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Distribution of endophytic fungi in the leaf and stem of *Cissus quadrangularis* Linn. - A comparative study

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Abstract: The present study focused on the diversity and distribution of endophytic fungal species in different plant parts (stems and leaves) of the plant *Cissus quadrangularis* Linn. collected from different locations of Assam, India. Plant samples were collected from three districts, Kamrup, Barpeta and Biswanath of Assam for endophytic fungal isolation. Variation was found in the distribution of fungal endophytes in stems and leaves of the plant collected from different locations under study. The percentage isolation of the endophytic fungal isolates was more from the stems collected from Kamrup (M) whereas the percentage isolation of endophytic fungal isolates was more from the leaves collected from Biswanath Chariali and Barpeta. Shannon-diversity and Simpson diversity indices of fungal endophytes varied in stems and leaves of the plant under study. This variation can be attributed to the soil conditions of the sampling site as well as the seasonal variation during sampling periods. *Cladosporium* sp. and *Fusarium* sp., are the most dominant and frequently occurring endophytic fungal species in *C. quadrangularis* of Kamrup (M) and Barpeta districts. *Colletotrichum* sp. and *Rhizopus* sp. were the dominant endophytic fungal species in Biswanath district. Since *C. quadrangularis* is used traditionally in the treatments of bone fracture, the study of endophytes associated to this plant could be of paramount significance with a scope of identifying the potent endophytes that are capable of producing bioactive compounds necessary in bone-healing.

Keywords: *Cissus quadrangularis*, fungal endophytes, medicinal plant, percentage isolation, Shannon diversity index, Simpson's diversity index.

Introduction

Plants and the microbial world share a plethora of relationship with each other. These relationships account for a number of benefits not only to the plants but in return the microbes also gets advantage out of this association. Endophytic relationship is one such interaction where the microbes reside inside the host plant without rendering any harm to the host plant. Endophytes have been defined as those organisms whose infections are inconspicuous where the infected host tissues do not show any symptoms (Stone et al. 2000). Endophytes have been isolated from all the parts of the plant where the nature of the endophytic community is determined by the various biotic and abiotic factors (Gaiero et al. 2013; Gupta et al. 2015). Colonization frequency and species richness in the plant parts may be attributed to the age of the tissue (Nayak 2015). The difference in the endophytic species and its frequency can also be attributed to the exposure to the sun rays (Scholtysik et al. 2012). Geographical conditions like temperature and humidity of an area (Chowdhary & Kaushik 2015) as well as the different seasonal changes (Nalini et al. 2014) can also affect the endophytic distribution in a host plant.

Endophytes have been isolated from a variety of plant species including medicinal plants. Bioactive substances extracted from the medicinal plants and the ones extracted from the endophytic species sometime shows similarity in their properties (Iyer & Rajkumar 2017). Apart from medicinal importance, endophytes have been found to impart stress tolerance in the host plant. In addition, it has also proved to be of importance in imparting bioremediation, phytoremediation, antifungal properties and anti-bacterial properties to the host plant (Chandra 2012; Pietro-Souza et al. 2020; Syranidou et al. 2017). *C. quadrangularis* Linn. is a magnificent plant having enormous beneficial role in being effective against *Diabetes mellitus* (Lekshmi et al. 2015). It has also found to be having antimicrobial and antioxidant property (Deshpande et al. 2023). Endophytic fungi isolated from this plant can prove to be of immense benefit for the mankind. Endophytic studies related to this plant is very less in the North Eastern part of India. Hence the present work is an attempt to elucidate more on the endophytic fungal diversity of *C. quadrangularis*. This study can later help in elucidating more on the biopotential aspects of the endophytic fungal species isolated from this plant

Materials and Methods

Collection of plant samples

Plant samples (stems and leaves) of *C. quadrangularis* were collected from three different districts viz., Kamrup (Metro), Barpeta and Biswanath of Assam (India). The plant samples were collected in sterilized bags and were then brought to the laboratory for surface sterilization (Rani et al. 2017). After sterilization the plant

samples were cut into small pieces and were then inoculated in Potato Dextrose Agar (PDA) medium for isolation of fungal endophytes.

Isolation and identification of fungal endophytes

Fungal colonies were obtained in PDA medium by direct tissue inoculation method. Pure colonies of the fungal endophytes were obtained by streaking method and then identified under the compound light microscope at 450× (10 x 45 = 450×) magnification. Identification was done as per the standard protocol for microscopic identification (Barnett & Hunter 1971; Nagamani et al. 2006).

Collection of soil samples and measurement of soil parameters

Soil samples were collected from *C. quadrangularis* sampling sites in sterile bags and were then tested for pH, organic carbon percentage and available NPK by Neoland technologies, Assam.

Diversity studies

Colonization frequency (CF) of the endophytic fungal isolates was calculated using the formula CF = Nf / Nt) x 100, where Nf = Number of tissue segments colonized by each isolate and Nt = Total number of tissue segments plated. Species richness refer to the number of species isolated from the plant samples. Frequency of dominant endophyte was calculated as per the protocol given by Suryanarayanan et al. (2002) and Rajamani et al. (2018). Shannon diversity index and Simpson's diversity index was calculated using Shannon diversity index calculator and Simpson's diversity index calculator, respectively (Rain 2022; Singh 2022).

Results and Discussion

In the present study variation was found in the endophytic fungal diversity and species richness in the stems and leaves of *Cissus quadrangularis* collected from the three study locations: Kamrup (M), Barpeta and Biswanath districts of Assam, India. The stems harbored more endophytic fungal isolates than the leaves (Tab. 2). However the percentage isolation of fungal endophytes was more from the leaves collected from Kamrup (M) and Barpeta than that of the stems collected from Biswanath (Fig. 1). The Shannon diversity was more in the stems of Barpeta district and was low in the Biswanath. The Simpson's diversity indices was highest in Barpeta and found to be same in Kamrup Metro and Biswanath. The species richness of endophytic fungal diversity was insignificantly less in the leaves than the stems in Kamrup Metro and Barpeta districts however there was no significant difference in the species richness in the samples collected from Biswanath district (Tab. 2). The distribution of endophytic microbial species differs among different tissues as well as the sampling sites (Chou et al. 2022; Sohrabi et al. 2022). The findings in the present work are in agreement with this statement. *Cladosporium* sp. and *Fusarium* sp., were the most dominant and frequently occurring endophytic fungal species recorded in *C. quadrangularis* of Kamrup (M) and Barpeta districts. *Colletotrichum*

sp. and *Rhizopus* sp. were the dominant endophytic fungal species in the samples from Biswanath district (Tab. 1). The distribution of endophytic fungal isolates varied in the stems and leaves of the plants collected from different study locations. *Trichophyton rubrum* and *Absidia fusca* were isolated from stems and leaves of *C. quadrangularis* collected from Biswanath but were absent in the plant parts taken from Kamrup (M) and Barpeta districts (Fig. 2).

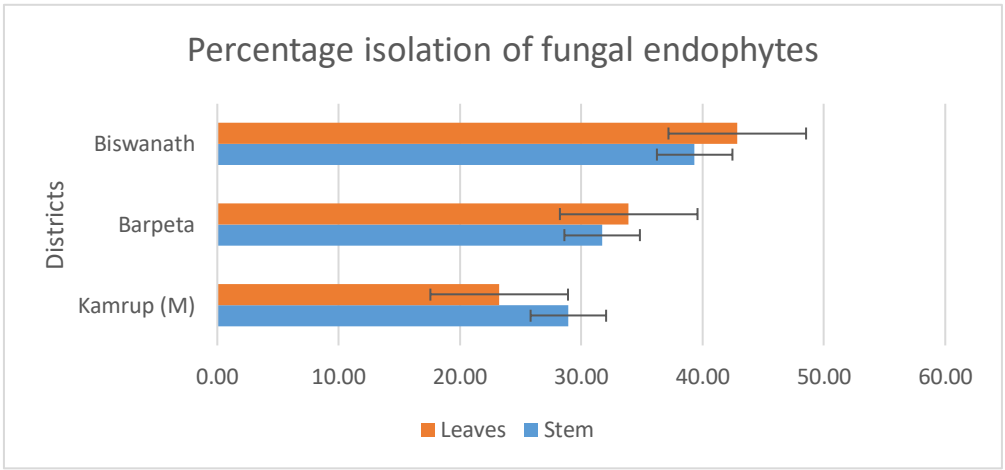

Fig. 1 Variation in the percentage isolation of endophytic fungi isolated from the stems and leaves of *C. quadrangularis* from different regions of Assam, India. Error bars represent standard error at 95% confidence level.

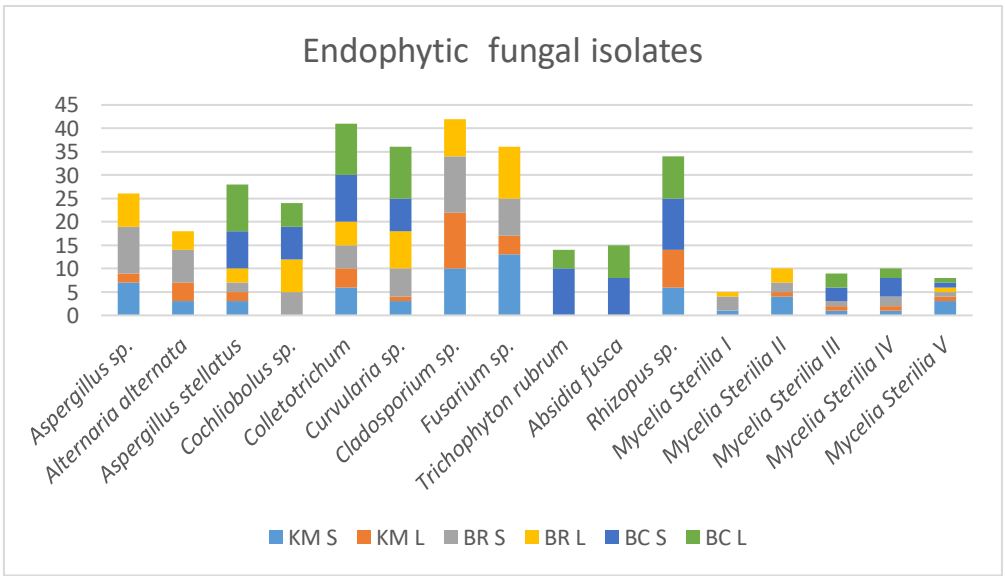

Fig. 2: Variation in the abundance of the fungal isolates in the stems and leaves collected from different study locations of Assam (*KM S and KM L refers to Kamrup Metro stem and leaves, BR S and BR L refers to Barpeta stem and leaves, BC S and BC L refers to Biswanath Chariali stem and leaves).

Tab. 1 Variation in the endophytic fungal isolates obtained from stems and leaves of *C. quadrangularis* collected from three districts of Assam. (*CF - colonization frequency; FDE- frequency of dominant endophyte).

Sl. No.	Endophytic Fungi	Kamrup(M)		Barpeta		Biswanath Chariali	
		CF%	FDE	CF%	FDE	CF%	FDE
1	*Aspergillus* sp.	1.38	8.82	2.62	13.93	Ab	Ab
2	*Alternaria alternata*	1.08	6.86	1.69	9.02	Ab	Ab
3	*Aspergillus stellatus*	0.77	4.9	0.77	4.1	2.77	13.63
4	*Cochliobolus* sp.	Ab	Ab	1.85	9.84	1.85	9.09
5	*Colletotrichum* sp.	1.54	9.81	1.54	8.2	3.23	15.91
6	*Curvularia* sp.	0.62	3.92	2.15	11.47	2.77	13.63
7	*Cladosporium* sp.	3.38	21.57	3.08	16.39	Ab	Ab
8	*Fusarium* sp.	2.62	16.67	2.92	15.57	Ab	Ab
9	*Trichophyton rubrum*	Ab	Ab	Ab	Ab	2.15	10.6
10	*Absidia fusca*	Ab	Ab	Ab	Ab	2.31	11.36
11	*Rhizopus* sp.	2.15	13.73	Ab	Ab	3.08	15.15
12	MS I	0.15	0.98	0.62	3.28	Ab	Ab
13	MS II	0.77	4.9	0.77	4.1	Ab	Ab
14	MS III	0.31	1.96	0.15	0.82	0.92	4.54
15	MS IV	0.31	1.96	0.31	1.64	0.92	4.54
16	MS V	0.62	3.92	0.31	1.64	0.31	1.51

Tab. 2 Variation in species richness and diversity of fungal endophytes isolated from different plant parts of *C. quadrangularis* collected from different locations of Assam.

Location/Part	Total isolates	Species richness	Diversity indices	
			Shannon	Simpson
Kamrup Metro				
Stem	60	13	2.3	0.89
Leaf	43	12	2.11	0.86
Barpeta				
Stem	63	13	2.32	0.9
Leaf	53	11	2.16	0.83
Biswanath				
Stem	67	10	2.17	0.89
Leaf	65	10	2.13	0.88

Tab. 3 Variation in the soil parameters of the soil collected from the three study locations of Assam and its relation with the Shanon-weiner diversity.

Soil parameters/ Districts	Kamrup (M)	Barpeta	Biswanath Chariali
H- index	2.205	2.24	2.15
pH	8	7.3	5.9
Organic carbon (%)	2.27	2.39	3.27
Available NPK (Kg/ha)	386.497	465.347	487.597

The relative abundance, diversity and richness of the endophytic community within the host plant is affected by both biotic and abiotic factors (Gaiero et al. 2013). The soil samples collected from the three study locations showed variation in the soil parameters. In the soil samples considered from Biswanath district, the pH was lower but the percentage of organic carbon and that of available NPK were higher than the other samples with higher pH (Tab. 3). Soil type can have an effect on the endophytic diversity is evident in the present findings. Shannon diversity index of fungal endophytes was highest in the soil sample with soil having pH value 8 which is basic whereas the Shannon diversity index was least in the soil sample collected from Biswanath with pH value 5.9 which is acidic. Alkaline pH of the soil may be attributed to the presence of certain chemicals like calcium or magnesium carbonate in the soil which increases its pH. More amount of available NPK can lower the pH value of the soil and thus can have an effect on the microbial community. This may in turn have an effect on the microbial diversity in the host plant. This has been observed in the present work where the soil with more organic carbon percentage and more available NPK had less pH and thus less H-index value (Shannon index value). Thus the type of agricultural practices, soil conditions and the type of plant organ may affect the endophytic diversity within the host plants (Gaiero et al. 2013; Maela & Serepa-Dlamini 2019).

Conclusion

Endophytes of medicinal plants occupy a unique habitat, are highly diverse and are important sources of secondary metabolites of pharmaceutical importance. There is no significant difference in the endophytic diversity in the three studied areas viz., Kamrup Metro, Barpeta and Assam as the Shannon diversity and Simpson's diversity indices shows less variation. However the distribution of the fungal endophytes varies with respect to the number of isolates obtained from the plant samples under study. This may be attributed to the difference in the sampling sites from where the plant samples of *C. quadrangularis* were collected. Soil pH of Assam is mostly acidic however alkaline pH of the soil may be attributed to the addition of fertilizers added to the soil as the plant samples were collected from the land used for gardening purpose. As deriving plant extracts of medicinal importance may threaten the existence and biodiversity of the medicinal plants in future hence it is important to isolate the endophytes from the medicinal plants and study them for their importance in human welfare. The plant *C. quadrangularis* has been documented to be effective in bone-healing practices. The relationship of endophytes with their host plant is very specific and is a complicated process. The endophytes isolated and recovered in the present work may not include all the endophytes of *C. quadrangularis*, hence detailed investigation into the endophytic fungi from the other plant parts i.e., roots and petiole is also needed which may lead to the discovery of novel endophytes as well.

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