



ENDOPHYTIC FUNGI ASSOCIATED WITH *BALANITES AEGYPTIACA* (L.) Delile

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ABSTRACT

During present study observed the diversity of endophytic fungi present in plant parts of *Balanites aegyptiaca* (L.) Delile like leaves, stems, roots and fruits. Plant parts were surface sterilized and cultured on potato dextrose agar for fungal growth. A total number of 144 segments were analysed, out of which 78 showed fungal growth, giving an overall colonization frequency of 54.16%. About 18 fungal taxa were identified based on morphological characteristics such as *Alternaria*, *Aspergillus*, *Fusarium*, *Cladosporium*, *Chaetomium*, *Cylindrocephalum*, *Phoma*, *Penicillium*, *Nigrospora*, *Trichoderma*, *Curvularia*, *Rhizoctonia*, *Drechslera* and *Bipolaris*. Leaves showed the maximum colonization frequency (66.67%) followed by stems, fruits and roots. The results showed that *Balanites aegyptiaca* (L.) Delile hosts diverse fungal endophytes that may have potential for producing biologically active compounds.

KEYWORDS: Endophytic fungi, *Balanites aegyptiaca* (L.) Delile, colonization frequency, fungal diversity.

INTRODUCTION

Endophytic fungi are live in internal plant tissues during at least a part of their life cycle without causing any disease to the host plant. These fungi form ecological relationships with plants which frequently contribute to the survival and adaptability of their hosts. In recent years, endophytic fungi have gained increasing scientific attention due to their remarkable ability to produce a wide variety of secondary metabolites with important biological activities.^[1] Endophytic fungi can enhance plant growth, improve tolerance to environmental stresses and provide protection against pathogen.^[2] Many compounds produced by endophytic fungi possess significant pharmacological properties such as antimicrobial, anticancer, antioxidant and anti-inflammatory activities. Because of these characteristics endophytic fungi associated with plants are considered valuable sources for the discovery of novel natural products and bioactive compounds.^[3]

Balanites aegyptiaca (L.) Delile commonly known as the desert date. This plant belongs to the family Zygophyllaceae and is widely distributed in arid and semi-arid regions of Africa, the Middle East and parts of

Asia. Different plant parts including leaves, fruits, seeds and stem have been used in traditional medicine to treat several diseases such as infections, inflammation, digestive disorders and metabolic diseases.^[4]

Numerous studies have investigated the pharmacological and phytochemical properties of *Balanites aegyptiaca* (L.) Delile relatively little information is available regarding the diversity of endophytic fungi associated with this plant. Understanding the microbial communities inhabiting medicinal plants is important because these microorganisms may also produce biologically active compounds similar to those found in their host plants.^[5] Therefore, the present study aims to isolate endophytic fungi from *Balanites aegyptiaca* (L.) Delile. These fungal endophytes may provide valuable insights into the microbial diversity associated with this medicinal plant and could lead to the discovery of bioactive metabolites with pharmaceutical and biotechnological applications.

MATERIALS AND METHODS

Collection of Plant Material

Healthy and disease-free plant parts of *Balanites aegyptiaca* (L.) Delile including fruit, leaves, stem and root were collected from Morryapuram, Shirur (Pune) MH, India. The collected plant samples were placed in polyethylene bags and transported to the laboratory under hygienic conditions for further study.^[6, 7]

Surface Sterilization of Plant Parts

Balanites aegyptiaca (L.) Delile plant materials were washed thoroughly under running tap water to remove dust and debris. The plant parts were then immersed in 70% ethanol for about 1 minute, followed by treatment with 1% sodium hypochlorite solution for 2 minutes. Then plant parts were rinsed 2-3 times with sterile distilled water. Finally, the sterilized plant parts were placed on sterile filter paper under aseptic conditions before culturing.^[8,9]

Isolation of Endophytic Fungi

After surface sterilization *Balanites aegyptiaca* (L.) Delile plant parts were cut into 1cm segments under sterile condition. Inoculate segments into plates containing Potato Dextrose Agar (PDA) medium. The inoculated plates were incubated at 25-27°C under controlled laboratory conditions for 5-10 days. During incubation period fungal hyphal growth emerging from the internal plant tissues were observed. Colonization frequency (CF %) was calculated as described by^[10, 2] Briefly, proper time of incubation was given for colonizing frequency counting. Colonization Frequency (CF) was calculated using the formula.

$$CF (\%) = (\text{Number of colonized segments} / \text{Total number of segments}) \times 100$$

Each distinct fungal colony appearing on the medium was carefully transferred to fresh culture plates for further purification.^[6,1] To obtain pure cultures, individual fungal colonies were subculture on PDA plates. Pure culture of fungi was maintained on PDA slants and stored under refrigeration for subsequent analysis.^[11]

Identification of Fungi

Identification of the isolated endophytic fungi was carried out based on macroscopic and microscopic characteristics. The identification of fungi was performed by comparing the observed morphological features with standard taxonomic descriptions and identification manuals.^[12, 13]

RESULTS AND DISCUSSION

Endophytic fungi were isolated from different tissues of *Balanites aegyptiaca* (L.) Delile including leaves, stems, fruits and roots. The plant sample were collected from Shirur (Pune). A total of 18 fungal taxa were recovered from the examined plant parts. Leaves showed the highest number of endophytic fungi compared with other plant parts. About 14 endophytic fungi were isolated

from leaves, stem, fruit and roots segments of plants such as *Penicillium sp.*, *Alternaria alternata*, *Aspergillus terreus*, *Aspergillus ochraceus*, *Phoma sp.*, *Fusarium solani*, *Cladosporium sp.*, *Chaetomium globosum*, *Nigrospora sphaerica*, *Trichoderma sp.*, *Curvularia lunata* and *Rhizoctonia sp.* During present study leaves exhibited the maximum diversity and colonization frequency of endophytic fungi. The maximum diversity observed in leaves may be related to their exposure to environmental spores which increases the chances of fungal colonization. Previous studies have reported that leaf-associated endophytes from medicinal plants are particularly sources of bioactive compounds with potential pharmaceutical applications.^[2] *Alternaria sp.*, *Curvularia sp.*, *Cladosporium sp.*, *Chaetomium sp.*, *Phoma sp.*, were reported from leaves segment of *Balanites aegyptiaca* (L.) Delile.^[1, 14]

Stem segments of *Balanites aegyptiaca* (L.) Delile yielded 12 endophytic fungi such as *Aspergillus terreus*, *Penicillium sp.*, *Fusarium solani*, *Chaetomium globosum*, *Nigrospora sphaerica*, *Trichoderma sp.*, *Mycelia sterilia*, *Bipolaris sp.*, *Cylindrocephalum sp.*, *Aspergillus flavus* and *Drechslera sp.* El-Bondkly et al., 2020 also reported endophytic fungi from stem bark such as *Aspergillus flavus*, *Nigrospora sphaerica*, *Fusarium oxysporum*, *Penicillium sp.*, from *Balanites aegyptiaca* (L.) Delile. The presence of endophytic fungi in stems indicates that vascular tissues provide a suitable internal environment for fungal colonization. Stem endophytes may contribute to plant health by producing metabolites that inhibit pathogen and enhance plant resistance to environmental stress.^[2]

Fruit segments contained 09 endophytic fungi, although the diversity was slightly lower than that observed in leaves and stems. Commonly reported fruit endophytes include *Penicillium sp.*, *Fusarium solani*, *Cladosporium sp.*, *Phoma sp.*, *Nigrospora sphaerica*, *Curvularia lunata*, *Aspergillus ochraceus* and *Aspergillus fumigatus*. Fruits contain sugars and other nutrients that may support fungal growth, but they also produce antimicrobial compounds that limit excessive colonization. Endophytic fungi present in fruits may contribute to the production of protective metabolites that pre-vent infection by pathogenic organisms. In some cases, these fungi are also capable of producing enzymes that assist in the degradation of plant tissues during fruit maturation.^[15] El-Bondkly et al., 2020 reported *Nigrospora sp.*, *Curvularia lunata*, *Fusarium sp.*, *Penicillium sp.*, *Aspergillus sp.* From fruit of *Balanites aegyptiaca*.^[16]

Root segments showed the presence of 07 fungal isolates, though the diversity was generally lower compared to aerial parts of the plant. Fungi recovered from roots including *Fusarium solani*, *Chaetomium globosum*, *Trichoderma sp.*, *Rhizoctonia sp.*, *Mycelia sterilia*, *Bipolaris sp.*, *Aspergillus flavus*, and *Drechslera sp.* Root-associated endophytes are known to play an

important ecological role in nutrient acquisition and plant health. They may assist the host plant in improving tolerance to environmental stress, including drought and soil-borne pathogens. Studies on medicinal plants have shown that root endophytes frequently produce enzymes and metabolites that contribute to plant defense and growth promotion.^[6,1] Kusari et al., reported *Fusarium solani*, *Rhizoctonia* sp., *Drechslera* sp., *Chaetomium* sp from *Balanites aegyptiaca* (L.) Delile.^[14]

When comparing the different plant parts, leaves generally exhibited the highest colonization frequency and fungal diversity, followed by stems, fruits and roots. The genus *Aspergillus* was particularly common, represented by several species such as *A. terreus*, *A. flavus*, *A. ochraceus* and *A. fumigatus*. Other frequently

occurring genera included *Fusarium*, *Penicillium*, *Trichoderma* and *Nigrospora*. The greater diversity in aerial tissues may be attributed to increased exposure to airborne fungal spores and favourable environmental conditions for colonization. The presence of diverse fungal endophytes in *Balanites aegyptiaca* (L.) Delile suggests a complex symbiotic relationship between the host plant and its associated microorganisms. Endophytes isolated from *Balanites aegyptiaca* (L.) Delile may represent promising sources of novel bioactive molecules for pharmaceutical and agricultural applications.^[1,17] Overall, the results indicate that different tissues of *Balanites aegyptiaca* harbor diverse endophytic fungi, with leaves and stems showing the greatest diversity.

Table 1: Colonization Frequency of Endophytic Fungi from *Balanites aegyptiaca* (L.) Delile

Plant Part	Total Segments Plated	Segments with Fungal Growth	Colonization Frequency (%)
Leaves	36	24	66.67%
Stems	36	20	55.56%
Fruit	36	18	50.00 %
Roots	36	16	44.45%
Total	144	78	54.16 %

Table 2: Endophytic fungi reported from *Balanites aegyptiaca* (L.) Delile plant parts.

Sr. No.	Name of Endophytic Fungi	Leaves	Stems	Root	Fruit
1	<i>Aspergillus terreus</i> Thom	+	+		
2	<i>Penicillium</i> sp.	+	+		+
3	<i>Fussarium solani</i> (Mert.) App	+	+	+	+
4	<i>Alternaria alternata</i> (Fr.) Keissl.	+			
5	<i>Cladosporium</i> sp.	+			+
6	<i>Phoma</i> sp.	+			+
7	<i>Chaetomium globosum</i> Kunze and Schmidt	+	+	+	
8	<i>Nigrospora sphaerica</i> (Sacc.) Mason	+	+		+
9	<i>Trichoderma</i> sp.	+	+	+	
10	<i>Curvularia lunata</i> (Wakker) Boedijn.	+			+
11	<i>Rhizoctonia</i>	+		+	
12	<i>Mycelia sterilia</i>	+	+		
13	<i>Aspergillus ochraceus</i> K.Wilhelm	+			+
14	<i>Aspergillus flavus</i> Link	+	+	+	+
15	<i>Cylindrocephalum</i> sp.		+		
16	<i>Bipolaris</i> sp.		+	+	
17	<i>Aspergillus fumigatus</i> Fresenius.		+		+
18	<i>Dreshslera</i> sp.		+	+	

CONCLUSION

The present investigation revealed that various parts of *Balanites aegyptiaca* (L.) Delile support a wide range of endophytic fungi. In total, 18 fungal taxa were isolated from the leaves, stems, fruits and roots of the plant. The level of colonization differed among the plant parts. Leaves showed the highest colonization frequency followed by stems, fruits and roots. Such differences may be related to factors such as exposure to the environment, availability of nutrients, and structural characteristics of the plant tissues. The fungal isolates obtained in this study belonged to several commonly occurring fungi including *Aspergillus*, *Fusarium*,

Penicillium, *Trichoderma* and *Nigrospora*. These genera are frequently reported as endophytic fungi in many plant species. The higher occurrence of *Aspergillus* species suggests that members of this genus are well adapted to survive and grow within internal plant tissues. The results of the present study indicate that *Balanites aegyptiaca* (L.) Delile can act as an important source of endophytic fungi. These endophytes may play a role in enhancing the biological and medicinal value of the host plant through the production of various secondary metabolites. Further studies are needed for molecular characterization of the isolated fungi and for the investigation of their antimicrobial, antioxidant, and

other bioactive properties, which may have potential applications in biotechnology and pharmaceutical research.

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