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Seasonal dynamics of phyllosphere fungi of *Camellia sinensis* (L.) O. Kuntze from the plantations of Tripura, Northeast India

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Abstract

The objective of this study is to investigate the seasonal composition of phylloplane and endophytic fungi in *Camellia sinensis*, as well as their seasonal flux. The results indicated that the peak isolation rate for both groups of fungi occurred prior to winter. For endophytic fungi, the lowest isolation rate was observed during the winter season, and for phylloplane fungi, during the monsoon season. Genera *Alternaria*, *Colletotrichum* and *Phyllosticta* exhibited the highest colonization frequency of fungal endophytes across all four seasons. *Penicillium*, followed by *Aspergillus* and *Mucor* reveals the highest proportion of phylloplane fungi across all four seasons. In the winter, phyllosphere fungi had the highest diversity. The similarity index of endophytic and phylloplane fungal genera isolated from *C. sinensis* for distinct seasons revealed the highest similarity during the pre-monsoon season and the lowest similarity during the monsoon season. The occurrences of both categories of fungi are discussed in terms of seasonal and environmental variation.

Keywords – Endophytes – Fungal diversity – Phylloplane – Seasonal fluctuations – Tea plant

Introduction

Plants house fungi, an essential component of biodiversity (Chandrasekhar et al. 2014), within their bodies and on leaf surfaces. The plant-fungal association is distinct in that it can be beneficial to both the fungus and the host plant, or it can be detrimental to the host plant by causing disease that can result in the plant's mortality. The relationship between fungi and their hosts varies based on the condition of the host plant (Balestrini 2021). The phyllosphere is the interior as well as the exterior part of an entire leaf (Carroll et al. 1977) that provides shelter to many microorganisms. Therefore, endophytic and phylloplane fungi are phyllosphere fungi that reside in different parts of foliage (Yao 2019).

Phylloplane fungi refer to fungi that grow on the surface of leaves. There are two types of phylloplane fungi: permanent and transient(Prabakaran et al. 2011). Permanents can proliferate on the leaf surface of the host in any discernible way. Transients, on the other hand, make contact with the

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leaf surface, but they are unable to proliferate (Prabakaran et al. 2011). Fungi that live inside the plant tissue for the entire or a segment of their life cycle without causing noticeable damage or infection are called endophytes (Petrini et al. 1992).

The coexistence of phylloplane and endophytic microorganisms may contribute to the well-being of the host plant (Andrews & Harris 2000) as well as microbial biodiversity (Hawksworth & Rossman 1997). It is believed that phylloplane fungi colonize leaves in order to modulate moisture and temperature conditions, as well as to protect the host from solar rays, pollution, competitors, and grazing (Kharwar et al. 2010). Arnold & Herre (2003) report a positive correlation between the colonization of foliar endophytes and the abundance of aerial and epiphytic propagules.

Camellia sinensis (L.) O. Kuntze is an evergreen shrub extensively cultivated in tropical and subtropical regions, including Asia and Africa (Mahmood et al. 2010, Tanti et al. 2016). *Camellia sinensis* is the second most consumed beverage in the world, after water. Tea has a variety of beneficial physiological and medicinal effects, according to multiple studies (Miller 1995, Sharangi 2009, Mahmood et al. 2010, Namita et al. 2012).

Camellia sinensis fungal endophytes have been studied (Agusta et al. 2006, Fang et al. 2013, Unterseher et al. 2018, Xie et al. 2020). However, no information exists regarding the phylloplane fungi of tea. The purpose of this study is to investigate the seasonal composition of phylloplane and endophytic fungi in *C. sinensis*, as well as their seasonal variation. This study may reflect the impact of ecological factors leading to the categorization and marking out of fungal species. Moreover, this study may give an idea about the phyllosphere fungal community of tea plants, which may serve as a source of many industrially important secondary metabolites and these also may be exploited as biocontrol candidates against important foliar pathogens.

Materials & Methods

Sampling site

The leaf samples of *C.sinensis* were collected from the Fatikchera tea garden (91°20′37″ E and 23°56′47″ N) in the West Tripura district of Tripura, India (Fig. 1). Tropical climate influences this region which can be generally divided into four seasons, i.e.,pre-monsoon (March to May), monsoon (June to September), prewinter (October to November), and winter (December to February). The average annual rainfall and temperature of this region are 2000 mm and 25 ºC, respectively (Jamatia & Chaudhuri 2017). From November 2020 through December 2021, samples were collected. The samples were collected from the same tea garden during the pre-monsoon, monsoon, pre-winter, and winter seasons of a single year. The disease-free, fresh leaves were harvested from 10 to 12 identically aged tea shrubs. These harvested leaves were placed in sterile zip-lock containers and transported to a laboratory for the isolation of phyllosphere fungi.

Isolation of endophytic fungi

During the period of November 2020 to December 2021, endophytic fungi were isolated from *C. sinensis* grown in the Fatikchera tea garden of west Tripura, as previously described (Nath et al. 2012). The plant samples were rinsed with faucet water to eliminate pollen and other particles. They were immersed in 70% ethanol for one minute, then in 3% sodium hypochlorite (NaOCl) for thirty seconds, and then in 70% ethanol for another minute. The leaf segments were then blotted dry with sterile blotting paper using autoclaved distilled water. Five leaf segments were plated on streptomycin-enhanced malt extract agar media containing 50 mg/l. Plates were sealed with parafilm and incubated in the dark at room temperature. The growth of endophytic fungal colonies from leaf segments was monitored daily by observing the plates. Appeared fungal colonies were isolated and cultured in their purest form. For further research, pure cultures of the isolates were maintained on MEA slants and agar

plates. By placing aliquots of sterilant on agar plates and observing fungal colonies for three weeks (Schulz et al. 1993), the efficacies of surface sterilization of samples were determined.

Isolation of phylloplane fungi

From the leaves of *C. sinensis*, phylloplane fungi were isolated (Santamaria & Bayman 2005). The medium used for cultivation was malt extract agar (MEA). Five leaf fragments from the middle of the mid-vein and the leaf margin were deposited on MEA plates supplemented with 50 mg/l streptomycin (ventral surface contacting MEA) and then imprinted. Plates with leaf impressions were incubated at room temperature for a week and observed for the growth of fungal colonies.

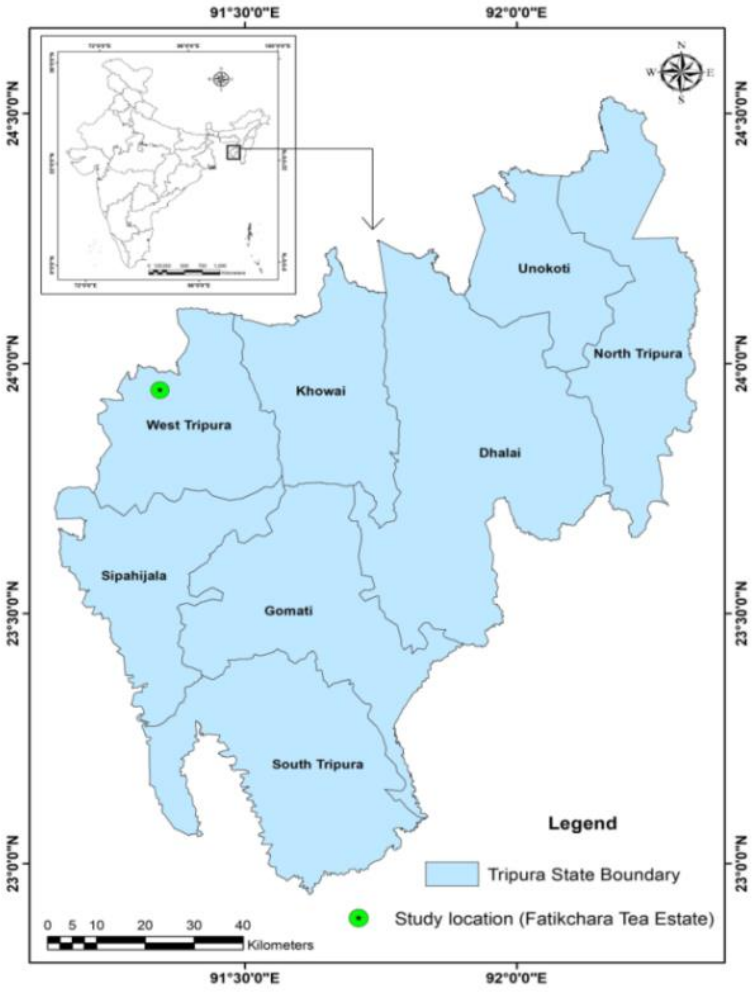

Fig. 1 – Location of the study site.

Identification of the isolated fungi

With the aid of a compound microscope (OLYMPUS-CX21i), the identified fungal colonies were stained with lactophenol cotton blue. Using standard taxonomic keys, macro and micro-morphological characteristics were examined for preliminary identification. The fungi were identified at genus level and species 1, 2 and so on were named due to the dissimilarity in their morphological structure of hyphae and conidia.

Data analysis

The colonization frequency (CF) was computed according to Hata & Futai (1995) instructions. Using the following formula, the colonization frequency (percent) of an endophyte species was calculated:

$$\text{CF } (\%) = \frac{\text{No of plant segments colonized by a single fungi}}{\text{Total no. of plant segment observed}} \times 100$$

The percentage of occurrence (PO) (Van Ryckegem & Verbeken 2005) was determined by:

$$\text{PO of taxon A} = \frac{\sum \text{Records of taxon}}{\text{No. of leaf segments observed}} \times 100$$

The relative frequency (RF) of isolation is used to characterize the diversity of fungi. According to the following equation and expressed as a percentage (Huang et al. 2008):

$$\text{Relative frequency} = \frac{\text{No. of isolates of one species}}{\text{Total no. of isolates}} \times 100$$

Isolation Rate (IR) is a measurement of fungal richness in a particular plant tissue sample, i.e., the incidence of numerous infections per segment or fragment. According to the formula given by Huang et al. (2008) but expressed as percentage, it is computed as follows:

$$\text{Isolation rate} = \frac{\text{No. of isolates obtained from segments}}{\text{Total no. of segments}} \times 100$$

For studying fungal species richness, we have calculated the Shannon diversity index (H'), evenness index (J') and Simpson diversity index (1/D) (Magurran 2004). The similarity index (Sorensen 1948) was used to compare the seasonal similarity of species.

Results

The isolation of phylloplane fungi using the media is depicted in Fig. 2. The isolation rate of endophytic fungi and phylloplane fungi is depicted in Table 1. Maximum isolate rate was observed in the pre-winter season for both groups of fungi. The isolated species of phylloplane fungi are provided in Figs. 3 & 4. The isolation of endophytic fungi using the media is depicted in Fig. 5. The pure cultures of isolated species of endophytic fungi are provided in Figs. 6–8. The lowest isolation rate was observed from the specimens collected in the winter season in the case of endophytic fungi and from the monsoon season for phylloplane fungi (Fig. 9).

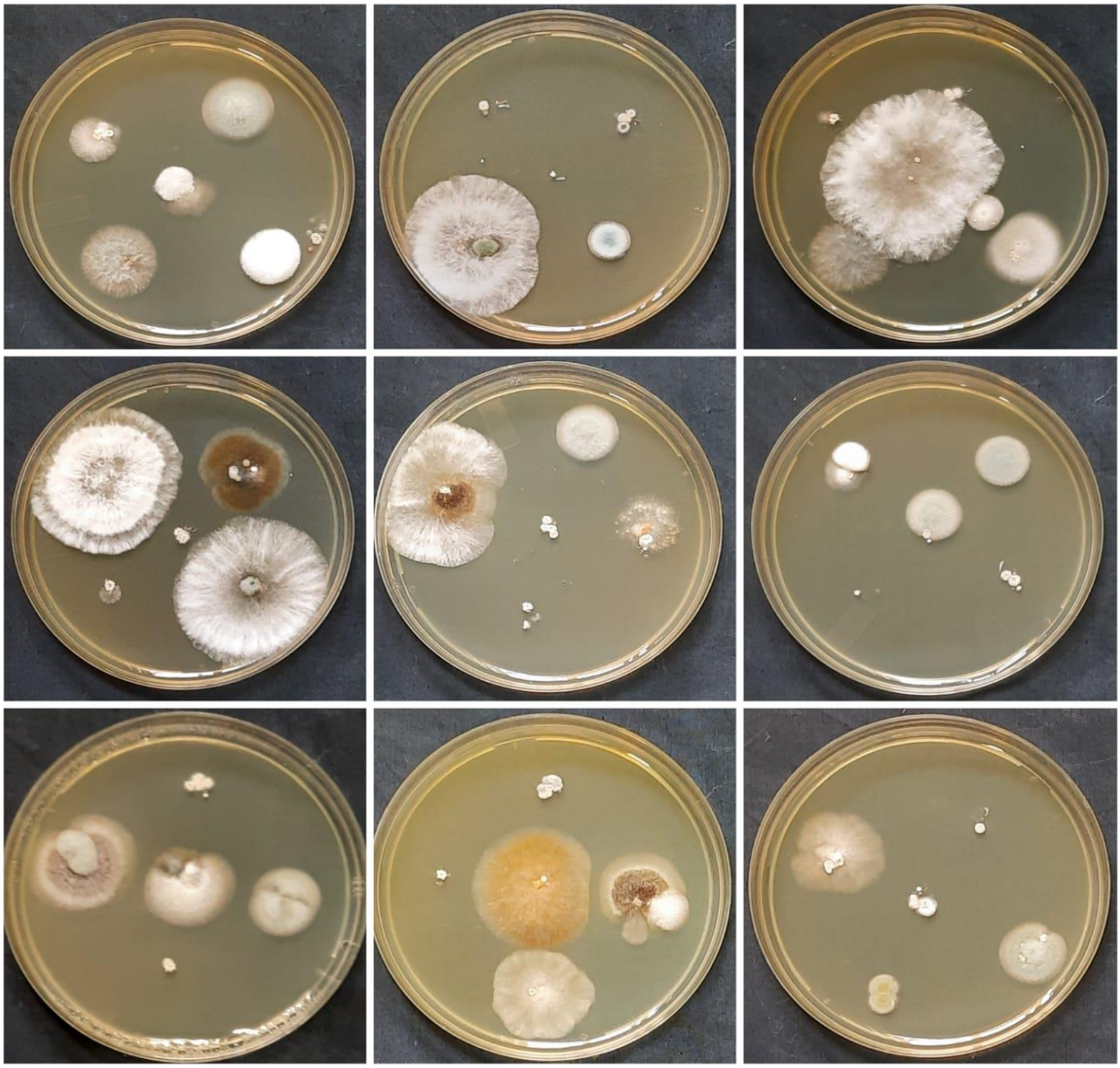

Fig. 2 – Isolation plates of phylloplane fungi.

Table 1 Isolation rate (%) of endophytic fungi and phylloplane fungi in four different seasons.

Season	Endophytic fungi	Phylloplane fungi
PM	71.60	63.30
M	72.80	48.00
PW	81.20	94.60
W	60.40	73.30

Pre-monsoon(PM), Monsoon (M), Prewinter (PW), Winter (W)

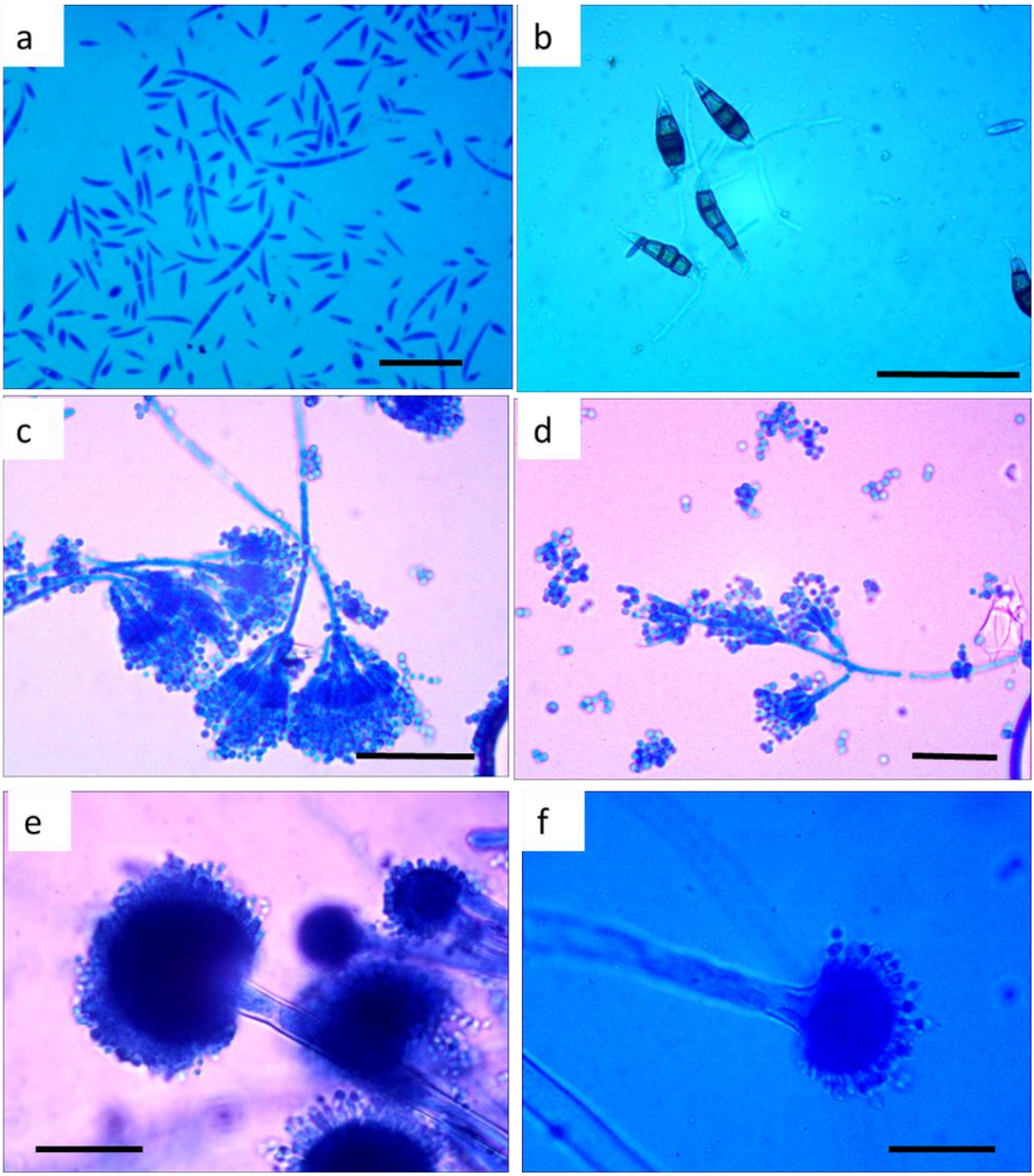

Fig. 3 – Reproductive structures of some phylloplane fungi. (a) Hyaline macro & micro conidia of *Fusarium* sp. (b) Conidia with 5 cells of *Pestalotiopsis* sp. (c) Catenulate conidia on each phialide of *Penicillium* sp.1 (d) Verticillate phialide of *Penicillium* sp. with conidia (e)Vesicle, phialide and conidia of *Aspergillus* sp.1 (f) Uniseriate phialides developed on clavate vesicles of *Aspergillus* sp. 2. Scale bars: a, d = 40 µm, b–c, e–f = 20 µm.

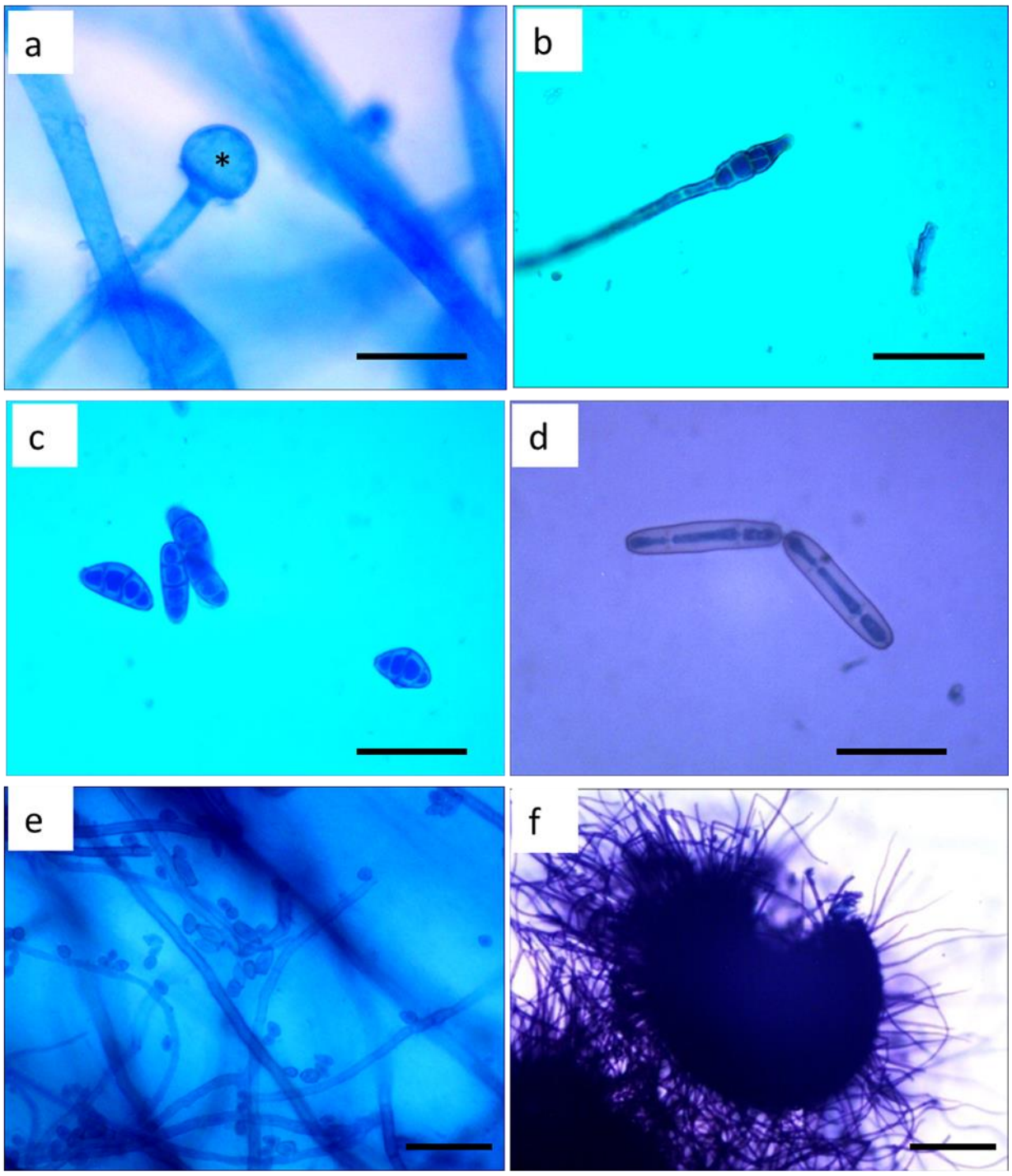

Fig. 4 – Reproductive structures of some phylloplane fungi. (a) Sporangiophore of *Mucor* sp. (b) Solitary obclavate and beaked conidia of *Alternaria* sp**.** with transverse/oblique/longitudinal septa. (c) Curved clavate conidia of *Curvularia* sp. with transverse septa. (d) Oblong conidia of *Drechslera* sp. with three pseudosepta. (e) Brown hyphae with ovoid conidia with a distinct scar in *Cladosporium* sp. l. (f) Perithecium of *Chaetomium* sp. with terminal hairs. Scale bars: a–d = 20 µm, e–f = 40 µm.

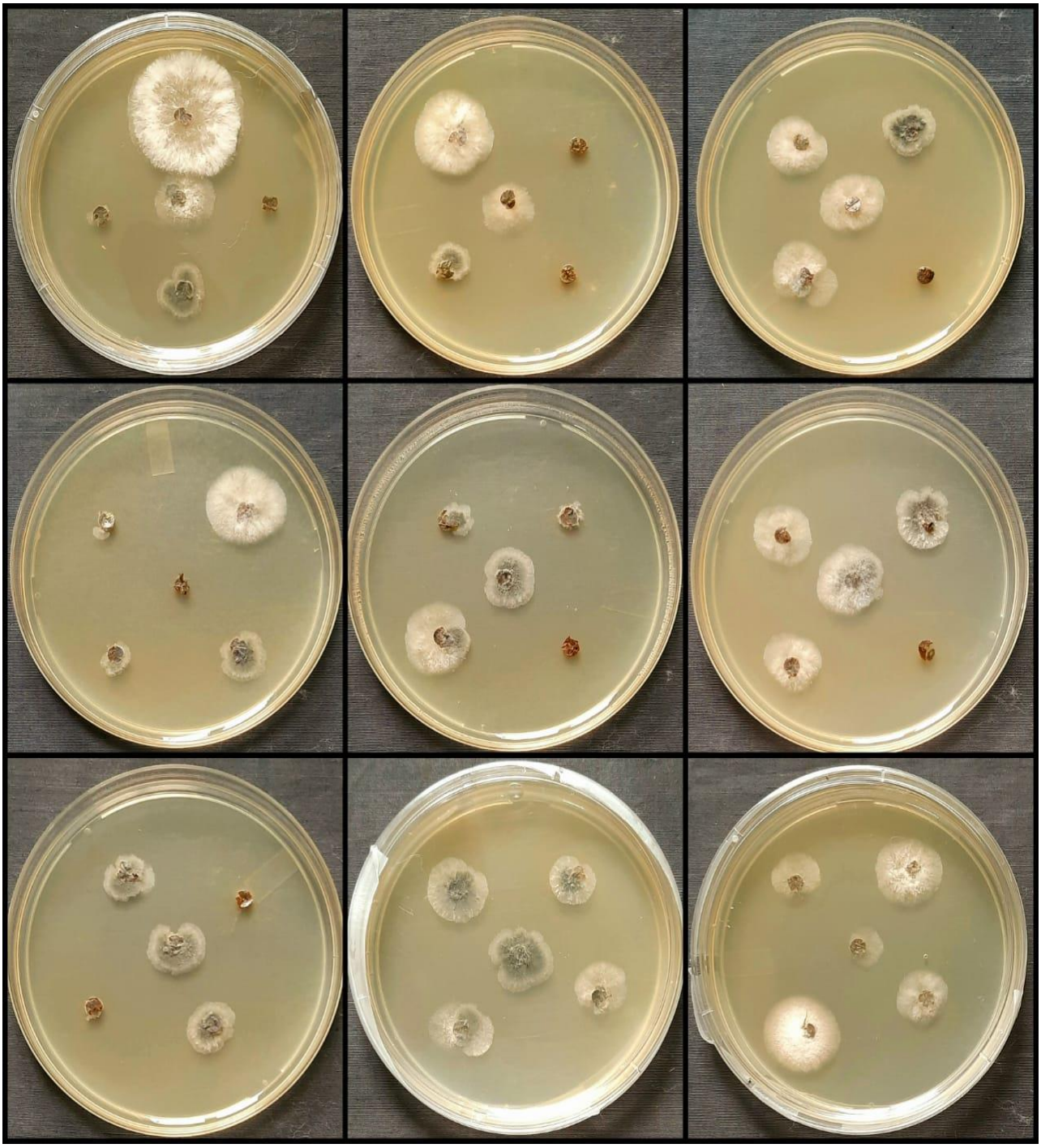

Fig. 5 – Isolation plates of endophytic fungi.

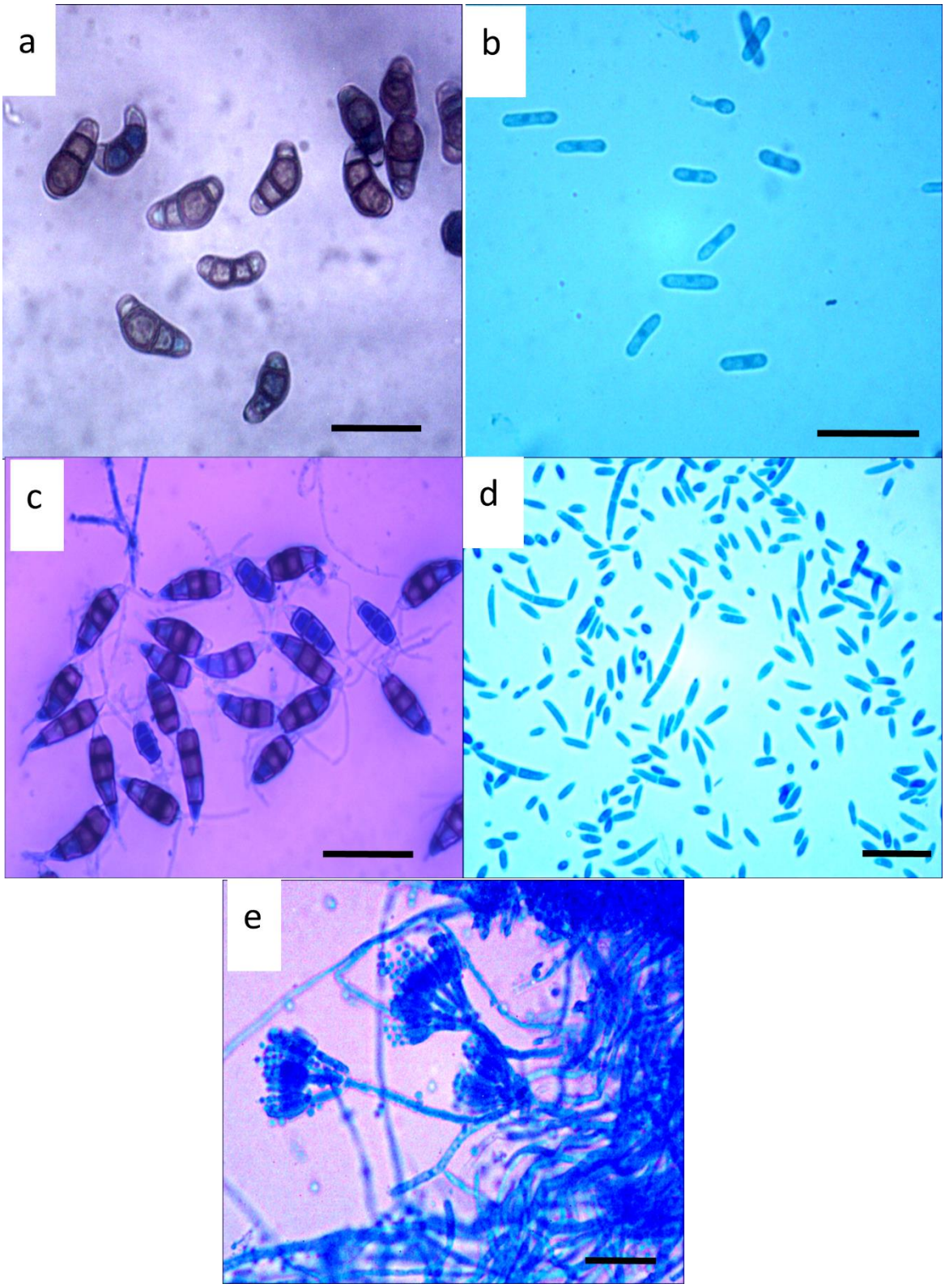

Fig. 6 – Reproductive structures of some endophytic fungi under the microscope. (a) Curved clavate conidia of *Curvularia* sp. with transverse septa. (b) Alpha conidia of *Diaporthe* sp. (c) Conidia with 5 cells of *Pestalotiopsis* sp. (d) Hyaline macro & micro conidia of *Fusarium* sp. (e) Phialide of *Penicillium* sp. with conidia. Scale bars: a–d = 20 µm, e = 40 µm.

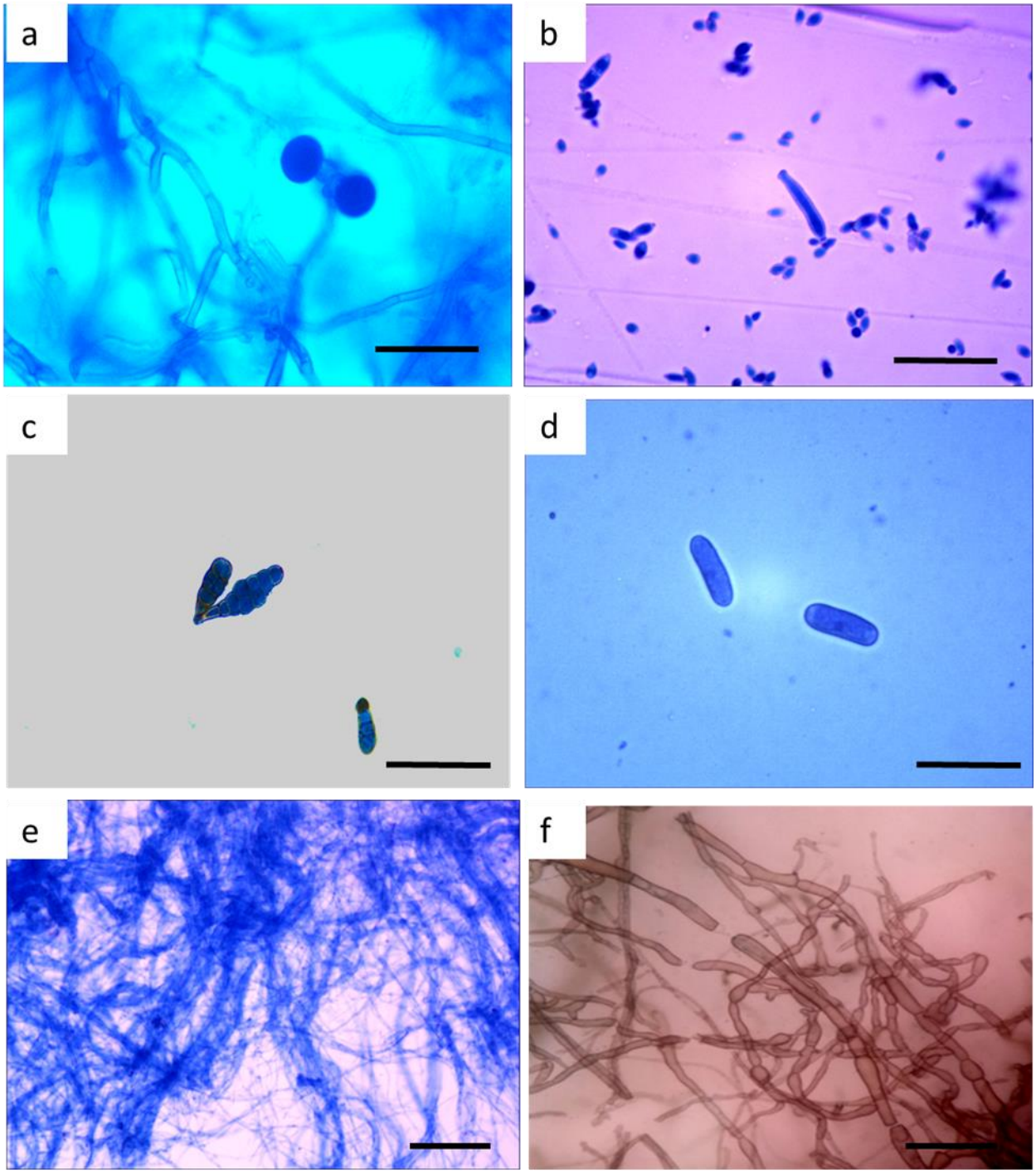

Fig. 7 – Reproductive structures of some endophytic fungi. (a) Aleuriosporous black disk-shaped conidia of *Nigrospora* sp. (b) Ovoid conidia of *Cladosporium* sp. (c) Conidia of *Alternaria* sp. with transverse/longitudinal septation. (d) Phialosporous hyaline cylindrical conidia of *Colletotrichum* sp. (e) White sterile (f) Black sterile. Scale bars: a–d = 20 µm, e–f = 50 µm.

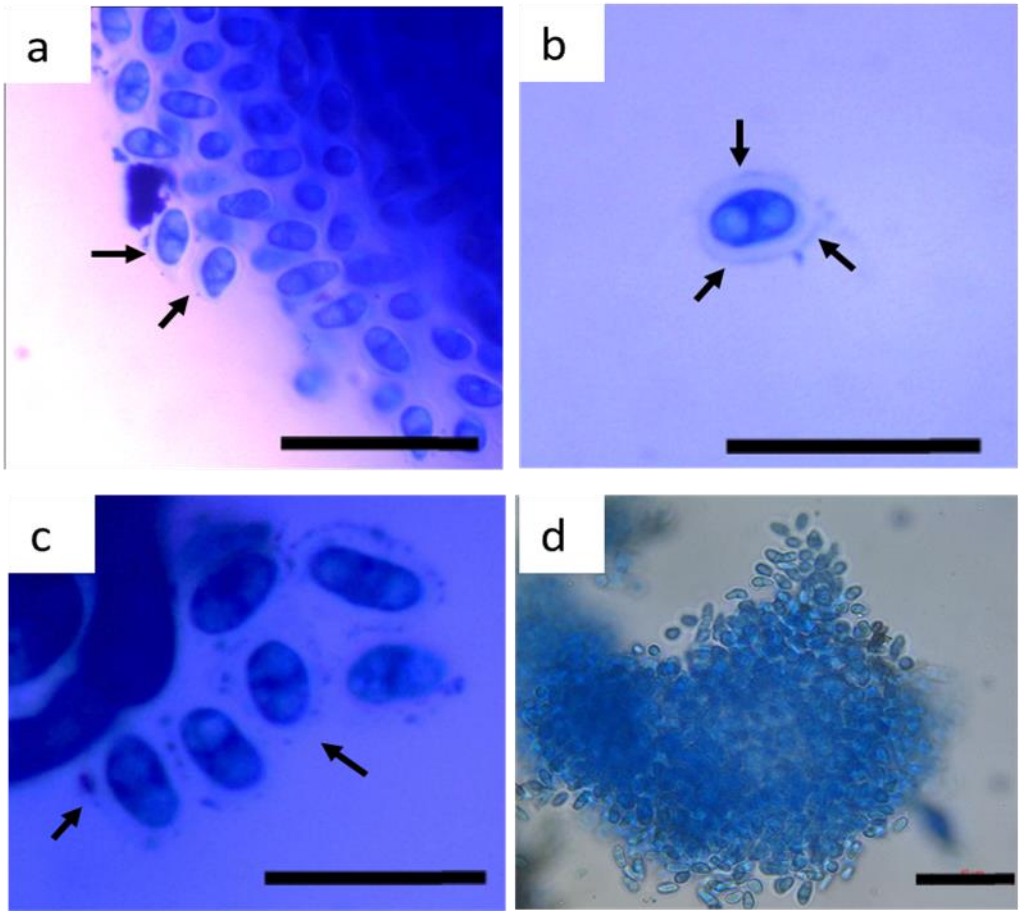

Fig. 8 – Reproductive structure of *Phyllosticta* sp. (a–c) Conidia with mucoid sheaths in *Phyllosticta* sp. (d)Aggregate of conidia of *Phyllosticta* sp. Scale bars: a–c = 5 µm, d = 50 µm.

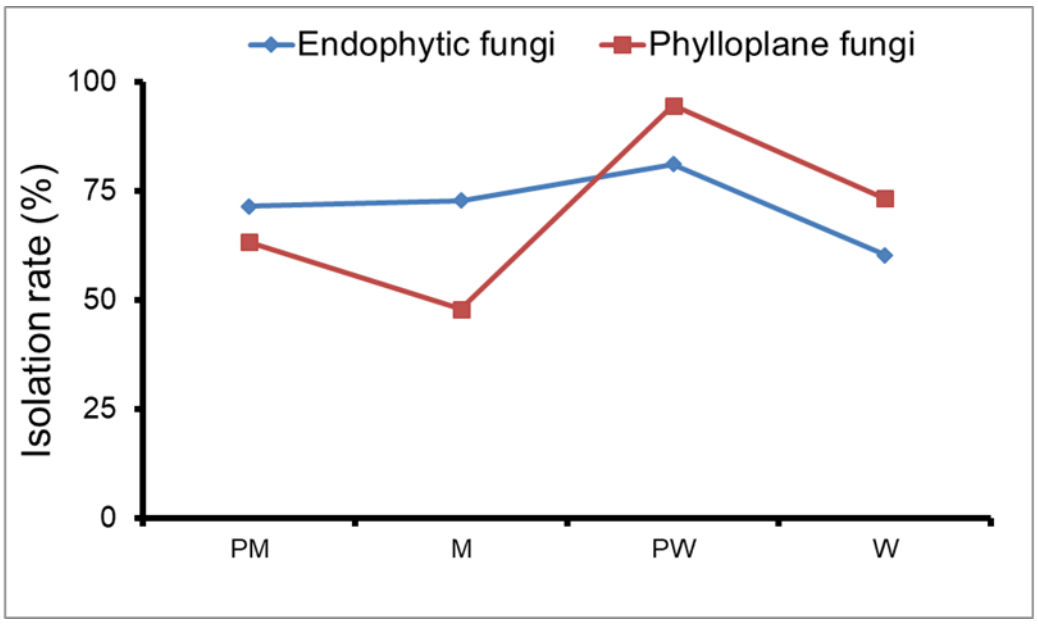

Fig. 9 – Seasonal representation of isolation rates of endophytic fungi and phylloplane fungi from *Camellia sinensis*. Pre-monsoon(PM), Monsoon (M), Prewinter (PW), Winter (W)

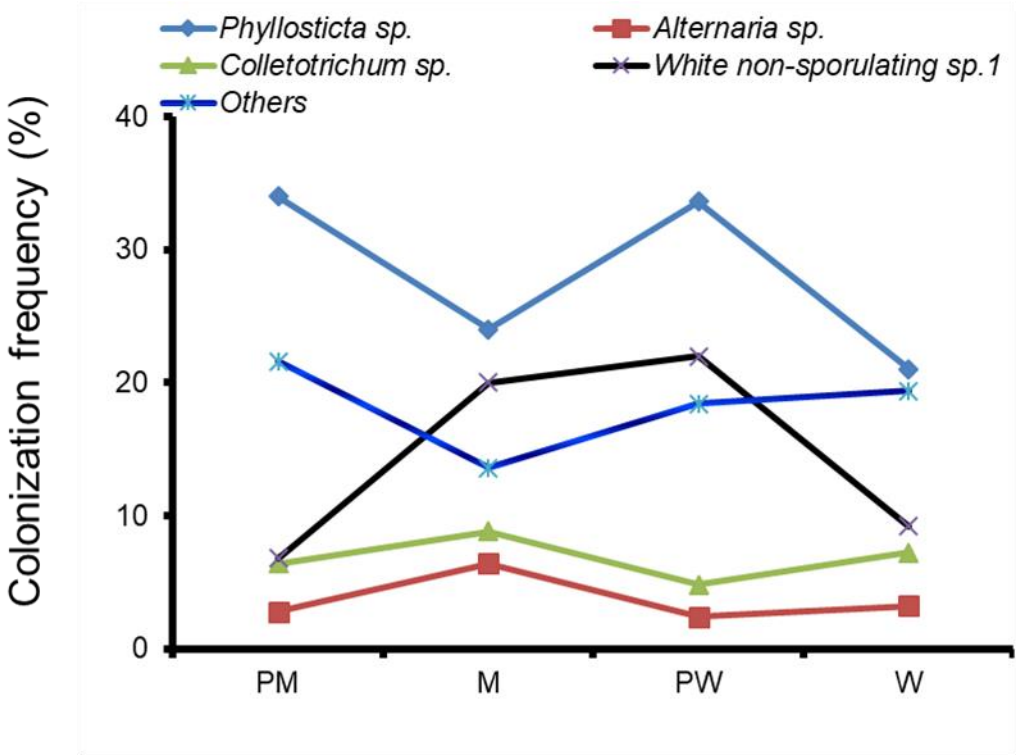

Fig. 10 – Colonization frequency of endophytic fungal genera isolated from all the seasons and other genera from *Camellia sinensis* in four different seasons. Pre-monsoon (PM), Monsoon (M), Prewinter (PW), Winter (W).

Table 2 Colonization frequency (CF %) of endophytic fungi isolated from *Camellia sinensis* in four different seasons.

Endophytic fungi	**PM**	**M**	**PW**	**W**
Phyllosticta sp.	34.0	24.0	33.6	21.0
Curvularia sp.	-	-	7.2	2.0
Diaporthe sp.	1.6	3.2	-	2.4
Cladosporium sp.	4.8	3.2	-	0.4
Pestalotiopsis sp.	6.0	-	0.8	4.0
Fusarium sp.	-	-	6.4	2.4
Alternaria sp.	2.8	6.4	2.4	3.2
Colletotrichum sp.	6.4	8.8	4.8	7.2
Penicillium sp. 1	0.4	-	0.4	1.6
Penicillium sp. 2	2.0	3.2	1.2	1.6
Nigrospora sp.	1.6	2.4	-	-
Pyricularia sp.	0.8	0.8	-	1.2
Unidentified sp.1	0.4	0.4	-	1.2
Unidentified sp.2	-	0.4	-	0.8
Unidentified sp. 3	-	-	0.4	0.8
White non-sporulating sp.1	2.0	4.8	6.0	3.2
White non-sporulating sp.2	4.8	15.2	16.0	6.0
Black non-sporulating sp.	4.0	-	2.0	1.6
Total	71.6	72.8	81.2	60.0

Pre-monsoon(PM), Monsoon (M), Prewinter (PW), Winter (W)

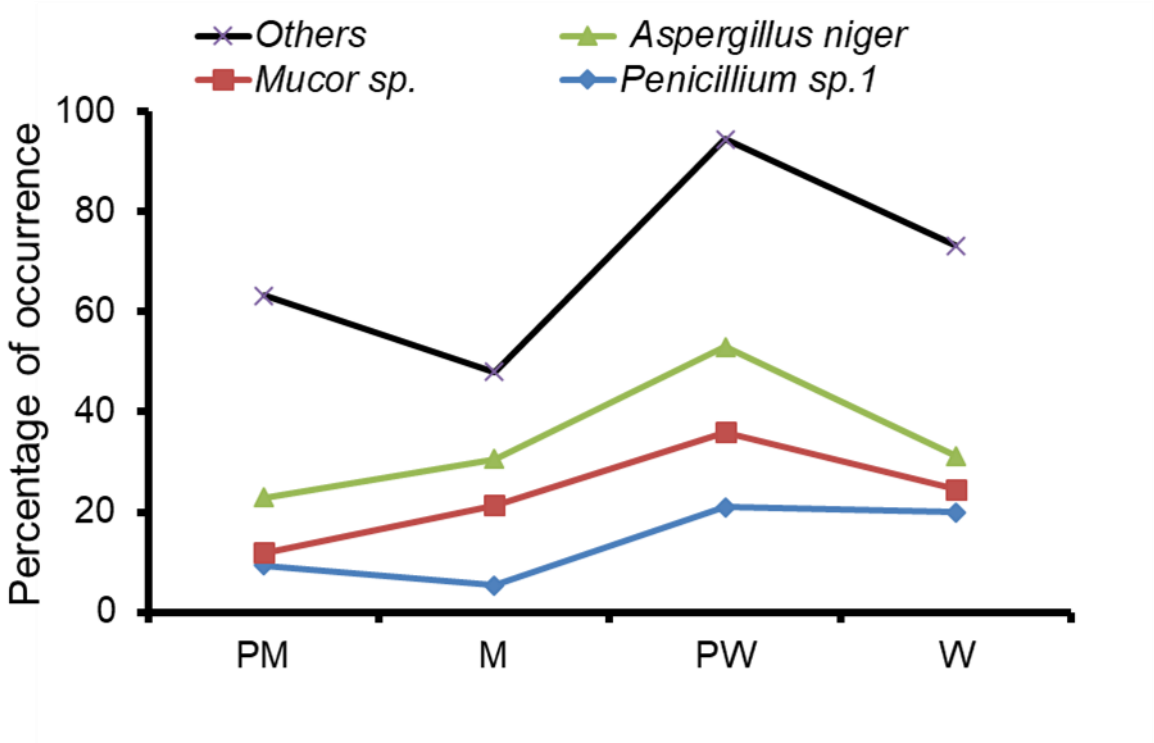

Fig. 11 – Graphical representation of the percentage of occurrence of phylloplane fungi from *Camellia sinensis* in four different seasons. Pre-monsoon (PM), Monsoon (M), Prewinter (PW), Winter (W).

The colonization frequency of endophytic fungi isolated from *C. sinensis* in four different seasons is shown in Table 2. Maximum colonization frequency was observed for *Phyllosticta* sp., followed by *Colletotrichum* sp., *Phyllosticta* sp., *Alternaria* sp. and *Colletotrichum* sp. from all four seasons. The seasonal fluctuation of the colonization frequency of three frequently occurring genera and other genera is provided in Fig. 10.

The percentage of occurrence of phylloplane fungi is exhibited in Table 3. *Penicillium* sp.1, followed by *Aspergillus* sp.1 and *Mucor* sp. reveals the maximum percentage of occurrence in all four seasons. The seasonal fluctuation of frequently occurring genera *Penicillium* sp.1, *Aspergillus* sp. 1 and *Mucor* sp., along with other fungal genera, is portrayed in Fig. 11.

The relative frequency of endophytic and phylloplane fungi isolated from *C. sinensis* from four different seasons is depicted in Table 4. Out of 28 morphotypes of phyllosphere fungi, eight were commonly isolated in the monsoon season. White non-sporulating sp. 2 exhibited the highest relative frequency isolated from phylloplane during the pre-monsoon. *Phyllosticta* sp. exhibited the highest relative frequency as an endophyte from the pre-monsoon season. White non-sporulating sp. 2 was the only common phyllosphere fungus recorded in the monsoon season. *Mucor* sp. has the highest relative frequency as phylloplane fungi recorded in monsoon, and white non-sporulating sp. 2 was recorded as the endophytic fungi having the highest relative frequency during monsoon. During the pre-winter season, four fungal genera were commonly identified from the phyllosphere of *C. sinensis*. *Penicillium* sp. 1 exhibited the highest relative frequency among the phylloplane fungi and *Phyllosticta* sp. Among the endophytic fungi. Nine morpho-genera were commonly isolated from the winter season. Maximum relative frequency was recorded by *Penicillium* sp. 1 among the phylloplane fungi and *Phyllosticta* sp. among the endophytic fungi, similar to the pre-winter season.

Table 3 Percentage of occurrence of phylloplane fungi isolated from *Camellia sinensis* in four different seasons.

	PM	M	PW	W
Penicillium sp.1	9.3	5.3	21.0	20.0
Penicillium sp.2	3.3	-	10.0	8.0
Mucor sp.	2.6	16.0	15.0	4.6
Aspergillus sp.1	11.0	9.3	17.0	6.6
Alternaria sp.	2.6	-	11.0	5.3
Pestalotiopsis sp.	1.3	-	5.3	8.0
Curvularia sp.	-	2.0	4.0	4.6
Drechslera sp.	-	2.0	1.3	-
Fusarium sp.	-	-	1.3	0.6
Phomopsis sp.	-	-	-	1.3
Xylaria sp.	-	-	1.3	2.6
Nigrospora sp.	1.3	-	2.6	-
Cladosporium sp.	2.6	-	-	1.3
Rhizopus sp.	1.3	-	1.3	0.6
Chaetomium sp.	-	-	-	2.0
Aspergillus sp.2	-	1.3	0.6	-
Unidentified sp.1	1.3	0	-	-
Unidentified sp.2	-	0	-	0.6
White non-sporulating sp.1	11.0	0	-	5.3
White non-sporulating sp.2	16.0	11.0	-	1.3
White non-sporulating sp.3	-	1.3	2.0	-
Total	63.3	48.0	94.6	73.3

Pre-monsoon (PM), Monsoon (M), Prewinter (PW), Winter (W)

Table 4 Relative frequency of endophytic fungi (E) and phylloplane (P) fungi isolated from *Camellia sinensis* in four different seasons.

	Pre-monsoon		Monsoon		Prewinter		Winter	
	P	E	P	E	P	E	P	E
Curvularia sp.	-	-	4.2	-	4.2	8.9	6.4	3.3
Cladosporium sp.	4.2	6.7	-	4.4	-	-	1.8	0.7
Fusarium sp.	-	0	-	-	1.4	7.8	0.9	4.0
Alternaria sp.	4.2	3.9	-	8.8	11.3	2.9	7.3	5.3
Penicillium sp. 1	14.7	0.6	11.1	-	22.5	0.5	27.3	2.6
Penicillium sp. 2	5.3	2.8	-	4.4	10.6	1.5	10.9	2.6
Nigrospora sp.	2.1	2.2	-	3.3	2.8	-	-	-
Phyllosticta sp.	-	47.5	-	33.0	-	41.4	-	34.4
Diaporthe sp.	-	2.2	-	4.4	-	-	-	4.0
Pestalotiopsis sp.	-	8.4	-	-	-	1.0	-	6.6
Colletotrichum sp.	-	8.9	-	12.1	-	5.9	-	11.9
Pyricularia sp.	-	1.1	-	1.1	-	-	-	2.0
Mucor sp.	4.2	-	33.3	-	16.2	-	6.4	-
Aspergillus sp.1	17.0	-	19.4	-	18.3	-	9.1	-
Pestalotia sp.	2.1	-	-	-	5.7	-	10.9	-
Drechslera sp.	-	-	4.2	-	1.4	-	-	-
Phomopsis sp.	-	-	-	-	-	-	1.8	-

Table 4 Continued.

	Pre-monsoon		Monsoon		Prewinter		Winter	
	P	**E**	**P**	**E**	**P**	**E**	**P**	**E**
Xylaria sp.	-	-	-	-	1.4	-	3.6	-
Rhizopus sp.	2.1	-	-	-	1.4	-	0.9	-
Chaetomium sp.	-	-	-	-	-	-	2.7	-
Aspergillus sp. 2	-	-	2.8	-	0.7	-	-	-
Unidentified sp. 1	2.1	0.6	-	0.5	-	-	-	2.0
Unidentified sp. 2	-	-	-	0.5	-	-	0.9	1.3
Unidentified sp. 3	-	-	-	-	-	0.5	-	1.3
White non-sporulating sp. 1	17.0	2.8	-	6.6	-	7.4	7.3	5.3
White non-sporulating sp. 2	25.0	6.7	22.2	20.9	-	19.7	1.8	10.0
White non-sporulating sp. 3	-	-	2.8	-	2.1	-	-	-
Black non-sporulating sp.	-	5.6	-	-	-	2.5	-	2.7

Table 5 Diversity indices of phylloplane fungi from *Camellia sinensis* in four different seasons.

	PM	M	PW	W
No**.** of species	12	8	14	16
No**.** of isolate	95	72	142	110
Shannon index (*H*')	2.111	1.729	2.177	2.351
D	0.143	0.793	0.133	0.120
Simpson's index (1-*D*)	0.857	0.207	0.867	0.880
Evenness *(J*')	0.897	0.735	0.926	1.000

Pre-monsoon(PM), Monsoon (M), Prewinter (PW), Winter (W)

Table 6 Diversity indices of endophytic fungi from *Camellia sinensis* in four different seasons.

	PM	**M**	**PW**	**W**
No**.** of species	14	12	12	17
No**.** of isolate	179	182	203	151
Shannon index (*H*')	1.906	1.969	1.816	2.302
D	0.249	0.178	0.227	0.152
Simpson's index (1-*D*)	0.751	0.822	0.773	0.848
Evenness *(J*')	0.828	0.855	0.789	1.000

Pre-monsoon(PM), Monsoon (M), Prewinter (PW), Winter (W)

Table 7 Similarity index of endophytic and phylloplane fungi isolated from *Camellia sinensis* in four different seasons.

Season	**Similarity index**
PM	61.54
M	10.00
PW	38.46
W	54.55

Pre-monsoon(PM), Monsoon (M), Prewinter (PW), Winter (W)

Table 8 Similarity index of endophytic fungi isolated from *Camellia sinensis*during four different seasons.

	PM	**M**	**PW**	**W**
PM	100.00	84.62	69.23	83.87
M		100.00	50.00	75.86
PW			100.00	82.76
W				100.00

Pre-monsoon(PM), Monsoon (M), Prewinter (PW), Winter (W)

Table 9 Similarity index of phylloplane fungi isolated from *Camellia sinensis* during four different seasons.

	PM	**M**	**PW**	**W**
PM	100.00	40.00	61.54	71.43
M		100.00	63.64	41.67
PW			100.00	66.67
W				100.00

The diversity indices of phylloplane fungi from *C. sinensis* from four different seasons are depicted in Table 5. Shannon index (H') was highest in the winter season. Dominance is the maximum in the monsoon season. Evenness was maximum in the winter season. The diversity indices of endophytic fungi from *C. sinensis* from four different seasons are depicted in Table 6. Shannon diversity index was also maximum in the winter season. The least diversity was recorded in the pre-winter season. Dominance was maximum in the pre-monsoon season. Evenness was maximum in the winter season.

The similarity index of endophytic and phylloplane fungal genera isolated from *C. sinensis* for different seasons is depicted in Table 7. Maximum similarity (61.54%) was recorded from the pre-monsoon season. Least similarity (10%) between phylloplane and endophytic fungi was recorded during monsoon season.

The similarity index of endophytic fungi isolated from *C.sinensis* in four different seasons is depicted in Table 8. Maximum similarity (84.62%) of endophytic fungi was recorded in pre-monsoon and monsoon. Endophytic fungi from the monsoon and pre-winter seasons exhibited the least similarity (50%). Pre-winter and winter seasons exhibited 82.76% of similarity in endophytic fungi composition.

The similarity index of phylloplane fungi isolated from *C.sinensis* in four different seasons is depicted in Table 9. Maximum similarity (71.43%) of phylloplane fungi was observed during pre-monsoon and winter seasons. Least similarity (40%) in phylloplane fungal composition was recorded in pre-monsoon and monsoon seasons.

Discussion

There is no information on the phylloplane fungi of tea and, most importantly, in general, on phyllosphere fungi; however, the studies concentrated on fungal endophytes from *C. sinensis* have been documented and characterized (Agusta et al. 2006, Fang et al. 2013, Unterseher et al. 2018, Xie et al. 2020). This work focuses on the seasonal aspects of phylloplane and endophytic fungi in *C. sinensis* along with their seasonal changes.

In this study, the majority of endophytic fungi isolated from *C. sinensis* leaves correspond to the phylum Ascomycota. Similar results were reported by Lu et al. (2007) for tea plants in China. They discovered endophytes to be a highly diverse group, with the majority belonging to the phylum Ascomycota and the remain to the phylum Basidiomycota. Tea plants are reportedly dominated by the

endophytic *Colletotrichum* (Lu et al. 2007, Fang et al. 2013). Numerous non-sporulating fungi were discovered in the leaf and root tissues of plants. This group of fungi (non-sporulating fungi) has been frequently isolated as leaf endophytes from numerous host plants (Suryanarayanan et al. 1998, Rajagopal et al. 2000, Huang et al. 2008).

The frequency of endophyte colonization was greater during the monsoon and pre-winter seasons than during the summer and winter. The greater number of fungal isolates suggested that colonization by endophytes is correlated with climatic factors, such as precipitation and atmospheric humidity, which may affect the occurrence of certain endophytic species (Wilson & Carroll 1994). Earlier studies also found that fewer isolates were recovered during the arid season (Rodrigues 1994), which was attributed to the effects of water. Under conditions of water deficiency, it is known that certain plants can accumulate non-structural carbohydrates. This accumulation typically results in a buildup of carbon-based defences such as tannins, rendering the plant more resistant to fungal endophyte colonization during the arid season (Rodrigues 1994, Suryanarayanan et al. 1998).

The sporulating phylloplane fungi with the highest population and relative abundance during pre-monsoon, monsoon, prewinter, and winter were *Aspergillus niger*, *Penicillium* sp.1, *Mucor* sp., *Penicillium* sp.1, *Penicillium* sp.2, and *Pestalotia* sp., in that order. *Aspergillus* and *Penicillium* may be dominant due to their higher rate of spore production and dispersion, as well as their resistance to the current environmental conditions (Schimel 1995). Low phylloplane fungal population densities on leaf surfaces collected during pre-monsoon and monsoon seasons may be attributable to increased temperature and precipitation, which may have a direct impact on the quantitative estimation of phylloplane microflora. The findings of this study corroborate the earlier findings (Tanti et al. 2012, Chauhan et al. 2014, Tanti et al. 2016).

The highest diversity of phyllosphere fungi was observed during the winter season. However, the isolation rate was highest prior to winter. In terms of adequate moisture and temperature, the pre-winter season can be regarded as a favourable time. Environmental factors that are conducive to hyphae and spore proliferation could be responsible for the increased isolation rate. Nonetheless, the lower isolation rate in winter may be attributable to unfavourable environmental conditions, which may result in the inability of spores to proliferate. Thus, the species diversity may vary. The diversity analysis of endophytes was found to be greater during winter due to the fact that the internal environment of plant tissues may serve as a cushion, and withstanding ambient abiotic conditions allow them to thrive within the plant. However, phylloplane fungi also exhibited higher diversity during winter, which may be due to the fact that the temperature during the collection of the samples does not fall below 15 ºC in the studied region. These fungi may be mesophilic in nature, thereby thriving in the winter season.

The greatest similarity between phylloplane and endophyte was observed during the pre-winter season and decreased during the monsoon season. This can be explained by the effect of rainfall, which may flush away the phylloplane fungi. Consequently, phylloplane fungi are characterized by a smaller number of species.

The monsoon has no effect on endophytic fungi, as evidenced by the smaller degree of similarity between the monsoon and pre-winter seasons. This could be due to the fact that endophytic fungi reside within the plant's tissue. Phylloplane fungi exhibited less similarity during pre-monsoon and monsoon, possibly due to the washing away of spores and hyphae from the leaf surface.

Conclusion

Our study sheds light on the diversity of the phyllosphere fungal community isolated from Tripura-grown *C. sinensis*. Seasonal diversity studies of both phylloplane and endophytic fungi inhabiting *C. sinensis* reveal seasonal influence on the fungal composition, isolation rate, and colonization frequency. Our findings are essential for better understanding the diversity and assemblages of phyllosphere fungi associated with *C. sinensis* in Tripura, which may provide a starting

point for the commercial and industrial use of these endophytes in particular the secondary metabolites and as biocontrol candidates.

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