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Studies on Colonization Frequency of Endophytic Fungi from *Coffea arabica* L. from Melghat Forest, Maharashtra, India

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Abstract

A medicinal plant *Coffea arabica* L. has been investigated to study the diversity of endophytic fungi and their colonization frequency. During this investigation endophytic fungi has been isolated in different seasons and their colonization frequency has been recorded. Entire period of study exhibited variations in colonization frequency of endophytic fungi.

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Keywords

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Introduction

The term "endophytes" was originally introduced by De Bary in 1866 to encompass all organisms inhabiting internal plant tissues. Endophytes, ubiquitous and diverse microorganisms, typically reside within plant tissues without inducing visible symptoms.

They represent one of the largest and underexplored groups of microorganisms, harboring abundant bioactive metabolites and chemically novel compounds. These attributes make endophytes a promising resource with significant potential for applications in medicine, agriculture, and industry (Tiwari, 2015).

Coffea arabica L. (Rubiaceae), commonly found in India, is esteemed for its significant medicinal value. The principal constituent of coffee, caffeine, is widely recognized for its stimulant properties. Additionally, compounds such as chlorogenic acid, caffeic acid, condensed proanthocyanidins, quinic acid, and ferulic acid have been identified in coffee, each associated with various beneficial health effects including antimicrobial, diuretic, and antioxidant activities (Mohammed and Bayati, 2009).

This study focuses on *Coffea arabica* L., investigating variations in the colonization frequency of endophytic fungi across different seasons.

Materials and Methods

Isolation of the Endophytic Fungi

The collected plant samples underwent initial washing under running tap water to remove surface adherents. Surface sterilization was conducted following the method outlined by Suryanarayanan *et al.,* (2001) to

eliminate epiphytes (Table no. 1). Subsequently, the surface-sterilized explants were inoculated at 26 ± 2°C onto Petri dishes containing water agar (WA). The plates were regularly monitored for fungal growth.

Fungi observed on the WA plates were then sub-cultured onto fresh Potato Dextrose Agar (PDA) plates to obtain pure isolates. These pure endophytic fungal cultures were transferred onto PDA slants and preserved as stock cultures for future investigations. All procedures were meticulously executed under aseptic conditions within a laminar airflow hood.

Microscopic Observation

Permanent slides were prepared from pure colonies of isolated endophytic fungi. Morphological characteristics such as pycnidia, conidia, and conidiogenous cells (for Coelomycetes); conidia and conidiophores (for Hyphomycetes); and ascocarp, ascospores, and asci (for Ascomycetes) were examined using a Carl Zeiss Trinocular Research Microscope (Axioscope-A-1) at magnifications of 5x, 10x, 40x, and 100x. Microphotography of the specimens was conducted using the same research microscope.

Mountants and Stain

In the present study, microscopic observation of isolated endophytic fungi initially utilized water mountant. However, for detailed examination of various fruiting structures, mounting in lactophenol-cotton blue was employed. This staining and mounting medium has been widely recognized and utilized for different taxonomic groups of fungi (Hawksworth, 1974; Purvis *et al.,* 1966).

Identification of Endophytic Fungi

All endophytic isolates were morphologically identified and classified into respective genera and species of fungi using established taxonomic keys and monographs. Identification followed references such as Ellis (1971, 1976); Sutton (1980); Subramanian (1971) and Barnett and Hunter (1972). Additional pertinent taxonomic literature on endophytes was consulted for comprehensive taxonomic analysis.

Data analysis

The Colonization Frequency (CF %) of fungal endophytes were calculated by using the following formula (Kumaresan and Suryanarayanan, 2001).

$$\text{Colonization Frequency (CF \%)} = \frac{\text{Total Number of segments colonized by Fungi}}{\text{Total Number of segments studied}} \text{X } 100$$

Observations and Results

Diversity of Endophytic Fungi from *Coffea arabica* L. During Season 2012-2013

Twelve fungi were systematically screened across all seasons in the study involving the specified host. Few fungi were recovered during summer, whereas the highest numbers were isolated during winter, followed by the monsoon season. Colonization frequency was notably higher during the monsoon and winter periods. Key fungi identified included *Alternaria alternata, Cladosporium cladosporioides, Curvularia lunata,* and *Penicillium chrysogenum*, all of which were consistently isolated throughout the seasons (Table 2). The petiole tissue of the host exhibited particular susceptibility to *Acremonium fusidioide* and *Curvularia lunata*. Notably, *Penicillium chrysogenum* demonstrated varying colonization patterns: it exhibited higher colonization in stem tissue during the monsoon season, while colonization rates were higher in leaf and petiole tissues during winter and summer, respectively (Fig. 1). Among all isolates, three species, *Nigrospora oryzae, Pestalotiopsis funerea,* and *Phoma crysanthemicola* were exclusively isolated during winter.

Diversity of Endophytic Fungi from *Coffea arabica* L. During Season 2013-2014

In the current year, the occurrence of fungal endophytes was more prevalent during the rainy season compared to other seasons. Thirteen endophytes were identified during this period, with ten fungi exhibiting the highest colonization frequencies during the rainy season, whereas only five fungi were recovered during summer with lower colonization frequencies. Some endophytes, such as *Alternaria alternata* and *Epicoccum nigrum*, displayed similar colonization behaviors as observed in previous investigations on the current host. *Nigrospora oryzae* showed high colonization frequencies in stem tissue during both the monsoon and winter seasons (Table 3). Additionally, certain endophytes like *Stachybotrys chartarum, Stachybotrys nilgirica,* and *Trimmatostroma hughesii* exhibited higher colonization rates during the monsoon but were absent during winter and summer (refer to Fig. 2).

Table.1 Surface sterilization of explants

Chemicals	Concentration	Time
Ethanol	70%	1 min
SDW	-	3 min × 4 times
NaOCl	4%	30 sec
SDW	-	3 min × 4 times
Ethanol	70%	30 sec
SDW	-	3 min × 4 times

SDW- Sterile Distilled Water; NaOCl - Sodium Hypochlorite

Table.2 Colonization frequency (%) of endophytic fungi isolated from *Coffea arabica* during 2012-2013.

Sr. No.	Endophytes	Monsoon			Winter			Summer		
		Stem	Leaf	Petiole	Stem	Leaf	Petiole	Stem	Leaf	Petiole
1.	*Acremonium fusidioide*	45.30	31.50	51.24	28.06	32.64	44.35	_	_	_
2.	*Alternaria alternata*	18.36	14.22	25.09	30.31	27.29	24.35	5.33	2.08	1.74
3.	*Aspergillus nidulans*	22.32	19.08	16.07	_	_	_	_	_	_
4.	*Cladosporium cladosporioides*	21.55	25.65	19.44	18.96	16.92	14.28	3.55	9.22	5.66
5.	*Colletotrichum gloeosporiodes*	21.94	26.09	17.54	_	_	_	_	_	_
6.	*Curvularia lunata*	22.99	21.87	32.26	38.65	21.33	41.22	3.99	5.21	6.25
7.	*Epicoccum nigrum*	41.25	25.38	49.23	13.20	18.20	15.25	_	_	_
8.	*Nigrospora oryzae*	_	_	_	31.94	36.03	21.85	_	_	_
9.	*Penicillium chrysogenum*	31.64	28.66	21.88	21.06	28.23	15.34	12.02	7.22	14.36
10.	*Pestalotiopsis funerea*	_	_	_	15.32	12.06	19.85	_	_	_
11.	*Phoma crysanthemicola*	_	_	_	24.05	16.35	27.01	_	_	_
12.	*Pithomyces chartarum*	34.75	36.12	28.41	22.66	24.11	18.64	_	_	_

–absent

Figure.1 Variation in colonization frequency of fungal endophytes isolated from *Coffea arabica* during 2012-13.

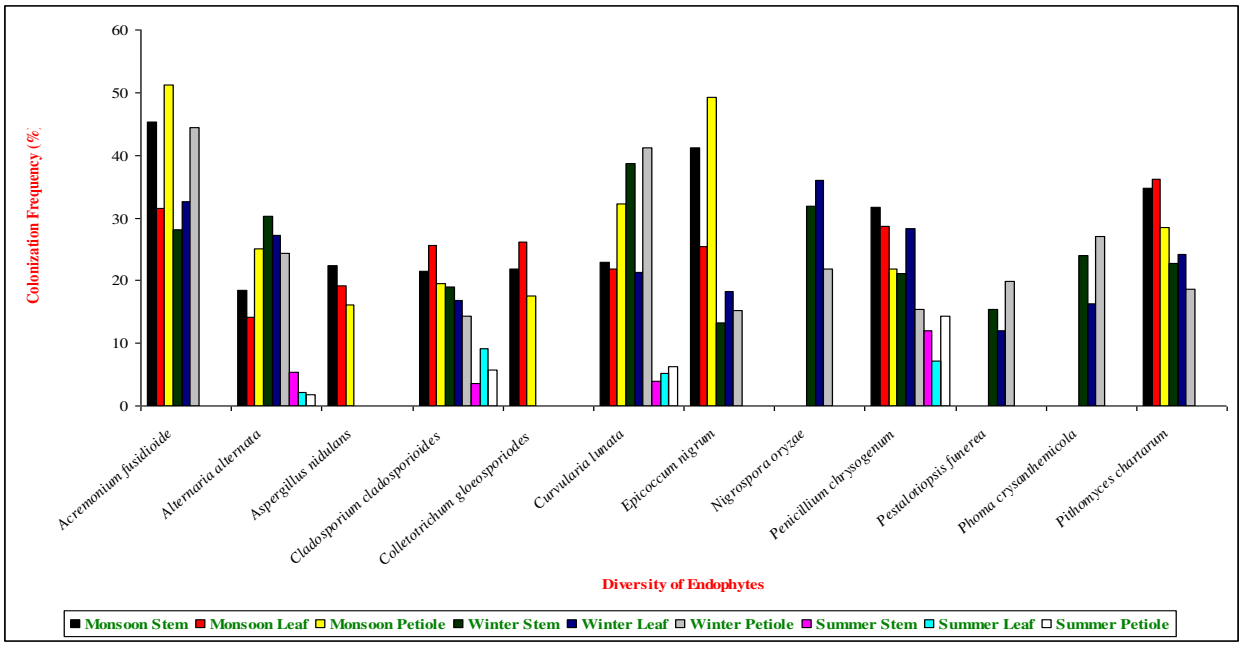
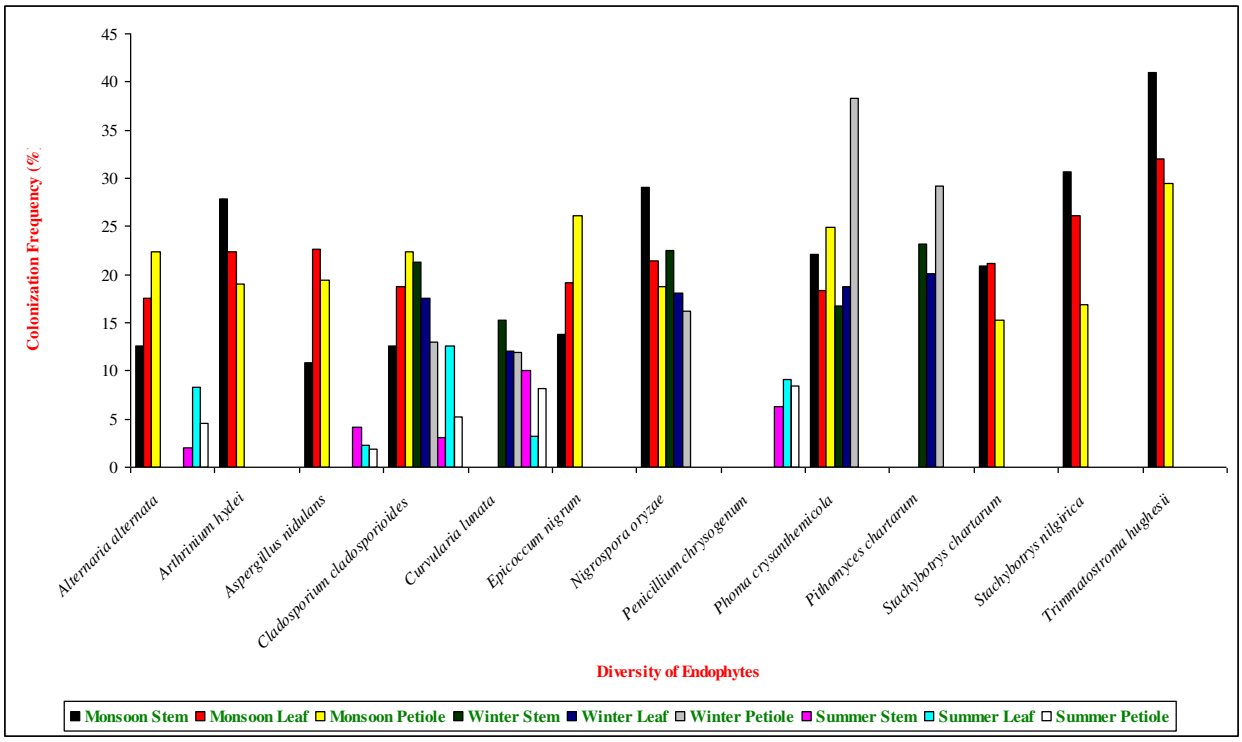


Table.3 Colonization frequency (%) of endophytic fungi isolated from *Coffea arabica* during 2013-2014.

Sr.No.	Endophytes	Monsoon			Winter			Summer		
		Stem	Leaf	Petiole	Stem	Leaf	Petiole	Stem	Leaf	Petiole
1.	*Alternaria alternata*	12.65	17.57	22.30	_	_	_	2.06	8.34	4.51
2.	*Arthrinium hydei*	27.85	22.41	19.04	_	_	_	_	_	_
3.	*Aspergillus nidulans*	10.88	22.64	19.42	_	_	_	4.09	2.30	1.88
4.	*Cladosporium cladosporioides*	12.54	18.73	22.31	21.33	17.54	12.99	3.06	12.55	5.26
5.	*Curvularia lunata*	_	_	_	15.32	12.08	11.87	10.02	3.24	8.21
6.	*Epicoccum nigrum*	13.84	19.20	26.10	_	_	_	_	_	_
7.	*Nigrospora oryzae*	29.00	21.44	18.77	22.51	18.06	16.24	_	_	_
8.	*Penicillium chrysogenum*	_	_	_	_	_	_	6.24	9.16	8.42
9.	*Phoma crysanthemicola*	22.05	18.33	24.88	16.77	18.73	38.33	_	_	_
10.	*Pithomyces chartarum*	_	_	_	23.21	20.15	29.16	_	_	_
11.	*Stachybotrys chartarum*	20.90	21.14	15.21	_	_	_	_	_	_
12.	*Stachybotrys nilgirica*	30.69	26.11	16.89	_	_	_	_	_	_
13.	*Trimmatostroma hughesii*	40.92	32.04	29.44	_	_	_	_	_	_

–absent

Figure.2 Variation in colonization frequency of fungal endophytes isolated from *Coffea arabica* during 2013-14.



Penicillium chrysogenum was detected in summer, whereas *Pithomyces chartarum* was exclusively present during the winter season.

Conclusion

This study investigated the potential influence of seasonal variations on the colonization frequency of endophytic fungi. Our findings revealed significant fluctuations in colonization rates throughout the year. These observations strongly suggest a potential link between environmental factors, such as temperature and humidity, and the establishment of endophytic fungal communities within plant hosts. Further research is warranted to elucidate the precise mechanisms by which

these environmental parameters influence fungal colonization dynamics. Understanding these relationships will not only enhance our knowledge of endophyte-plant interactions but also provide valuable insights into the potential impact of climate change on these ecologically important symbioses.

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