MANSOURA JOURNAL OF BIOLOGY

Official Journal of Faculty of Science, Mansoura University, Egypt

E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Isolation and Characterization of Fungal Endophytes from Medicinal Plant *Reichardia tingitana*

Basma S. El-Nabawy, Hoda M. Soliman, El-Sayed F. El-Halawany, and Yasser A. El-Amier

Botany Department, Faculty of Science, Mansoura University, Mansoura - 35516, Egypt

**** Correspondence to:** yasran@mans.edu.eg; Tel. +201017229120*

Received:22/6/2022
Accepted: 18/7/2022

Abstract *Reichardia tingitana* is a glabrous annual herb growing wildly in the coastal and inland desert of Egypt. The current study intended to evaluate the biological potency, and characterize the chemical constitutes of the extracted *Reichardia tingitana* shoots by standared methods. In this study, thirteen species of endophytic fungi were isolated from the four parts of *Reichardia tingitana* and from the soil supporting the growth collected from different habitats (Delta coast and inland desert). 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. Different kinds of fungus will form colonies that appear different from one another; some colonies may be colored, while others may be irregularly shaped or round. Common colony kinds including colony hue, morphology, height, and colony edge are described using specialized language.

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

1. Introduction

Endophytic fungi are many polyphyletic groupings of microorganisms that may live asymptomatically in the stems, leaves, and/or roots of living plants as well as in other healthy plant tissues above and/or below the earth. More than a million endophytic fungus species are thought to exist in nature [1,2]. Plants in scorching deserts, Arctic tundra, mangroves, temperate and tropical forests, grasslands and savannas, and croplands have all produced endophytic fungus [3].

Numerous varieties of fungus are involved in the long-standing relationship between plants and fungi. Fewer than 10% of all known fungus can colonize live plants, making fungi a significant category of plant pathogens that cause the majority of plant illnesses [4]. Fungus that are related with plants make up a relatively modest portion of the total number of fungi. The majority of fungus are decomposers that feed on the leftovers of dead plants and other living things. The function of fungi as decomposers, as advantageous symbionts, and as endophytes cryptic plant colonizers are examples of other forms of relationships [5, 6].

Due to their capacity to secrete antiviral compounds, act as Bio control agents, antimicrobial agents, antitumor agents, and immunosuppressants, as well as the production of natural antioxidants, antidiabetic agents, antibiotics, and insecticidal products, endophytes are becoming more important in biotechnology and industry [7-9]. Endophytes must create a large number of enzymes that help the hydrolysis of the plant cell wall in order to colonize the surface of plants. These enzymes assist in the indirect reduction of phytopathogens as well as the breakdown of fungal cell walls. Endophytes exploit competition as a potent defense against pathogen colonization of the host tissue [10].

Only two *Reichardia* species, *R. picroides* (L.) Roth and *R. tingitana* (L.) Roth, were identified in the Egyptian flora. The latter is a glabrous annual plant that grows freely in Egypt's interior and coastal desert. False sowthistle, mrara, maknn, murr, halawla, and huwwa are some of its common names [11]. The plant has a taproot that may grow up to 40 cm long and produces a rosette of huge radical leaves initially. The stem branches out from the

base, and its flowering period lasts from March to May [12]. In desert wadis, the Mediterranean area, Southwest Asia, Tropical East Africa, and Australia, *Reichardia tingitana* grows naturally [12, 13]. Our research focused on isolation and identification of fungal endophytes from medicinal plant *Reichardia tingitana* which thrives in the extremely dry Egyptian Desert.

2. Materials & Methods

2.1. Plant material

The parts of *Reichardia tingitana* L. species were collected during the flowering stage from two different habitats in Egypt (Delta coast and inland desert). The first samples of *R. tingitana* was collected from sandy habitat near Gamasa City, coastal desert; however, the second sample was collected from Wadi Araba, the eastern desert of Egypt for endophytes isolation.

Healthy mature green plants of *Reichardia tingitana* L., each sample divided into four parts; shoot, leaf, flower and root. Each sample was placed in a separate sterilized bag and brought back to the laboratory for processing. The plant species were identified, authenticated and maintained in our Laboratory.

2.2. Isolation and Purification of endophytic fungi

Samples from the shoot and root were split into multiple pieces that were each approximately 5 mm in diameter and then washed under running water. Pieces were immersed in 70% ethanol for 1 minute, sodium hypochlorite (2 percent available chlorine v/v) for 3 minutes, then 70% ethanol for 30 seconds to surface sterilize them. They were then washed twice in sterile distilled water .

Each Petri dish included ten pieces of each organ along with 250 mg/l of chloramphenicol. Each sample organ was replicated five times (in Petri plates). Then, dishes were incubated at 26±2°C [14].

The segments' developing hyphae were moved to a fresh PDA Petri dish for purification after one

week. The isolated endophytic fungi were sub cultured once every month and kept on a PDA slant. Slants underwent a 5-day incubation period at 28 °C before being put into storage at 4 °C.

3. Results and Discussion

Our understanding of the precise interactions between endophytic fungus and their host plants is still quite limited, despite the fact that they are one of the most crucial components of plant micro-ecosystems and should have a considerable impact on the growth and development of hosts plants.

3.1. Isolation of endophytic fungi

Endophytic fungi were isolated from apparently healthy *Reichardia tingitana* samples growing in two habitats and from soil supporting the growth of this plant. As illustrated in Figures 1-3, the isolation was performed on four components (root, stem, leaf, and flower) of two fresh plant samples.

3.2. Purification of endophytic fungi

Thirteen species of endophytic fungi were isolated from the four parts of Reichardia tingitana and from the soil supporting the growth collected from different habitats (Delta coast and inland desert) as shown in Table 1 and Figures 4-6.

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

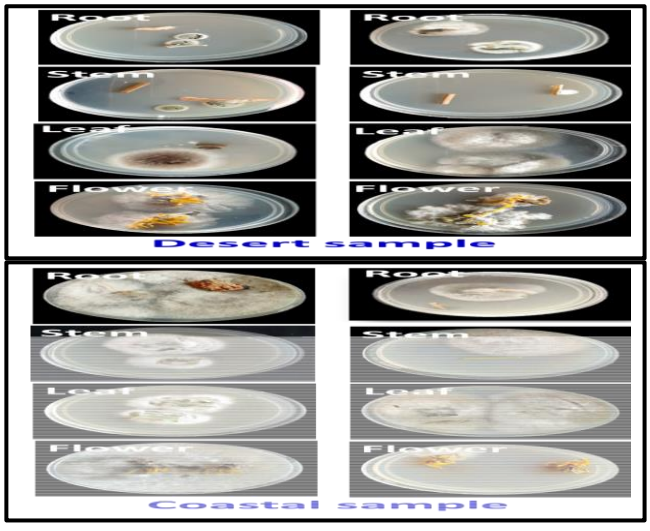

Figure 1. Isolation of endophytic fungi from *Reichardia tingitana* collected from coastal and inland habitats

Table 1: Number of fungi endophytes isolated from *Reichardia tingitana* collected from different habitats.

NO	Fungus name	Coastal sample						Inland sample				
		Root	Stem	Leaf	Flower	Soil		Root	Stem	Leaf	Flower	Soil
1	*Alternaria brassicicola*	+	+	-	+	-		-	-	-	-	-
2	*Aspergillus flavus*	-	-	-	-	-		-	-	+	-	-
3	*Aspergillus niger*	-	-	-	-	-		-	-	+	-	-
4	*Mucor mucedo*	+	-	-	-	-		-	-	-	-	-
5	*Penicillium digitatum*	-	-	-	-	-		-	-	-	-	+
6	*Penicillium janthinellum*	-	-	-	-	-		-	-	-	-	+
7	*Penicillium lanosum*	-	-	-	-	+		-	+	-	-	-
8	*Penicillium notatum*	-	-	+	-	+		+	+	-	+	+
9	*Phytophthora cinnamomi*	-	-	-	-	+		-	-	-	-	-
10	*Phytophthora infestans*	-	-	-	-	+		-	-	-	-	-
11	*Rhizopus stolonifer*	-	-	-	-	+		-	-	-	-	-
12	*Rizoupus oryzae*	-	+	+	-	-		-	-	-	-	-
13	*Fusarium semitectum*	-	-	-	+	-		-	-	-	+	-

Values are mean of triplicates ± standard error.

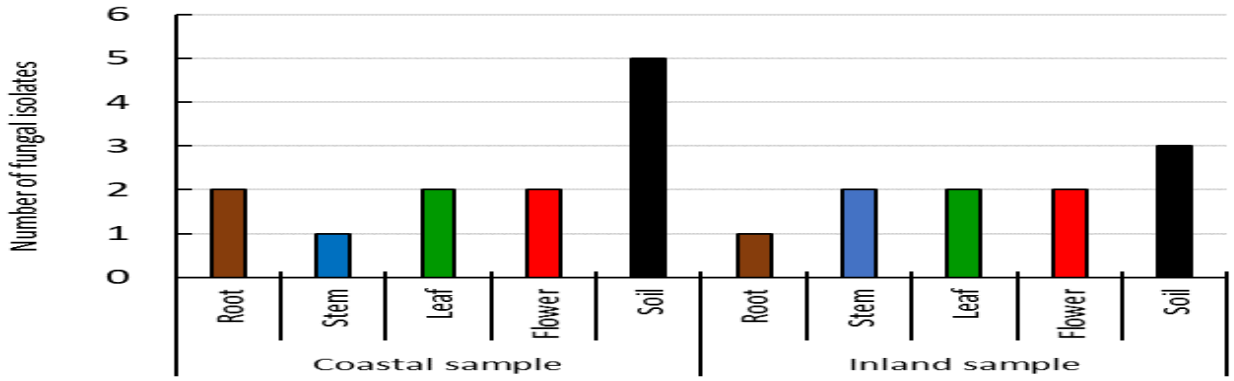

Figure 2. Number of fungi endophytes isolated from Rumex spp. collected from different habitats.

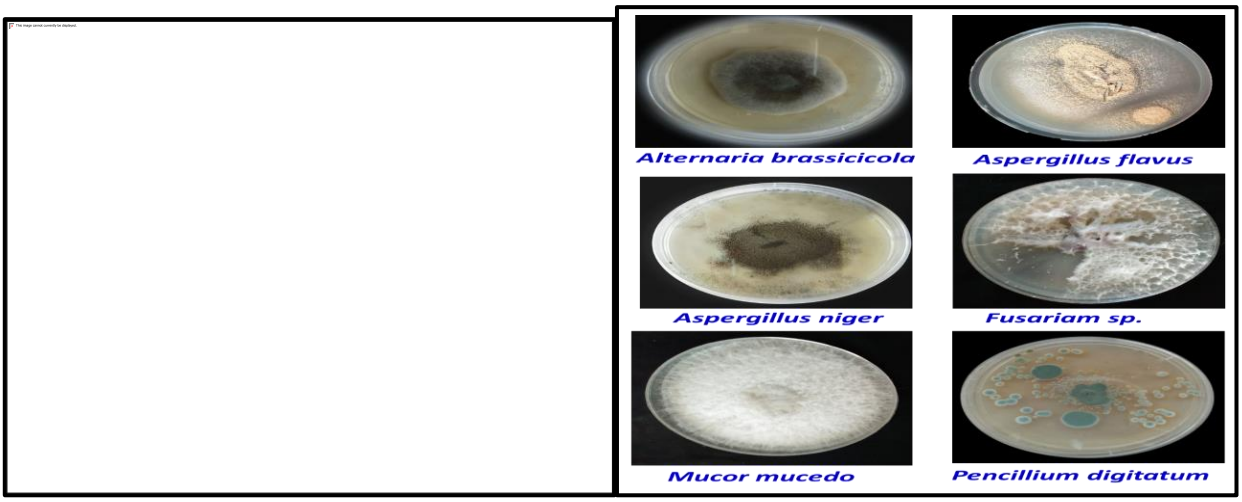

Figure 3. Purification of endophytic fungi isolated from *Reichardia tingitana* in coastal and inlanddesert habitats

Different kinds of fungus will form colonies that appear different from one another; 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

.4. Conclusion

The current study shown that the therapeutic plant *Reichardia tingitana*, which is native to arid and semi-arid environments, serves as an ecological niche for a variety of endophytic fungus. So, based on morphological characterization, 13 fungal strains were discovered from healthy *Reichardia tingitana* sections (root, stem, leaf, and flower). The detected fungal strains also shown biological activity in agricultural, industrial, and medicinal processes, according to the literature study. For the assessment of isolated fungal strains for purposes, their sustainability, and their safety, more experimental research is advised

4. References

1 Knogge, W., (1996). Fungal infection of plants. The Plant Cell, **8(10)**, p.1711.

2 Saikkonen, K., Ahlholm, J., Helander, M., Lehtimäki, S. and Niemeläinen, O., 2000. Endophytic fungi in wild and cultivated grasses in Finland. Ecography, **23(3)**, pp.360-366.

3 Faeth, S.H. and Fagan, W.F., (2002). Fungal endophytes: common host plant symbionts but uncommon mutualists. Integrative and Comparative Biology, **42(2)**, pp.360-368.

4 El Mansy, S.M., Nouh, F.A.A., Mousa, M.K. and Abdel-Azeem, A.M., (2020). Endophytic fungi: diversity, abundance, and plant growth-promoting attributes. In Agriculturally Important Fungi for Sustainable Agriculture (pp. 21-59). Springer, Cham.

5 Heilmann-Clausen, J., Barron, E.S., Boddy, L., Dahlberg, A., Griffith, G.W., Nordén, J., Ovaskainen, O., Perini, C., Senn-Irlet, B. and Halme, P., (2015). A fungal perspective on conservation biology. Conservation biology, **29**(1), pp.61-68.

6 Raj, K., Wati, L. and Kumar, A., (2021). Fungal diversity and its role in sustainable agriculture. In Biocontrol Agents and Secondary Metabolites (pp. 519-535). Woodhead Publishing.

7 Gouda, S., Das, G., Sen, S.K., Shin, H.S. and Patra, J.K., (2016). Endophytes: a treasure house of bioactive compounds of medicinal importance. Frontiers in microbiology, 7, p.1538.

8 Yadav, A.N., (2018). Biodiversity and biotechnological applications of host-specific endophytic fungi for sustainable agriculture and allied sectors. Acta Sci Microbiol, **1**(**5**), pp.01-05.

9 Adeleke, B.S. and Babalola, O.O., (2021). Pharmacological Potential of Fungal Endophytes Associated with Medicinal Plants: A Review. *Journal of Fungi*, **7(2)**, p.147.

10 Martinuz, A., Schouten, A., Menjivar, R.D. and Sikora, R.A., (2012). Effectiveness of systemic resistance toward Aphis gossypii (Hom., Aphididae) as induced by combined applications of the endophytes Fusarium oxysporum Fo162 and Rhizobium etli G12. Biological Control, **62(3),** pp.206-212.

11 Tackholm V., (1974). Students' Flora of Egypt, (2nd ed.), Cairo University Press, Cairo, Egypt.

12 Boulos, L. (2002). Flora of Egypt, vol **3.** Al Hadara Publishing, Cairo, Egypt. pp. 1-4

13 Abd-ElGawad, A.M., El-Amier, Y.A., Assaeed, A.M. and Al-Rowaily, S.L., (2020). Interspecific variations in the habitats of Reichardia tingitana (L.) Roth leading to changes in its bioactive constituents and allelopathic activity. Saudi journal of biological sciences, **27(1)**, pp.489-499..