First report of *Athelia rolfsii* causing stem rot disease on Vasaka (*Justicia adhatoda*)

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Abstract

Athelia rolfsii is a facultative plant pathogen which develops characteristic white mycelial mats and sclerotia at the crown infection sites when conditions are favourable. In July 2019, stem rot symptoms such as dark brown necrotic lesions were observed at collar of the stem base. The infected plants showed dark brown symptoms which later turned into light white mycelial growth with immature white sclerotia, while the isolated fungus was initially white in colour and later turned black. The identification of the isolate as *Athelia rolfsii* was also confirmed by using internal transcribed spacer region sequencing. To the best of our knowledge, this is the first report of stem rot of *Justicia adhatoda* caused by *Athelia rolfsii* in the world.

Keywords Stem rot · *Athelia rolfsii* · *Justicia adhatoda* · Sclerotia · Pathogenicity

Vasaka or Malabar nut (*Justicia adhatoda*), family Acanthaceae, is a shrub found throughout the tropical regions of Southeast Asia (Chakrabarty and Brantner 2001). It is a perennial, evergreen and highly branched shrub with an unpleasant smell and bitter taste. It is a highly valuable Ayurvedic medicinal plant used to treat cold, cough, asthma and tuberculosis (Dhankhar et al. 2011). In July 2019, a Vasaka plant was observed showing signs of stem rot disease in the research field, College of Agriculture, Lembucherra, Agartala, Tripura (India) with disease incidence ranging from 3 to 6%. In the early stage of disease, the symptoms of dark brown necrotic lesions first appeared on the stem base (Fig. 1). In the later stage, light white mycelial growth with immature white sclerotia was observed on the outer surface collar region. Mycelia and immature sclerotia were collected from the collar region of the infected plant for isolation and pathological studies. Collected plant samples were first surface sterilised in 0.25% v/v sodium hypochlorite for 1 min, washed thrice with sterilised distilled water, air dried and placed in Petri

plates containing potato dextrose agar (PDA) media and incubated at 25 °C. After 1 week of incubation, mycelia and sclerotia were formed on PDA and the mycelium was transferred onto fresh PDA plates to obtain pure culture. Pure cultures with abundant aerial and white mycelia formed typical clamp connections during microscopic observation. Initially the sclerotia were white in colour but later turned brown in colour with size of 0.8 × 1.2 mm in diameter (Fig. 2). Initial growth of sclerotia took 11 days on PDA but its complete maturation took place at 14 day approximately and sclerotia scattered throughout the Petri plate. Based on above cultural and morphological characteristics, the pathogen was identified as *Athelia rolfsii* (Aycock 1966). The established culture was deposited in the Indian Type Culture Collection, Division of Plant Pathology, ICAR - Indian Agricultural Research Institute, New Delhi, India (ITCC-8664). Morphological identification was further confirmed by molecular characterisation. Pure fungal sclerotia were transferred into flasks containing potato dextrose broth media for mycelia mat preparation and kept at 25 °C. The genomic DNA was extracted from a mycelia mat of *A. rolfsii* using Zymo Research fungal/bacterial DNA miniprep kit (Irvine, CA) and the internal transcribed spacer (ITS) region of rDNA was amplified using universal primers ITS1 and ITS4 (White et al. 1990). A PCR product of 650 bp was amplified, sequenced and deposited on GenBank (MT119282). The sequence showed 99% similarity with *Athelia rolfsii* strain CBS 115.22 GenBank accession MH854711. The phylogenetic

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Fig. 1 Sign on collar region of Vasaka plant infected by *A. rolfsii*

Fig. 2 Morphological features of the pathogen: **a** Mycelia growth **b** Initial white colour sclerotia formation **c** Formation of mature sclerotia after 14 days of inoculation (light brown colour)

tree were constructed using partial sequences of ITS region of rDNA using maximum likelihood method with 1000 bootstrap value and Tamura-Nei nucleotide substitution model. The tree was constructed by using MEGA 6 software (Tamura et al. 2013). DNA sequence obtained from infected plants was closely grouped with sequence of *A. rolfsii* strain CBS 115.22 (MH854711), with a bootstrap value of 96% compared to several members of the clade (Fig. 3). For the pathogenicity test, healthy detached leaves and stems of *Justicia adhatoda* were collected and single mature sclerotia were inoculated on the detached leaf and

stem and placed in the moist chamber with controlled temperature (27 °C) and humidity (95%). Hyphae was observed from 2 days post-inoculation onward and sclerotia were formed after 11 days post-inoculation (Fig. 4). The fungus was re-isolated from inoculated plant samples and transferred onto a PDA Petri dish and identity was confirmed based on morphological characteristics, that is colony growth, mycelia mat colour, sclerotia colour and size and presence of clamp connections in mycelium. To the best of our knowledge, this is the first report of stem rot of Vasaka caused by *A. rolfsii* in India.

Fig. 3 Phylogenetic tree inferred from internal transcribed spacer (ITS) sequences using the Maximum Likelihood method, obtained from one sequence of *A. rolfsii* (isolate 8664), one CBS strain 115.22 (MH854711) and three other *A. rolfsii* sequences retrieved from NCBI GenBank along

with one sequence of *S. Delphinii* (MH856163) and one sequence of *Rhizoctonia solani* (MH863394) as outgroups. ClustalW alignment function was used to align the sequences. Bootstrap values are shown next to the branches. GenBank accession numbers are indicated in parentheses

Fig. 4 Pathogenicity test on detached leaf and stem of healthy plant of Vasaka, mycelia growth started (3DAI), growth and colonisation of mycelia on plant parts (5DAI), sclerotia initiation (7DAI), sclerotia maturation (11DAI). *(DAI – Days after Inoculation)

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