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Morphotaxonomic Study of Some Endophytic Fungi from Andrographis paniculata (Burm. f.) Wall. ex Neesfrom a few regions ofAmravati District

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Abstract:

Endophytic fungi contribute significantly to the production of bioactive substances with a variety of uses. The diversification of endophytic fungal communities linked to Andrographis paniculata (Burm. f.) Wall. ex Nees, a commonly used medicinal plant with well-known therapeutic benefits, is the main subject of this study. The study examined the colonization patterns of fungal endophytes isolated from diverse plant parts collected from different places in the Amravati district of Maharashtra, India.The identification of 10 fungal species*Alternaria alternata, Aspergillus flavus, Cladosporium longissimum, Colletotrichum gloeosporioides, Fusarium oxysporum, Fusarium solani, Penicillium spp., Curvularia lunata, Chaetomium spp., and Nigrospora spp.,*showed significant variations in the frequency of colonization across roots, stems, and leaves. *Aspergillus flavus*(70%), *Cladosporium longissimum* (50%), and*Fusarium oxysporum* (70%) had the highest colonization rates in stem, roots, and leaves. According to our results, the endophytic fungal population of A. paniculata is unique and diverse, and it may be a viable source of new bioactive chemicals.

Keywords: Endophytic Fungi, *Andrographis paniculata*, Colonization Frequency, Distribution

Introduction:

Endophytic fungi, are asymptomatic microorganisms that live inside plant tissues without causing harmful effects to the host plant and generate a variety of bioactive secondary metabolites with possible uses in industry, agriculture, and medicine. These havedrawn a lot of attention (Schulz et al., 2002; Strobel & Daisy, 2003). Endophytes are found ubiquitously in all plant species forming a communalistic or mutualistic association with respective plants, about 300,000 plant species on earth harbor one or more endophytes(Ejaz et al., 2020). Endophytes are found ubiquitously in all plant species forming a communalistic or mutualistic association with respective plants, about 300,000 plant species existing on earth harbor one or more endophytes(Ejaz et al., 2020). Endophytes can be isolated from various plant parts such as seeds, leaves, and stems. The collected plants for studying endophytic communities should look like healthy and disease-freeplants, i.e. they do not display any visual symptoms of diseases(Jariwala & Desai, 2018).In recent decades, the exploration of endophytic fungal diversity has become a promising approach for the discovery of novel natural products, including antibiotics, and anticancer agents(Kusari et al., 2012).

According to the World Health Organization, more than 80% of the world's population receives primary healthcare from traditional plant-based medicine, demonstrating the long history of using medicinal plants to treat human illnesses (Jena & Tayung, 2003).*Andrographis paniculata* (Burm. f.) Wall. ex Nees, commonly known as "Kalmegh" or "King of Bitters," is a widely used medicinal plant in traditional systems of medicine such as Ayurveda, Unani, and Traditional Chinese Medicine(Akbar, 2011). It belongs to the family Acanthaceae. It is used as a traditional herbal medicine in Bangladesh, China, India, Pakistan, Philippines, Malaysia, Indonesia, and Thailand and is ethnobotanically used for the treatment of snake bites, bug bites, diabetes, dysentery, fever, and malaria(Hossain et al., 2014). The study of endophyte distribution, biodiversity, and their biochemical characteristics are of immense importance in plant biology to understand and improve plant fitness (Selvanathan et al., 2011).This research contributes to the understanding of plant-microbe interactions in medicinal plants and highlights the unrealized potential of endophytic fungi for biotechnological applications.The diversity of fungal endophytes has been investigated in this ethnomedicinal plant and the same is discussed in this research article.

Material and Methods:

A. Collection of plants:

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Healthy leaves, stems, and root segments of *Andrographis paniculata* (Burm. f.) Wall. ex Neeswere collected from different localities of Amravati, district Amravati. The plant material was brought to the laboratory in sterile bags and processed immediately to reduce the chances of contamination.

B. Isolation of endophytic fungi:

The plant materials were taken and first rinsed in running tap water to remove the dust and other debris. Segments were cut by sterile lancet blades and surface sterilized by agitating in 70% ethanol (1min), followed by treatment with 4% NaOCl (90 s) and then rinsed in sterile distilled water (4 times). 40 (leaf, stem, and root samples) segments from the*Andrographis paniculata* (Burm. f.) Wall. ex Neesplants were processed for the isolation of endophytic fungi. Leaf, stem, and root segments were then placed onto Potato dextrose agar (PDA) amended with streptomycin. The Petri dishes were sealed using parafilm and incubated at 25°C. The fungi that grew out from the tissues were isolated and stocked. The cultures were maintained on PDA slants at 4°C for further screening(Rajeswari et al., 2017).

C. Identification of endophytic isolates:

The endophytic fungal isolates were identified up to the genus level based on morphological features such as colony morphology, pigmentation, growth pattern, spore structures, and other hyphal characteristics with the help of the standard mycological manuals. The microscopic examination was also done to study their reproductive spores. Cultures that failed to produce spores were grown on different minimal media and incubated for several weeks to months (Rana, 2008).

D. Statistical analysis:

Colonization frequency (CF)

To know the endophyte richness, the frequency of fungal endophytes harbored in plant species was calculated by the number of segments colonized by endophyte species divided by the total number of segments examined ×100 (Saranya et al., 2017).

$$\text{Colonization frequency of endophyte(CF\%)} = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segments observed}} \times 100$$

Results:

About 120 segments (40 segments of each part respectively) of the medicinal plant were screened for the isolation of the endophytic fungi. A total of 10 endophytic fungi were isolated and identified from the medicinal plant *Andrographis paniculata* (Burm. f.) Wall. ex Nees The leaf segments showed a greater repository for endophytic fungi than the root and stem segments. Among the 10 endophytic fungi, the predominant endophytic fungi isolated belonged to the genera *Alternaria alternata*,*Aspergillus flavusCladosporium longissimum.*,*Colletotrichum gloeosporioides, Fusarium oxysporum,* Tables (2,3and 4) showed the taxonomic position, colonization frequency,and abundance in particular plant parts of isolated endophytic fungi.The predominant endophytic fungi isolated belonged to the genera *Alternaria alternata, Aspergillus flavusCladosporium longissimum, and Fusarium oxysporum.*

Table: -1. Endophytic Fungi Isolated from Medicinal Plant (per sample) *Andrographis paniculata* (Burm. f.) Wall. ex Nees

Sr.No.	Site of Location	No. of Samples	No. of Fungi Isolated
1	Leaves	40	10
2	Root	40	09
3	Stem	40	09

Table: - 2. Taxonomic Position of Endophytic fungi isolated from medicinal plant *Andrographis paniculata* (Burm. f.) Wall. ex Nees

Sr.No.	Endophytic Fungi	Class
1	*Alternaria alternata*	Ascomycetes
2	*Aspergillus flavus*	Ascomycetes
3	*Cladosporium longissimum*	Ascomycetes
4	*Colletotrichum gloeosporioides*	Ascomycetes

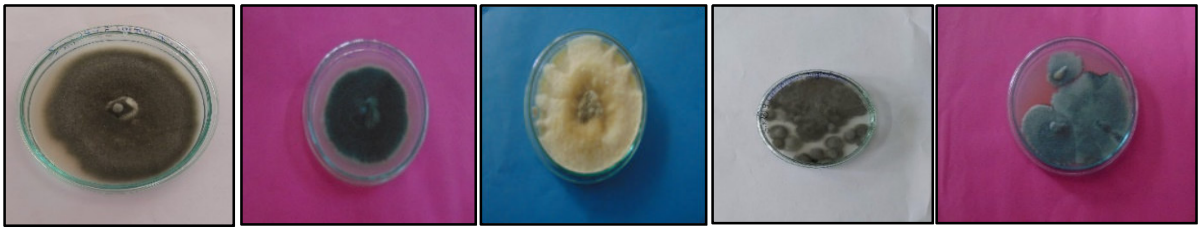
5	*Fusarium oxysporum*	Ascomycetes
6	*Fusarium solani*	Ascomycetes
7	*Penicillium spp*	Ascomycetes
8	*Curvularia lunata*	Ascomycetes
9	*Chaetomium spp*	Ascomycetes
10	*Nigrospora spp*	Ascomycetes

Table: -3. Endophytic fungi isolated from Leaves, roots, and stems from the medicinal plant *Andrographis paniculata*(Burm. f.) Wall. ex Nees

Sr.No.	Endophytic Fungi	Leaves	Roots	Stem
1	*Alternaria alternata*	+	+	+
2	*Aspergillus flavus*	+	+	+
3	*Cladosporium longissimum*	+	+	+
4	*Colletotrichum gloeosporioides*	+	+	+
5	*Fusarium oxysporum*	+	+	+
6	*Fusarium solani*	+	-	+
7	*Penicillium spp*	+	-	+
8	*Curvularia lunata*	+	+	-
9	*Chaetomium spp*	+	-	+
10	*Nigrospora spp*	+	+	-

Table: - 4. Colonization Frequency (CF%) of Endophytic fungi isolated from Leaves, roots, and stems from the medicinal plant *Andrographis paniculata* (Burm. f.) Wall. ex Nees

Sr.No.	Endophytic Fungi	(CF%) Leaves	(CF%) Roots	(CF%) Stem
1	*Alternaria alternata*	62.5	25	50
2	*Aspergillus flavus*	75	37.5	50
3	*Cladosporium longissimum*	70	50	25
4	*Colletotrichum gloeosporioides*	37.5	50	25
5	*Fusarium oxysporum*	50	50	62.5
6	*Fusarium solani*	50	25	37.5
7	*Penicillium spp*	70	37.5	50
8	*Curvularia lunata*	50	30	62.5
9	*Chaetomium spp*	50	-	75
10	*Nigrospora spp*	37.5	20	37.5



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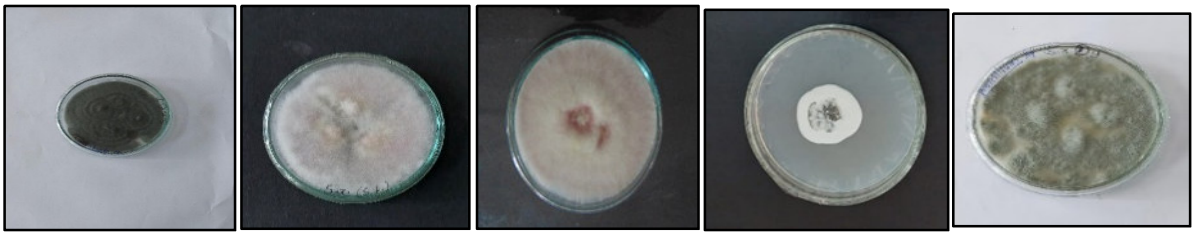

Fig: -Colony Morphology of Isolated Endophytic Fungi from Medicinal Plant *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Isolates name as mentioned in the table)

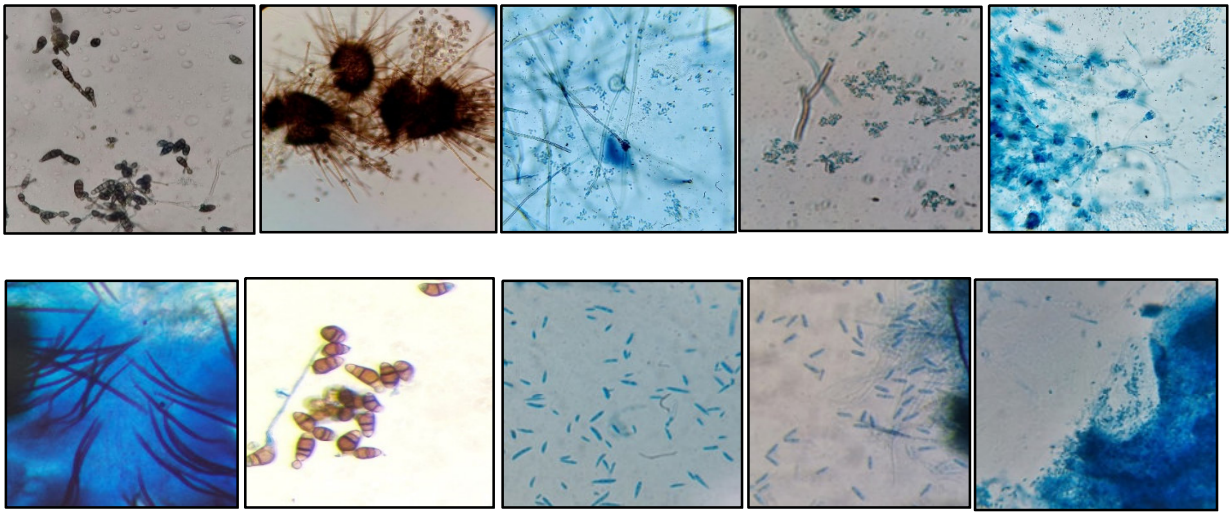

Fig: -Microscopic images of Isolated Endophytic Fungi from Medicinal Plant *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Isolates name as mentioned in the table)

Discussion:

This study was carried out to isolate and identify endophytic fungi from the Amravati district. In the study, a total of 10 fungal colonies were isolated from 120 segments. Among the 10 endophytic fungi, the predominant endophytic fungi isolated belonged to the genera.*Aspergillus flavus*, *Cladosporium longissimum*, and *Fusarium oxysporum* . The same results showed the presence of endophytic fungi in Avicennia officinalis, which isolated the endophytic fungi namely reported *Aspergillus*, *Penicillium*, *Curvularia*, *Cladosporium*, and *Fusarium* species(Gao et al., 2005).

Conclusion:

Medicinal plants are a good source for the isolation of endophytic fungi that colonize the tissue without causing apparent symptoms. Endophytic organisms have received considerable attention as they are found to protect their hosts against pests, pathogens and even domestic herbivores. In this study, a total of 10 endophytic fungi were isolated from the*Andrographis paniculata* (Burm. f.) Wall. ex Nees a well-known medicinal plant contains various chemical compounds. Isolation of endophytic fungi from this plant produces novel bioactive compounds.

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