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Isolation and Screening of Endophytic Microorganisms Isolated from Leaves of Three Medicinal Plants Growing in Marsa Matrouh

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Abstract: ***This study aimed to investigate the biodiversity of endophytic microorganisms isolated from leaves of Lycium shawii, Urginea maritima and Nicotiana glauca medicinal plants growing in Marsa Matrouh. After 21 days incubation period on different media viz potato dextrose agar, starch casein agar, nutrient agar and yeast peptone dextrose solid media. The results showed twenty one endophytic microorganisms have been isolated from the three selected plants. Fungal endophytic isolates were dominant isolates with eight isolates followed by actinomycetes, yeast and bacteria with 6, 5 and 2 isolates, respectively.***

Keywords: Endophytes – medicinal plants - *Lycium shawii -Urginea maritima - Nicotiana glauca.*

1. Introduction

Endophytes are microorganisms that inhabit plant hosts for all or part of their life cycle. They colonize the internal plant tissues beneath the epidermal cell layers without causing any apparent harm or symptomatic infection to their host, living within the inter-cellular spaces of the tissues and its seems that they may penetrate the living cells **(Strobel & Daisy, 2004).**

Endophytes are hidden in plant tissues, but they have important implications for communities, agriculture and biodiversity. In addition, certain endophytes are an excellent model system for studying interactions between organisms. No plant is an island. Each plant is a community, including diverse types of microorganisms **(Stainely & William, 2016)** The study was carries in one of the richest area in the Egyptian desert with the medicinal plants, Marsa Matroh, the region has typical Mediterranean flora with the weather being between pleasant and hot during summer and mild in winter. The coastal land, with its various ecosystems, offers important sites for natural resources and their development.
Three medicinal plants known for their traditional medicinal and economic importance in the local area were selected for study. These plants are *Lycium shawii, Urginea maritime* and *Nicotiana glauca*. Endophytes were isolated from these plants.

2. Materials and Methods

Plant Samples
Three healthy medicinal plants species were collected from, Marsa Matrouh, during spring season April, 2013. The plant species were identified in the field by Dr. Atia Eisa, lecturer of plant Taxonomy, Damanhur University and by Department of Ecology and Taxonomy in the Desert Research Center.
Leaves tissues were collected and placed in sterile polyethylene bags, labeled, transported in ice box to the laboratory, and placed in a refrigerator at 4^{o}C for further investigations. All plant samples were processed within 24 hrs. of collection.

Isolation of endophytes from plant samples
Plant samples were washed thoroughly in distilled water, blot dried, and immersed in 75% ethanol (v/v) for 2min, followed by immersion in sodium hypochlorite NaOCl, 5.3% (v/v) for 5min.They were rinsed three times in changes of sterile distilled water and dried on sterile filter paper under aseptic condition. Fragments of 2 mm size were excised with the help of a sterile blade. The fragments of plant samples were placed on sterile petri-dishes contain different isolating media (mention before). The plates were wrapped in clean parafilm wrap and incubated at 27 ^{o}C for 21 days **(Saini, Dudeja, & Kumar, 2015)**

3. Results and Discussion

Twenty one (21) endophytic microorganisms have been isolated from three medicinal plant samples growing in Marsa Matrouh as recorded **in Table (1)**. All endophytic microorganisms were isolated from leaves tissue of the three plant samples on their specific media. All colonies of different forms and colors showing separate growth on media were picked up as recorded in **Table (2), Plate (1).** Consecutive transfers and technical purification steps were

carried out for the isolated microorganisms under investigation according to the recommendation of **Colleins and Lyne (1989)**.

Table 1: Plant name, family and GPS location with site

Plant	Family	Location	Site
Lycium shawii	Solanaceae	N 31° 21' 20" ,E27° 10' 22"	Ageba Plateau
Urginea maritime	Liliaceae	N31° 25' 28", E26° 58' 32"	International Costal Road
Nicotiana glauca	Solanaceae	N 31° 22' 40", E 27° 4' 22"	El-Kasr Road

Table 2: Plant name, endophytic isolate code, medium used, part of plant used in isolation and type of endophytic microorganism

Plant Name	Isolate number	Medium used	Plant part	Isolate Taxa
Urginea maritime	UM1	PDA	LEAF	F
	UM2	PDA	LEAF	F
	UM3	SC	LEAF	A
	UM4	SC	LEAF	A
	UM5	PDA	LEAF	F
	UM6	PDA	LEAF	F
Nicotiana glauce	N1	NA	LEAF	B
	N2	NA	LEAF	B
	N3	NA	LEAF	A
	N4	PDA	LEAF	F
	N5	YPD	LEAF	Y
	N6	YPD	LEAF	Y
	N7	YPD	LEAF	Y
	N8	PDA	LEAF	F
	N9	SC	LEAF	A
Lycium shawii	LS1	SC	LEAF	A
	LS2	YPD	LEAF	Y
	LS3	SC	LEAF	A
	LS4	YPD	LEAF	Y
	LS5	PDA	LEAF	F
	LS6	PDA	LEAF	F

Where: LS_n, *Lycium shawii*; AM_n, U_n, *Urginea maritima*; N_n, *Nicotiana glauca*, n (1, 2, 3…) code of isolates, Y, yeast isolates; B, Bacterial isolates; A, Actinomycetes isolates and F fungal, isolates.

Most isolates were obtained from *Nicotiana glauca* (9 isolates) followed by *Lycium shawii* (6 isolates) and *Urginea maritima* (6 isolates) as recorded in **Table (3).**

Based on previous results, differences in endophytes between various plants and tissue might be related with the hosts' phytochemistry **(Cohen, 2013).** It has been known that medicinal plants used in the treatment of various diseases for several ages, so it contains a bioactive compound (**Niero *et al.*, 2008**). While host plant and it's endophytic microorganisms are symbionts, in which host plant and endophytes benefit from each other, host plant provide nutrition and protection to their endophytes, in return, endophytes excrete function product to host and increase their resistance to biotic and/or abiotic stresses. (**Zhang *et al.*, 2006**).

Rudgers *et al.* (2007) suggested that plant phenolic compounds influenced the community of endophytes. So the higher the content of active compound in host plant, the more richness with endophytic microorganisms.

In view of the present result, *Nicotiana glauca* (9 isolates), *Lycium shawii* (6 isolates) and *Urginea maritima* (6 isolates) have medical significance according to their content of active constituents such as phenols, flavonoids and carbohydrates that make them suitable host for endophytes **(Panghal *et al.*, 2011**).

In this study, three medicinal plants have been collected from Marsa Matrouh. They were selected for the isolation of endophytes on the basis of medicinal importance and availability. All the medicinal plants in this study were found to colonize with various endophytes, similar trend was observed by previous researchers where **Selim *et al.* (2011)** isolate 132 endophytic species from 18 medicinal plants growing in Saint Katherine, Egypt and **Sette *et al.* (2015)** recovered a total of 42 endophytic actinomycetes from different organs of seven selected medicinal plants. Only 56 endophytic isolates have been isolated from some medicinal plants growing in Saint Katherine by **Hesham *et al.* (2015)**.

This achieve the fact of endophytes existence in all growing plants that stated by **Strobel *et al.* (2004)** who indicated that " *Endophytes are to be found in virtually every plant on earth. They reside in the living tissues of the host plant and do so in a variety of relationships ranging from symbiotic to pathogenic*".

Endemic plants in desert habitat exposed to difficult climatic conditions of high temperature and water scarcity and poor nutrition of sandy soil necessary for the development of plant. This desert habitat and its flora led to a significant variation in endophytes and their biological properties **(Strobel, 2002 and Banerjee, 2014)**. It was previously suggested that, differences in chemical composition of soil could influence the endophytic microbial communities **(Shulkla *et al.*, 2014).** Furthermore, many biological and environmental factors affected the endophytic populations in plants, such as plant cultivar, plant age, tissue type and time of sampling **(Prabavathy & Valli, 2015).**

Diverse endophytes found in plants, representing a rich resource of bioactive natural products with potential for utilization in pharmaceutical and agricultural fields **(Shekhawat, Rao, & Batra, 2013)**. The colonization and propagation of endophytes may in some ways offer important benefits to their host plants by producing excess of substances that provide protection or increase the fitness of the hosts, such as detraction of stress-, insect- or disease-resistance, and productivity progress (**Tan & Zou, 2001; Banerjee, 2014**). However, it is thought that most of the endophytes diversity remains to be discovered.

In the present investigation, fungi were dominant endophytes with a number of 8, while actinomycetes, yeast and bacteria endophytic microorganisms gave 6, 5 and 2 respectively as

showed in **Figure (2)**.

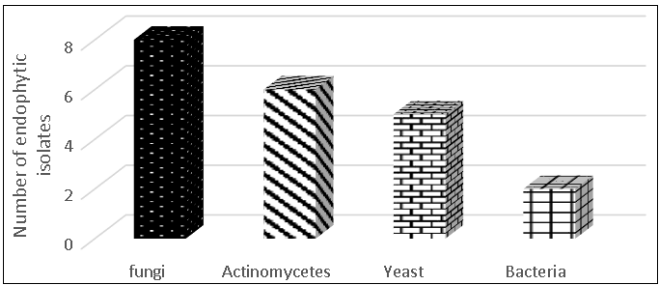

Figure 2: Total number of endophytes (fungi, actinomycetes, yeast and bacteria present in three medicinal plants

In view of the result of other investigators**,** fungal endophytes were dominant endophytic microorganisms in medicinal plants. Plants and fungi are the chief source of natural compounds used for medicine, in which medicinal plants and endophytes have attracted considerable interest and most attention for their wide variety of bioactive metabolites. Endophytic fungi represent an important and quantifiable component of fungal diversity. **(Krings *et al.*, 2007 and Selim *et al.*, 2011)**.

In the view of the present work, bacteria gave the least number of other taxa. This may be due to the addition of Nystatin which may be has inhibitory effect on bacterial isolate. In view of the findings of other authors, Growth of bacterial endophytes in the presence of antibiotic in medium may reveal that these bacterial endophytes have special resistance to antibiotic (**Saikkonen *et al.*, 1998; Bryn & Strobel, 2003)**

The plant tissues especially leaves are excellent reservoirs for endophytic microorganisms **(Qin *et al.*, 2011; Kandpal *et al.*, 2012)**. This may be because of *plant leaves* are the part of the shoot system in the angiosperms which serve as the primary food-producing organ with richness of bioactive primary compound which can be used as substrate for different endophytic microorganisms. Successful achievement of endophytic microorganism isolation from leaves of leaf tissue was strong evidence on success of the surface sterilization procedures. It is proposed that the epiphytic microbes were completely removed. Ethanol and sodium hypochlorite are often used in plant surface sterilization procedures which build suitable settings to isolate and characterize endophytic microorganisms that was in accordance with **(Cai *et al.* 2004)**

On the other hand **Bhagat *et al.* (2015)** has another opinion that root segments are predominantly inhabited by endophytes that ascribed to the ability of endophytes to enter into the host plant mainly from openings or wounded parts of the plant.

4. Conclusion

In the systematic investigation reveals a wide diversity of endophytes from medicinal plants. Endophytes are shown to be a rich source of bioactive natural products.

5. Acknowledgement

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