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Ecological diversity of Phylloplane Mycoflora of medicinal plants in Naharlagun, Papumpare District, Arunachal Pradesh, India

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ABSTRACT

Leaves constitute the major part of exposed plant surface. They are open to infestation or sporotropic colonization by air dispersed or splash dispersed mycoflora. The leaves provide unique environment to their surface occupants. The topography of the leaf surface, the microclimate around the leaves and the typical leaf exudate influence the growth and development of varieties of leaf surface micro-organisms and in turn determine the vitality of the plant concerned. In this context survey on the occurrence of phylloplane fungi on leaf surfaces of eight important medicinal plants viz; *Catharanthus roseus* (L.) G.Don, *Houttuynia cordata* Thunb., *Solanum lycopersicum* Lam., *Murraya koenigii* (L.) Spreng. , *Eryngium foetidum* L., *Clerodendrum glandulosum* Lindl., *Ocimum sanctum*, *Capsicum chinense* Jacq. From the present study it is being concluded that most abundant phylloplane fungi are *Alternaria* sp, *Penicillium* sp, and *Aspergillus* sp with high value of relative abundance. The number of identified fungal strains were analyzed to obtain relative abundance, species richness, evenness, Shannon Wiener diversity index, Simpson dominance index, and community similarity index. The Shannon Wiener diversity index, Simpson dominance index species richness index, species evenness index was estimated high in *Solanum lycopersicum* followed by *Houttuynia cordata*, *Ocimum* sp, *Eryngium foetidum* etc. In all the selected medicinal plants same fungal community structure was obtained based on the calculation of similarity index.

Kew words: *Medicinal plants, Phylloplane, Shannon Wiener diversity index, Simpson dominance index, Traditional medicine, similarity index*

Introduction

Plants have been used for medicinal purposes since pre-historic period, it has occupied an important position in maintaining human health and civilizing the quality of human life for many years. The term 'herb' derived from a latin word "herba" and old French word "herbe", herbs are plant or plant part used for its scent, flavor or therapeutic properties. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value, traditional systems of medicine continue to be widely practiced on many accounts. Due to increase in population rise and inadequate supply of drugs for infectious diseases,medicinal plants are regarded as the excellent source of alternate medicines for a wide variety of human ailments. Different plants have different medicinal properties they may be used directly or after being processed. The earliest historical record of herbs was

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found from Sumerian civilization, the World Health Organization estimated that 80% of the world's population depends on mainly on traditional medicines. The monetary significant use of therapeutic plants is higher in Asian countries like India and China (Kumar and Arunai, 2019). In India a large number of plants are used as traditional medicine, Ayurveda, the Traditional Indian Medicine remains the most ancient yet living tradition. *Curcuma longa, Cuminum cyminum, Elettaria cardamomum longa, Piper nigrum* are some commonly used medicinal plants in India. *Phyllanthus amarus* is one of the main herb of Indian Ayurvedic Medicine System (Lokare and Fatima, 2020). These therapeutic plants are destructed by different microorganisms, which contaminates roots, stems, leaves, blossoms and natural products. Different parts of *Taraxacum officinale* are traditionally used for treatment of ailments including gallstone, joint pain, muscle ache, eczema, loss of appetite, intestinal gas.Some people use *T. officinalle* to treat infection, especially viral infection and cancer. *Catharanthus roseus* has been used for relieving muscle pain, depression of the central nervous system, wasps stings, nose bleed, bleeding gums, mouth ulcers and sore throats. It has also been used internally for the treatment of the loss of memory, hypertension, cystitis, gastritis, enteritis, diarrhoea and the raised blood sugar levels (Shamsi and Sultana, 2014).

Numerous phytochemical with potential or established biological activity has been identified and are extracted from medicinal plants, these may be from a certain parts of the plant or the whole plant. Phytochemical are natural bioactive compound found in plants such as vegetables, fruits, flowers, leaves and that work with nutrients and fibers to act as defense system which protect against diseases (Garg *et al*., 2019). According to their functions in plant metabolism phyto chemicals are divided into two groups, they are primary constituents and secondary constituents. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, phenolic compounds and many more such as flavonoids, tannins.

Phylloplane Microflora and its role in soil conservation

The medicinal plants are infected by microorganism in their phyllosphere and rhizosphere region, they affect various parts of the leaves, stem, roots, flowers, fruits etc. Huge number of microorganisms occupies the phyllosphere of plants, the environment of phyllosphere includes physical, chemical and biological components occupying the surrounding space (Chauhan and Jain, 2020). The non- pathogenic fungi that inhabit the phyllosphere depends on the nutrients exuded from the atmosphere (Thakur, 2017). Phylloplane, or leaf surface, represents an important terrestrial habitat that harbors a wide range of microorganisms (Andrews and Harris, 2000 and Lindow and Brandle, 2003). Fungi, encompassing both filamentous and yeast taxa, are a major component of the phylloplane microbiota. Most of the endophytes colonize different compartments of the plant apoplast, including the intercellular spaces of the cell walls and xylem vessels (Malfanova *et al*., 2013). The leaf surface being very rich in nutrients offer a suitable substratum for the colonization of various microorganisms both the parasites and saprophytes. In addition to nutrient level, growth and abundance of Phylloplane micro flora influenced the plant population and ecosystem functioning, fungi are also influence by environmental conditions such as temperature, humidity, light intensity, wind speed, UV radiation and presence of air pollutants. These microorganisms includes a variety of epiphytic and endophytic that colonizes the surface and internal tissues of the plants, respectively (Yadav, 2015).

The competition among the native microbial communities of plants exhibit an important role affecting the plant microbe interaction in leaf surface and thereby contribute significantly for beneficial plant growth and disease suppression(Tanti *et al*., 2016). The phylloplane is considered as the hostile environment for microbial growth. The leaf surface is most important substrate for growth of microorganisms, as it provides essential nutrients required for the growth, the leaf surface contains different types of stimulatory and inhibitory substances that regulate the microbial colonization, the filamentous fungi are present predominantly as spores whereas rapidly sporulating species, bacteria and yeast colonize this habitat more actively (Ray *et al*., 2014)

The size, density of hairs and sculptures of leaf surface seem to be most reliable factors of fungal biodiversity on the studies plant species. Phylloplane microorganisms interact with each other by means of antibiosis, competition and parasitism and due to this interaction they protect the plants against pathogens.

Several researchers reported various fungi from leaf surfaces (phyllosphere and phylloplane) and observed most common fungi grow in leaf surfaces as*Alternaria, Chaetomium, Cladosporium, Cochliobolus, Curvularia, Mycosphaerella, Setosphaeria* and *Satchybotrytis* (Abdel, 1981; Mazen *et al*., 1985 and El-Said, 2001). The mycoflora of phylloplane is essential to understand microbial diversity because they provide information about their occurrence in the niche. It also suggests the role of such association to the health and wellbeing of the plant as well as on members of the food chain (Hajong *et al*., 2019).

Prabakaran *et al*. (2011) isolatedsignificant number of fungal speciesfrom *Ocimum sanctum, Phyllanthus amarus* and *Azadirachta indica* by dilution plating technique. *Some important strains of Penicillium janthinellum, Aspergillus fumiculosis, Aspergillus sp., Curvularia lunata and Fusarium moniliforme* were also isolated from the phylloplane of *Azadirachta indica* (Prabakaran *et al*., 2011; Ray *et al*., 2014., Jalender and Gachande, 2012)

A total number of four genera were isolated from medicinal plant *Ocimum sanctum*among them *Aspergillus, Mucor and Rhizopus* were isolated from the phylloplane. *Aspergillus* and *Fusarium* were isolated from phyllosphere. *Aspergillus, Mucor* and *Rhizopus* were isolated from rhizoplane part but he was only able to isolate *Mucor* from rhizosphere of *Ocimum sanctum* (Yadav, 2015).

Phylloplane mycoflora of 11 types of medicinal plants *Abrus precatorius, Abutilon indicum, Cercospora avicenna. Boerhavia diffusace,Acalypha indica, Catharanthus roses, Desmodium gangetium, Euporbia hirta, Gloriosa superb, Oxalis corniculata, Tinospora cordifolia* and concluded that some are fungi associated with particular plant but they does not show pathogenecity with that particular plants like *Fusarium* and *Phyllachora* present on *Boerhavia* but they does not show pathogenity. *Cladosporium* and *Colletotrichum* are present on *Abrusprecatorius* but does not show pathogenecity it means they are only associated fungi present on but does not show pathogenecity it means they are only associated fungi present on phyllosphere (Girdharilal and Fatima, 2006 and Angela *et al*., 2016).

Phylloplane mycoflora of some medicinal plants like *Azadirachta indica, Centella asiatica, Justicia adhatoda, Ocimum tenuiflorum* and *Plectranthus amboinicus* in Mahe were also carried out. The survey showed altogether 18 fungal species under 13 genera of which *Aspergillus* had the highest mean incidence followed by *Cercospora, Cladosporium, Curvularia* and *Diplococcium* (Mari *et al*., 2015, 2017).

Various workers investigated medicinal plant nurseries during the rainy season in and around Dehradun, Uttarakhand for disease severity *Aspergillus niger, Trichoderma harzianum, Trichoderma piluliferum, Penicillium sublateritium, Penicillium tardum, Penicillum herquei, Penicillium frequentans, Penicillium citreo-viride* and *Cladosporium cladosporioides* were reported and he reported that *Aspergillus niger, Trichoderma harzianum, Penicillium sublateritium* and *Cladosporium* were the most common isolatedfungi in all the season (Andrews and Harris, 2000).

Eleven number of fungi were isolated and identified from the leaf of oak tasar plant (*Q.serrat*a L.) in different ages of leaves namely tender, semimature and mature leaves during the two rearing seasons spring and autumn. They were *Aspergillus niger, A. fumigatus, A. flavus, Alternarea alternata, Curvularia sp., Mucor sp., Penicillium sp., Verticillium sp., Fusarium sp., Colletotricum sp., Cladosporium sp*.

A total of 35 fungal species comprising Zygomycetes, Ascomycetes and Deuteromycetes including sterile forms were isolated during from the various medicinal plants.A total of thirteen fungal species viz. *Aspergillus niger, A. flavus, A.fumigatus, Penicillium cyclopium, P. chrysogenum, Cladosporium elatum, C. cladosporioides*, Mucor etc. were isolated from mature leaves (Chauhan and Navneet, 2019).

A total number of 44 fungal species were isolated from surface sterilized leaf segments of five medicinal plants such as *Ocimum sanctum, Ficus bengalensis, Datura metel, Butea monosperma* and *Stevia rebaudiana*, Among them 04 species and 03 genera were isolated from the phylloplane of *Ocimum sanctum*, 17 species and 07 genera from *Butea monosperma*, 03 species and 03 genera from *Datura metel*, 05 species and 05 genera from *Ficus bengalensis* and 15 species and 10 genera were isolated from phylloplane of leaves surfaces (Thakur, 2017).

Phyllanthus amarus as it is one of the significant herb, there were a total of six fungi were isolated as *Aspergillus niger, A. nidulans, Fusarium sp, Cercospora sp, Alternaria sp*[2]. So, it is very essential to pay attention to characterize the phyllosphere fungi from the medicinal plantswhich are very rare and endangered in the NE region.

Materials and Methods

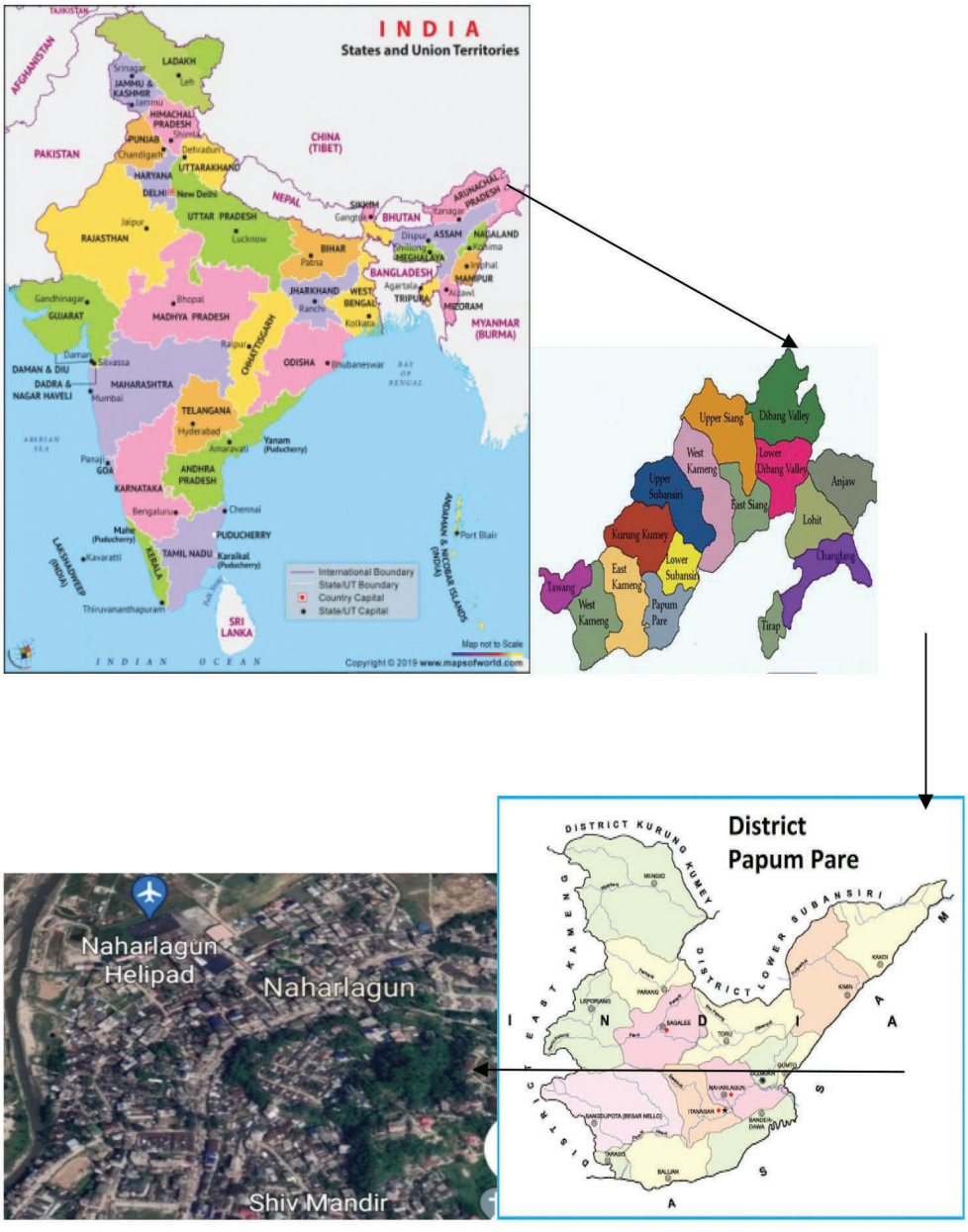
Description of the Study Area

Naharlagun is a small town in the district of Papum Pare in Arunachal Pradesh located at a height of 200 meters from sea level. The district is situated in the North Easter part of Arunachal Pradesh situated between 26°552 and 28°402 North latitude and 92°402 and 94°212 East longitudes. The district is bounded by Kra Daadi district in the north,

The natural vegetation comprises mainly tropical semi-evergreen and sub-tropical evergreen forests. The Capital City of Arunachal Pradesh, Itanagar located at an altitude of about 1700 ft. above the sea level between 93° East Longitude and 27° North Latitude (Figure 1).

Sample collection

The plant disease samples were collected during the



Courtesy- Google Map

Fig. 1. Location of the study area

month of March to May. The infected leaves of various plants were used in this study comes in abundant source, easily available and they are utilized in traditional medicine. Nine different types of medicinal plants which are common were selected for the - *Catharanthus roseus* (L.) G.Don, *Houttuynia cordata* Thunb., *Solanum lycopersicum* Lam., *Murraya koenigii* (L.) Spreng. , *Eryngium foetidum* L., *Clerodendrum glandulosum* Lindl., *Ocimum sanctum*, *Capsicum chinense* Jacq.

Blotter paper method (BPT)

The blotter paper technique was developed by Doyer in 1938. In this method Petri plates of size 90 mm were wrapped in brown paper. Each leaf sample was taken in separate Petri plates. The leaf fragment of 1 cm was cut out using sterile scissor. 10 pieces of leaf samples of each variety were placed in different Petri plates with potato dextrose Agar (PDA) and were incubated at 25 ± 2 °C. After 7 days of incubation different colonies were developed on leaves that were observed with the help of compound microscope.

Isolation of phylloplane fungi

Leaf samples of *Catharanthus roseus*(L.) G.Don, *Houttuynia cordata* Thunb., *Solanum lycopersicum* Lam., *Murraya koenigii*(L.) Spreng., *Eryngium foetidum* L., *Clerodendrum glandulosum* Lindl., *Ocimum sanctum* , *Capsicum chinense* Jacq.were collected and placed in sterile plastic bags, and immediately brought to the laboratory. From the basal part of the leaf, a fragment of 1cm of leaf blade was cutout and shaken in flasks filled with 200 ml of distilled water. From the suspension of microorganisms prepared in this way 0.2 ml was transferred into petridishes containing potato dextrose agar medium with streptomycin. The inoculum was spread uniformly and kept undisturbed in an incubator at 26±2! for a period of 3-5 days. The fungal colonies were observed and pure cultures were maintained.

Statistical analysis

The composition and diversity of the phylloplane diversity fungi measures such as the relative abundance, species richness (S), and Shannon-Wiener diversity index (H'), Evenness index, Simpson dominance index and Similarity index were calculated. The Shannon-Wiener diversity index considers relative abundance between species, species richness and evenness, and species distribution. However, the Shannon-Wiener diversity index focuses more on species richness (Odum, 1969; Obermaier, 2014; Kim *et al.*, 2017; Fan *et al.*, 2020; Kasi *et al.*, 2021). The relative abundance/proportion was estimated as a percentage of the number of fungal species, operational taxonomic units (OTUs) or phylum divided by its total number. Species richness (S) among the isolates was calculated as the number of species recovered from the particular sampling site or tree tissue. All the data were calculated by using the following formulas:

Relative Abundance (RA)

Formula for relative abundance is:

$$RA = \frac{ni}{N}\ 100\%$$

Where, N is the total number of species and ni is the number of individuals in species i.

Species richness (SR) index:

Formula for species richness (SR) is:

$$SR = \frac{s-1}{\log eN}$$

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

Shannon-Wiener Diversity Index (H')

Formula for Shannon-Wiener diversity index (H') is

$$\boldsymbol{H'} = \sum_{l=0}^{s} (\frac{ni}{N} log2\frac{ni}{N})$$

Where, N is the total number of species and ni is the number of individuals in species i

Evenness index (J)

Formula for Pielou's evenness index (J) is:

$$J = \frac{H'}{logeS}$$

Where H' is Shannon Weiner diversity and S is the total number of species in a sample

Similarity index (SI)

Formula for Similarity index (SI)

$$SI = \frac{2c}{A+B}$$

Where, C is the number of shared species between the two location and Aand B are the number

of species unique to each location

Results and Discussion

Fungal pathogens are able to infect various parts such as roots, stems, leaves, flowers and fruits including visible characteristic visible symptoms like spot, blight, anthracnose, wilts and rots etc. A total of eight medicinal plants were studied and according to their symptoms the pathogen were identified from the plants, *Catharanthus roseus* (L.) G. Don, *Houttuynia cordata* Thunb., *Solanum lycopersicum* Lam., *Murraya koenigii* (L.) Spreng. , *Eryngium foetidum* L., *Clerodendrum glandulosum* Lindl., *Ocimum sanctum* , *Capsicum chinense* Jacq. Diversity of phylloplane fungi isolated from the medicinal plants was calculated. Various fungi isolated from the medicinal plants were identified as *Alternaria sp., Aspergillus fumigatus, Aspergillusniger., Rhizopus sp., Fusarium sp., Penicillium sp., and Cercospora sp*. The diversity of fungi was found to be more in *Solanum lycopersicum* followed by *Murraya koenigii, Houttuynia cordata, Ocimum sanctum, Catharanthus roseus, Murraya koenigii, Eryngium foetidum* and *Capsicum chinense Jacq.* Leaf samples of different medicinal plants showed a varied level of incidence of mycoflora. From the study of the above medicinal plants it can be observed that *Alternaria sp* showed the highest O.T.U and relative abundance from the above eight medicinal plants followed by *Aspergillus sp*. *Rhizopus* sp and *Penicillium* sp. (Thakur, 2017, Mari *et al*., 2017 and Lokare *et al*., 2020) (Figs. 2, 3).

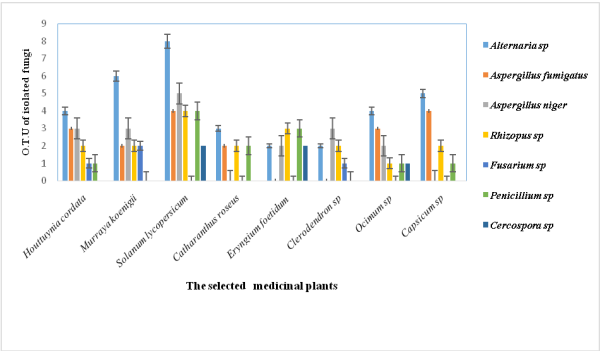


Fig. 2. Diversity of Phylloplane fungi isolated from the medicinal plants

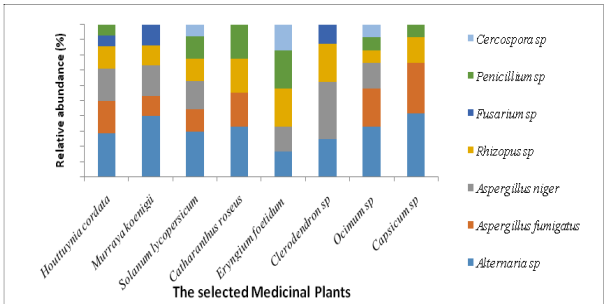


Fig. 3. The relative abundance of Phylloplane fungi isolated from the medicinal plants

Shannon-Wiener diversity index, Simpson dominance index, species richness index, species evenness index and Similarity index of isolated fungi in the selected medicinal plants are also estimated. The highest Shannon-Wiener diversity index was found in *Solanum lycopersicum* (1.71), followed by *Houttuynia cordata* (1.67), *Ocimum* sp (1.63), *Eryngium foetidum* (1.59) (Table 1). The lowest Shannon-Wiener diversity index was estimated in *Capsicum* sp (1.24). Simpson dominance index was also observed high in *Solanum lycopersicum* (0.48), followed by *Murraya koengii* (0.50), *Houttuynia cordata* (0.46), *Ocimum* sp (0.36). The lowest value was observed in *Clerodendron* sp (0.01) and *Catharanthus roseus* (0.13*)*. The species richness and species evenness index were found more in *Solanum lycopersicum*, *Ocimum* sp, *Houttuynia cordata* etc. The similarity index was estimated as 0.57 (Table 1). The relative abundance of isolated fungi was found to be more higher in *Solanum lycopersicum Houttuynia cordata*, *Murraya koengii* and less in *Clerodendron* sp,

Table 1. Shannon-Wiener diversity index, Simpson dominance index, species richness index, species evenness index and Similarity index of isolated fungi in the selected medicinal plants.

	The selected medicinal plants							
	Houttuynia cordata	*Murraya koenigii*	*Solanum lycopersicum*	*Catharanthus roseus*	*Eryngium foetidum*	*Clerodendron sp*	*Ocimum sp*	*Capsicum sp*
Shannon-Wiener diversity index	1.67	1.49	1.71	1.37	1.59	1.32	1.63	1.24
Simpson Dominance Index	0.46	0.50	0.48	0.13	0.36	0.01	0.36	0.36
Species Richness Index	1.79	1.61	1.79	1.61	1.61	1.61	1.79	1.39
Species Evenness Index	0.93	0.93	0.96	0.85	0.99	0.82	0.91	0.89
Similarity Index	0.57							

Capsicum sp and *Catharanthus roseus* (Figure 3).

The Shannon-Wiener diversity index was calculated to study the relative abundance between species, species richness and evenness, and species distribution. Thus, the Shannon-Wiener diversity index focuses more on species richness. Simpson index is generally estimated to observe the diversity of species in a community and it also emphasizes species evenness. If the Simpson index was high, it means that the diversity in the community was low. The Simpson dominance index value ranges from 0-1, where if the index value was close to zero, means that there are no dominant species and illustrate the stability of the community. Meanwhile, if the index value is close to 1, it means that there are dominant species and indicated that the ecosystem was in an unstable condition and there was ecological pressure. The Simpson dominance index value for all the selected medicinal plants were close to zero, so it can be stated that the phylloplane fungal community structure in the medicinal plants were stable without any ecological pressure (Cavender *et al*., 2009; Fierer *et al*., 2012; Bahram *et al*., 2015; Jiang *et al*., 2021; Lin *et al*., 2021; Liu *et al*., 2022; Wang *et al*., 2022)

Conclusion

The study on Phylloplane mycoflora of medicinal plants is of great importance to conserve these plants for future use. From this preliminary investigation various phylloplane pathogens were reported in the medicinal plant in Naharlagun Area and dominant species were identified as *Alternaria sp., Aspergillus niger*., *Rhizopus* sp., *Penicillium* sp. The relative abundance of isolated fungi was found to be significant in *Solanum lycopersicum Houttuynia cordata*, *Murraya koengii*. The Shannon-Wiener diversity index and Simpson index showed significant values which gives the clear idea about the stable phylloplane fungi community structure in the medicinal plants. It is also important for further molecular analysis for their characterization up to species level. Future research work can be carried out to isolate and identification of surface fungi growing in these medicinal plants and thereby to suggest the control measures for conservation and fruitful commercial utilization by the people of this region.

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Conflict of interest

The authors declare that there is no conflict of interest.

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