First evidence of Chaetomium globosum infection on Strobilanthes kunthiana.(Neelakurinji), a vulnerable and endemic species of tropical montane grassland in the Western Ghats, Kerala, India

Arjun M S

arjun-2022-27-001@student.kau.in

Research Article

Keywords: Chaetomium globosum, Strobilanthes kunthiana, invasive species, leaf blight, Western Ghats

Posted Date: November 4th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-8015490/v1

Additional Declarations: The authors declare no competing interests.

Abstract

Strobilanthes kunthiana (Nees) T. Anderson ex Benth., locally known as Neelakurinji, is a vulnerable and endemic species restricted to the tropical montane shola grassland ecosystems of the Western Ghats in southern India. It exhibits a gregarious 12-year mass flowering cycle and has substantial ecological and ecotourism value. Despite its conservation importance, the phytopathological and invasion threats are poorly documented. During field surveys 2022–2024 in Eravikulam National Park, leaf blight was detected in scattered Neelakurinji populations, with symptoms ranging from severe foliar necrosis to plant death. This study investigates the fungal associates of *S. kunthiana* and invasive plant co-occurrence across its habitats to support disease management and conservation. Fungal Pathogens isolated from infected plant material were characterized based on their morphological features and further confirmed through multi-gene phylogenetic analysis, including sequencing of the internal transcribed spacer (ITS1–ITS4), β-tubulin (tub2), and translation elongation factor 1-alpha (TEF1-α) gene regions. The causal organism was conclusively identified as *Chaetomium globosum* validated through Koch's postulates. Further, *in vitro* assessments were conducted to evaluate the influence of different growth media includes PDA (Potato Dextrose Agar), SDA (Sabouraud Dextrose Agar), RB (Rose Bengal) and temperature regimes (15, 22, and 30°C) on the radial mycelial growth of the pathogen.

Introduction

The Nilgiri Hills (Blue Mountains) of southern India, derive its name from *Strobilanthes kunthiana*, (locally Neelakurinji) a well-known species among Strobialnthes genus. Belonging to Acanthaceae, this plant is characterised by its plietesial behavior flowering after long intervals, followed by death of the plant. The semelparous life cycle of Neelakurinji has been extensively documented, with synchronized mass blooming events reported at 12-year intervals since 1838 (Robinson, 1935; Matthew,1959; Matthew, 1971; Lockwood, 2006; Singh & Arigela, 2019) in various regions in southern Western Ghats. The synchronized mass flowering and mast seeding serve as an adaptive reproductive strategy, ensuring population persistence and regeneration (Singh & Arigela, 2019). Eravikulam National Park, located in the Western Ghats, is renowned for the endangered Nilgiri Tahr (*Nilgiritragus hylocrius*) and the mass flowering events of Neelakurinji. Extensive botanical surveys have documented the occurrence of approximately 22 species of *Strobilanthes* within the park's montane grassland and shola ecosystems landscape covers (97 km²) (Augustine, 2008).

Neelakurinji was recently evaluated for the IUCN Red List assessment (2024) and is classified as Vulnerable (VU) under criterion A2c. It is estimated to have undergone approximately a 40% decline in both population size and Area of Occupancy (AOO) over the last three generations, with ongoing losses driven by habitat deterioration and human-induced disturbances (Hyder et al., 2024). Various invasive species like *Calicotome spinosa*, *Cestrum aurantiacum* and *Lantana camara* pose major threats to *Strobilanthes* species in Nilgiris through habitat encroachment and hindering their survival (Kiruthika et al., 2024).

Neelakurinji is known to possess a diverse array of bioactive phytochemicals (Everlyne et al., 2015; Prabakaran & Kirutheka, 2018; Balasubramaniam et al., 2020b). Fernandes & Krishnan (2019) detected forty-three different compounds in the extracts obtained from its leaves and stems. Aqueous leaf extract of *S. kunthiana* contains significantly higher levels of phenolics, flavonoids, and tannins when compared to the ethanolic extract (Chandran &Indira, 2016).

Several studies have also demonstrated that *Strobilanthes* species possess a wide range of therapeutic properties including analgesic and anticancer (Arya et al., 2022), in-vitro antioxidant and cytotoxic activities (Balasubramaniam et al., 2021); analgesic activity (Desu et al., 2012); antioxidant property (Brijyog et al., 2014); antibiofilm properties (Everlyne et al., 2016); central nervous system depressant activity (Rajasekaran et al., 2000); and effective hepatoprotective activities (Balasubramaniam et al., 2020a). Preethi & Suseem (2014) reported that the plant possesses a broad spectrum of therapeutic properties, including antimicrobial (antibacterial, antiviral, and antifungal), anti-inflammatory, and pharmacological activities relevant to the treatment of conditions such as respiratory and stomach ailments, rheumatism, anxiety, diabetes, arthritis, and cancer, along with diuretic and laxative effects. Recently researchers explore the potential natural reducing properties of *Strobilanthe*s species for the green synthesis of various nanoparticles like gold (Sridharan et al., 2025); copper and silver ions (Murugan et al., 2025).

Chaetomium globosum has been reported as a causal agent of leaf blight or leaf spot disease in several economically important plants, including cabbage (*Brassica oleracea*; Zhu et al., 2021), pomegranate (*Punica granatum*; Guo et al., 2016a), olive (*Olea europaea* 'Gemlik'; Avan & Atay, 2024), star fruit (*Averrhoa carambola*; Reddy et al., 2024), fiber hemp (*Cannabis sativa*; Chaffin et al., 2020), and brinjal (*Solanum melongena*; Hassan et al., 2022).

Previous surveys from the Western Ghats have reported *C. globosum* as an endophyte of *Hypericum mysorense* (Samaga et al., 2014; Samaga & Rai, 2016). Building on this context, we have now isolated *C. globosum* from *S. kunthiana* (Neelakurinji), an endemic shrub of the tropical montane grassland–shola mosaic, which so far as the first record of this fungus from *Strobilanthes* and extends its known host range within this high-elevation landscape, threatening the vulnerable species in the prestigious ecosystem.

Conversely, some strains of *Chaetomium globosum* function as biocontrol agents against phytopathogens. Reported modes of action include antibiosis, competition, mycoparasitism, and induction of plant defenses, mediated by antifungal metabolites such as chaetoviridins, chaetoglobosins, and chaetomin (Aggarwal, 2015; Madbouly & Abdel-Wareth, 2019). In crops, *C. globosum* has reduced potato late blight caused by *Phytophthora infestans* (Shanthiyaa et al., 2013) and suppressed Fusarium crown rot of wheat caused by *Fusarium pseudograminearum* (Feng et al., 2023). Beyond biocontrol, *C. globosum* can also promote plant growth for example, in tomato it increased height, root length, and photosynthetic performance, especially when applied as combined seed

treatment plus soil drench (Singh et al., 2022). These effects are strain and host-dependent, which helps explain why *C. globosum* can be pathogenic in one system yet beneficial in another.

Almost all Kurinjis are highly specialized to thrive only within specific habitats, making their survival intricately linked to the integrity of montane grasslands and shola forests. Any disruption to these pristine ecosystems inevitably threatens the persistence of these remarkable shrubs, which possess just a single opportunity for reproduction in their life cycle (Augustine et al., 2017; Bera et al., 2020). The Government of Kerala designated the Kurinjimala Sanctuary in 2006 with the primary objective of conserving *Neelakurinji* and securing the long-term protection of the region's unique biodiversity. Notably, it represents the first protected area in the state established specifically for the conservation of a flowering plant (Department of Forests and Wildlife, Government of Kerala (2011). To date, there have been no documented reports of pathogenic diseases affecting *Strobilanthes kunthiana*; the present study is the first to report and identify *Chaetomium globosum* as a fungal pathogen associated with leaf blight symptoms in this vulnerable endemic species. The present study conducted on the tropical montane grassland ecosystems, in Kerala with a particular focus on populations within Eravikulam National Park. Neelakurinji is not only a vulnerable species under the IUCN Red List, but also a major tourist attraction, contributing significantly to revenue generation for the forest department, making its conservation critically important.

Materials and methods

Study area

The study was conducted in Eravikulam National Park located in the High Ranges (Kannan Devan Hills) of the Southern Western Ghats in the Devikulam Taluk of Idukki District, Kerala State, India between 10º 05' – 10º 20' N Latitude and 77º 0' – 77º 10' E Longitude.

Fungal isolation and detailed morphological analysis

Diseases symptoms on S*trobilanthes. kunthiana* leaf were noticed during the period of 2022–2025 during a field survey in Eravikulam National Park, Munnar, Kerala. The leaf samples showing disease symptoms were collected from different locations within the landscape and transported to pathology lab immediately for further isolation. Leaf segments (~ 5 × 5 mm) from symptomatic leaves were surface-sterilized with (70% ethanol for 30–60 s) followed by 1–2% sodium hypochlorite 30–60 s and then three sterile-water rinses) and plated on solidified potato dextrose agar (PDA) in sterilized Petri dishes (Schulz et al., 1993; Atlas, 2004; Sinclair & Dhingra, 1995). Plates were incubated at 25 ± 2°C under a 12 h light/dark cycle for one week, emerging mycelia were purified using the hyphal-tip method and subcultured on PDA. Since the isolates displayed uniform growth patterns on PDA, a representative culture was selected for subsequent analyses Colony characters (pigmentation, texture, zonation, growth rate) and micromorphology were examined after one week using stereomicroscopy and compound

microscopy (Leica LAS software, version 4.12.0) in lactophenol cotton blue, following standard mycological keys (Simmons, 2007). The diseased samples were deposited in the Mycological Herbarium of the Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala, under the accession number KFRIMH 692, and the representative culture was deposited in the KFRI Microbial Culture Collection under the accession number KFRIMCC 692.

Pathogenicity assessment

Pathogenicity assessment

Pathogenicity assessment were done by mycelial bit inoculation method following (Dreischhoff et al., 2023). Five mm plugs of actively growing hyphae from a seven-day-old culture was aseptically excised and placed on the adaxial and abaxial leaf surfaces of *S. kunthiana* plants that had been pinpricked with a sterile needle. Sterile moistened cotton was placed around the inoculation site to maintain local humidity, and the inoculated plants were enclosed in a transparent plastic sleeve and lightly misted with sterile distilled water to maintain high humidity (≈ 95% RH) at 25 ± 2°C. Controls received sterile PDA plugs on similarly wounded leaves and maintained no-plug (wounded only) controls to account for any wound effects. Leaves were monitored daily for symptom development, lesion formation, and pathogen progression, following standard protocols (Pettitt et al., 2011; Guo et al., 2016b). Each treatment was performed in triplicate. To complete Koch's postulates, the causal organism was re-isolated from symptomatic tissue and its identity verified by comparing macro and micromorphological characters.

DNA extraction, amplification, and sequencing.

DNA was isolated using QIAGEN DNeasy UltraClean Microbial Kit (Cat. No. / ID: 12224-50) from a seven-day old mycelial mat of the *Chaetomium globosum*. The purity and integrity of the extracted DNA were assessed through agarose gel electrophoresis using a Cleaver Scientific system. Amplification of the ITS region was performed using the ITS1 and ITS4 primer set (White et al., 1990), and the resulting PCR products were purified to remove residual contaminants with the QIAGEN QIAquick PCR Purification Kit (Cat. No./ID: 28104). Bidirectional sequencing of the purified amplicons was conducted with the same primer pair using the BDT v3.1 Cycle Sequencing Kit on an Applied Biosystems™ MiniAmp™ Plus Thermal Cycler, and fragments were subsequently analysed on an ABI 3730xl Genetic Analyzer. In addition, the β-tubulin (tub2) locus was amplified with Bt2a/Bt2b primers (Glass & Donaldson, 1995), and the translation elongation factor 1-alpha (TEF1/EF1α) region was amplified following Carbone & Kohn (1999) and Matheny et al. (2007) to achieve species-level resolution. While the ITS region serves as the primary barcode for fungal identification, tub2 and TEF1 markers provide enhanced discriminatory power (Schoch et al., 2012; Rehner & Buckley, 2005).Sequences have been deposited in GenBank and accession number .

Phylogenetic analysis

The GenBank accession numbers assigned to *Chaetomium globosum* were PX148901 for the internal transcribed spacer (ITS) region and PX170153 for the translation elongation factor 1-alpha ((TEF1/EF1α) gene. In total, 36 nucleotide sequences were included in the analysis, comprising 34 reference sequences from related taxa within the genus retrieved from the NCBI GenBank database, along with a single outgroup sequence. Multiple sequence alignment was carried out using ClustalW (Thompson et al., 1994), yielding a final matrix of 838 aligned characters. Phylogenetic inference was performed using the Maximum Likelihood (ML) approach in MEGA v12 (Kumar et al., 2024), applying the Tamura–Nei evolutionary model (Tamura & Nei, 1993) within a General Time Reversible (GTR) framework, coupled with a gamma distribution (+ G) to accommodate rate variation among sites. Rate heterogeneity was modeled using a discrete Gamma distribution with five categories and a shape parameter of 0.1683, reflecting substantial variation in substitution rates across the alignment. Node support was assessed through a bootstrap analysis of 1000 pseudoreplicates, and the resulting bootstrap values are presented at their respective branching points in the ML tree.

For heuristic tree searching, two initial topologies were compared: one derived from a Neighbor-Joining (NJ) method using a pairwise distance matrix under the GTR model, and the other from Maximum Parsimony (MP) using 10 independent searches with random starting trees. The topology yielding the highest log likelihood was selected for the final ML tree. All evolutionary analyses were executed in MEGA12, employing up to four parallel threads to optimize computational performance.

Effect of culture medium and temperature on mycelium growth of Fungus

The effect of three culture media includes PDA, sabouraud dextrose agar (SDA), and rose bengal agar (RBA) and three temperature regimes (15 ± 1 °C, 22 ± 1 °C, and 30 ± 1 °C) on the radial mycelial growth of different fungal isolates was evaluated assessed to determine the optimal growth conditions of the fungal isolate. The mycelial discs were inoculated onto petri plates under sterile conditions, incubating them in the incubator, and recording mean colony diameters at regular intervals, with data analysed using ANOVA under a completely randomized factorial design (Yadav & Chandra, 2014).

Result and Discussion

Field observations revealed leaf blight disease in *S. kunthiana*. associated with *C. globosum*. Symptoms were characterized by irregular necrotic lesions with brown to dark brown discoloration, tissue collapse, and progressive marginal blighting, indicative of a rapidly spreading foliar pathogen (Figure. 2 a) and were typically linked to serious necrosis and often leading to plant death (Figure. 2b). Healthy plants without symptoms were also recorded for comparison (Figure. 2c).

Pathogenicity tests conducted under controlled conditions further validated the disease symptoms, with progressive stages of leaf blight development observed in inoculated plants, as depicted in Figure (3). By day 5 post-inoculation, symptoms covered ~40% of the leaf area; by day 8, leaves were fully affected by blight.

Morphological analysis

After two weeks of incubation on PDA medium, colonies of *C. globosum* exhibited characteristic growth features, including dense, woolly, white mycelium on the frontal side, and a distinct yellow to brown pigmentation on the reverse side. Microscopically, dark brown to black conidiomata were observed, indicating active sporulation typical of the genus in Figure (4).

Microscopic observation revealed *C. globosum* produced lemon-shaped (limoniform), bilaterally-flattened to subglobose ascospores, frequently clustered or associated with loosely coiled perithecial hyphae, characteristic of its ascomycetous nature (Figure 5). Microscopy showed ostiolate perithecia clothed in flexuous/loosely coiled ascomatal hairs and lemon-shaped to subglobose, bilaterally flattened, pigmented ascospores, consistent with the diagnostic characters of *C. globosum* reported in the taxonomic literature (Asgari & Zare, 2011; Wang et al., 2016).

Molecular characterisation

The ITS-rDNA sequence analysis via NCBI-nBLAST showed up to 98.41% similarity (72–75% query coverage) to *C.globosum* and related taxa within the species complex (e.g., *C. subglobosum*, *C. globosum var. griseum*). Additionally, the tub2 gene sequence showed 100% similarity (≥82% query coverage) to multiple *C. globosum* strains (e.g., KX976958.1, OK245521.1), the tef1 gene sequence displayed 100% similarity (96–97% query coverage) to isolates such as OP373438.1 and MW588207.1, confirming the identity as *C. globosum*. The present isolates KFRIMCC 837 were represent as *Chaetomium globosum KAU* in Maximum likelihood phylogenetic tree (Figure 6).

Effect of culture medium and temperature on mycelium growth of Fungus

Chaetomium globosum exhibited distinct medium dependent morphological variations (Figure 7). On PDA, colonies were compact, radially symmetrical, and characterized by a dense, cottony to woolly mycelial mat with smooth margins. SDA supported floccose to woolly growth with irregular radial expansion and lobed margins. In contrast, RB medium induced irregular, floccose colonies with lobate margins and numerous raised, globose structures, suggestive of sporulating bodies.

The extent of sporulation in *C. globosum* varied markedly with the culture medium, with the highest sporulation observed on SDA, followed by RB, while PDA supported the lowest degree of sporulation (Figure 7). This differential sporulation pattern highlights the influence of medium composition on the reproductive physiology of the fungus, particularly in relation to nutrient availability and stress-induced morphogenesis.

The number of days required to reach full radial growth (≥9 cm) varied significantly between *C. globosum* across different temperature regimes and culture media (Figure 8). In *C. globosum*, a clear temperature dependent response was observed, with full growth achieved by day 17–18 at 15°C, and markedly earlier at 22°C and 30°C on days 11 and 10, respectively (Figure 8a). Kedves et al., 2021 reported that *C. globosum* grows between ~15–40 °C with an optimum around 25–30 °C. Growth also differed notably

by medium. *C. globosum* exhibited the fastest and most consistent growth on RB medium (mean: 12–13 days), followed by SDA (~13 days), and slower, more variable growth on PDA (~14 days). These results highlight RB medium as optimal for *C. globosum* (Figure 8b). Several studies culture, *C. globosum* successfully on SDA and PDA, with SDA often supporting greater mycelial growth and/or excellent sporulation (Uikey et al., 2020; Kim et al., 2013), though sporulation can be strain and medium-dependent and is sometimes poor on SDA/PDA (Najafzadeh et al., 2014).

Conclusion

This study for the first time identifies *C. globosum* as a key foliar pathogen affecting the health of *S. kunthiana* in tropical montane grassland ecosystem in Western Ghats, India. By documenting a previously unreported disease in this endemic and vulnerable plant, the study provides critical insights for future ecological monitoring and underscores the importance of proactive conservation efforts to protect this species. Additionally, preliminary field evidence suggests that controlled burning may serve as an effective management strategy to limit disease spread. These findings offer vital insights for conserving this iconic and vulnerable species in the tropical montane ecosystems of the Western Ghats.

Declarations

Ethical approval

This article does not contain any studies with human participants or animals by any of the authors.

Conflict of interest

The authors declare that they have no conflicts of interest

References

1. Aggarwal, R. (2015). *Chaetomium globosum: a potential biocontrol agent and its mechanism of action. Indian Phytopathology, 68*(1), 8–24.
2. Arya, G. C., Jakhmola, V., Mehla, S., & Bainsal, N. (2022). Mechanistic study of analgesic and anticancer activity of unexplored plant *Strobilanthes kunthiana* phytoconstituents using molecular docking studies. *Research Square*. https://doi.org/10.21203/rs.3.rs-2272936/v1
3. Asgari, B., & Zare, R. (2011). The genus *Chaetomium* in Iran: A phylogenetic study including six new species. *Mycologia, 103*(4), 863–882. https://doi.org/10.3852/10-349
4. Atlas, R. M. (2004). *Handbook of microbiological media* (4th ed.). CRC Press.
5. Augustine, J. (2008). 20.0 Genus *Strobilanthes* in high ranges of Kerala: Diversity, distribution and endemism. *Special Habitats and Threatened Plants of India*, 11(1), 139.
6. Augustine, J., Josekutty, E. J., & Biju, P. (2017). *Strobilanthes sainthomiana* — a new species of *Strobilanthes* Blume (Acanthaceae) from Western Ghats, India. *Taiwania, 62*(1), 63–66.

https://doi.org/10.6165/tai.2017.62.63

7. Avan, M., & Atay, M. (2024). Occurrence and importance of *Chaetomium globosum* as a fungal pathogen on olive leaves in Adıyaman Province of Türkiye. *Applied Fruit Science, 66*(4), 1601–1607. https://doi.org/10.1007/s10341-024-01124-6
8. Balasubramaniam, G., Sekar, M., & Badami, S. (2020b). Pharmacognostical, physicochemical and phytochemical evaluation of *Strobilanthes kunthianus* (Acanthaceae). *Pharmacognosy Journal*, 12(4), 731–741. https://doi.org/10.5530/pj.2020.12.106
9. Balasubramaniam, G., Sekar, M., & Badami, S. (2021). In-vitro antioxidant and cytotoxic properties of *Strobilanthes kunthianus*. *Research Journal of Pharmacy and Technology, 14*(5), 2522–2528. https://doi.org/10.52711/0974-360X.2021.00444
10. Balasubramaniam, G., Sekar, M., Varadarajan, M., & Badami, S. (2020a). Antioxidant and hepatoprotective activities of *Strobilanthes kunthianus* against carbon tetrachloride-induced hepatotoxicity in rats. *Pharmacognosy Journal, 12*(5), 1143–1151. https://doi.org/10.5530/pj.2020.12.161
11. Bera, S., Das, S., & Kalindi, D. (2020). Botany, pharmacology and conservation status of wonder flower: Neelakurinji (*Strobilanthes kunthiana* (Nees) T. Anderson ex Benth). *Bulletin of the National Research Centre, 44*(1), 124. https://doi.org/10.1186/s42269-020-00379-9
12. Brijyog, B., Singh, B., Das, S., & Maithi, A. (2014). Antioxidant property for lipophilic extract of *Strobilanthes kunthiana* flowers. *Indian Journal of Research in Pharmacy and Biotechnology*, 2, 1005–1009.
13. Carbone, I., & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia, 91*(3), 553–556. https://doi.org/10.1080/00275514.1999.12061051
14. Chaffin, A. G., Dee, M. E., Boggess, S. L., Trigiano, R. N., Bernard, E. C., & Gwinn, K. D. (2020). First report of *Chaetomium globosum* causing a leaf spot of hemp (*Cannabis sativa*) in Tennessee. *Plant Disease, 104*(5), 1540. https://doi.org/10.1094/PDIS-08-19-1697-PDN
15. Chandran, K. C. I., & Indira, G. (2016). Quantitative estimation of total phenolic, flavonoid, tannin and chlorophyll content of leaves of *Strobilanthes kunthiana* (*Neelakurinji*). *Journal of Medicinal Plants Studies, 4*(4), 282–286.
16. Desu, B. S. R., Elango, K., Kumar, M. N. S., Suresh, B., Manimaran, S., & Nanjan, M. J. (2012). Analgesic activity of *Strobilanthes kunthianus* and *Strobilanthes cuspidatus*. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 3, 435–438.
17. Dreischhoff, S., Das, I. S., Häffner, F., Wolf, A. M., Polle, A., & Kasper, K. H. (2023). Fast and easy bioassay for the necrotizing fungus *Botrytis cinerea* on poplar leaves. *Plant Methods, 19*, 32. https://doi.org/10.1186/s13007-023-01011-3
18. Everlyne, I. M., Darsini, D. T. P., & Yadav, S. A. (2016). Unraveling antibiofilm potency of *Strobilanthes kunthiana* (Nees) T. Anderson ex Benth against throat-infectious methicillin-resistant *Staphylococcus aureus*. *Indo American Journal of Pharmaceutical Research*, 6, 5707–5716.

19. Everlyne, I. M., Sangilimuthu, A. Y., & Darsini, D. T. P. (2015). Spectral analyses of the bioactive compounds present in the ethanolic leaf extract of *Strobilanthes kunthiana* (Nees) T. Anderson ex Benth. *Advances in Bioscience*, 6, 65–71.
20. Feng, C., Xu, F., Li, L., Zhang, J., Wang, J., Li, Y., Liu, L., Han, Z., Shi, R., Wan, X., & Song, Y. (2023). Biological control of *Fusarium* crown rot of wheat with *Chaetomium globosum* 12XP1-2-3 and its effects on rhizosphere microorganisms. *Frontiers in Microbiology, 14*, 1133025. https://doi.org/10.3389/fmicb.2023.1133025
21. Fernandes, M. C., & Krishnan, S. (2019). Anatomical characterization of *Strobilanthes* (Acanthaceae) species from the northern Western Ghats of India and its implication for identification at vegetative state. *Nordic Journal of Botany, 37*(7), e02550. https://doi.org/10.1111/njb.02550
22. Glass, N. L., & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology, 61*(4), 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995
23. Guo, J.-W., Cheng, J.-S., Yang, L.-F., Zhou, Y.-L., Wang, H.-F., Chen, J.-Y., Abdala Mohamad, O., Tian, X.-J., Liu, Y.-H., & Li, W.-J. (2016a). First report of a leaf spot disease caused by *Chaetomium globosum* on pomegranate from Yunnan, China. *Plant Disease, 100*(1), 223. https://doi.org/10.1094/PDIS-03-15-0243-PDN
24. Guo, Y., Olsen, R. T., Kramer, M., & Pooler, M. (2016b). Use of mycelium and detached leaves in bioassays for assessing resistance to boxwood blight. *Plant Disease, 100*(8), 1622–1626. https://doi.org/10.1094/PDIS-01-16-0016-RE
25. Hassan, S., Nisa, M., Wani, A. H., Majid, M., Jan, N., & Bhat, M. Y. (2022). First report of *Chaetomium globosum* causing leaf spot disease of *Solanum melongena* in Kashmir Valley, India. *New Disease Reports, 46*(1), 22–26. https://doi.org/10.1002/ndr2.12119
26. Hyder, A. B. K., & Devika, M. A. (2024). *Strobilanthes kunthiana. The IUCN Red List of Threatened Species 2024: e.T239578615A239579829.* https://doi.org/10.2305/IUCN.UK.2024-1.RLTS.T239578615A239579829.en
27. Jobin, V., Das, A., Harikrishnan, C. P., Chanda, R., Lawrence, S., & Robin, V. V. (2022). *Patterns of secondary invasion in the understory of exotic, invasive timber stands*. *bioRxiv*. https://doi.org/10.1101/2022.11.29.518341
28. Kedves, O., Kocsubé, S., Bata, T., Andersson, M. A., Salo, J. M., Mikkola, R., Salonen, H., Szűcs, A., Kedves, A., Kónya, Z., Vágvölgyi, C., Magyar, D., & Kredics, L. (2021). *Chaetomium* and *Chaetomium*-like species from European indoor environments include *Dichotomopilus finlandicus* sp. nov. *Pathogens, 10*(9), 1133. https://doi.org/10.3390/pathogens10091133
29. Kerala Forests and Wildlife Department. (2013). *Third management plan of Eravikulam National Park, 2012–2013 to 2021–2022*. Government of Kerala, 205 pp.
30. Kim, D. M., Lee, M. H., Suh, M. K., Ha, G. Y., Kim, H., & Choi, J. S. (2013). Onychomycosis caused by *Chaetomium globosum*. *Annals of Dermatology, 25*(2), 232–236. https://doi.org/10.5021/ad.2013.25.2.232

31. Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: Molecular Evolutionary Genetics Analysis version 12 for adaptive and green computing. *Molecular Biology and Evolution, 41*(12), msae263. https://doi.org/10.1093/molbev/msae263
32. Lockwood, I. (2006). Kurinji crown. *Frontline*, 23, 66–72
33. Madbouly, A. K., & Abdel-Wareth, M. T. A. (2020). The use of *Chaetomium* taxa as biocontrol agents. In A. Abdel-Azeem (Ed.), *Recent developments on genus Chaetomium* (*Fungal Biology* series, pp. 251–266). Springer, Cham. https://doi.org/10.1007/978-3-030-31612-9_10
34. Matheny, P. B., Wang, Z., Binder, M., Curtis, J. M., Lim, Y. W., Nilsson, R. H., Hughes, K. W., Hofstetter, V., Ammirati, J. F., Schoch, C. L., & Langer, E. (2007). Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution, 43*(2), 430–451. https://doi.org/10.1016/j.ympev.2006.08.024
35. Matthew, K. M. (1959). The vegetation of Kodaikanal grassy slopes. *Journal of the Bombay Natural History Society*, 56, 387–422.
36. Matthew, K. M. (1971). The flowering of *Strobilanthes* (Acanthaceae). *Journal of the Bombay Natural History Society*, 67, 502–506.
37. Murugan, R. V., Sridharan, G., Atchudan, R., Arya, S., Nallaswamy, D., & Sundramoorthy, A. K. (2025). A facile synthesis of bimetallic copper–silver nanocomposite and their application in ascorbic acid detection. *Current Nanoscience, 21*(2), 309–318.https://doi.org/10.2174/0115734137281377240103062220
38. Najafzadeh, M. J., Fata, A., Naseri, A., Keisari, M. S., Farahyar, S., Ganjbakhsh, M., Ziaee, M., Dolatabadi, S., & de Hoog, G. S. (2014). Implantation phaeohyphomycosis caused by a non-sporulating *Chaetomium* species. *Journal de Mycologie Médicale, 24*(2), 161–165. https://doi.org/10.1016/j.mycmed.2013.09.007
39. Pettitt, T. R., Wainwright, M. F., Wakeham, A. J., & White, J. G. (2011). A simple detached leaf assay provides rapid and inexpensive determination of pathogenicity of *Pythium* isolates to 'all year round' (AYR) chrysanthemum roots. *Plant Pathology, 60*(5), 1000–1009. https://doi.org/10.1111/j.1365-3059.2011.02451.x
40. Prabakaran, R., & Kirutheka, E. (2018). GC–MS, phytochemicals and antioxidant activities of *in vitro* callus extracts of *Strobilanthes kunthiana* (Nees) T. Anderson ex Benth: An endemic plant of Acanthaceae. *Brazilian Journal of Biological Sciences, 5*(10), e322. https://doi.org/10.21472/bjbs.051015
41. Preethi, F., & Suseem, S. R. (2014). A comprehensive study on an endemic Indian genus — *Strobilanthes*. *International Journal of Pharmacognosy and Phytochemical Research*, 6, 459–466.
42. Rajasekaran, A., Loganathan, V., Jaewanth, A., & Jayakar, B. (2000). Central nervous activity of *Strobilanthes kunthiana* leaf. *Hamdard Medicus*, 43, 38–40.
43. Reddy, D. B., Kumar, H. J., Srilatha, P., Pavani, T., Murthy, K. G. K., Neelima, P., Reddy, I. V. S., Reddy, M. S., & Ramakrishna, A. (2024). First report of *Chaetomium globosum* causing leaf spot on *Averrhoa carambola* in India. *Plant Disease, 108*(7), 2222. https://doi.org/10.1094/PDIS-02-24-0353-PDN

44. Rehner, S. A., & Buckley, E. (2005). A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia, 97*(1), 84–98. https://doi.org/10.1080/15572536.2006.11832842
45. Robinson, M. E. (1935). The flowering of *Strobilanthes* in 1934. *Journal of the Bombay Natural History Society*, 38, 117–122.
46. Sajeev, T. V., Sankaran, K. V., & Suresh, T. A. (2012). *Are alien invasive plants a threat to forests of Kerala? KFRI Occasional Paper*. Forest Health Programme Division, Kerala Forest Research Institute, Peechi.
47. Samaga, P. V., & Rai, V. R. (2016). Diversity and bioactive potential of endophytic fungi from *Nothapodytes foetida*, *Hypericum mysorense* and *Hypericum japonicum* collected from the Western Ghats of India. *Annals of Microbiology, 66*(1), 229–244. https://doi.org/10.1007/s13213-015-1099-9
48. Samaga, P. V., Rai, V. R., & Rai, K. M. L. (2014). Production of an antimicrobial cytochalasan by an endophytic *Chaetomium globosum* HYML55 from *Hypericum mysorense* and its RNA secondary structure analysis. *Mycology*, 5(4), 566–578. https://doi.org/10.1080/02757540.2013.878335
49. Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., *Fungal Barcoding Consortium*, Bolchacova, E., & Voigt, K. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences, 109*(16), 6241–6246. https://doi.org/10.1073/pnas.1117018109
50. Schulz, B., Wanke, U., Draeger, S., & Aust, H. J. (1993). Endophytes from herbaceous plants and shrubs: Effectiveness of surface sterilization methods. *Mycological Research, 97*(12), 1447–1450.
51. Shanthiyaa, V., Saravanakumar, D., Rajendran, L., Karthikeyan, G., Prabakar, K., & Raguchander, T. (2013). Use of *Chaetomium globosum* for biocontrol of potato late blight disease. *Crop Protection, 52*, 33–38. https://doi.org/10.1016/j.cropro.2013.05.006
52. Simmons, E. G. (2007). *Alternaria: An identification manual – fully illustrated and with catalogue raisonné* (CBS Biodiversity Series No. 6). CBS Fungal Biodiversity Centre, Utrecht.
53. Sinclair, J. B., & Dhingra, O. D. (2017). *Basic plant pathology methods* (2nd ed.). CRC Press.
54. Singh, J., Aggarwal, R., Bashyal, B. M., Darshan, K., Meena, B. R., Yadav, J., Aditya, S., & Hussain, Z. (2022). Physiological and growth responses of tomato plants to different delivery methods of biocontrol agent *Chaetomium globosum*. *Journal of Community Mobilization and Sustainable Development, 17*(2), 420–428.
55. Singh, R. K., & Arigela, R. K. (2019). Gregarious flowering of two endemic *Strobilanthes* species (Acanthaceae) in southern Western Ghats, India, and typification of two names. *NeBIO*, 10(4), 227–232.
56. Sivarajan, V. V., & Pradeep, A. K. (1995). *Aristea ecklonii* Baker (Iridaceae) — a new record for India. *Rheedea, 5*(2), 173–176.
57. Sridharan, G., Murugan, R. V., Atchudan, R., Arya, S., & Sundramoorthy, A. K. (2025). Electrochemical detection of dopamine using green-synthesized gold nanoparticles from *Strobilanthes kunthiana* leaf extract. *Nano LIFE, 15*(1), 2450015. https://doi.org/10.1142/S1793984424500154

58. Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution, 10*(3), 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023
59. Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research, 22*(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673
60. Uikey, W., Raghuwanshi, K. S., & Uikey, D. W. (2020). Influence of culture media on growth, colony character and sporulation of *Chaetomium globosum* fungus. *International Journal of Current Microbiology and Applied Sciences, 9*(6), 2567–2572.
61. Wang, X. W., Lombard, L., Groenewald, J. Z., Li, J., Videira, S. I. R., Samson, R. A., Liu, X. Z., & Crous, P. W. (2016). Phylogenetic reassessment of the *Chaetomium globosum* species complex. *Persoonia, 36*, 83–133. https://doi.org/10.3767/003158516X689657
62. White, T. J., Bruns, T., Lee, S. J. W. T., & 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). Academic Press, San Diego.
63. Yadav, M. K., & Chandra, R. (2014). Effect of culture media, pH and temperature on mycelial growth of *Agaricus bisporus* strains. *Journal of Pure and Applied Microbiology, 8*(3), 2497–2500.
64. Zhu, M., Fang, Z., Zhuang, M., Zhang, Y., Lv, H., Ji, J., Yang, L., & Wang, Y. (2021). First report of *Chaetomium globosum* causing leaf blight on *Brassica oleracea* in China. *Plant Disease, 105*(4), 1204. https://doi.org/10.1094/PDIS-07-20-1540-PDN

Figures

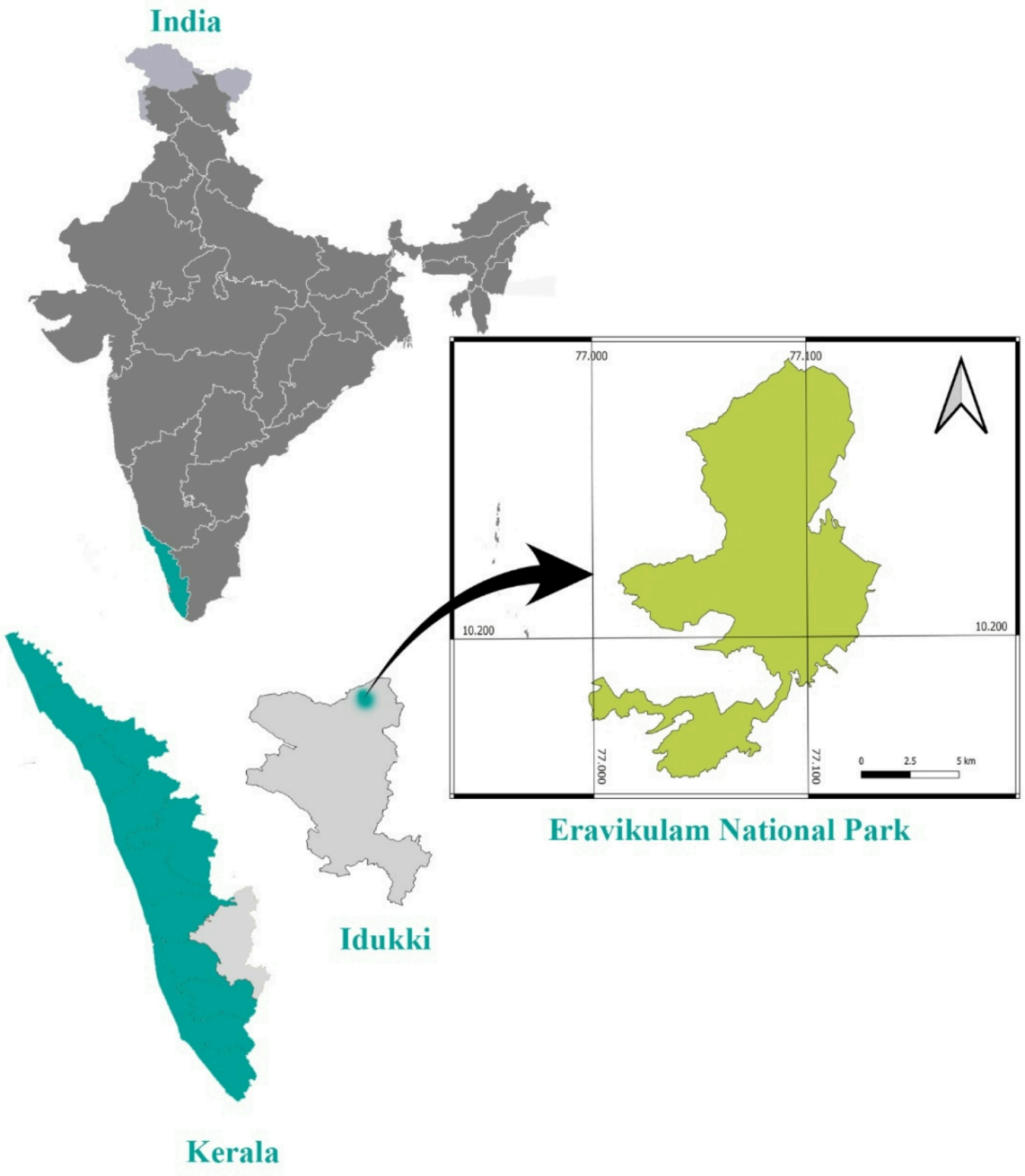

Figure 1

Map of study area

Figure 2

Various symptoms in the field; (a) leaf blight (*Chaetomium globosum*); (b) Field observation of severe foliar blight symptoms on *Strobilanthes kunthiana*under natural conditions in the shola-grassland ecosystem; (c) healthy *Strobilanthes*plant with flower

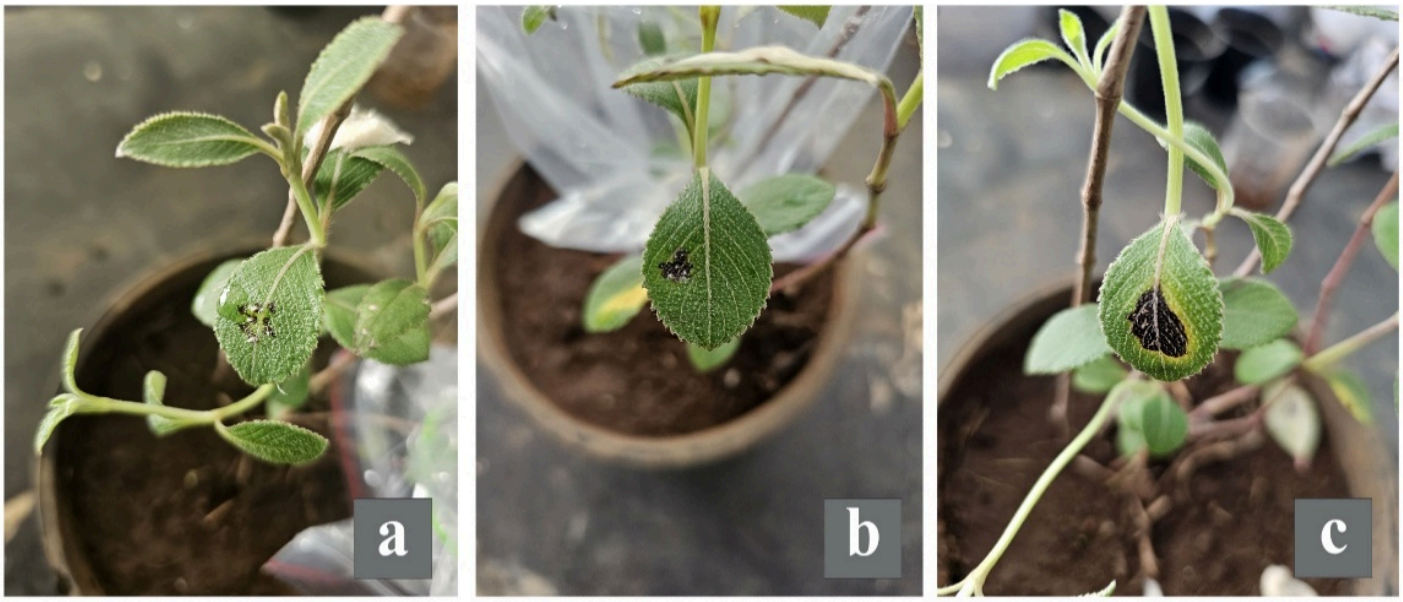

Figure 3

Various stages of pathogenicity assessment. of leaf blight disease (a: 2nd day; b: 3th day and c: 5th day).

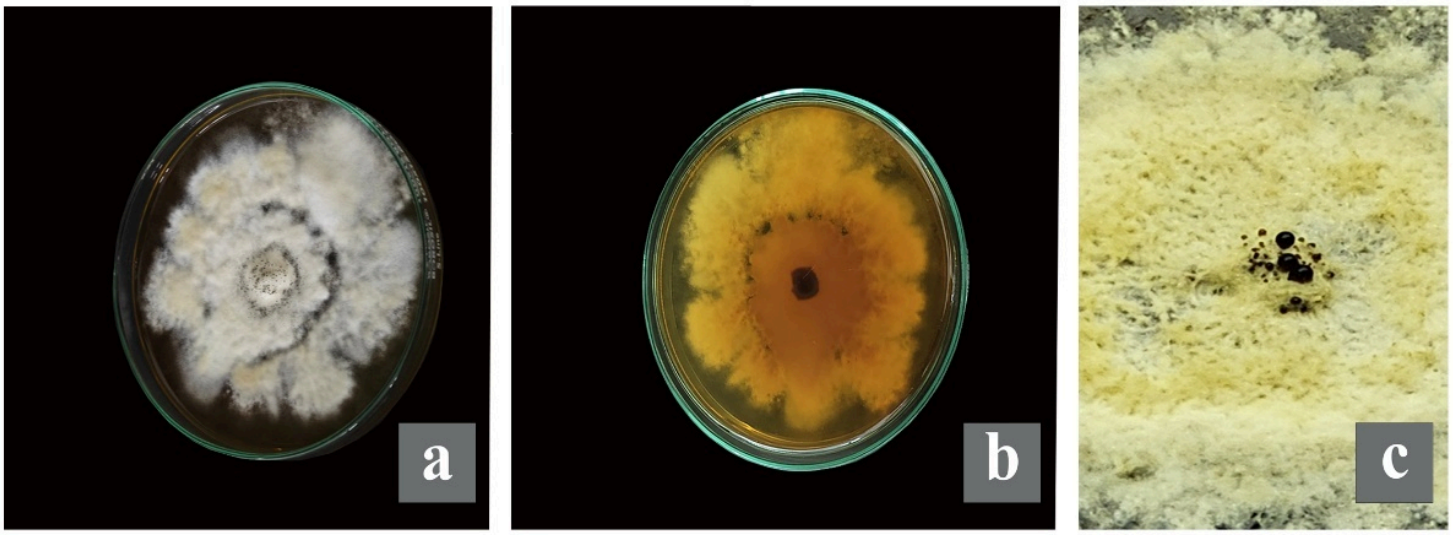

Figure 4

Photographs of *C. globosum* culture at 2 weeks on PDA medium (a) frond side; (b) back side and (c) conidiomata

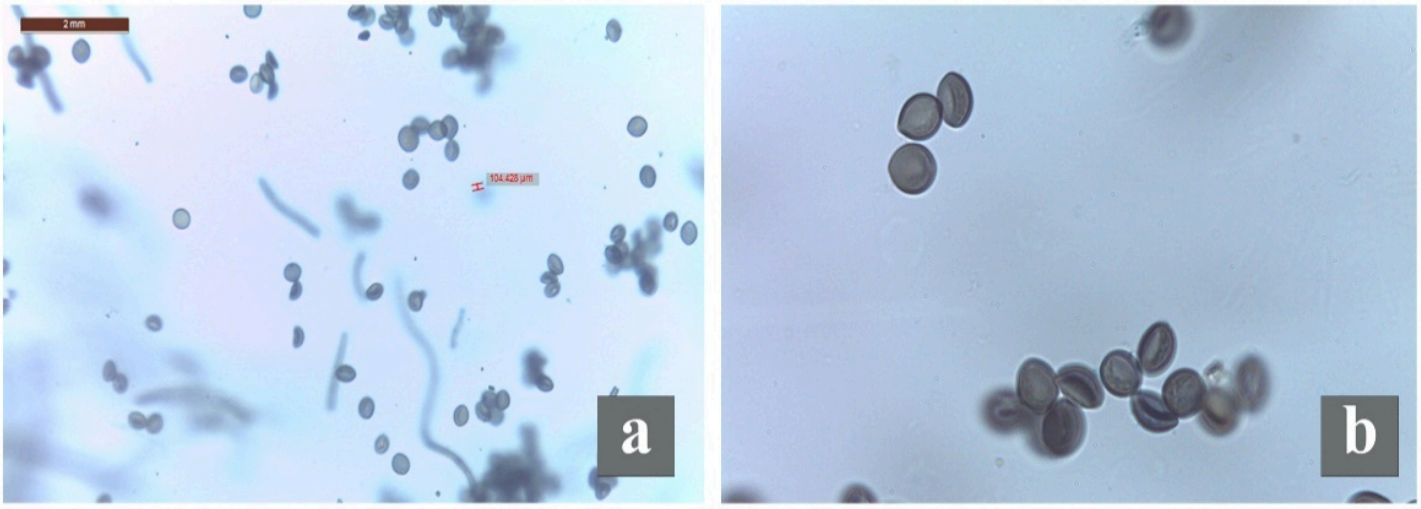

Figure 5

Spores of *C. globosum* 40X (a) and 100X (b) observed under microscope.

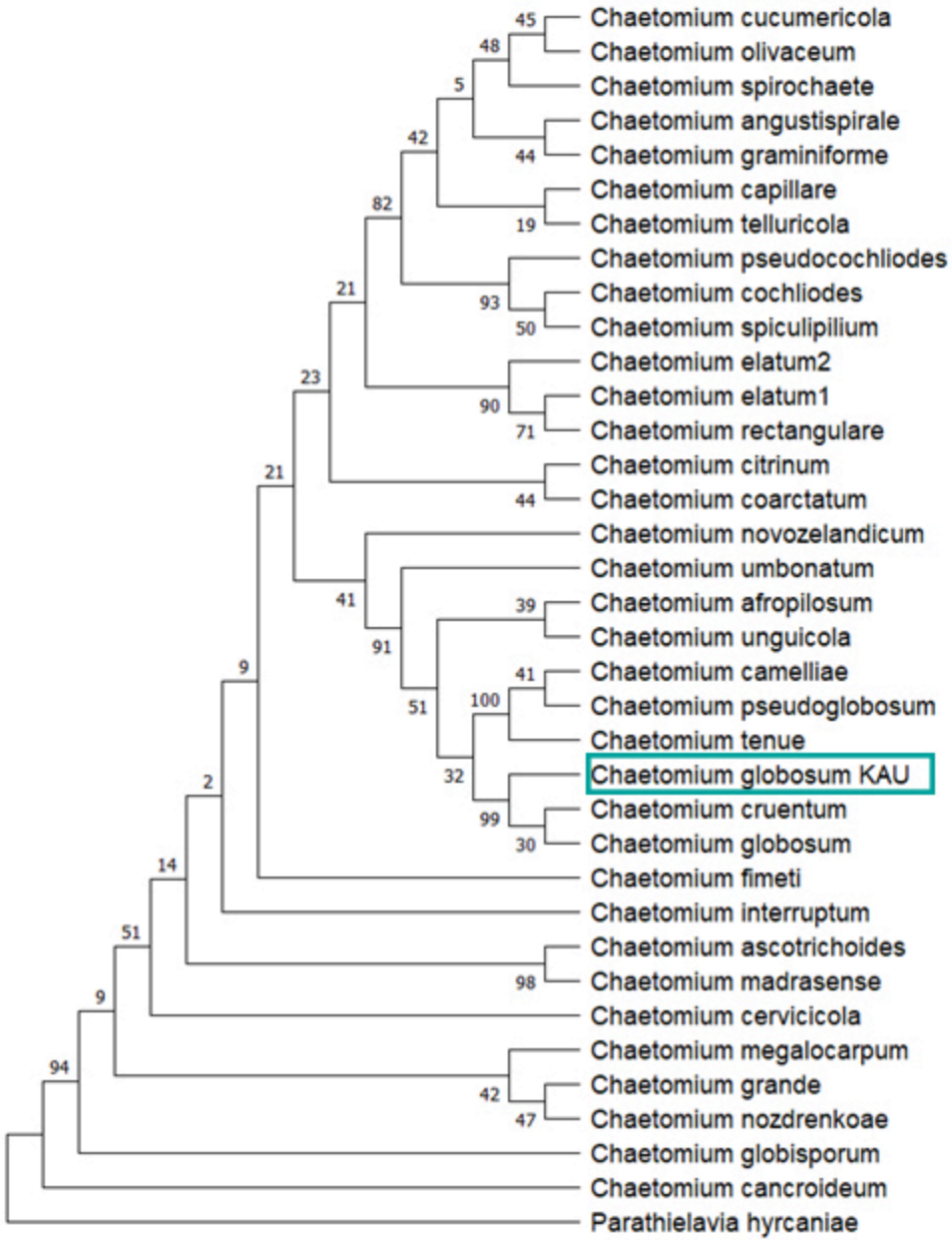

Figure 6

Maximum likelihood phylogenetic tree generated through analysis of concatenated alignment of ITS, tub2 and tef1 gene sequenced data of isolates of *Chaetomium*spp. along with other similar taxa. Bootstrap values from 1000 replicates are shown above the nodes.

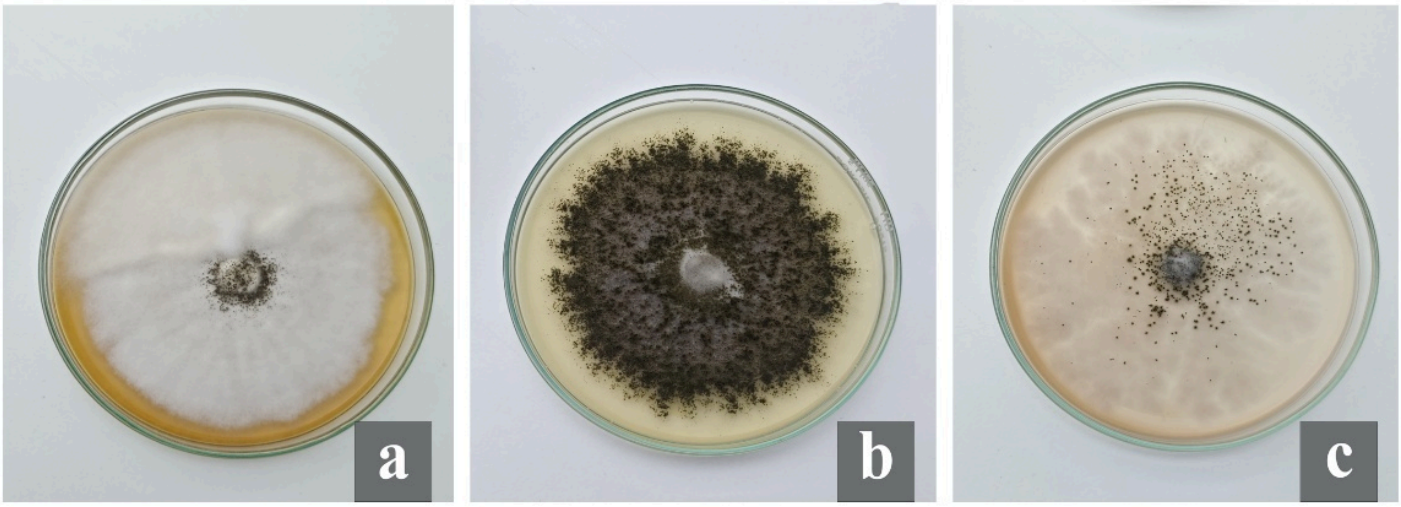

Figure 7

Cultural characteristics of *C. globosum* on different media (a; SDA, b:PDA and c:RB)

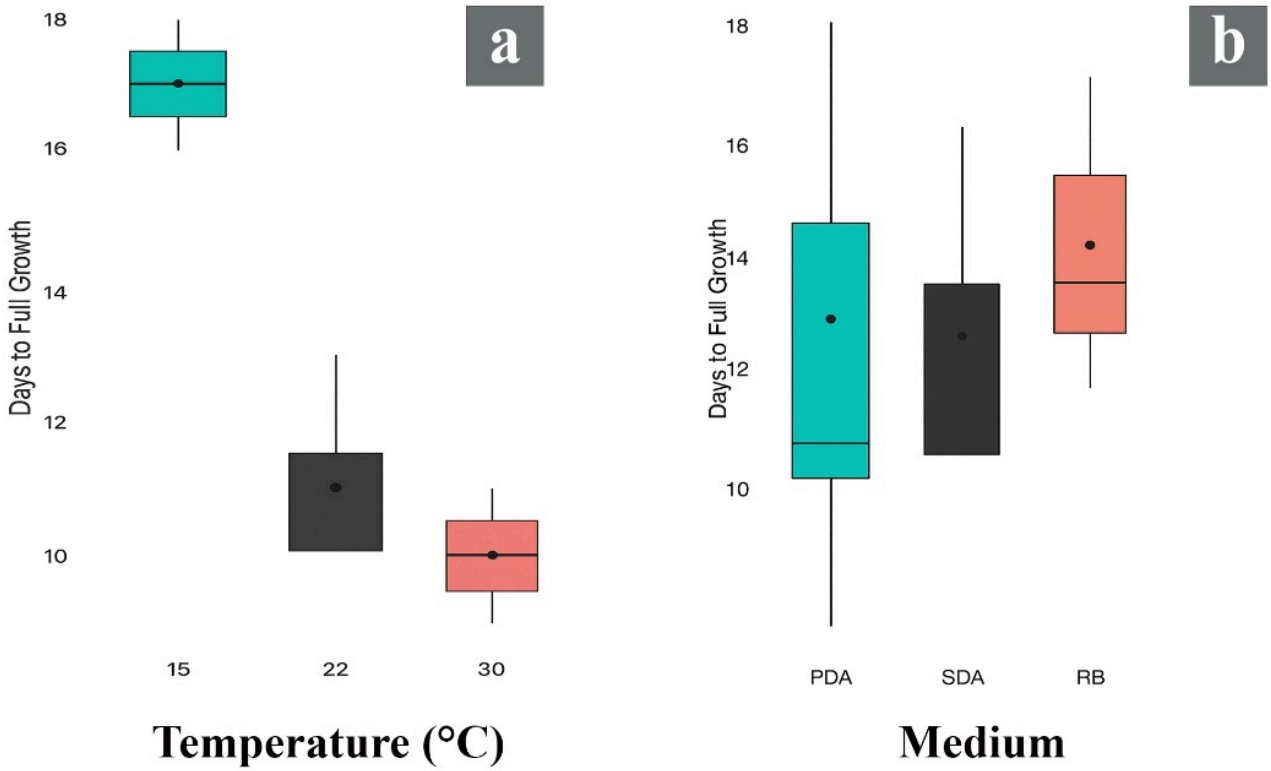

Figure 8

Effect of temperature (a) and culture medium (b) on days to full growth of *C. globosum*

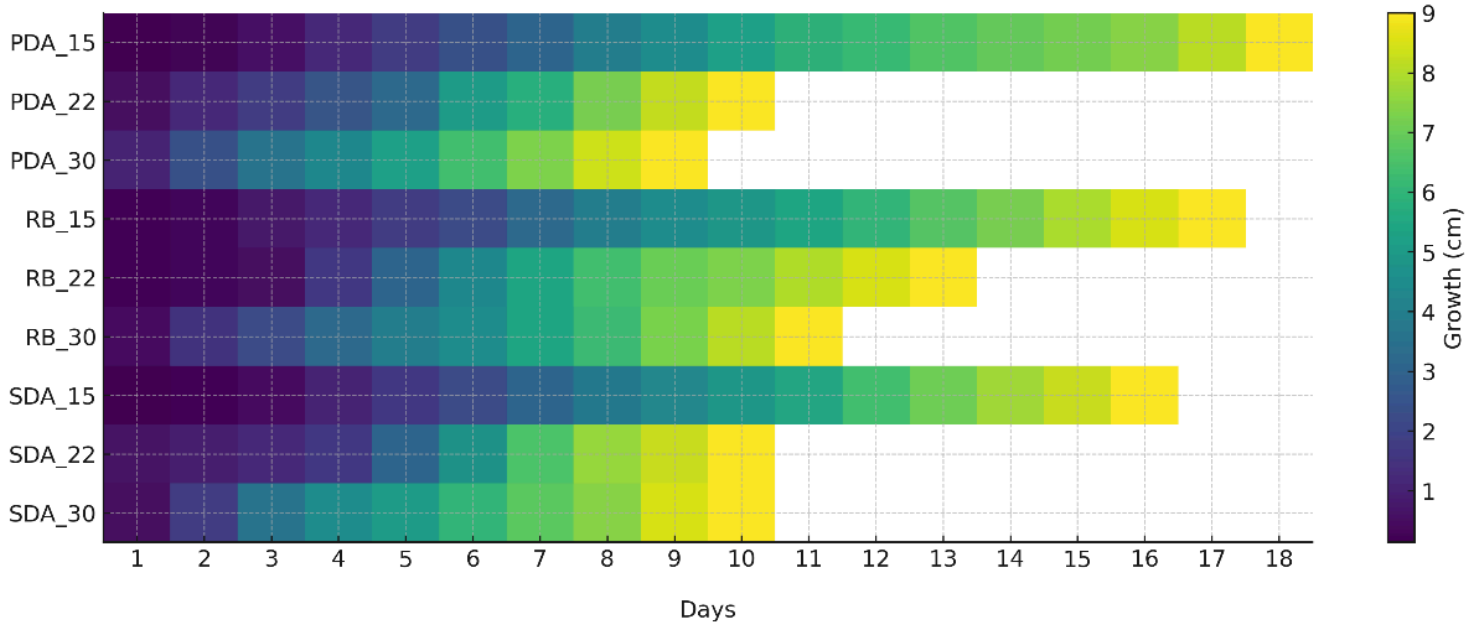

Figure 9

Daily radial growth (cm) of *C. globosum* across different media and temperatures