Oxford University Press, New York, 2006, pp. 183–203.
6. Levin, D. A., *Ann. Bot.*, 2012, **109**, 613–620.
7. Wright, S. I., Kalisz, S. and Slotte, T., *Proc. R. Soc. London, Sec. B.*, 2013, **280**; http://dx.doi.org/10.1098/rspb.2013.0133
8. Shivanna, K. R., *Proc. Natl. Acad. Sci., India, Sect. B.*, 2014, **84**, 681–687; doi: 10.1007/s40011-014-0307-x
9. Shivanna, K. R. and Rangaswamy, N. S., *Pollen Biology: A Laboratory Manual*, Springer, Berlin, Heidelberg, 1992.
10. Dukas, R. and Dafni, A., *Plant Syst. Evol.*, 1990, **169**, 65–68.
11. Ahmed, T., Sarwar, G. R., Ali, T. and Qaiser, M., *Pak. J. Bot.*, 1995, **27**, 93–99.
12. Raju, A. J. S., Zafar, R. and Rao, S. P., *Curr. Sci.*, 2005, **80**, 1378–1380.
13. Stebbins, G. L., *Am. Nat.*, 1957, **91**, 337–354.

ACKNOWLEDGEMENTS. I thank the Indian National Science Academy, New Delhi for awarding the INSA Honorary Scientist position.

Received 6 June 2014; accepted 22 July 2014

K. R. SHIVANNA

Ashoka Trust for Research in Ecology and the Environment,
Royal Enclave, Srirampura, Jakkur Post,
Bengaluru 560 064, India
e-mail: shivanna@atree.org

Assessment of endolichenic fungal diversity in some forests of Kumaun Himalaya

The statement by Hammer[1] that 'Biodiversity studies depend upon biogeography and biogeography depends upon biodiversity', emphasizes that without insights into biogeographical patterns we cannot fully understand the evolution of species and without some knowledge of what grows where, our attempts at something as simple as identification may prove fruitless. Henceforth, if we accept Hawksworth's hypothesis[2] that there are 1.5 million species of fungi known from the world of which only 100,000 are described, then a question arises 'Where are all the undescribed fungi?' Hawksworth & Rossman[3] identified three categories where we can find these undescribed species: (1) fungi in tropical forests, (2) fungi in unexplored habitats, and (3) lost or hidden species. The second category (fungi in unexplored habitats) includes hypogeous fungi in Australia, fungi in the guts of other beetles and insects, lichenicolous fungi and endophytic fungi.

Endophytes are organisms which live inside other organisms without producing any apparent disease symptoms. They are a polyphyletic group of highly diverse, primarily ascomycetous fungi defined functionally by their occurrence within asymptomatic tissues of plants[4,5], mosses and ferns[6,7], marine algae[8,9], and seed plants from the Arctic to the tropics, and from agricultural fields to the most biotically diverse tropical forests. Their population depends on host species, location and environmental conditions in which the host is growing[10]. Commonly, a single plant can be a host of numerous endophyte species, amongst which at least one species shows host specificity.

Fungal symbionts resembling endophytes have also been reported from healthy lichen thalli forming persistent and symptomless infections[11–16]. Miadlikowska *et al.*[17] used the term 'endolichenic' fungi for endophytes isolated from lichens. These endolichenic fungi represent lineages of Ascomycota that are distinct from lichen mycobionts (the primary fungal component of the lichen thallus), lichenicolous fungi (which fruit or are otherwise symptomatic on thalli), and incidental fungi on thallus surfaces[11,18,19]. They are known from every lichen species sampled to date at sites ranging from the Arctic to the tropics[11], but have been characterized in only a few communities[11,13,14,16].

These endolichenic fungi colonize either inter- or intracellularly and may be either localized or systemic. Microdissection demonstrates that they live in close association with photobionts and are relatively rare in the mycobiont-dominated cortices and medulla[11]. Majority of these isolates belong to ubiquitous genera (e.g. *Acremonium*, *Alternaria*, *Cladosporium*, *Coniothyrium*, *Epicoccum*, *Fusarium*, *Geniculosporium*, *Phoma*, *Pleospora*), but some genera are common in both tropical and temperate climates (e.g. *Fusarium*, *Phomopsis*, *Phoma*), while members of the family Xylariaceae along with *Colletotrichum*, *Guignardia*, *Phyllosticta* and *Pestalotiopsis* predominate as endophytes in the tropics.

In India, studies on endolichenic fungi have been initiated recently[16,20,21]. Suryanarayanan *et al.*[16] have isolated endolichenic fungi from tropical regions of South India and reported 33 taxa along with mycelia sterilia. In contrast, Tripathi *et al.*[20,21] worked on endolichenic fungi of temperate regions of Kumaun Himalaya and isolated seven taxa, excluding mycelia sterilia as endophytes from *Physcia dilatata* and *Heterodermia flabellata*.

This further led authors to work on endolichenic fungi of some Kumaun Himalayan macrolichens. For isolating endolichenic fungi the macrolichens were collected from different forests of Kumaun Himalaya and taken in sterile polythene bags to the laboratory and processed within 24 h of collection. For each lichen, 100 segments were randomly cut from the thallus and surface sterilized following the modified protocol of Suryanarayanan *et al.*[16]. The efficacy of surface sterilization was confirmed by pressing the sterilized lichen thallus segments onto the surface of PDA (potato dextrose agar) medium. The absence of growth of any fungi on the medium confirmed that the surface sterilization procedure was effective[22]. The samples were cultured on PDA medium supplemented with streptomycin sulphate (150 mg/l), incubated at 25°C and left for 4 weeks for sporulation. Endophytic fungal species were identified on the basis of cultural characteristics and morphology of fruiting bodies and spores using standard texts and keys[23–29]. Cultures that failed to sporulate were recorded as

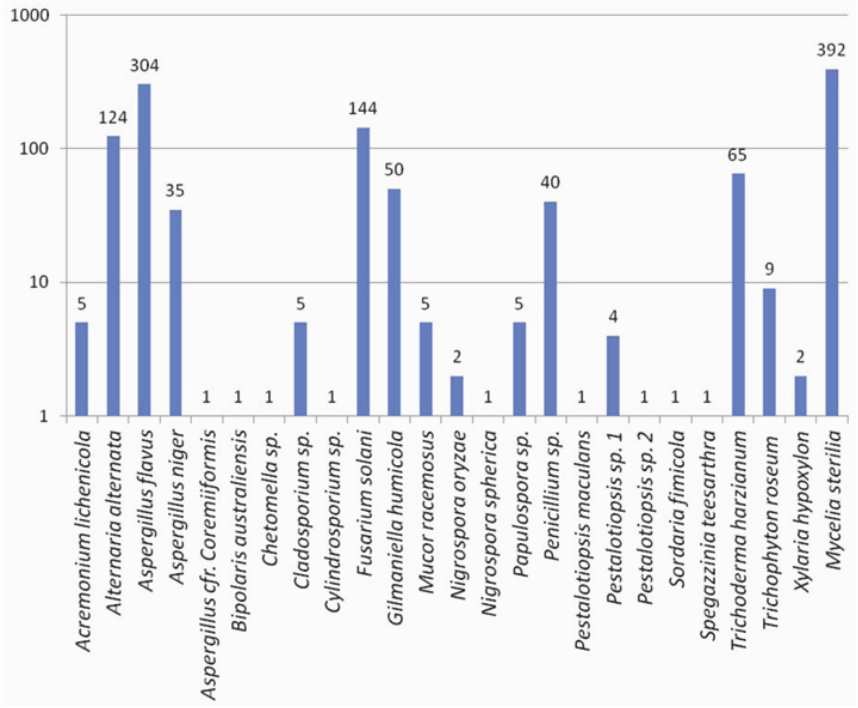

Figure 1. Number of colonies of endolichenic fungi isolated from various macrolichens.

mycelia sterilia. The samples are deposited in the herbarium of Kumaun University (ALM).

Colonization rate (CR) was calculated as the total number of lichen segments affected by fungi divided by the total number of segments incubated × 100. Relative frequency (RF) was calculated as the total number of a taxa divided by the total number of taxa obtained from lichen thalli incubated. Shannon–Weiner Biodiversity index (H') was calculated using the formula

$$H' = \log Ni/N \times 3.322 \times \log Ni/N,$$

where Ni is the number of individual fungal species and N is the total number of different fungi species.

A total of 24 isolates of endolichenic fungi belonging to 20 genera [*Acremonium lichenicola* W. Gams, *Alternaria alternata* (Fr.) Keissl., *Aspergillus* cfr. *coremiiformis*, *Aspergillus flavus* Link, *Aspergillus niger* Tiegh., *Bipolaris australiensis* (M.B. Ellis) Tsuda & Ueyama,

Table 1. Endolichenic fungi isolated from macrolichens

Lichens species	Family	Endolichenic fungus	Reference
Bulbothrix meizospora (Nyl.) Hale	Parmeliaceae	*Alternaria alternata*, *Aspergillus flavus*, *Cylindrosporium* sp., *Fusarium solani*, *Gilmaniella humicola*, *Mycelia sterilia*, *Penicillium* sp.	16
Flavoparmelia caperata (L.) Hale	Parmeliaceae	*Alternaria alternata*, *Aspergillus* cfr. *coremiiformis*, *Aspergillus flavus*, *Fusarium solani*, *Mycelia sterilia*	–
Heterodermia flabellata (Fée) D.D. Awasthi	Physciaceae	*Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Bipolaris australiensis*, *Fusarium solani*, *Pestalotiopsis* sp. 1, *Pestalotiopsis* sp. 2, ***Spegazzinia tessarthra***, *Trichoderma harzianum*	14, 16, 20, 21
Heterodermia hypochraea (Vain.) Swinscow & Krog	Physciaceae	*Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Papulospora* sp.	16
Leptogium burnetiae Dodge	Collemataceae	*Alternaria alternata*, *Aspergillus flavus*, *Fusarium solani*, *Gilmaniella humicola*	–
Parmelaria thomsonii (Stirton) D.D. Awasthi	Parmeliaceae	*Acremonium* sp., *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, ***Nigrospora sphaerica***, *Pestalotiopsis* sp., *Trichoderma harzianum*	14, 16
Parmotrema crinitum (Ach.) Choisy	Parmeliaceae	*Alternaria alternata*, *Aspergillus flavus*, *Fusarium solani*, *Mycelia sterilia*, *Trichoderma harzianum*	14, 16
Parmotrema graynum (Hue) Hale	Parmeliaceae	*Alternaria alternata*, *Aspergillus flavus*, *Gilamniella humicola*, *Fusarium solani*, *Trichophyton roseum*	–
Parmotrema nilgherrense (Nyl.) Hale	Parmeliaceae	*Alternaria alternata*, *Aspergillus flavus*, *Chaetomella* sp., *Cladosporium* sp., *Gilmaniella humicola*, *Fusarium solani*, *Mycelia sterilia*	16
Parmotrema praesorediosum (Nyl.) Hale	Parmeliaceae	*Alternaria alternata*, *Aspergillus flavus*, *Cladosporium* sp., *Fusarium solani*	16
Parmotrema reticulatum (Taylor) Choisy	Parmeliaceae	*Acremonium lichenicola*, *Alternaria alternata*, *Aspergillus flavus*, *Fusarium solani*, ***Nigrospora oryzae***, *Papulospora* sp., *Penicillium* sp., ***Pestalotiopsis maculans***, ***Sordaria fimicola***, *Xylaria hypoxylon*	16
Physcia dilatata Nyl.	Physciaceae	*Alternaria alternata, Aspergillus flavus*, *Aspergillus niger*, *Bipolaris australiensis*, *Cladosporium* sp., *Fusarium solani*, *Trichoderma harzianum*	14, 16

*Bold indicates specialized species.

Chaetomella sp., *Cladosporium* sp., *Cylindrosporium* sp., *Fusarium solani* (Mart.) Sacc., *Gilmaniella humicola* G.L. Barron, *Mucor racemosus* Bull., *Nigrospora oryzae* (Berk. & Broome) Petch, *Nigrospora sphaerica* (Sacc.) F.W. Masson, *Papulospora* sp., *Penicillium* sp., *Pestalotiopsis* sp. 1 & 2, *Pestalotiopsis maculans* (Corda) Nag Raj, *Sordaria fimicola* (Robberge ex Desm.) Ces. & De. Not., *Spegazzinia tessarthra* (Berk. & M.A. Curtis) Sacc., *Trichoderma harzianum* Rifai., *Trichophyton roseum* E. Boddin. and *Xylaria hypoxylon* (L.) Greb.] were recovered from 1200 lichen segments incubated from 12 macrolichens (Figure 1), and comprised Hyphomycetes (56.0%), Plectomycetes (16.0%), Coelomycetes (12.0%), Pyrenomycetes (8.0%) and Zygomycetes (4.0%) (Figure 2). So for the Zygomycetes population is represented by *Mucor racemosus*. Earlier studies across the globe have shown that Hyphomycetes dominates the endophytic assemblages and the incidence of Zygomycetes appears to be low. This is true in the present study too, as Zygomycetes population is 4.16% and Basidiomycetes was totally absent. Nine fungal species (*Acremonium lichenicola*, *B. australiensis, N. sphaerica*, *Papulospora* sp., *Pestalotiopsis maculans, Sordaria fimicola, Spegazzinia tessarthra*, *Trichophyton roseum, X. hypoxylon*) are being reported across the world as true endolichenic fungi. Generally it has been noticed that members of Xylariaceae predominate as endophytes in tropical regions, but the occurrence of *X. hypoxylon* in lichen samples of temperate region extends its geographical distribution.

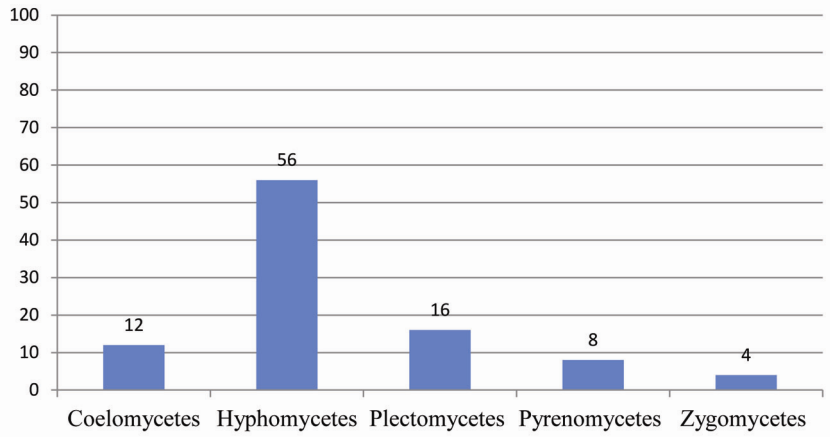

Figure 2. Percentage of endolichenic fungal classes in the study area.

Table 2. Colonization rate (CR), relative frequency (RF) and Shannon–Weiner biodiversity index (*H′*) of endolichenic fungi isolated from macrolichens of Kumaun Himalaya

Endolichenic fungus	No. of colonies	CR (%)	RF	*H′*
Hyphomycetes				
Acremonium lichenicola	05	0.41	0.20	0.0317
Alternaria alternata	124	10.33	4.96	0.3322
Bipolaris australiensis	01	0.08	0.04	0.0082
Cladosporium sp.	05	0.41	0.20	0.0317
Cylindrosporium sp.	01	0.08	0.04	0.0082
Fusarium solani	144	12.00	5.76	0.3667
Gilmaniella humicola	50	4.16	2.00	0.1847
Nigrospora oryzae	02	0.16	0.08	0.0148
Nigrospora sphaerica	01	0.08	0.04	0.0082
Papulospora sp.	05	0.41	0.20	0.0317
Spegazzinia tessarthra	01	0.08	0.04	0.0082
Trichoderma harzianum	65	5.41	2.60	0.2260
Trichophyton roseum	09	0.75	0.36	0.0528
Mycelia sterilia	392	32.66	15.68	0.5208
Plectomycetes				
Aspergillus flavus	304	25.33	12.16	0.4983
Aspergillus niger	35	2.91	1.40	0.1473
Aspergillus cfr. *coremiiformis*	01	0.08	0.04	0.0082
Penicillium sp.	40	3.33	1.60	0.1622
Pyrenomycetes				
Chaetomella sp.	01	0.08	0.04	0.0082
Sordaria fimicola	01	0.08	0.04	0.0082
Xylaria hypoxylon	02	0.16	0.08	0.0148
Coelomycetes				
Pestalotiopsis maculans	01	0.08	0.04	0.0082
Pestalotiopsis sp. 1	04	0.33	0.16	0.0251
Pestalotiopsis sp. 2	01	0.08	0.04	0.0082
Zygomycetes				
Mucor racemosus	05	0.41	0.20	0.0317

The occurrence of *Aspergillus niger*, *Cladosporium* sp., *N. oryzae*, *Penicillium* sp. and *Pestalotiopsis* sp. as endolichenic fungi in the present study corroborates with earlier investigations[16].

The frequently isolated fungi such as *Alternaria alternata*, *Aspergillus flavus* and *Fusariun solani* are generalist species which grow rapidly on culture medium[30–33]. *Aspergillus*, *Penicillium* and *Cylindrosporium* species isolated in this study are common soil or airborne fungi, but they also have the potential to live endophytically in lichens. Besides this, some species of endolichenic fungi (viz. *Spegazzinia tessarthra*, *N. sphaerica*, *N. oryzae*, *Pestalotiopsis maculans* and *Sordaria fimicola*) are specialized and reported from a single lichen species (Table 1). A single taxon of coprophilous fungi (*Sordaria fimicola*) was recorded as endolichenic, while the rest of the endolichenic fungi isolated in the present study were previously reported as saprophytes from Kumaun Himalaya.

In the present study mycelia sterilia has been frequently isolated as endophytes from all the macrolichens and was found having highest colonization rate (32.66%), relative frequency (15.68) and Shannon–Wiener biodiversity index (0.5208), followed by *Aspergillus flavus* > *Fusarium solani* > *Alternaria alternata* > *Trichoderma harzianum* > *Gilmaniella humicola* > *Penicillium* sp. > *Aspergillus niger* (Table 2). As reported earlier[34], many sterile fungi do not

sporulate in culture and due to the existence of non-culturable endophytes, the real number of endophytic species can be underestimated.

Recent studies have successfully used molecular techniques such as DNA cloning, DGGE and T-RLFP[35–37] to give taxonomic placements for mycelia sterilia. In spite of these techniques, the evaluation of fungal diversity is a major challenge to mycologists due to the scarcity of fungal and related eukaryotic sequences in databases[38]. Meanwhile, the last decade has bought significant advancements to the understanding and appreciation of the kingdom Fungi. Now we have a much clearer picture of how fungi evolve, assemble and interact. However, some questions in this new branch of endolichenic fungi need special attention and answer in near future: (1) What are they doing there and how do they co-exist? (2) What is their mode of nutrition? (3) Do these endophytes have some role in lichenization of a fungi? (4) Do they play a key role in host tolerance to stressful conditions? The use of genomics certainly will resolve this problem and enable mycology to flourish in near future.

1. Hammer, S., *Diversity and Distributions*, 2003, **9**, 487–488.
2. Hawksworth, D. L., *Mycol. Res*., 1991, **95**, 641–655.
3. Hawksworth, D. L. and Rossman, A. Y., *Phytopathology*, 1997, **87**, 888–891.
4. Sturz, A. V., Christie, B. R. and Nowak, J., *Crit. Rev. Plant Sci.*, 2000, **19**, 1–30.
5. Arnold, A. E., Maynard, Z., Gilbert, G. S., Coley, P. D. and Kursar, T. A., *Ecol. Lett.*, 2000, **3**, 267–274.
6. Petrini, O., Fischer, P. J. and Petrini, L. E., *Sydowia*, 1992, **44**, 282–293.
7. Raviraja, N. S., Sridhar, K. R. and Bärlocher, F., *Sydowia*, 1996, **48**, 152–160.
8. Smith, C. S., Chand, T., Harris, R. F. and Andrews, J. H., *Appl. Environ. Microbiol.*, 1989, **55**(9), 2326–2332.
9. Stanley, S. J., *Can. J. Bot*., 1992, **70**, 2089–2096.
10. Hata, K., Futai, K. and Tsuda, M., *Can. J. Bot.*, 1998, **70**, 2089–2096.
11. Arnold, A. E. *et al.*, *Syst. Biol*., 2009, **58**, 283–297.
12. Girlanda, M., Isocrono, D., Bianco, C. and Luppi-Mosca, A. M., *Mycologia*, 1997, **89**, 531–536.
13. Kannangara, B. T. S. D. P., Rajapaksha, R. S. C. G. and Paranagama, P. A., *Lett. Appl. Microbiol*., 2009, **48**, 203–209.
14. Li, W. C., Zhou, J., Guo, S. Y. and Guo, L. D., *Fungal Divers.*, 2007, **25**, 69–80.
15. Petrini, O., Hake, U. and Dreyfuss, M. M., *Mycologia*, 1990, **82**, 444–451.
16. Suryanarayanan, T. S., Thirunavukkarasu, N., Hariharan, G. N. and Balaji, P., *Sydowia*, 2005, **57**, 120–130.
17. Miadlikowska, A., Arnold, A. and Lutzoni, F., *Ecol. Soc. Am. Annu. Meet*., 2004, **89**, 349–350.
18. Lawrey, J. D. and Diederich, P., *Bryologist*, 2003, **106**, 80–120.
19. Lutzoni, F. L., Pagel, M. and Reeb, V., *Nature*, 2001, **411**, 937–940.
20. Tripathi, M., Gupta, R. C. and Joshi, Y., *Indian Phytopathol.*, 2014, **67**(1), 109–110.
21. Tripathi, M., Gupta, R. C. and Joshi, Y., *Proc. Natl. Acad. Sci*., 2014; DOI: 10.1007/S40009-014-0271-2.
22. Schulz, B., Wanke, U., Draeger, S. and Aust, H. J., *Mycol. Res*., 1993, **97**, 1447–1450.
23. Subramaniam, C. V., *Hyphomycetes: An Account of Indian Species except Cercosporae*, ICAR, New Delhi, 1971, p. 930.
24. Sutton, B. C., *The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata*, CABI Publications, Commonwealth Mycological Institute, Kew, London, 1980, p. 696.
25. Barnett, H. L. and Hunter, B. H., *Illustrated Genera of Imperfect Fungi*, Burgess Publishing Co, 1972, 3rd edn, p. 234.
26. Ellis, M. B., *Dematiaceous Hyphomycetes*, CABI Publications, Commonwealth Mycological Institute, Kew, London, 1971, p. 608.
27. Ellis, M. B., *More Dematiaceous Hyphomycetes*, CABI Publications, Commonwealth Mycological Institute, 1976, p. 507.
28. Chowdhry, P. N., *Manual on Identification of Plant Pathogenic and Biocontrol Fungi of Agricultural Importance*, IARI, New Delhi, 2000.
29. Gilman, J. C., *Manual of Soil Fungi*, Oxford and IBH Publishing Co., 1967, p. 450.
30. Fröhlich, J. and Hyde, K. D., *Biodivers. Conserv.*, 1999, **8**, 977–1004.
31. Cannon, P. F. and Simmons, C. M., *Mycologia*, 2002, **94**, 210–220.
32. Suryanarayanan, T. S., Murali, T. S. and Venkatesan, G., *Curr. Sci.*, 2003, **85**, 489–493.
33. Krishnamurthy, Y. L. and Shankarnaik, B., *Microbes Environ.*, 2008, **32**, 24–28.
34. Hyde, K. and Soytong, K., *Cryptog. Mycol.*, 2007, **28**(4), 1–9.
35. Seena, S., Wynberg, N. and Barlocher, F. R., *Fungal Divers.*, 2008, **30**, 1–14.
36. Duong, L. M., Jeewon, R., Lumyong, S. and Hyde, K. D., *Fungal Divers.*, 2006, **23**, 121–138.
37. Nikolcheva, L. G. and Barlocher, F., *Environ. Microbiol*., 2005, **7**, 270–280.
38. Hyde, K. and Soytong, K., *Fungal Divers.*, 2008, **33**, 163–173.

ACKNOWLEDGEMENTS. We thank the Head, Department of Botany, S.S.J. Campus, Kumaun University, Almora for providing laboratory facilities, and University Grants Commission, New Delhi for providing financial assistance.

Received 29 January 2014; revised accepted 7 August 2014

MANISH TRIPATHI[1]
YOGESH JOSHI[1,*]
RAMESH CHANDRA GUPTA[2]

[1]*Lichenology Division, and*
[2]*Mycology Division,*
Department of Botany,
S.S.J. Campus, Kumaun University,
Almora 263 601, India
**For correspondence.*
e-mail: dryogeshcalo@gmail.com