

Original Research Article

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First Report of *Alternaria alternata* (Fr.) Keissler Causing Leaf Blight on *Anthurium andraeanum* in India

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ABSTRACT

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Leaf blight was observed in anthurium crop in Tamil Nadu and Kerala states of India during 2011-2012. Initially, the symptoms were small circular to irregular brown spots with concentric rings on the leaves that gradually enlarged. Fungal isolates, obtained from infected leaf tissues were grown in pure culture and on the basis of cultural and morphological characteristics and ribosomal DNA spacer sequences, the organism was identified as *Alternaria alternata* (Fr.) Keissler. To our knowledge this is the first report of *Alternaria* leaf blight disease in *Anthurium andraeanum* in India.

Introduction

Anthurium is an important cutflower crop which draws the attention of floriculturists in recent years and cutflower industry in India is fast expanding. Anthurium ranks eleventh in the global flower trade and commands a respectable price both for its cut flower and whole plant. Presently the Netherlands is the world's leading producer and exporter followed by Mauritius where it is also the national flower. In India, the anthurium cut flower industry is still in its infancy. The perusal of literature indicated that there have been a few reports on occurrence of fungal, bacterial and viral diseases on this crop. However, the occurrence of leaf blight disease

was not mentioned in the literature. Taking clue from these, the roving survey was conducted in few major growing areas of Tamil Nadu and Kerala. In few isolated pockets, occurrence of unusual leaf blight with concentric rings symptom was observed. The disease was found to hamper the keeping quality of flower. With these back drop, this study was planned to characterize pathogen associated with the melody.

Materials and Methods

Survey and collection of diseased samples

Survey was conducted during 2010-2011 to assess the severity of leaf blight and

anthracnose in different anthurium growing areas of Tamil Nadu and Kerala. Disease severity was recorded in 0-9 scale (Anonymous, 1980) and per cent disease index (PDI) was calculated. Infected samples of leaves were collected from these areas.

Isolation and identification of pathogens

The pathogens causing leaf blight in anthurium were isolated from the collected samples by tissue segment method (Rangaswami, 1958) and they were purified by single spore isolation and maintained on potato dextrose agar (PDA). The causal organisms were identified based on spore morphology. Identification of *A. alternata* being the first report on anthurium was further confirmed (ID.NO. 8580.12) by Indian Type Culture Collection Centre (ITCCC) of Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi. The identification of leaf blight pathogen was further confirmed using the ITS primer (Jung *et al.*, 2002). Genomic DNA was extracted from a suspension culture of *A. alternata* by the cetyltrimethyl ammonium bromide (CTAB) method as described by Knapp and Chandlee (1996). Specific primers for 18S rDNA, ITS-F (5'- GTCCTAACAAG GTTCCGTA-3'; AJ297952) and ITS-R (5'- TTCTCCGCTTATTGATATGC-3'; AJ297953) were used to amplify an ~ 650bp PCR amplicon of ITS region.

Pathogenicity test

The pathogenicity of purified cultures of *A. alternata* was confirmed by Koch's Postulates. Sporulating cultures of *A. alternata* were inoculated on PDA and incubated at room temperature ($28 \pm 2^\circ\text{C}$) to attain full growth. After incubation, the dishes were flooded with 10 ml of distilled water and the spores were collected using a small brush. The spore suspension was filtered through a six-layer-sterile cheese cloth to remove mycelial debris.

Using a haemocytometer spore concentration of *A. alternata* was adjusted to 6×10^4 spores ml^{-1} respectively with distilled water. Single leaf inoculation technique was followed and the spore suspension was sprayed using a syringe until run-off on to the leaves of anthurium raised in glasshouse. Such single leaves were covered with polythene bags and symptom expression was observed regularly. Proper controls were also maintained.

Results and Discussion

Survey, collection and assessment of PDI

A Survey was conducted during 2010-2011 at five locations in Tamil Nadu and two places at Kerala to assess the intensity of leaf blight in anthurium. The leaf blight severity as per cent disease index (PDI) ranged from 12.20 to 56.34. The highest incidence of leaf blight was found in Coimbatore district of Tamil Nadu (56.34 PDI) followed by Wynad district of Kerala (44.33 PDI). The lowest incidence of leaf blight was observed in Thadiyankudisai, Dindugal district of Tamil Nadu (12.20 PDI).

Symptomatology

Symptoms were observed only on leaves and not on any other parts of plants. Initially small and brown spots were observed on leaves. Later, these spots were circular to irregular and brown to black with concentric rings and marginal blight with concentric rings (Fig. 1a and 1b).

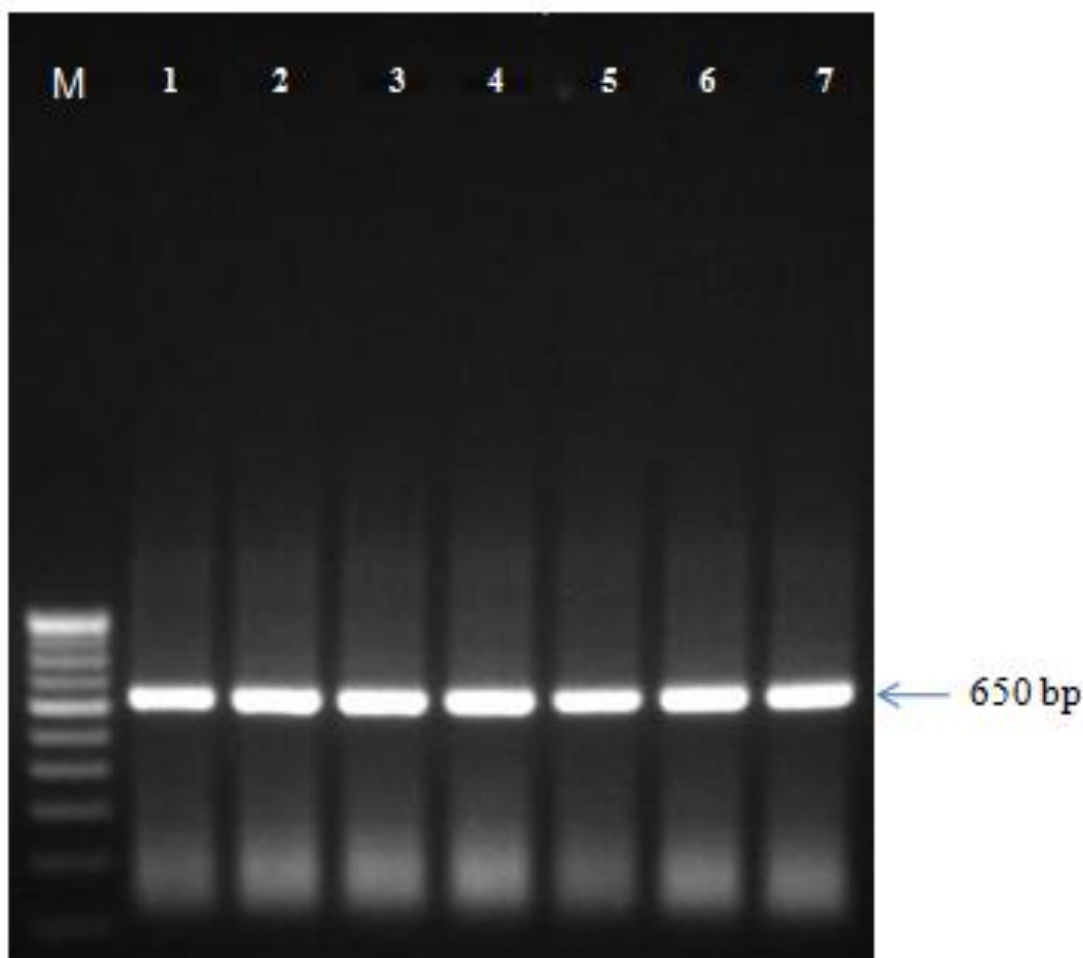
Isolation and identification of the pathogen

A total of ten times the isolation was repeated to confirm the association of pathogen with symptom. The causal organism was identified based on spore characters and colony morphology and they were purified by single spore isolation and maintained on PDA.

Fig.1 Symptoms of leaf blight disease of anthurium and morphology of *Alternaria alternata* and morphology of *A. alternata* a brown spots with concentric rings b marginal blight with concentric rings c colony of *A. alternata* on PDA d conidial mass of *A. alternata* e single conidia of *A. alternata* f healthy anthurium plant g leaf blight of anthurium 14 day after inoculation with *A. alternata*



Fig.2 PCR amplification of ITS region of *Alternaria alternata* M 100 bp DNA ladder; Lanes 1-7, isolates of *A. alternata*



The fungus produced flat, downy to woolly and grey colonies with olive green peripheries. The *Alternaria* grew well on PDA and formed an olivaceous black colony with dark olive margins, reverse black at $28 \pm 2^{\circ}\text{C}$ after 10 days. The conidiophores were branched, straight pale brown to olive brown in colour. The conidia were pale brown to light brown in colour (Fig. 1c, 1d, and 1f). Based on the characters, the organism was identified as *Alternaria alternata* (Fr.) Keissler and further, the identification was confirmed by the Indian Type Culture Collection Centre, IARI, New Delhi, India (ID.NO. 8580.12). The ITS region of

Alternaria was amplified with the *Alternaria* 18S rDNA specific primers ITS-F and ITS-R to get 650 bp PCR amplicon of ITS regions. All the seven isolates of *Alternaria* used in the study showed amplified product of the size 650 bp (Fig. 2).

Pathogenicity

Pathogenicity was performed by spraying leaves of 9 healthy 3 month-old potted *A. andraeanum* plants with a spore suspension of 6×10^4 spores per ml. Control plants were sprayed with sterile water. Plants were covered with polythene bags for 2 weeks and

kept on the glass house at $30 \pm 20^{\circ}\text{C}$. After 2 weeks typical symptoms were produced on the inoculated leaves (Fig. 1f and 1g). Koch's postulates were fulfilled by consistently reisolating *A. alternata* from the lesions, where the control plants were remained symptomless. Inoculations were repeated twice with the same results. According to our studying thesis the first report of a disease caused by *A. alternata* on anthurium in India and we propose the name leaf blight.

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