

## A New Endophytic *Thielavia Icacinacearum* (Ascomycete) Isolated from Medicinal Plant *Nothapodytes Nimmoniana*

Sanjay K. Singh, Varsha Prakash Gaikwad and Vimal Waingankar

Mycology and Plant pathology group, Agharkar Research Institute, G.G. Agarkar Road, Pune 411 004,  
Email: sksingh@aripune.org

### ABSTRACT

*Thielavia icacinacearum* was isolated from healthy leaves of *Nothapodytes nimmoniana* (Grah.) Mabb. (Icacinaceae). This fungus is described and illustrated as new species based on pure culture grown on Potato Dextrose Agar. This is the first report of *Thielavia* Zopf. known to occur on its host as an endophyte.

**Key words:** Biodiversity, Endophyte, *Thielavia icacinacearum*, *Nothapodytes nimmoniana*, India.

### Introduction

Endophytic fungi are a poorly investigated group and represent potential source of bioactive and chemically novel compounds for exploitation in medicine, agriculture and industry. With this background we screened the number of important medicinal plants and isolated associated endophytic mycoflora in our laboratory. In this investigation we have isolated an ascomycetous fungus *Thielavia* Zopf from *Nothapodytes nimmoniana* (= *Mappia foetida*), an important plant used in medicine for centuries. The genus *Thielavia* now contains about 26 species reported from all over the world<sup>1-3</sup>. Recently, Manoharachary (1994)<sup>4</sup> has compiled a monographic account of species of this genus reported from India and expanded generic circumscription. Most of its species are known to occur as saprophytes and rarely parasites. Upon comparison with the allied species of this genus the present collection was found to be new. This is addition to a few endophytic fungi described as new strain/ species from India<sup>5</sup>

### Materials and methods

**Sampling and Isolation:** A few small and healthy looking twigs with leaves were cut from 3-4 individual plants of *Nothapodytes nimmoniana* located in Tilarighats (Maharashtra). Samples were transported to the laboratory in sterile polythene bags within 24 hrs after harvesting. Twigs were removed from the polythene bags and thoroughly washed with sterile water. Small segments (5 x 5 cm) were cut separately from leaves and twigs and subjected to surface sterilization following the technique by Rodrigues

and Samuels<sup>6</sup>. As the fungal colonies appeared from the edges of the inoculated plant segments and extended onto agar medium, were transferred onto PDA slants. Type culture is deposited in National Collection of Industrial Micro-organisms (NCIM), National Chemical Laboratory, Pune (INDIA).

**Microscopy:** The fungal fruiting bodies growing on Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA) and Malt Extract Agar (MEA) (all Hi-media) were studied by binocular light and stereomicroscopes. The other morphological details of asci and ascospores were observed using suitable stain and photomicrograph were taken from the OLYMPUS (CX41) microscope attached with Digital Camera. The identity of the fungus was confirmed with the help of relevant literature<sup>1,4</sup>.

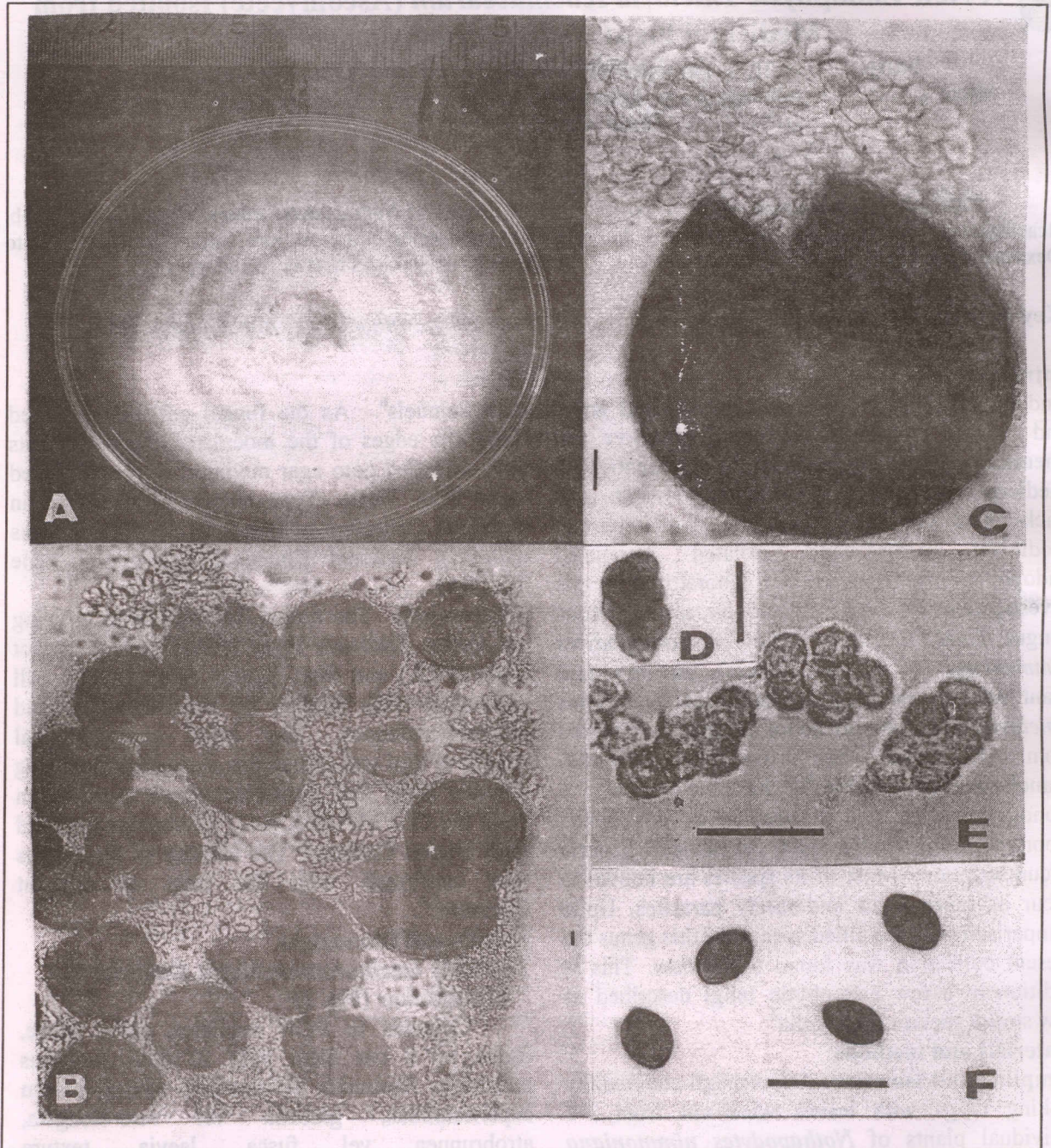
### Results and Discussions

#### *Thielavia icacinacearum* Singh, Gaikwad & Waingankar (Fig. 1)

Colonies in PCA regulares, sicca, albae, usque ad 8 cm diam post 10 dies. Reversus creameus. Ascōmata cleistothecia sparsa, partim superficialibus, globosa vel raro-elongata, atrobrunnea vel fusca, laevia, textura epidermoidea, 80-200 µm diam. Asci, unitunicati, octospori, clavati vel irregulares, 22-29 x 14-18 µm. Paraphysibus absentibus. Ascosporae evanescentae, ellipsoideae, aseptatae, brunneae, 10-12 x 7-8.5 µm.

**HOLOTYPE:** Cultura in PDA exiccata, extractis in foliis vivis *Nothapodytes nimmoniana*, Tilarighat, India, leg. S.K. Singh,





**Figs. A-F: *Thielavia icacinacearum* sp. nov.**

**A.** Colony with concentric rings on Potato Carrot Agar medium **B.** Asci released from ruptured mature ascoma **C.** Enlarged view of ascoma showing *textura epidermoidea* Bar = 20  $\mu$ m **D. & E.** Asci and irregularly arranged immature ascospores, Bar = 20  $\mu$ m **F.** Enlarged view of mature ascospores with a single apical germ pore Bar = 20  $\mu$ m.



Jan.2004, NCIM 1320 holotypus, ARIFCC E 532 isotypus.

Colonies on PCA regular in outline, dry, white, floppy, forming concentric rings, later becoming darker due to development of dark brown to black fruiting bodies, attaining a diameter of 8 cm after 10 days. Reverse creamy or off-white. Ascomata cleistothecia scattered, partly immersed in the aerial mycelium, spherical to rarely slightly elongated, non-ostiolate, smooth, dark brown to brown or black with the wall consists of textura epidermoidea, 80-200  $\mu\text{m}$ . Asci unitunicate, 8 spored, clavate to irregular, 22- 29 x 14 -18  $\mu\text{m}$ . Paraphyses absent. Ascospores irregularly arranged, evanescent, ellipsoidal, 1-celled, with a single apical germ pore at one end, brown, 10-12 x 7-8.5  $\mu\text{m}$ .

The formation of ascomata was observed after 7 days of incubation on MEA, PCA at  $35 \pm 1$   $^{\circ}\text{C}$ , while after 15 days on Potato Dextrose Agar. The culture was submerged to grow at  $45$   $^{\circ}\text{C}$  on PDA showed reduced growth (2 cm after 7 days) with sulcate colonies. Overall the MEA supported the luxuriant growth and earlier formation of ascomata in culture. Survey of literature indicates that 25 species are currently accepted in the genus *Thielavia* Zopf<sup>7</sup>. Out of which 18 species has been reported from India<sup>4</sup>. The present collection needs to be compared with *T. basicola* Lodha and *T. terricola* (Gilman and Abbott) Emmons based on

the morphological features. It differs from *T. terricola* in having smaller and comparatively slender ascospores, while it differs from *T. basicola* in having smaller ascostromata. In addition, present collection is the first report of *Thielavia* sp. isolated as an endophyte in pure culture from surface sterilized healthy leaves and stem of *Nothapodytes nimmoniana* (= *Mappia foetida*).

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