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ISOLATION OF ENDOPHYTES FUNGI FROM PROSOPIS JULIFLORA LEAVES

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Abstract
Prosopis juliflora (commonly known as mesquite) belongs to fabaceae family. It is a thorny evergreen tree with a large, flat-topped crown and open canopy; this report aimed to investigate the isolation of endophyte fungi from Prosopis juliflora leaves, a hardy leguminous shrub known for its adaptability to arid environments. The research explored the ecological and economic significance of Prosopis juliflora and the roles played by endophyte fungi in plant's health and ecology and to lay the foundation for exploring their functional roles and bioactive potential. Endophyte fungi are microorganisms that colonize the internal tissues of living. These fungi enhance host resistance against abiotic stress and disease by producing various bioactive.Notable fungi, including Alternaria alternata, Aspergillus nidulans, Aspergillus sydowii, and Trichoderma harzianum, were isolated and characterized from mesquite's leaves. The study highlights the diverse presence of endophyte fungi, indicating potential contributions to nutrient uptake, stress tolerance, and disease resistance. This research provides future prospectives for a deeper exploration of the specific roles of identified fungi, particularly Trichoderma harzianum and Aspergillus nidulans, providing valuable insights for ecological studies and potential agricultural applications. Overall, the study underscores the significance of endophyte fungi in supporting the resilience and vitality of Prosopis juliflora in various environments.

INTRODUCTION

Prosopis juliflora, commonly known as mesquite, is a perennial leguminous shrub native to Mexico, South America and has adapted to a wide range of arid and semi-arid climate. The significance of *Prosopis juliflora* lies in its ability to thrive in various scientific disciplines. it grows in harsh

and challenging environments, including regions with limited water resources and poor soil conditions. Its deep root system enables it to access groundwater, contributing to its resilience in arid landscapes. Moreover, *Prosopis juliflora* shows nitrogen-fixing capabilities through its symbiotic relationship with nitrogen-fixing bacteria, which enhances soil fertility and supports surrounding vegetation [1].

Beyond its ecological adaptations, *Prosopis juliflora* holds economic importance in many regions. The plant has been utilized for forage, wood, and traditional medicines by local communities. Additionally, its pods serve as a valuable source of nutrition for livestock, further emphasizing its multifaceted significance [2].

However, endophyte fungi, residing within plant tissues without causing apparent harm, have emerged as key players in shaping plant health, adaptation, and ecological interactions. These fungi are crucial for biodiversity by maintaining to overall ecosystem dynamics. Several research studies have shed light on the multifaceted roles of endophyte fungi in promoting plant health and influencing ecological processes.

Endophyte fungi are known to facilitate nutrient uptake by plants, including essential elements such as nitrogen and phosphorus. Through symbiotic relationships, these fungi contribute to the improved nutrient status of their host plants, enhancing their growth and development [3]. Additionally, they have been associated with increased tolerance of plants to environmental stressors, such as drought and salinity [4].

Research indicates that certain endophyte fungi play a crucial role in protecting plants from pathogens. These fungi can produce secondary metabolites with antimicrobial properties, acting as a defense mechanism against harmful microbes and potentially enhancing the plant's resistance to diseases [5].

Endophyte fungi have been linked to the promotion of plant growth and productivity. They can produce growth-promoting substances, such as auxins and cytokinins that influence plant growth and development positively. This aspect is of particular interest for sustainable agriculture and the potential reduction of reliance on synthetic fertilizers [6].

Endophytes contribute to the overall biodiversity of ecosystems. They participate in complex ecological interactions, including mutualistic relationships with plants and other microorganisms. Understanding these interactions is crucial for unraveling the dynamics of natural ecosystems [7].

Certain endophyte fungi assist plants in adapting to challenging environmental conditions, such as extreme temperatures or nutrient-poor soils. These adaptations contribute to the resilience of plant communities in diverse ecological niches [8]. The present study focused on the isolation of endophytic fungi from surface-sterilized leaves of *Prosopis juliflora*.

1. Materials and Methods

1.1 survey and collection of *Prosopis juliflora* leaves

Fresh leaves of *Prosopis juliflora* were collected from Plant Pathology Department, university of Karachi. Leaves were selected from different parts of the plant to ensure representation across various developmental stages.

1.2 Surface sterilization of collected leaves

Surface sterilization of collected leaves was carried out by washing them with 70% ethanol for 1 minute, followed by a rinse with sterile distilled water. Subsequently, the leaves were immersed in a 5% sodium hypochlorite solution for 2-3 minutes to remove surface pathogens and then rinsed thoroughly with sterile distilled water to remove chemical residues.

1.3 preparation of culture medium

According to Westphal (2021), a custom-made culture medium was made by boiling 200g of peeled potatoes in distilled water with a slight bubbling, filtering the resulting potato extract through muslin cloth, adding 20g of sucrose, and then cooling the potato broth before adding 16g of agar to prevent the agar from clotting in the media, distilled water was added to makeup 1-liter media. The media was transferred into an autoclaved flask, and kept at 121°C and 15 psi for 15 minutes. Before pouring, the media

were left to cool down and the media was amended with streptomycin sulphate (0.2 g/liter) and benzyl penicillin (100,000 units/liter), then poured into 90 mm Petri plates.

After that, surface-sterilized leaves of *Prosopis juliflora* were cut into smaller sections (approximately 1 cm^2) using sterile scalpels and forceps. The leaf segments were placed on the prepared culture media in Petri dishes. Each Petri dish. The plates were covered with Parafilm tape to prevent contamination from airborne spores.

2.4 Isolation and Purification of the endophype fungi:

In order to isolate, purify, identify, and conduct further tests, the endophyte fungal hyphae growing colonies were repeatedly sub-cultured on new PDA media plates after being cut from the margin of growing colonies. The isolation process was conducted under controlled environmental conditions. The temperature was maintained at 25-28°C representing optimal conditions for the growth of endophyte fungi. Humidity was regulated to prevent contamination.



Fig 1 # Different endophype fungi isolated from leaves of *Prosopis juliflora*

2.5 Preservation of endophyte fungi culture

Cultures that had been isolated from leaves of *Prosopis juliflora* were preserved in PDA slants. A disc of agar containing fungal mycelium from pure culture was put into the PSA slant and stored at 15oC after being filled with 10 ml of sterilized PDA medium and plugged with cotton wool.

2.6 Identification of endophyte fungi

Fungi were grown on PDA medium with 2% water agar at 26°C 2 for two weeks to investigate cultural morphology. Light microscopy was used to analyze microscopic characteristics, which were then compared to a taxonomic key (Barnett & Hunter, 1972). Eleven different endophype fungi isolate from leaves of mesquite.

By employing these materials and methods, the study aimed to obtain a diverse collection of endophyte fungi from *Prosopis juliflora* leaves, facilitating subsequent analyses and characterization.

2. Results and Discussion

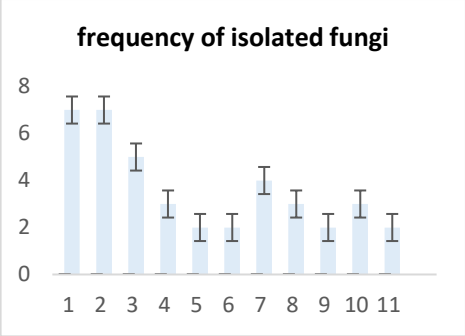
Table 1: Morphological Characteristics of Isolated Endophyte Fungi. [11] [12]

Fungal Species	Colony Appearance	Hyphal Structures	Spore Morphology
Alternaria sp. 1	Greenish-black, fluffy	Septate, branched hyphae	Conidiophores with multiple chains of spores
Alternaria sp. 2	Olive-green, velvety	Septate, branched hyphae	Brown, muriform conidia
Alternaria alternata	Dark brown, woolly	Septate, dichotomously branched hyphae	Oblong, multicellular conidia
Alternaria tenuissima	Grayish-green, powdery	Septate, branched hyphae	Ellipsoidal to ovoid conidia
Aspergillus sydowii	Green to yellow-green, powdery	Septate, conidiophores with vesicles	Globose conidia in chains
Aspergillus nidulans	Bluish-green, powdery	Septate, conidiophores with vesicles	Globose conidia in chains
Chaetomium sp.	White to gray, velvety	Septate, mycelium with perithecia	Asci containing ascospores in perithecia
Cladosporium sphaerospermum	Black, olive to brown, velvety	Septate, branched hyphae	Brown to black, spherical conidia
Trichoderma harzianum	White to yellow, cottony	Septate, branched hyphae, conidiophores	Globose to pyriform conidia
Nigrospora sp.	Dark brown to black, powdery	Septate, branched hyphae	Dark brown, ovoid to ellipsoidal conidia
Drechslera sp.	Grayish-brown, velvety	Septate, mycelium with conidiophores	Brown, curved conidia in chains

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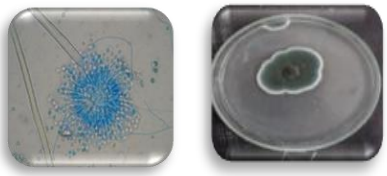
Table 2: Endophyte Fungi Isolation from Leaves of *Prosopis juliflora.*

S. no	Fungus isolated	No. of isolations
1	*Trichoderma harzianum*	7
2	*Aspergillus nidulans*	7
3	*Nigrospora sp.*	5
4	*Alternaria alternata*	3
5	*Alternaria sp1*	2
6	*Alternaria sp2*	2
7	*Alternaria tenuissima*	4
8	*Aspergillus sydowii*	3
9	*Chaetomium sp.*	2
10	*Cladosporium sphaerospermum*	3
11	*Drechslera sp.*	2



a. Aspergillus sydowii

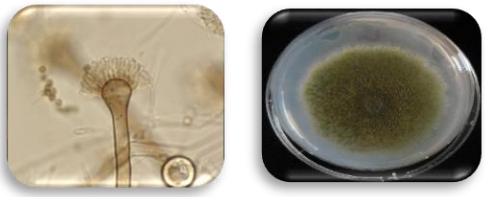
Aspergillus sydowii has fast-growing colonies with powdery appearance. Its conidiophores formation is Vesiculate and uniseriate. Whereas Conidia is formed in chains, typically smooth-walled. Conidia sizes range of 2.5 to 4.5 micrometers in diameter.



Fig#2 *Aspergillus sydowii*

b. Aspergillus nidulans

A. nidulans contains fast-growing colony and typically green in color. Conidiophores are uniseriate and arise from the vesicle. Conidia are produced in chains and are usually globose or ellipsoidal. Conidiophores are uniseriate, meaning conidiophores are arranged in a single row. Conidia are typically smooth-walled, initially green and turn brown with age. Conidial heads can have a characteristic radiate arrangement. The conidia size falls within a range of 3.5 to 5.5 micrometers in diameter.



Fig#3 *Aspergillus nidulans*

c. Alternaria tenuissima

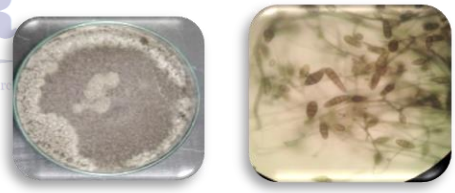
Colonies are fast-growing, initially white or pale, and later turning dark olive to black color. Mycelium is septate, and conidiophores are brown, branched, and bear conidia. Conidia are typically ellipsoidal or obclavate. The size of conidia ranges from approximately 20-60 µm in length and 7-15 µm in width. Branched conidiophores arise from the substrate and bear conidia on short branches.



Fig#4 *Alternaria tenuissima*

d. Alternaria alternata

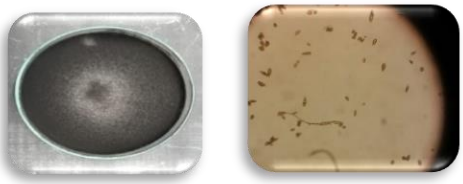
Colonies are typically dark brown to black with a velvety or woolly texture. Mycelium is septate, and conidiophores are branched, brown, and bear conidia. Conidia are typically multicellular, dark, and typically have a beak-like or elongated shape. The size of conidia is variable, ranging from approximately 15-25 µm in length. Branched conidiophores arise from the substrate and bear conidia on short branches.



Fig#5 *Alternaria alternata*

e. Alternaria sp. 1

Colonies are typically dark brown to black with a velvety or woolly texture. Mycelium is septate, and conidiophores are branched, brown, and bear conidia. Conidia are typically multicellular, dark, and typically have a beak-like or elongated shape.The size of conidia is variable, with a length ranging from approximately 20-50 µm and a width ranging from 5-10 µm.The beak length ranges from approximately 5-12 µm. Branched conidiophores arise from the substrate and bear conidia on short branches.



Fig#6 *Alternaria sp. 1*

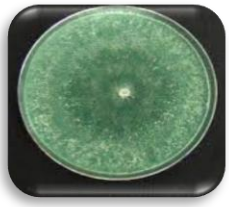
f. *Alternaria sp.2*

Colonies are typically dark brown to black with a velvety or woolly texture. Mycelium is septate, and conidiophores are branched, brown, and bear conidia. Conidia are typically multicellular, dark, and typically have a beak-like or elongated shape.The size of conidia is variable, with a length ranging from approximately 15-29 µm and a width ranging from 7.5-12.5 µm.The beak length ranges from approximately 2.5-7.5 µm. Branched conidiophores arise from the substrate and bear conidia on short branches.

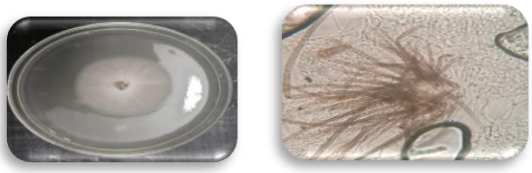


Fig#7 *Alternaria sp. 2*

g. *Chaetomium sp.*



Colonies are typically fast-growing, initially white and cottony, later becoming dark-colored and powdery.The mycelium is septate, and it produces characteristic perithecia. Flask-shaped structures containing asci and ascospores. Simple or branched conidiophores may also be present, producing asexual spores (conidia). Asexual spores (conidia) are typically fusiform or ellipsoidal in shape. Conidia Length: approximately 10 to 30 micrometers. Conidia Width: approximately 5 to 15 micrometers.



Fig#8 *Chaetomium sp.*

h. *Drechslera sp.*

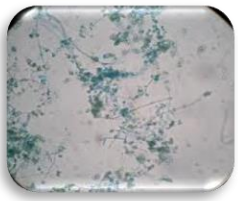
Colonies are fast-growing and typically have a cottony or velvety texture.Mycelium is septate, and conidiophores arise from the substrate. Asexual spores (conidia) are produced on conidiophores. Conidia are usually dark, multicellular, and may have a distinctive shape. Branched conidiophores bearing conidia on short branches. Conidia are typically multicellular, dark Conidia Length: ranging from approximately 20 to 100 micrometers. Conidia Width: generally around 5 to 15 micrometers.



Fig#9 *Drechslera sp.*

i. *Tricoderma harzianum*

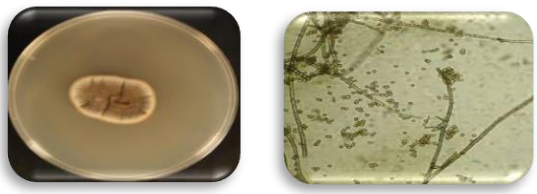
Colonies are fast-growing, usually white, and have a cottony or woolly appearance. Mycelium is septate, and conidiophores are typically branched and bear conidia. Asexual spores (conidia) are typically hyaline, smooth-walled, and produced on conidiophores. Conidia are often borne in distinctive structures called phialides. Branched conidiophores with phialides that produce conidia. Colonies can turn green or other colors with age due to sporulation. Conidia Length: Typically around 3 to 4.5 micrometers. Conidia Width: Typically around 2 to 3 micrometers.



Fig#10 *Tricoderma harzianum*

j. *Cladosporium sphaerospermum*

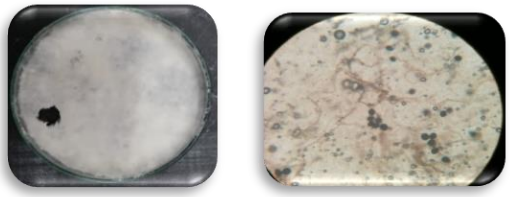
Colonies are typically dark green to olive-brown in color. Mycelium is septate, and conidiophores are usually branched. Conidia are typically dark, single-celled, and spherical to ellipsoidal in shape. Conidia are usually borne in chains. Branched conidiophores with conidia produced at the tips of short branches. Conidia Diameter: Typically ranges from approximately 2 to 5 micrometers.



Fig#11 *Cladosporium sphaerospermum*

k. *Nigrospora sp.*

Colonies are often fast-growing and may appear initially white, becoming darker with age. Mycelium is septate, and conidiophores are branched. Conidia are typically dark-colored, single-celled, and may have a distinctive shape. Conidia are borne on conidiophores. Branched conidiophores with conidia produced at the tips of short branches. Conidia Length: Typically ranges from approximately 20 to 60 micrometers. Conidia Width: generally around 5 to 10 micrometers.



Fig#12 *Nigrospora sp.*

3. Discussion

The study on the isolation of endophyte fungi from *Prosopis juliflora* leaves has revealed valuable insights into the intricate relationship between this hardy shrub and its microbial inhabitants. The ecological significance of *Prosopis juliflora* in arid and challenging environments, attributed to its nitrogen-fixing capabilities and deep root system [13], underscores its adaptability and resilience. Furthermore, the economic importance of *Prosopis juliflora* in diverse regions for forage, wood, and traditional medicines emphasizes its multifaceted role in supporting local communities [14].

Turning our attention to endophyte fungi, the findings align with existing research on the multifaceted roles they play in plant health and ecology. Enhanced nutrient uptake and tolerance, as demonstrated by the isolated endophytes, echo previous studies indicating the contribution of these fungi to improved nutrient status and stress resistance in plants [15][16]. The observed biocontrol and disease resistance properties of certain endophytes, such as *Aspergillus nidulans*, resonate with literature emphasizing their role in protecting plants from pathogens [17].

The improvement of plant growth and productivity, evident in the isolated *Trichoderma harzianum* and other species, corresponds with previous research highlighting the growth-promoting substances produced by endophytes, offering potential applications in sustainable agriculture [18]. The ecological interactions and biodiversity implications of endophytes underscore their contribution to the overall dynamics of ecosystems, aligning with the broader understanding of these intricate relationships [19].

The environmental adaptation exhibited by certain endophytes, assisting plants in coping with challenging conditions, resonates with studies emphasizing their role in enhancing the resilience of plant communities in diverse ecological niches [20].

In comparison with similar studies, the diversity of endophyte fungi isolated from *Prosopis juliflora* leaves corresponds with findings in other plant species [21][22]. Noteworthy commonalities with previous research include the presence of Alternaria alternata, Aspergillus nidulans, and Trichoderma harzianum, suggesting a consistent association of these fungi with various host plants.

However, further investigations are warranted to delve into the specific roles of these identified fungi, especially *Trichoderma harzianum* and *Aspergillus nidulans*, which emerged as common inhabitants. A comparative analysis with studies elucidating the functions of these fungi in plant growth and environmental adaptation would contribute to a deeper understanding of their significance [23].

4. Conclusion

This study revealed the presence of diverse endophyte fungi from the leaves of, with notable

isolations including *Alternaria alternate, Aspergillus nidulans, Aspergillus sydowii, Trichoderma harzianum,* and others. These fungi, residing within the plant without causing harm, are like unseen plant assistants, contributing to nutrient uptake, stress tolerance, and potential disease resistance. This shrub tree can be used to isolate endophytes in order to use them as bio -control agents against plant pathogens.

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