

Morphotaxonomy of endophytic fungi from *Cissus quadrangularis* L.

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Abstract

During present investigation extensively utilised medicinal plant *Cissus quadrangularis* L. was investigated to isolate the endophytic fungi. Ten endophytes were observed from aerial parts i.e. 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.

Keywords: Taxonomy, endophyte, Amravati, *Cissus quadrangularis* L.

1. Introduction

The word 'endophyte' means "inside the plant" (endon Gr. = within, phyton = plant) for all microbes (including fungi, bacteria, cyanobacteria and actinomycetes) that reside within plant tissue [1].

Characterization and identification of endophytic fungi are challenging tasks. Near about 70,000 fungal species had been isolated, identified and characterized [2, 3]. Manoharachary suggested that about 27,000 fungal species have been illustrated in India [4]. Researcher is continuously striving to look for into the diversity of fungi and their natural potentials. The multiplicity of endophytic fungi and their role in various biochemical reactions occupy most important place in the nature.

In morphotaxonomical study of Melghat Forest of Amravati District numerous authors collected and reported many rare and interesting fungal forms they are new to Maharashtra [5-11].

Taxonomy of fungi is based on morphological features like shape, size and colour of various fruiting bodies in traditional taxonomy [12]. During present investigation isolated fungi were identified macroscopically and microscopically by studying their morphotaxonomical characteristics.

2. Materials and Methods

Collection of plant samples were done from Melghat forest of Amravati district. The samples were collected in sterilized polythene bags and brought to the laboratory and processed within 24 hrs.

Surface sterilization was done to remove the epiphytes. The surface sterilized explants then inoculated at $26 \pm 2^\circ\text{C}$ into the Petri dishes containing potato dextrose agar and observed for fungal growth periodically [13].

Isolated endophytes were identified morphologically and placed in appropriate genera and species of fungi using standard taxonomic keys and monographs [14-18].

3. Observations and Results

3.1 *Arthrimum hydei* (Fig. 1)

Mycelium smooth, hyaline to pale brown, branched, septate, 2-3 μm diameter. Conidiophores pale brown smooth, cylindrical, septate, branched, $22-34 \times 3-5 \mu\text{m}$. Conidiogenous cell aggregated in clusters on hyphae, smooth, hyaline, doliiform. Conidia unicelled, brown, globose to lenticular with pale equatorial slit 10-22 μm diameter in side view [19].

Remark: The species under study matched with *A. hydei*, and new to this region. *A. hydei* was isolated and cultured on PDA.

3.2 *Arthrimum phaeospermum* (Corda) (Fig.2)

Colonies dark brown to greenish, round, oval or irregular in shape. Mycelium hyaline to pale brown, smooth hyphae, 3-4 μm in diameter. Conidiophores are cylindrical, narrow, erect or flexuous, straight, simple, smooth, hyaline 5-12 \times 3-5 μm thick, dark brown with transverse septa 48-120 μm long, 2-4.5 μm 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 10-16 μm long, 4-7 μm wide in surface view [15].

Remark: The colonies were dark brown to greenish in color, round, oval and irregular in shape.

3.3 *Epicoccum nigrum* Ehrenb.Ex.Schlecht. (Fig.3)

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 9-11 μm , producing a single dark gangliosporer terminally. Young conidia rounded, non septate and pale in color. Mature gangliosporer golden brown or brown or olivaceous or black, 40 globosed or pyriform or sometimes of irregular angular shape, septate, muriform, verrucose, 15-54 μm in diameter. Mature conidia contain multiple transverse and vertical septa [17].

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

3.4 Nigrospora oryzae (Fig. 4)

Order: Moniliales; Class: Hyphomycetes.

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 24-28×21-24 µm.

Remark: The species understudy found to be similar morphologically with *Nigrospora oryzae*.

3.5 Pestalotiopsis funerea (Fig.5)

Order: Sphaeropsidales; Class: Coelomycetes

Pustules black, punctiform, globose-lenticular, 110-290 µm in diameter. Conidia broad, tapering towards the base, clavate-fusoid, straight, 5-celled, 15.5-28.5×6.6-9.2 µm, intermediate coloured cells guttulate, umber or olivaceous, equally coloured, lowest coloured cell sometimes slightly paler, slightly constricted at septa, apical appendages 1-2, 4.8-6.5 µm.

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

3.6 Phoma crysanthemicola

Order: Sphaeropsidales; Class: Coelomycetes

Colour of colony grey to dark brown, mycelia was branched, septate and brown. Pycnidia unilocular to globose. Conidiomata was pycnidial, smooth walled and brown in colour. Conidia hyaline, smooth, ellipsoid to cylindrical cylindrical, straight 4-7 × 1.5-2.5 µm.

Remark: The species understudy match with *Phoma crysanthemicola*.

3.7 Pithomyces chartarum. (Fig. 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, 21-32 µm long, 12-35 µm wide, each conidium carrying away a part of conidiophores [15].

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

3.8 Stachybotrys chartarum

Order: Moniliales; Class: Hyphomycetes.

At beginig colonies colorless or whitish then becoming black, mycelium is septate, hyaline, branched hyphae 4-6 µm wide conidiophores arising from hyphae laterally, hyaline at the base, fuliginous near the tip, simple or branched, 1-2 septate, 64-109 µm long, 5 µm wide, terminating in a cluster of phialides. Phialides 11-19 × 4.5-6.5 µm, dark colored at tip as compared to base. Conidia smooth, single, oval at young stage, elliptical when mature, dark green to black colored at maturity 15-16.5 µm long, 3.2-7.3µm wide.

Remark: The species under study correlate with *Stachybotrys chartarum*

3.9 Stachybotrys nilgirica. (Fig.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, 68.7-93.1 µm long smooth, terminating in a cluster of about 6-7 phialides, apical cell of philophore 13.3-17 µm long and 2-6 µm 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 15.3-27.8 µm in diameter [17].

Remark: The characters of the species understudy were allied with *S. Nilgirica*.

3.10 Trimmatostroma hughesii. (Fig.8)

Colony olivaceous, brown, conidiophores macronematous, septate, sporogenous cell bears on top of conidiophores, 40-156 µm long and 4-7µm wide. Conidiophores simple, sptate, hyaline. Conidia small, simple, catenulate, only one longitudinal septa over single transverse septa, 6-11µm × 4-9 µm [20].

Remark: Characters of the present specimen match with *Trimmatostroma hughesii*.

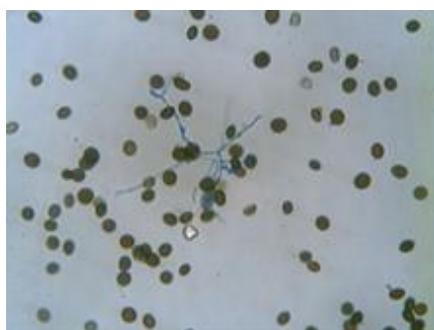


Fig 1: Mycelium with conidia of *A. hydei* **Fig 2:** Mycelium with conidia of *A. phaeospermum* **Fig 3:** Mycelium with conidia of *E. nigrum*

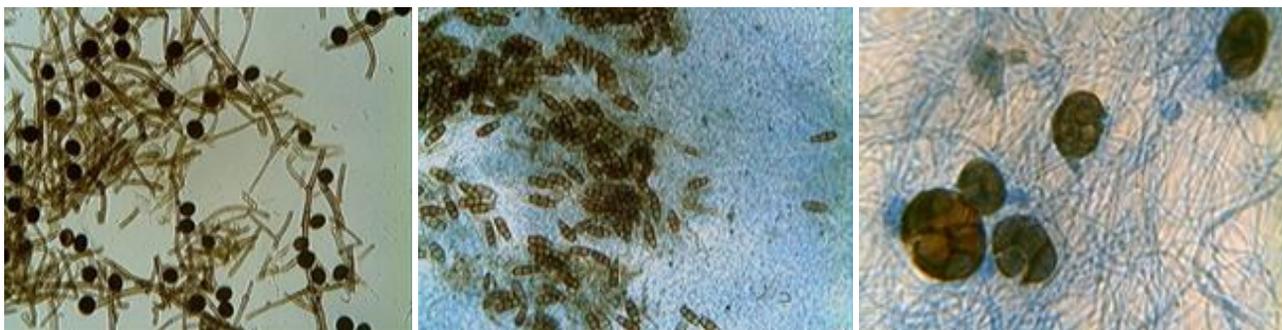


Fig 4: Mycelium with conidia of *N. oryzae* Fig 5: Mycelium with conidia of *P. funereal* Fig 6: Mycelium with conidia of *P. chartarum*



Fig 7: Mycelium with conidia of *S. nilgirica* Fig 8: Mycelium with conidia of *T. hughesii*

4. Discussion

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.

Anamorphic ascomycetes *Epicoccum nigrum* distributed globally which colonizes on different types of soil and different host plants. Large difference in pigmentation; spore-size and other morphological features, together with frequent sectorizations in culture plates indicate that *E. nigrum* has variable species [21]. 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 [22].

In present study, two species of *Arthrinium* such as *A. pheospermum* and *A. hydei* were obtained from different hosts in different seasons. Both the species were identified by morphological characteristics. The *A. hydei* has conidiophores 21.65-33.22 μm long and 3.2-4.8 μm in diameter. Conidia lenticular, globose, 9.6-21.4 μm in diameter while *Arthrinium pheospermum* having dark brown to greenish colored colony, mycelium hyaline, conidiophores are narrow, cylindrical, erect 4.7-11.6 \times 2.8-5.1 μm . Conidia sessile or sometimes born on short peg alongside of conidiophore with 10.1- 15.9 \times 3.9-7.2 μm size. Some specific differences were observed in shape and size of conidia and conidiophores which provided identity to isolated species.

During present investigation *Stachybotrys nilgirica* was identified by comparing their characteristics with other species. 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 [23]. The conidia of *S. chartarum* and *S. chlorohalonata* are ellipsoidal to oval while conidia of *S. nilgirica* are circular and small.

Trimmatostroma hughesii showed colony olivaceous, brown, conidiophores macronematous, septate, sporogenous cell bears on top of conidiophores, 40- 156 μm long and 4-7 μm wide. Conidiophores simple, septate, hyaline. Conidia small, simple, catenulate, only one longitudinal septa over single transverse septa, 6-11 μm \times 4-9 μm . 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. Taxonomical characters 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, [14, 24].

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6. References

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