# Isolation And Characterisation Of Active Fungal Endophytes From *Aegle Marmelos*

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## 1.0 INTRODUCTION:

Developing eco-friendly and sustainable methods for synthesizing metal nanoparticles has gained significant attention in recent years. Among the various metal nanoparticles, silver nanoparticles have been extensively studied due to their unique physicochemical properties, including antimicrobial, anti-inflammatory, and wound-healing activities, making them promising for various biomedical applications. The conventional chemical and physical methods for the synthesis of silver nanoparticles are often energy-intensive, costly, and involve the use of hazardous chemicals, which can have adverse environmental and health implications. (Nahar et al., 2015) (Hossain et al., 2019) To address these concerns, the use of biological systems, such as microorganisms, has emerged as a viable alternative for producing silver nanoparticles in an environmentally friendly manner. (Jeyaraj et al., 2014) (Nahar et al., 2015)

Fungal endophytes, have been identified as a great source for producing silver nanoparticles and are known to exist within plant tissues without appearing to cause any harm. These fungi can convert silver ions into metallic silver, which produces nanoparticles with distinct sizes and shapes. Furthermore, because endophytic fungi are easily cultivated, widely accessible, and don't require complicated extraction or purification procedures, using them to synthesize nanoparticles has several benefits.

In this research paper, we aim to explore the synthesis and characterization of silver nanoparticles using endophytic fungi and the preparation of nano-emulsions incorporating these nanoparticles.

Fungal endophytes, residing within plant tissues without causing visible harm, represent a ubiquitous and often overlooked facet of plant biology. Far from being passive inhabitants, these microscopic fungi engage in complex interactions with their hosts, influencing a wide array of plant processes, from growth and development to defense against pathogens and herbivores. Despite their inconspicuous nature, the pervasive influence of fungal endophytes demands a thorough understanding of their diversity, ecology, and evolutionary history. This review delves into the multifaceted world of fungal endophytes, exploring their roles as hidden drivers of plant health, resilience, and ecological interactions. This vast and often overlooked group of fungi plays a crucial role in plant ecology and evolution, shaping plant-microbe interactions, and community dynamics, and even influencing the success of invasive species. The intimate association between endophytes and their hosts has sparked a burgeoning field of research, unveiling the multifaceted nature of these symbiotic relationships. One of the primary areas of interest lies in understanding the mechanisms by which endophytes colonize their hosts. While traditional methods for detecting endophytic fungi rely on invasive techniques such as direct isolation or molecular detection recent advancements have explored non-invasive approaches. The impact of endophytes on plant physiology and defense has garnered significant attention. Remarkably, the presence of specific endophytes - enhanced the growth of adapted neighbors while suppressing the growth of evolutionary naive neighbors, highlighting the intricate role of endophytes in shaping plant community dynamics. Beyond their ecological implications, endophytic fungi have emerged as a promising source of bioactive compounds with potential applications in agriculture and medicine. This finding underscores the potential of endophytes as biocontrol agents and sources of novel antimicrobial compounds.

*Aegle marmelos,* also known as “Bael” belongs to Rutaceae. It has the potential to play a vital role in primary medical care. It has been into use for over 5000 years by various ethnic populations that live in the Indian subcontinent. In the Indian traditional medicine system, it has the potential to treat various ailments and diseases and also has various bioactive compounds present in them. The several components are used for their various medicinal properties like anaemia, wound healing, swollen joints, etc. They are reported to have chemical compositions like alkaloids, coumarins, terpenoids, etc. This plant also can cure various fungal

## 2.0 METHODOLOGY:

### 2.1 Collection of plant samples:

i] (*Aegle marmelos*): Ten (10) symptomless leaf samples were collected from CSIR-IIM, Jammu & Kashmir & local areas of Jammu in January 2023. All of these samples were then collected in clean and sterilized bags and to keep the leaves fresh, the bark of the leaves was inserted in wet sponges. Also, these bags were punched with small holes for proper aeration. Later on, these samples were brought to the Student Research Laboratory at Sharda School of Allied Health Sciences, Sharda University.

The surface of the leaves and stems was properly sterilized. They were washed with running tap water for almost 10 minutes to remove dirt and debris. They were then allowed to air dry for another 15 minutes.

### 2.2 Media Preparation and autoclaving:

Potato Dextrose Agar [PDA] was prepared to isolate and grow fungal endophytes from the plant. This prepared media and Petri plates were autoclaved for 45 minutes at 121℃.

### 2.3 Isolation of fungal endophyte from *Aegle marmelos:*

The surface sterilization of leaves was done with purified water to lower the microbial load from the sample. It was also washed with 70 % ethanol. The plant sample(leaves) was first dipped properly in distilled water and was kept like that for 5 minutes. Later the leaves were allowed to air dry. These leaves were then dipped in 70% ethanol for approximately 3-5 minutes, followed by air drying. The leaves were again dipped in distilled water for 5 minutes and were then allowed for air drying. The surface of the leaves was sterilized with sodium hypochlorite (NaOCl) and 75% ethanol. These steps were carried out in a Laminar air Flow chamber. With the help of a sterilized blade, leaves were cut into small pieces.

These cut leaves are then inoculated into the Potato Dextrose Agar medium (PDA). The plates were incubated at 25 degrees Celsius for 4-5 days. The plates were then sealed with parafilm to avoid desiccation and contamination. Later, hyphal tips were transferred to fresh media to obtain pure cultures for further identification.

### 2.4 Preservation of isolated endophytes:

The purified fungal isolates were aseptically transferred separately to potato dextrose slants at 4 degrees Celsius.

### 2.5 Identification of fungal endophytes:

All the isolated strains were identified with the help of lactophenol cotton blue staining and were observed under 40 x resolution.

### 2.6 Colonizing Frequency of plant endophytes:

The colonizing frequency of each fungal endophyte was calculated –

$$CF\ (\%) = \frac{\text{Number of plant segments colonized by single fungus}}{\text{The total number of plant segments observed}} \times 100$$

## 3.0 RESULTS AND DISCUSSION:

### 3.1 Collection of samples:

10 samples were collected from different locations in Jammu & Kashmir.

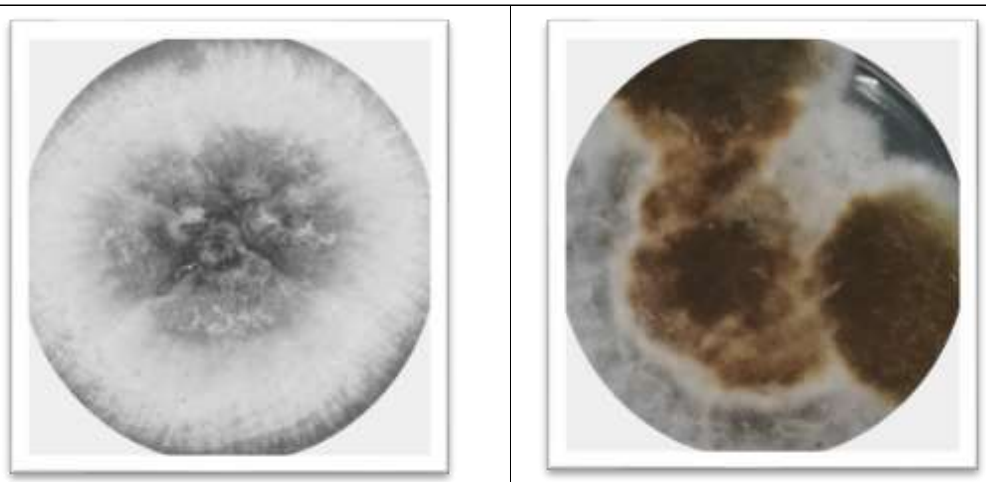
### 3.2 Isolation and characterization:

Endophytes were isolated on Potato Dextrose Agar. Proper identification and characterization of these isolated endophytes were carried out. The identification was done based on the appearance, morphology, hyphae, and mycelial arrangement.

**Table 1- Identification and characterization of endophytic fungi**

| **Location** | **Number of Isolates** | **Species** |
|---|---|---|
| Jammu & Kashmir (Location 1) | 2 | *Alternaria spp.* (1), *Aspergillus spp.* (1) |
| Jammu & Kashmir (Location 2) | 3 | *Alternaria spp.* (1), *Muscodor spp.* (1), *Fusarium spp.* (1) |
| Jammu & Kashmir (Location 3) | 2 | *Alternaria spp.* (1), *Aspergillus spp.* (1) |
| Jammu & Kashmir (Location 4) | 3 | *Fusarium spp.* (1), *Ulocladium spp.* (1), *Rhizopus spp.* (1) |

Various strains of endophytic fungi that were isolated from different locations- *Alternaria spp.* (3), *Aspergillus spp.* (2), *Fusarium spp.* (2) and *Ulocladium spp.* (1), *Muscodor spp*. (1), *Rhizopus spp.* (1).



**(a)** **(b)**

**Figure 1: (a) This shows the endophytic growth of *Alternaria spp.* (b) This shows the endophytic growth of *Muscodor spp.***

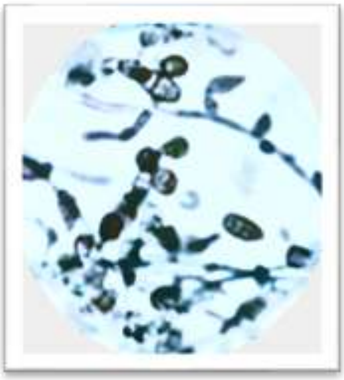
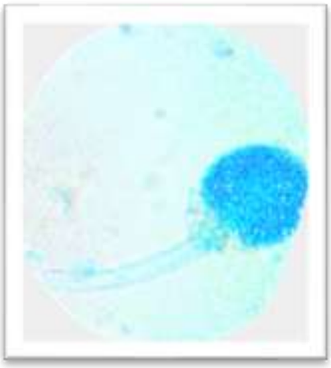
| | |
|---|---|
|  **(a)** |  **(b)** |

**Figure 1: (a) This shows the endophytic growth of *Alternaria spp.* (b) This shows the endophytic growth of *Aspergillus spp.***

**3.3Calculation of Colonization Factor:**

**Table 2: Calculating the colonization factor percentage**

| **Location** | **Part of Plant** | **CF %** |
|---|---|---|
| Location 1 | Leaves | 40 % |
| Location 2 | Leaves | 30 % |
| Location 3 | Leaves | 60 % |
| Location 4 | Leaves | 20 % |
| Location 5 | Leaves | 30 % |

The highest colonization frequency was observed by Location 3 (60%), followed by Location 1, Location 2, and Location 5 (30%). The lowest value was observed by Location 4 (20%).

**4. CONCLUSION:**

This study's results demonstrated that various endophytic fungi occupy an ecological niche in the natural regions inhabited by the medicinal plant *Aegle marmelos*. As a result, 10 fungal strains were identified by ITS sequence analysis after being isolated from healthy *Aegle marmelos* leaves. These endophytes exhibited a range of actions that encouraged plant growth. Among these, the ability to enter tissues is provided by making various interstitial lytic enzymes, including pectinase, amylase, cellulase, and catalase. Additionally, the fungal strains showed activity against many pathogenic microorganisms, potentially enhancing plant tolerance to infections.

The various endophytes isolated from several ethnomedicinal plants can exhibit scavenging effects at different levels. It is seen that the most effective strains can be exploited as the reservoir of novel bioactive molecules. Later on, this research can help focus on various other associated factors. At this point, we have more precise knowledge about things like this group of fungi's enormous diversity, where they are found in different plant tissues and compartments, how they vary seasonally, and how the structure & composition of the population of endophytes vary and evolve along with their host plants.

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