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Seasonal Variation and Distribution Patterns of Endophytic Community in *Withania somnifera*

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Abstract:- ***Withania somnifera*** **also known as** ***Ashwagandha*** **used as** ***rasayana*** **(tonic) in** ***Ayurvedic*** **system of medicine. It is widely considered as** ***Indian Ginseng*** **and possesses medicinal properties like adaptogenic, anti-stress, anxiolytic, anti-arthritic, anti-inflammatory etc. There is long history of microbes having stable symbiotic relationship with plants aiding them in growth and survival. This endophytic relationship is also been observed in medicinal plants. This study is an attempt to observe the biodiversity of endophytic fungal community in** ***Withania somnifera*** **and to understand their colonization. The study was aimed to isolate endophytes from different organs of** ***Withania somnifera*** **with seasonal variations. A total of 131 endophytic fungi were isolated from 450 explants from different organs like leaves, stems and roots of** ***W. somnifera.*** **The overall colonization rate of endophytes in winter, summer and monsoon were 42.67%, 7.33% and 37.33% respectively. Among the identified isolates, most abundant and frequently isolated genera were** ***Alternaria, Fusarium and Chaetomium sps.*** **The dominant species isolated in winter were the** ***Fusarium moniliformae*** **and** ***Chaetomium globosum*** **from leaves with 17.24% and 13.79% dominant frequency, respectively. While** ***Alternaria raphani*** **and** ***Chaetomium globosum*** **were most dominant species in summer from leaves with 40% dominant density. In the monsoon,** ***Microsporum ferruginem*** **was the most dominant species isolated from leaves and stem with 61.29% and 42.86%, respectively. The most recurring endophytic species of Ascomycetes group were** ***Alternaria alternata*** **and** ***Chaetomium globosum*** **seen in all seasons.**

Keywords:- *Endophytic fungi, Withania somnifera, Seasonal Diversity, Colonization frequency.*

I. INTRODUCTION

Microorganisms living inside the plant tissues without causing any noticeable symptoms of the disease to their host are called “Endophytes” (Tejesvi, M. et al., 2007). The Fungal microbes resides inside the plant tissues is called “Mycoendophytes”. Sometimes microbes are host specific which defines the specific relation to single host or group of related species in same habitat. Also, single fungal endophyte can form selective relationship depending upon its own preference (Petrini et al., 1993, Cohen, 2006). These endophytes are believed to be accompanied with production of bioactive secondary metabolites secreted by them to protect the host from adverse conditions and interact mutualistically to play role in augmenting host defense response against infectious agents (Azevedo et al., 2000). Endophytes may also act as an opportunistic pathogens and saparotrophs (Promputtha et al., 2010). Fungal endophytes are believed to be associated with the production of many bioactive natural compounds including flavonoids, alkaloids, terpenoids, peptides, steroids and phenols etc. which may have great medical, agricultural and pharmaceutical values (Zhang et al., 2006). *Withania somnifera* generally known as Ashwagandha belonging to family Solanaceae used in Ayurvedic polyherbal preparations. It has medicinal properties like anti-cancerous, anti-oxidant, anti-inflammatory, antistress and immunomodulatory (Alanazi & Elfaki, 2023). It contains various secondary metabolites but active principles of this plant are steroidal lactones viz. Withanolides and Withaferin A (Mir et al., 2012).

The degree of mutualism between plant and endophytes may be alter due to climate change that can lead to change in the efficacy of transmission of the endophytes from one plant to the another plant (Lindow & Brandl, 2003). Distribution of endophytes in plants is controlled by genes of both the plants and endophytes and altered by the environment as well. Different environmental parameters like rainfall, temperature, humidity, terrain, or season may play an important role in the distribution pattern of endophytes within a host (Moricca & Ragazzi, 2008). The aim of the present study was to investigate the diversity of fungal endophytes and seasonal

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colonization pattern of fungal endophytes from *Withania somnifera.*

II. MATERIAL AND METHODS

➢ *Collection of Plant Materials*

The plant samples of *Withania somnifera* were collected from cultivated species at Government science college Vankal. Different organs of *W. somnifera* were collected *viz*., leaf lamina, petiole, stem (nodes and internodes) and root in different seasons like monsoon (July-Oct), winter (Nov-Feb), and summer (Mar-June). Selected healthy organs were cut with sterile scalpel and stored in sterile polythene bag at 4^0 C till they were processed.

➢ *Isolation and Identification of Endophytic Fungi*

Healthy samples went for sterilization treatments. Different sterilizing agents were used for different selected organs; stem and root segments were treated with 70% Ethanol, 4% Sodium hypochlorite, 0.1% Mercury chloride and finally with sterile distilled water. For leaves 0.01% Mercury chloride was used. Evaluation of sterilization was done by impression method. The petri dishes were incubated at 27±2°C in dark for 15 days and monitored regularly to check the colonial growth. Pure fungal culture was transferred and cultured.

Identification was done on the basis of morphological characters like colony shape, colour and texture, type of spores and its structure. Lactophenol blue was used as stain for microscopic examination. Fungal cultures that failed to sporulate were recorded as sterile form. The identification was authenticated by National Fungal Culture Collection of India (NFCCI), Agharkar Institute, Pune, India.

➢ *Diversity Study*

Diversity study was done using dominant frequency and colonization frequency. Comparison of frequencies was assessed by Shannon-Wiener and Simpson's indices to understand the order or disorder of diversity and isolates belongs to same or different species respectively. Species richness and evenness relates the different species found and abundance of different species in isolates community respectively.

The percent colonization frequency of occurrence and Dominant fungi frequency was calculated using the method of (Wang and Guo, 2007, Kumar and Hyde, 2014)

$$\text{Colonization frequency (CF \%)} = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segments inoculated}} \times 100$$

$$\text{Dominant frequency (DF \%)} = \frac{\text{\% Colonization frequency}}{\text{Sum of \% colonization frequency of all endophytes}} \times 100$$

Comparison in percentage of colony frequency in the different tissues (leaf, stem, root) in different seasons, were determined by computing Shannon–Wiener indices and Simpson's Diversity indices (Cordero-Bueso et al., 2011).

Simpson's Index of diversity was calculated according to the following formula

$$Ds = \Sigma ni(ni-1) / N(N-1)$$ (Simpson, 1951)

Where, *n* is the total number of organisms of a particular species, *N* is the total number of organisms of all species

The Shannon- Wiener biodiversity index (H′) was calculated using the following formula:

$$H_S = \Sigma (Pi) (In\ Pi)$$ (Shannon, 1948)

Where, ***i* = 1**, Hs is the Symbol for the diversity in a sample of S species or kinds, S is the number of species in the sample, Pi is the relative abundance of i^{th} species or kinds measures, N is the total number of individuals of all kinds ni is the number of individuals of i^{th} species, In is the Log to base 2

Species evenness was calculated with Hill's formula as following:

Hill's evenness: $$EH = \frac{H}{In(N)}$$ (Hill, 1973)

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Where *Ni* is number of individuals of species *i* in the community, *N* is the total number of individuals in the community, *S* is the number of species encountered in the community and *Pi* is the proportional abundance of the i^{th} species.

III. RESULTS AND DISCUSSION

A total of 131 endophytic fungi were isolated from 450 samples of leaves, stems and roots of *W. somnifera.* Different nutritional media were used for endophytes isolation from which PDA (Potato dextrose Agar) gives best results with full strength as well as half strength in all plant parts. Figure 1 shows colonial growth of different individual isolates and figure 2 shows its microscopic characteristics.

- *Diversity Study*

Table-I suggests from 450 explants 64 endophytes were isolated in winter season with colonization frequency 42.67%; in summer season 11 endophytes with 7.33% colonization frequency and 56 isolates with 37.33% colonization frequency.

Organ colonizing frequency in leaves, stem and roots were 51.67%, 35% and 13.33% in monsoon season; 48.33%, 31.67% and 53.33% in winter season; while 8.34%, 10% and 0% in summer season respectively. Thus, more colonizing frequency was observed during monsoon and winter season.

Among the identified isolates as in table II, most abundant and frequently isolated genera's in all the three seasons were *Alternaria alternata*, *Chaetomium globosum* and *Aspergillus glaucus*. Most of the identified endophytes mainly belong to class Ascomycota, Basidiomycota and Deuteromycota. The dominant species isolated in winter were the *Fusarium moniliformae* and *Chaetomium globosum* from leaves with dominant frequency 17.24% and 13.79%, respectively. While *Alternaria raphani* and *Chaetomium globosum* were most dominant species in summer from leaves with same 40% dominant frequency. In monsoon, *Microsporum ferruginem* was the most dominant species isolated from leaves with dominant frequency 61.29%.

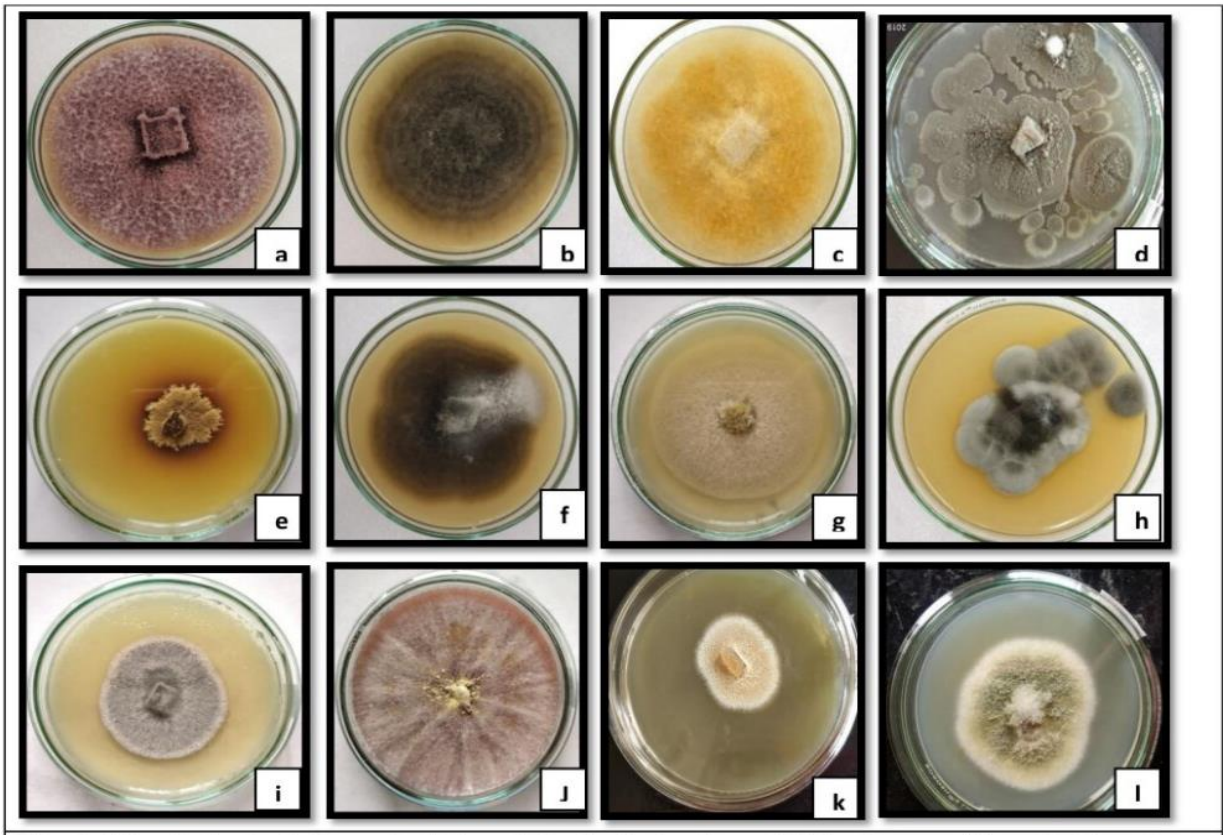

Fig. 1 Morphological characteristics of Endophytes from *Withania somnifera* (a) *Fusarium moniliformae* (b) *Alternaria alternata* (c) *Coprinellus radians* (d) *Penicillium citrinum* (e) *Aspergillus glaucus* (f) Alternaia raphani (g) *Fusarium solani* (h) *Cladosporium sp.* (i) *Alternaria raphani* (i) *Chaetomium globosum* (j) *Mycelia sterilia* (k) *Aspergillus sp.* (l) *Aspergillus nidulans*

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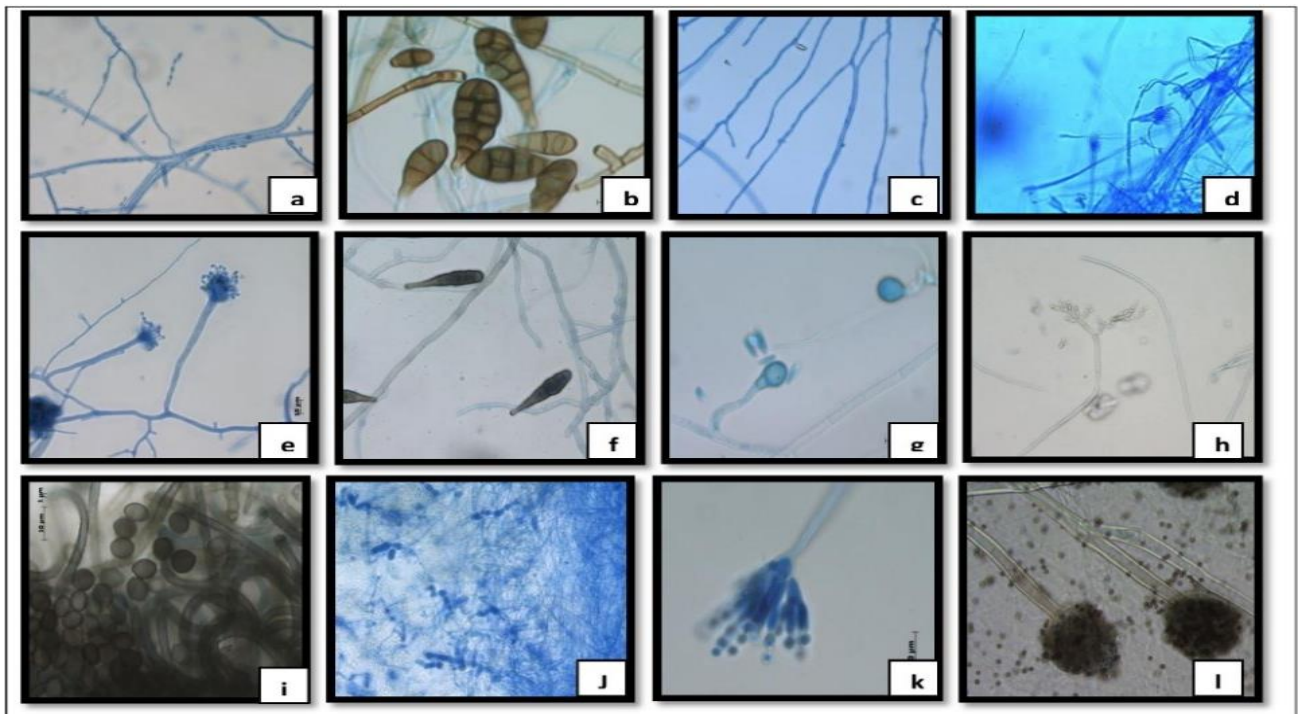

Fig. 2 Microscopic characteristics of Endophytes from *Withania somnifera* (a) *Fusarium moniliformae* (b) *Alternaria alternata* (c) *Coprinellus radians* (d) *Penicillium citrinum* (e) *Aspergillus glaucus* (f) Alternaia raphani (g) *Fusarium solani* (h) *Cladosporium sp.* (i) *Alternaria raphani* (i) *Chaetomium globosum* (j) *Mycelia sterilia* (k) *Aspergillus sp.* (l) *Aspergillus nidulans*

Table 1: Frequency of Endophytic Fungi Isolated from Different Parts of *Withania somnifera*

Season	Leaf		Stem		Root			
	NI	CF (%)	NI	CF (%)	NI	CF (%)	Total (NI)	Total (CF) %
Winter	29	48.33	19	31.67	16	53.33	64	42.67
Summer	5	8.34	6	10	0	0	11	7.33
Monsoon	31	51.67	21	35.00	4	13.33	56	37.33

NI- No. of Isolates; CF- Colonization frequency

Table 2: Identified Species with Number of Isolates, CF%, and DF% of Different Organs According to Seasonal Variation.

Winter									
Endophytic fungi	**Leaf**			**Stem**			**Root**		
	NI	**CF (%)**	**DF (%)**	**NI**	**CF (%)**	**DF (%)**	**NI**	**CF (%)**	**DF (%)**
Fusarium solani	4	6.67	13.79	0	0.00	0.00	2	6.67	12.50
Alternaria alternata	3	5.00	10.34	0	0.00	0.00	6	20.00	37.50
Alternaria raphani	1	1.67	3.45	1	1.67	5.26	6	20.00	37.50
Fusarium moniliformae	5	8.33	17.24	4	6.67	21.05	-	-	-
Microsporum ferrugineum	0	-	0.00	0	0.00	0.00	-	-	-
Coprinellus radians	2	3.33	6.90	2	3.33	10.53	-	-	-
Aspergillus glaucus	2	3.33	6.90	1	1.67	5.26	-	-	-
Penicillium griseofulvum	1	1.67	3.45	1	1.67	5.26	-	-	-
Penicillium citrinum	0	-	0.00	1	1.67	5.26	2	6.67	12.50
Aspergillus niger	0	-	0.00	3	5.00	15.79	-	-	-
Cladosporium	2	3.33	6.90	2	3.33	10.53	-	-	-

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cladosporium cladosporoides	1	1.67	3.45	0	0.00	0.00	-	-	-
Chaetomium globosum	4	6.67	13.79	2	3.33	10.53	-	-	-
Fusarium oxysporum	2	3.33	6.90	0	-	0.00	-	-	-
Aspergillus spp.	0	-	0.00	1	1.67	5.26	-	-	-
sterile Mycelia	2	3.33	6.90	0	0.00	0.00	-	-	-
E2 hylain form	0	-	-	1	1.67	5.26	-	-	-
Summer									
Endophytic fungi	Leaf			Stem			Root		
	NI	CF (%)	DF (%)	NI	CF (%)	DF (%)	NI	CF (%)	DF (%)
Fusarium solani	0	0	0	0	0	0	0	0	0
Alternaria alternata	1	1.66	20	1	1.66	16.66	-	-	-
Alternaria raphani	2	3.33	40		0	0	-	-	-
Fusarium moniliformae	-	-	-	-	-	-	-	-	-
Microsporum ferrugineum	-	-	-	-	-	-	-	-	-
Coprinellus radians	-	-	-	-	-	-	-	-	-
Aspergillus glaucus	-	-	-	1	1.66	16.66	-	-	-
Penicillium griseofulvum	-	-	-	-	0	-	-	-	-
Penicillium citrinum	-	-	-	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-	-	-	-
Cladosporium	-	-	-	2	3.33	33.33	-	-	-
Cladosporium cladosporoides	-	-	-				-	-	-
Chaetomium globosum	2	3.33	40	2	3.33	33.33	-	-	-
Fusarium oxysporum	-	-	-	-	-	-	-	-	-
Aspergillus spp.	-	-	-	-	-	-	-	-	-
sterile Mycelia (unidentified)	-	-	-	-	-	-	-	-	-
hylain form (unidentified)	-	-	-	-	-	-	-	-	-
Monsoon									
Endophytic fungi	Leaf			Stem			Root		
	NI	CF (%)	DF (%)	NI	CF (%)	DF (%)	NI	CF (%)	DF (%)
Fusarium solani	4	6.67	12.90	-	-	-	-	-	-
Alternaria alternata	0	-	0	1	1.67	4.76	3	10.00	75.00
Alternaria raphani	1	1.67	3.23	3	5	14.29	-	-	-
Fusarium moniliformae	2	3.33	6.45	0	0.00	0	-	-	-
Microsporum ferrugineum	19	31.67	61.29	9	15	42.86	-	-	-
Coprinellus radians	2	3.33	6.45	1	1.67	4.76	-	-	-
Aspergillus glaucus	-	-	-	0	0.00	0	1	3.33	25.00
Penicillium griseofulvum	-	-	-	1	1.67	4.76	-	-	-
Penicillium citrinum	-	-	-	-	0.00	0	-	-	-
Aspergillus niger	1	1.67	3.23	2	3.33	9.52	-	-	-
Cladosporium	2	3.33	6.45	-	-	-	-	-	-
Cladosporium cladosporoides	-	-	-	1	1.67	4.76	-	-	-
Chaetomium globosum	-	-	-	3	5	14.29	-	-	-
Fusarium oxysporum	-	-	-	-	-	-	-	-	-
Aspergillus spp.	-	-	-	-	-	-	-	-	-
sterile Mycelia (unidentified)	-	-	-	-	-	-	-	-	-
hylain form (unidentified)	-	-	-	-	-	-	-	-	-

NI- No. of Isolates; CF- Colonization frequency; DF- Dominant frequency

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Table 3: Ecological Index Analysis of Three Seasons in Different Plant Organs

	Winter			Summer			Monsoon		
Index analysis	**L**	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**
Abundance	29	19	16	5	6	1	31	21	4
Species richness(S)	12	11	4	3	4	1	7	8	2
Simpson diversity(D)	0.93	0.93	0.73	0.2	0.87	0	0.61	0.8	0.5
Shannon diversity(H')	1.98	1.95	1.25	1.05	1.36	0	1.31	1.72	0.56
Species evenness(E)	0.80	0.81	0.90	0.96	0.99	0	0.67	0.83	0.81

(L: Leaf; S: Stem; R: Root)

Seasonal variation observed for winter, summer, and monsoon samples were 42.67%, 7.33%, and 13.33% respectively (Table I). From 450 segments of plant materials a total of 131 isolates were obtained. Mycelia sterilia the fungal group which fails to sporulate, were also reported in this study. The interaction between type of plant tissue and season had a significant effect on the frequency of endophytic fungi. Moreover, in simple correspondence analysis of most frequently isolated endophyte species, *Alternaria alternata* was exclusively categorized with all plant parts i.e. leaf, stem and root; *Chaetomium globosum* and *Cladosporium* with stem and leaf. The total colonization frequency 36.11% of isolates from leaves, 25.56% from stem and 22.22% from root. Morphological characteristics were examined for all of the fungal isolates which were assigned to 17 fungal species (Table II).

Fungal species diversity analyzed by Simpson index (D) and Shannon–Wiener Index (H′) shows considerable difference between fungal communities associated with the type of tissue to be colonized in different seasons. Dominant frequency showed by *Alternaria alternata* (11.45%), *Alternaria raphani* (10.69%), and *Chaetomium globosum* (9.92%) in all seasons. Colonizing frequency showed by *Alternaria* alternata was 3.33%, *Alternaria raphani* was 3.11%, and *Chaetomium globosum* was 2.88%. Thus *Alternaria* spp. represents the dominant fungal species (Table II).

Differences in seasonal variation of species richness and evenness in fungal endophytes was analyzed as listed in Table 3, where Shannon- Weiner diversity index for leaves, stem and root were H'=1.98, H'=1.95 and H'=1.25 for winter season; H'=1.05, H'=1.36 and H'=0 for summer season and H'=1.31, H'=1.72 and H'=0.56 for monsoon season, respectively. Simpson index showed D=0.93 for both stem and leaves; while D=0.73 for roots during winter season; for leaf, stem and root D=0.2, D=0.87 and D=0 respectively for summer season, while D=0.61, D=0.8 and D=0.5 for monsoon season, respectively. *Withania somnifera* cultivated in Vankal was ecosystem for our endophyte isolates. Thus, richness and evenness of the species analyzed showed evenly distribution of the species in all the organs of the plant with less diversity in the isolated species. According to table 3, shows evenness of the species in all the three organs viz., leaves, stem and root were 0.80, 0.81, and 0.90 for winter season; 0.96, 0.9, and 0 for summer season; whereas 0.67,0.83 and 0.81 for monsoon season respectively.

IV. CONCLUSION

Withania somnifera an important plant medicinally, in Indian Ayurvedic system, harbors diverse class of microbes as endophytes when studied with seasonal variation. Major class involves are Deuteromycetes, Ascomycetes and Basidiomycetes. The maximum numbers of endophytes have been found in monsoon season from July to October. The present study could be further employed for investigation of secondary metabolites produced by them. Therefore traditional methods from medicinal plants for production of metabolites can be replaced by endophytes in future.

- *Data Availability*

All the supplementary information and relevant data are included in the paper.

- *Conflicts of Interest*

The authors agree with the contents of the manuscript and declare that they have no conflicts of interest regarding the publication of this paper.

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REFERENCES

[1]. Alanazi, H. H., & Elfaki, E. (2023). The immunomodulatory role of withania somnifera (L .) dunal in in fl ammatory diseases. February: 1–10. https://doi.org/10.3389/fphar.2023.1084757

[2]. Azevedo, J. L., Maccheroni, W., Pereira, J. O., & De Araújo, W. L. (2000). Endophytic microorganisms: A review on insect control and recent advances on tropical plants. Electronic Journal of Biotechnology, 3(1):40–65. https://doi.org/10.2225/vol3-issue1-fulltext-4

[3]. Cohen, S. D. (2006). Host selectivity and genetic variation of Discula umbrinella isolates from two oak species: analyses of intergenic spacer region sequences of ribosomal DNA. Microbial Ecology, 52(3):463–469. https://doi.org/10.1007/s00248-006-9073-5

[4]. Cordero-Bueso, G., Arroyo, T., Serrano, A., Tello, J., Aporta, I., Vélez, M. D., & Valero, E. (2011). Influence of the farming system and vine variety on yeast communities associated with grape berries. International Journal of Food Microbiology, 145(1):132–139. https://doi.org/10.1016/j.ijfoodmicro.2010.11.040

[5]. Hill, M. O. (1973). Diversity and Evenness: A Unifying Notation and Its Consequences. Ecology, 54(2): 427–432. https://doi.org/10.2307/1934352

[6]. Kumar, D. S. S., & Hyde, K. D. (2014). Biodiversity and tissue-recurrence of endophytic fungi in Tripterygium wilfordii Biodiversity and tissue-recurrence of endophytic fungi in Tripterygium wilfordii. May.

[7]. Lindow, S. E., & Brandl, M. T. (2003). Microbiology of the phyllosphere. Applied and Environmental Microbiology, 69(4): 1875–1883. https://doi.org/10.1128/AEM.69.4.1875-1883.2003

[8]. Mir, B. A., Khazir, J., Mir, N. A., Hasan, T.-U., & Koul, S. (2012). Botanical, chemical and pharmacological review of Withania somnifera (Indian ginseng): an ayurvedic medicinal plant. Indian Journal of Drugs and Diseases, 1. www.iseeadyar.org/ijdad.html

[9]. Moricca, S., & Ragazzi, A. (2008). Fungal endophytes in mediterranean oak forests: A lesson from Discula quercina. Phytopathology, 98(4):380–386. https://doi.org/10.1094/PHYTO-98-4-0380

[10]. Petrini, O., Sieber, T. N., Toti, L., & Viret, O. (1993). Ecology, metabolite production, and substrate utilization in endophytic fungi. Natural Toxins, 1(3): 185–196. https://doi.org/10.1002/nt.2620010306

[11]. Promputtha, I., Hyde, K. D., McKenzie, E. H. C., Peberdy, J. F., & Lumyong, S. (2010). Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? Fungal Diversity, 41(March):89–99. https://doi.org/10.1007/s13225-010-0024-6

[12]. Shannon, C. E. (1948). A Mathematical Theory of Communication. Bell System Technical Journal, 27(4): 623–656. https://doi.org/10.1002/j.1538-7305.1948.tb00917.x

[13]. Simpson, E. H. (1951). The Interpretation of Interaction in Contingency Tables. Journal of the Royal Statistical Society: Series B (Methodological), 13(2): 238–241. https://doi.org/10.1111/j.2517-6161.1951.tb00088.x

[14]. Tejesvi, M. VTejesvi, M. V., Nalini, M. S., Mahesh, B., Prakash, H. S., Kini, K. R., Shetty, H. S., & Subbiah, V. (2007). New hopes from endophytic fungal secondary metabolites. Bol Soc Quím Méx, 1(1): 19–26.

[15]. Wang, Y., & Guo, L. D. (2007). A comparative study of endophytic fungi in needles, bark, and xylem of Pinus tabulaeformis. Canadian Journal of Botany, 85(10):911–917. https://doi.org/10.1139/B07-084

[16]. Zhang, H. W., Song, Y. C., & Tan, R. X. (2006). Biology and chemistry of endophytes. Natural Product Reports, 23(5): 753–771. https://doi.org/10.1039/b609472b