

DISEASE NOTE

FIRST REPORT OF *ALTERNARIA ALTERNATA* CAUSING LEAF SPOT OF *POLYALTHIA LONGIFOLIA* IN PAKISTAN

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In October 2014, plants of *Polyalthia longifolia*, an ornamental growing on the premises of University of the Punjab, Lahore were observed, that showed brown circular spots 1 to 2 mm in size covering 30 to 40% of the leaf blade. Small tissue pieces from a number of spots were surface-sterilized, plated in malt extract agar (MEA) and incubated at 25 ± 2°C. Morphological observations were made on 7-day-old cultures. Fungal colonies, deposited in the First Fungal Culture Bank of Pakistan (FCBP1518), were olive-green and did not show exudates or zonation. Conidia were olive brown in colour, arranged in long chains, had prominently geniculate beaks with protuberant scars. Some were 4-celled, 10-15 × 20-30 µm in size, but the majority were 6-celled, ellipsoidal and up to 10-15 × 40-50 µm in size. These traits concur with those of *Alternaria alternata* (Simmons, 2007). Amplification of the ITS region of ribosomal RNA (White *et al.*, 1990) yielded a product (GenBank accession No. KT283682) with 100% similarity to comparable sequences of several *A. alternata* isolates: FL7-PL4 (KP900243.1), EIODSF001 (KJ173524.1), KSH53T (KF380814.1). Pathogenicity tests were performed three times by inoculating autoclaved sterilized soil with 2 × 10⁵ spores of three different isolates of the pathogen separately. Leaves were also sprayed with the same spore inoculum. Similar necrotic spots appeared 15 days post inoculation on the plants that were inoculated through the soil and after 21 days on those that were sprayed with spore suspension. Control plants remained asymptomatic. The fungus was successfully isolated from symptomatic plants, fulfilling Koch's postulates. To the best of our knowledge, this is the first report of *P. longifolia* leaf spot by *A. alternata* from Pakistan.

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FIRST REPORT OF *ZUCCHINI YELLOW MOSAIC VIRUS* ON *CUCURBITA MOSCHATA* IN INDIA

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Zucchini yellow mosaic virus (ZYMV), genus *Potyvirus*, infects and causes severe yield losses to cucurbits, including *Cucurbita moschata*, which is widely grown and consumed in the Indian subcontinent. During 2012-2014, while conducting survey for virus diseases of cucurbits in Tamil Nadu, plants exhibiting mosaic and malformation of leaves and fruits recalling symptoms induced by ZYMV were observed in pumpkin crops at Udumalpet (Tiruppur, isolate TN UDU PUM1) and Anamalai (Coimbatore, isolate TN ANA PUM1) with incidence ranging from 40 to 70%. Ten symptomatic samples (five each from Udumalpet and Anamalai) selected randomly were tested by DAS-ELISA for the presence of ZYMV using a commercial kit (DSMZ, Germany) and ZYMV-specific immunostrips (Agdia, USA). ZYMV was detected only in symptomatic plants. Likewise, RT-PCR using the primer pair (GK ZYMV F/R2) constructed on the coat protein sequence of the virus (Nagendran *et al.*, 2015) amplified a product of ca. 1,000 bp only from symptomatic samples, which was cloned and sequenced in both orientations. Multiple alignment of the nucleotide sequences of both viral isolates (KJ866937 and KJ729043) with comparable sequences from GenBank revealed 98-99% identity with ZYMV isolates from India (HQ529776, KJ866939, KJ729041, KJ729044, JF797206), Iran (JN183062), Israel (EF062582), Syria (KF056805) and Germany (AJ420019) confirming the presence of this virus in symptomatic pumpkin plants. The occurrence of ZYMV in pumpkin has been reported from Germany, Serbia, Korea and Australia (Vučurović *et al.* 2012), and in cucumber, gherkins, snake gourd, bottle gourd, zucchini and *Amaranthus viridis* in India (Nagendran *et al.*, 2015). This is then the first report of ZYMV occurrence in pumpkin in India.

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