Eur J Plant Pathol (2018) 151:1081–1087
https://doi.org/10.1007/s10658-017-1415-2

Pathological studies on the southern blight of China aster (*Callistephus chinensis*) caused by *Sclerotium rolfsii*

S. Mahadevakumar · C. Chandana · Y. S. Deepika · K. S. Sumashri · Vandana Yadav · G. R. Janardhana

Accepted: 27 December 2017 / Published online: 12 January 2018

Abstract A severe outbreak of southern blight disease of China aster was observed during the post rainy season (September–November 2015) in the Mysore and Mandya Districts of Karnataka, Southern India. The disease incidence ranged between 12 and 47%. The typical disease symptoms include water-soaked lesions on leaves, stems and on the lower stem surfaces followed by quick wilting of the whole plant with abundant production of sclerotia near the stem-soil interface. The associated fungal pathogen was isolated on potato dextrose agar (PDA) medium, on which numerous reddish-brown sclerotia were seen. A total of 26 fungal isolates were isolated and studied for the mycelial compatibility. Isolate SrCCM 1 was used for pathogenicity analysis. The results of the study showed that, there was no variation among the isolates tested. Molecular identification of the pathogen by ITS-rDNA sequences of *S. rolfsii* showed 100% similarity with reference sequences. Based on the cultural, morphological and molecular characteristics, the fungal pathogen was identified as *Sclerotium rolfsii* Sacc. (Sexual morph: *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr). Pathogenicity tests were performed on healthy leaves, roots and stems. Typical disease symptoms on leaves, stems and roots were evident after 5, 8 and 10 days of post-inoculation. *Sclerotium rolfsii* is known to cause diseases in economically important crop plants. However, no reports are available on the occurrence of *S. rolfsii* on China aster in India.

Keywords *Sclerotium rolfsii* · *Callistephus chinensis* · Southern blight · Pathogenicity · Molecular identification

S. Mahadevakumar · C. Chandana · Y. S. Deepika · K. S. Sumashri · G. R. Janardhana (✉)
Mycology and Phytopathology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka 570 006, India
e-mail: grjbelur@gmail.com

V. Yadav
Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore, Karnataka 570 006, India

China aster (*Callistephus chinensis* (Linn.) Nees) is one of the most important floriculture crops grown in most parts of the world. It is cultivated owing its market preference and attractive longer shelf life of cut flowers (Navalinskien et al. 2005). In Karnataka it was cultivated in 2194 ha, with a production of 20,646 mt during 2009 (Anon. 2009). Recent observation revealed that as the cultivation area increases, the incidence of diseases also increased. The important diseases known to affect *C. chinensis* include *Fusarium* wilt (*Fusarium oxysporum* f. sp. *callistephi* (Beach) Snyder & Hansen), Gray mold (*Botrytis cinerea* Pers.), leaf spot (*Stemphylium callistephi* Baker & Davis) and root rots (*Pythium* sp. and *Phytophthora* sp.) (Anon. 2014). Association of 16SrII-D group phytoplasma with virescence and phyllody of China aster has been reported from South Korea and India (Win et al. 2011; Mahadevakumar et al. 2017a). Owing to a recent outbreak of southern blight disease on *C. chinensis*, the present

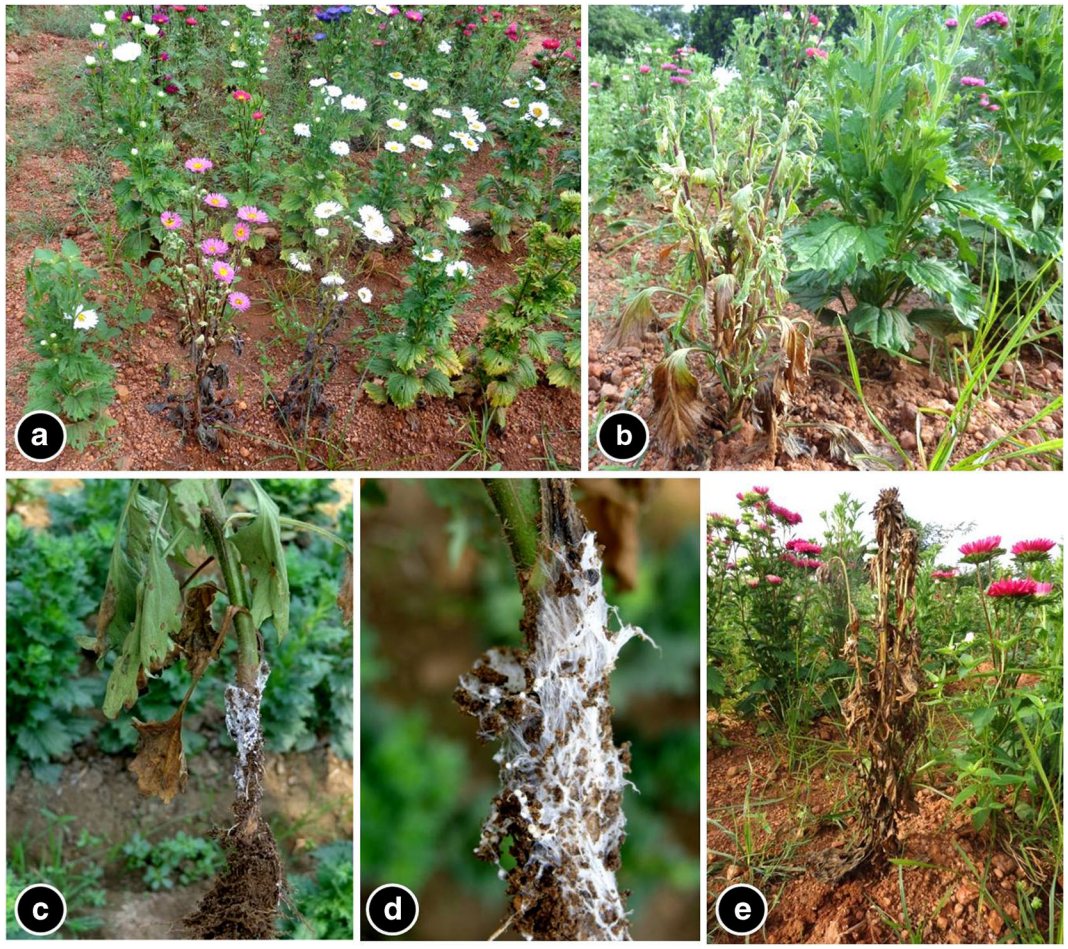

Fig. 1 Southern blight disease on China aster: **a** Diseased China aster; **b** Wilt symptoms; **c** & **d** Mycelial cords on the stem during early and later stages of disease development; **e** Death of China aster plant due to foot rot disease

investigation was undertaken to evaluate the disease incidence and the identification of pathogen based on morphological, cultural and molecular analysis.

Disease incidence

The study was carried out in southern region of Karnataka. A rowing field survey was conducted in major China aster growing regions of Mysore and Mandya Districts during 2014–2015. China aster plants associated with sclerotial fungus causing foot rot, leaf blight and stem blight symptoms were found in a total of 72 fields out of 160 fields studied (approx. 45 ha). Disease incidence was estimated in two regions by selecting 12 fields randomly in each region. In each field, the disease incidence was determined by the number of plants infected for every 100 plants examined (Mahadevakumar et al. 2017b). The disease incidence (%) was found maximum in Doddamaragowdanahally region (47.25 ± 6.29 SD) and minimum in Krishnarajasagara region (12.08 ± 2.57 SD). The disease symptoms initiated with the appearance of water-soaked lesions at the point of infection followed by the appearance of mycelial strands with small globoid sclerotia of varied size (Fig. 1a–e). The disease symptoms were severe during high humid and rainy periods than in rest of the seasons.

Isolation of the fungal pathogen

Associated fungal pathogens from the infected plant samples ($n = 20$) were isolated on potato dextrose agar (PDA) medium amended with chloramphenicol

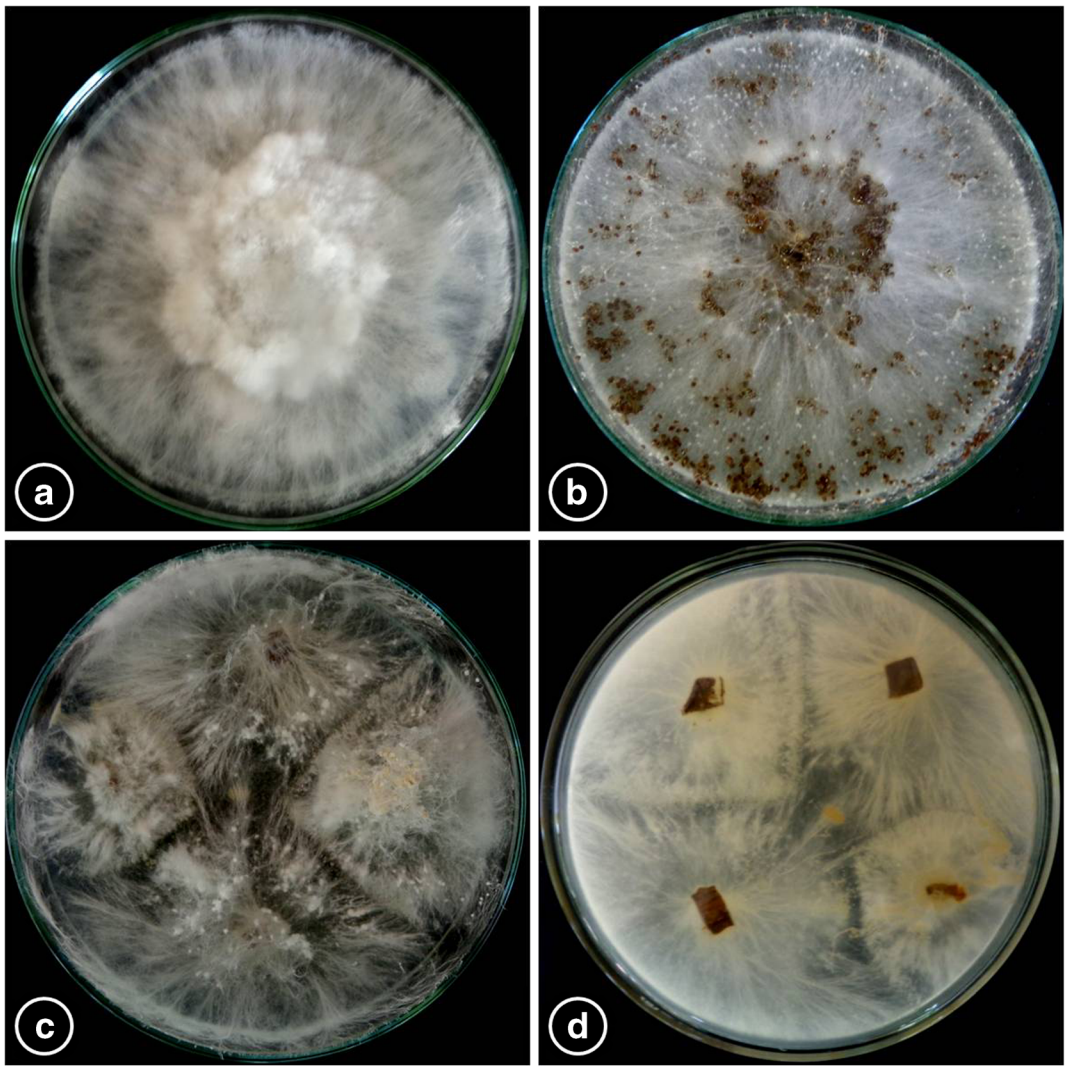

Fig. 2 *Sclerotium rolfsii* on culture media and mycelial compatibility: **a** & **b** Pure culture of *Sclerotium rolfsii* grown on PDA medium; **c** & **d** Mycelial compatibility of *S. rolfsii* isolates paired on PDA medium

(100 mg/L). The fungal colonies were sub-cultured and their colony morphology, number of sclerotia, mycelial compatibility grouping, pathogenicity and molecular identification were performed. The associated fungal pathogen was consistently isolated from all the symptomatic samples. The fungal colonies showed characteristic dense, aerial, fluffy, whitish, cottony mycelia with globoid sclerotia after 12–15 days of inoculation. Sclerotia measured 0.5–2.5 mm in diameter and were whitish in the beginning and turned to brownish on maturity (Fig. 2a–b). The number of sclerotia per plate ranged from 288 to 350 (mean 329 ± 16, $n = 8$). Based on the morphological and cultural characteristics, the fungal pathogen was identified as *Sclerotium rolfsii* Sacc. (Sexual morph: *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.) (Saccardo 1931; Mordue 1974; Punja et al. 1982). The morphological features that differentiated *S. rolfsii* from *Sclerotium delphinii* Welch. were the fluffy nature of mycelia; abundant sclerotia (>300 to 400/plate) and smaller size (2–3 mm) of sclerotia (Stevans 1931; Punja and Damiani 1996; Mahadevakumar and Janardhana 2016).

Molecular identification

The genomic DNA was isolated by CTAB method (Zhang et al. 1998; Mahadevakumar and Janardhana

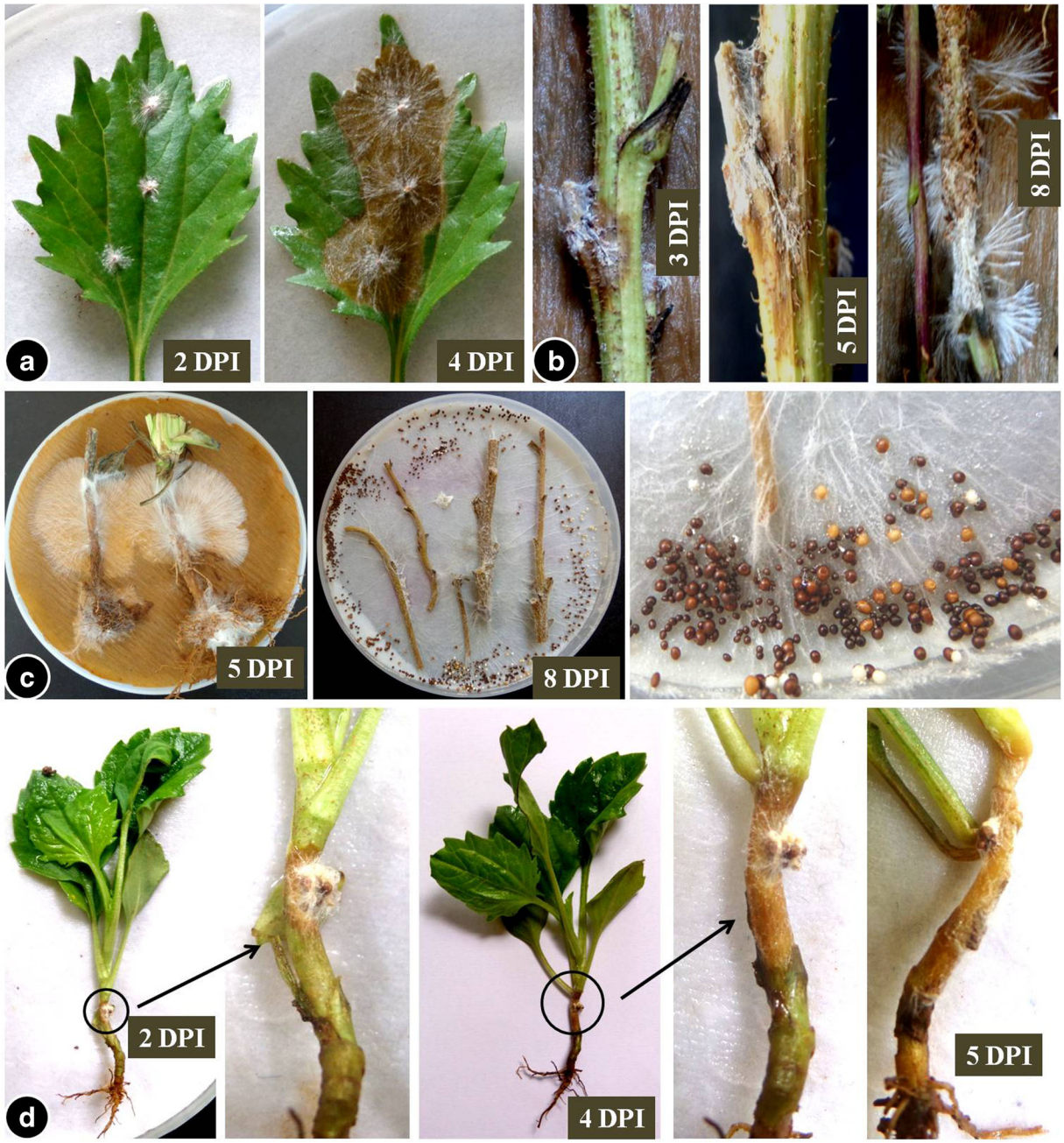

Fig. 3 Detached leaf and stem assay: **a** & **b** Assay showing the growth of *S. rolfsii* after 4 dpi; **b** Stem blight symptoms developed after 5 dpi; **c** Detached stem assay **s**howing typical stem blight; **d** Typical collar rot symptoms after 2 and 4 dpi (*DPI-days of post inoculation)

2016). The ITS-rDNA region was amplified employing ITS1-ITS4 primers (White et al. 1990). The PCR was performed using Thermocycler (Advanced Primus 25, Peqlab, Germany). An expected amplicon size of 550–600 bp was detected on 1.5% agarose gel. The PCR products were purified and sequenced using an AB3730xI DNA analyser (Applied Biosystems, Foster City, CA, USA) from both the directions. The sequences obtained were aligned with ITS sequences retrieved from GenBank. The nucleotide blast analysis of sequence revealed, 100% homology with reference sequences of *S. rolfsii* (AB075298.1, AB075299.1 and JF966208.1). A phylogenetic tree constructed using Neighbour-Joining (NJ) method showed that the

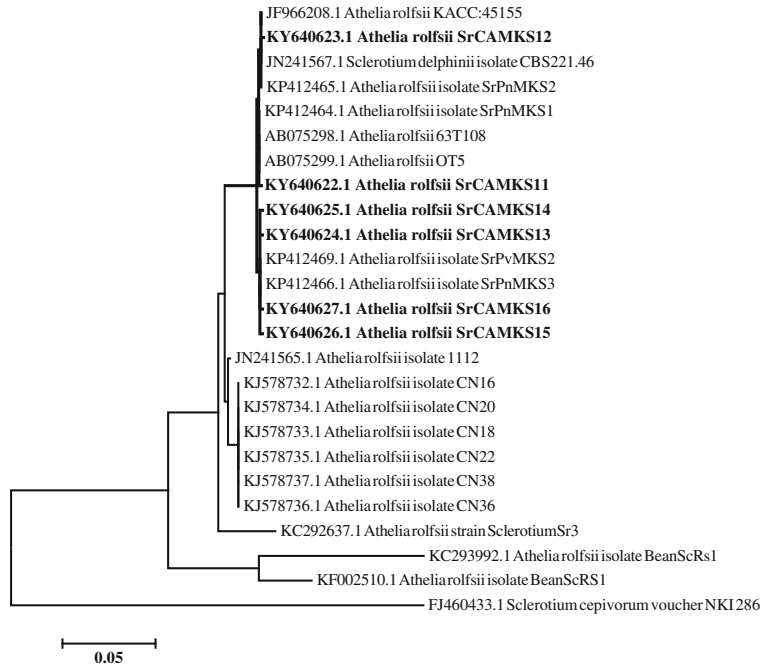

Fig. 4 Phylogenetic tree constructed for ITS region of *S. rolfsii* (*Athelia rolfsii*) isolates by Neighbour Joining method and the tree was rooted with *S. cepivorum*. Tamura-Nei Substitution model and nearest neighbour-interchange search options with 1000 bootstrap replicates were used

sequences grouped into a single clade along with reference sequence of *S. rolfsii* (JN241567.1, JF966208.1, KP412465.1, KP412464.1, AB075298.1, AB075299.1) and rooted to *Sclerotium cepivorum* (FJ460433.1) with 1000 boot strap replications (Fig. 4). A total of six DNA sequences from the present study were deposited in GenBank (KY640622 to KY640627).

Mycelial compatibility grouping

Mycelial compatibility grouping (MCG) among 26 isolates of the fungal pathogen was performed by placing mycelial discs (5 mm diam) from the edge of an actively growing fungal isolates and transferred onto a Petri dish containing PDA medium and incubated for seven days at room temperature (Le et al. 2012; Xei et al. 2014). The MCG studies revealed that all the tested isolates were compatible with each other and there was no clear barrage zone formation (Fig. 2c–d).

Pathogenicity studies

Detached healthy leaves (100), cut stems (100) and roots (30) of China aster were washed with running tap water and used for challenge inoculation under in-vitro conditions. The leaves stem and roots were inoculated by placing mature sclerotia on the surface of leaves, stem and roots. Inoculated stems, leaves and roots were incubated in a moist chamber for seven days at 26 ± 2 °C. Samples inoculated with sterile water served as control. In another set of experiment, 30-days-old china aster seedlings (30) were inoculated with 15-days-old *S. rolfsii* (SrCCM 1) by wrapping 10 sclerotia around the stem near stem-soil interface. The China aster seedlings inoculated only with sterile water were considered as control. The appearance of southern blight symptoms on seedlings was recorded after five days-post-inoculation period.

The detached leaf and stem assay produced typical symptoms after 4–5 days post-inoculation period with an incidence of 80% and 78% (mean lesion length of 2.47 ± 0.34 cm and 1.93 ± 0.12 cm), respectively (Fig. 3). Healthy seedlings inoculated with the fungal pathogen showed typical symptoms after 8–10 days post-inoculation with an incidence of 73.3% (mean lesion length 1.06 ± 0.06 cm). No such disease symptoms were observed on water inoculated seedlings. The fungal pathogen was isolated from the inoculated plants and its identity was reconfirmed based on cultural and morphological features (Fig. 4).

Sclerotium rolfsii is a potent fungal pathogen causing diseases on a wide variety of plants, including cereals,

vegetables, fruits, ornamentals and turfs at various stages of their growth and development (Aycock 1966; Punja 1985; Mullen 2001). The disease caused by this fungus is generally referred to as foot rot, southern blight or southern stem blight. The pathogen is also known to infect seedlings, herbaceous plants, woody plants, fleshy roots, bulbs, and fruits (Mullen 2001). The most important crop plants associated with southern blight and leaf spot disease include southern blight of common bean (*Phaseolus vulgaris* Linn.), leaf spot of Indian Jasmine (*Jasminium multiflorum* (Burm. f) Andrews), boll rot of cotton (*Gossypium hirsutum* L.), fruit rot of pumpkin (*Cucurbita maxima* Duchesne) and southern blight of wild coffee (*Psychotria nervosa* Sw.) (Mahadevakumar et al. 2015a, b, c; Mahadevakumar and Janardhana 2015, 2016). The pathogen is soil-borne and the inoculum persists in the soil for 2–3 years and responsible to cause new infection when the crop comes up in the next season (Aycock 1966; Punja 1985; Smith et al. 1989).

Acknowledgements First author (MKS) acknowledges the Department of Science and Technology (DST), Government of India, New Delhi for providing INSPIRE Fellowship (IF110422). We thank Professor K. R. Sridhar, UGC-BSR Faculty, Mangalore University, Mangalore, India for their help and critical comments.

Compliance with ethical standards

Conflict of interest No conflict of interests reported by the authors.

Human studies and participants There was no involvement of human participants and/or animals in the present study.

References

Anonymous (2009). *Statistical data on horticultural crops in Karnataka state: Department of Horticulture, Lalbagh, Bengaluru, Karnataka.*

Anonymous (2014). *UC-IPMM Pest management guidelines: Floriculture an ornamental nurseries*. UCANR Publications, 3392.

Aycock, R. (1966). *Stem rot and other diseases caused by Sclerotium rolfsii or the status of Rolfs' fungus after 70 years* (pp. 202). North Carolina Agric. Expt. St. Tech. Bull. No. 174.

Le, C. N., Mendes, R., Kruijt, M., & Raaijmakers, J. M. (2012). Genetic and phenotypic diversity of *Sclerotium rolfsii* in groundnut fields in central Vietnam. *Plant Disease, 96*(3), 389–397.

Mahadevakumar, S., & Janardhana, G. R. (2015). First report of leaf spot disease caused by *Sclerotium rolfsii* on *Jasminium multiflorum* in India. *Journal of Plant Pathology, 98*(1), 355.

Mahadevakumar, S., & Janardhana, G. R. (2016). Morphological and molecular characterization of southern leaf blight of *Psychotria nervosa* (wild coffee) incited by *Sclerotium rolfsii*. *Journal of Plant Pathology, 98*(2), 351–354.

Mahadevakumar, S., Tejaswini, G. S., Shilpa, N., Janardhana, G. R., Dharanendraswamy, S., & Yadav, V. (2015a). First report of *Sclerotium rolfsii* associated with boll rot of cotton in India. *Plant Disease, 100*(1), 229.

Mahadevakumar, S., Tejaswini, G. S., Yadav, V., & Janardhana, G. R. (2015b). First report of southern blight and leaf spot (*Sclerotium rolfsii*) on common bean (*Phaseolus vulgaris*) in India. *Plant Disease, 99*(9), 1280.

Mahadevakumar, S., Yadav, V., Tejaswini, G. S., & Janardhana, G. R. (2015c). Morphological and molecular characterization of *Sclerotium rolfsii* associated with fruit rot of *Cucurbita maxima*. *European Journal of Plant Pathology, 145*, 215–219.

Mahadevakumar, S., Thorat, V., Yadav, V., Yadav, A., & Janardhana, G. R. (2017a). First report on the occurrence of '*Candidatus* phytoplasma aurantifolia' (16SrII-D) associated with virescence and Phyllody diseases of Chinese aster (*Callistephus chinensis* (L.) Nees.) in India. *Plant Disease, 101*(1), 241.

Mahadevakumar, S., Amruthavalli, C., Sridhar, K. R., & Janardhana, G. R. (2017b). Prevalence, incidence and molecular characterization of *Phomopsis vexans* (*Diaporthe vexans*) causing leaf blight and fruit rot disease of brinjal in Karnataka (India). *Plant Pathology and Quarantine, 7*(1), 29–46.

Mordue, J. E. M. (1974). *CMI descriptions of pathogenic fungi and bacteria*. No. 410.

Mullen, J. (2001). Southern blight, southern stem blight, white mold. *The Plant Health Instructor*. https://doi.org/10.1094/PHI-I-2001-0104-01.

Navalinskien, M., Samuitien, M., & Jomantiene, R. (2005). Molecular detection and characterization of phytoplasma infecting Callistephus Chinensis plants in Lithuania. *Phytopathology Polonica, 35*, 109–112.

Punja, Z. K. (1985). The biology, ecology and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology, 23*, 97–127.

Punja, Z. K., & Damiani, A. (1996). Comparative growth, morphology and physiology of three *Sclerotium* species. *Mycologia, 88*(5), 694–706.

Punja, Z. K., Grogan, R. G., Adams, G. C., & Jr. (1982). Influence of nutrition, environment and the isolate on basidiocarps formation, development and structure in *Athelia* (*Sclerotium*) *rolfsii*. *Mycologia, 74*(6), 917–926.

Saccardo, P. A. (1931). *Sclerotium rolfsii (1500 pp)*. Pavia: Sylloge Fungorum XXII.

Smith, V. L., Jenkins, S. F., Punja, Z. K., & Benson, D. M. (1989). Survival of sclerotia of *Sclerotium rolfsii*: Influence of sclerotial treatment and depth of burial. *Soil Biology and Biochemistry, 21*(5), 627–632.

Stevans, L. (1931). A comparative study of *Sclerotium rolfsii* and *Sclerotium delphinii*. *Mycologia, 23*(3), 204–222.

White, T., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for

phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols, a guide to methods and applications* (pp. 315–322). New York: Academic Press.

Win, N. K. K., Kim, Y., Chung, H., & Jung, H. (2011). Detection of 16SrII group phytoplasma in China aster (*Callistephus chinensis*). *Tropical Plant Pathology, 36*(3), 186–189.

Xei, C., Huang, C., & Vallad, G. E. (2014). Mycelial compatibility and pathogenic diversity among *Sclerotium rolfsii* isolates in the southern United States. *Plant Disease, 98*(12), 1685–1694.

Zhang, Y. P., Uyemoto, J. K., & Kirkpatrick, B. C. (1998). A small scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. *Journal of Virological Methods, 71*(1), 45–50.