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DIVERSITY OF FUNGAL ENDOPHYTES IN *CUCUMIS DIPSACEUS* EHRENB. EX SPACH.

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ABSTRACT

The need for new and active bio- metabolites to treat various human ailments is ever increasing. Endophytic fungi were considered as potential source for producing secondary metabolites. Endophytic fungi are organisms which reside inside the plant tissues without causing damage to the host plants. The present investigation was carried out to study the diversity of endophytic fungi in an ethanomedicinal plant of South Africa namely Cucumis dipsaceus which is collected from Coimbatore district. Leaf and stem tissues were used as explants. They were surface sterilized and segmented. The segments were inoculated into four different medium like Potato dextrose agar (PDA), Czapek's Dox agar (CDA), Sabouraud's dextrose agar (SDA) and Malt extract Agar (MEA) for isolation of endophytic fungi. Totally, 500 segments were taken for isolation of endophytic fungi. Twenty fungal species were isolated and identified. Various parameters like colonization rate, colonization frequency and isolation rate was noted. Leaf segments showed more fungal diversity when compared to stem. Endophytic fungi like Aspergillus terreus, Aspergillus flavus, Aspergillus fumigatus and Paecilomyces sp., were found to be dominant. In this study, we concluded that, 20 endophytic fungi were isolated and further studies will be carried out to screen their bioactive metabolite production.

KEY WORDS

Aspergillus, colonization rate, Cucumis dipsaceus, Endophytic fungi

INTRODUCTION

The discovery of natural products have been exploited for human use for thousands of years and among them plants have been the chief source of compounds used for medicine. From earlier historical periods till date medicinal plants play a major role in providing health care to human population. An ethno biological survey revealed that about 8,000 species of medicinal plants are used as food supplements, medicines, biocides and other phytochemicals. Natural products are naturally derived compounds present as metabolites or byproducts from microorganisms, plants or animals [1]. The quality and quantity of secondary metabolites obtained from plants were affected by some factors like genetic background of the plants, soil nutrients, habit and habitat etc., [2]. In recent years, it is recognized that the secondary metabolites from plants are affected by endophytes through host-endophyte relationship.

Endophytes are microorganisms, which reside in plant tissues without causing any negative effect on host plants. Endophytic fungi are one of the major potential sources for new metabolites. The novel secondary

metabolites obtained from endophytes are widely used in industries such as pharmaceutical, agricultural, etc., [3]. Screening of diverse endophytic fungi from various plants with different ecological conditions help to isolate more number of metabolites. In the present investigation, diversity of endophytic fungi was studied in stem and leaves of *Cucumis dipsaceus* collected from Coimbatore district. *Cucumis dipsaceus* (Cucurbitaceae) is an annual herb without a woody root stock, stems are climbing with hispids, leaves with long petiole possess less hispids on the upper surface. Tendrils are present. Flowers are monoecious [4]. *Cucumis dipsaceus* is an ethanomedicinal plant of South Africa which helps to treat gonorrhoea, urinary retention and skin infections caused by fungus [5].

MATERIALS AND METHODS

Collection of plant sample

Leaf and stem tissues of *Cucumis dipsaceus* were collected from various places in Coimbatore district. Plant samples were identified by Botanical survey of India (BSI/SRC/5/23/2014-15/Tech./663), Coimbatore. Samples were collected in sterile polythene bags and processed within 24 hours of collection (Fig.-1).

Isolation and identification of endophytic fungi

Surface sterilization was carried with slight modifications of the methods proposed by Fisher et al., [6]. Healthy plant materials were taken and rinsed with running tap water to remove dust and debris present in it. Stem and leaf segments were separated and dipped in 70% ethanol for 2minutes, then it was immersed in 4% sodium hypochlorite for 3minutes and finally rinsed with sterile distilled water for 1minute. The effectiveness of surface sterilization in leaf and stem segments were checked using imprint method [7].

The excess moisture in sterilized plant tissues were blotted on sterile filter paper. The leaf and stem tissues were sectioned using sterile scalpel blade. The sections were then placed onto petri dish containing four different medium [8] like potato dextrose agar (PDA), Malt extract agar (MEA), Czapeks dox agar (CDA) and Sabouraud's dextrose agar (SDA) (Fig.-2) which are amended with Chloramphenicol (150mg/l). The petri dishes were sealed and incubated at 28±2˚C for 3 weeks in a light chamber. Tissues were observed for mycelial growth at regular intervals. Actively growing fungal mycelia were subcultured in PDA/SDA/MEA/CDA (Fig.-3and4). The potential fungal isolates were identified morphologically by Agharkar Research Institute, Pune.

Data analysis

During isolation process, following parameters were noted [9].

$$\text{Colonization rate} = \frac{\text{Total number of segments infected by fungi}}{\text{Total number of segments incubated}}$$

$$\text{Colonization frequency} = \frac{\text{Number of plant segments colonized by single endophyte}}{\text{Total number of segments observed}} \times 100$$

$$\text{Isolation rate} = \frac{\text{Number of isolates obtained from plant segments}}{\text{Total number of segments incubated}}$$

RESULTS

About 500 segments of stem and leaf tissues of *Cucumis dipsaceus were* screened for the presence of endophytic fungi. Both sporulating and sterile forms were isolated. A total of 20 isolates were obtained from plant tissues (Table-1). The overall fungal composition includes 16 isolates in stem and 17 isolates in leaf. Among the 20 isolates the most predominant endophytic fungi belong to genera Aspergillus with 8 different species viz, *Aspergillus aculeatus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus* and *Aspergillus ustus*. Isolated endophytic fungi belong to families like *Aspergillaceae, Ceratostomataceae, Ophiocordycipitaceae, Trichocomaceae, Chaetomiaceae and Xylariaceae.*

Colonization rate of stem and leaf tissues were 0.76 and 0. 92 respectively and isolation rate was 0.08 and 0.09 respectively (Table-2).

Colonization frequency was dominant in *Aspergillaceae* followed by *Ceratostomataceae, Chaetomiaceae, Ophiocordycipitaceae, Trichocomaceae* and *Xylariaceae* (Table-3). Isolation rate on both the tissues indicated that about 95% of organisms were common in leaf and stem tissues. Endophytes like Aspergillus aculeatus, *Aspergillus fumigatus1* and *Chaetomium* sp. were present only in stem tissues. Likewise, *Aspergillus* sp., *Aspergillus ustus 1, Aspergillus fumigatus 2* and *Paecilomyces* sp. were screened only in leaf tissues.

Table-1: List of endophytic fungi from *Cucumis dipsaceus*

S.No.	Endophytic fungi	Family	Source
1.	*Aspergillus* sp.		S/L
2.	*Aspergillus aculeatus*		L
3.	*Aspergillus flavus*1		S/L
4.	*Aspergillus flavus*2		S/L
5.	*Aspergillus fumigatus1*		L
6.	*Aspergillus fumigatus*2		S
7.	*Aspergillus nidulans*		S/L
8.	*Aspergillus niger*	*Aspergillaceae*	S/L
9.	*Aspergillus ochraceus*		S
10.	*Aspergillus terreus*1		S/L
11.	*Aspergillus terreus*2		S/L
12.	*Aspergillus terreus*3		S/L
13.	*Aspergillus ustus*1		L
14.	*Aspergillus ustus*2		S/L
15.	*Aspergillus ustus*3		S
16.	*Chaetomium* sp.	*Chaetomiaceae*	L
17.	*Melanospora zamiae*	*Ceratostomataceae*	S/L
18.	*Nodulisporium gregarium*	*Xylariaceae*	S/L
19.	*Paecilomyces* sp.	*Trichocomaceae*	S/L
20.	*Purpureocillium lilacinum*	*Ophiocordycipitaceae*	S/L

S- Stem; L- Leaf

Table-2: Colonization and isolation rate of endophytic fungi from *Cucumis dipsaceus*

	Stem	Leaf	Total
No.of segments	250	250	500
No.of segments colonized by fungi	192	232	424
No. of isolates	16	17	33
Colonization rate	0.76	0.92	0.84
Isolation rate	0.08	0.09	0.08

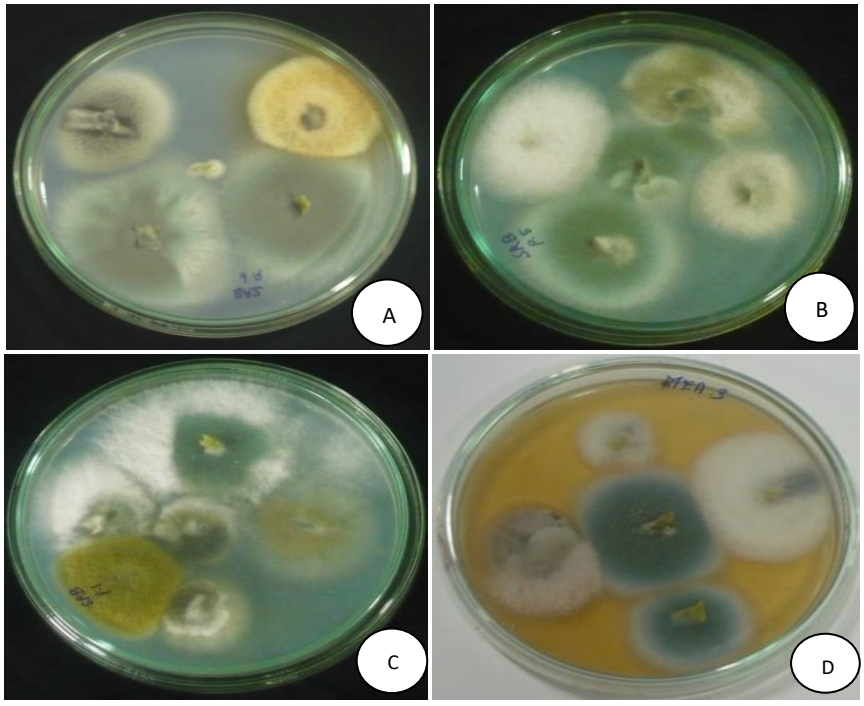
Table-3: Colonization frequency of endophytic fungi

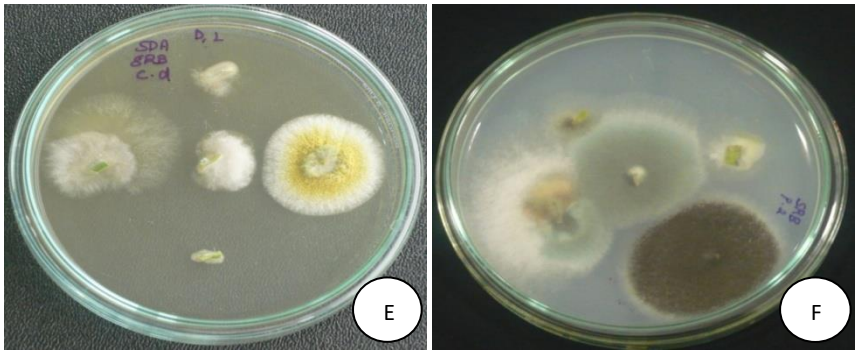
ENDOPHYTE	STEM	CF (%)	LEAF	CF (%)
Aspergillaceae				
Aspergillus sp.	12	4.8	13	5.2
Aspergillus aculeatus	-	-	20	8.0
*Aspergillus flavus*1	16	6.4	12	4.8
*Aspergillus flavus*2	11	4.4	11	4.4
*Aspergillus fumigatus*1	-	-	18	7.2
*Aspergillus fumigatus*2	22	8.8	-	-
Aspergillus nidulans	9	3.6	13	5.2
Aspergillus niger	12	4.8	14	5.6
Aspergillus ochraceus	13	5.2	-	-
*Aspergillus terreus*1	17	6.8	16	6.4
*Aspergillus terreus*2	11	4.4	18	7.2

*Aspergillus terreus*3	6	2.4	15	6.0
*Aspergillus ustus*1	-	-	24	9.6
*Aspergillus ustus*2	9	3.6	6	2.4
*Aspergillus ustus*3	11	4.4	-	-
Chaetomiaceae				
Chaetomium sp.	-	-	17	6.8
Ceratostomataceae				
Melanospora zamiae	19	7.6	5	2.0
Xylariaceae				
Nodulisporium gregarium	7	2.8	9	3.6
Trichocomaceae				
Paecilomyces sp	11	4.4	14	5.6
Ophiocordycipitaceae				
Purpureocillium lilacinum	6	2.4	7	2.8

Fig.1: *Cucumis dipsaceus*

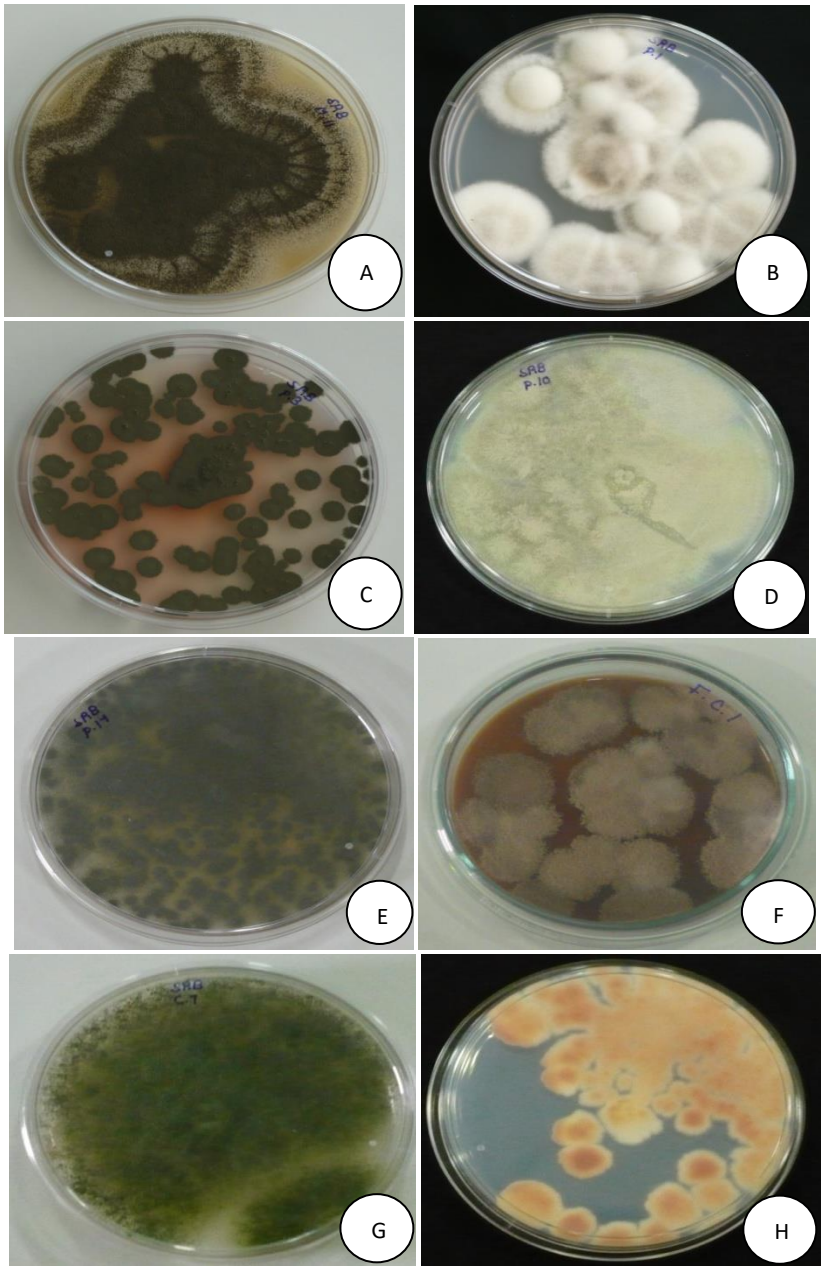
Fig.2 : Endophytic fungi emerging from plant tissues





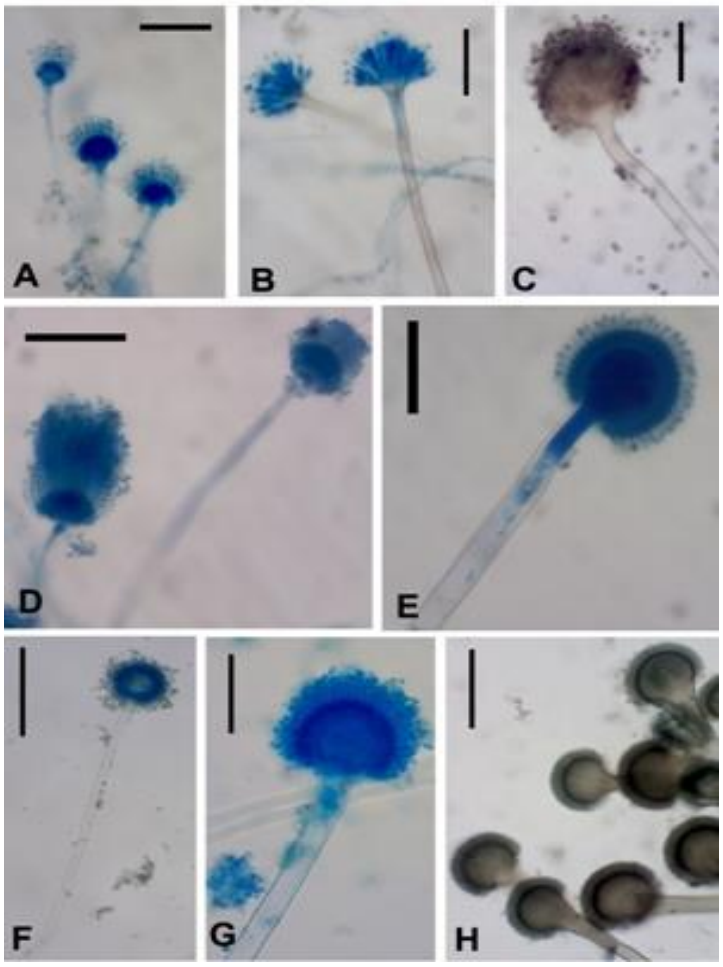
A, B-Emergence of endophytic fungi from leaves and stem on PDA
C-Emergence of endophytic fungi from leaves on CDA
D-Emergence of endophytic fungi from leaves on MEA
E, F- Emergence of endophytic fungi from leaves and stem on SDA

Fig. 3: Pure cultures of Endophytic fungi



***A-Aspergillus niger*; B- *Paecilomyces* sp.; C- *Chaetomium* sp.; D- *Aspergillus fumigatus* 1; E- *Aspergillus fumigatus* 2; F- *Aspergillus terreus* 1; G- *Aspergillus flavus*; H- *Aspergillus terreus* 2.**

Fig.4: Microphotograph of Endophytic fungi



***A-Aspergillus fumigatus* 1(Bar= 100µm); B- *Paecilomyces* sp. (Bar= 100µm); C-*Aspergillus niger* (Bar= 50µm); D- *Aspergillus terreus* 1(Bar= 50µm); E- *Aspergillus terreus* 2 (Bar= 20µm); F- *Aspergillus ochraceus* (Bar= 100µm); G- *Aspergillus flavus* (Bar= 20µm); H- *Aspergillus fumigatus* 2 (Bar= 50µm).**

DISCUSSION

Endophytic fungi are ubiquitous in nature. Each and every plant species examined to date have been found to colonize with fungal endophytes. A single plant species may harbor one to hundreds of endophytes which are distributed in all their tissues namely leaves, petioles, stems, twigs, bark, xylem, roots, fruit, flowers, and seeds [10]. In the present investigation, genus *Aspergillus* showed highest colonization frequency and consisted of eight different species with different strains in both the plant tissues. Maximum number of endophytes were obtained from leaf segments which is in accordance with the earlier reports that showed the dominance of endophytic fungal population in leaves compared to other tissues [11,12]. Dominance of endophytes in leaf tissues is due to their anatomical structure, supply of nutrient elements on which the endophyte depends and entry of endophytes through penetration, wounding and natural openings (stomata). Leaves interact mostly with external microbes, so it can harbor more isolates when compared to stem [13]. Abundance, diversity, species richness and frequencies of its occurrence vary by tissue type and age [14]. Similar results have been observed in present study also and most of the isolates were found both in stem and leaf tissues. Composition of isolates and frequencies of occurrence vary in same host plant [15]. Earlier no reports have been published in *Cucumis dipsaceus.* But endophytic fungi like *Chaetomium* sp. and *Aspergillus* sp. have been reported in *Cucumis sativus* L. seedlings which were collected from different soil samples [16].

CONCLUSION

Intensive research on endophytes have been carried out for more than a century but, the relationship behind host and the endophyte is yet to be explored. Almost all the plant species in earth harbor one to hundreds of endophytes, which can be screened through various

biological processes. The endophytes has the capability to alter the metabolism of host plants through their secondary metabolite production. These secondary metabolites can produce valuable antibiotics, biocontrol agents etc., which can be utilized in various fields like industries, agriculture, medicines etc., In the present study, some isolates have been screened from *Cucumis dipsaceus* which will be further evaluated for their secondary metabolite production.

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