First report of *Sclerotium delphinii* causing stem rot disease on green chireta (*Andrographis paniculata*)

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Abstract

Green chireta (*Andrographis paniculata*) a popular medicinal plant found infected with *Sclerotium delphinii* causing stem rot disease with 5 to 20% incidence. The rotting symptoms were first observed on outer surface of the stem base along with white mycelium and sclerotia. The causal organism was identified as *Sclerotium delphinii* based on its morphological and molecular characterization. *Sclerotium delphinii* causing stem rot disease in Green chireta is being reported for the first time in India and worldwide.

Keywords *Andrographis paniculata* · Molecular identification · Pathogenicity · *Sclerotium delphinii* · Stem rot

Green chireta (*Andrographis paniculata*) belongs to the family Acanthaceae, and is commonly known as Kalmegh or King of bitters. It is an annual herbaceous plant and is used to treat several human diseases due to its biochemical compounds (Joselin and Jeeva 2014; Nyeem et al. 2017). Usually, it is not affected by any pest or pathogen, but during January 2017, many plants exhibited symptoms of stem rot in the experimental field of the College of Agriculture, University of Tripura, Lembucherra, Agartala, Tripura, India, with the disease incidence ranging from 5 to 20%. In the early stage of disease development, the outermost leaves began to turn yellow and then dark brown necrotic lesions appeared on the stem base. Later white mycelial growth with immature white sclerotia was observed on collar region of the plant (Fig. 1).

The pathogenic fungus was isolated from stem tissues of Green chireta plants exhibiting stem rot disease in the field. Diseased plant samples were collected and surface sterilised in 0.25% v/v sodium hypochlorite for 1 min and then washed thrice with sterilised distilled water. The samples were air dried and placed in Petri plates containing PDA (Potato Dextrose Agar) media

and incubated at 25 °C. Mycelium started growing in two days and sclerotia were formed i.e., after one week of incubation. The mycelium further transferred to PDA slants to obtain pure culture. Consistently, similar abundant, fluffy and dense white to creamish white mycelial colonies were appeared on PDA from all the collected stem rot diseased samples (Fig. 2a). All these isolates produce sclerotia which were spherical in shape, 1.0 to 3.5 mm in diameter, white when young turning reddish brown when mature (Fig. 2b, c).

On the basis of the above morphological characteristics, the fungus was identified as *Sclerotium delphinii* (Xu et al. 2008). The representative culture of the isolate was deposited in the Indian Type Culture Collection, ICAR-Indian Agricultural Research Institute, New Delhi, India (ITCC No. 8394). The identification of the fungus was further confirmed by molecular characterisation. The pure fungal mycelium was transferred to flask containing potato dextrose broth medium for the development of mycelial mat and incubated at 25 °C for one week. Mycelial mat was filtered through Whatman filter paper and genomic DNA was extracted from fungal mycelial mat using Zymo Research fungal/bacterial DNA miniprep kit (Irvine, CA). The internal transcribed spacer (ITS) region of rDNA was amplified by polymerase chain reaction (PCR) using universal forward primer ITS1 (5'-TCCGTAGG TGAACCTGCGG-3') and reverse primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). A PCR product of 650 bp was amplified, sequenced and deposited in GenBank (MK131036). The ITS sequence of the

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Fig. 1 Rotting disease symptoms on Green chireta infected by *Sclerotium delphinii*. (a) Healthy plant (b) diseased plant (c) sclerotia formation near the collar region

cultured specimen showed 99% sequence homology with *S. delphinii* strain CBS 221.46 (MH856168).

The phylogenetic tree was constructed using partial sequences of ITS region of rDNA using maximum likelihood method with 1000 bootstrap values and Tamura-Nei nucleotide substitution model with the help of MEGA 6 software (Tamura et al. 2013). The ITS sequence obtained from the above fungus was closely grouped with the sequences of *S. delphinii* strains (CBS 272.30, MH855140; CBS 221.46, MH856168; CBS843.84, JN241566), with a bootstrap value of 61% compared to several members of the clade (Fig. 3).

The pathogenicity test was conducted using detached leaf method (Sharma et al., 2012). Healthy detached leaves and stems of green chireta were collected and agar plugs containing single mature sclerotia of *S. delphinii* were inoculated on the detached leaf and stem and placed in the moist chamber which was prepared with

Petri plates and moist Whatman filter paper at controlled temperature (27 °C) and humidity (95%) for one week. The non-inoculated detached leaf and stem were maintained to serve as control. Mycelium was observed after two or three days of inoculation and sclerotia formation was observed after 7 days and was fully matured from 10 to 15 days (Fig. 4). The fungus was re-isolated from the inoculated plant parts exhibited the similar morphology of the earlier described one. No symptoms were observed on control plant parts. According to USDA fungal database (<https://nt.ars-grin.gov/fungalDATABASES/fungushost/fungushost.cfm>) and other available literatures (Bilgrami et al. 1991, Sarbhoy et al. 1996), no report is available for *S. delphinii* causing stem rot disease on Green chireta (*Andrographis paniculata*). This study provides the evidence of first identification of *S. delphinii* as a causal organism of stem rot of Green Chireta in India and world.

Fig. 2 Morphological features of the pathogen: (a) Mycelial growth and formation of young sclerotia at 4–5 days of inoculation (B) Mature sclerotia after 10 Days of inoculation

Fig. 3 Phylogenetic tree inferred from internal transcribed spacer (ITS) sequences using the Maximum likelihood method, obtained from one sequence of *S. delphinii* (isolate 8394), three CBS strains (CBS 272.30, MH855140; CBS 221.46, MH856168; CBS843.84, JN241566), four *Athelia rolfsii* sequences (MH854711, MN121365, MK936164, MK926449), five sequences of different species of *Athelia* (MT594783,

MH860532, MH860510, MH558297, MH201278), three sequences of different species of *sclerotium* (NR145331, FJ231393, FJ231396) retrieved from NCBI GenBank and one sequence of *Rhizoctonia solani* (MH863394) as an out group. 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 the stem of healthy plant of Green chireta, initial mycelial growth started (2 to 3 DAI), growth and colonization of mycelia on plant parts (5DAI), sclerotia initiation (7DAI), and sclerotia maturation (10DAI). *(DAI – Days after Inoculation).

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