**ABSTRACT**

*Gardenia latifolia* is commonly known as Indian boxwood or Ceylon boxwood. Various parts of this plant are utilized to treat several cases of inflammatory pain, skin diseases, caries in humans, snake bite, stomachache, haemorrhage along with the ephemeral fever in live stocks. During the routine survey of Dr. Harisingh Gour Vishwavidyalaya Campus, Sagar for mycotaxonomic evaluation of terrestrial plants an interesting fungal specimen was encountered on *G. latifolia* Aiton which upon detailed morphological observations and mycotaxonomic treatment, proved to be a novel fungal species *Gyrothrix sagarensis*. It is also noteworthy that most of the species of this genus *Gyrothrix* (Corda) Corda, are reported on dead plant parts while the present novel species was collected and examined on living plant parts (i.e. on twig) of *G. latifolia*.
Introduction

Fungi cause the deterioration and spoilage of crops, vegetables, fruits, forest plants, and other materials. Plants and their various products are invariably used variously in every walks of day to day human life and therefore, the associated fungi as the causal organisms are of prime importance (Prabakaran et al., 2011). Dr. Harisingh Gour Vishwavidyalaya Campus is situated 5 Km east of Sagar city and its campus covers an area of 1312.89 acres over Pathariya Hills connected to the Vindhya Range, surrounded by tropical dry deciduous and miscellaneous type forests (Vyas et al., 2014; Reshi et al., 2017) (about 100 acres) within its campus and has effectively contributed to the maintenance and conservation of ecosystem and its associated biodiversity (http://dhsgsu.ac.in/profile.html). Gardenia latifolia is commonly present in the deciduous forests along the water springs in the major portion of the Indian sub-continent. Different parts of this plant are utilized and treated for many cases of inflammatory pain, skin diseases, caries in humans, snake bite, stomach ache, haemorrhage along with ephemeral fever in live stocks. This plant is referred to as Indian boxwood or Ceylon boxwood (Tamilselvi et al., 2018; Md et al., 2019). Fungal species Gyrothrix sagarensis was encountered on G. latifolia Aiton (Family Rubiaceae) during the routine survey for the collection, identification, and study of microfungi on terrestrial plants of Dr. Harisingh Gour Vishwavidyalaya Campus, Sagar. Most of the species of Gyrothrix (Corda) Corda, are reported on dead plant parts while the present novel species was collected from the living twig of G. latifolia Aiton. The genus Gyrothrix is characterized by superficial, effuse, grey to dark or black, velvety, colonies; profusely branched straight setae; conidiophores micronematous; conidia solitary, hyaline, smooth, without any septa developing on conidiogenous cells (Ellis, 1971). A review of the previously published literature revealed that thirty species of Gyrothrix were recorded over the world while five species namely G. cornuta, G. grisea, G. indica, G. kigeliae and G. thevetiae were reported from India. Out of these thirty Gyrothrix species, two have been made to synonymies such as Vermiculariopsiella cornuta and V. pediculata (http://www.indexfungorum.org/names/Names.asp). The frequent survey, collection, identification and systematic study of the associated fungi are the urgent need of the hour to know the correct nature and status of the fungi and their fungal propagules.

2 Materials and Methods

Infected plant samples were collected from Dr. Harisingh Gour Vishwavidyalaya Campus (23°49’25.3” N 78° 46’16.0” E) in zip lock polythene bags. During sampling irregular scattered blackish lesions, were observed on the twigs. The samples were brought to the laboratory, examined under a dissecting microscope (Magnus MS13/MS24). Slides were prepared in lactophenol cotton blue for detailed microscopic observations (Khalkho et al., 2021). Morphological characters were studied using an Olympus CX2Li Trinocular light microscope. Measurements were taken by MICAPS-MicroView software. Scanning Electron Microscopic (SEM) images were taken using NOVA NANO SEM 450 by coated fungal sample with gold-pladium and examined with FEI double beam (Dube et al., 2019). The holotype (AMH-10300) of the new species was deposited in Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute, Pune, India and an isotype (RJ-215) was deposited in the Mycological Herbarium, Department of Botany, Dr. Harisingh Gour University, Sagar, M.P., India.

3 Results

Based on velvety black symptoms, branched setae, polyblastic lageniform conidiogenous cells, aseptate hyaline conidia this fungus was identified as species of genus Gyrothrix (Corda) Corda (Ellis, 1971; Cunningham 1974; Bhardwaj et al., 2019).

3.1 Taxonomic details and Description

Gyrothrix sagarensis S. Jain, A. N. Rai and S. Bhardwaj sp. nov.

3.1.1 Etymology

The specific epithet sagarensis is based on the name of the place Sagar. (Figure 1-3)
Figure 2 (a-h) *G. sagarensis*: a. Setae with conidiophores and mycelium; b. Setae with conidiophores bearing conidia; c. Fungal attachment with host cells; d. Attachment of conidia with conidiophores; e,f,g. Detached conidia; h. Fragmented conidia.

(Scale bars a-h 20µm)
Gyrothrix sagarensis sp. nov.: A Novel Hyphomycetous Fungus on Medicinal Plant Gardenia latifolia Aiton From Central India

Figure 3 (a-c) G. sagarensis: SEM images: a. Mass of setae at 800X  b. Attachment of mycelium with host cells at 1608X magnification; c. Enlarged image of setae surface at 24000X magnification.
3.1.2 Index Fungorum no

IF558183

3.2 Diagnosis

Proposed species of *Gyrothrix* can be identified based on attachment with living host cells, structure and dimensions of conidia, an uncommon feature of fragmenting conidia.

3.3 Taxonomic description

The symptom of lesions is irregular, scattered and blackish. Colonies effuse, velvety and black. Mycelium partly immersed, olive-brown, 2–6.5 μm diameter. Setae erect, septate, repeatedly branched in the above half, verrucose, coiled at the tip, arising solitary from mycelium up to 90μm long swollen at the base 8.9–11 (9)μm, dark brown becoming pale brown toward the apex, 3.5 μm wide in the middle and 1.3μm at the tip. Conidiophores semi-macronematous, sub-hyline, smooth 3.1–11.6μm x 1.8–5μm. Conidigenous cells represent the reduction of conidiophores. The hyphae of superficial mycelium show the polyblastic, ampulliform conidal ontogeny. Conidia solitary, adhering together, scattered, sometimes detached in bundles of two, three and more, straight to slightly curved, aceros, fusiform, smooth, aseptate, hyaline 6.1–13.3 (10) × 1.6–2.2 (1.8) μm.

3.3.1 Specimen examined

On living twig of *G. latifolia*, Dr. H. S. Gour Central University Campus, Sagar, Madhya Pradesh, India, 15 September 2020, leg. S. Jain, (Holotype, AMH- 10300, Isotype RJ-215).

3.4 Key for *Gyrothrix* species reported from India

Setae branched, septate, brown, erect; conidigenous cells produced on superficial mycelium or on basal cells of setae; conidia hyaline continuous, smooth.................................1

1. Setae smooth..........................................................2

1. Setae minutely verrucose or sparsely verrucose, nearly smooth..............................................................3

2. Setae 100-160 μ. tall; conidia mostly 10–18 × 1–1.5μ..........................................................*G. grisea*

3. Setae 120-180 μ. tall; branches not regularly dichotomous; conidia 12–16×2.2–5.0μ...............................................*G. indica*

4. Setae coiled or spirally twisted, branches long, flagellate or spiral.................................................................5

5. Setae coarsely verrucose or spinulose, at least in the branches.................................................................*G. kigeliae*

5. Setae verrucose throughout, moderately branched; branches circinate.............................................................6

6. Setae 100-250 μ. tall; conidia 2.5–4.3 × 1.5–2μ.................................................................*G. kigeliae*

6. Setae 100-150μ. Tall; apices 1.0–1.5 μ.; conidia 7–10 × 1.3-1.8 μ.................................................................*G. thevetiae*

6. Setae upto 90μm tall; apices 1.3μm; conidia 6.1–13.3 (10) × 1.6–2.2 (1.8) μ..................................................*G. sagarensis* sp. nov.

7. Conidia straight;..................................................................8

8. Conidia long; 2.5–4.3 × 1.5–2μ.............................................*G. thevetiae*

8. Conidia long; 6.1–13.3 (10) × 1.6–2.2 (1.8) μ....................*G. sagarensis* sp. nov.

9. Conidia falcate; 7–10 × 1.3-1.8 μ long..................................................*G. thevetiae*

4 Discussion and Conclusions

A thorough survey of mycological literature and available information from Index Fungorum shows that thirty species of *Gyrothrix* world over and five species namely *G. cornuta*, *G. grisea*, *G. indica*, *G. kigeliae* and *G. thevetiae* from India (http://www.indexfungorum.org/names/Names.asp). Previous literature also revealed that a large number of *Gyrothrix* species are found on dead plant parts while current species *G. sagarensis* sp. nov. is distinctly collected from living plant parts. Based on key for *Gyrothrix* species reported from India and for others data is presented in Table 1 with *G. encephalarti* (Crous 2020), *G. eucalypti* (Crous 2019), *G. oleae* (Crous 2019) for comparison with the new taxon *G. sagarensis* sp. nov. which indicate that the new proposed taxon differs morphologically from the tabular species showing distinct association and attachment with living host tissues and other characters of taxonomic value (i.e. colonies, setae, conidiophores and conidia). Uncommon features of fragmenting conidia are also reported rarely (Pirozynski, 1962). The foregoing discussions justify the distinct identity of *G. sagarensis* and its disposal as a new species.
Table 1: Comparative account of *G. sagarensis* sp. nov. with allied species

| Name of species | Symptoms/Colonies | Setae | Conidiogenous cells & Conidiophores | Conidia |
|-----------------|-------------------|-------|-------------------------------------|---------|
| *G. encephalarti* Crous, 2020 | Mycelium consisting brown, smooth, septate, branched, 1.5–2 µm diam hyphae. | Setae erect, 80–130 µm long, 3–4 µm diam, brown, multiseptate, thick walled, verrucose, sub-cylindrical with apical taper, base bulbous, 5–6 µm diam, apex spirally twisted with twisted lateral branches in apical region. | Conidiophores reduced to conidiogenous cells around base of setae, ampulliform to subcylindrical, pale brown, 6–10 × 3–4 µm, prolofrating at apex. | Conidia hyaline, smooth, aseptate, fusoid, innequilateral, inner plane flat, outer plane convex, apex subobtuse, base truncate, (7–10)–12(–14) × (3–)5(–7)µm. |
| *G. eucalypti* Crous, 2019 | Mycelium internal and external, consisting of branched, septate, hyaline to pale brown, 2–3 µm diam hyphae. | Setae erect, straight to geniculatesinous, dark brown, thick walled, verrucose to warty, 100–180 µm tall, 4–5 µm wide at base, 4–10 septate, branched, forming 2–6 lateral branches. | Conidiophores reduced to conidiogenous cells (rarely with a supporting cell), arranged on hyphae around base of setae, smooth, olivaceous, pampuliform, 5–10 × 3–4 µm, giving rise to conidia via conspicuous annellations. | Conidia forming in a slimy mass, hyaline, smooth, falcate, aseptate, with excentric hilum, 0.5–1 µm diam, (8–)10–13(–15) × (2–)2.5(–3)µm. |
| *G. oleae* Crous, 2019 | Mycelium consisting of hyaline, smooth, branched, septate, 2–3 µm diam hyphae. | Setae erect, 100–150 µm long 3–4 µm diam, brown, multiseptate, thick-walled, verruculose to warty, subcylindrical with apical taper, base bulbous, 4–6 µm diam, apex spirally curved, apical region frequently with curved lateral branches. | Conidiophores reduced to conidiogenous cells arranged around the base of setae, subcylindrical to amphpulliform hyaline to subhyaline, smooth, 7–13 × 2–3 µm, proliferating percurrently at apex. | Conidia hyaline, smooth, aseptate, fusoid, innequilateral, inner plane flat, outer plane convex, apex subobtuse, tapering toward inner plane, base with excentric, truncate hilum, tapering towards inner plane, (7–)9–10(–11) × (1.5–)2(–2.5)µm. |
| *G. sagarensis* sp. nov. | The symptoms of lesions, irregular by scattered, blackish. Colonies effuse, velvety and black. Mycelium partly immersed, olive brown, 2–6.5 µm diam hyphae. | Setae erect, septate, repeatedly branched in the above half, verrucose, coiled at the tip, arising solitary from mycelium upto 90 µm long, swollen at the base 8.9–11 (9) µm, dark brown becoming pale brown toward the apex, 3.5 µm wide in the middle and 1.3 µm at tip. | Conidiogenous cells represent the reduction of conidiophores. The hyphae of superficial mycelium shows the polyblastic, ampulliform conidia ontogeny. Conidiophores semi-macrornatous, sub-hyaline, smooth. Conidiogenous cells polyblastic, lageniform 3.1–11.6 µm × 1.8–5 µm. | Conidia solitary, adhering together, scattered, sometimes detached in bundles of two, three and more, straight to slightly curved, smooth, aseptate, hyaline 6.1–13.3 (10) × 1.6–2.2(1.8) µm. |

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Conflict of interest

The authors declare that they have no conflict of interest.

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