Asian Journal of Mycology 4(2): 144–158 (2021) ISSN 2651-1339

www.asianjournalofmycology.org **Article**

Doi 10.5943/ajom/4/2/10

Endophytic fungal diversity associated with roots of *Angelica glauca*: An endangered medicinal plant of Northwest Himalaya

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Gaur A, Parkash V, Kumar V, Thakur A 2021 – Endophytic fungal diversity associated with roots of *Angelica glauca*: An endangered medicinal plant of Northwest Himalaya. Asian Journal of Mycology 4(2), 144–157, Doi 10.5943/ajom/4/2/10

Abstract

Angelica glauca Edgew. is an endangered medicinal plant species that is known for its roots containing valuable essential oil. It is overexploited for its roots and has become endangered and nearing its threshold. This study is aimed at exploring the endophytic fungal diversity associated with its roots to further study the conservation aspects with the help of the interaction of host plant species and endophytes. About 24 different root fungal endophyte species were isolated among which *Geotrichum candidum* (32.03 ± 7.95 %) was found to be the most dominant followed by *Fusarium oxysporum* (11.49 ± 21.69 %). The average root colonization of all the endophytes in all four collection fields was 61.195 ± 9.67 % wherein the maximum colonization was observed in wild root samples (74%) and the least root colonization by fungal endophytes was observed in two years old cultivated plants root system (52.36%). Positive Pearson's correlation (r = 0.801) was observed between the samples/fields and root colonization by fungal endophytes, indicating the colonization increases in roots as the age of plant species advances. Both the Simpson's Diversity Index (D) and Shannon Wiener diversity index (H) indicate relatively lower diversity in Field 3 (D = 0.43 & H = 1.07) and Field 4 (D = 0.57 & H = 0.97) *i.e.* in two years old plantations and wild plants. Menhinick Index (D_{mn}) and Margalef Richness Index (M_f) are based on species richness, and both of them indicate Field 3 (D_{mn} = 0.95 & M_f = 2.04) to be the richest. Both Buzas and Gibson's Index (E) and Equitability Index (E_H) are a measure of evenness and yield similar results *viz*. Field 1 (one year old plantations) (E = 0.65 & E_H = 0.76) and Field 4 (E = 0.66 & E_H = 0.70) being the most even in terms of species distribution. Berger-Parker Dominance Index (B) shows field 3 (B = 0.75) having the highest values.

Keywords – Diversity index – Endophytic strains – Essential oil – Root fungal endophytes.

Introduction

Endophytes are microorganisms (often fungi or bacteria) that live inside a living host for at least a part of their life cycle without causing any apparent disease (Pirttilä & Frank 2018). Plant endophytic fungi have been reported from every plant ever examined and are known to play an important role in plant defence mechanisms by producing secondary metabolites. These secondary metabolites have proved to be a major source of medicinally important compound/s for use in the

Submitted 1 September 2021, Accepted 7 December 2021, Published 31 December 2021
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pharmaceutical industry (Tejesvi & Pirttilä 2018). One of the valuable metabolites 'Taxol', which is an important anticancer drug, has been obtained from an endophytic fungus, *Taxomyces andreanae*. There are many other examples of commercially valuable products isolated from endophytic fungi, such as 'Radicicol' from an endophytic fungus, *Chaetomium chiversii* isolated from *Ephedra fasciculata*, terpenoids from *Phomopsis* species and many more (Bano et al. 2016).

Plant endophytes are also potential plant growth promoters and are, therefore, utilized as biofertilizers in crop cultivation. There have been many studies where endophytic fungi are used for plant growth promotion. Zhou et al. (2014) worked on the diversity and plant growth-promoting ability of endophytic fungi from five flowering plant species collected from Yunnan, Southwest China and concluded that endophytes may have potential uses in floriculture to alleviate environmental stress and reduce agricultural costs in the future.

Angelica glauca (smooth angelica) commonly known as '*Chora'* (family Apiaceae), is endemic to India (Butola & Vashistha 2013). It is found along with the western Himalayan ranges and grows in the altitudinal range of 1800-3700 amsl. The roots are rhizomatous, exhibiting great medicinal importance, and used for gastric and stomach-related problems by the folk (Gaur 1999). The roots of *A. glauca* are used as a stimulant, cardioactive, carminative, sudorific and expectorant and yield a pale to a brownish-yellow essential oil (0.4-1.3% dry basis) (Kaul et al. 1996). Being aromatic, the roots are traditionally used as a spice by the natives of the Himalayas. The herb is used in various diseases in women and is known as 'female ginseng' in Asia. *Chora* is propagated both by seeds and vegetatively in fields. Alternate photoperiod (16 hrs. dark and 8 hrs. light), temperature (25°C), and soil depth not more than 1.0 cm is an effective treatment to achieve optimum germination of *A. glauca* (Butola et al. 2014). The species has been given an endangered (global) status appeared in International Union for Conservation of Nature (IUCN) Red List 2015 (Ved et al. 2015). Due to unsustainable *in-situ* harvesting and various other ecological and environmental factors, this plant species has become globally endangered for the Himalayan regions as per IUCN (CAMP 2003; Butola & Badola 2004; Vashistha et al. 2006; Butola 2008; Butola et al. 2010).

The plant species has immense medicinal usage but on the other hand, its medicinal usage leads to its overexploitation resulting in it being endangered and nearing a threshold. Therefore, in this study, the associated root fungal endophytes are investigated. Thus, the basic knowledge of associated fungal endophytes in unexplored plants is an important aspect that will form a base for future experiments regarding their use as bio-inoculants and as a source of medicinally important metabolites.

Material & Methods

The root samples of target plant species for endophyte isolation were collected from Shimla, Himachal Pradesh (Fig. 1). The map of the collection site was prepared using QGIS Desktop 3.16.3 with GRASS 7.8.5 with the help of Geospatial data retrieved from DIVA-GIS. Four different types of fields were chosen for sample collection (Table 1). Field 1 consisted of plantations that were one year old and were being cultivated in large numbers. Plantations of Field 2 were from a local cultivated field. Two years old plantations were also collected from a forest nursery (Field 3). Some wild samples (Field 4) were also collected from a plant in the nearby forest area but seeing the endangered status of the plant and very less availability of wild populations only 3-4 plants were uprooted for their root assessment within the locality.

Isolation of fungal endophytes from roots was done by the modified protocol given by Hallmann et al. (2006). PDA (Potato dextrose agar) was used as the culture medium. Roots were first thoroughly washed in tap water to remove most of the dirt and soil from the surface. Then, these were dipped in 95% ethanol for 15 seconds followed by sterilization with 4% sodium hypochlorite for 30 seconds and then dipped in 95% ethanol for 15 seconds. The roots were then finally washed with sterilized distilled water (SDW) two to three times, and the water collected from the final washing was used to inoculate PDA plates to set a control plate for checking that the isolated fungi have not been isolated from the surface due to erroneous surface sterilization. The

surface-sterilized roots were then cut into small pieces such that the inner portion/root tissues were exposed to the artificial culture media. These root pieces were placed in the petri-dish containing PDA and were incubated at 28 ± 2 °C for 10 days. Further, sub-culturing was done for obtaining pure strains of endophytic fungi. Identification was done with the help of microscopic studies and available literature (Barnett & Hunter 1998; Domsch et al. 1980). The confirmed identification of the most dominant endophytic fungi was done from Agharkar Research Institute (National Fungal Culture Collection of India (NFCCI)- A National Facility) with reference letter no. 3/426/2019/Myc/471 dated 25.06.2019. The colonizing frequency (CF%) of endophytic fungal species was calculated using Hata & Futai (1995). Endophytes colonizing frequency calculation was done.

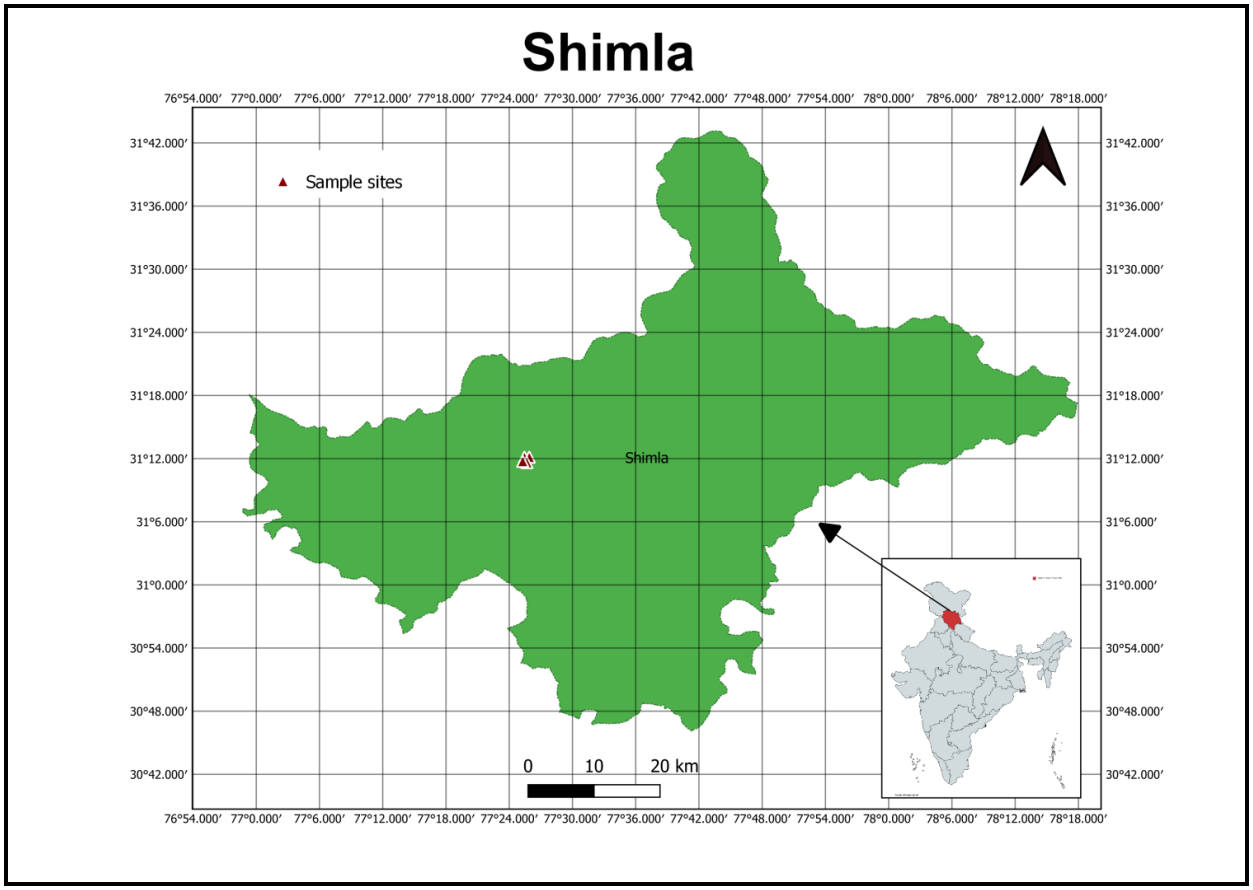

Fig. 1 – Map of collection sites Shimla, Himachal Pradesh, India (made by QGIS software)

Table 1 Collection of root samples for endophytic isolation.

Site	GPS Coordinates	Altitude	Type of sample collected
Field 1	31°12'08"N 77°25'28"E	2437m	One year old plantations collected from Shillaru, Shimla, Himachal Pradesh (H.P.), India.
Field 2	31°12'08"N 77°25'29"E	2434m	Sample collected from cultivated field in Shillaru, Shimla, H.P., India
Field 3	31°12'13"N 77°25'35"E	2415m	Two years old plantations from Shillaru Nursery of HFRI, Shimla, H.P., India
Field 4	31°11'55"N 77°25'33"E	2353m	Wild plants from the forest area of nearby places of Shillaru, Shimla, H.P.

Endophytes colonizing frequency

Colonizing frequency was calculated for each fungi by using the following formula.

$$\%\text{Colonizing frequency} = \frac{\text{No. of segments colonized by specific fungus}}{\text{Total no. of segments examined}} \times 100$$

T_{cf} = Total % Colonization frequency of samples

$$\text{Dominant Endophytic fungi} = \frac{\%CF}{T_{cf}} \times 100$$

Pearson's Correlation index

Pearson's Correlation was also studied between the sources of endophytes and colonization by endophytes. For studying Correlation analysis, the colonization percentage at each field was taken into consideration. The Pearson's correlation coefficient (r) was calculated through statistical analysis by using the following formula.

$$r = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}}$$

Where

r = correlation coefficient

x_i = values of the x-variable in a sample

bar{x} = mean of the values of the x-variable

y_i = values of the y-variable in a sample

bar{y} = mean of the values of the y-variable

The diversity analysis of the observed results was done using the following indices:

Simpson's Diversity Index (Simpson 1949)

$$D = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

Where

n = number of individuals of each species

N = total number of individuals of all species

Shannon-Wienner diversity index (Shannon & Weaver 1963)

$$H_s = -\sum_{i=1}^{s} (Pi)(\ln Pi)$$

Where

H_s = Symbol for the diversity in a sample of S species or kind.

S = The number of species in the sample.

Pi = Measures of relative abundance of i^{th} species or kinds = ni/N

N= Total number of individuals of all kinds.

Ln = log to base 2.

Berger–Parker index (Berger & Parker 1970)

$$B = \frac{1}{\infty D}$$

Menhinick Index (Whittaker 1977)

$$D_{mn} = \frac{S}{\sqrt{N}}$$

Where N is the total number of individuals in the sample and S is the species number.

Buzas and Gibson's Index (Buzas & Gibson 1969)

$$E = \frac{e^H}{S} (0 < E \leq 1)$$

Where e is the natural logarithm base

Equitability Index

$$E_H = \frac{H}{H_{max}} = \frac{H}{\ln S}$$

Where H = Shannon's diversity index

S = total number of species in the community (richness)

Margalef Richness Index (Margalef 1958)

$$M_f = \frac{S-1}{\ln N}$$

Where S = total number of species; N = total number of individuals in the sample; ln = natural logarithm.

Lorenz graph, rarefaction curve, Hill Graph and Preston's diagram were made with the help of online available Biodiversity calculator by Al Young Studios (Young 2021).

Results

Fig. 2 shows the images of all four fields from where the root samples were collected.

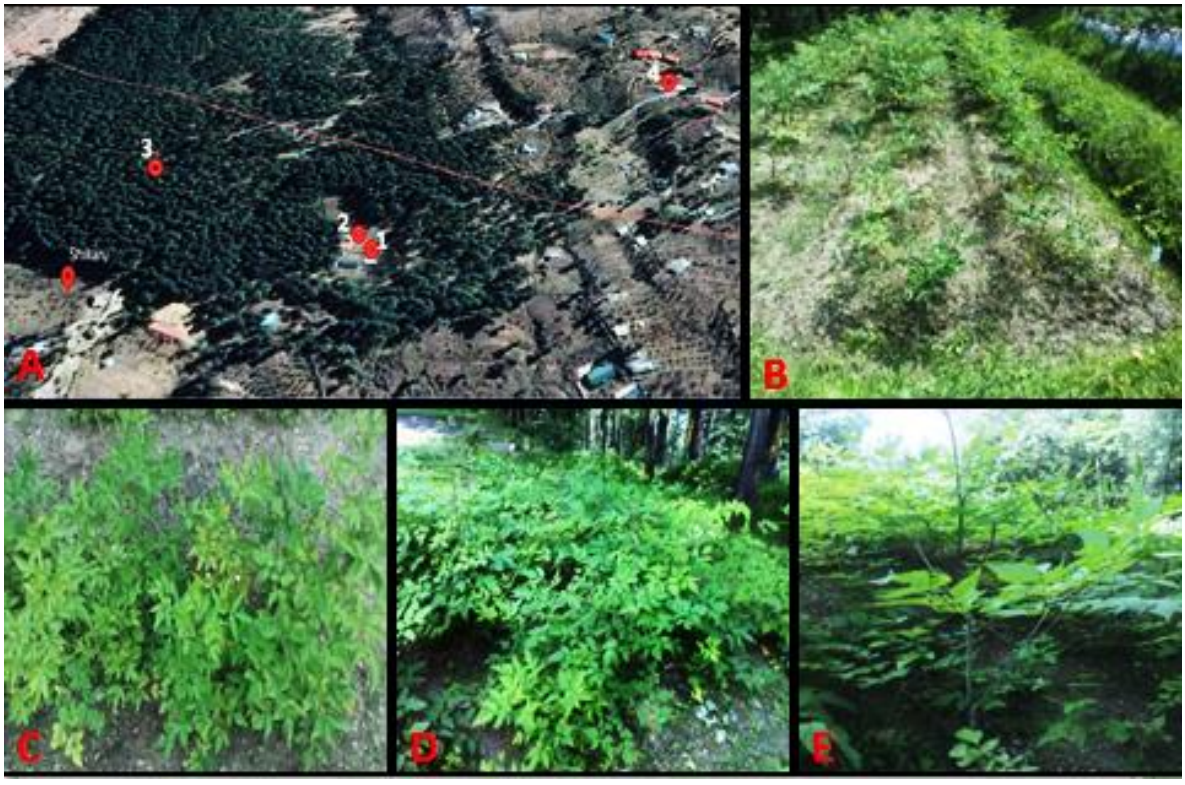

Fig. 2 – A: Satellite view of the collected sites (Source: Google Earth); B: Field 1; C: Field 2; D: Field 3; E: Field 4.

A total of 24 fungal endophytes were isolated, among which 15 were identified, and 9 produced sterile mycelia and thus could not be identified. The isolated endophytes are listed in Table 2. The total % colonization at every field can be observed in the table. Field 4, which consists of plant samples of wild habitat were the most colonized (74%), whereas the least colonization was observed in 2 years old plantations (52.36%). Overall average colonization of the plant species was found to be 61.12 ± 9.67% which shows the plant is heavily colonized by endophytic fungi.

Table 2 List of endophytes isolated from different fields and their colonization percentages.

S. no.	Endophytes	Field 1	Field 2	Field 3	Field 4	Average colonization (%)	Standard deviation	Dominant endophyte (%)
1.	*Fusarium compactum*	0	0	0.39	0	0.10	0.20	0.16
2.	*Aureobasidium* sp.	0	0	0.39	0	0.10	0.20	0.16
3.	*Ceratorrhiza* sp.	0	0	0.39	0	0.10	0.20	0.16
4.	*Chrysonilia sitophilia*	0	0	1.18	0	0.30	0.59	0.48
5.	*Cladosporium* sp.	0	1.38	0.79	0	0.54	0.67	0.89
6.	*Corynespora cassicola*	2.35	0	0	6	2.09	2.83	3.41
7.	*Fusarium chlamydosporium*	0	7.37	0	0	1.84	3.69	3.01
8.	*Fusarium oxysporum*	0	0	1.97	44	11.49	21.69	18.78
9.	*Geotrichum candidum*	29.41	37.33	39.37	22	32.03	7.95	52.34
10.	*Humicola* sp.	0	0	0	2	0.67	1.15	1.09
11.	*Penicillium citrinum*	0	0	1.18	0	0.30	0.59	0.48
12.	*Phialospora* sp.	0	0.92	0	0	0.23	0.46	0.38
13.	*Rhizoctonia* sp.	0	1.84	0	0	0.46	0.92	0.75
14.	*Scedosporium* sp.	0	0	0.79	0	0.20	0.40	0.32
15	*Stemphylium* sp.	0	0	3.15	0	0.79	1.58	1.29
16.	UD/C1/09/a	5.88	0	0	0	1.47	2.94	2.40
17.	*Penicilium chrysogenum*	10.59	0	0	0	2.65	5.30	4.33
18.	UD/C1/10/II	4.71	0	0	0	1.18	2.36	1.92
19.	UD/C1/12/II	2.35	0	0	0	0.59	1.18	0.96
20.	UD/C2/04/1/b	0	4.15	0	0	1.04	2.08	1.70
21.	UD/C2/06/I	0	2.76	0	0	0.69	1.38	1.13
22.	UD/C2/08/a	0	5.07	0	0	1.27	2.54	2.07
23.	UD/C2/08/b	0	2.3	0	0	0.58	1.15	0.94
24.	UD/C3/15/II	0	0	2.76	0	0.69	1.38	1.13
Total % Colonization		55.29	63.13	52.36	74	61.195	9.67	

Among the isolated endophytic fungi, only one species *viz. Geotrichum candidum* was observed at all the collection sites. This was also the most dominant fungus (52.34 ± 7.95 %) among the colonized endophytic fungal species. Among the wild populations, *Fusarium oxysporum* was observed to be the dominant fungus followed by *G. candidum*. After *G. candidum*, *F. oxysporum* was the second most dominant fungus (18.78 ± 21.69 %) in the roots of *Angelica glauca*. Fig. 4 shows pictures of some of the isolated endophytes with their microscopic images.

Table 3 gives detailed descriptions of isolated and identified fungal endophytes with respect to their microscopic features, the families that they belong to and if they have been previously reported as an endophyte from other plant species. It is visible that almost all the isolated endophytes have previously been isolated from different hosts as endophytes.

Table 3 Detailed description of isolated and identified fungal endophytes.

S. no.	Endophytes	Family	Division	Microscopic features	Previous report as endophyte
1.	*Fusarium compactum*	Nectriaceae	Ascomycota	White colony growth on PDA with orange red pigment release on aging. Clustered macroconidia present.	
2.	*Aureobasidium* sp.	Dothioraceae	Ascomycota	Cream colored colony turning greenish with age. Blastoconidia present. Cylindrical conidia with size varying from 3-5×2-3µm	From *Boswellia sacra* (Khan et al. 2016).

Table 3 Continued.

S. no.	*Endophytes*	Family	Division	Microscopic features	Previous report as endophyte
3.	*Ceratorhiza* sp.	Ceratobasidiaceae	Basidiomycota	Whitish yellow colony was observed. Polynucleate hyaline haphae with diameter of 7-11 µm	From *Habenaria dentata* (Shan et al. 2002)
4.	*Chrysonilia sitophila*	Sordariaceae	Ascomycota	Cottony white colony with arthroconidias present. Conidia varying from 4-8 µm × 9-12 µm	From. *Cereus jamacaru* (Bezerra et al. 2013)
5.	*Cladosporium* sp.	Davidiellaceae	Ascomycota	Olive green colonies with conidiophores 200 µm in length and 2–5 µm in width.	From *Needles of pine tree* (Wang et al. 2007).
6.	*Corynespora cassicola*	Corynespora	Ascomycota	Olive green to greyish colony with conidia varying in size from 9 - 180 µm.	From *Hevea brasiliensis* (Qi et al. 2011)
7.	*Fusarium chlamydosporium*	Nectriaceae	Ascomycota	White colony with pale brown pigment. Abundant terminal and intercalary chlamydospores. Both microconidia and macroconidia were observed.	*Anvillea garcinii* (Ibrahim et al. 2016)
8.	*Fusarium oxysporum*	Nectriaceae	Ascomycota	White mycelia turning pale violet from centre. Macroconidia varying from 17–45 × 2– 4 µm.	*Juniperus recurva* (Kour et al. 2007)
9.	*Geotrichum candidum*	Dipodascaceae	Ascomycota	Yeast-like white velvety culture. Arthroconidias ranging from 5-13 x 3-5 µm.	*Phyllanthus reticulatus* (Pai & Chandra 2018)
10.	*Humicola* sp.	Trichocomaceae	Ascomycota	Green colored colony with round conidia ranging from 4-8 µm.	*Elaeocarpus sphaericus* (Shukla et al. 2012)
11.	*Penicillium citrinum*	Trichocomaceae	Ascomycota	Greyish-green culture with whitish edges. Biverticillate conidiophores with small conidia of 2–3 µm.	From *Clerodendron cyrtophyllum* (Doan et al. 2019)
12.	*Phialospora* sp.	Herpotrichiellaceae	Ascomycota	Green to black colored colony with dark brown thick walled hyphae. Phialides with condia at the apices ranging from 1-3 µm.	From Pine bark (Iwatsu et al. 1981)
13.	*Rhizoctonia* sp.	Ceratobasidiaceae	Basidiomycota	Light brown colored culture with angled hyphae. Hyphae ranged between 5 to 7 µm wide.	From *Cucumis sativus* . L. (Al-Fadhal et al. 2019)
14.	*Scedosporium* sp.	Microascaceae	Ascomycota	Cottony culture yellow to brown-grey. Conidia varying in size from 5–9×3-4 µm.	From lichen *Parmotrema* (Chauhan et al. 2013).
15	*Stemphylium* sp.	Pleosporaceae	Ascomycota	Brownish white culture with brown hyphae. Conidia ranging from 11-18 x 14-28 µm.	*Taxus baccata* (Mirjalili et al. 2012)
16.	*Penicilium chrysogenum*	Trichocomaceae	Ascomycota	Blue-green colony with conidia 2-5µm in diameter. Terverticillate conidiophores.	From *Plectranthus amboinicus* L. (Tiwari et al. 2020)

Table 4 shows different diversity indices calculated in each field. Both Simpson's Diversity Index and Shannon Wiener diversity index indicate relatively lower diversity in Field 3 and Field 4, *i.e.*, in two years old plantations and wild plants. Menhinick Index and Margalef Richness Index are based on species richness, and both of them indicate Field 3 to be the richest as seen from Table 2 also. Both Buzas and Gibson's Index and Equitability Index are a measure of evenness, and both the indexes yield similar results *viz*. Field 1 and Field 4 are the most even in terms of species distribution. Berger-Parker Dominance Index shows the dominancy of the most common species, and Field 3 has the highest index. The most common species *Geotrichum candidum* is the most dominant in Field 3 and this can be confirmed from raw data in Table 2.

Table 4 Diversity indices of isolated fungal endophytes with respect to collection sites

S.No.	Diversity indices	Field 1	Field 2	Field 3	Field 4
1.	Simpson's Diversity Index	0.67	0.63	0.43	0.57
2.	Shannon Wiener diversity index	1.37	1.45	1.07	0.97
3.	Menhinick Index	0.87	0.77	0.95	0.66
4.	Buzas and Gibson's Index	0.65	0.47	0.27	0.66
5.	Equitability Index	0.76	0.66	0.45	0.7
6.	Berger-Parker Dominance Index	0.53	0.59	0.75	0.6
7.	Margalef Richness Index	1.29	1.63	2.04	0.83

The graphs, namely Preston's Diagram, Renyi/Hill graph, Rarefaction graph and Lorenz graph, were made for all the fields (Fig. 3). Preston's Diagram shows species abundance. The most species abundance in Field 3 were found in classes of 1 and 2-4. At first, the rarefaction curve grows rapidly when the most common species (fungal endophytic) are found but the curve plateus when the rarest species (fungal endophytic) are sampled. When the rarefaction graphs were compared of all the fields, it can be seen that the graph plateus only after a comparatively high value. The highest area covered in the Lorenz curve is seen in Field 3, depicting the most unequal distribution of endophytic species.

Table 5 describes the occurrence of endophytes in different fields studied. It is evident from the Table that the highest occurrence of different endophytes was observed in Field 3, which had 2-year old plantations, followed by Field 2 with plantations from the house of a local resident. The least occurrence was observed in wild populations.

Table 5 Occurrence of endophytes in different fields

S. No.	Endophytes	Field 1	Field 2	Field 3	Field 4
1.	*Fusarium compactum*	-	-	+	-
2.	*Aureobasidium*	-	-	+	-
3.	*Ceratorrhiza* sp.	-	-	+	-
4.	*Chrysonilia sitophilia*	-	-	+	-
5.	*Cladosporium* sp.	-	+	+	-
6.	*Corynespora cassicola*	+	-	-	+
7.	*Fusarium chlamydosporium*	-	+	-	-
8.	*Fusarium oxysporum*	-	-	+	+
9.	*Geotrichum candidum*	+	+	+	+
10.	*Humicola* sp.	-	-	-	+
11.	*Penicillium citrinum*	-	-	+	-
12.	*Phialospora* sp.	-	+	-	-
13.	*Rhizoctonia* sp.	-	+	-	-
14.	*Scedosporium* sp.	-	-	+	-
15.	*Stemphylium* sp.	-	-	+	-
16.	*Penicilium chrysogenum*	+	-	-	-
17.	UD/C1/09/a	+	-	-	-

Table 5 Occurrence of endophytes in different fields

S. No.	Endophytes	Field 1	Field 2	Field 3	Field 4
18.	UD/C1/10/II	+	-	-	-
19.	UD/C1/12/II	+	-	-	-
20.	UD/C2/04/1/b	-	+	-	-
21.	UD/C2/06/I	-	+	-	-
22.	UD/C2/08/a	-	+	-	-
23.	UD/C2/08/b	-	+	-	-
24.	UD/C3/15/II	-	-	+	-

Pearson's correlation was also calculated to study the relation between sample source type and their root colonization by endophytes (Table 6). Fig. 5 shows the positive linear correlation between the two factors studied.

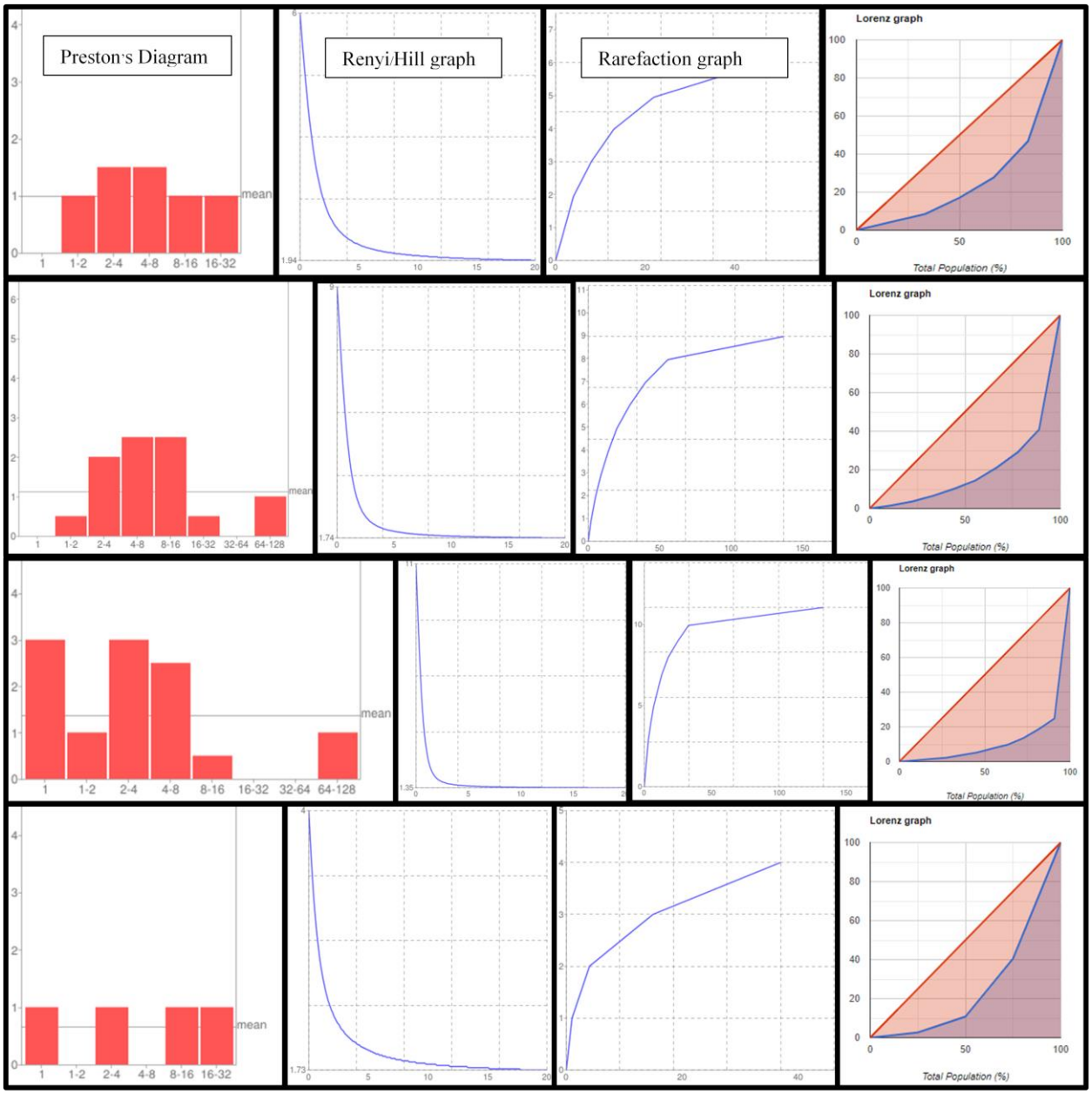

Fig. 3 – (Left to right) Preston's Diagram; Renyi/Hill graph; Rarefaction graph; Lorenz graph. (Top to bottom row) Field 1, Field 2, Field 3, Field 4.

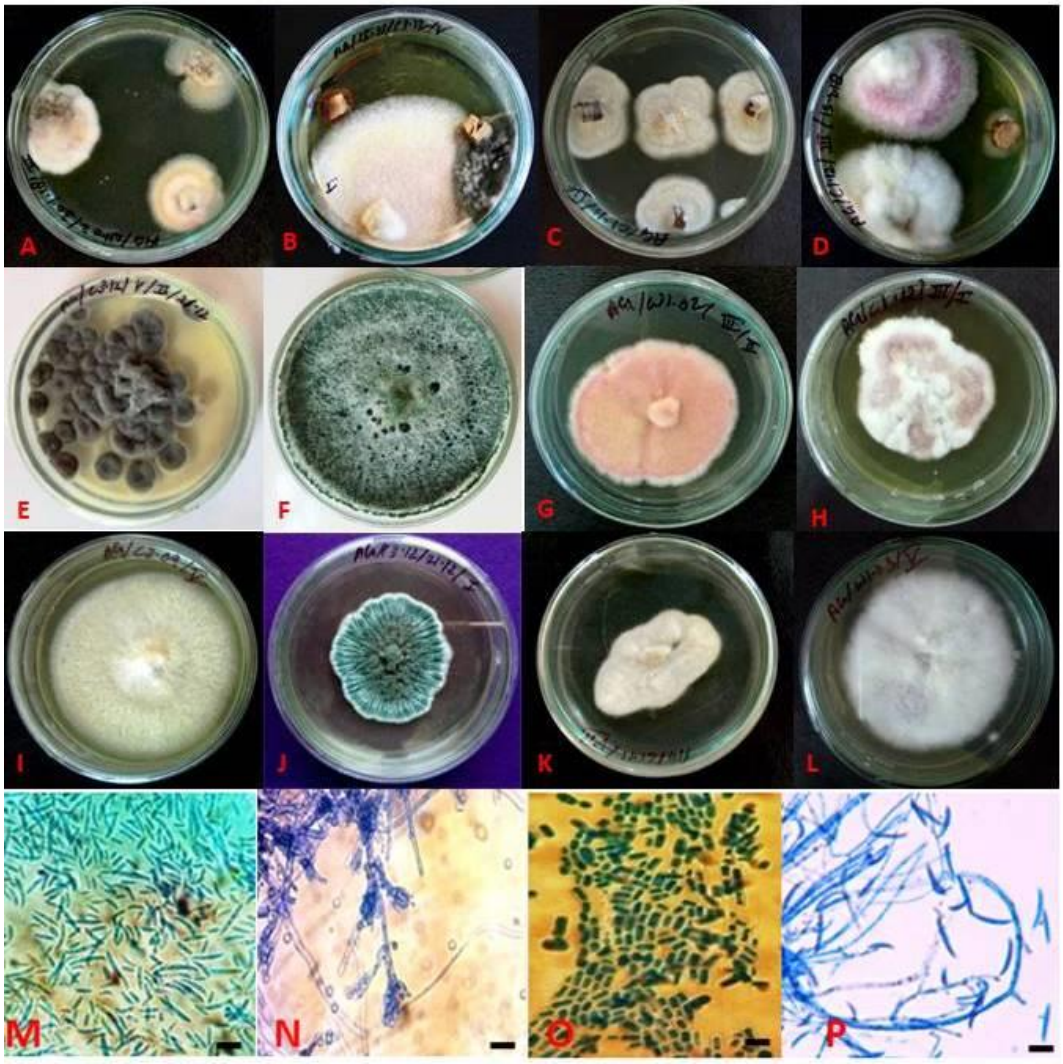

Fig. 4 – A-D Rhizospheric Enodphytic isolates. E *Cladosporium* sp. F *Humicola* sp. G *Scedosporium* sp. H *Corynospora cassicola*. I *Fusarium chlamydosporium*. J *Penicillium citrinum*. K *Geotrichum candidum*. L *Fusarium oxysporum*. M *Fusarium chlamydosporium*. N *Penicilium citrinum*. O *Geotrichum candidum*. P *Fusarium oxysporum* (scale bar=10 µm)

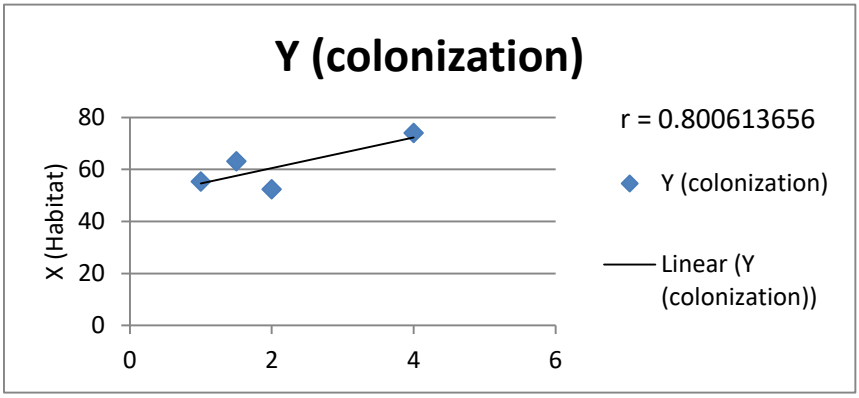

Fig. 5 – Scatter graph of correlation

Discussion

The percentage of endophytic colonization among each field was calculated, and no significant difference in colonization was observed among plants under cultivation. However, when

the fields with cultivated plantations were compared to wild plant samples considerable difference in endophytic colonization was observed. The difference may be attributed to the fact that the plants under cultivation are subjected to doses of fertilizers and pesticides which may affect the colonizing potential of fungi (Zhou et al. 2016). When each field was considered, *G. candidum* was the most dominant in all fields except the wild populations. Instead, *F. oxysporum* was the dominant in the wild populations. The reason that *F. oxysporum* was not dominant in cultivated plant samples can be related to the use of organic/inorganic fertilizers that inhibit/control the growth of fungi. Zhou et al. (2016) stated that the usage of long-term fertilizers affects the fungal community structure, and Zhao et al. (2014) worked on how bio-organic fertilizer application alters the composition of fungal communities and observed that biofertilizers application could suppress the population of *F. oxysporum* and change the fungal community structure in the rhizosphere soils.

Table 6 Pearson's Correlation between the type of sample source and root colonization by fungal endophytes.

	X (habitat)	*Y (colonization)*
X (sample)	1	
Y (colonization)	0.800613656	1

The isolated endophytes have been recorded previously in other plant species as well and are known to contribute significantly in biological activities of human welfare on their own or through their interaction with their host/s.

The most dominant mould *Geotrichum candidum* has been recorded as an endophyte from various medicinal plants such as *Phyllanthus reticulatus* (Pai & Chandra 2018). The fungus also has an important application in the cheese industry, and its commercial strains are used for cheese ripening in the industry (Boutrou & Guéguen 2005).

Fusarium oxysporum, which was one of the endophytes in this study, has the second highest colonizing frequency and have previously been isolated from *Juniperus recurva* (Kour et al. 2007), banana, as well as from tomato roots (Vu et al. 2006). It was recorded to be a crucial source of podophyllotoxin (Kour et al. 2007) and also provided systemic resistance to *R. similis* (Vu et al. 2006). Ibrahim et al. (2016) recorded *Fusarium chlamydosporium* as an endophyte on *Anvillea garcinii* which possessed antimicrobial and cytotoxic benzamide derivatives. Different species of *Humicola* have been isolated as endophytic fungi from the stem of Rudraksh (*Elaeocarpus sphaericus*) (Shukla et al. 2012), fruits of Indian neem plant *Azadirachta indica* (Verma et al. 2011) and only a few reports with respect to its isolation from roots are available. Endophytic *Penicillium* species observed in the present study have previously been known to be a source of a range of secondary metabolites listed by Nicoletti et al. (2014), thus, proving its importance as an endophyte.

Different *Stemphylium* species as endophytic fungi have been already isolated from medicinal plants. *Stemphylium sedicola* was isolated from *Taxus baccata* (Mirjalili et al. 2012) and *S. globuliferum* from *Mentha pulegium* (Debbab et al. 2009). In both plant species, the endophytic fungi isolated were examined and found to be a good source of bioactive metabolites *viz.* taxol in *T. baccata* (Mirjalili et al. 2012) and five new secondary metabolites from *M. pulegium* exhibiting kinase inhibitory activity (Debbab et al. 2009). Thus, proving the fact that *Stemphylium* sp. as an endophyte can hold a promising aspect in *Angelica glauca* as a source of secondary metabolite producer. Bezerra et al. (2013) also reported *Chrysonilia sitophilia* from cactus, *i.e., Cereus jamacaru*. Another endophytic fungus, *i.e., Corynespora cassicola* was isolated majorly from wild seedlings of *A. glauca* which is known to possess aromatase inhibitory and antioxidant activities as reported by Chomcheon et al. (2009) and acts as a source of depsidones and diaryl ether derivatives which help in the inhibition of inflammatory mediators and reactive oxygen (Okoye et al. 2013). The *Ceratorhiza* species has mostly been isolated from the roots of orchids (Shan et al. 2002), thus,

its presence in the roots of *A. glauca* reveals that this endophyte can also act as plant promoting potential as *Ceratorhiza* species is acting as orchidoid mycorrhizal association.

The important observation from Table 2 and Table 3 was that the most diverse fungal endophytic population was found in the field with the least root colonization percentage, whereas the least diverse fungal endophytic population was found with the highest colonization in roots.

Pearson's correlation value between sample source type and their root colonization by endophytes was positive (r = 0.800613656). Thus, it can be interpreted that colonization of endophytes is directly related to the age and type of samples from where endophytes are being isolated.

Conclusion

With this study, we can conclude that *Angelica glauca*, an endangered medicinal plant, is heavily colonized with a large number of different fungal endophytes in the roots. *Geotrichum candidum* was found to be the most dominant fungus in all the samples. All the isolated endophytes have previously been reported as endophytes from other plant species. Higher endophytic colonization was observed in wild samples compared to cultivated plant samples. The most diverse fungal endophytic population was found in the field with the least root colonization percentage whereas the least diverse fungal endophytic population was found with the highest colonization in roots. Relatively lower diversity was observed in wild plants when the Simpson's Diversity Index and Shannon Wiener diversity index was taken into account. The roots of the plant species yield essential oil, which is of great medicinal importance. Thus, the presence of fungal endophytes in the roots can be correlated to its medicinal value, and this concept can be further explored in future. The dominant fungi should be studied for their role in the essential oil formation of the plant as *Geotrichum candidum* already have a wide range of applications and is a promising fungus for carrying out this study of fungus-plant interaction. Moreover, this study forms a base for carrying out further plant growth promotion experiments with endophyte as bio-inoculant to better implement the concept of conservation through bio-inoculation technology.

Acknowledgement

The author Dr. Vipin Parkash is thankful to National Medicinal Plant Board, New Delhi for financial assistance in the project. All the authors are also thankful to the Director, Forest Research Institute (Indian Council of Forestry Research & Education), Dehradun for facilitating the research activities in laboratories of Forest Pathology Discipline. The authors are thankful to the Himalayan Forest Research Institute, Shimla, Himachal Pradesh, India for providing assistance and guidance in survey work.

Funding

National Medicinal Plant Board, New Delhi, India; Project No.: R&D/UK-02/ 2018-19-NMPV-IV.

Conflicts of interest/Competing interests

The authors declare no competing interests for this research work.

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