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Diversity, Scale and Effects of Endophytes on Roots and Leaves

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ABSTRACT

Altogether 160 segments from 15 plants of Ruta graveolens Linn. were collected from University Botanical Garden. And they were screened for the presence of endophytic fungi. A total of 7 fungal species viz Asper-gillus flavipes, Bipolaris nodulosa , Aspergillus niger, Cunninghamella blacksleeana, Rhizopus nodusus , Nigrospora spha-erica , Fusarium avenaceum isolated and identified based on the morphology of the fungal culture and spores with the help of manuals. The present investigation was the diversity of endophytes and colonization frequency was more dominated in host leaves had been determined. Importance of endophytes in medicinal plant has been discussed.

Keywords— Endophytes, Host Specificity, Colonization Frequency, Ruta graveolens Linn., Rhizopus nodusus Namyslowski.

I. INTRODUCTION

Endophytic fungi, defined as those species that occur within the living tissues of plants, without causing symptoms and have been isolated from every organ of almost every plant species sampled (Stone et al., 2000). Fungal endophytes colonize within plant organs and recently endophytes are viewed as outstanding source of secondary metabolites, bio-active compounds, antimicrobial natural products and nutri-ents uptake process. One of the most compelling features of fungal endophytes is their exceptional diversity. Several fungi showed differences in the same plant (Jayashree et al., 2011), but these differences were not consistent between sites. The aim of this paper is to provide recent data about diversity of fungal endophytes, colonization frequency of particular site of plant and their dominancy in their host.

Ruta graveolens Linn. is a strong-scented, erect, glabrous shrub. The whole plant parts or leaves are used for the isola-tion. Medicinal uses of Ruta graveolens Linn (Satapa) is re-solvent, diuretic, emmenagogue, antispasmodic, stimulant, acro-nacrotic, poison, irritant and abortifacient. It is useful in hysteria and amenorrhoea. Juice of the herb relieves earache and toothache. Leaves are used in rheumatic pains, in treat-ing hysteria, worms, colic and atonic amenorrhoea and men-orrhagia. Herb and the oil act as stimulants, their influence being chiefly directed to the uterine and nervous systems. It is anthelmintic and in excessive doses it is acro-nacrotic. Oil is used externally as rubefacient. Ruta graveolens is a Ho-meopathic remedy. The chief symptoms noted are pains in bones, joints and cartilages. It is used in the treatment of rheumatism especially of the wrist and ankle; and to avert abortion or to stop bleeding during pregnancy. It is indicated in many eye troubles. Its Chemical compositions having alka-loids, graveolin; graveolinine, rutamine, dictamine, aeborine and arborinine. Glucoside rutin and essential oil.

II. MATERIALS AND METHODS

Ruta graveolens Linn. is a most promising medicinal plants was selected for the present investigation of endophytic fun-gal diversity, where plants are growing in Karnatak University Botanical Garden Dharwad. Location of the studied site is lying in between 14°15¹ to 15°5¹ North longitude and 74°49¹ and 76° 21¹ east latitude. There is a marked diurnal tempera-ture difference. That can be below as 20.2°C in June and high as 34.42°C in March. The annual rain fall is 600-850 mm. The climate is semi humid to humid. Soil is covered with a hard, compact crust having dark brown colour.

Collection of plant samples:

The samples of 15 Ruta graveolens Linn. plants were col-lected from medium sized healthy plants from Karnatak Uni-versity Botanical Garden Dharwad. The plant materials were collected in closed sterile polythene bag with labeled and brought to the laboratory and they were processed within 24 hours of collection.

The samples were rinsed gently under running tap water to remove dusts and debris. The leaves, petiole, stem and roots cut into segments (0.5-1cm). The samples were surface steri-lized accordingly proposed by Dobranic et al., (1995). The samples were immersed in 70% ethanol for 5 seconds fol-lowed by 4% sodium hypochlorite for 90 seconds and then rinsed in sterile distilled water for 10 seconds. The excess moisture was blotted in a sterile filter

paper. The surface steri-lized segments were placed in petridishes containing PDA and MEA medium. The petridishes were sealed using para-film type and incubated at 26 ± 10c at 12 hours light and dark cycle. The petridishes were monitored every day to check the growth of endophytic fungal colonies from the segments.

% of CF =Number of segments colonized by an endophyte X 100 Total number of segments analyzed

The hyphal tips which grew out from the segments were iso-lated and sub cultured on PDA and MEA medium. The pure cultures were maintained on PDA slants. The endophytic fungi were identified according to their macroscopic and mi-croscopic characteristics such as the morphology of fruiting structures and spore morphology with the help of identifica-tion manuals (Ellis, 1971; Subramanin; 1971; Sutton, 1980; Nag Raj, 1993).

III. RESULTS

Altogether 160 segments (40 segments are leaves, 40 seg-ments are petiole, 40 segments are stem, 40 segments are roots) of Ruta graveolens Linn, and they were processed for the isolation of endophytic fungus. A total of 7 fungus was obtained (Table 1) in root samples there is no significance occurrence of fungi. All the isolated and identified fungus was stored in Microbiology Laborotary Karnatak University Dharwad (MLKUD).

Description of Endophytic Fungi:

Species 1: Aspergillus flavipes Bainier and Sartory.Colonies on MEA or PDA media white at first, becoming yellowish, in some strains forming more or less abundant, closely woven, yellow masses containing many helicoids to horseshoe-shaped, thick-walled cells. Heads mostly colum-nar or calyptri form masses, commonly persistently white, but with some strains in pale avellaneous shades to deep avel-laneous. Conidiophores 300-500× 4-5µ, or up to 2-3mm. in length and 8-10 µ in diameter, smooth; vesicles subglobose or elliptical up to 20×30 µ; phialides in two series; primary 4-7 µ or 8×2µ or 3µ, secondary 5-8 ×1.5-2µ.Conidia 2-3 µ. Smooth, subglobose, colorless or nearly so. (plate II-2)

Species 2: Aspergillus niger Tiegh. Colonies growing moderately on PDA or MEA, 3.5-4.5cm in 10 days, with abundant submerged mycelium, conidial heads carbon black, exudates lacks, conidial heads large and black, at first globose and then radiate or splitting in well defined columns in age, up to700-800µmin diam; conidiophores aris-ing directly from the substratum, smooth, non septet, thick walled, 1-2mm ×15-20µm; vesicles globose, walls thick, commonly 45-75µm in diam, occasionally longer, bear-ing two series of fully packed phialides, brownish; metulae mostly 20-30×5-6µm, often reaching 60-80×8-10µm,rarely septate; phialides7-10×3-3.5µm;conidia globose, spinulose with colouring substance, black, 4-5µm; globose to subglo-bose (Plate II-1)

Species 3: Bipolaris nodulosa (Bert. And Curt.ex Sacc.) Shoemaker. B.nodulosa (Bert. And Curt.ex Sacc.) Shoemaker. Conidi-ophores simple, branched, hyaline at tip dark brown at lower parts usually broader toward the apex, swollen and genicu-late at the conspicuous circular conidial scars occurring in close succession in the upper portion; conidia straight, el-lipsoid or ovate or typically obclavate,. Thin-walled pale to moderately dark brown or olivaceous brown, with a circular basal hilum included within the coutous of theroundedba-sal wall and often surrounded by a hyaline area, 3-7 septate, 28-70× 10-18µ, mostly 48.4 ×56.6 ×13-15.7µ(21.64× 12-17 µ mean 48.6× 14µ) in the type 1 produced singly and ac-rogenously at the tips of the conidiophores. And successive growing points, with a length/ breadth ratio of about 5.5-3.7 (Plate II-5).

Species 4: Cunninghamella blakesleeana Lender. Colonies growing rapidly on PDA and MEA, white, later becoming yellow, loose, erect,2-4cm in height; sporangio-phores long, simple or regularly verticillately branched, lat-eral branches of the sporangiophores variable in length and number, usually less than 50µm long; terminal vesicals, glo-bose to subglobose, 40-60µm; lateral vesicles usually smaller than terminal vesicles, 19-28µm;sporangioles hyaline, echi-nulate or smooth, ovoid ones 7.5-10µ; in diam (excluding spines), ellipsoidal ones 10-12.5×7-8µm(excluding spines); (Plate II-3).

Species 5: Fusarium avenaceum sacc. Mycelium highly septate, branched, conidia produced on false heads, conidia long filiform. Conidia mostly 2-3 septate 1- septate 14-16X3µm, 3 septate 30-33X 4-5µm (Plate III-4).

Species 6: Nigrospora sphaerica (Saccardo)Mason. Both sterile and fertile hyphae creeping, at first hyaline, then dark; sterile hyphae septate 18µ in diameter; fertile hyphae septate, 4µ in diameter, much branched bearing swollen jar-like cells terminally and laterally on which are borne single the sub spherical, smooth, black conidia. Conidia one-celled, 11-14µ in diameter. (Plate II-6)

Species 7: Rhizopus nodusus Namyslowski. The mycelium is cottony, white when young, then tinted ochre-yellow. In midst of the mycelium and on the stolons, branches ending in sporangia occur. These branches 1-2mm. in height×12-28µ in diameter have thick, smooth walls, color-less at first, then becoming pale ochre or brown. They are simple or branched, the branches ending in sporangia. The branchesmay swollen at any point. When these swellings are terminal they give rise to a group of three to five.

Table 1: Colonization frequency (%) of endophytes in Ruta graveolens Linn., on different media

S.N	Endophytic fungi	Colonization frequency (%)						Total iso-lates
		MEA			PDA			
		L	P	S	L	P	S	
1	Aspergillus flavipes Bainer and Sartory.	-	13.3	-	6.66	20	6.66	46.64
2	Aspergillus niger Tiegh.	6.66	-	20	20	13.3	13.3	73.26
3	Bipolaris nodulosa (Bert and Curt. ex. Sacc.) Shoemaker.	-	-	6.66	6.66	-	-	13.32
4	Cunninghamella blacksleeana Lender.	26.6	20	33.3	33.3	26.6	26.6	133.1
5	Fusarium avenaceum Sacc	6.66	6.66	13.3	6.66	-	20	53.28
6	Nigrospora sphaerica (Saccardo) Mason.	6.66	-	-	-	-	-	6.66
7	Rhizopus nodusus Namyslowski.	33.3	26.6	26.6	20	20	26.6	153.1
	TOTAL							479.36

* L-Leaf , P-petiole , S-stem

Fig.6: Colonization frequency (%) of endophytes in Ruta graveolens Linn. on different media sporangiophores, each terminating in a sporangium. Sporan-giophores 1-2mm. high, the sporangia are globose 100-200µ in diameter (Plate I-7).

IV. DISCUSSION

Medicinal plants are one of the oldest forms of health care known, every plant on earth is known to harbor at least one endophytic microbe. These are one of the most unexplored and diverse group of organisms having symbiotic associa-tion with higher life forms and may produce benificial sub-stances for host (Weber,1981). Present studies revealed that the leaves of the host plant exhibited the highest endophytic diversity than the petiole and stem samples. Thus the present finding have suported that the endophytes isolated from leaf samples exhibited

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greater diversity and high colonization fre-quincy compared to the endophytes of the other plant parts examined.

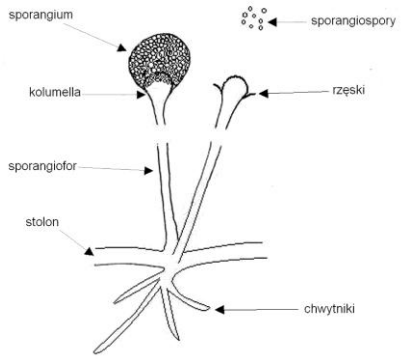
Leaves, petiole, stem and roots of a single plant often differ greatly in the dominant members of their endophytic com-munities (Chaverri et al.,2010, Gazis et al.,2010, Hoffman et al.,2008, Pocasangre.,2000). and may even show functional differences. As in case of Alfalfa plants of leaves, stems and roots are colonized by distinct fungi that produce different ranges of secondary metabolites (Weber et al., 2006). Even with a single plant different leaves may differ significantly in community composition (Gamboa et al.,2001, Fisher et al., 1996). Single leaves of a tropical forest tree, manilkara bi-dentata, showed fine scale variation of endophyte isolation rates and identity. In this respect, plants are genetic mosa-ics because each organ may have a unique combination of genes in its micro biome (Herre et al., 2007.). However, some endophytes are restricted to single cell and tissues in the leaf endophytes in different tissues may not interact (Stone JK. 1987). This potential goldmine of undescribed biodiver-sity has the issue of host specificity, particularly in tropical plants. A recent meta-analysis found that leaf endophytes are indeed more species-rich in the tropics than in temperate regions (Arnold et al., 2007). This report consistent to earlier workers as, leaf samples finding more number of endophytic diversity in the plants. One of the possible reasons for the differences in the colonization rates between plants is the structure and substrate which influence the colonization and distribution of endophytic fungi (Okane et al., 1997). Kumar and Hyde (2004) also stated that the overall colonization rate in the leaves was found to be significantly higher than those in root, stem and petiole. Present studies clearly exhibited that the number of endophytic fungi was higher in leaves followed by petiole, stem and roots. However, the overall colonization frequen-cies differed with different organs. Similar results have been observed in the endophytic diversity of Thalavaipandian et al., 2011.

The most prevalent endophytes were recorded among Rhizopus nodusus Namyslowski was dominant followed by Cunninghamella blacksleeana Lender. The total coloniza-tion frequency higher from Rhizopus nodusus (153.1%) and low from Nigrospora sphaerica (Saccardo) Mason followed by Bipolaris nodulosa (Bert and Curt. ex. Sacc.) Shoemaker. (13.32% & 6.66%). The other endophytes recovered from Fusarium avenaceum sacc Aspergillus flavipes Bainer and Sartory Aspergillus niger Tiegh. These endophytes have also been reported as endophytes in earlier studies.

PLATE I

Ruta graveolens Linn

R.g leaf in MEA medium



Rhizopus nodusus Namyslowski

Pure culture
PLATE II
1 Aspergillus niger Tigeh

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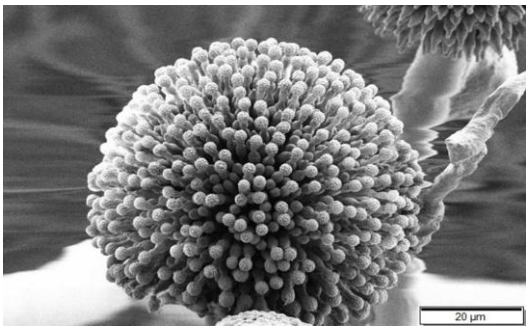
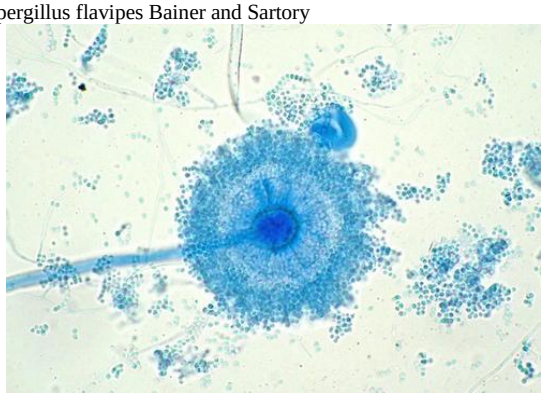


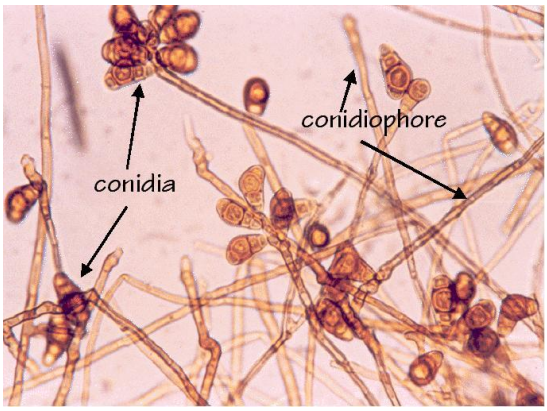
2 Aspergillus flavipes Bainer and Sartory



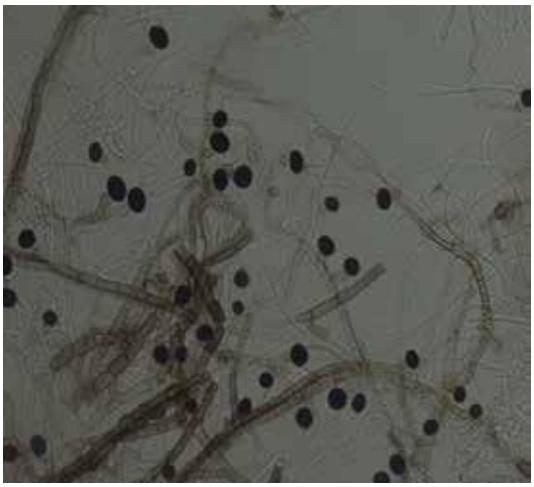
3 Cunninghamella blacksleeana lender

4 Fusarium avenaceum sacc

5 Bipolaris nodulosa (Bert&cart cart



6 Nigrospora sphaerica (saccurdo)



V. CONCLUSION

The understanding of the number of endophyt-ic diversity associated with a Ruta graveolens Linn. plant of different sites of leaves, petiole and stem parts finding is a significant variation was detected in the colonization frequen-cy of endophytic species with 15 medicinal plants. However, high colonization frequency was in leaves. Endophytic flora in roots examined but do not get results, in some samples shows results as only one or two endophytic fungi. Similarly made an attempt to culture in different culture media results shows as endophytes grows more in MEA compare to PDA media. In Other finding have supported that the number of endophyte species present in Ruta graveolens Linn we now has an accumulation of studies suggesting that their diversity among there fungal endophytes were immense.

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