

## Biodiversity of fungal endophytes from *coleus aromaticus* (benth.)

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### Abstract

All plants in natural ecosystems appear to be symbiotic with fungal endophytes. Herbal plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. The new prospective area on agriculture and forestry are the use of microorganisms to promote plant growth and to protect the plant hosts from pests and diseases. One group of the microorganisms is endophytic fungi. The research aims to isolate of fungal endophytes from *Coleus aromaticus*. *C. aromaticus* (Benth) belonging to the family Lamiaceae are well known for their therapeutic potentials. It is used in traditional medicine because of their antimicrobial, antioxidant, antiseptic and other pharmacological activities. In endophytic fungi presences of large amount of secondary metabolites or bioactive compounds. Many of these compounds are being used for the treatment of a number of diseases in this plant all ten fungal species were isolated by using Potato Dextrose Agar medium.

**Keywords:** *coleus aromaticus*, endophytic fungi, secondary metabolites, bioactive compound

### 1. Introduction

The use of local plants in medical practices has a long history. The resource base of the traditional medical practices prevalent in rural and tribal villages of India is mainly the plants. There is a need to search new ecological niches for potential of natural bioactive agents for different pharmaceutical, agriculture and industrial application; these should be renewable, eco-friendly and easily obtainable natural products discovery in the search for new drugs, and is the most potent source for the discovery of novel bioactive compounds. Therefore, a large number of bioactive compounds are isolated from the plants, bacteria, fungi and many other organisms. Endophytic fungi being the most promising of these have been a source of various such bioactive compounds. Many of these compounds are being used for the treatment of a number of diseases (Sandhu *et al.* 2014). Endophytic fungi that reside inside plant tissues without making any symptoms of disease to the host plant.

*Coleus aromaticus* also known as a *Plectranthusamboinicus*. It is large succulent aromatic perennial herb, *Coleus aromaticus* is an annual herb about 2 to 4 feet in height. An erect, glabrous or minutely pubescent, branched annual, up to 180 cm. tall, it is found throughout India (Rashmi *et al.* 2011). The leaves of the plant are bitter, acrid and were being widely used traditionally for various purposes. *C. aromaticus* is a common medicinal herb in India the leaves are used in treatment of common cold, cough and headache, Disorders of the digestive system are treated by using *C. aromaticus* for stomach pain, nausea, vomiting, and mouth infections (Mnish and Iffat, 2012) [15]. The plant has been worked out very well and isolated several chemical constituents and had shown various biological properties. The Lamiaceae plants are usually aromatic. This aromatic smelling medicinal plant contains many phytochemical components are presents. In the present study, it is an attempt to isolate endophytic fungi from the leaves of *Coleus aromaticus* collected from Botanical garden of Yeshwant

Mahavidhyalaya Nanded Maharashtra India. Endophytic fungi will be identified by using pathological literature.

### 2. Materials and Methods

#### Collection of plant Samples

For host species symptomless and apparently healthy individual plants were randomly chosen from their respective sites. The samples were collected in sterilized polythene bags.

#### Surface sterilization and incubation

Isolation of endophytic fungi was done according to the method described by (Petrini, 1986) [17, 18]. The plants samples were rinsed gently in running water to remove dust and debris. Leaves samples were cut into 0.5-1.0 cm long pieces. Each sample was disinfected with 75% ethanol for 1 min followed by immersion in Sodium hypochlorite (NaOCl 1-13% for 3-10 minutes, depending on the type of samples) and then once again in 75% ethanol for 30 seconds. The segments were then rinsed three times in sterile distilled water and the pieces were blotted-dry on sterile blotting paper. The efficiency of surface sterilization procedure was ascertained for every segment of tissues as per method of (Schulz *et al.* 1993). About 5-6 segments were placed on Potato dextrose agar (PDA) supplemented with streptomycin (100 µg ml<sup>-1</sup>) in aseptic condition. The petridishes were sealed with parafilm and incubated at 27±2oC for 4-6weeks. The Petridishes were monitored every day to check the growth of endophytic fungal colonies from the segments. Most of the fungal growth was initiated within 10 days of inoculation. The fungi that grew out from the segments were periodically isolated and identified by transferring the hyphal tips to fresh PDA plates.

Pure cultures were maintained on PDA slants. Endophytic fungi were identified according to their macroscopic and microscopic characteristics such as the morphology of fruiting structures and spore morphology.

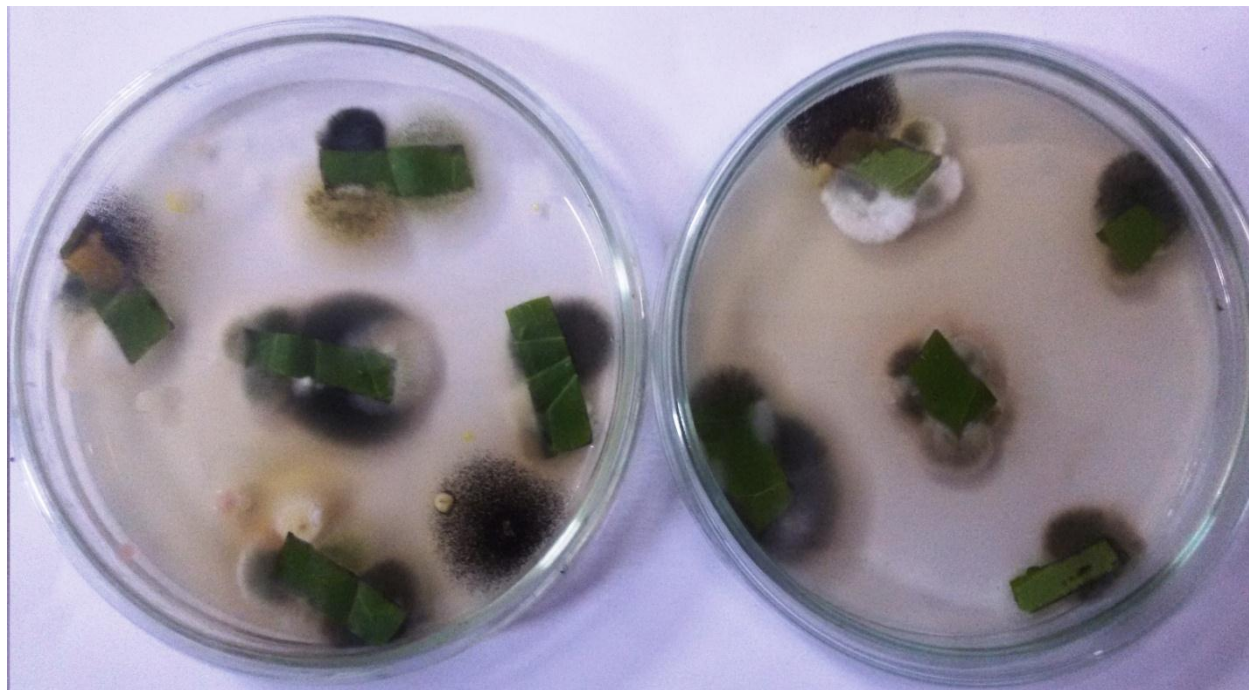
Colonization Frequency (CF) was calculated as described by (Suryanarayanan *et al.* 2003) <sup>[30]</sup>. Briefly proper time of incubation was given for CF counting.

$$\text{Colonization frequency of endophytes } 0/0 = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segment observed}} \times 100$$

Fungi were grown on specified media under specified culture condition, for identification. The fungi were identified on the basis of their morphological and cultural characteristics (Ellis, 1971; Kenneth *et al.* 1965 and Sutton, 1980) <sup>[32]</sup>.

### 3. Result

The present reaserch isolation of endophytic fungi occurs in *Coleus aromaticus* collected from Botanical garden of Yeshwant Mahavidyalaya, Nanded, Maharashtra, India. The study of endophyte biodiversity and their biochemical characteristics are of immense importance in plant biology to understand and to improve plant fitness. Ten species belonging to 12 genera of fungi were isolated during the present studies. Most dominant endophyte genera were *Aspergillus*, *fusarium sp* and *Alternaria*.



**Fig 1:** Isolation of endophytic fungi

**Table-1:** Fungal endophytes isolated from leaves of *Coleus aromaticus* and their colonization frequency.

Sr. No	Fungal Endophytes	Number of isolates	Frequency of Colonization (%)
1	<i>Aspergillus flavus</i>	6	10.0
2	<i>A. fumigatus</i>	3	05.0
3	<i>A.niger</i>	4	06.6
4	<i>A.nidulans</i>	5	08.3
5	<i>Alternaria alternata</i>	4	06.6
6	<i>Alternaria solani</i>	5	08.3
7	<i>Fusarium spp</i>	3	05.0
8	(unidentified mycelium)	1	01.6
9	(unidentified mycelium)	1	01.6
10	(unidentified mycelium)	1	01.6

Sixty segments were plated for analyzing the frequency in respectively

### 4. 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. Endophytic organisms have received considerable attention as they are found to protect their host against pest, pathogens and even domestic herbivorous (Weber, 1981) <sup>[34]</sup>. Only a few plants have been investigated for their endophytic flora and their potential to produce bioactive compounds. Some studies have been conducted about the endophytic biodiversity, taxonomy, reproduction, host ecology and their effects on host (Petrini, 1986 <sup>[17, 18]</sup>;

Redman *et al.* 2002 and Clay & Schardl, 2002) <sup>[8]</sup>. Currently, endophytes are considered as unexplored source of bioactive natural compounds. They have been found to play a crucial role in the production of beneficial chemical compounds and it is use as antimicrobial agents (Ananda K *et al.* 2012). Diverse endophytic population was detected to colonize this plant. Fungal strains of 10 different species were isolated.

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<sup>[34]</sup> Rezwana *et al.* 2007) <sup>[21]</sup>.

*Alternaria alternata*, *Aspergillus Niger*, *Aspergillus flavus*, *penicillium citrinum*, *mucor* (Blodgett *et al.* 2000; Suryanarayanan *et al.* 2002) [6, 29]. were isolated as endophytes. The present study which have been previously reported as endophytes. An endophytic fungus, *Pestalotiopsis terminaliae* was isolated from *Terminalia arjuna* produces taxol, an important chemotherapeutic drug used in the treatment of breast, lung and ovarian cancers (Gangadevi and Muthumary, 2009) [13]. *Aspergillus flavus*, *Trichoderma spp* and *Pestalotiopsis spp.* were isolated from *Carissa carandas* from central region of Madhya Pradesh, India (Rajesh *et al.* 2012) [20]. Similar studies were carried out and endophytic fungi like *Aspergillus sp*, *Alternaria sp* *Fusarium sp* were isolated during the present work.

## 5. Acknowledgement

We are very much thankful to the Principal of Yeshwant Mahavidyalya, Nanded for kind support and providing the facility for the present research work.

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