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Characterization of some Endophytic fungi Isolated from some halophytes

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Abstract Endophytic fungi are widely present in internal plant tissues and provide different benefits to their host. Halophytes have unexplored diversity of functional fungal association; therefore this work aimed to isolate and identify some endophytic fungi present in certain halophytes growing in coastal regions of the Mediterranean Sea during April 2022. In the present work, ten isolated fungal endophytes belonging to Ascomycota and Mucoromycota with six different genera, *Penicillium*, *Alternaria*, *Fusarium*, *Cladosporium*, *Mucor* and *Aspergillus*, were obtained from healthy selected four halophytes. These fungal endophytes have unique appearance in colony unit on agar media which range from dark to white. The colonies that are produced by various species of fungi have irregular or failamentous in form. The isolated endophytic fungi were elevated or nmbonate in terms of elevation, and their margins were curled, whole, lobate, and filiform. The findings are important because isolated fungal species can effectively promote plant development in environments under stress.

keywords: Endophytic fungi, *halophytes*, Wild plants; Desert; Isolation.

1. Introduction

Endophytic fungi are microorganisms that may be found in a wide variety of plant tissues above and below ground, including the stems, leaves, and roots of healthy plants. It is estimated that there are over a million different species of endophytic fungi in the wild [1,2]. Endophytic fungi have been found on plants from a wide variety of habitats, including hot deserts, Arctic tundra, mangroves, temperate and tropical forests, grasslands, savannas, and croplands [3].

The long-standing interaction between plants and fungi involves many different kinds of fungi. Only around 10% of all fungi are able to colonize living plants, yet they nonetheless represent a key group of plant pathogens responsible for the vast majority of plant diseases [4]. There are a lot more kinds of fungus than people realize, but only a small fraction of them are plant relatives. Most fungi are decomposers, meaning they thrive on the decaying matter of other organisms. Other types of connections include the roles that fungi play as decomposers, beneficial symbionts, and endophytes, which invade plants in a stealthy manner[6 ،5] .

Endophytes are gaining prominence in biotechnology and industry, due to their ability to secrete antiviral compounds, act as biocontrol agents, antimicrobial agents, antitumor agents, and immunosuppressants, .As well as, they produce natural antioxidants, antidiabetic agents, antibiotics, and insecticidal products [7-9]. In order to colonize plant surfaces, endophytes must produce a plethora of enzymes that aid in the hydrolysis of the plant cell wall. These enzymes aid in the degradation of fungal cell walls and the indirect destruction of phytopathogens. When a pathogen tries to colonize the host tissue, endophytes use competition as a powerful defense [10].

Halophytes are plants that can grow and reproduce in environments with salt concentrations of 200 mM NaCl or more and tolerate salt concentrations that kill 99% of other species (Jiménez-Becker et al., 2019). In addition, halophytes have specific

morphological and anatomical traits, as well as physiological processes, that make them well-suited to saline environments (Saharan et al., 2022). In the course of our study, we concentrated on isolating and identifying fungal endophytes that are found in certain halophytes that are found in coastal areas of the Mediterranean Sea.

2. Materials & Methods

2.1. Plant material

The parts of halophytes species were collected during the flowering stage from different habitats in coastal area Egypt (Delta coast) for endophytes isolation. Healthy mature green plants of *Arthrocnemum macrostachyum, Atriplex halimus, Atriplex portulacoides,* and *Bassia indica*, each plant sample divided into four parts; shoot, leaf, flower and root. After placing each specimen in its own sterile bag, we transported the bags to the lab so that we could begin the analysis procedure. Our laboratory was able to correctly identify the plant species, verify their authenticity, and care for them.

2.2. Isolation and Purification of endophytic fungi

After cutting the samples from the shoot and the root into several pieces that each had a diameter of roughly 5 millimeters, the pieces were washed under running water. For the surface sterilization of the pieces, they were first submerged in 70% ethanol for one minute, then in sodium hypochlorite containing 2% available chlorine v/v for three minutes, and finally in 70% ethanol for thirty seconds. After that, they were given two washes in sterile water that had been distilled .

In every single Petri dish, there were 10 pieces of each organ in addition to 250 mg/l of chloramphenicol. Five identical copies of each organ sample were created (in Petri plates). After that, the plates were placed in an incubator at 262°C [4].

After one week, the growing hyphae of the segments were transferred to a clean PDA Petri dish so that they could be purified. The endophytic fungi that were isolated were propagated by subculturing them once a month and storing them on PDA slants. After being incubated for five days at a temperature of 28 degrees Celsius, the slants were then placed in storage at a temperature of four degrees Celsius.

3. Results and Discussion

Even though they are one of the most important components of plant micro-ecosystems and should have a significant influence on the growth and development of hosts plants, our knowledge of the specific interactions that occur between endophytic fungi and their host plants is still infancy state.

3.1. Isolation of endophytic fungi

Endophytic fungi were isolated from apparently healthy halophytes samples growing in costal habitats. As illustrated in Figure 1, the isolation was performed on three components (root, stem, and leaf) of two fresh plant samples.

3.2. Purification of endophytic fungi

Ten species of endophytic fungi were isolated from the four parts of some halophytes collected from different habitats (Delta coast) as shown in Table 1 and Figure 1.

3.3. Morphological characterization of isolated endophytic fungi

A technique used by scientists to define the properties of a specific colony of fungus growing on agar in a Petri dish is called colony morphology. It may be utilized to assist in locating them

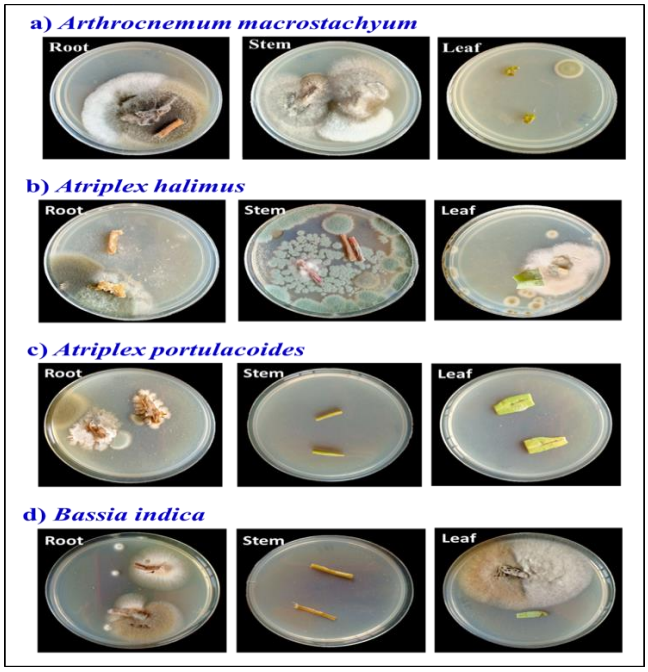

Figure 1. Isolation of endophytic fungi from some halophytes in coastal desert habitats.

Table 1: Numbers of endophytic fungi isolated from some halophytes in coastal desert habitats.

Fungus name	Arthrocnemummacrostachyum				Atriplex halimus				Atriplex portulacoides				Bassia indica			
	Root	Stem	Leaf	Flower	Root	Stem	Leaf	Flower	Root	Stem	Leaf	Flower	Root	Stem	Leaf	Flower
Alternaria brassicicola	*															
Penicillium notatum									*							
Penicillium digitatum									*							
Fusarium oxysporum	*	*							*						*	
Cladosporium oxysporum	*															
Alternaria helianthi					*											
Alternaria alternata		*														
Mucor mucedo							*									
Aspergillus flavus													*		*	
Aspergillus fijiensis									*							

Table 2. Morphological characterization of isolated endophytic fungi from halophytes species growing in coastal habitats.

No.	Fungus name	Colony tint		Form	Elevation	Margin
		Surface	Reverse			
1	*Alternaria brassicicola*	Dark olivaceous brown	Green	Irregular	Raised	Curled
2	*Penicillium notatum*	Greenish blue	Orange	Irregular	Raised	Entire
3	*Penicillium digitatum*	Dark green	Orange	Irregular	Nmbonate	Lobate
4	*Fusarium oxysporum*	White cottony	Whitish orange	Failamentous	Raised	Filiform
5	*Cladosporium oxysporum*	Greenish olive	Brownish	Failamentous	Raised	Filiform
6	*Alternaria helianthin*	Whitish brown	Green	Irregular	Nmbonate	Filiform
7	*Alternaria alternata*	Greenish olive	Dark olive	Irregular	Raised	Curled
8	*Mucor hiemalis*	Grayish white	Yellowish	Failamentous	Corvex	Filiform
9	*Aspergillus flavus*	Light yellowish green	Yellow	Failamentous	Raised	Filiform
10	*Aspergillus fijiensis*	Black	White	Failamentous	Raised	Filiform

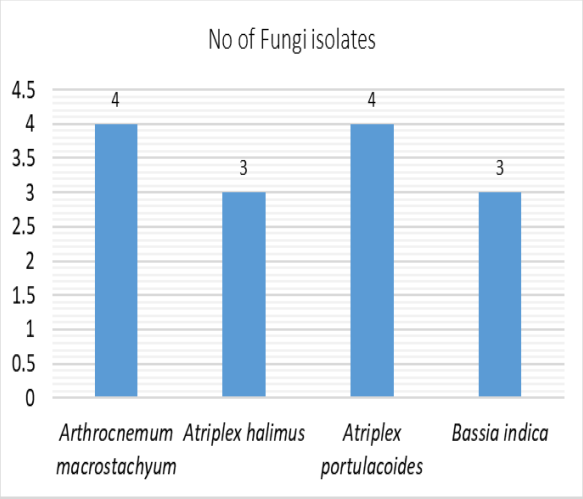

Figure 2. Numbers of endophytic fungi e isolated from some halophytes in coastal desert habitats.

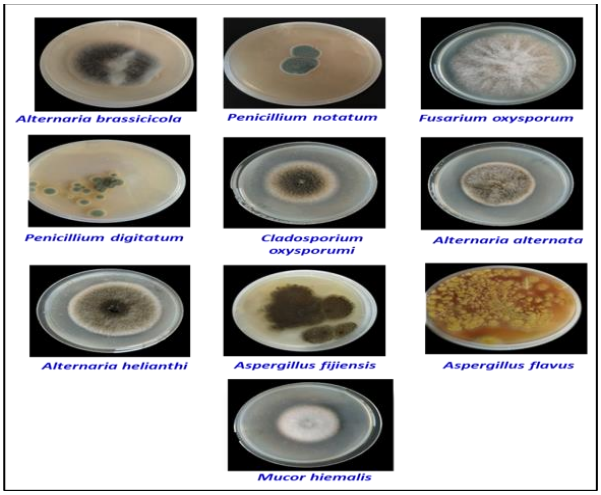

Figure 3. Purification of endophytic fungi isolated from some halophytes occupied in coastal desert habitats

Different kinds of fungi will form colonies that appear differentiality from another one; some colonies may be colored, while others may be irregularly shaped or round. Common colony characteristics such colony colour, shape, height, and border are described using specialized terms, as illustrated in Table 2

Conclusion

Halophytes, the plants that can survive in extreme salty environments. These plants have a capacity to be atolerant salt,due to many eco-physiological processes. Thus, 10 fungal isolates were isolated from i sections of healthy halophytes(root,stem,leaf and flower) and identified

depending upon morphological characteristics In a prospective study, the biological activities of such these endophytic fungi could be carried out ,to illustrate how to exploit these fungi to be effective in some biotechnological processes

4. References

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