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A Comparative Study of Fungal Endophytes - Endomycorrhizal Organs in Ocimum Sanctum and Santalum Album

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Abstract

The study of Endophytic fungi in the medicinal plants showed quite a variation. The plant Ocimum sanctum showed six types of fungi Trichoderma viridae, Fusarium solani, Gliocladium roseum, Cladosporium sp., Cladorrhinium sp. and Nigrospora sp. that were isolated from rhizosphere and found to be 42.8 % of the population of total fungi isolated, while only one type of endophyte, Mycelia sterilia was 14.2% of the total population of the isolates. The four fungi Fusarium solani, Gliocladium roseum, Mycelia sterilia and Nigrospora sp. were isolated as endophytes. with 42.8% of the total population of the isolates. In the plant Santalum album five fungi Trichoderma viridae, Stachylidium extorre, Pseudobotrytis sp., Fusarium oxysporum, Mycelia sterilia, were isolated from rhizosphere and found to be 55.55% of the population of total fungi isolated. While four fungi Chalaropsis sp., Phoma glomerata, Collectotrichum sp. and Fusarium solani were isolated as endophytes which accounted for 33.33%. The fungi common both as rhizospheric & endophytic include one isolate Collectotrichum sp. with 11.11% of total population of isolates. The presence of vesicles was observed within the cortical cells of both the plants Ocimum sanctum & Santalum album.

Keywords: *Ocimum sanctum, Santalum album. endophyte, endomycorrhizal organs, vesicles*

1. Introduction

With an estimated few million species, fungi endophytes are found throughout the plant world. Numerous organisms known as endophytes may be repositioned by plants (Bacon and White, 2000; Strobel, 2002). By definition, an endophyte is a type of organism that lives in tissues beneath the layers of epidermal cells and does not appear to harm the host (Petrini, 1991). Since 2000, there has been a lot of research done on tropical endophytes to learn more about these varied endophyte groupings and their increased biodiversity. One million species of endophytic fungi may be found in a variety of therapeutic plants, according to Dreg Fuss and Chapela (1994). For all or almost all of their life cycle, they create undetectable infections inside the tissues of healthy plants (Limsuwan et al., 2009). Even under stressful circumstances, host-plants have been found to benefit from fungal associations that promote plant growth. Higher levels of macro and micronutrients, including calcium, magnesium, potassium, phosphorus, and sulphur, are obtained by these endophytes. [1–5] Site of Sampling: Dhanvantri Vana, which is situated in Jnana Bharathi, Department of Forestry, Government of Karnataka, Bengaluru, Karnataka, India, was selected as the study location for the collection of endophytic fungal samples. (See Table 1)

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Table 1 Dhanvantri Vana

Locality	Latitude	Longitude	Habitat/ Forest type
Dhanvantri Vana.	77.498159° W	12.942061° S	Cultivated

2. Materials and Methods

2.1. Isolation of Rhizosphere Fungi

The serial dilution technique involved homogenising the soil samples after they had been suspended in 90 millilitres of saline to ensure consistent mixing. To achieve various dilutions of the sample, ranging from lower to higher dilutions, such as 10-1 to 10-7, respectively, the sample was diluted in a series of 9ml saline tubes. The dilution tube approach makes it easier to isolate colonies for characterisation by lowering the quantity of organisms. Using the pour plate technique, 1 millilitre of the chosen dilution tube was put onto sterile Petri dishes. After adding 20 millilitres of potato dextrose agar medium, the media plate was rotated both clockwise and anticlockwise to mix it. This facilitates the even growth of the fungi on the plate. These inoculation plates were coated with lactophenol cotton blue to allow observation of the essential characteristics of the fungi and then incubated for one week at 28°C to monitor fungal growth. [11–14]

2.2. Isolation of Endophytic Fungi

The extracted root samples from Santalum album and Ocimum sanctum were cleaned, chopped into tiny pieces, and then surface sterilised. After being sanitised for one minute in 75% alcohol, the processed root parts were submerged in 5% sodium hypochlorite for eight minutes. To get rid of any remaining sterilant residue on the surface of the sterilised root bits, they were again subjected to 75% alcohol for 30 seconds. After that, they were suspended in sterile distilled water. Lastly, using a modified technique, the root pieces were blot dried on sterile blotting paper. (Wang and others, 2008; Samaga and others, 2014; Guo and others, 2008).The processed root bits were placed on sterilized Potato Dextrose Agar (PDA) medium containing streptomycin and incubated at 28°C for 21 days and observed for growth of fungus.

2.3. Identification of Fungal Isolates

The isolated fungal colonies were studied for their colony characteristics like growth characteristics, pigmentation and the morphological characters using lactophenol cotton blue.

2.4. Staining Endophytic Fungal Structure in Roots to Study Endomycorrhizal Organs

The root bits were collected and stored in 10% KOH for 2 days at room temperature. The root bits were rinsed with tap water and incubated in 5% Ink-vinegar solution (Black ink, Sheaffer) for 30minutes at 80°C. At a high temperature the root bits become soft and the endomycorrizal structures like vesicles can be stained and observed. The root bits were subsequently de-stained by draining out excess of stain. The stained root bits were acidified with weak acids like vinegar for at least 12 hours, sectioned and mounted for observation. The study of Vesicles and Arbuscles in the cortical cells of the medicinal plant roots were observed at 45x magnification. It was observed for the presence of Hartig's net within the cortical cells. This would help to understand whether medicinal plant roots have special structures even in endophytic associations.

3. Results and Discussions

Studies in the plant Ocimum sanctum and Santalum album. Figur2 (2-9) and Figure (11-17).

3.1. Studies in The Isolation of Rizosphere Fungi from Ocimum Sanctum

A total six fungi Trichoderma viridae, Fusarium solani, Gliocladium roseum, Cladosporium sp., Cladorrhinium sp. and Nigrospora sp. were isolated from rhizosphere while four fungi Fusarium solani, Gliocladium roseum, Mycelia sterilia and Nigrospora sp. were isolated as endophytes from Ocimum sanctum. Three types of rhizosphere fungi Trichoderma viridae, Cladosporium sp, Cladorrhinium sp. were found to be 42.8 % of the population of total fungi isolated, while only one type of endophyte, Mycelia sterilia was 14.2% of the total population of the isolates. Three fungal isolates Gliocladium roseum, Fusarium solani and Nigrospora sp. were common both as rhizospheric

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and endophytic fungi with 42.8% of the total population of the isolates. The results are presented in Table 2 and Figure 1

Table 2 Rhizospheric and Endophytic Fungi Isolated from *Ocimum Sanctum*

Sl No	Fungal isolates	Rhizosphere fungi	Endophytic fungi
1	*Trichoderma viridae*	+	-
2	*Fusarium solani*	+	+
3	*Gliocladium roseum*	+	+
4	*Cladosporium* sp.	+	-
5	*Mycelia sterilia*	-	+
6	*Cladorrhinium* sp.	+	-
7	*Nigrospora* sp.	+	+

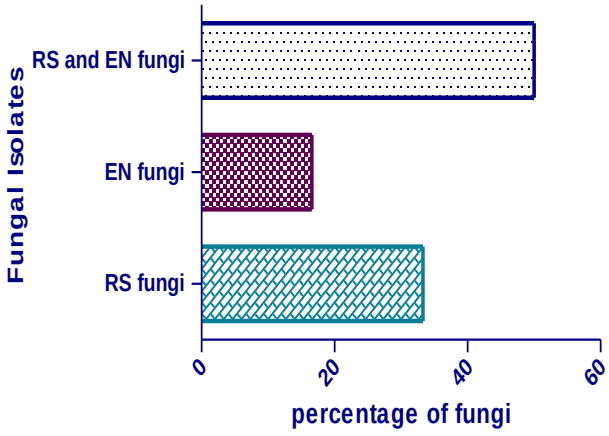

Figure 1 Percentage of Rhizospheric (RS), Endophytic (EN) Fungi and Total Fungal Isolates from *Ocimum Sanctum*

3.1.1. Fungal Isolates of Rhizospheric (RS), Endophytic (EN) Fungi from Ocimum Sanctum

Figure 2 *Ocimum Sanctum*

Figure 3 *Trichoderma Viridae*

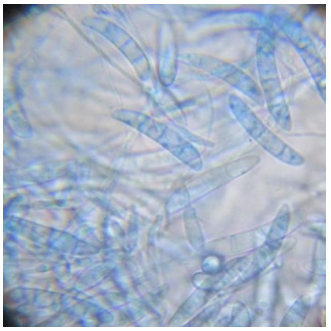

Figure 4 *Fusarium Solani*

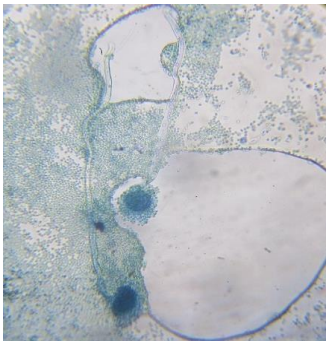

Figure 5 *Gliocladium Roseum*

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Figure 6 ***Cladorrhinium*** **Sp**

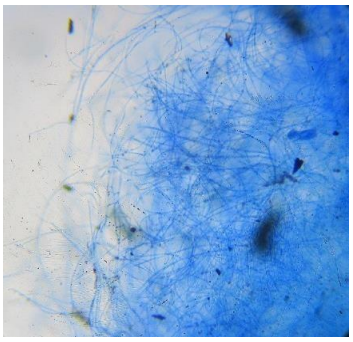

Figure 7 ***Mycelia Sterilia***

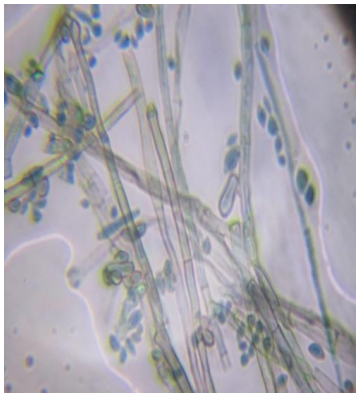

Figure 8 ***Cladosporium*** **Sp**

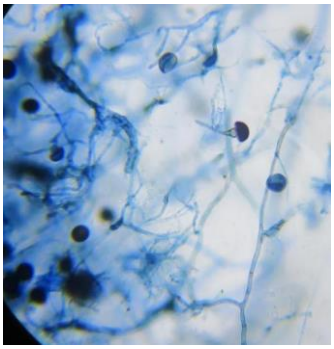

Figure 9 ***Nigrospora*** **Sp.**

3.2. Studies in The Plant Santalum Album

Table 3 Rhizospheric and Endophytic Fungi Isolated from Santalum Album

Sl No	Fungal isolates	Rhizosphere fungi	Endophytic fungi
1	*Trichoderma viridae*	+	-
2	*Stachylidium extorre*	+	-
3	*Chalaropsis* sp.	-	+
4	*Pseudobotrytis* sp.	+	-
5	*Phoma glomerata*	-	+
6	*Colletotrichum* sp.	+	+
7	*Fusarium oxysporum*	+	-
8	*Fusarium solani*	-	+
9	*Mycelia sterilia*	+	-

In total five fungi Trichoderma viridae, Stachylidium extorre, Pseudobotrytis sp., Fusarium oxysporum, Mycelia sterilia, were isolated from rhizosphere while four fungi Chalaropsis sp., Phoma glomerata, Collectotrichum sp. and Fusarium solani were isolated as endophytes from the plant Santalum album. Five types of rhizosphere fungi Trichoderma viridae, Stachylidium extorre, Pseudobotrytis sp. Fusarium oxysporum, and Mycelia sterilia were isolated which were found to be 55.55% of the population of total fungi isolated while three isolates of endophytes Chalaropsis sp. Phoma glomerata, and Fusarium solani were isolated which were 33.33%. The fungi common both as rhizospheric and endophytic include one isolate Collectotrichum sp. which was 11.11% of the total population of the isolates. The results are presented in Table 2 and Figure 10.

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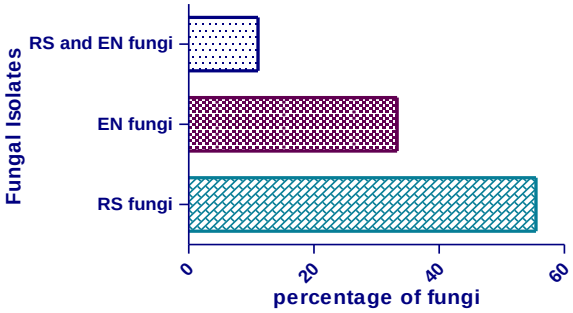

Figure 10 Percentage of Rhizospheric (RS), Endophytic (EN) Fungi and Total Fungal Isolates from Santalum Album

3.2.1. Fungal Isolates of Rhizospheric (RS), Endophytic (EN) Fungi from Santalum Album

Figure 11 *Santalum Album*

Figure 12 *Stachyladium* sp.

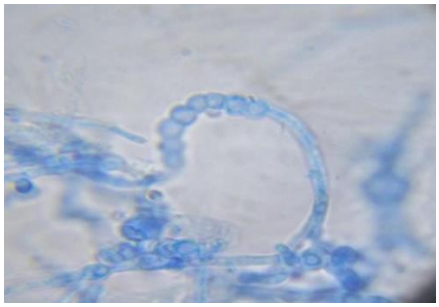

Figure 13 *Chalaropsis* sp.

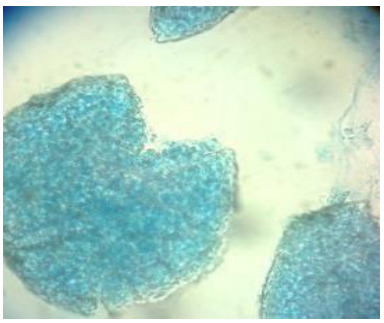

Figure 14 *Phoma* sp.

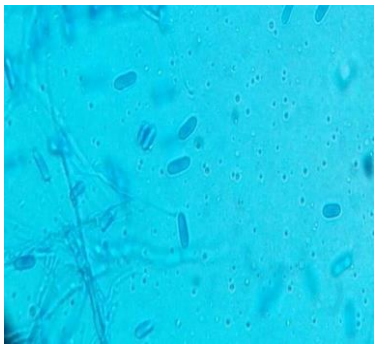

Figure 15 *Colletotrichum* sp

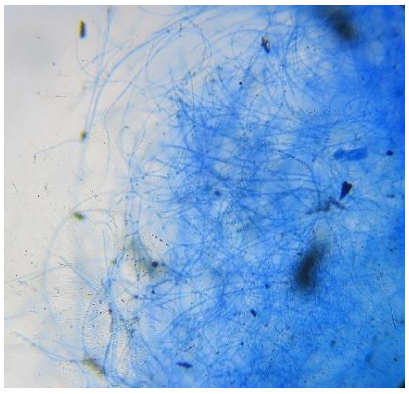

Figure 16 *Mycelia sterilia*

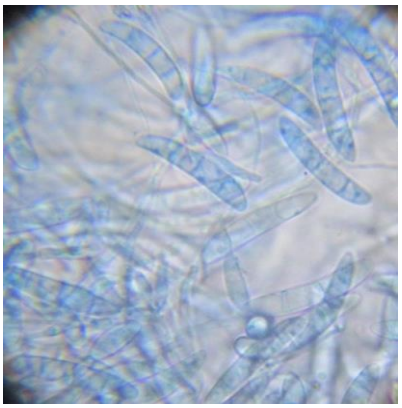

Figure 17 *Fusarium solani*

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3.3. Endophytic Fungal Structure in Roots – The Endomycorrhizal Organs

Table 3 Plant Roots Showing Endo Mycorrhizal Organs

Sl No	Name of the Plant	Mycorrhizal Organs		
		Hartig's Net	Vesicles	Arbuscles
1	***Ocimum sanctum***	-	+	-
2	***Santalum album***	-	+	+

(+ denotes presence) (- denotes absence)

3.3.1. Microscopic View of the Fungal Endomycorrhizal Orgns in Roots of Ocimum Sanctum and Santalum Album

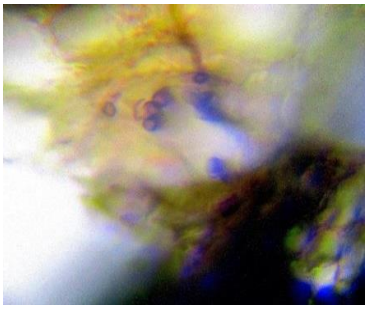

Figure 18 ***Ocimum Sanctum***

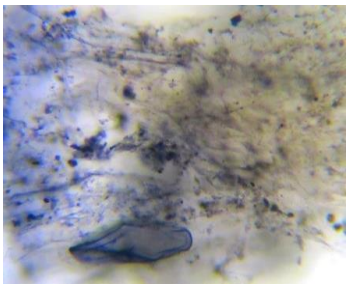

Figure 19 ***Santalum Album***

3.4. Discussion

A comparative study of Rhizosphere and Endophytic fungal study was done for both the medicinal plant species- *Ocimum sanctum* and *Santalum album* showed variation between the two plant species. The fungal species were diverse in habitat in both the plant species of *Ocimum sanctum* and *Santalum album* with few acting as endophytes too. In the studies of endomycorrhizal organs, both the plant species showed the presence of hartigs net between the cortical cells. There was formation of vesicles in both the plant samples of *Ocimum sanctum* and *Santalum album.* (Refer Figure 18,19 and Table 3)

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