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Studies on Tissue Specificity of Endophytic Fungi from *Withania somnifera* Linn.

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Abstract

During the current study on *Withania somnifera* Linn., the tissue specificity of endophytic fungi was investigated. A total of thirteen endophytes were isolated from various plant parts, including leaves, stems, and petioles. Species such as *Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Penicillium chrysogenum, Stachybotrys chartarum*, and *Stachybotrys nilgirica* were found exclusively colonizing the leaf and petiole tissues of this host throughout the study period."

Keyword- Endophytic fungi, Tissue specificity, *Withania somnifera* Linn.

Introduction

Association of plants and microorganisms is very complicated to study and manipulate as compare to the association of plants and animals. Most of the plants are always infected like leguminous plants by rhizobium bacteria, trees by mycorrhiza. So microorganism can be consider easily just as a plant organ (Clay, 1990). The study and manipulation of plant-microorganism associations are considerably more complex compared to plant-animal associations. Many plants, such as leguminous plants with rhizobium bacteria and trees with mycorrhiza, are commonly infected. Microorganisms can thus be considered akin to plant organs (Clay, 1990).

In recent years, endophytic fungi have gained widespread acceptance as ubiquitous microbes that reside within plant tissues without causing noticeable symptoms. These fungi exhibit great diversity and may vary depending on the host species or tissue type (Fang et al., 2019). In the relationship between endophytic fungi and plants, the host plant provides a habitat for the endophyte, which in turn aids plant growth by enhancing nutrient uptake and utilization

(Yadav et al., 2017). Therefore, understanding the association between endophytic fungi and plants is crucial for assessing the potential benefits of these fungi (Sandberg et al., 2014).

Previous studies have examined tissue specificity of endophytes in grasses (Clay, 1990), orchids (Bayman et al., 1997), and various tree species (Arnold, 2001). In the current investigation, we explored the tissue specificity of endophytic fungi in *Withania somnifera* Linn."

Methods

Isolation of the Endophytic Fungi:

The collected plant samples were washed under running tap water to remove surface adherents. Surface sterilization were done according to the method described by (Suryanarayanan *et al.,* 2001) to remove the epiphytes (Table No.1). The surface sterilized explants then inoculated at 26 ± 2°C into the growth medium (PDA). The pure endophytic fungal cultures were transferred on PDA slant and stored as stock culture for further studies.

Table No. 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

Identification of Endophytic Fungi

All the endophytic isolates were identified morphologically and placed in appropriate genera and species of fungi using standard taxonomic keys and monographs. (Ellis, 1971, 1976 Sutton, 1980, Subramanian, 1971 and Barnett and Hunter, 1972) were referred for identification of endophytes. In addition, other taxonomic relating papers of endophytes were also referred.

Data analysis

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

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

Observations and Results-

Among the thirteen endophytes recovered across all seasons (Table 2, Fig. 1), *Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Penicillium chrysogenum, Stachybotrys nilgirica*, and *Stachybotrys chartarum* exhibited tissue specificity. These fungi exclusively colonized leaf and petiole tissues of the host plant, while the stem tissues remained uncolonized. They also stood out from other isolates due to their higher colonization frequencies.

Colletotrichum gloeosporiodes showed seasonal specificity, being present only during seasons with higher atmospheric moisture levels. The colonization potential of *Epicoccum nigrum* and *Trimmatostroma hughesii* appeared to be affected by high temperatures, as they were absent during the summer.

Table. 2. Colonization frequency (%) showing tissue specificity of endophytic fungi isolated from *Withania somnifera*.

Sr.No.	Endophytes	Monsoon			Winter			Summer		
		Stem	Leaf	Petiole	Stem	Leaf	Petiole	Stem	Leaf	Petiole
1.	*Alternaria alternata*	_	22.99	24.11	_	21.98	29.51	_	3.58	2.74
2.	*Cladosporium cladosporiode*	15.54	21.56	17.28	16.23	19.54	22.99	12.35	16.89	8.04
3.	*Colletotrichum gloeosporiodes*	24.05	27.99	18.02	_	_	_	_	_	_
4.	*Curvularia lunata*	_	28.06	18.08	_	30.25	27.95	_	7.46	4.38
5.	*Epicoccum nigrum*	22.98	15.56	23.54	51.64	44.89	36.57	_	_	_
6.	*Fusarium oxysporum*	_	26.51	29.55	_	18.24	20.06	_	8.64	3.54
7.	*Nigrospora oryzae*	_	_	_	51.75	26.33	32.49	_	_	_

8.	*Penicillium chrysogenum*	_	29.54	21.64	_	16.74	20.54	_	9.22	6.35
9.	*Pestalotiopsis funereal*	_	_	_	_	31.44	41.23	_	_	_
10.	*Pithomyces chartarum*	_	_	_	16.11	19.46	26.07	_	_	_
11.	*Stachybotrys chartarum*	_	26.08	18.04	_	20.26	18.66	_	8.30	9.21
12.	*Stachybotrys nilgirica*	_	34.05	27.32	_	24.51	28.88	_	6.22	10.24
13.	*Trimmatostroma hughesii*	12.65	17.01	19.35	29.47	27.29	46.30	_	_	_

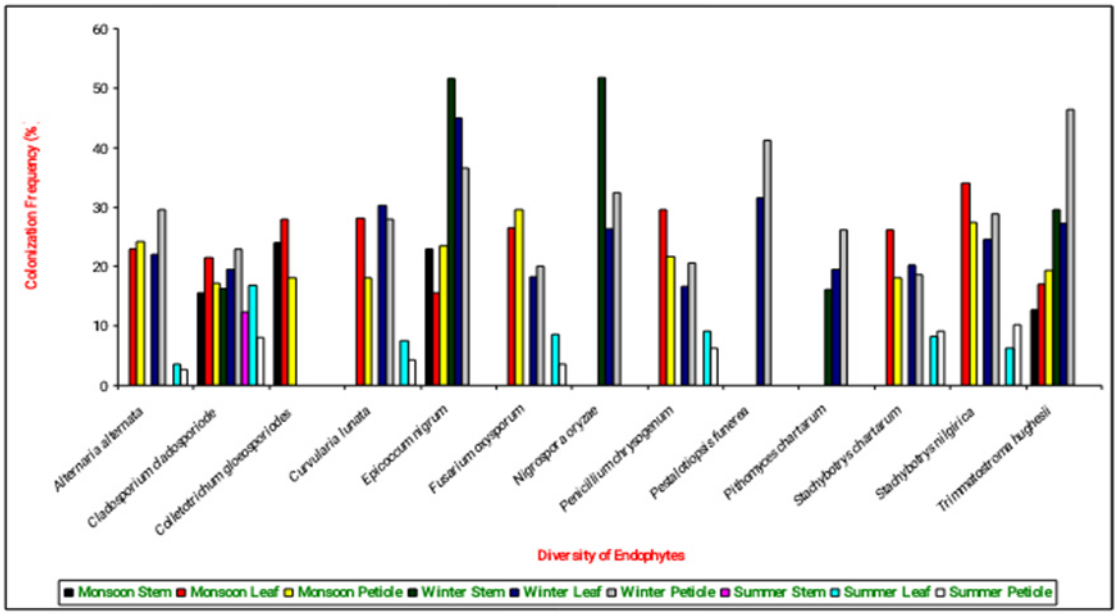

Fig.1: Variation in colonization frequency and tissue specificity of fungal endophytes isolated from *Withania somnifera*

Conclusion

In the course of this investigation, the endophytic fungi isolated from *Withania somnifera* displayed distinct patterns of tissue specificity in their colonization behavior. Interestingly, a significant observation was the general avoidance of stem tissue colonization by most of the endophytes identified. This specificity suggests that certain environmental or host-related factors may influence the preference of these fungi for particular plant tissues.

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