

**MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF  
ENDOPHYTIC FUNGI ISOLATED FROM *ANDROGRAPHIS  
PANICULATA* (BURM. F.) WALL. EX NEES  
AND *CENTELLA ASIATICA* (L.) URBAN**

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**Keywords:** Morphological identification; ITS sequencing; Medicinal plant; Fungi; Bangladesh.

**Abstract**

Fungal endophytes were isolated from the leaves, stems and roots of two widely used medicinal plants viz., *Andrographis paniculata* (Burm. f.) Wall. ex Nees and *Centella asiatica* (L.) Urban. A total of 28 endophytic fungi were identified based on morphological and molecular analyses. The identified fungi were: *Aspergillus flavus* Link, *A. fumigatus* Fresen., *A. niger* Tiegh., *A. terreus* Thom, *Cladosporium* sp., *Colletotrichum* sp., *Curvularia chonburiensis* (ibpromma & K.D. Hyde, *C. hominis* Da Cunha, Madrid, Gené & Cano, *C. lunata* (Wakker) Boedijn, *C. lycopersici* Tandon & Kakkar, *Curvularia* sp., *Fusarium falciforme* (Carrión) Summerb. & Schroers, *F. phaseoli* (Burkh.) T. Aoki & Donnell, *F. solani* (Mart.) Appel & Wollenw, *F. udum* (Berk.) Wollenw, *Fusarium* sp., *Lasiodiplodia theobromae* (Pat.) Gri. & Maubl., *Monodictys paradoxa* (Corda) Hug., *M. putredinis* (Wallr.) Hug., *Penicillium commune* Thom, *P. chrysogenum* Thom, *P. oxalicum* Currie & Thom, *Penicillium* sp. 1, *Penicillium* sp. 2, *Penicillium* sp. 3, *Penicillium* sp. 4, *Scytalidium lignicola* Pesante and *Talaromyces trachyspermus* (Shear) Stolk & Samson. Among them *Aspergillus flavus*, *A. niger*, *A. terreus*, *Cladosporium* sp., *Colletotrichum* sp. and *Penicillium* sp. 1 were isolated from both the plants. *Curvularia chonburiensis*, *C. hominis*, *C. lycopersici*, *Fusarium falciforme*, *F. phaseoli*, *Monodictys paradoxa*, *Penicillium commune* and *Scytalidium lignicola* were found to be new records for Bangladesh. Findings of this study will be helpful for better understanding of endophytic fungal diversity and the species richness in those medicinal plants.

**Introduction**

The term endophytic fungi refers to the fungi that live within the plant tissues throughout their entire or partial life cycle by establishing a mutually beneficial symbiotic relationship with its host plant without causing any adverse effect or disease (Hyde *et al.*, 2019; Patchett and Newman, 2021). Endophytic fungi have been isolated from many plants, including trees, vegetables, fruits and other crops (Rosenblueth and Martinez-Romero, 2006).

Medicinal plants harbor endophytic microflora and they are valuable source of bioprospecting endophytes. *Andrographis paniculata* (Burm. f.) Wall. Ex Nees and *Centella asiatica* (L.) Urban are two widely used medicinal plants. The whole plant of *Andrographis paniculata* has been used for several applications such as anti-dote for snake-bite and poisonous stings of some insects and to treat dyspepsia, influenza, dysentery, malaria and respiratory infections (Chopra, 1980; Jarukamjorn *et al.*, 2010). Aside from healing wounds, *C. asiatica* is used for the treatment of various skin conditions such as lupus, leprosy, varicose ulcers, eczema, and psoriasis. (Brinkhaus *et al.*, 2000) and also as a blood purifier (Gohil *et al.*, 2010). Endophytic fungi from medicinal plants have significant role in pharmacology and in industries. They can also promote their

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accumulation of secondary metabolites. In the present study these two important medicinal plants, *Andrographis paniculata* and *Centella asiatica* were used for the isolation of endophytic fungi. This study will lead to evaluate the potential bioactive metabolites of the endophytes, relation between endophytes and host plants and also to study the endophytic fungal diversity and the species richness in those medicinal plants.

### Materials and Methods

*Centella asiatica* and *Andrographis paniculata* were collected from the Botanical Garden, University of Dhaka and used for the present investigation.

#### *Isolation and morphological identification of fungi*

Endophytes associated with selected samples were isolated by following “Tissue planting method” on Potato Dextrose Agar medium (CAB, 1968). Morphological identities of the fungal isolates were determined following the standard literature (Thom and Raper, 1945; Booth, 1971; Ellis, 1971,1976; Barnett and Hunter, 1972; Sutton, 1980).

#### *Molecular characterization of fungi*

Molecular identification was done by following Amer *et al.* (2009) with some modifications.

#### *DNA extraction*

Fungi were grown on PDA medium at 28°C for 10 days. Fungal mycelium was harvested by scraping the surface of 10 days old cultures with a sterile spatula from the Petri plates. One gram of fungal mycelium of each isolate was taken in a 1.5 ml sterile Eppendorf tube. The mycelium was immediately ground with a homogenizer with 400µl sterile extraction buffer (200mM Tris-HCl, 250mM NaCl, 25mM EDTA, 0.5% SDS) in each Eppendorf tube. Then 6 µL of 20 mg/ml RNase was added in each Eppendorf. Tubes were stirred with a vortex mixer so that the mixture became homogenous. The tubes were transferred to 65°C preheated water bath for 10 minutes. The samples were taken from the water bath and cooled down to room temperature. 130 µL of 3M sodium acetate, pH 5.2 was added in each tube. Tubes were vortexed for 30 seconds at maximum speed and incubated at -20° C for 10 minutes. The samples were centrifuged at 13,000 rpm for 15 minutes. The supernatants were transferred to fresh tubes and equal volume of chloroform: isoamyl alcohol mixture (24:1) was added and mixed by gentle inversion and then tubes were centrifuged at 12000 rpm for 5 minutes. The supernatant was discarded and the pellet was washed twice with 700 µL of 70% ethanol. The DNA pellets were subsequently air-dried. The resultant DNA pellet was then resuspended in 100 µL of 1 x TE (10 mM Tris- HCl, 1 mM EDTA) buffer (pH 8.0). The DNA was allowed to dissolve overnight at 4°C. Then it was stored at -20°C for further analyses.

#### *Polymerase chain reaction amplification, sequencing and phylogenetic analysis*

Molecular identification of the isolates was performed using the internal transcribed spacer (ITS) regions. PCR amplification was conducted using the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers for the ITS regions. The PCR was carried out in 0.2 ml PCR tube with 25 µl reaction volume containing 2.0 µl template DNA, 12.5 µl master mix, 1.0 µl forward primer, 1.0µl reverse primer and 8.5 µl nucleus free H<sub>2</sub>O. Reaction mixture was vortexed and centrifuged in a microcentrifuge. The PCR was initiated by an initial denaturation step at 94°C for 5 minutes following 30 cycles of 94, 54 and 72°C each for 30 sec, with a final extension step of 5 min at 72°C and ended with 4°C. PCR amplified products were stored in - 20°C freezer for analysis by resolving in 1% agarose gel. The gel was prepared using 1.0 g agarose powder containing ethidium bromide. Agarose gel

electrophoresis was conducted in 1× TAE buffer at 90 Volts and 300 mA for 40 minutes. DNA bands were photographed by a gel documentation system (model: DI-HD, UK). The purified DNA samples were sequenced through automated sequencer in the Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka, 1000. Sequences were aligned with CLUSTAL W alignment using the Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 (Kumar *et al.*, 2016). The phylogenetic tree was constructed with help of same software using the neighbor-joining method—with relative branch support of 1000 bootstrap replications.

## Results and Discussion

### *Morphological identification*

Fungal endophytes were isolated from the leaves, stems and roots of *Andrographis paniculata* and *Centella asiatica*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Cladosporium* sp., *Colletotrichum* sp., *Curvularia* sp. 1, *Curvularia* sp. 2, *Curvularia* sp. 3, *Fusarium* sp. 1, *Fusarium* sp. 2, *Fusarium* sp. 3, *Fusarium* sp. 4, *Fusarium* sp. 5, *Lasiodiplodia* sp., *Monodictys paradoxa*, *M. putredinis*, *P. chrysogenum*, *P. oxalicum*, *Penicillium* sp. 1, *Penicillium* sp. 2, *Penicillium* sp. 3, *Penicillium* sp. 4, *Penicillium* sp. 5, *Scytalidium lignicola*, and *Talaromyces trachyspermus* were identified morphologically.

### Key morphological features of the isolated fungi

***Aspergillus flavus*** Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 3 (1): 16 (1809) **(Fig. 1a)**

Olive-green colony, flat at their borders while raised in the middle. Conidiophore hyaline, coarsely roughened, up to 1.0 mm in length. Vesicles globose to sub globose, 25-45 µm in diameter. Conidia pale green, globose to sub globose, 3-4 µm in diameter.

*Specimen examined:* Isolated from *Centella asiatica* and *Andrographis paniculata* from Curzon Hall Campus botanical garden, University of Dhaka. 23 March, 2021. F. Nessa 1.

***Aspergillus fumigatus*** Fresen., Beiträge zur Mykologie 3: 81 (1863) **(Fig. 1b)**

Colonies attain a diameter of 3-5 cm within 7 days, consisting of a dense felt of dark green conidiophores intermixed with aerial hyphae bearing conidiophores. Conidiophores short, smooth-walled. Vesicles broadly clavate, 20-30 µm in diameter. Phialides directly borne on the vesicle, often greenish pigmented, 6-8 x 2-3 µm. Conidia globose to subglobose, 2.5-3.0 µm in diameter, green, rough-walled to echinulate.

*Specimen examined:* Isolated from *Centella asiatica* and *Andrographis paniculata* from Curzon Hall Campus botanical garden, University of Dhaka. 23 March, 2021. F. Nessa 2.

***Aspergillus niger*** Tiegh., Annales des Sciences Naturelles Botanique 8: 240 (1867) **(Fig. 1c)**

Colonies black powdery with conidial production. The reverse is pale yellowish white. Conidiophores arise from long, broad, thick-walled, mostly brownish, sometimes branched foot-cells. Conidia in large, radiating heads, mostly globose, irregularly roughened, 4.0-5.0 µm in diameter, uninucleate.

*Specimen examined:* Isolated from *Centella asiatica* and *Andrographis paniculata* from Curzon Hall Campus botanical garden, University of Dhaka. 23 March, 2021. F. Nessa 3.

***Aspergillus terreus*** Thom, American Journal of Botany 5 (2): 85 (1918) **(Fig. 1d)**

Colonies yellowish-brown to cinnamon-brown, consisting of a dense felt of conidiophores. Conidiophore stipes smooth-walled, hyaline. Vesicles subspherical, 10-20 µm diameter. Metulae

as long as the phialides. Conidia smooth-walled, striate with SEM, spherical to broadly ellipsoidal, 1.5-2.5  $\mu\text{m}$ , hyaline.

*Specimen examined:* Isolated from *Centella asiatica* and *Andrographis paniculata* from Curzon Hall Campus botanical garden, University of Dhaka. 16 June, 2021. F. Nessa 14.

***Cladosporium* sp. (Fig. 1e)**

Colonies raised at the center, umbonate, circular, ash to blackish ash color, thinly hairy. Conidiophore solitary, slightly flexuous, mid brown, smooth 4 – 5.6  $\mu\text{m}$  thick. Conidia arising in simple or branched chains, cylindrical, ellipsoidal, sub-hyaline, smooth, 5 – 6.5  $\times$  2 – 3.5  $\mu\text{m}$ .

*Specimen examined:* Isolated from *Centella asiatica* and *Andrographis paniculata* from Curzon Hall Campus botanical garden, University of Dhaka. 5 January, 2022. F. Nessa 32.

***Colletotrichum* sp. (Fig. 1f)**

Colony cottony white, fluffy, front side white with light orangish shade, reverse side orangish- white. Conidia hyaline, aseptate, straight to falcate, smooth, thin walled. Conidia length 9.58  $\mu\text{m}$  & width 2.41  $\mu\text{m}$ .

*Specimen examined:* Isolated from *Centella asiatica* from Curzon Hall Campus botanical garden, University of Dhaka. 11 November, 2021. F. Nessa 24.

***Curvularia hominis* Da Cunha, Madrid, Gené & Cano, Persoonia 33: 55 (2014) (Fig. 1g)**

Colonies on PDA reaching 70-80 mm diam in 1 week, white in color, with moderate aerial mycelium giving the colony a slightly cottony appearance, lobulate; reverse pale to darker luteous towards periphery. Conidia 4–5-celled, slightly curved, ellipsoidal to obovoid, the third cell from the base often larger and unequal sided, end cells subhyaline to pale brown and smooth-walled.

*Specimen examined:* Isolated from fresh and healthy root of *Centella asiatica* from the botanical garden of Curzon Hall Campus, University of Dhaka. 22 July, 2021. F. Nessa 19.

***Curvularia lunata* (Wakker) Boedijn, Bull. Jard. Bot. Buitenzorg 13 (1): 127 (1933) (Fig. 1h)**

Colonies on PDA covering the surface of the Petri dish in 1 week, center white to colourless towards periphery; abundant aerial mycelium giving the colony a cottony appearance, lobulate with a fimbriate margin; reverse pale gray. Conidia smooth-walled, pale brown, end cells paler; conidia obovoidal to broadly clavate, curved at the subterminal cell.

*Specimen examined:* Isolated from healthy stem of *Centella asiatica* from the Botanical Garden of Curzon Hall Campus, University of Dhaka. 22 July, 2021. F. Nessa 18.

***Curvularia* sp. (Fig. 1i)**

Colonies on PDA white or pale grey when young, orange to brown. Colonies effuse orangish black, fluffy, cottony, raised. Conidiophore solitary, mostly unbranched, straight or slightly undulating. Conidia mostly 4-5 septate, brown, slightly curved.

*Specimen examined:* Isolated from *Centella asiatica* from the botanical garden of Curzon Hall Campus, University of Dhaka. 11 November, 2021. F. Nessa 26.

***Fusarium falciforme* (Carrión) Summerb. & Schroers, Journal of Clinical Microbiology 40 (8): 2872 (2002) (Fig. 2a)**

Colonies off-white to pale cream, velvety or slightly fluffy, with a slightly raised centre and adpressed margin, growing slowly. Conidia colourless, ellipsoidal to reniform, aseptate or septate.

*Specimen examined:* Isolated from fresh and healthy leaves and root of *Centella asiatica* from

botanical garden of Curzon Hall Campus, University of Dhaka. 13 April, 2021. F. Nessa 10.

***Fusarium phaseoli*** (Burkh.) T. Aoki & O'Donnell, Mycologia 95 (4): 671 (2003) **(Fig. 2b)**

Colony color on PDA white with grayish tint; conidial pustules sometimes present, grayish-green to dark green under fluorescent; macro-conidia septate, 2-4 celled.

*Specimen examined:* Isolated from fresh and healthy stem of *Centella asiatica* from botanical garden of Curzon Hall Campus, University of Dhaka. 22 July, 2021. F. Nessa 19.

***Fusarium solani*** (Mart.) Appel & Wollenw., Arbeiten aus der Kaiserlichen Biologischen Anstalt für Land- und Forstwirtschaft 8: 64-78 (1910) **(Fig. 2c)**

Colonies growing rapidly, covering the surface of the Petri dish in 1 week, Aerial mycelium generally abundant, white cottony; Conidiophores arising laterally from aerial hyphae. Monophialides mostly with a rather distinct collarette. Macroconidia produced on shorter, branched conidiophores which soon form sporodochia, usually moderately curved, with short, blunt apical and indistinctly pedicellate basal cells, mostly 3-septate. Microconidia usually abundant, Chlamydo spores frequent.

*Specimen examined:* Isolated from fresh and healthy leaves and root of *Andrographis apiculata* from botanical garden of Curzon Hall Campus, University of Dhaka. 22 July, 2021. F. Nessa 21.

***Fusarium udum*** (Berk.) Wollenw., Phytopathology 1: 206 (1913) **(Fig. 2d)**

Colony brownish-black to colorless towards periphery, growth rate medium, raised at center; reverse orange center to white towards periphery with colorless border. Conidia initially produced on simple or verticillately branched conidiophores; variable in size, with a curved apex; there is no clear distinction between microconidia and macroconidia.

*Specimen examined:* Isolated from fresh stem and root of *Andrographis apiculata* from botanical garden of Curzon Hall Campus, University of Dhaka. 13 April, 2021. F. Nessa 11.

***Fusarium* sp.** **(Fig. 2e)**

Colonies growing rapidly, with white to cream-coloured aerial mycelium, reverse usually colourless. Conidiophores arising laterally from aerial hyphae. Macroconidia produced on shorter, branched conidiophores. Microconidia usually abundant, produced on elongate. Chlamydo spores frequent, singly or in pairs, terminal, rough-walled.

*Specimen examined:* Isolated from fresh and healthy leaves and root of *Andrographis apiculata* from botanical garden of Curzon Hall Campus, University of Dhaka. 16 November, 2021. F. Nessa 27.

***Lasiodiplodia theobromae*** (Pat.) Griffon & Maubl., Bulletin de la Société Mycologique de France **25: 57 (1909)** **(Fig. 2f)**

Colonies on agar greyish sepia to mouse grey to black, fluffy with abundant aerial mycelium; reverse fuscous black to black. Conidiogenous cells hyaline, simple, cylindrical to subobpyriform, holoblastic, annelidic. Conidia initially unicellular, hyaline.

*Specimen examined:* Isolated from fresh and healthy leaves of *Andrographis paniculata* from botanical garden of Curzon Hall Campus, University of Dhaka. 16 June, 2021. F. Nessa 16.

***Monodictys paradoxa*** (Corda) S. Hughes, Canadian Journal of Botany 36(6): 786 (1958) **(Fig. 2g)**

Colonies white, effuse, dotted with bundles of black conidia, reverse side black and brownish. Conidiophora micronematica. Conidiophore cells inflated. Conidia ellipsoidal, oval, wall-shaped,

Blackish, smooth, often with one or more basal cells, paler than the others, 20-43 x 17-30  $\mu\text{m}$ , black-soot, basal cell subhyaline.

*Specimen examined:* Isolated from fresh and healthy leaf of *Andrographis paniculata* from Curzon Hall Campus botanical garden, University of Dhaka. 23 June, 2021. F. Nessa 17.

***Monodictys putredinis*** (Wallr.) S. Hughes, Canadian Journal of Botany 36 (6): 785 (1958)

(Fig. 2h)

Colonies effuse, beige in color. Reverse side reddish brown. Mycelium mostly superficial. Stroma none. Setae and hyphopodia absent. Vegetative hyphae hyaline or sooty, 1-2.5  $\mu\text{m}$  wide. Conidiophora micronematic, aggregated. Conidiophore cells not markedly swollen. Conidia individually acrogenous, subglobose or ellipsoid, multicellular, wall-shaped, sometimes slightly constricted at the septa, 20-30 x 15-25  $\mu\text{m}$ , smooth.

*Specimen examined:* Isolated from fresh and healthy root of *Centella asiatica* from botanical garden of Curzon Hall Campus, University of Dhaka. 12 April, 2021. F. Nessa 8.

***Penicillium commune*** Thom, U.S.D.A. Bur. Animal Industr. Bull. 118: 56 (1910) (Fig. 2i)

Colony growing rapidly, olive-gray in color, white towards the periphery; reverse white to yellow, Conidiophore stipes rough-walled, penicilli terverticillate. Phialides flask-shaped, tapering into a narrow neck. Conidia spherical to subspherical, smooth-walled.

*Specimen examined:* Isolated from fresh and healthy stem of *Centella asiatica* from botanical garden of Curzon Hall Campus, University of Dhaka. 1 December, 2021. F. Nessa 29.

***Penicillium chrysogenum*** Thom, U.S.D.A. Bur. Animal Industr. Bull. 118: 58 (1910) (Fig. 3a)

Colonies bright green with yellow pigmentation in center, velutinous to floccose, exuding a bright yellow pigment into the medium; reverse yellow. Conidiophore stipes smooth-walled, 200-300  $\mu\text{m}$  long; penicilli usually terverticillate. Metulae 8-12  $\mu\text{m}$  long. Phialides flask-shaped, 7-10  $\mu\text{m}$  long. Conidia smooth-walled, ellipsoidal, 2.5-4.0  $\mu\text{m}$  long, blue or bluish-green.

*Specimen examined:* Isolated from *Centella asiatica* and *Andrographis paniculata* from Curzon Hall Campus botanical garden, University of Dhaka. 1 December, 2021. F. Nessa 30.

***Penicillium oxalicum*** Currie & Thom, J. Biol. Chem. 22: 289 (1915) (Fig. 3b)

Colony white, soluble pigment lacking, reverse pale to yellow. Conidial heads irregularly biverticillate. Conidiophores smooth, 200-400 x 3-3.5  $\mu\text{m}$  long. Metulae appressed. Phialides in verticils of 6-10, acerose, 10-15 x 3-3.5  $\mu\text{m}$ . Conidia elliptical, smooth.

*Specimen examined:* Isolated from fresh leaves and root of *Andrographis apiculata* from botanical garden of Curzon Hall Campus, University of Dhaka. 12 April, 2021. F. Nessa 9.

***Penicillium sp. 1*** (Fig. 3c)

Colonies olive in color, with clear white border, moderate growth; Conidiophores arising from the mycelium singly, smooth-walled. Spores minute, globose, white.

*Specimen examined:* Isolated from *Andrographis apiculata* from botanical garden of Curzon Hall Campus, University of Dhaka. 13 April, 2021. F. Nessa 12.

***Penicillium sp. 2*** (Fig. 3d)

Colony yellow in colour with bright orange pigmentation in center, reverse orange. Multiple phialides on each metullae grouped in brush-like clusters (penicilli) at the ends of the conidiophores; conidia unicellular, round.



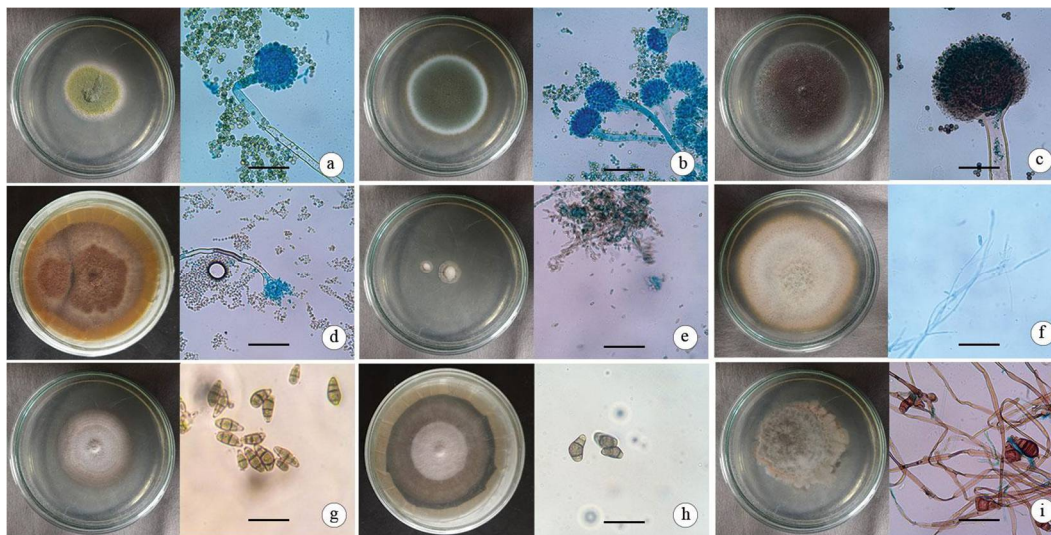


Fig 1. Colony on PDA medium and conidia under microscope: a. *Aspergillus flavus*, b. *A. fumigatus*, c. *A. niger*, d. *A. terreus*, e. *Cladosporium* sp., f. *Colletotrichum* sp., g. *Curvularia hominis*, h. *C. lunata* and i. *Curvularia* sp. (Bar = 50  $\mu$ m)

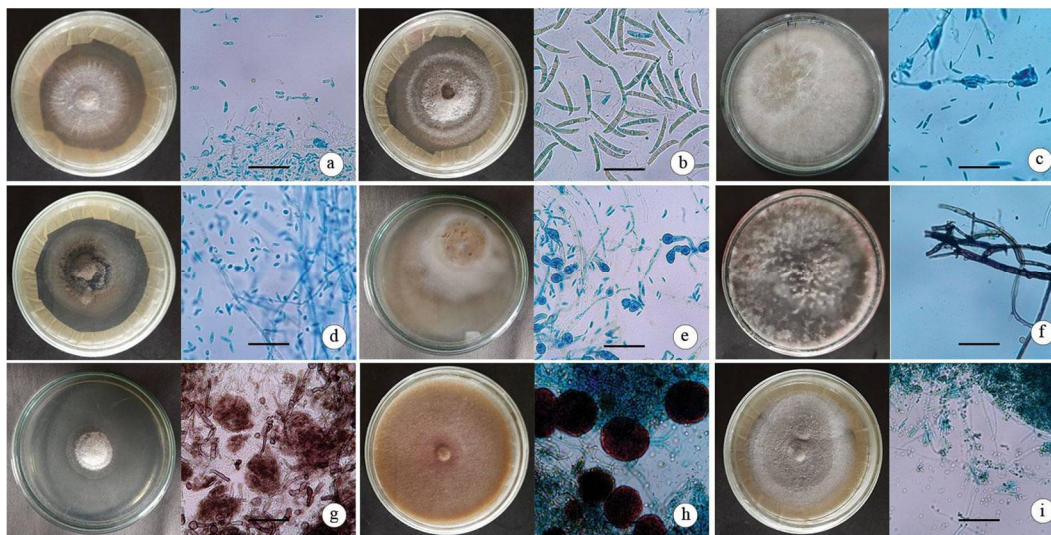


Fig 2. Colony on PDA medium and conidia under microscope: a. *Fusarium falciforme*, b. *F. phaseoli*, c. *F. solani*, d. *F. udum*, e. *Fusarium* sp., f. *Lasiodiplodia theobromae*, g. *Monodictys paradoxa*, h. *M. putredinis*, and i. *Penicillium commune*. (Bar = 50  $\mu$ m)

*Specimen examined:* Isolated from *Centella asiatica* from botanical garden of Curzon Hall Campus, University of Dhaka. 1 December, 2021. F. Nessa 31.

***Penicillium* sp. 3****(Fig. 3e)**

Colony yellow in colour with green center, raised at center. No pigmentation present. Conidia round, hyaline, rough walled.

*Specimen examined:* Isolated from fresh and healthy leaves and root of *Andrographis apiculata* from botanical garden of Curzon Hall Campus, University of Dhaka. 5 January, 2022. F. Nessa 35.

***Penicillium* sp. 4****(Fig. 3f)**

Colony pale yellow in color, orange in center, reverse orange; reddish orange pigmentation. Conidiophore greenish in colour. phialides grouped in brush-like clusters (penicilli) at the ends of the conidiophores. Conidia round, greenish yellow.

*Specimen examined:* Isolated from *Andrographis paniculata* from botanical garden of Curzon Hall Campus, University of Dhaka. 5 January, 2022. F. Nessa 35.

*Scytalidium lignicola* Pesante, Annali della Sperimentazione Agaria 11 (suppl.): 265 (1957)

**(Fig. 3g)**

Colonies effuse, flat with raised folds, cottony to woolly, initially whitish, finally becoming dark grey to black. Microscopy. Hyphae hyaline at first, later becoming brown. Arthroconidia hyaline, thin-walled, rectangular, about 5-8 x 2 µm. Chlamydospore-like conidia single or in chains, dark brown, thick-walled, swollen up to 7 µm wide.

*Specimen examined:* Isolated from fresh and healthy leaf of *Andrographis paniculata* from botanical garden of Curzon Hall Campus, University of Dhaka. 23 March, 2021. F. Nessa 4.

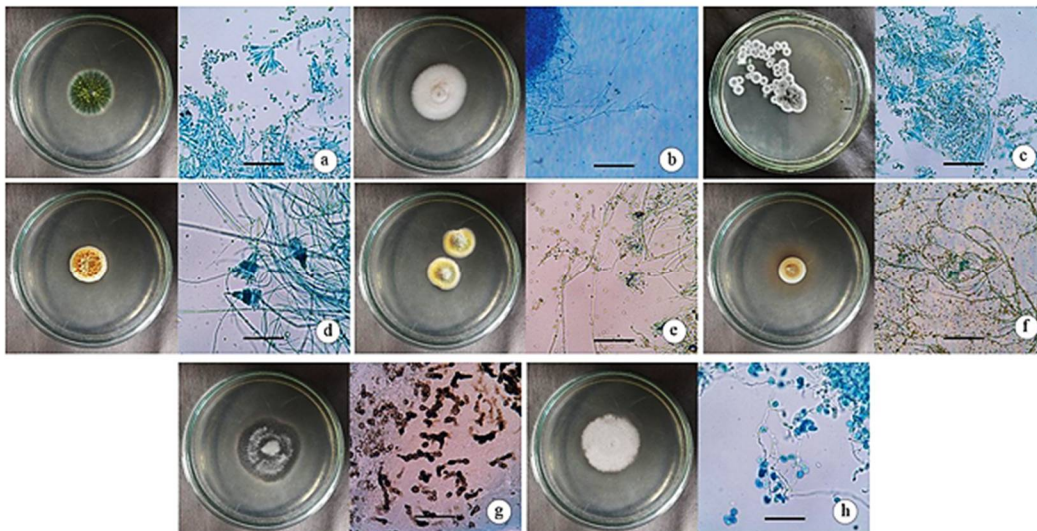


Fig 3. Colony on PDA medium and conidia under microscope: a. *Penicillium chrysogenum*, b. *Penicillium oxalicum*, c. *Penicillium* sp. 1, d. *Penicillium* sp. 2, e. *Penicillium* sp. 3, f. *Penicillium* sp. 4, g. *Scytalidium lignicola* and h. *Talaromyces trachyspermus*. (Bar = 50 µm).

*Talaromyces trachyspermus* (Shear) Stolk & Samson, Stud. Mycol. 2: 32 (1972)

**(Fig. 3h)**

The front side of the colony white and reverse side light brown, texture floccose, sporulation moderately dense to dense, and conidia numerous, colonies grew slowly. Phialides lanceolate,



metulae in small verticils. Conidia ellipsoidal to ovoidal and 5.90~ 7.87  $\mu\text{m}$  in diameter.

*Specimen examined:* Isolated from the fresh and healthy root of *Centella asiatica* from botanical garden of Curzon Hall Campus, University of Dhaka. 16 June, 2021. F. Nessa 15.

#### Molecular identification

Molecular characterization of the fungal species was conducted for proper identification using sequence analysis of ITS region. Ten isolates were identified by analyzing ITS regions sequences using the ITS1 and ITS4 as forward and reverse primers. In order to confirm at the genomic sequence level, PCR amplified bands (~550 bp) from ten samples were subjected to automated sequencing followed by BLAST analysis (Table 1).

The endophytic fungi of this study were identified on the basis of sequence similarity of ITS region. PCR amplification of internal transcribed spacer (ITS) regions generated a sharp band of approximately 550 bp in 1% agarose, confirming the presence of the desired region from each of the isolates (Fig 4).

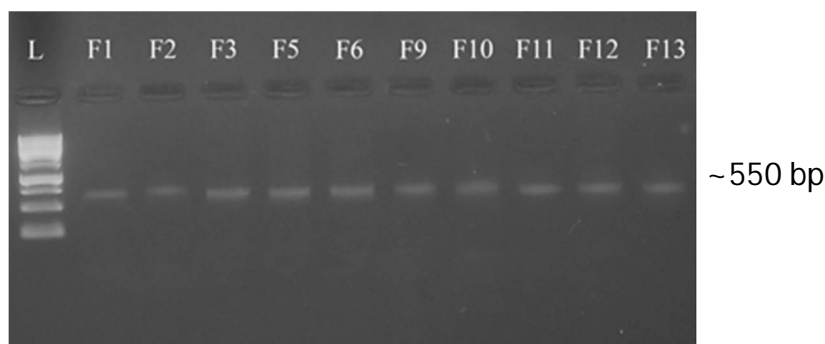


Fig 4. Gel electrophoresis of amplified ITS region of the isolated endophytic fungi. (L represents 1kb DNA ladder)

**Table 1. BLAST analysis of the amplified sequences from the isolated DNA of endophytic fungi.**

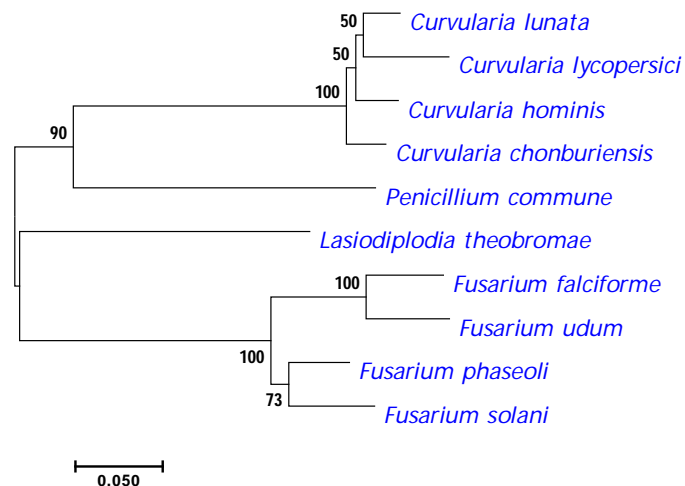
Sample ID	Name of Fungi	Max score	Total score	Query coverage (%)	E value	Identity (%)	NCBI Gene Bank Acc. No.
F12	<i>Curvularia chonburiensis</i>	704	704	75	0.0	98.47	NR168176
F9	<i>Curvularia hominis</i>	798	798	96	0.0	93.62	MN540244
F10	<i>Curvularia lunata</i>	778	778	96	0.0	92.69	OL699891
F11	<i>Curvularia lycopersici</i>	470	470	100	6E-128	99.23	MT590310
F2	<i>Fusarium falciforme</i>	656	656	84	0.0	91.53	OM372820
F5	<i>Fusarium phaseoli</i>	771	771	92	0.0	94.20	OM839786
F1	<i>Fusarium solani</i>	798	798	94	0.0	94.65	KU939057
F6	<i>Fusarium udum</i>	621	621	84	4e-173	91.01	MW647689
F13	<i>Lasiodiplodia theobromae</i>	753	753	85	0.0	97.56	OL453207
F3	<i>Penicillium commune</i>	714	714	73	0.0	98.53	EU436692

To confirm identity, the obtained DNA sequences of the isolated endophytic fungi were matched with the already available sequences in National Center for Biotechnology Information database. The obtained DNA sequences showed 98.47% identity with *Curvularia chonburiensis*, 93.62% identity with *Curvularia hominis*, 92.69% identity with *Curvularia lunata*, 99.23% identity with *Curvularia lycopersici*, 91.53% identity with *Fusarium falciforme*, 94.20% identity with *Fusarium phaseoli*, 94.65% identity with *Fusarium solani*, 91.01% identity with *Fusarium udum*, 97.56% identity with *Lasiodiplodia theobromae*, 98.53% identity with *Penicillium commune* (Table 1). Molecular analysis showed species identification of all the fungal genera studied morphologically (Table 2).

**Table 2. Comparison between morphological and molecular identification of ten fungal isolates.**

Isolates No.	Morphological identification	Molecular identification
F9	<i>Curvularia</i> sp. 1	<i>Curvularia hominis</i>
F10	<i>Curvularia</i> sp. 2	<i>Curvularia lunata</i>
F2	<i>Fusarium</i> sp. 1	<i>Fusarium falciforme</i>
F5	<i>Fusarium</i> sp. 2	<i>Fusarium phaseoli</i>
F1	<i>Fusarium</i> sp. 3	<i>Fusarium solani</i>
F6	<i>Fusarium</i> sp. 4	<i>Fusarium udum</i>
F13	<i>Lasiodiplodia</i> sp.	<i>Lasiodiplodia theobromae</i>
F3	<i>Penicillium</i> sp.5	<i>Penicillium commune</i>
F12	Unidentified	<i>Curvularia chonburiensis</i>
F11	Unidentified	<i>Curvularia lycopersici</i>

Neighbor-joining tree based on ITS sequences of ten endophytic fungi was also constructed to see the phylogenetic relationship among them (Fig. 5). From this tree, it was demonstrated that fungi belonging to same genera form same cluster.



**Fig 5.** Phylogenetic tree based on ITS sequences of ten endophytic fungi. Scale bar indicates the number of nucleotide substitution per site

Among the total 28 fungal endophytes, 18 species were recovered from different parts of the *Andrographis paniculata* plant. The fungi were *Aspergillus flavus*, *A. niger*, *A. terreus*, *Cladosporium* sp., *Colletotrichum* sp., *Curvularia chonburiensis*, *Curvularia lycopersici*, *Fusarium solani*, *Fusarium udum*, *Fusarium* sp., *Lasiodiplodia theobromae*, *Monodictys paradoxa*, *Penicillium chrysogenum*, *P. oxalicum*, *Penicillium* sp. 2, *Penicillium* sp. 3, *Penicillium* sp. 4 and *Scytalidium lignicola*. Earlier 6 fungal endophytes were isolated from the same plant to study their potential for the production of plant growth promoters and enzymes. (Adhikari Mukhopadhyay, 2022)

Sixteen species of endophytic fungi were isolated from the *Centella asiatica* plant. The fungi were *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Cladosporium* sp., *Colletotrichum* sp., *Curvularia hominis*, *C. lunata*, *Curvularia* sp., *Fusarium falsiforme*, *F. phaseoli*, *Penicillium commune*, *Penicillium* sp. 1 and *Penicillium* sp. 2. This work will lead to the study of the endophytic fungal diversity and the species richness in those medicinal plant.

Among the isolated fungi, *Curvularia chonburiensis*, *Curvularia hominis*, *Curvularia lycopersici*, *Fusarium falsiforme*, *Fusarium phaseoli*, *Monodictys paradoxa*, *Penicillium commune* and *Scytalidium lignicola* have been reported as new records for Bangladesh as these were not documented in relevant literature (Siddiqui *et al.*, 2007; Shamsi S, 2017; Nahar *et al.*, 2019; Khatun *et al.* 2022). The present investigation suggests that molecular technique is more accurate and rapid means of fungal identification. ITS-based molecular identification methods might be an important complement to conventional mycological detection by culture.

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