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Diversity of foliar mycobionts on *Strychnos nux-vomica* L. (Loganiaceae) from Vettikulam sacred grove, Pathanamthitta, Kerala

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Abstract
Fungi are ubiquitous and are known to occur on diverse habitats. Among them the leaf inhabiting fungi are known as 'foliar fungi' (Dix and Webster, 1995). These fungi are mostly host specific and produce spots or lesions of varied types such as mildews, tar spots, rusts, scab etc. The nature of colonization by these fungi depends on season, age of the plant and nutritional status of the leaf (Last and Deighton, 1965; Dix and Webster 1995). The present study deals with diversity of foliar mycobionts on *Strychnos nux-vomica* L. (Loganiaceae) from Vettikulam sacred grove, Pathanamthitta, Kerala. This paper deals with the detailed microscopic examination of the fungi namely; *Meliola strychni-multiflorae* Hansf., *Meliola strychnacearum* Hosag. & Abraham, *Meliola strychnigena* Hosag. & Manoj., *Meliola strychni* Mibey and *Questieriella strychni* Hosag. The collections were identified and deposited in the fungal herbarium of S. G. College, Kottarakara.

Keywords: foliar fungi, *Strychnos nux-vomica* L., Loganiaceae *Meliola*, *Questieriella*, mycobionts

Introduction
Sacred groves are patches of virgin forest with rich diversity and have been protected by local people on the basis of religious faith for centuries. Most of these groves are veritable sanctuaries of flora and fauna (Kannan and Rekha, 2019). Many floristic studies have been conducted in these groves. But the foliar fungal studies are lacking here.
Fungi inhabiting the leaves are referred as phylloplane or foliicolous fungi. Foliicolous fungi constitute a major part of microbial diversity which includes rusts, smuts, powdery mildews, black mildews etc. (Jayashankara, 2012). Inhabiting on the surface of the leaves, they produce special organs and attain special adaptation and act as necrotrophs or biotrophs. Black mildews are group of fungi which maintain a parasitic symbiotism (Hosagoudar, 2012) with their host plants and do not cause any severe symptomatic appearance on their host. These fungi produce black colonies on the surface of host plants (Hosagoudar, 2011) [2]. Black mildews belongs to different taxonomic groups *viz*. Meliolaceous fungi, Asterinaceous fungi, Schiffnerulaceous fungi, Hyphomycetous fungi (Hosagoudar, 2011) [2].
The present study is the result of work carried out in the Vettikulam sacred grove, Pathanamthitta, Kerala. During the exploration of foliar mycobionts on *Strychnos nux-vomica* L. from the grove, five fungal species were found namely; *Meliola strychni-multiflorae*, *Meliola strychnacearum* Hosag. & Abraham., *Meliola strychnigena* Hosag. & Manoj., *Meliola strychni* Mibey and *Questieriella strychni* Hosag..

Materials and Methods
Field trips were conducted to Vettikulam sacred grove, Pathanamthitta, Kerala for the collection of fungal infected host plants. The foliar mycobionts mainly infect leaves, soft stems and tender shoots of herbaceous plants to trees of 30-40 metres in height. Fungal infected plants were collected from the field. The collected plants were placed individually in polythene bags. Field notes were prepared regarding pathogenicity of fungi, nature of colonies, nature of infection, locality, altitude, etc. These infected plant parts were pressed neatly and dried in-between blotting papers. After ensuring their dryness, they were kept in manifold or butter paper folders. Such materials were later used for the microscopic study.
In case of ectophytic or superficial fungi, scrapes were made directly from the infected host parts and were mounted in Lactophenol (prepared according to Rangaswamy, 1975). Dematiaceous fungi were first mounted in 10% KOH solution and later transferred to Lactophenol so as to make the septa visible. Fungal colonies on infected leaves were analysed with the help of hand lenses for morphological studies. Nail polish technique was used to study the colonies in situ (Hosag. & Kapoor, 1985). Permanent slides were prepared using DPX as mountant. These permanent slides were then used for further studies. For the innate fungi, free hand sections were made, mounted in lactophenol, a tinge of cotton blue stain were used to stain the hyaline fungal parts. Observations were made under compound microscope. Measurement was taken with the help of ocular micrometer on a calibrated microscope. Previous collections were observed. Recent nomenclature was verified

and experts were consulted. Description of the fungal species along with photomicrographs was done. All the fungal specimens are documented and deposited in the St. Gregorios College Herbarium, Kottarakara (SGCH).

Description to the species

1. *Meliola strychni-multiflorae* Hansf., Sydowia 11 (1-6): 59, 1958

Colonies amphigenous, thin to subdense, scattered to conjoint, up to 9 mm in diameter. Hyphae substraight, flexuous to undulate, branching opposite at acute angle, loosely to closely reticulate, cells 19 – 41.8 × 3.42 - 7.6 µm. Appressoria two celled, alternate, unilateral to irregular, straight to curved, antrorse to rarely reflexed, 15-33.44 µm long; stalk cells cuneate to cylindrical, 3.8 - 15 µm long; head cells ovate, entire, angulose to rarely sublobate, 11.4 - 19 × 7.6 – 11.4 µm. Phialides mixed with appresoria, opposite, ampulliform, 9.5- 26.6 × 3.8 - 7.6 µm. Mycelial setae scattered to grouped around perithecia, simple,straight to curved, acute to obtuse at the apex, up to 555 µm long. Perithecia scattered to grouped, globose, verrucose up to 129 µm in diameter; ascospores obovoidal, 4- septate, slightly constricted at the septa 34 -38 × 11.4-14.44 µm.

Materials examined: On leaves of *Strychnos nux-vomica* L. (Loganiaceae) Vettikulam Ammommakavu, Ikkadu vadakk, Kerala, November 27, 2020; Soumya Prasad

Based on Beeli formula *Meliola strychni-multiflorae* Hansf. and *Meliola strychnigena* Hosag. & Manoj. are similar. However, it differs from *Meliola strychnigena* Hosag. & Manoj. in having opposite appressoria.

2. *Meliola strychnacearum* Hosag. & Abraham, in Hosag., Abraham & Goos, *Mycotaxon* 66: 118, 1998.

Colonies amphigenous, thin to subdense, scattered to conjoint, up to 14 mm in diameter. Hyphae flexuous, undulate to curved, branching opposite at acute angle, loosely to closely reticulate, cells 7.6 -38 × 3.8- 7.6 µm. Appressoria two celled, alternate, unilateral, irregular to rarely opposite, straight to curved, antrorse rarely subantrose to retrose, 17 - 28.5 µm long; stalk cells cuneate to cylindrical, 3.8 - 11.4 µm long; head cells ovate, oblong, entire 11.4 - 17 × 7.6– 11.4 µm. Phialides mixed with appresoria, opposite to irregular, ampulliform, 13 - 22.8 × 3.8 -7.6 µm. Mycelial setae scattered to grouped around perithecia, simple, straight to curved, acute to obtuse at the apex, up to 480 µm long. Perithecia scattered to grouped, globose, verrucose, up to 159.6 µm in diameter; ascospores obovoidal to cylindrical, 4-septate, slightly constricted at the septum, 32 -38 × 11.4- 15 µm.

Materials examined: On leaves of *Strychnos nux-vomica* L. (Loganiaceae) Vettikulam Ammommakavu, Ikkadu vadakk**,** Kerala, November 27, 2020; Soumya Prasad

Meliola strychnacearum Hosag. & Abraham differs from *Meliola strychni-multiflorae* Hansf. in having opposite appressoria (few) and longer mycelial setae.

3. *Meliola strychnigena* Hosag. & Manoj., *Indian Phytopath.* 57: 463, 2004.

Colonies epiphyllous, thin, up to 5mm in diameter,confluent. Hyphae flexuous to curved, branching opposite at acute angle, closely reticulate, cells 17- 43.7 × 3.8- 7.6 µm. Appressoria two celled, alternate, unilateral, irregular to rarely opposite, straight to curved, antrorse, subantrose to rarely reflexed, 19- 28.5 µm long; stalk cells cuneate to cylindrical, 7.6- 11.4 µm long; head cells ovate, oblong entire to rarely angulose, 11.4- 19 × 7.6 – 9.5 µm. Phialides mixed with appresoria to few separated, opposite to irregular, ampulliform, 11.4- 30.4 × 3.8 -7.6 µm. Mycelial setae scattered to grouped around perithecia, simple, curved, mostly acute to rarely obtuse at the apex, up to 600 µm long. Perithecia scattered to grouped, globose, verrucose up to 152 µm in diameter; ascospores obovoidal to cylindrical, 4-septate, slightly constricted at the septum, 34.2 - 36 × 11.4 -13 µm.

Materials examined: On leaves of *Strychnos nux-vomica* L. (Loganiaceae) Vettikulam Ammommakavu, Ikkadu vadakk, Kerala, November 27, 2020; Soumya Prasad

Meliola strychnigena Hosag. & Manoj. differs from *Meliola strychnacearum* Hosag. & Abraham in having longer mycelial setae.

4. *Meliola strychni* Mibey in Mibey & Hawksworth, *Mycol. Pap.* 174: 75, 1997.

Colonies amphigenous, thin, confluent up to 7 mm in diameter. Hyphae flexuous, undulate to curved, branching opposite at acute angle, loosely to closely reticulate, cells 19- 41.8 × 3.8- 7.6 µm. Appressoria two celled, alternate, irregular, straight to curved, antrorse, rarely subantrorse to retrose, 15 - 30.4 µm long; stalk cells cuneate to rarely cylindrical, 3.8 - 11.4 µm long; head cells ovate, oblong, entire to sublobate, 9.5 - 19 × 6.84 – 14.44 µm. Phialides mixed with appresoria, opposite, alternate, unilateral, ampulliform, 11.4 - 34.8 × 3.8 -7.6 µm. Mycelial setae scattered, simple, straight to curved, acute to obtuse at the apex, up to 450 µm long. Perithecia scattered to grouped, globose, verrucose up to 190 µm in diameter; ascospores obovoidal to cylindrical,4-septate, slightly constricted at the septum, 33.44 - 36 × 11.4 -15 µm.

Materials examined: On leaves of *Strychnos nux-vomica* L. (Loganiaceae) Vettikulam Ammommakavu, Ikkadu vadakk, Kerala, November 27, 2020; Soumya Prasad

Meliola strychni Mibey differs from *Meliola strychnacearum* Hosag. & Abraham in lacking opposite appressoria and it differs from *Meliola strychni-multiflorae* Hansf. in having shorter mycelial setae.

5. *Questieriella strychni* Hosag., J. Econ. Taxon. Bot. 28:196, 2004. Hosag., The genus *Schiffnerula* in India. Plant Pathology & Quarantine 1(2), 140, 2011.

Colonies amphigenous, dense, up to 6mm in diameter, confluent. Hyphae, flexuous to undulate, branching alternate, unilateral to irregular at acute angle, loosely to closely reticulate, cells 11.4 - 26.6 × 3.8 - 5.7 µm. Appressoria opposite, alternate, unilateral, globose, entire, 7.6 - 9.5 µm. Questieriella type of conidia produced from the pore of hyphal cells, scattered, fusiform, pale brown, distal cells were much smaller and paler than the central two cells, acute to obtusely rounded at the apices, 3- septate, constricted at the septum, 41.8 - 53 × 10.6 - 11.4 µm.

Materials examined: On leaves of *Strychnos nux -vomica* L. (Loganiaceae) Vettikulam sacred grove, Ayckadu Vadakk, Kerala, November 27, 2020; Soumya Prasad
This fungus was associated with the dark colonies of *Meliola strychni* Mibey.

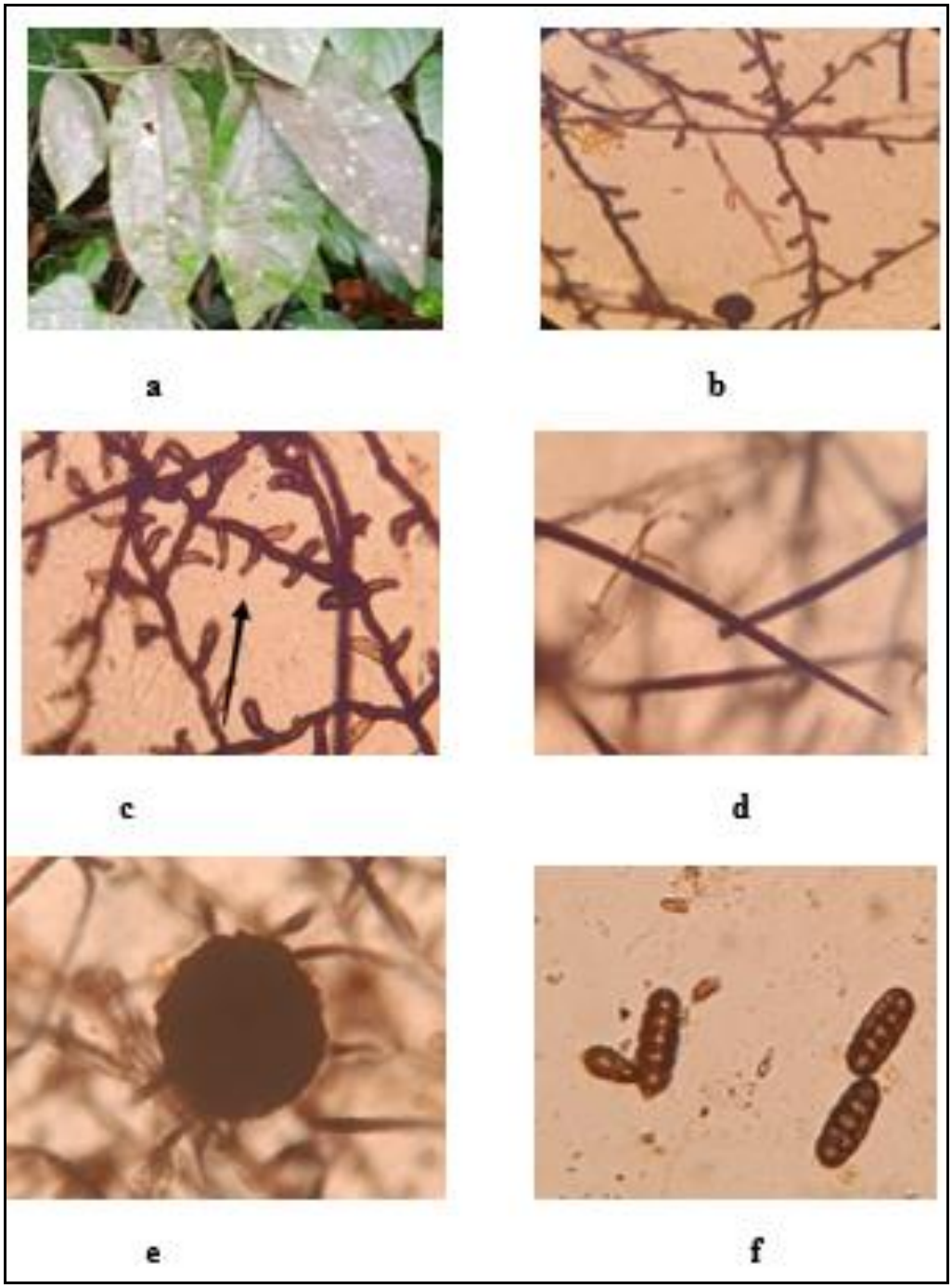

Fig 1: *Meliola strychni-multiflorae* Hansf. a. Infected host plant b. Hyphae with appressoria c. Phialide d. Mycelial setae e. Perithecium f. ascospores.

Table 1: A comparative account of morphotaxonomic features of *Meliola strychni-multiflorae* Hansf., *Meliola strychnacearum* Hosag. & Abraham, *Meliola strychnigena* Hosag. & Manoj., *Meliola strychni* Mibey

Sl No.	Fungal species	Infection spot	Hyphae	Appressoria	Mycelial setae
1	*Meliola strychni-multiflorae* Hansf.	amphigenous, thin to subdense, scattered to conjoint 9 mm in diameter	substraight, flexuous to undulate cells 19 – 41.8 × 3.42 - 7.6 µm	alternate, unilateral to irregular antrorse to rarely reflexed; head cells ovate, entire, angulose to rarely sublobate	acute to obtuse at the apex, up to 555 µm long.
2	*Meliola strychnacearum* Hosag. & Abraham	amphigenous, thin to subdense, scattered to conjoint 14 mm in diameter	flexuous, undulate to curved cells 7.6 -38 × 3.8- 7.6 µm	alternate, unilateral, irregular to rarely opposite, antrorse rarely subantrose to retrose; head cells ovate, oblong, entire	acute to obtuse at the apex, up to 480 µm long
3	*Meliola strychnigena* Hosag. & Manoj	epiphyllous, thin, confluent up to 5mm in diameter,	flexuous to curved cells 17-43.7 × 3.8- 7.6 µm.	alternate, unilateral, irregular to rarely opposite, antrorse, subantrose to rarely reflexed; head cells ovate, oblong entire to rarely angulose	mostly acute to rarely obtuse at the apex, up to 600 µm long
4	*Meliola strychni* Mibey	amphigenous, thin, confluent up to 7 mm in diameter	flexuous, undulate to curved cells 19- 41.8 × 3.8- 7.6 µm	alternate, irregular, antrorse rarely subantrorse to retrose head cells ovate, oblong, entire to sublobate	acute to obtuse at the apex, up to 450 µm long

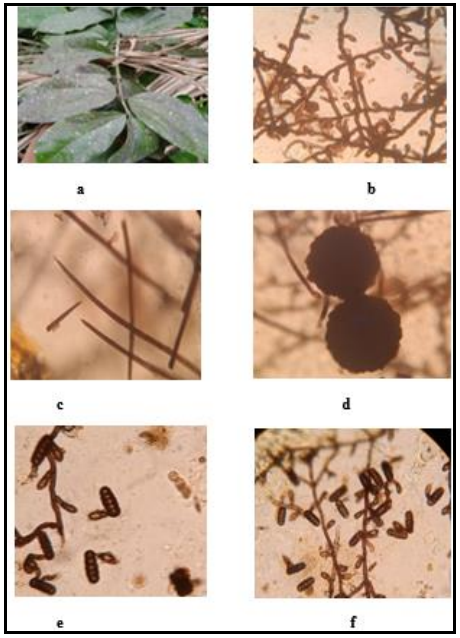

Fig 2: *Meliola strychnacearum* Hosag. & Abraham a. infected host plant b. hyphae with appressoria c. mycelial setae d. perithecium e. & f. ascospores

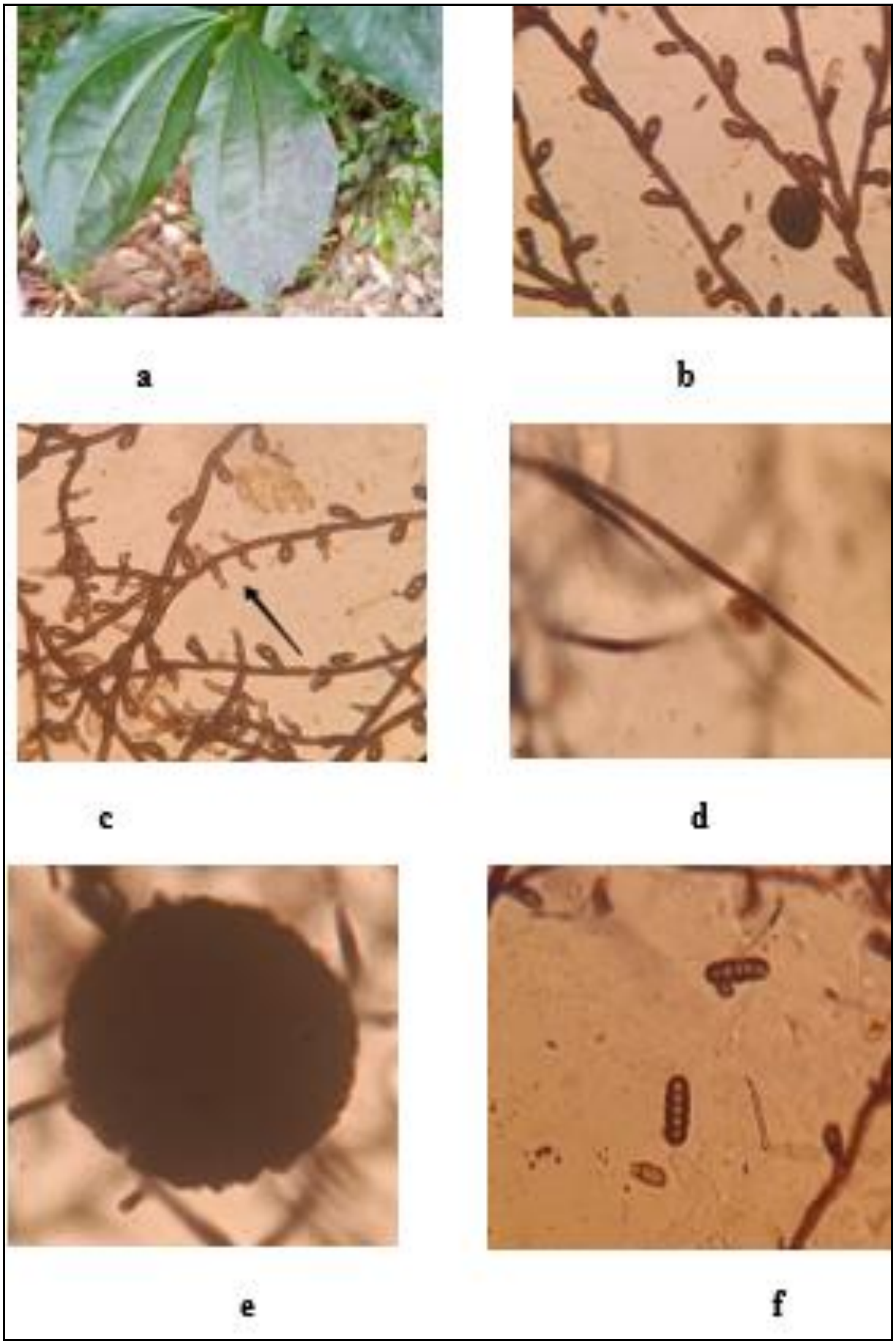

Fig 3: *Meliola strychnigena* Hosag. & Manoj a. infected host plant b. hyphae with appressoria c. phialide d. mycelial setae d. perithecium f. ascospore

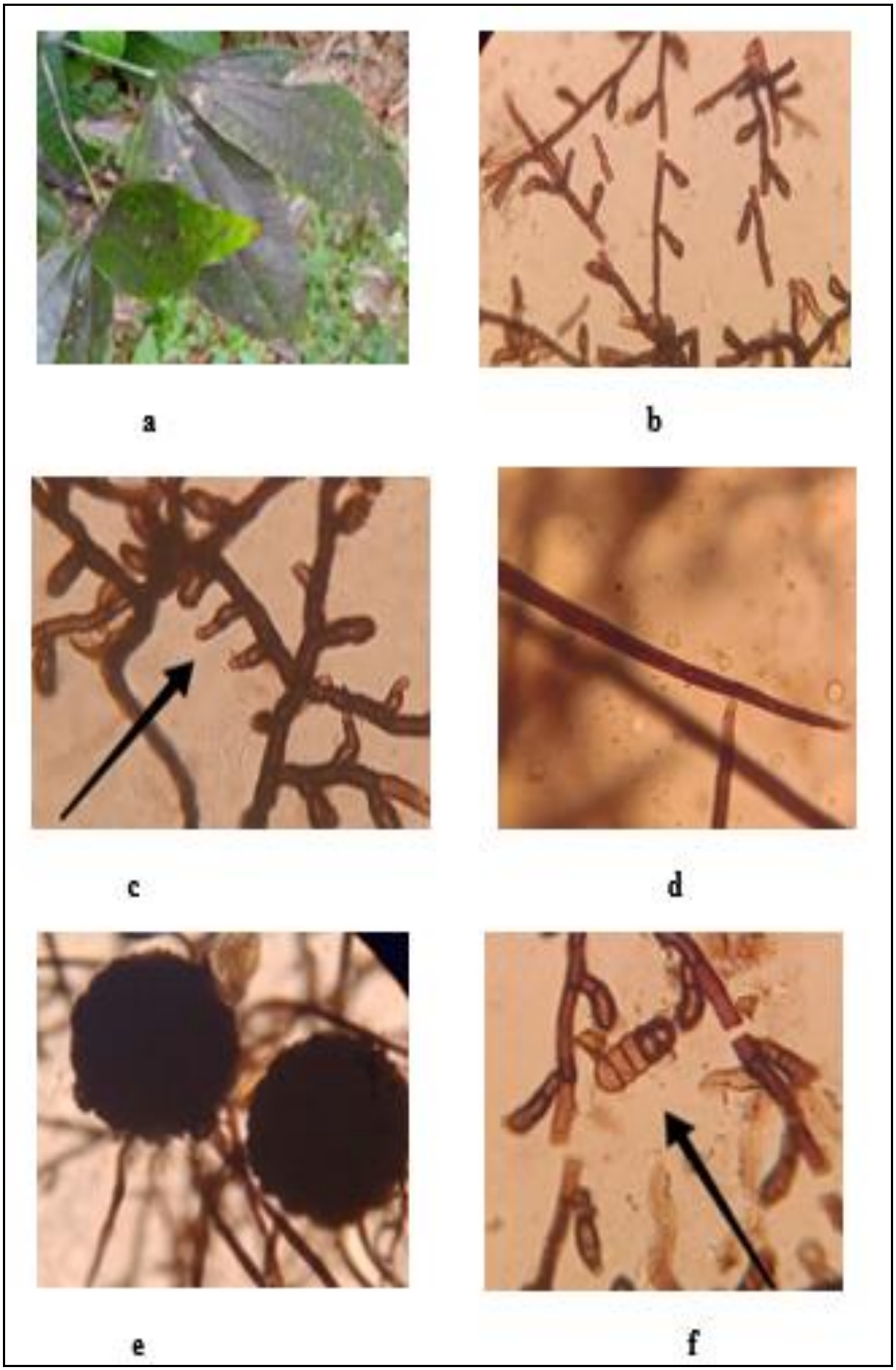

Fig 4: *Meliola strychni* Mibey. a. infected host plant b. hyphae with appressoria c. phialide d. mycelial setae e. perithecium f. ascospore

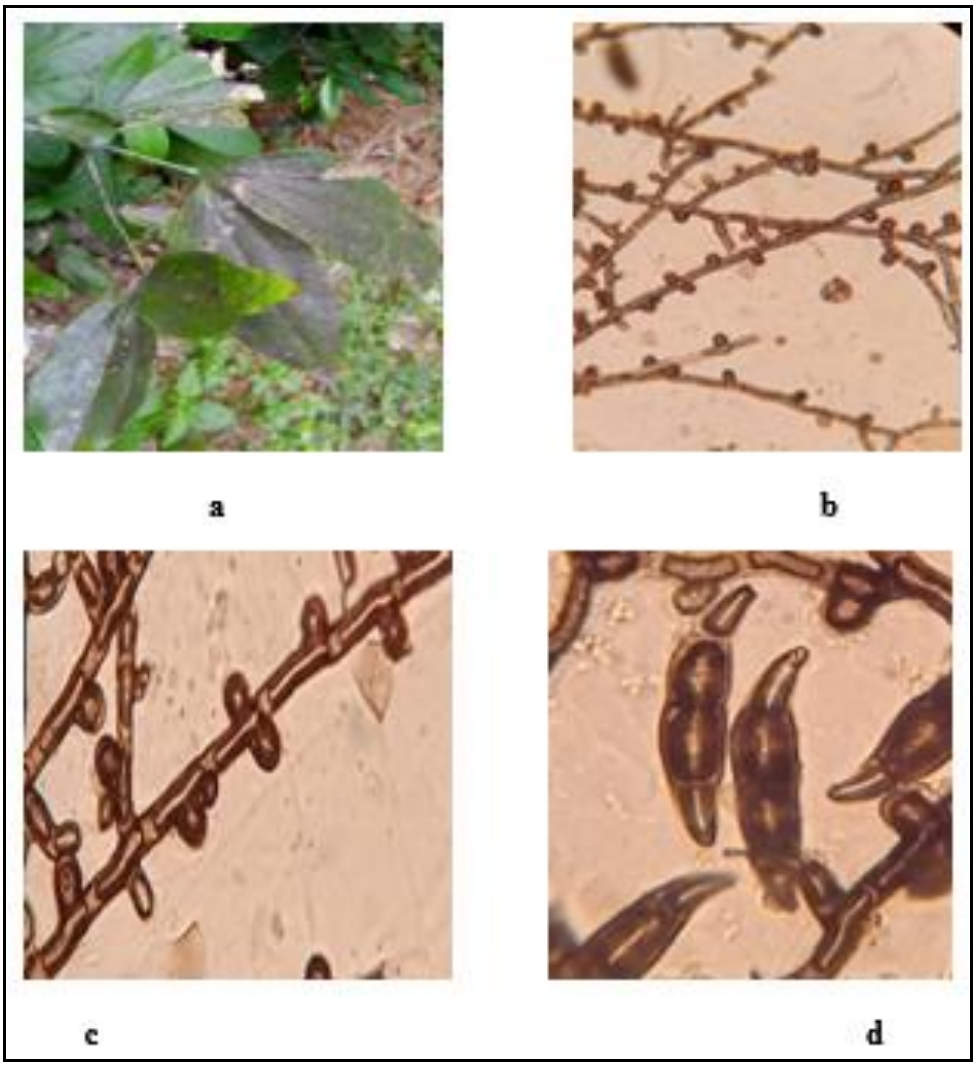

Fig 5: *Questieriella strychni* Hosag. a. infected host plant b. hyphae c. hyphae with appressoria d. conidia of *Questieriella*

Conclusion

The present study aimed to study the foliar fungal wealth on *Strychnos nux-vomica* of Vettikulam sacred grove, Pathanamthitta, Kerala. This sacred grove is associated with Karuvilakkodu malanada kavu and is located at Ikkadu Vadakk in Kodumon Panchayat of Pathanamthitta district. The vegetation in Vettikulam sacred grove is luxuriant and comprises several trees mixed with shrubs, lianas and herbs. The flora of this grove is represented by *Chasselia curviflora* (Wall.) Thwaites, *Ziziphus* sp., *Uvaria narum* (Dunal) Bl, *Ixora coccinea* L., *Tabarnaemontana* sp., *Memecylon* sp., *Strychnos nux- vomica* L., *Xanthophyllum flavescens* Roxb., *Chrysophyllum cainito* L., *Hydnocarpus pentandra* (Buch. -Ham.) Oken, *Artocarpus hirsutus* Lamk. etc. The present study resulted in the identification and documentation of five foliar mycobionts on *Strychnos nux-vomica* L. namely; *Meliola strychni-multiflorae*, *Meliola strychnacearum* Hosag. & Abraham, *Meliola strychnigena* Hosag. & Manoj., *Meliola strychni* Mibey and *Questieriella strychni* Hosag.

During the course of evolution, some of these mycobionts and their host plants developed a special relationship with one and another, which can significantly influence the formation of metabolic products in plants. Therefore, a better understanding of biology of these fungi is greatly needed. The present work will serve both as a reference and stimulus for further work aimed at disclosing the diversity of foliar fungi of Pathanamthitta district of Kerala state. It will also be helpul for identifying the diversity of foliar mycobionts on *Strychnos nux-vomica* L.

The cultural practices, religious belief systems and associated taboos helped to prevent the exploitation of natural flora present in the Vettikulam sacred grove. However, human interaction may lead to the degradation of the natural resources. So, in order to avoid such a condition, necessary actions are needed. Thus by conserving floristic wealth of these groves, the foliar fungal wealth can also be conserved.

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