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Diversity of The Endophytic Fungi Isolated from *Ocimum Basilicum* Linn. - A Promising Medicinal Plant

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ABSTRACT

Altogether 105 segments from 15 plants of Ocimum basilicum Linn.were collected from Karnatak University Botanical Garden; Dharwad. And they were screened for the presence of endophytic fungi. A total of 7 fungal species viz, Aspergillus sydowi (Bainier & sartory) Thom & Church. Mycelia sterilia., Alternaria burnsii Uppal, petel., Geotrichum candidum Link., Torula alli (Harz) Saccardo., Fusarium poae (peck) Wr., and Aspergillus clavatus Desm. 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, Colonization Frequency, Alternaria burnsii Uppal, petel., Geotrichum candidum Link.

INTRODUCTION:

Endophyte and endophytic are used with particular meaning by different workers and for particular groups of hosts and microbes. Generally endophytes apply to microbes capable of symptomless occupation of apparently healthy plant tissue (Chanway,1996). Endophytic fungi was first described by De bery (1866). And he, broadly defined as "fungi that live for all or at least significant part of their life cycle internally and asymptomically inside plants." These fungi colonize within plant tissue and remain the tissue, except that fruiting structures may emerge through the surface of the plant tissue. The study of endophyte distribution, biodiversity and their biochemical characteristics are of immense importance in plant biology to understand and to improve plant fitness. Endophytic fungi occurrence in Lichens, Moss,, Ferns, Gymnosperms and Angiosperms. They shows biotechnological importance as new pharmaceutical compounds, secondary metabolites would be found by further exploration of endophytes. Dreyfuss and Chapela (1994) estimated that endophytic fungi from the 2.70,000 species of plants existing on this planet could account for 1.38X106 unique fungal species. The enormity of this estimation is due to the fact that fungal endophytes have been isolated from every plant species examined to up to date and these are important components fungal biodiversity.

Ocimum basilicum Linn. (Kannada:Kamkasturi) plant is native to tropical areas of Asia and Africa and grows wild as perennial on some pacific islands. Chemical composition of O.basilicum Linn. are Linalol-54.94%, Methylchavikol-(11.98%), Methylcinna-(7.24%), Linolen-(0.14%), Essential oil is also found in sweet basil, along with rosmarinic acid, citral, eugenol and geranic. Medicinal uses of O.basilicum Linn. is considered to be an antibacterial, antifungal and stimulant. Fresh leaves are used in case of fevers, abdominal, poor digestion. Leaves decoction helpful remedy in treatment of respiratory disorders. Leaves promotes expulsion of kidney stones. Plant is also useful in reduction of blood cholesterol (Lakshman et al., 2012). Fungal endophytic studies on this plant is considered to be first time therefore to evaluate the fungal endophytic association the present study was undertaken.

MATERIALS AND METHODS:

Ocimum basilicum Linn. is a most promising medicinal plants was selected for the present investigation of endophytic fungal 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 temperature 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 Ocimum basilicum Linn. plants were collected from medium sized healthy plants from Karnatak University 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.

Isolation of endophytic fungus: 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 sterilized accordingly proposed by Dobranic et al., (1995). The samples were immersed in 70% ethanol for 5 seconds followed 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 sterilized segments were placed in petridishes containing PDA and MEA medium. The petridishes were sealed using parafilm type and incubated at 26 ± 1°c at 12 hours light and dark cycle. The petridishes were monitored every day to check the growth of endophytic fungal colonies from the segments.

$$\% \text{ of colonization Rate} = \frac{\text{Number of segments infected}}{\text{Total number of tissues segments Inoculated}} \times 100$$

$$\% \text{ of CF} = \frac{\text{Number of segments colonized by an endophyte}}{\text{Total number of segments analyzed}} \times 100$$

The hyphal tips which grew out from the segments were isolated 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 microscopic characteristics such as the morphology of fruiting structures and spore morphology with the help of identification manu-

als (Ellis, 1971; Subramanin; 1971; Sutton, 1980; Nag mani; 2005).

RESULTS:

A study was conducted from February 2012 to May 2013 at P.G. Department of studies in Botany, Karnatak University, Dharwad, Karnataka, India. The medicinal plant samples of Ocimum basilicum L. yielded 7 endophytic fungal isolates, belongs to 6 genus. Based on a microscopic observation of mycelia and conidia, these endophytes were identified as Alternaria burnsii Uppal, petel., Aspergillus clavatus Desm., Aspergillus sydowi (Bainier & sartory) Thom & Church., Fusarium poae (peck) Wr., Geotrichum candidum Link., Mycelia sterilia and Torula alli (Harz) Saccardo. Endophytic fungi were identified on the basis of cultural characteristics and morphology of fruiting bodies and spores. Some cultures fail to sporulet on the culture media were recorded as sterile forms. Using standard manuals for the identification of endophytic fungi (Ellis, 1971; Subramanin; 1971; Sutton, 1980; Nag Raj, 1993).

A total of 105 isolates belonging to 7 fungal species were isolated from Ocimum basilicum L. The high % of colonization rate and colonization frequency is found Aspergillus sydowi (Bainier & sartory) Thom & Church. High endophytic colonization isolates were observed in PDA media compare to MEA media and the dominating fungi are Aspergillus sydowi (Bainier & sartory) Thom & Church., Mycelia sterilia., Alternaria burnsii Uppal, petel., Geotrichum candidum Link., Torula alli (Harz) Saccardo., Fusarium poae (peck) Wr., followed by Aspergillus clavatus Desm (table 1 shows colonization rate and table 2 shows colonization frequency of endophytic fungi in the host plant).

Illustration for the identified endophytic fungi

Species1: Altarnaria burnsii Uppal, petel.

Mycelium septate, Hyaline when young later becominglight, olive green, 1.7-5.8µ in diameter ,conidiophores are solitary or fasciculate,erect,simple or sometime branched, light olive septate straight,or somewhat irregular bent,occasionally with a singleterminal scar,geniculate, 3-5 celled,15-52×1-9-37µ, conidia borne singly on the host but in chains of 2-10 in culture, mostly obovator,rarely obclavate,with rounded base, tapering to the apex. Which may be drawn a septate or non septate beak, often as long as the spore, conidia with trasverse,longitudinal and oblique septa,transverse septa1-9 but mostly 3-5 constricted at septa (except in the reason of the bark)olive brown in colour,13.8-55.1×6.9-25.2µ(without beaks and 23-118.9×7-29.9µ including beak) (Plate III 13).

Species 2: Aspergillus sydowi (Bainier and sartory) Thom and church.

Colonies on Czapek's agar blue green, with the bluish effects, prominent,velvety with some aerial interlacking and trailing hyphae reverse and substratum shades of orange red, becoming almost black. Conidiophores mostly arise from submerged µdiameter. Phialides radiate in to 2 series primary up to 7 2-3 , secondary 7-10 2 conidia globose 2.5-3.5 spinulose no sclerotia or cleistothecia found. (Plate III 15)

Species 3: Aspergillus clavateus Desm.

Colonies on CZA growing well, 3-4cm in in 10 days;mycelia felt thin,plane,conidialheads abundant,large,clavate,-blue green when young,later slate olive,1-4mm in length; revese colourlessat first, light brown in algae ;exudates absent;odour strongly fetid in some strains;conidial heads clavate,250-400×135-175µm, often splitting into divergent colums at maturity;conidophores thin walled, gradually enlarging at the apex into a clavate vesicle; vesicle 180-250×38-60 µm,fetile over the entire surface;phiaides in a single series,basal ones smaller,2.5-3.4×1.8-3µm,those at the apex of the vesicle larger, 6.5-10×2.6-3.3µm;conidia elliptical,smooth,somewhat heavy walled,3-4.8×2.5-3.5µm,light green in mass. (Plate III 14)

Species 4: Torula allii (Harz) saccurdo.

Hyphae prostrate; septate richly branched,with, here and there,short hyaline lateral branchlet's, on which the conidial chains occur, conidia from 5-10in slightly curved chains, becoming larger at the tip,14µ in diameter, globose, hyaline at first becoming brownish- black. (Plate III 18)

Species 5 : Fusarium poae (peck) Wol.

Mycelium composed of branched, septate, hyaline, hyphae about 2-6µ wide, frutinglayer cobwebby or wooly to felt- like, white to rose,with many dichotomous or whorled branches and conidiophores, stroma carmine purple or ochre yellow or violet. Conidia usually in abundanceon the conidiophores,Sometimes bound in chains,discharged &then seen as an isabellin or whitish powder scaltered over the mycelium or giving a somewhat scandy or mealy appearance to the substrate microconidia typically globose,limoniform, pyriform,1-or 2 celled, microconidia, loneg,fusiform,ellipsoid or falcate. (Plate III 18)

Species 6 : Geotrichum Candidum Link.

Key : Colonies creamy white, arthro conidial produced in chain by fragmentation of hyphae, variously shaped 3-20 x 3-6 upto 9 µ commonly 6-12 x 3-5 µm.

Colonies on agar media thin, spreading, creamy cohite, soft and of some what yeast like texture. Mycelium hyaline, septate, more or less specialized into broad, radiating vegetative hyphae branching dichotomously and narrower, lateral, sporulating hyphae which may also branch, on some media dichotomous branching, depressed or absent spores (arthrospores) formed in chains by disarticulation of the leteral hyphae at septate some times intercalary in the broad vegetative hyphae as well, usually maturing in rapid basipetal succession. Spore chains aerial and erect, or decumbent or flat on the surface of the medium or under the surface, spores cylindrical at first, later varying from barell shaped to ellipsoidal or subglobose, 2-8 x 3-50µ, commonly 3-6 x 6-12µ. (Plate III 16) .

DISCUSSION:

Herbal medicine is one of the oldest forms of health care known; every plant on earth is known to harbor at least one endophytic microbe. Plants have a long history of use in treatment of cancer (Hartwell, 1982). Endophytic fungi are one of the most unexplored and diverse group of organisms having symbiotic associations with higher life forms and may produce beneficial substances for host (Weber, 1981). In the present investigation, a significant variation was detected in the colonization frequency of endophytic species with medicinal plants and revealed the variation in distribution of fungal endophytes in different organs, clearly shows that endophytes were not restricted to single species, genera or family and even particular organs of the same species.

In present study total of 7 different species have been recorded. The similar results were reported by Suryanayanan et al., (2003), and they have reported diversity of fungal species varing from 10-26 in the host. Results revealed that, the predominant endophyte genera found in plant were Aspergillus., Mycelia sterilia and Alternaria. These results strongly support the report made by Bayman et al., Gamboa and Bayman (2001); Lodge et al., (1996) and Rodriguez et al., (1993). Previous studies reported distinct endophytes community compositions and in different host plants suggesting host preference (Cannon and Simmons, 2002; Cohen, 2006). This study shows such a trend was apparent with leaves, root, stem and flower parts of Ocimum basilicum L. medicinal plant.

In agreement with previous studies, endophyte communities at small and large spatial scales were characterized by a large number of singletons (species that occurred only once) (Arnold et al., 2000) including 138 of the 277 species recorded. The host and geographic specificity of these rare species remain unknown. More generally, the ecological roles of the vast majority of endophytes recovered in this study have yet to be explored. Isolation of only sixteen taxa of endophytic fungi shows that the medicinal property of the plant has some role to play in the colonization of endophytic fungi. This low

rate of colonization may be attributed to the secretion of the phyto-chemicals, since they contain certain antifungal and antibacterial components (Rajgopal et al., 2010). Endophytic fungi from tropical plants have recently gained importance in biological control of plant diseases and also as a source of pharmacologically active compounds. Only a few plant species have been investigated for their endophytic fungal population (Strobe and Daisy 2003). Therefore, any information and/or research on endophyte-plant symbiosis, such as in this study is of value. Effective extracts could provide potential leads towards the development of novel and environmental friendly biologically active agents. Previously documented, growth responses to leaf chemistry, and the correlation of these results with endophyte isolation frequencies from different hosts ; Arnold et al., 2003, suggest that interaction between given plant and fungal taxa are likely unique. Whether the outcomes of such interactions are stable in time and space, and are influenced by other endophytes that may co-occur with focal species in individual leaves, remain to be evaluated.

Conclusion:

Infact, these endophytes play important role by producing lots of secondary metabolites when infect or colonize with different parts of plant organs. We examined flowers system in different media to isolate endophytic flora but we do not get significant results in flowers system compare to the roots, stem and leaves. However we made an attempt to culture endophyte in viable media. The endophytic fungi were isolated by placing segments (leaf, root, flower and stem) on appropriate growth media (PDA and MEA). Plant organs (leaf, root, flower and stem) may contain many different species of endophytic fungi. Segments of Ocimum basilicum L. medicinal plant showed positive results with respect to the presence of endophytic fungi. The % of colonization rate and frequency was recorded and the highest colonization rate and frequency was A. sydowi. A total of 105 isolates belonging to 7 species occurred in the MEA media. All the 7 fungal endophytes belong to 6 genera. Among the isolated endophytic fungal genera, genus Aspergillus sydowi was the predominant from medicinal plant. The genus Mycelia sterilia had shown to be predominant endophytes in all the medicinal plants with respect to colonization frequency. From the experimental results, it can be concluded that, there is variation in occurrence of endophytic fungi in all the organs of the medicinal plant selected in this study. Results clearly indicated that, the fungal endophytes were not restricted to any plant species but, they showed host specificity and organ to organ variations. The isolated endophytic fungi showed variation with respect to development and formation of colony on different media. The PDA media showed highest number of total isolates belonging to 7 endophytic fungal species.

Table 1: % Colonization Rate of endophytes in Ocimum basilicum L. on different Media

Sl No.	Host plant part used	Colonization Rate		Total isolates
		PDA	MEA	
1	Leaf	55	70	125
2	Root	95	100	195
3	Stem	86.66	80	166.66
4	Flower	80	70	150

Fig 1: % Colonization Rate of endophytes in Ocimum basilicum L.on different media

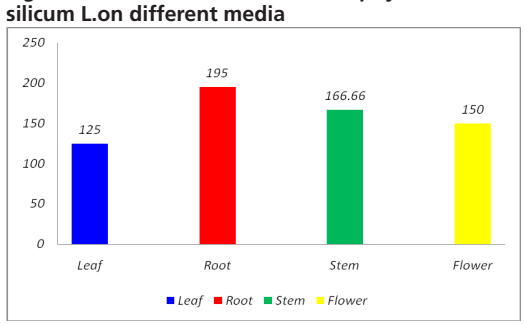
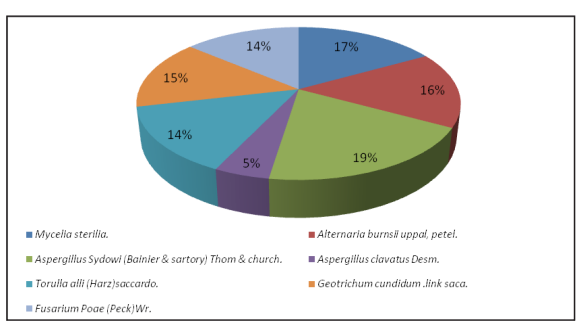


Table 2: Colonization frequency (%) of endophytes in Ocimum basilicum L. on different Media

Sl. No	Endophytic Fungi	Colonization frequency (%)		Total Isolates
		PDA	MEA	
1	Mycelia sterilia.	148.33	140	288.33
2	Alternaria burnsii uppal, petel.	145	130	275
3	Aspergillus Sydowi (Bainier & sartory) Thom & church.	123.33	210	333.33
4	Aspergillus clavatus Desm.	60	20	80
5	Torulla alli (Harz)saccardo.	123.33	120	243.33
6	Geotrichum cundidum .link saca.	93.33	160	253.33
7	Fusarium Poae (Peck)Wr.	176.66	160	236.66
	Total			1466.65

Fig 2: Colonization frequency (%) of endophytes in Ocimum basilicum L. on different media



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