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Assessment of Endophytic Fungal Flora Responsible for Plant Growth Promotion and Survival in Sanganer Region of Rajasthan

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ABSTRACT

The present paper discusses the highest colonization of fungal endophytes as Alternaria speciesin comparison with Colletotrichumspecies and Fusarium species in all three plants Pongamia pinnata, Securinega leucopyrus and Rhus mysorensis. These endophytic fungi protect these plants from various environmental factors such as temperature, moisture and other environmental factors. The survival of these plants depends upon the colonization of these fungi in nodes, internodes and leaves of these plants, which not only exchange enzymes but also gives protection against extreme conditions.

Keywords: *Endophytes, Mycelia sterilia, Relative colonization rate, Potato dextrose agar.*

INTRODUCTION

Sanganer region of Rajasthan is one of twenty five hot spots of global biodiversity with approximately 5,000 species of flowering plants the mean temperature during the study period was 30 ± 2°C. Sanganer region of Rajasthan is having large number of plants such as Prosopsis cineraria L., (Khejdi,Khejra) Holoptelia integrefoli L.,(Papri, Kanjeri, Churil) Mimusops hexandra L.,(Khirni) Anogeissus latifolia L.,(Dhauri,Dhao) Anogeissus pendulaL., (Dhok, Dhao)Rhus mysorensis L.,(Darsan)Acacia Senegal L.,(Kumatiyo, Kumbat) Boswellia serrat L.,Acacia nilotica L., (Babul, Kikar) Butea monospermaL., (chhola,Palas, Khankhera, Kesu) Grewia tenax L., (Gagrain, Gangeti) Commiphora wightii L., (guggul) Securinega leucopyrus L., (Willd.). Muell. (Ghat-bor) and Pongamia pinnataL. Pierre (Karanja), which have shown their economic and medicinal importance. (Fröhlich et al., 2000; Collado et al., 2001; Taylor et al., 2001; Kumar and Hyde, 2004; Tiwari and Chittora, 2013).

Some of the plants has long ancient history for the colonization in this region. The adaptation in adverse conditions and unique mode of survival, made these plants grow and flourish in seasonal variations. One of the important factor is endophytic fungal colonization in the roots of these plants. The relationship between endophytes and the host plants may represent a continuum of interaction, ranging from latent phyto-pathogenesis to mutualistic symbiosis. (Fröhlich *et al*., 2000; Collado *et al*., 2001; Taylor *et al*., 2001; Kumar and Hyde, 2004; Tiwari and Chittora, 2013).

The dynamics and evolutionary response of a host population to both biotic and abiotic environmental factors should depend on potential interactive effects of the host genotype and endophyte infection. It is generally accepted that endophytic microbial communities play an important beneficial role in the physiology and ecological adaptability of host plants. Some of the endophytes protect their host plants from insect attack because they produce unique metabolites of pharmaceutical importance. Permanent colonization with fungal endophytes in the roots of these plants protected the plants from various environmental factors and insects.

BACKGROUND

Author worked for seven years in Sanganer region of Rajasthan as part of their Ph.D. research work. In this time duration explored this region and studied the endophytic fungal association in plants which has unique strategy of survival in extreme conditions.

MATERIALS AND METHODS

Criteria for Plant Selection

A specific rationale for the collection of each plant for endophyte isolation and colonization is used.

- Plants from unique environmental niches especially those with an unusual biology, and possessing novel strategies for survival.
- Plants that have an ethno botanical history which are used in specific uses.
- Plants that are endemic, that have an unusual longevity, are more likely to lodge endophytes with active natural products than other plants.
- Plants growing in areas of great biodiversity have the more number of entophytes.

Plants Selected for Study

From the preliminary isolation experiments it was found that the three plants Securinega leucopyrus L., (Willd.). Muell. (Ghat-bor) Pongamia pinnataL. Pierre (Karanja) and Rhus mysorensis L., (Darsan) have shown the maximum endophytic colonization, therefore these plants were selected for study and the samples were collected from the different sites of Sanganer region of Jaipur, during the months of July-November, 2010. Leaves, nodes and internodes were collected randomly from the plants and were first washed with running water. The leaves were cut into segments (5×5 mm), and stems (nodes and internodes) were cut into pieces (10 mm in length).

Surface Sterilization

All segments and pieces were successively surface-sterilized by dippingin 75% ethanol for 1 minute, 4 % sodium hypochlorite for 5 minutes followed by rinsing three times in sterilized distill water. In each petri dish (9 cm diameter), a total of four-five processed segments or pieces were evenly spaced onto the surface of potato dextrose agar (PDA) media supplemented with 200 µg /ml tetracycline.

Isolation of Fungal Endophytes

All the inoculations were carried out in laminar air flow cabinet. The laminar air flow was swabbed with cotton dabbed in rectified spirit and then irradiated with ultra violet light for 15-20 minutes before use. The stainless steel instruments and other items such as forceps, scalpel, scissors, coupling jar etc. were autoclaved before use. Petri plates, flasks containing distilled water, were also autoclaved prior to use. After keeping all the required material for inoculation except the explants in the laminar air flow cabinet, an ultra violet irradiation was given for 30-40 minutes. The surface sterilized explants (Schulz et al., 1993) were then inoculated into the culture vessel containing potato dextrose agar medium supplemented with 200 µg /ml tetracycline.

Incubation

After inoculation, cultures were incubated in the culture room which was provided with one air conditioner and temperature controller to regulate temperature and humidity of culture room at 26 ± 2°C and 55 ± 5% respectively. Fluorescent tubes and incandescent bulbs were fitted in culture shelves to render constant high intensity of 2000-3000 lux. A photo period of 12 hours light and 12 hours of darkness was regulated with the help of a timer.

Observation

Daily observations were recorded and sporulating mycelia around the explant were (Nodes / Internodes / Leaves) subsequently transferred in the potato dextrose agar media for further study.

Identification of Fungal Entophytes

The identification of endophytic fungal strains was based on the morphology of the fungal culture colony or hyphae, the characteristics of the spores and reproductive structures if these features were discernible. Measurements of all fungal characters were made in water mounts, and the slides were subsequently mounted in lactophenol and sealed with nail vanish. All experiments and observations were repeated at

least twice. Those cultures which failed to sporulate were named as mycelia sterilia, and divided into different morpho-species according to their cultural characteristics.

Data Analysis

Data analysis was carried out by calculating the frequency of colonization rate (CR) and relative colonization frequencies (RF) as described by Surya narayanan et al., (2005). Briefly proper time of incubation was given for colonization rate and relative colonization frequency counting. Colonization rate (%) of an endophyte genus was equal to the number of segments colonized by a single endophyte divided by the total number of segments observed x 100.Relative colonization frequency is the overall comparison (A total % of colonization rate in all ten sites) in all ten sites for analysis.

Preservation of Cultures

The fungi in the pure culture were preserved on the slant and preserved at 4°C with proper labeling; each tube was labeled with the code number of the host plant, batch number and full name of fungi, and date of storage. Several replicate were made for each isolate and appropriate media was used according to the need of the organisms.

Results and Discussion

From the table [Table – 1 and 2, Figure-1, 2 and 3] it is clear that Alternaria species was dominant in all ten sites of the plant samples (15.28 %) followed by Colletotrichum species (14.61 %) and Fusarium species (13.94 %). Many previous reports indicate that the endophytic fungi may exhibit the evidences of tissue specificity (Fröhlich et al., 2000; Collado et al., 2001; Taylor et al., 2001; Kumar and Hyde, 2004; Tiwari and Chittora, 2013). The endophytic fungi in these plants samples also exhibited tissue specificity since various endophytic compositions and abundances were found in different tissues.

The difference in endophyte assemblages from various tissues indicated that some individual dominant endophytic taxa have an affinity for different tissue types, and this might reflect their capacity for utilizing or surviving within a specific substrate in connection with the location of the plant sample (Rodrigues, 1994).

The present study also showed host recurrence, tissue specificity, and spatial heterogeneity of endophytic fungi from different plants of Sanganer area of Jaipur. In fact these endophytic fungi remain residsent with these plants in all seasonal variations and gives protection from insects, temperature variations and other environment factors.

Table1. *Comparison of endophytic fungal genera distribution and relative colonization frequency (RF) from different sites of Sanganer region of Jaipur.*

Location	Type of plant Sample (Leaves, Nodes and Internodes)	Number of Plant Samples	Sample yielding Isolates	Colonization Rate (%)	Fungal Genera
Site - I	03	160	145	90.62	18
Site - II	03	160	137	85.62	16
Site - III	03	160	143	89.37	16
Site – IV	03	160	145	90.62	16
Site - V	03	160	127	79.37	13
Site - VI	03	160	125	78.12	16
Site - VII	03	160	142	88.75	13
Site - VIII	03	160	131	81.87	15
Site - IX	03	160	124	77.5	15
Site – X	03	160	129	80.6	16
Total	30	1600	1348	84.25	18

Sampling sites for endophytic fungi collection from plants (Pongamia pinnata, Rhus mysorensis & Securinega leucopyrus)

Site – I Khonagorion *Site – II Muhana* *Site – III Jagatpura* *Site – IV Goner*

Site – V Watika *Site – VI Powlia* *Site – VII Neola* *Site – VIII Kalwara*

Site – IX Bagru *Site – X Rampura*

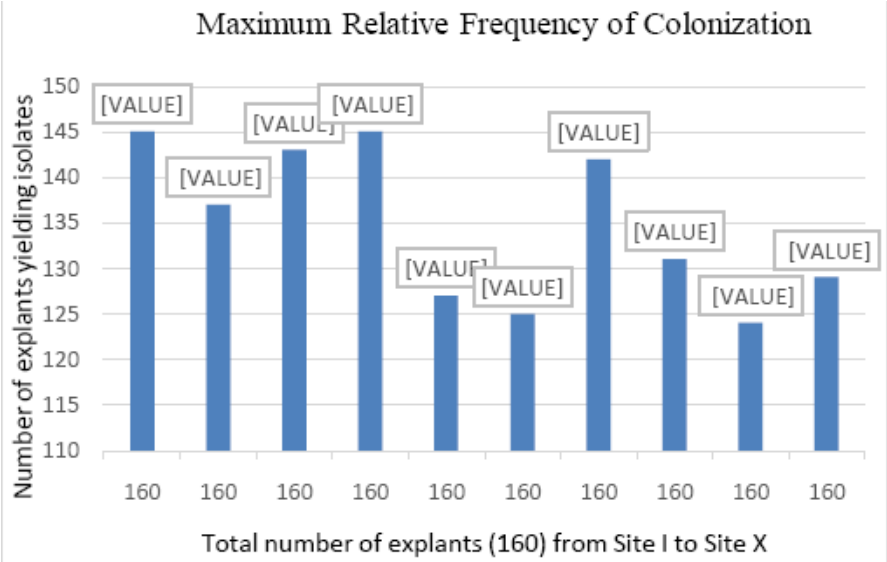

Figure1. *Comparison of endophytic fungal genera distribution and relative colonization frequency (RF) from different sites of Sanganer region of Jaipur.*

Table2. *The relative colonization frequencies (%) of different endophytic fungal taxa isolated from ten different sites.*

Location	Al	Co	Fu	As	Ph	He	Cl	Cu	MS
Site I	16.7	16.7	12.7	4.02	1.34	4.02	1.34	4.69	33.55
Site II	15	15.71	13.57	5	-	9.28	-	3.5	34.28
Site III	13.9	13.28	17.4	3.49	3.49	4.1	2.09	3.4	34.9
Site IV	19.3	15.17	12.4	2.7	-	6.2	1.35	4.8	33.1
Site V	11	10.2	11	3.1	0.78	3.1	0.78	7.08	45.6
Site VI	11.2	14.4	17.6	4.8	0.8	4	1.6	3.2	48.8
Site VII	10.5	14	14.7	2.11	-	2.11	0.70	6.33	40.8
Site VIII	12.05	10.6	11.3	3.5	3.5	6.3	1.4	4.96	32.6
Site IX	18.5	12.9	12.9	4.03	0.8	5.6	-	4.03	26.6
Site X	22.4	20.9	13.9	0.02	3.8	4.65	-	3.1	25.5
Total	15.28	14.61	13.94	3.56	1.48	5.04	0.96	4.59	40.5

Al: Alternaria sp.; Co: Colletotrichum sp.; Fu: Fusarium sp.; As: Aspergillus sp. ; Ph: Phomopsis sp.; He:Helminthosporium sp.; Cl: Cladosporium sp.; Cu: Curvularia sp.; MS: Mycelia Sterilia.

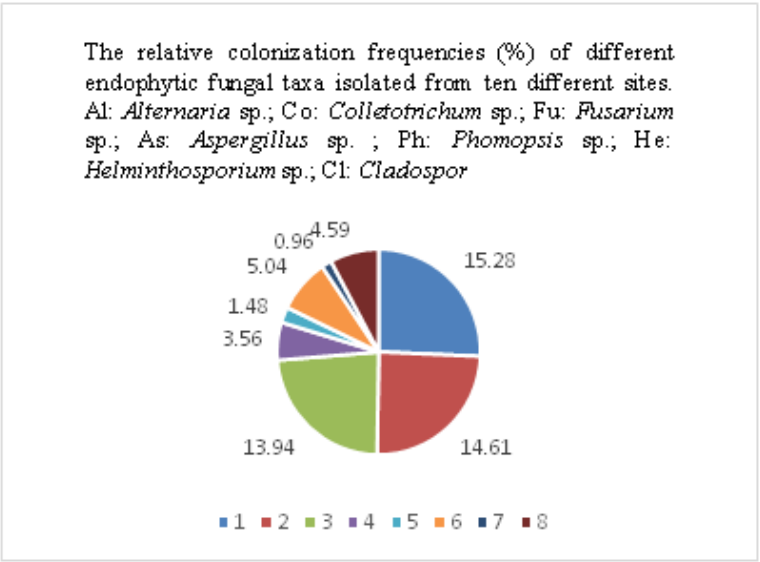

Figure2. *The relative colozination frequencies (%) of different endophytic fungal taxa (8 Taxa and 1 Mycelia Sterilia) isolated from ten different sites*

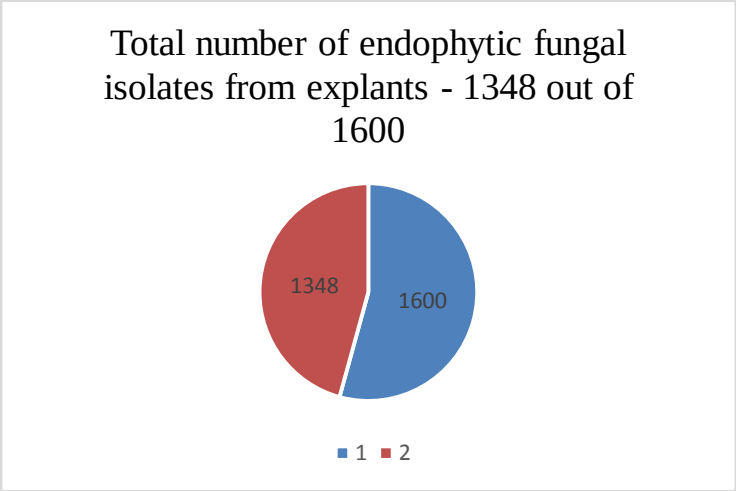

Figure3. *Comparison of endophytic fungal genera distribution and relative colonization frequency (RF) from different sites of Sanganer region of Jaipur.*

CONCLUSION

Various mechanisms for survival hired by the plants. These mechanisms include mycorrhiza, rhizosphere and plant growth promoter microorganisms. This research work confirms that endophytic fungal association in plants within various parts of their body such as leaves, nodes, and internodes, also protect the plants from various environmental factors. This is also one of the reasons of long time history of survival and adaptation in these plants

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