

Morphotaxonomy of endophytic fungi on *Cissus quadrangularis* from Amravati (MS) India

Abstract

Endophytes play significant role to establish fungal diversity. The research interest has been increasing in ecology, biology and applications of endophytic fungi. It is believed that endophytic fungi are diverse in those areas where diversity of plants are diverse and certainly Melghat Forest is one among these areas which has huge plant diversity but studies on endophytic fungal diversity from this region extremely inadequate. In the present study widely used medicinal plant *Cissus quadrangularis* was investigated to isolate the endophytic fungi. Total eight endophytes were observed from different parts (stem, leaf and petiole) of the host by using standard methods. For the specific identification of species; morphological characters and dimensions of various fruiting bodies were studied microscopically.

Keywords: taxonomy, fungal endophytes

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Introduction

The word 'endophyte' means "inside the plant" (endon Gr.=within, phyton=plant) and term was coined by De Bary in 1866 to define all microbes (including fungi, bacteria, cyanobacteria and actinomycetes) that reside within plant tissue.

The association between fungal endophytes and host plants may be symbiotic or antagonistic or slightly pathogenic in nature.¹ Major impact of endophytes is observed on ecology, distribution and physiology along with immunity of plants. Almost all the plant species (~400,000) harbour one or more endophytic organisms.² To date, only a few plants have been extensively investigated for their endophytic biodiversity and their potential to produce bioactive secondary metabolites. It is, therefore, important to determine endophytic biodiversity of medicinal plants. Identification, taxonomic position and mapping of fungi are challenging tasks. Out of many fungi about 70,000 fungal species had been isolated, identified and characterized.^{3,4} Manoharachary et al.,⁵ are of the opinion that about 27,000 fungal species have been described in India. Scientific community is continuously striving to search into the diversity of fungi and their natural potentials. The multiplicity of endophytic fungi and their role in various biochemical processes occupy most important place in the biological world and India has been the cradle for such fungi.

During mycological investigation of Melghat Forest of Amravati District several author collected and reported many rare and interesting fungal forms they are new to Maharashtra.⁶⁻¹² The traditional taxonomy of fungi is based on morphological features like shape, size and colour of various fruiting bodies.¹³ In the present study isolated fungal species were also identified morphotaxonomically by studying their macroscopic and microscopic characteristics.

Materials and methods

Collection of plant samples

The plant samples were collected from Melghat forest of Amravati district. The samples were collected in sterilized polythene bags. The

collected samples were brought to the laboratory and processed within 24hrs of collection.

Isolation of the endophytic fungi

Surface sterilization were done according to the method described by Suryanayanan¹⁴ (Table 1) to remove the epiphytes. The surface sterilized explants then inoculated at 26±2°C into the Petri dishes containing potato dextrose agar (PDA). The plates were periodically observed for fungal growth.

Table 1 Surface sterilization of explants

Chemicals	Concentration	Time
Ethanol	70%	1min
SDW	-	3min×4times
NaOCl	4%	30sec
SDW	-	3min×4times
Ethanol	70%	30sec
SDW	-	3min×4times

SDW, sterile distilled water; NaOCl, sodium hypochlorite

Microscopic observation

Permanent slides were prepared from pure colonies of isolated endophytic fungi. Morphological characters such as pycnidia, conidia and conidiogenous cells (Coelomycetes); conidia and conidiophores (Hyphomycetes) were studied under Carl Zeiss, Trinocular Research Microscope (Axioscope-A-1) with magnification of 5x, 10x, 40x and 100x.

Mountants and stain

In the present study microscopic observation of isolated endophytic fungi was initially done in water mountant. However, various fruiting structures were observed by mounting in lactophenol-cotton blue. This stain-cum-mounting medium has been used for different taxonomic groups of fungi.^{15,16}

Identification of endophytic fungi

All the endophytic isolates were identified morphologically and placed in appropriate genera and species of fungi using standard taxonomic keys and monographs¹⁷⁻²¹ were referred for identification of endophytes.

Observations and results

Arthrinium hydei Crous and Groenewald (Plate I, Figure 1)

Mycelium smooth, hyaline to pale brown, branched, septate, 2-3µm diameter. Conidiophores pale brown smooth, cylindrical, septate, branched, 22-34×3-5µm. Conidiogenous cell aggregated in clusters on hyphae, smooth, hyaline, doliiform. Conidia unicelled, brown, globose to lenticular with pale equatorial slit 10-22µm diameter in side view.

Remark: The species under study matched with *A. hydei*, and new to this region. *A. hydei* was isolated and cultured on PDA. Colonies developed were olive white with patches of grey to black.

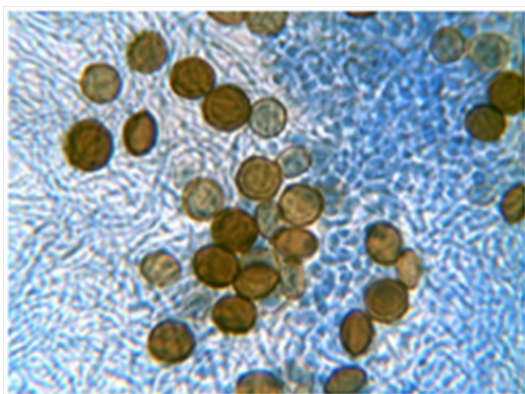


Figure 1 Mycelium with conidia of *A. hydei*.

Table 2 Comparison between species of *Arthrinium*

Species	Colony character(color)	Conidiophores	Conidia
<i>A. caricicola</i> Kunze ex Fries	Black, pulvinate	150µm×4µm	36-54µm×9-12µm
<i>A. hydei</i> Crous	Olive white with patches of grey to black	21.65-33.22µm×3.2-4.8µm	10-22µm
<i>A. marii</i> Larrondo and Calvo	Whitish- black to olivaceous grey	6-10× 2.5×4µm	7-10µm
<i>A. phaeospermum</i> (Corda) Ellis	Dark brown to greenish in color	4.7-11.6µm×2.8-5.1µm	10-16µm×3.9-7.2µm

Dense mycelial growth on PDA, colonies with uniform edges, color ranges from dark purple red to green brown in PDA. Sporodochia develop after approximately after 20 days on PDA. Sporodochia powdery, brownish to grey black in color. Mycelium hyaline, smooth, septate, brown on maturation. Short conidiophores originated on hyphae in clusters. These conidiophores branched repeatedly and are visible as dense masses. Conidiophore hyaline, claviform, 1-3 septate, smooth up to 9-11µm, producing a single dark gangliospor

Table 3 Comparison between species of *Epicoccum*

Species	Colony character (color)	Conidiophores	Conidia
<i>Epicoccum andropogonis</i> (Ces) Schol- Schwarz	Greyish black sometime radish grey	9×12µm	22-28µm
<i>Epicoccum nigrum</i> Ehrenb.Ex.Schlecht.	Dark purple red to green brown	8-11.5µm	14.5-53.9µm

Epicoccum nigrum Ehrenb.Ex.Schlecht. (Plate I, Figure 3)

Arthrinium phaeospermum (Corda) Ellis (Plate I, Figure 2)

Colonies dark brown to greenish, round, oval or irregular in shape. Mycelium hyaline to pale brown, smooth hyphae, 3-4µm in diameter. Conidiophores are cylindrical, narrow, erect or flexuous, straight, simple, smooth, hyaline 5-12×3-5µm thick, dark brown with transverse septa 48-120µm long, 2-4.5µm in diameter between septa, basal cell somewhat flattened and round or irregular in shape. Conidia sessile or sometimes borne on short hyaline pegs along the sides of the conidiophores, which are somewhat flattened, lemon shape in surface view, triangular in side view but outer edge is curve and the corners round, brown pale at tips, smooth 10-16µm long, 4-7µm wide in surface view.

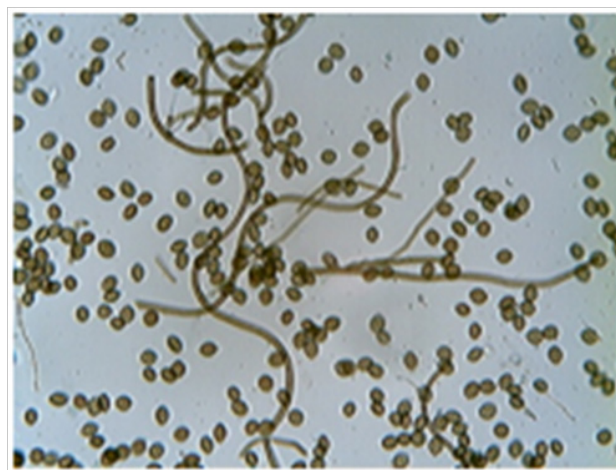


Figure 2 Mycelium with conidia of *A. phaeospermum*.

Remark: Isolated specie cultured on PDA. The colonies were dark brown to greenish in color, round, oval and irregular in shape (Table 2).

terminally. Young conidia rounded, non septate and pale in color. Mature gangliospor golden brown or brown or olivaceous or black, 40 globosed or pyriform or sometimes of irregular angular shape, septate, muriform, verrucose, 15-54µm in diameter. Mature conidia contain multiple transverse and vertical septa.

Remark: The characters of the specimen understudy were allied with *Epicoccum nigrum*, hence assigned to the said species (Table 3).



Figure 3 Mycelium with conidia of *E. nigrum*.

***Nigrospora oryzae* (Berk & Br.) Petch. (Plate I, Figure 4)**

Mycelium septate, branched, brown in colour. Conidiophore short, ampiliform, somewhat brown in colour, bearing single conidium at the tip. Conidia borne singly at the tip of the 113 conidiophores,

globose or somewhat flattened, absolutely opaque black, with hyaline membrane on the upper side 24-28×21-24µm.

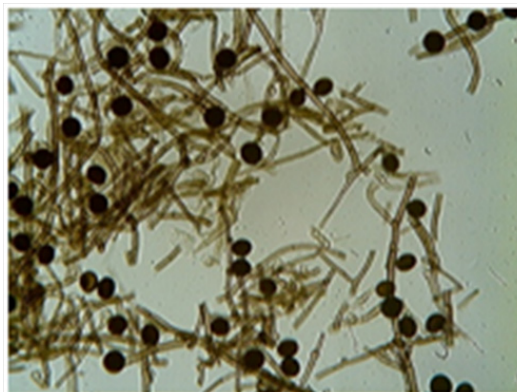


Figure 4 Mycelium with conidia of *N. oryzae*.

Remark: The species understudy found to be similar morphologically with *Nigrospora oryzae* (Table 4).

Table 4 Comparison between species of *Nigrospora*

Species	Colony character (color)	Conidiophores	Conidia
<i>Nigrospora oryzae</i> (Berk & Br) Petch.	Brown	Short, ampiliform	24-28µm ×21-24µm
<i>Nigrospora panici</i> Zimm.	Yellowish	Short, slightly inflated	25-30µm× 22-25µm
<i>Nigrospora padwickii</i> Prasad, Agnihotri and Agarwal	Pale brown	Short, swollen below apex	33.5-41.8µm ×31.8-40.2µm

***Nigrospora oryzae*(Berk & Br) Petch (Plate I, Figure 4)**

***Pestalotiopsis funerea* Stey. (Plate I, Figure 5)**

Pustules black, punctiform, globose-lenticular, 110-290µm in diameter. Conidia broad, tapering towards the base, clavate-fusoid, straight, 5-celled, 15.5-28.5×6.6-9.2µm, intermediate coloured cells guttulate, amber or olivaceous, equally coloured, lowest coloured

cell sometimes slightly paler, slightly constricted at septa, apical appendages 1-2, 4.8-6.5µm.

Remark: On comparison with known species, the present specie proved to be *P. funerea* (Table 5).

Table 5 Comparison between species of *Pestalotiopsis*

Species	Colony character(color)	Apical appendages	Conidia
<i>Pestalotiopsis funerea</i> Stey.	Dark brown	2-Jan	15.5-28.5×6.6-9.2µm
<i>Pestalotiopsis guepinii</i> (Desm) Stey.	Olivaceous brown	3-Jan	12-15µm×5.5-6.6µm
<i>Pestalotiopsis maculans</i> (Corda) Nag Raj	Pale brown to moderate brown	3-Feb	10-15.5µm

Pestalotiopsis funerea Stey (Plate I, Figure 5).

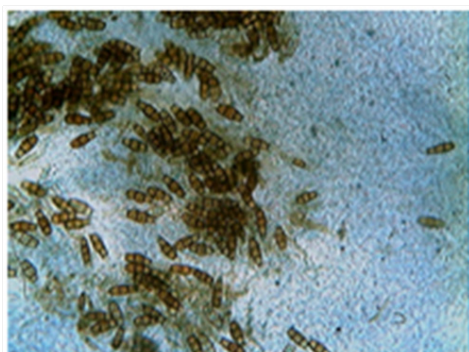


Figure 5 Mycelium with conidia of *P. funerea*.

***Pithomyces chartarum* (Berk and Curtis) Ellis (Plate I, Figure 6)**

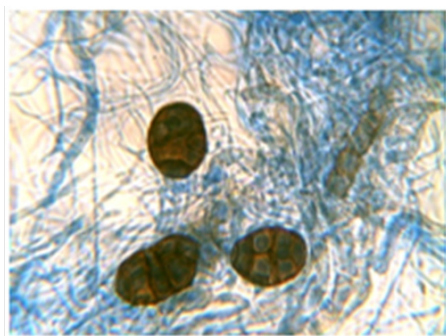
Colonies effused faint yellow, olive-green, shiny. Mycelium composed of a network of brown, smooth or rough walled hyphae. Conidiophores short peg like, 2-4µm wide, arising laterally on hyphae, subhyaline. Conidia produced singly as blunt out ends at the apex of conidiophores, oval, elliptical, obovoid, pale brown at young stage, dark brown at mature stage and often one or more oblique or longitudinal septa, 21-32µmlong, 12-35µm wide, each conidium carrying away a part of conidiophores.

Remark: The specimen shows similar characters with *Pithomyces chartarum*, hence assigned to the said species (Table 6).

Table 6 Comparison between species of *Pithomyces*

Species	Colony character(color)	Conidiophores	Conidia
<i>Pithomyces chartarum</i> (Berk and Curtis) Ellis	Olive-green	1.8-3.9µm	20.6-31.8µm×11.7-34.9µm
<i>Pithomyces atro-olivaceus</i> (Cooke & Harkn) Ellis	Dark olivaceous brown	1-5µm×1-3µm	15-35µm×7-10µm
<i>Pithomyces flavus</i> Berk.& Br.	At first yellow to olive green later dark olivaceous	2-5µm×1.5-2µm	28-45µm×15-26µm

Pithomyces chartarum(Berk and Curtis) Ellis (Plate I, Figure 6)

**Figure 6** Mycelium with conidia of *P. chartarum*.

Stachybotrys nilgirica Subram. (Plate I, Figure 7)

Mycelium composed of creeping hyaline or pale colored, branched hyphae. Conidiophores (phialophores) scattered on substratum, erect, straight, hyaline, slightly narrow above, 2-3 septate (septa 21.28-39.9µm apart), swollen at base, 68.7-93.1µm long smooth, terminating in a cluster of about 6-7 phialides, apical cell of phialophore 13.3-17µm

Table 7 Comparison between species of *Stachybotrys*

Species	Colony characters	Conidiophore	Phialides	Conidia
<i>S. chartarum</i> (Ehrenb.) Hughes	Colorless or whitish then becoming black	64-109µm×5µm	11-19µm×4.5-6.5µm	15-16.5µm×3.2-7.3µm.
<i>S. chlorohalonata</i> Andersen and Thrane	Colonies thick dark black coloured	73-91µm ×13-16µm	11-16µm×4-6µm	11-16µm×4-6µm
<i>S. nilgirica</i> Subram.	Black	68.7µm -93.1µm	13.3-17µm×2-6µm	15.3µm-27.9µm

*Stachybotrys nilgirica*Subram (Plate I, Figure 7).

Trimmatostroma hughesii Rao and Subhedar (Plate I, Figure 8)

Colony olivaceous, brown, conidiophores macronematous, septate, sporogenous cell bears on top of conidiophores, 40- 156µm long and 4-7µm wide. Conidiophores simple, septate, hyaline. Conidia small, simple, catenulate, only one longitudinal septa over single transverse septa, 6-11µm×4-9µm.

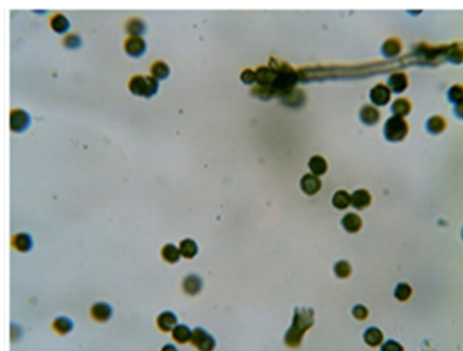
Remark: Characters of the present specimen match with *Trimmatostroma hughesii*, hence assigned to the same (Table 8).

Table 8 Comparison between species of *Trimmatostroma*

Species	Colony character(color)	Conidiophores	Conidia
<i>Trimmatostroma eriodictyonis</i> (Dearn. and Barthol) Ellis	Dark brown	45-68µm	18- 57µm×5- 26µm
<i>Trimmatostroma hughesii</i> Rao and Subhedar	Olivaceous, brown	39.6-155.7µm×3.8-7.2µm	6.2-11.4µm×3.9-8.8µm
<i>Trimmatostroma scutellare</i> (Berk and Br.) Ellis	Pale brown to moderate brown	30-50µm long, 2-4µm	13.5-32.3µm×29-39.5

Trimmatostroma hughesii Rao and Subhedar (Plate I, Figure 8)

long and 2-6µm wide and subhyaline. When young pale olive green, at maturity conidia borne singly acrogenously at the tip of phialides, 1-celled, globose, tuberculate, dark greenish black 15.3-27.8µm in diameter.

**Figure 7** Mycelium with conidia of *S. nilgirica*.

Remark: The characters of the species under study were allied with *S. Nilgirica* (Table 7).

**Figure 8** Mycelium with conidia of *T. hughesii*.

Discussion

Medicinal plants are reported as great reservoir of endophytes.²² One to several fungi could be isolated from single host.²³ In the present study total eight endophytic fungi were isolated from single host. Previous study proved that out of the total groups of fungi, anamorphic fungi are prevalent as endophytes in the plants screened throughout the world.^{24–26} The fungi found in the present investigation also showed dominance of anamorphic fungi.

Taxonomy is the discipline of classifications i.e. the assemblage of organisms into definite category (taxa). Morphological characters are very much useful in the field of taxonomy to give the special identity to the organism. In the fungal taxonomy morphological as well as microscopical characters play an important role to identify them. Colony morphology, type of hypha, spore and reproduction are characteristics which can be used to identify the fungi.

Arthrimum genus observing similarities in all the two species studied and some specific differences in structure and colour etc of conidia, the investigator through same may assist in finding morphological differences. Conidiogenesis is particularly interesting. Conidiogenous cells tend to be dolliform to subcylindrical, pale brown with clear periclinal thickening. On further development these cells become ampulliform, with a prominent elongated neck. The neck can terminate in conidia either sympodially or in some species percurrently while in others with annelation etc. One must note that variation in conidiogenesis makes it difficult to compare these characters among taxa, as conidiophores can either be hyphae with lateral loci or be reduced to dolliform conidiogenous cells. Conidial ontogeny is holoblastic. The apical holoblastic conidium, initially spherical, changes to lenticular.²⁷ When conidium matures the neck of the conidiophores becomes narrower and a circular breaking can be seen on the outer wall conidiophores (this is initial of basauxic growth). This holoblastic nature and sizes of conidia vary as per species. The conidia of *Arthrimum* can develop a spontaneous break of the wall, thus, releasing protoplasmic contents.

Epicoccum nigrum is an anamorphic ascomycete distributed globally which colonizes on different types of soil and different host plants. Morpho-cultural characters make two groups of *E. nigrum*. The first group is showing yellow to orange mycelium while second group shows grey, pink, red or brown mycelium^{28,29} in her classic paper stated that large difference in pigmentation; spore-size and other morphological features, together with frequent sectorizations in culture plates indicate that *E. nigrum* has variable species.

In present study *Stachybotrys nilgirica* was identified by comparing their characteristics with other species. The conidia of *S. chartarum* and *S. chlorohalonata* are ellipsoidal to oval while conidia of *S. nilgirica* are circular and small. The conidial wall of *S. chartarum* is smooth, while conidial wall in *S. chlorohalonata* is rough but wall of conidia of *S. nilgirica* is verrucose to tuberculate.³⁰

Morphology of *Trimmatostroma* shows variation in size of conidia. *T. scutellare* showed moderate brown to dark brown colony, mycelium subhyaline, conidiophore irregularly branched, septate, conidia obovate to subglobose, multicellular, dark brown.^{17,31} *Trimmatostroma hughesii* showed colony olivaceous, brown, conidiophores macronematous, septate, sporogenous cell bears on top of conidiophores, 40–156µm long and 4–7µm wide. Conidiophores simple, septate, hyaline. Conidia small, simple, catenulate, only one longitudinal septa over single transverse septa, 6–11µm×4–9µm.^{32–34} The conidia of *Trimmatostroma hughesii* small, catenulate and only

one longitudinal septa over single transverse septa is present while in *Trimmatostroma scutellare* having conidia large, with many cross and longitudinal septation.

Conclusion

The present investigation aims to study basic taxonomic study of endophytic fungi from *Cissus quadrangularis* from Melghat Forest of Amravati district of Maharashtra. Study concluded that several endophytes can be associated with single host. The present findings reported first time from this region.

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Conflict of interest

The author declares no conflict of interest.

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