubated at 25 °C in darkness and monitored daily until blight symptoms were seen, typically 10–14 days after inoculation (Fig. 3). However, no symptoms were seen on leaves of the control. To accomplish Koch's postulates, pieces of diseased leaves collected from the edge of blight lesion and healthy tissue were surface sterilised and

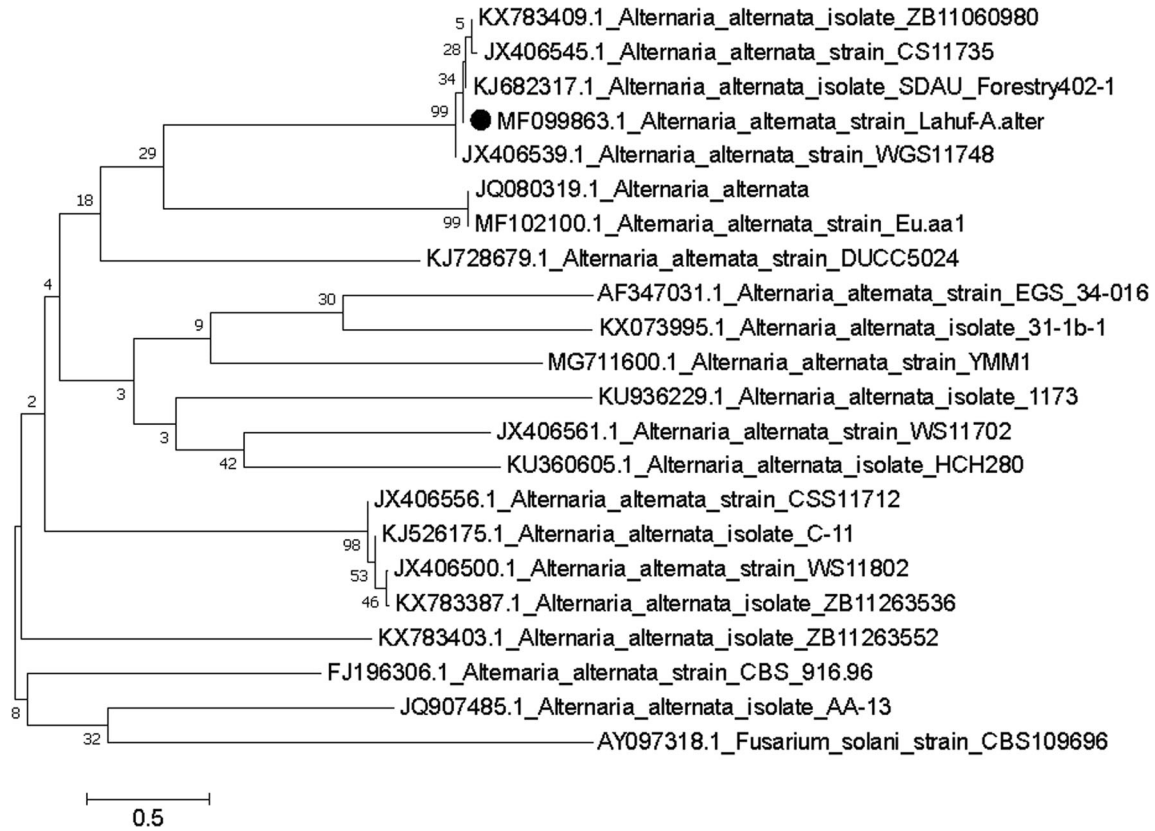

Fig. 2 Phylogenetic analysis of the fungus isolated from the diseased rosy periwinkle plant (MF099863.1; indicated with a black dot) with different strains of *A. alternata* depending on the alignment of the nucleotide sequences using MEGA7.0 applying neighbour-joining tree process. The out-group of this tree was *Fusarium solani* strain AY097318.1

Fig. 3 Detached leaf assay with pathogenic *A. alternata* inoculation, (left) symptoms of leaf blight on rosy periwinkles leaf (right) control leaf

placed on PDA as mentioned above. The fungus was re-isolated consistently from all inoculated leaves with morphological characterisations identical to those described above.

The fungal pathogen isolated from symptomatic leaves of rosy periwinkle was characterised to species level based on its morphological and molecular characteristics, which were similar to those of *A. alternata.*

Alternaria alternata has been reported as a fungus causing various diseases mainly leaf spot and blight on a wide range of plant hosts. It was, for example, reported as the cause of leaf spot of *Musa* spp., *Actinidia deliciosa*, *Rumex vesicarius* and *Juglans regia* (Parkunan et al. 2013; Corazza et al. 1999; Sankar et al. 2012; Belisario et al. 1999) and blight of *Zinnia acerosa*, *Artemisia annua*, *Incarvillea emodi* and *Zanthoxylum piperitum* (Colbaugh et al. 2001; Samanta et al. 2009; Shanmugam et al. 2011; Yang et al. 2013). Additionally, *A. alternata* was reported as an endophytic fungus on *Catharanthus roseus* (Kharwar et al. 2008; Momsia and Momsia 2013; Palem et al. 2015). However, there is no previous report relating this pathogen to disease on rosy periwinkle plants in Iraq. Hence, this is the first report of *A. alternata* causing leaf blight of rosy periwinkle.

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