J. Indian bot. Soc. e-ISSN:2455-7218, ISSN:0019 - 4468 Vol. 99 (1&2) 2020:46-55

DIVERSITY OF FUNGAL ENDOPHYTES FROM *PHLOGACANTHUS THYRSIFORMIS* (ROXB.EX HARDW.) MABB., A PROMISING MEDICINAL PLANT IN ARUNACHAL PRADESH, NORTHEAST INDIA.

RICHA SHARMA[1], SUMPAM TANGJANG[1] AND AMRITESH C. SHUKLA[2]

[1]Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh, Itanagar- 791112 Arunachal Pradesh, India, [2]Department of Botany, University of Lucknow, Lucknow, Uttar Pradesh- 226007, India.
E-mail: amriteshcshukla@gmail.com
Date of online publication: 31st March 2020
DOI:10.5958/2455-7218.2020.00004.2

The findings of the present study deals with the first type of its report, as presented in the current study, on the diversity of fungal endophyte from ethno medicinal plant- *Phlogacanthus thyrsiformis* (Roxb.ex Hardw.) Mabb., (Acanthaceae). The plant is growing wildly in the forest of Papum Pare, Arunachal Pradesh, India. The samples of the fungal endophytes were collected randomly from the study area and identified based on the morphological, cultural, and reproductive structures (hyaline, ellipsoidal, aseptate, pycnidia, beta conidia, perithecia, asci and ascospores). Further, the phylogenetic analysis of the isolated species was made, using the sequences of 5.8S and 28S rDNA internal transcribed spacer sequence 1 and 4. Overall, 41 fungal spp have been isolated, out of which 37 species belong to the class Ascomycetes; 01 species belong to the each class Zygomycota, Oomycota and Basidiomycota, respectively; however 01 fungal endophyte couldn't identified properly. The maximum number of fungal isolates were recorded from leaves (37%), followed by stems (33%) and root (30%). The observations shows that *Colletotrichum siamense* was the most dominant endophytic fungi, isolated from the selected plant (*P. thyrsiformis*); followed by *Chaetomium globosum*, *Sordaria fimicola, Aureobasidium* sp., *Pythiopsis cymosa* etc. However, *Colletotrichum siamense, Aspergillus fischeri, Plenodomus wasabiae, Aspergillus fumigatus, Nigrospora oryzae,* Saccharomycetales sp., *Epicoccum nigrum., Curvularia borreriae, Colletotrichum guajavae* were the common fungal endophytes recorded from all the plant parts viz., leaf, stem and roots. Besides this, the density of colonization (rD%) was also recorded chronologically as *Colletotrichum siamense* (12.66%) < *Chaetomium globosum* (3.33%) < *Sordariomycetes* sp., *Sordaria fimicola* and *Aureobasidium* sp. (2.66%) followed by the other remaining endophytes (0.66% to 2.00%), respectively.

Keywords: Biodiversity, ethno medicinal plant, endophytic fungi, Molecular identification, Phylogenetic analysis.

De Barry (1866) coined the term 'endophyte' to detect fungi which lives intercellularly and intracellularly in plants tissues, without causing any harm to the plant (Compant *et al.* 2017, Jeewon *et al.* 2017). Further, on the bases of their interaction with host plants, fungal endophytes may be divided into three groups- commensalists, parasites and mutualists (Jia *et al* 2016, Kirschner 2018). In mutualism, definite fungal endophytes gives tolerance to abiotic and biotic stresses on their host plants, improve their growth, and restrain diseases (Redman 2002, Rodriguez 2008). These Endophytes protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites (Nath *et al.* 2012, Nongalleima *et al.* 2013). Endophytic fungi were reported in almost all the plants spp viz. algae, ferns, mosses and mainly in gymnosperms, angiosperms, reported from various parts of the world (Radic and Strukelj 2012, Doilom *et al.* 2017). Many researchers have proven that endophyte are the potential source of novel natural products for exploitation in modern medicine, agriculture and industry (Kaul *et al.* 2013).Till date it has been found that taxol can be produced by endophytic fungi *Metarhizium anisopliae* and *Cladosporium cladosporioides* (El-Maali *et al* 2018). Moreover, since, fungal endophytes can easily be grown in laboratories under routine culture techniques, hence, the potential for discovering virtually inexhaustible supply of metabolites is high. Fungal endophytes are capable of producing compounds similar to their host plants, and are capable in the preservation of world's diminishing biodiversity (Bender *et al.* 2016, Mane *et al.* 2017). However, the practicality of commercial production of compounds by

Received on October 31, 2019 www.indianbotsoc.org Accepted on February 15, 2020

endophytic fungi still remains unproven. The reduction of secondary metabolite production on repeated sub-culturing under axenic monoculture conditions is one of the key challenges that need to be addressed in order to establish, restore and sustain the *in vitro* biosynthetic potential of endophytes (Strobel and Daisy 2003).

Studies on fungal endophytes concentrated on medicinal plants have shown that the curative property of the medicinal plant is not only because of the chemicals present in the plant but also because of the fungal endophytes that present in the plant (Verma 2011, Suryanarayanan 2013). Thus, there is enormous need to isolate and identify more number of fungal endophytes and explore the potency of their secondary metabolites. Although, number of studies have already been reported in the association of fungal endophytes with medicinal plants from India viz., *Clerodendron serratum, Pongamia, Withania somnifera (*Ashwagandha*) Taxus brevifolia, Azadirachta indica, Terminalia arjuna, Trigonella foenum, Labelia nicotinifolia, Adhatoda zeylanica, Bauhinia phoenicea,Catharanthus roseus, Parthenium hysterophorus, Ficus religiosa, Coffea arabica, Crataeva magna, Silybum marianum, Allium sativum, Mamordica chaeantia,* and *Nothapodytes nimmoniana*, (Mane *et al.* 2017, Mane and Vedamurthy 2018).

It is useful for curing coughs, colds and asthma and is easy to administer. It has also been reported to have antimicrobial properties (Singh and Singh 2010). Fruits and leaves are taken after burning as specific for fevers. Fruits and leaves are taken by the Karbi tribes of Assam after burning them as a specific treatment for fever (Patwari 1992); and also been used in jaundice (Khanikar 2005).

MATERIALS AND METHODS

Study area and Sample collection

The study area Papum Pare is located at the N 26°56′11″ to 27°35°44″ and E 93°12′45″ to 94°13′30″ is one of the important hotspots in

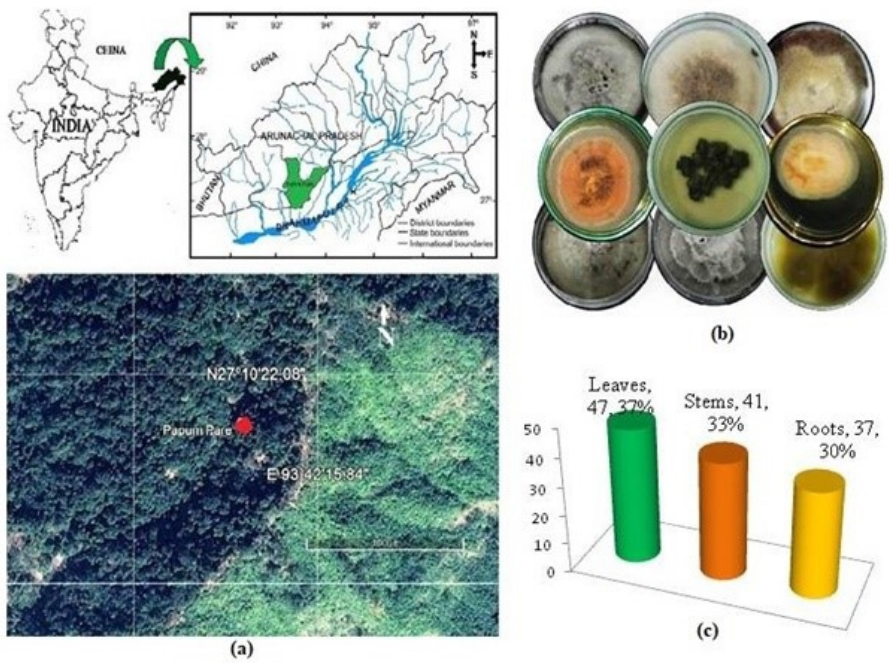

Figure 1 (a) Sample collection site of *Phologacanthus thyrsiformis* Roxb.ex Hardw.Mabb., in Arunachal Pradesh, India (satellite image from Google Earth), where, red circle indicates collection site. **(b**) Isolated endophytic fungi (some) on the culture plates, separately **(c)** Percentage fungal endophytes isolated from different parts of *P. thyrsiformis*

the world, ranked 25th (Chowdhery 1999) and among the 200 globally important eco-regions (Olson and Dinerstein 1998). It covers an area of 2875 sq. km, having annual rainfall 2694 mm. Major part (75%) of this district is sheltered by thick forest which has sub-tropical, deciduous and humid type of vegetation (Fig.1-a). The selected plant, *P. thyrsiformis* is an evergreen shrub grows up to 2.4 m high, branchlets quadrangular; leaves 13 to 35 cm long, oblanceolate, elliptic-oblong, acute or acuminate, entire. Flowers in terminal elongated, thyrsoid panicles, up to 30 cm long; corolla tubular, curved; orange or brick red villous. Capsule 3.8cm long, linear clavate (Phurailatpam *et al* 2014).

Samples of leaves, stems and roots were collected during the period of Dec 2016 to Dec 2018 from the healthy plant of *P. thyrsiformis*; in sterile bags and brought to the laboratory for processing within 24 hours after sampling.

Surface sterilization and isolation: During investigation, total 150 sample segments (50 leaf, 50 stem, and 50 root) were collected from 15 different *P. thyrsiformis* plants. The samples thus collected were washed gently in running tap water to remove the soil and debris. Further, isolation of fungal endophytes was determined, using the method of Suryanarayanan *et al* (1998). Samples were chopped into small pieces of (0.5-1.0 cm), and surface-sterilized by dipping them serially in 70% ethanol for 5 sec followed by 4% NaOCl for 90 sec; and finally rinsed in sterile distilled water for 10 sec. The samples thus processed (150 segments) were placed in petriplates (7.5 cm diam; @ ten samples in each plate) already contained potato dextrose agar (PDA) medium (supplemented with 150 mg/L chloramphenicol). The petriplates were sealed properly using parafilm and incubated (12 h dark: 12 h light cycle) for 25 days at the temperature 25±2°C, following the method of Suryanarayanan (1992). After incubation period, the germinated hyphal tips were isolated and sub cultured on PDA medium, and preserved at 4°C to get the purified sample(s), for further investigation.

Besides this, the density of colonization (rD%) of a single endophyte species was calculated, using the formula as suggested by Fisher and Petrini (1987).

$$rD\% = (Ncol/Nt) \times 100$$

Where,

Ncol = Number of segments colonized by each fungus

Nt = Total number of segments inoculated

Morphological identification: The endophytes thus isolated, were identified based on their macroscopic and microscopic characteristics. The fruiting structures and spore morphology were assessed, as per the expertise available at the laboratory as well as confirmed using primary and secondary literature (Ellis 1976, Domsch *et al.* 1980, and Nagmani 2005, Jia *et al*. 2016).

Molecular Identification:DNA Extraction And PCR Amplification of rDNA : Genomic DNA was extracted from fungal mycelia (5-8 days old culture) growing on PDA plates (at 25± 2°C), using Qiagen microbial extraction kit. Fragments of 18S rRNA gene were amplified by PCR, using forward ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and revers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primer (White *et al.* 1990). Each PCR amplification reaction was performed in a final volume of 25 µL containing 25 ng template DNA, 10X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM MgCl2, 200 µM of each dNTP, 50 pM of each primer, 1 unit of Taq polymerase (Genei, Banglore) and distilled water (Sigma, USA). The conditions of the PCR was initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 45 s, annealing at 56 °C for 1 min and primer extension at 72 °C for 1 min (again 35 cycles), final extension at 72 °C for 7 min (1 cycle) and hold (cooling) at 4 °C. PCR

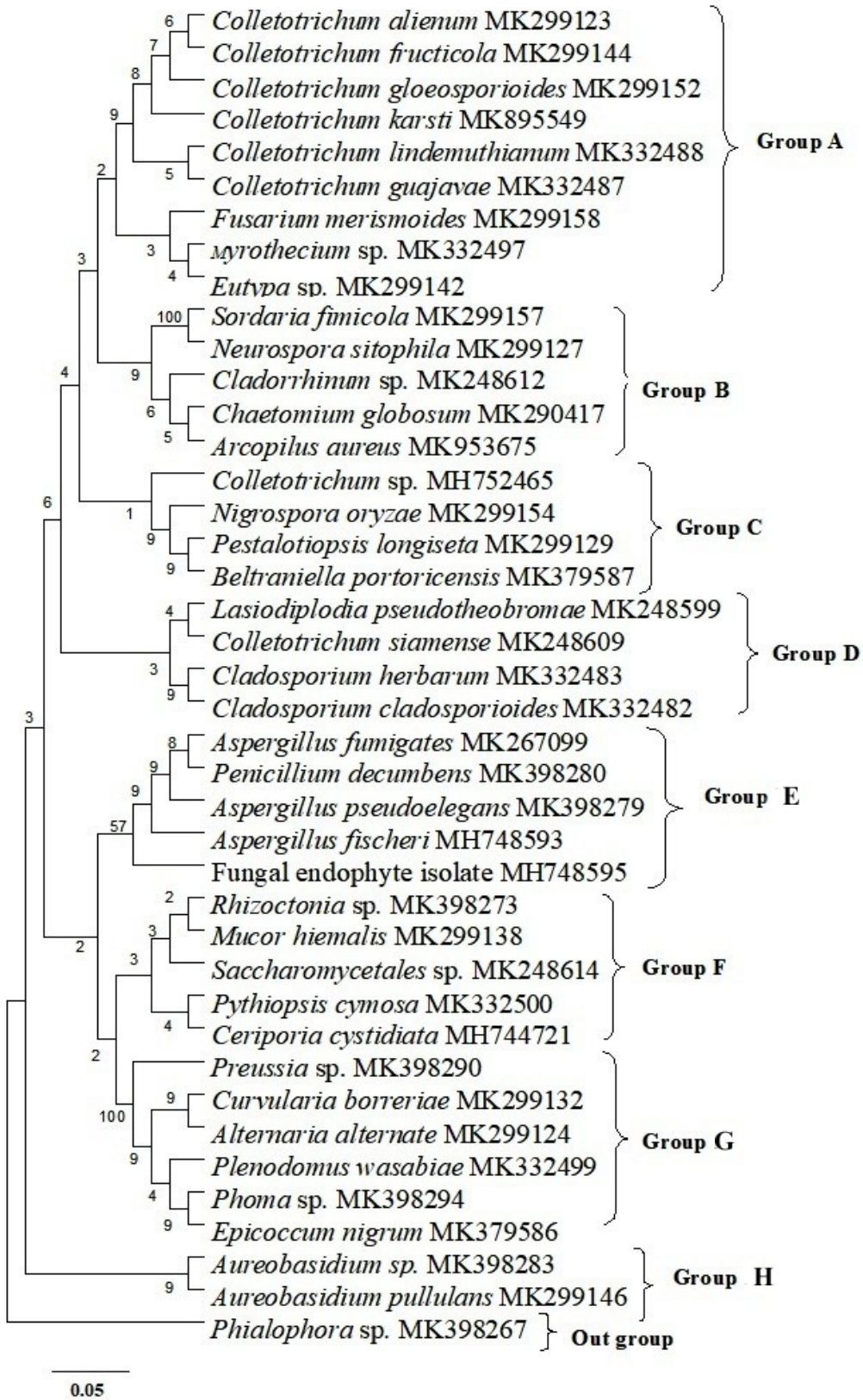

Figure 2 Joining tree based on rDNA of ITS sequences of the different fungal endophytes species isolated of the present study. Numerical value indicates bootstrap percentile from 1000 replicates.

Table 1: Fungal endophytes isolated from different parts of *P. thyrsiformis*., its number of isolated fungal endophytes spp and density (rD%)

S.N	Isolated of fungal Endophytes	Plant parts			No. of isolated fungal spp	rD (%)
		Leaf	Stem	Root		
1	*Colletotrichum siamense*	Leaf	Stem	Root	19	12.66
2	*Aspergillus fischeri*	Leaf	Stem	Root	2	1.33
3	*Ceripora cyctidia*	---	Stem	---	1	0.66
4	*Eutypa* sp.	Leaf	---	---	1	0.66
5	*Plenodomus wasabiae*	Leaf	Stem	Root	2	1.33
6	*Neurospora sitophila*	Leaf	Stem	---	2	1.33
7	*Colletotrichum fructicola*	Leaf	---	---	1	0.66
8	*Alternaria alternata*	---	Stem	Root	2	1.33
9	*Pythiopsis cymosa*	Leaf	---	Root	3	2.00
10	*Colletotrichum gloeosporioides*	---	Stem	---	1	0.66
11	*Colletotrichum* sp.	Leaf	---	---	2	1.33
12	*Arcopilus aureus*	Leaf	Stem	---	2	1.33
13	*Mucor hiemalis*	Leaf	---	Root	1	0.66
14	*Colletotrichum alienum*	Leaf	Stem	---	2	1.33
15	*Aspergillus pseudoelegans*	Leaf	Stem	---	1	0.66
16	*Penicillium decumbens*	Leaf	---	Root	3	2.00
17	*Cladosporium cladosporioides*	---	Stem	Root	2	1.33
18	*Myrothecium* sp.	Leaf	---	Root	3	2.00
19	*Cladosporium herbarum*	Leaf	Stem	---	2	1.33
20	*Aspergillus fumigatus*	Leaf	Stem	Root	1	0.66
21	*Chaetomium globosum*	Leaf	---	---	5	3.33
22	*Fusarium merismoides*	---	Stem	---	3	2.00
23	*Nigrospora oryzae*	Leaf	Stem	Root	2	1.33
24	Saccharomycetales sp.	Leaf	Stem	Root	4	2.66
25	*Cladorrhinum* sp.	---	---	Root	1	0.66
26	*Epicoccum nigrum*	Leaf	Stem	Root	3	2.00
27	*Colletotrichum karstii*	---	Stem	---	2	1.33
28	*Beltraniella portoricensis*	Leaf	---	---	2	1.33
29	*Sordaria fimicola*	---	Stem	Root	4	2.66
30	*Curvularia borreriae*	Leaf	Stem	Root	1	0.66
31	Fungal Endophyte sp.	Leaf	---	Root	1	0.66
32	*Pestalotiopsis longiseta*	---	Stem	---	3	2.00
33	*Aureobasidium pullulans*	Leaf	---	Root	3	2.00
34	*Colletotrichum guajavae*	Leaf	Stem	Root	2	1.33
35	*Colletotrichum lindemuthianum*	---	Stem	---	3	2.00
36	*Rhizoctonia* sp.	Leaf	---	Root	1	0.66
37	*Lasiodiplodia pseudotheobromae*	---	Stem	Root	1	0.66
38	*Preussia* sp.	Leaf	---	Root	3	2.00
39	*Phialophora* sp.	Leaf	---	---	2	1.33
40	*Phoma* sp.	---	Stem	Root	2	1.33
41	*Aureobasidium* sp.	Leaf	Stem	---	4	2.66

--- indicates absences of the fungal endophytes.

products were checked on 1.2 % Agarose gel in Tris–acetate-EDTA buffer (TAE) at pH 8.0, stained with ethidium bromide (0.3 lg/mL) and visualized under UV light by using Gel documentation system. Sequencing was done by GeNei Labs Private Limited, Bengaluru; using PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase, as per the manufacturer's instructions.

Phylogenetic analysis: The sequences thus obtained, were subjected to BLAST and submitted to Genbank of NCBI as well as got

the accession number. Further, to get the homology, search and multiple sequence alignments were made using Clustal W (Thompson *et al.* 2003). However, sequences were analyzed for generating phylogenetic tree, using neighbour joining methods of MEGA 5 (Tamura *et al.* 2011). The robustness of the inferred phylogeny was assessed using bootstrap value at 1,000 replications (Fig. 2).

RESULTS

The findings of the present investigation deals with the isolation of endophytic fungi, from *P. thyrsiformis*; an ethnomedicinal plants commonly used for the treatment of various ailments in human beings. The observations showed that total 105 isolates of endophytic fungi were isolated from the 150 sample segments. The maximum number of fungal isolates were recorded from leaves (37%), followed by stems (33%) and root (30%) (Fig.1-c), respectively. Overall 41 fungal endophytes were recorded, which belongs to 29 genera. *Colletotrichum siamense* was the most dominant endophytic fungi, followed by *Chaetomium globosum* , *Sordaria fimicola, Aureobasidium* sp., *Pythiopsis cymosa* and others. However, *Colletotrichum siamense, Aspergillus fischeri, Plenodomus wasabiae, Aspergillus fumigatus, Nigrospora oryzae,* Saccharomycetales sp., *Epicoccum nigrum., Curvularia borreriae, Colletotrichum guajavae* were the common fungal endophytes recorded from all the plant parts viz., leaf, stem and roots (Fig. 1-b). Besides, the density of colonization (rD%) was recorded in the chronology of *Colletotrichum siamense* (12.66%) < *Chaetomium globosum* (3.33%) < *Sordariomycetes* sp., *Sordaria fimicola* and *Aureobasidium* sp. (2.66%) and 0.66% to 2.00% for the remaining endophytes (Table 1).

Further, it was observed that out of 41 fungal endophytes; 20 belongs to the class-Sordariomycetes; 11 belongs to Dothideomycetes; 05 belongs to Eurotiomycetes; 02 belongs to Agaricomycetes; and 01 belongs to class Zygomycetes and Oomycetes, each; however, 01 couldn't identified properly. While categorizing up to the order level; it was recorded that *Colletotrichum siamense*, *C. fructicola*, *C. gloeosporioides, Colletotrichum sp., C. alienum, C. karstii, C. guajavae* and *C. lindemuthianum,* belongs to the order Glomerellales; *Plenodomus wasabiae*, *Alternaria alternata Epicoccum nigrum, Curvularia borreriae, Preussia* sp. and *Phoma* sp. belongs to the order Pleosporales; *A. fischeri, A. pseudoelegans, Penicillium decumbens* and *A. fumigatus* belongs to the order Eurotiales; *Neurospora sitophila*, *Arcopilus aureus*, *Chaetomium globosum* and *Sordaria fimicola* belongs to order Sordariales; *Eutypa* sp., *Beltraniella portoricensis* and *Pestalotiopsis longiseta* belongs to order Xylariales; *Cladosporium cladosporioides* and *C. herbarum* belongs to order Capnodiales; *Myrothecium* sp. and *Fusarium merismoides* belongs to order Hypocreales; *Aureobasidium* sp. and *A. pullulans* belongs to order Dothideales; *Sordariomycetes* sp. and *Cladorrhinum* sp. belongs to order Saccharomycetales. Besides this, 01 fungal endophytes belong to order Cantharellales viz. *Rhizoctonia* sp.; *Ceripora cyctidia* belongs to order Polyporales; *Mucor hiemalis* belongs to order Mucorales; *Pythiopsis cymosa* belongs to order Saprolegniales; *Lasiodiplodia pseudotheobromae* belongs to order Botryosphaerial; *Phialophora* sp. belongs to order Chaetothyriales and *Nigrospora oryzae* belongs to order Trichosphaeriales, respectively.

Furthermore, the isolated samples of the fungal endophytes (41 samples) were used for extraction of the DNA, molecular characterization as well as phylogenetic analysis. The observations of the neighbour joining tree as constructed based on the sequence structure alignment, shows clearly

eight well separated groups. Group 'A' consisted *Colletotrichum fructicola, C. gloeosporioides, C. alienum, C. karstii, Colletotrichum guajavae*, *C. lindemuthianum, Fusarium merismoides, Myrothecium* sp. and *Eutypa* sp. Similarly, group 'B' consisted *Sordaria fimic, Neurospora sitophila, Cladorrhinum* sp*., Chaetomium globosum, Arcopilus aureus*; group 'C' consisted *Colletotrichum* sp*., Nigrospora oryzae, Pestalotiopsis longiseta, Beltraniella portoricensis*; group 'D' consisted *Lasiodiplodia pseudotheobromae, Colletotrichum siamense, Cladosporium herbarum***,** *Cladosporium cladosporioides*; group 'E' consisted *Aspergillus fumigatus, Penicillium decumbent, Aspergillus pseudoelegans, Aspergillus fischeri,* fungal endophyte isolate; group 'F' consisted *Rhizoctonia* sp*., Mucor hiemalis, Saccharomycetales* sp*., Pythiopsis cymosa, Ceriporia cystidiata*; group 'G' consisted *Preussia* sp*., Curvularia borreriae, Alternaria alternata, Plenodomus wasabiae, Phoma* sp*., Epicoccum nigrum*; and group 'H' consisted *Aureobasidium sp., Aureobasidium pullulans;* however *Phialophora* sp. was used for outer group (Fig. 2).

DISCUSSION AND CONCLUSION

Literature revels that diversity of fungal endophytes have already been reported from several medicinal plant species viz., *Fomitopsis* sp. *Penicillium* sp., *Diaporthe* sp., *Arthrinium* sp. *Phomopsis* sp. and *Schizophyllum* sp. have been reported from *Garcinia mangostana* and *G .parvifolia* (Sim *et al* 2010); *Mortierella minutissima, N. sphaerica, Acremonium strictum, Humicola Grisea, Mortierella hyaline, Oidiodendron echinulatum, O. griseum, Humicola fuscoatra* and *Arthroderma tuberculatum* reported from *Elaeocarpus sphaericus* (Shukla *et al.* 2012); *P.citrinum, A. alternata, Aspergillus niger, Cladosporium* sp*., Rhizopus* sp*., C.vermiformis* reported from *Helicteres isora* (Gayathri and Chandra 2017); *Periconia hispidula, Allomyces arbuscula, N. sphaerica, A. falciforme, P. chrysogenum Aureobasidium* sp. *Chaetomium* sp. from *Litsea cubeba* (Deepanwita and Dhruva 2018); and *Fusarium* spp, *Aspergillus* sp*., Chaetomium* sp*., Penicillium* sp*., Setosphaeria rostrata*, *F.solani*, *Bipolaris maydis, D.phaseolorum, Rhizoctonia bataticola,* and *Macrophomina phaseolina* reported from *Chlorophytum borivilianum* (de Carvalho *et al*. 2019) as well as *Alternaria alternata*, *Aspergillus terreus*, *Alpestrisphaeria* reported from *Vitex rotundifolia* (Yu-Hung and Roland 2019). Similarly, in the current study, we have reported total 41 fungal endophytes, out of which *Aspergillus fischeri, A. fumigatus, Colletotrichum guajavae, Colletotrichum siamense, Curvularia borreriae, Epicoccum nigrum., Nigrospora oryzae, Plenodomus wasabiae, Saccharomycetales sp.,* were the common fungal endophytes recorded from all the plant parts viz., leaf, stem and roots. *Colletotrichum siamense* was the most dominant endophytic fungi having 12.66% density of colonization (rD%) (Table 1). Besides this, extraction of the DNA and its molecular characterization as well as phylogenetic analysis based on the ITS1-ITS4 sequencing data of the isolated 41 fungal endophytes, have also been investigated and recorded (Figure 2).

The authors are thankful to the tribal peoples of Arunanchal Pradesh, for providing ethnomedicinal information about the particular plant and its uses for various ailments. Further, authors are also thankful to the Head, Department of Botany, Rajiv Gandhi University, Arunanchal Pradesh as well as to the Head, Department of Botany, University of Lucknow for providing various facilities during the course of the present study. Besides this, one of the authors is thankful to the MHRD, Govt of India; for providing financial support.

REFERENCES

Bender SF, Wagg C, Van Der and Heijden MGA 2016 An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends *Ecol Evol* **31(6)** 440-452.

Chowdhery HJ 1999 *Arunachal Pradesh; in Floristic diversity and conservation strategies in India, Vol. II: In the context of states and Union Territories,* edition V Mudgal and PK Hajra Kolkata: Botanical Survey of India - Pp 1059.

Compant S, Saikkonen K, Mitter B, Campisano A and Mercado-Blanco J 2017 Editorial special issue: soil, plants and endophytes. *Plant Soil* **405(1–2)** 1–11.

De Barry 1866 *Morphologie und physiologie der pilze, flechten and myxomyceten,* (Vol. II), Hofmeister's Handbook of Physiological Botany, Leipzig, Germany.

de Carvalho CR 2019 Bioactive compounds of endophytic fungi associated with medicinal plants. In: Yadav A, Singh S, Mishra S, Gupta A Recent Advancement in White Biotechnology Through Fungi. *Fungal Biol.* Springer, Cham.

Deepanwita D and Dhruva KJ 2018 Antimicrobial activity of Endophytic Fungi from leaves and barks of *Litsea cubeba* Pers., a Traditionally Important Medicinal Plant of North East India, Jordan *J Biol sci.* Pp 73-79.

Doilom M, Manawasinghe IS, Jeewon R, Jayawardena RS, Tibpromma S, Hongsanan, S, Meepol W, Lumyong S, Jones EBG and Hyde KD 2017 Can ITS sequence data identify fungal endophytes from cultures? A case study from *Rhizophora apiculata*. *Mycosphere* **8** 1869–1892.

Domsch KH, Gamas W and Anderson TH 1980 Compendium of Soil Fungi, Academic press, New York, Vol. **1** 168-169, 540, 559-560.

Ellis MB 1976 More Dematiaceous Hyphomycetes. Commonwealth Mycological institute Kew, Surrey, England.

El-Maali N, Mohrram A, El-Kashef H and Gamal K 2018 Novel resources of Taxol from endophytic and entomopathogenic fungi: isolation, characterization and LC-Triple mass spectrometric quantification. *Talanta* **190** 466–474.

Fisher PJ and Petrini O 1987 Location of fungal endophytes in tissues of *Suaeda frutcosa*: A preliminary study. *Trans. Brit. mycol. Soc* **89** 246-249.

Gayathri Pai and Chandra M 2017 Screening of Phytochemicals and Isolation of Endophytic Fungi from Medicinal plant *Helicteres isora* L." IOSR *Journal of Pharmacy* (IOSRPHR), **7** 12, pp. 01- 05.

Jeewon R, Wanasinghe DN, Rampadaruth S, Puchooa D, Zhou LG, Liu AR and Wang HK 2017 Nomenclatural and identification pitfalls of endophytic mycota based on DNA sequence analyses of ribosomal and protein genes phylogenetic markers: a taxonomic dead end? *Mycosphere* **8(10)** 1802–1817.

Jia ML, Chen HL, Xin CJ, Zheng K, Rahman T, Han and LP and Qin 2016 A Friendly Relationships between Endophytic Fungi and Medicinal Plants: A Systematic Review. *Front Microbiol* **7** 906.

Kaul S, Ahmed M, Zargar K, Sharma P and Dhar MK 2013 Prospecting endophytic fungal assemblage of digitalis *Ehrh lanata*, (foxglove) as a novel source of digoxin: a cardiac glycoside, *Biotechnology* **3** 335-40.

Khanikar G, Sahajlavya Bandarabar Gun 2005 Rev. edition 7, Puthitirtha Prakashan, Guwahati, India, 34.

Kirschner R 2018 Fungi on the leaf a contribution towards a review of phyllosphere microbiology from the mycological perspective. *Biosyst Ecol Ser* **34** 433–448

Mane and Vedamurthy 2018 The fungal endophytes: Sources and future prospects, *J Med Plants Stud* **6, 2** 121-126.

Mane RS, Shinde MB, Wagh PR and Malkar HM 2017 Isolation of endophytic microorganisms as a source of novel secondary metabolite producers against Tuberculosis. *IJSART* **3** 1267-1269.

Nagmani AI and Kunwar Manoharachary KC 2005 *Hand book of soil fungi.* I. K. International Pvt. Ltd.

Nath A, Raghunatha P and Joshi S R 2012 Diversity and biological activities of endophytic fungi of *Emblica officinalis*, an ethnomedicinal plant of India. *Mycobiology* **40** 8-13.

Nongalleima K H, Dey A, Deb L, Singh C, Biseshwori T, Devi HS and Devi SI 2013 Endophytic fungus isolated from *Zingiber zerumbet* (L.) Sm. inhibits free radicals and cyclooxygenase activity. *Int J Pharm Tech Research,* **5** 301-307.

Olson D M and Dinerstein E 1998 The Global 200: A representation approach to conserving the Earth's most biologically valuable ecoregions. *Cons Biol* **12** 502–515

Patwari B 1992 In: *A glossary of medicinal plants of Assam and Meghalaya.* 1st ed. Guwahati, India. M.N. Printers 98.

Phurailatpam AK, Singh SR, Chanu TM, and Ngangbam P 2014 Phlogacanthus - An important medicinal plant of North East India: A review. *Afr J Agric Res* 9 **26** 2068–2072.

Radic N and Strukelj B 2012 Endophytic fungi-the treasure chest of antibacterial substances. *Phytomedicine* **19** 1270–1284.

Redman RS, Sheehan KB, Stout RG, Rodriguez RJ and Henson JM 2002 Thermotolerance conferred to plant host and fungal endophyte during mutualistic symbiosis. *Science* **298** 1581.

Rodriguez RJ, Henson J, Volkenburgh EV, Hoy M, Wright L, Beckwith F, Kim YO and Redman RS 2008 Stress tolerance in plants via habitat-adapted symbiosis. *ISME* **2** 404–416.

Shukla AK, Yongam Y and Tripathi P 2012 Distribution of endophytic fungi in different parts of Rudraksh (*Elaeocarpus sphaericus*) plants. In: Microbes: *Diversity & Biotechnology*, Pp. 37-42.

Sim JH, Khoo CH, Lee LH and Cheah YK 2010 Molecular diversity of fungal endophytes isolated from *Garcinia mangostana* and *Garcinia parvifolia*. *Journal of J.Microbiol. Biotechnol* **20** 651-658.

Singh SA and Singh NR 2010 Antimicrobial activity of Cassia didymobotrya and Phlogacanthus thyrsiflorus. *J Chem Pharm Rese* **2** 304.

Strobel G and Daisy B 2003 Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev* **67** 491–502.
Suryanarayanan TS 1992 Light-incubation: a neglected procedure in mycology. *Mycologist* **6** 144.

Suryanarayanan TS 2013 Endophyte research: going beyond isolation and metabolite documentation. *Fungal Ecol* **6** 561-568.

Suryanarayanan TS, Kumaresan V and Johnson JA 1998 Foliar fungal endophytes from two species of the mangrove *Rhizophora*. *Can J Microbiol* **44** 1003–1006.

Tamura KD, Peterson N, Peterson G, Stecher M and Nei S Kumar 2011 "MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods" *Mol Biol Evo* **l28** 2731- 2739.

Thompson JD, Higgins DG and Gibson TJ 2003 "Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice" *Nucleic Acid Res* **22** 4673-80.

Verma VC, Gond SK, Kumar A, Kharwar RN, Boulanger LA and Strobel GA 2011 Endophytic fungal flora from roots and fruits of an Indian neem plant *Azadirachta indica*, A. Juss and impact of culture media on their isolation. *Indian Journal of Microbiology* **51** 469-476.

White TJ, Bruns T, Lee S and Taylor J 1990 *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. *PCR protocols: a guide to methods and applications*. New York: Academic Press, 315–322.

Yu-Hung Yeh and Roland Kirschner 2019 Diversity of Endophytic Fungi of the Coastal Plant *Vitex rotundifolia* in Taiwan, *Microbes Environ* Vol. **34 (1)** 59-63.