Northeast Journal of Contemporary Research, December 2022. Vol. 9 No. 1, pp 16-24
ISSN 2349-3089

Diversity of endolichenic fungi associated with three lichens of Assam

Dipanjali Devi
Postgraduate Department of Botany, Darrang College, Tezpur-784001, Assam, India

ABSTRACT

The study was undertaken to isolate endolichenic fungi from three selected lichens viz., *Pyxine*sp., *Graphis* sp. and *Cryptothecia* sp. collected from Assam. Healthy looking lichen thallus were collected from Tezpur and Kaliabor of Assam. Lichen thallus were surface sterilized and were cut into smaller fragments. Surface sterilized fragments were then plated on PDA(Potato Dextrose Results showed that *Trichoderma viridae, Phoma* sp. and Morphotype 1 were the dominant endolichenic fungi recovered from *Pyxine* sp*., Cryptothecia* sp. and *Graphis* sp. respectively collected from Tezpur however Aspergillus, Morphotype 1 and Aspergillus niger were the dominant endolichenic fungi associated with *Pyxine* sp., *Cryptothecia* sp. and *Graphis* sp. respectively collected from Kaliabor, Nagaon. Study showed that there is a wide diversity of endolichenic fungi in these lichens. Further study might result in the discovery of unique endolichenic fungal diversity. This is the first recorded study on endolichenic fungi from Assam , no work has been reported yet.

Keywords: Endolichenic fungi, *Graphis, Pyxine, Cryptothecia*.

INTRODUCTION

Fungi represent a consortium of various biologically potent metabolites having wide importance be it as antimicrobial, anti-inflammatory etc. Fungi are ubiquitous in nature,they can be found in terrestrial, fresh waterand marine environments where they function as saprobes, symbionts, and pathogens (Kellogg and Raja, 2015). A Group of highly diversified fungi reside within the internal tissue of other organisms, living asymptomatically without any obvious sign of infection. Multiple reviews have highlighted the bioactive metabolite diversity and potential of endophytic fungi to produce pharmaceutically valuable natural products (Kaul *et al*. 2012; Nisa *et al*. 2015; Proksch *et al*. 2010; Strobel *et al*. 2004; Tan and Zou 2001). An analogous group of fungi inhabit the thalli of lichen in a similarly asymptomatic manner: the endolichenic fungi.

Lichen thalli is a combined structure of symbiotic association between a fungal organism (mycobiont) and at least one chlorophyll-containing photosynthetic organism (photobiont) such as a micro alga, a cyanobacterium, or both (Lutzoni and Miadlikowska 2009). In addition to the fungal partner of the lichen, the thallus is also a home to numerous, asymptomatic, cryptic microfungi that live in close association with the photobiont (Arnold *et al*. 2009). These diverse groups of fungi, which reside in the interior of a lichen thallus, have been termed as 'endolichenic fungi' (Arnold *et al*. 2009; Miadlikowska *et al*. 2004). Endolichenic fungi were discovered when

**Corresponding author's Email: sumidipa13@gmail.com*

attempts were compelled to isolate the Mycobiont of lichen into pure culture (Crittenden *et al*. 1995; McDonald *et al*. 2013; Petrini *et al*. 1990). These fungi are very much similar to the endophytic fungi (sometimes also referred to as endophyte-like fungi) (Arnold *et al*. 2009; U'Ren *et al*. 2016), which reside within healthy tissues of plants and they are phylogenetically and ecologically diverse without causing any diseased symptoms (Arnold 2001, 2007; Petrini 1991).

The endolichenic fungi, however, are dissimilar from mycobionts (Lutzoni and Miadlikowska 2009), which make up almost more than half part of the lichen thallus, and from lichenicolous fungi, an ecological group of meiosporic and mitosporic fungi that can often be observed on living lichens (Arnold *et al*. 2009). The endolichenic fungi consist of a number of horizontally transmitted, advantageous fungi, and include abundant taxa belonging to diverse classes, orders and families within the Ascomycota (Pezizomycotina), Deuteromycotina. (Arnold *et al*. 2009; Girlanda *et al*. 1997; Kannangara *et al*. 2009; Li *et al*. 2007; Petrini *et al*. 1990; Suryanarayanan *et al*. 2005; Tripathi and Joshi 2015; U'Ren *et al*. 2010, 2012). Endolichenic fungi have become a new approach for evaluation of bioactive secondary metabolite chemistry in natural products research, behind time.

Assam is rich in floral and faunal diversity and comes under the northeastern Indian biogeographic zone. The extraordinary physiographic topography makes the region suitable to colonize diverse organisms including many lichens. Despite being rich in biodiversity, the exploratory work on lichens of Assam is meagre. Floristic study on lichens in Assam was introduced by Stirton (1881), a Scottish lichenologist who described 39 lichen species only from tea plants. A few researchers made their contributions to the lichen biota of the state (Awasthi & Singh 1973; Pant & Upreti 1993; Rout *et al*. 2005, 2010; Gupta & Sinha 2011, 2016; Sinha *et al*. 2013; Daimari *et al*. 2014, Gogoi *et al*., 2019). Recently, Gupta & Sinha (2018) reported 300 species of lichen belonging to 83 genera and 26 families from Assam. Work on endolichenic fungi is limited from North east India,recently a work has been reported on *Cryptothecia* sp. collected from Arunachal Pradesh (Devi *et al*., 2022).However no work has been reported from Assam. This is the first study of endolichenic fungi reported from three selected lichens of Assam.

MATERIALS AND METHODS

Study area

Kaliabor is in Nagaon district and is located in Brahmaputra valley agro climatic zone, therefore the region is under subtropical humid climatic belt and it essentially enjoys characteristics of monsoonal climate. The district is characterized by excessive humidity, heavy summer rainfall, and cool dry winter. The mean annual rainfall was 141.5 mm and the mean annual temperature was 24.8□ during the period of 2010- 2013. During this period the major portion of rainfall received from May toSeptember and July is considered as the rainiest month of the year by receiving average rainfall as 377 mm. Tezpur is a city and urban agglomeration in Sonitpur district, Assam state, India. Tezpur is located on the banks of the river Brahmaputra, 175 kilometres northeast of Guwahati, and is the largest of the north bank cities with a population exceeding 100,000 as per Metropolitan Census 2011.Summer, winter and rainy season are experienced in the region. Variations of climate in the region are experienced due to the intermixing of hills and plains of different elevation. North east and south west monsoon governed the rainfall of the region. About more than three fourth of the total annual rainfall are influenced by the south west monsoon which operates from May/June to September/October. Rainfall during the month of November to April is governed by north east monsoon. Owing to its climatic conditions luxuriant growth of different lichen species was encountered in this region. For the present investigation two abundantly found lichens species were collected for study of endolichenic fungi.

Lichen identification

Healthy lichen thallus of 3 selected lichens viz., *Cryptothecia* sp. ,*Pyxine* sp., and *Graphis*sp.,was collected from Tezpur region (27.0274° N, 92.6102°E) and Kaliabor region (27.2109° N, 92.5067° E) of Assam which encloses parts of Indo-Burma belt. Morphological characterization was done under a Leica EZ4 and Leica S9i stereo-zoom microscope while anatomical details were examined under Leica DM2500 compound microscope. Chemical characterization was done through Spot tests and Thin layer chromatography was performed in solvent system C (Toluene:Acetic acid; 85:15 ml) (Orange *et al*., 2001). Identification of taxa was done by relevant published literature (Awasthi, 1991; 2001).

Isolation and identification of endolichenic fungi

The three lichen thallus was first surface sterilized following standard protocol (Guo *et al*., 2003). The surface sterilized thallus was cut into smaller fragments (0.5 × 0.5 cm) and was air dried. The dried surface sterilized lichen fragments were placed on PDA (Potato Dextrose Agar) which were supplemented with 0.01% Streptomycin sulphate. The plates were incubated at 28±2°C in BOD incubator until the growths of endolichenic fungi were appeared. The endolichenic fungi were identified on the basis of colony morphology and reproductive structures referring standard identification manuals (Barnett and Hunter, 1998 ; Gilman, 1971) and were inoculated in PDA slants and stored at 4°C.

Endolichenic fungi diversity data analysis

The relative colonization frequency (CF %) of endolichenic species was calculated using the same formula as applied to endophytic fungi:

CF % = (Ncol / Nt) × 100

Where, Ncol stands for the number of segments colonized by each endolichenic fungal isolates, and Nt stands for the total number of segments plated (Hata and Futai, 1995 and Tayung and Jha,2006).

RESULTS AND DISCUSSION

Identification of the lichen species

Identification of the collected lichen species was done by following the literatures of Awasthi (1991, 2007) and Jagadeesh Ram and Sinha (2016). Based on morphological and microscopic observations the selected lichen species were identified as *Cryptothecia* sp., *Graphis* sp. and *Pyxine*sp. Brief description and identifying features of each lichen species are presented below.

A) *Cryptothecia* sp.

Description: Thallus corticolus, epiphloeodal, greenish-grey, ecorticate; prothallus white, well developed; photobiont*Trentepohlia*.Ascigeros tissue scattered in the thallus, slightly elevated fertile areas; paraphysoids densely branched and interwoven, enclosing the asci; asci aggregated in ascigerous areas, globose to broadly clavate thick walled muriformascospores with wavy septa.

Chemistry: Thallus K–, C+ red, KC+ red, P–, medulla I+ blue; gyrophoric acid reported.

B) *Graphis* sp..

Description: Thallus crustose, epiphloeodal, whitish grey with greenish tinge, smooth to uneven, sparsely rimose; apothecialirellate, lirellae dense, semiemergent to emergent, prominent, simple, straight to slightly curved to flexuous, 0.5 –3.5 mm long, ends round, margin thin, disc concealed, black epruinose,round; labia entire, black; exciplulum convergent with lateral thalline margin, laterally carbonized; hymenium hyaline, inspersed, 50–70 µm high; paraphyses simple, anastomosing; asci cylindrico clavate, 8- spored; ascospores hyaline, elongate ellipsoid, 5–7-septate, 15–25 × 4–6 µm, I+ blue.

Chemistry: Thallus K−, C−, KC−, P−; no lichen substances detected by TLC.

C) *Pyxine* sp.

Description: Thallus foliose, corticolous, orbicular to suborbicular, 3–6 cm diam., pale grey, tightly adnate to the substrate; lobes linear, discrete, 0.5–1 mm wide, plane to concave, with diffused pruina mostly in the apical region; maculae marginal and laminal, distinct in the apical region, developing into pseudocyphellae and then into soralia; soralia orbicular, ellipsoid, linear or irregular in outline; soredia farinose to granular; lobes 110–220 µm thick; upper cortex paraplectenchymatous, 12–20 µm thick; medulla white; lower cortex brown to black, paler towards the margin, prosoplectenchymatous, 15–30 µm thick; rhizines ± dense, furcated. Apothecia not seen.

Chemistry: Spot tests: Cortex K−, C−KC−, P−, UV+ yellow; medulla K−, C−KC−, P−; TLC: lichexanthone.

3.2 Isolation and identification of endolichenic fungi

In the present study a total of about 162 number of isolates have been recovered from a total of 300 surface sterilised lichen fragments of *Cryptothecia* sp., *Pyxine* sp. and *Graphis* sp. Out of all the isolates Morphotype 1 is the highest occurring endolichenic fungal isolate from Tezpur, which is followed by *Trichoderma harzianum* (21 number of isolates).*Trichoderma harzianum* is the commonly occurring isolate among all the three lichens. Out of all the three lichens it has been found that maximum number of isolates has been recovered in *Pyxine* sp. (70 isolates) , followed by *Graphis* sp.(65 isolates) and lowest in *Cryptothecia* sp.(27 isolates).

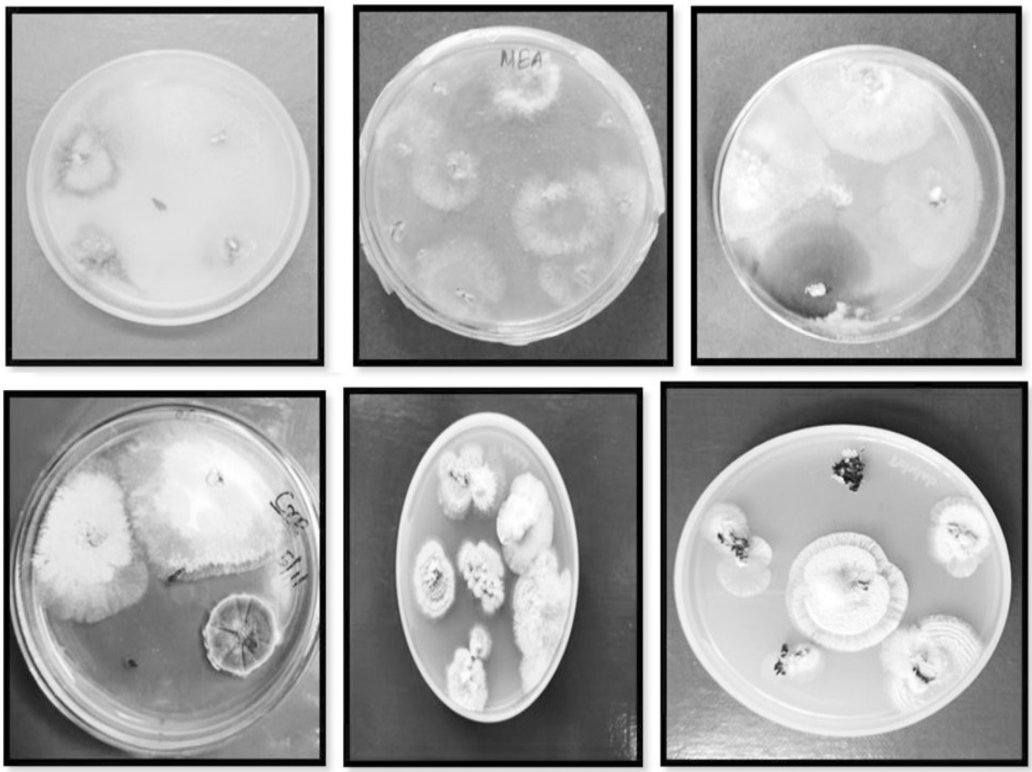

Figure 1. Photoplates representing endolichenic fungal isolates from surface sterilized lichen fragments of *Cryptothecia* sp., *Pyxine* sp. and *Graphis* sp.

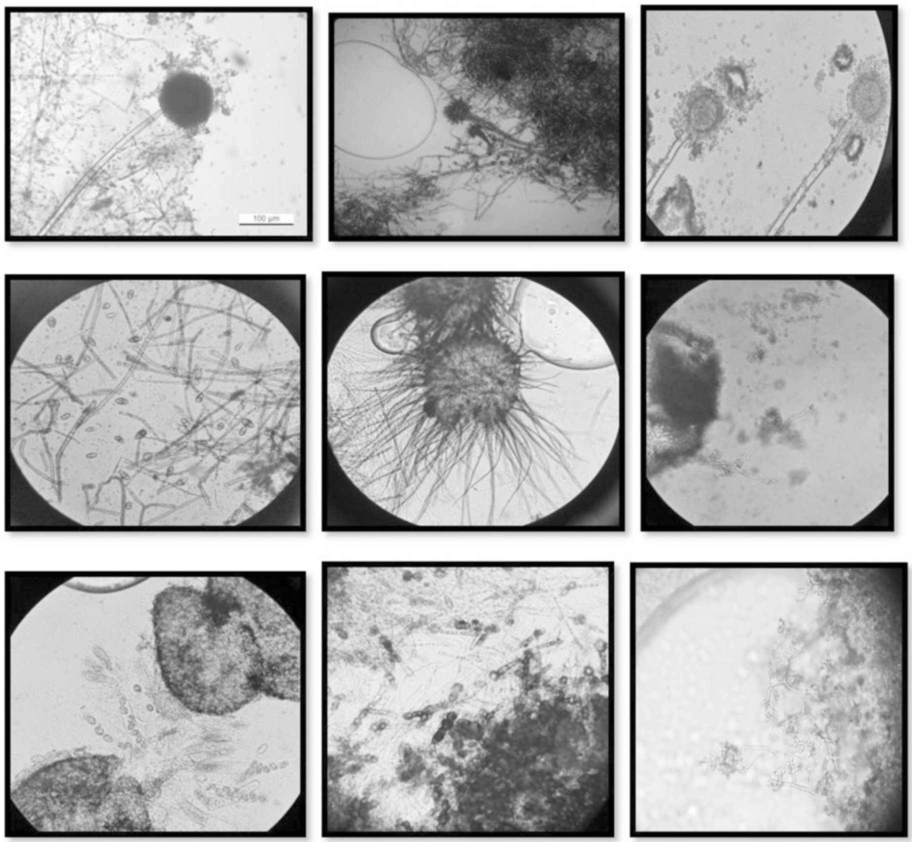

Figure 2. Photoplates showing microscopic image of the isolated endolichenic fungi
A- *Aspergillus niger* B- *Aspergillus* sp. C- *Aspergillus flavus* D-*Dreschlera* sp., E- *Chaetomium* sp. F-*Penicillium* sp. G- *Sordaria* sp. I-*Rhizoctonia* sp. J- *Trichoderma* sp. (Size-100 µm)

Table 1. Study of diversity pattern in endolichenic fungi from Tezpur

Lichen	Endolichenic fungal isolate	Number of isolates	Colonization rate (%)	Relative frequency
Pyxine sp.	*Rhizoctonia* sp.	2	2	3.50%
	Chaetomium sp.	1	1	1.75%
	Trichoderma harzianum	9	9	12.20%
	Trichoderma viridae	12	12	17.50%
	Penicillium sp.	2	2	3.50%
	Periconia sp.	1	1	1.75%

*Graphis*sp.	Mycelia sterilia	31	31	54.38%
	Phoma sp.	20	20	38.16%
	Trichoderma harzianum	6	6	11.53%
	Trichoderma viridae	8	8	15.30%
Cryptothecia sp.	Mycelia sterilia	12	12	23.07%
	Trichoderma harzianum	6	6	9.30%
	Rhizopus sp.	1	1	1.56%
	Aspergillus flavus	1	1	1.56%
	Sordariafimicola	1	1	1.56%
	Penicillium sp.	1	1	1.56%
	Morphotype 1	25	25	39.06%
	Morphotype 2	9	9	14.06%
	Morphotype 3	12	12	18.75%
	Acremonium sp.	2	2	3.12%

Isolation of endolichenic fungi from Kaliabor

In the present study a total of about 190 number of isolates have been recovered from a total of 300 surface sterilised lichen fragments of *Cryptothecia* sp., *Pyxine* sp. and *Graphis* sp. Out of all the isolates Mycelia sterilia is the highest occurring endolichenic fungal isolate from Kaliabor, which is followed by *Phoma*(20 number of isolates), whereas Morphotype 1 is the highest occurring endolichenic fungi from Tezpur . Out of all the three lichens it has been found that maximum number of isolates has been recovered in *Cryptothecia* sp. (74 isolates), followed by *Graphis* sp.(67 isolates) and lowest in *Pyxine* sp.(49).

Table 2. Study of diversity pattern in endolichenic fungi from Kaliabor

Lichen	Endolichenic fungal isolate	Number of isolates	Colonization rate (%)	Relative frequency
Cryptothecia sp.	*Bipolaris* sp.(Crp 04)	6	6	8.11%
	Bipolaris sp. 1 (Crp 08)	12	12	16.22%
	Bipolaris sp.2 (Crp 12)	6	6	8.11%
	Aspergillus sp.(Crp 03)	3	3	4.16%
	Mycelia sterilia	31	31	1.38%
	Penicillium chrysogenum	2	2	2.77%
	Penicillium chreasum	14	14	19.44%
Pyxine sp.	Myeceliasterilia	29	29	59.19%
	Aspergillus sp.	13	13	26.53%
	Sordariafimicola	05	05	10.20%
	Chaetomium sp.	02	02	4.08%

*Graphis*sp.	Mycelia sterilia	52	52	77.61%
	Aspergillus sp.	12	12	17.91%
	Penicillium sp.	2	2	2.96%
	Sordariafimicola	1	1	1.49%

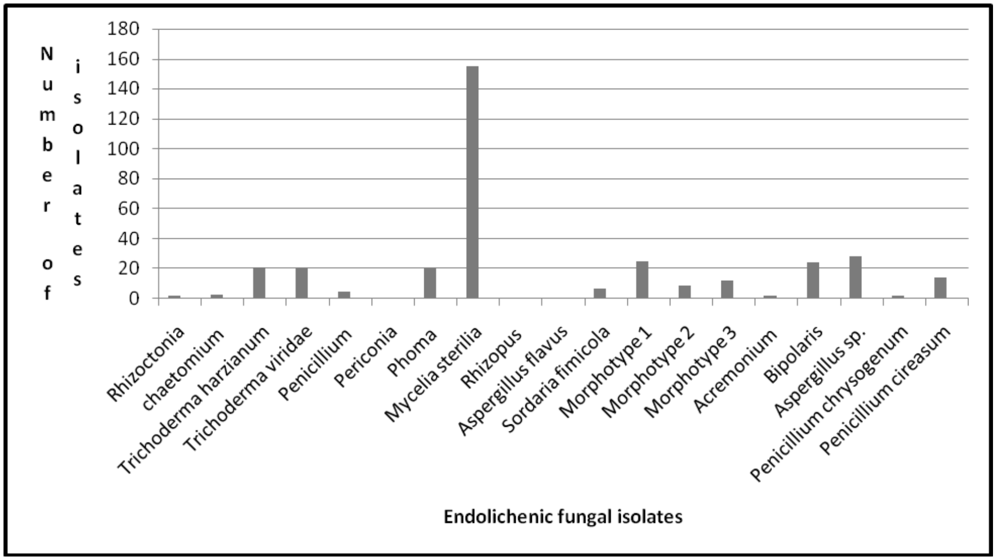

Figure 3. Graphical representation to the occurrence of endolichenic fungal isolates from two different sites

CONCLUSION

Endolichenic fungi are ubiquitous in nature as like endophytic fungi. Present study reveals the occurrence of endolichenic fungi from the two different sites of Assam particularly Tezpur and Nagaon.Study shows that both the region harbors many different endolichenic fungi.These three selected lichens basically known for their antimicrobial potential represents wide occurrence of endolichenic fungi.Studies have indicated that they produce potent bioactive metabolites with wide therapeutic applications. Considering the diminishing plant diversity which harbors maximum lichen flora research priority should be directed to study them especially in developing countries like India, because once lichens get extinct so will be the associated endolichenic fungi. The current study is an endeavor in this direction, and our study suggests that endolichenic fungi could be a potential source of antimicrobial agents.

ACKNOWLEDGEMENTS

The author is thankful to IQAC Darrang college for providing financial assistance to carry out the project work. Author also thanked Department of Botany for providing laboratory facilities in completing the project work.

REFERENCES

Arnold AE (2001) Fungal endophytes in neotropical trees: abundance, diversity, and ecological implications. In: Ganeshaiah KN, Shaanker RU, Bawa KS (eds) In tropical ecosystems: structure, diversity and human welfare. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp 739–743

Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biol Rev* 21 (2–3):51–66

Arnold AE, Miadlikowska J, Higgins KL *et al* (2009) A phylogenetic estimation of trophic transition networks for ascomycetous Fungi: Are lichens cradles of symbiotrophic Fungal diversification? *Syst Biol* 58(3):283–297

Awasthi, D.D. & K.P. Singh (1973). A note on the revision of some graphidaceous lichen taxa. *Current Science* 42(18): 656−657.

Awasthi, D.D. (1991). A key to the microlichens of India, Nepal and Sri Lanka. *Bibliotheca Lichenologica* 40: 1–136.

Awasthi, D.D. (2007). A Compendium of the Macrolichens from India, Nepal and Sri Lanka. Bishen Singh Mahendra Pal Singh, Dehradun, India, 580pp

Barnett, HL and Hunter BB. 1998. Illustrated Genera of Imperfect Fungi (APS press, St. Paul Minnesota).

Crittenden P, David J, Hawksworth D *et al* (1995) Attempted isolation and success in the culturing of a broad spectrum of lichen-forming and lichenicolous fungi. *New Phytol* 130(2):267–297

Daimari, R., N. Hazarika, R.R. Houqe, D.K. Upreti& S. Nayaka (2014). New records of epiphytic lichens from three districts of Assam. *The Indian Forester* 140(8): 807–811.

Devi D, Gogoi R, Yasmin F, Tayung K. 2022 Antimicrobial activity of Endolichenic fungi isolated from *Cryptothecia* sp. against some human test pathogens. *Research Journal of Pharmacy and Technology;* 15 (5):2193-7.

Gilman JC. 1971. A Manual of Soil Fungi. Iowa : Iowa State College Press.

Girlanda M, Isocrono D, Bianco C *et al* (1997) Two foliose lichens as microfungal ecological niches. *Mycologia* 89:531–536

Gogoi, R., S. Joseph, S. Nayaka & F. Yasmin (2019). Additions to the lichen biota of Assam State, India. *Journal of Threatened Taxa* 11(6): 13765-13781

Guo LD, Huang GR, Wang Y, He WH, *et al*; 2003. Molecular identification of white morphotype strains of endophytic fungi from Pinus tabulaeformis. *Mycological Research*; 107(6): 680–688.

Gupta, P. & G.P. Sinha (2018). Lichen Flora of Assam. Bishen Singh Mahendra Pal Singh, Dehradun, India, 274pp.

Hata K and Futai K. 1995. Endophytic fungi associated with healthy pine needles and needles infested by the pine needle gall midge, Thecodiplosis japonensis. *Canadian Journal of Botany* ;73(3): 384–390.

Jagadeesh Ram, T. A. M. & Sinha, G. P. (2016). A world key to *Cryptothecia* and *Myriostigma* (Arthoniaceae), with new species and new records from the Andaman and Nicobar Islands, India.*Phytotaxa,* 266(2), 103-114.

Kannangara BTSDP, Rajapaksha RSCG, Paranagama PA *et al* (2009) Nature and bioactivities of endolichenic fungi in Pseudocyphellaria sp., Parmotrema sp. and Usnea sp. at Hakgala montane forest in Sri Lanka. *Lett Appl Microbiol* 48(2):203–209

Kaul S, Gupta S, Ahmed M *et al* (2012) Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. *Phytochem Rev* 11(4):487–505

Kellogg JJ, Raja HA. 2016. Endolichenic fungi: a new source of rich bioactive secondary metabolites on the horizon. *Phytochem Rev*. doi:10.1007/s11101-016-9473-1

Li W-C, Zhou J, Guo S-Y *et al* (2007) Endophytic fungi associated with lichens in Baihua mountain of Beijing, China. *Fungal Di-*

vers 25:69–80

Lutzoni F, Miadlikowska J (2009) Lichens. Quick guide. Curr Biol 19:R502–R503.

McDonald TR, Gaya E, Lutzoni F (2013) Twenty-five cultures of lichenizing fungi available for experimental studies on symbiotic systems. *Symbiosis* 59(3):165–171

Miadlikowska J, Arnold AE, Lutzoni F (2004) Diversity of cryptic fungi inhabiting healthy lichen thalli in a temperate and tropical forest. *Ecol Soc Am Annu Meet* 89:349–350

Nisa H, Kamili AN, Nawchoo IA *et al* (2015) Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: a review. *MicrobPathog* 82:50–59

Orange, A., P.W. James & F.J. White (2001). Microchemical Methods for the Identification of Lichens. British Lichen Society, London, 101pp.

Pant, G. & D.K. Upreti (1993). The lichen genus Diploschistes in India and Nepal. *Lichenologist* 25(1): 33–50

Petrini O (1991) Fungal Endophytes in tree leaves. In: Hirano SS (ed) Microbial ecology of leaves. Springer, New York, pp 179–197

Petrini O, Hake U, Dreyfuss M (1990) An analysis of fungal communities isolated from fruticose lichens. *Mycologia* 82:444–451

Proksch P, Putz A, Ortlepp S *et al* (2010) Bioactive natural products from marine sponges and fungal endophytes. *Phytochem Rev* 9(4):475–489

Rout, J., P. Das & D.K. Upreti (2010). Epiphytic lichen diversity in a reserve forest in south Assam, northeast India. *Tropical Ecology* 51(2): 281–288.

Rout, J., R. Rongmei& P. Das (2005). Epiphytic lichen flora of a pristine habitat (NIT Campus) in southern Assam, India. *Phytotaxonomy* 5: 117–119.

Sinha, G.P., P. Gupta, T.A.M.J. Ram & C.M. Solanki (2013). A contribution to the lichen flora of Assam, India. *Indian Journal of Forestry* 36(3): 393–400.

Stirton, J. (1881). On the vegetable parasites of the tea plant, more especially of Assam. *Proceedings of Royal Philosophical Society of Glasgow* 13: 181–193

Strobel GA, Daisy B, Castillo U *et al* (2004) Natural products from endophytic microorganisms. *J Nat Prod* 67:257–268

Suryanarayanan T, Thirunavukkarasu N, Hariharan G *et al* (2005) Occurrence of non-obligate microfungi inside lichen thalli. *Sydowia* 57(1):120

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. *Nat Prod Rep* 18:448–459

Tayung K and Jha DK. 2006. Antimicrobial evaluation of some fungal endophytes isolated from the bark of Himalayan yew. *World Journal of Agriculture Science*; 2: 489–494.

Tripathi M, Joshi Y (2015) Endolichenic fungi in Kumaunhimalaya: a case study. Recent advances in lichenology. Springer, Berlin, pp 111–120

U'Ren JM, Lutzoni F, Miadlikowska J *et al* (2010) Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. *MicrobEcol* 60(2):340–353

U'Ren JM, Lutzoni F, Miadlikowska J *et al* (2012) Host and geographic structure of endophytic and endolichenic fungi at a continental scale. *Am J Bot* 99(5):898–914

U'Ren JM, Miadlikowska J, Zimmerman NB *et al* (2016) Contributions of North American endophytes to the phylogeny, ecology, and taxonomy of Xylariaceae (Sordariomycetes, Ascomycota). *Mol Phylogenet Evol* 98:210–232