Turkish Journal of Agriculture - Food Science and Technology, 10(10): 1934-1943, 2022
DOI: https://doi.org/10.24925/turjaf.v10i10.1934-1943.5388

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Isolation of Endophytic Fungi from Algerian Plant *Salicornia arabica* and Screening of their Antimicrobial Activity

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ARTICLE INFO

Research Article

Received : 19/07/2022
Accepted : 09/09/2022

Keywords:
Acremonium sp.
Endophytic fungi
Antibacterial activity
Antifungal activity
Salicornia arabica

ABSTRACT

The present study was aimed at isolating endophytic fungi from the Algerian medicinal plant *Salicornia arabica* and analyzing its antifungal and antibacterial effects. The morphological analysis allowed us to identify endophyte isolates at the genus level as belonging to 8 different genera, *Aureobasidium* sp., *Ulocladium* sp., *Acremonium* sp., *Stemphylium* sp., *Penicillium* sp., *Aspergillus* sp., *Phoma* sp., and *Chrysosporium* sp. with frequency percentages 6.7%, 13.3%, 20%, 13.3%, 13.3%, 13.3%, 6.7%, 6.7%, respectively. The antimicrobial activity was caried out using the agar plug diffusion method. The three isolates of the genus *Acremonium* sp. were highly active against all tested bacteria except *Enterococcus faecalis.* Comparison of the means of inhibition zones of the active isolates showed that the three fungal isolates of *Acremonium* were the most active, followed by *Chrysosporium* sp., *Penicillium* sp.1, *Aureobasidium* sp., *Stemphylium* sp.1, *Penicillium* sp. .2, and *Ulocladium* sp.2. The widest zones of inhibition were 22.33 and 20.33mm for *Acremonium* sp.3, 18.33 and 15.33mm for *Aureobasidium* sp., 19.33mm for *Penicillium* sp.1, and 19 and 15mm for *Stemphylium* sp.1 obtained against *Bacillus cereus* and *Staphylococcus aureus* bacteria, respectively. Regarding the antifungal activity, the best inhibitory activity was 80 and 64.70% obtained with the isolate *Penicillium* sp.2 against *Fusarium oxysporum* f.sp. *ciccri* and *Fusarium oxysporum* f.sp. *albedinis*, respectively, and of 63.29 and 58% observed against *Phytophthora infestans* and *Fusarium oxysporum* f.sp. *ciccri*, respectively, with the endophytic isolate *Aspergillus* sp.2. These results indicated the possible prospect of endophytes fungi isolated from *Salicornia arabica* as a promising resource of antimicrobial compounds and in the quest for the potential starting points for the development of new antibiotics.

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Introduction

The rapid evolution of the drug resistance mechanisms of bacteria has become a severe public health problem due to the idea that antibiotics were as “magic bullets” that could be consumed without the fear of probable affliction and side effects (Elkady et al., 2022; Pasrija et al., 2022). On the other hand, the use of agrochemicals to enhance food production due to the unceasing needs of the growing world population has become a threat to the ecosystem (Adeleke et al., 2022). Thus, new antimicrobial compounds are urgently needed in this modern era to fill the drug development pipeline. In order to find lasting solutions to these problems, these last year’s researchers came into focus on obtaining and producing bioactive natural products from endophytic fungi and not directly from the plant tissue because the indiscriminate exploitation in the search for remedies endangered many plant species (Gómez and Luiz, 2018).

Microorganisms known as endophytic fungi live in different plant organs, tissues, or intercellular spaces without causing any symptoms of infection in the host plant (Zheng et al., 2021; Benslama et Nouiri 2022). They are recognized for their production of a wide range of biologically active compounds and their ability to inhibit several pathogens in plants and humans (Sadrati et al., 2020). In this context, we focused on the isolation, identification, and evaluation of the potential antimicrobial activity of endophytic fungi associated with *Salicornia arabica.*

Materials and methods

Samples Collection and Endophyte Isolation

For the isolation of endophytic fungi, healthy roots, stems, and young branches of *Salicornia arabica* were collected from Chott El Hodna-M'sila (Algeria), and transported to the laboratory on ice. The samples were well washed with running tap water to remove dust and debris. The samples were then surface sterilized by immersion in 70% ethanol for 2 min, 4% NaOCl for 1 min, 70% ethanol for 30 s, and washed two times with sterile distilled water (1 min) to remove the excess surface sterilants. The sterilized samples were dried on pre-sterile filer paper and then cut aseptically into 1 × 1 cm pieces, inoculated on Potato Dextrose Agar (PDA) plates (five segments for each plate) amended with streptomycin (50 µg/ml) to eliminate bacterial growth, and incubated at 28 °C ± 2 °C until the growth of fungal hyphae. Each hypha emerging from the inoculated segments was isolated by transferring it onto a fresh PDA medium several times until a pure endophytic fungal strain is obtained. Pure fungal cultures were maintained on PDA slants at 4°C (Zerroug et al., 2020 ; Khalil et al., 2021).

Colonization frequency (FC) (%) was determined as the ratio of the number of plant fragments colonized by fungi and the total number of fragments×100. Isolation rate (IR) was determined as the ratio of the number of isolates obtained from plant segments by the total number of segments incubated. The relative frequency (RF) (%) was calculated by dividing the total number of isolates representing a single taxon by the total number of taxa obtained from all tissues×100 (Zuo et al., 2022).

Identification of Endophytic Isolates

The identification of endophytic isolates was based on their morphological characteristics both macroscopic and microscopic. These morphological studies were done by plating the fungi on PDA and incubating it for 7 days. The macroscopic observation was based on color in colony surface and reverse, colony surface texture, colony margin, pigment exuded, whereas for microscopic observations, mycelial and conidial structures were observed using a light microscope. Based on these observations, the endophytic isolates were identified up to at least the genus level using classification guides for fungi (Oktarina et al., 2022 ; Al-Maghraby et al., 2022).

Screening of Antimicrobial Activity

A rapid and qualitative selection of the active microorganisms was made using the agar plug diffusion method against three Gram-positive bacteria (*Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 49452 and *Staphylococcus aureus* ATCC 25923), and three Gram-negative bacteria (*Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922). For the antifungal activity, the dual culture method against four phytopathogenic fungi *Fusarium oxysporum* f.sp. *albedinis*, *Phytophthora infestans*, *Fusarium solani* var. *coeruleum* and *Fusarium oxysporum* f.sp. *ciccri*. The standard used to adjust bacterial cultures was 0.5 McFarland standard, while the inoculum size of fungal cultures was adjusted to 1×10^6 spores/mL (Nurhaida et al., 2019).

Antibacterial Activity

From the 7-day-old endophyte cultures, disks were cut (6 mm diameter) using a sterile cork borer and transferred to the surface of Petri dishes containing Mueller Hinton Agar (MHA) previously spread with cultures of test bacteria in triplicate. The plates were kept at 4°C for 6 hours for the diffusion of bioactive compounds in the agar, then incubated at 37°C for 24 h for bacterial growth. Antibacterial activity was determined by measuring inhibition zones produced by endophytic fungi against pathogenic bacteria (Nurhaida et al., 2019).

Antifungal Activity

For each combination of the pathogen and endophytic fungus, mycelial plugs (6 mm in diameter), three for endophytic fungus and one for phytopathogenic fungus were placed respectively in the border and center of the Petri dish containing PDA. Petri dishes inoculated only with phytopathogenic fungi were used as negative controls. All plates were incubated at 28 °C for 5 days. The growth of the pathogen and the endophyte was observed and the mean colony diameter was measured on the 5th day of inoculation. The percent of inhibition of the test phytopathogenic fungi was then calculated using the formula:

$A\ (\%) = [(R_1-R_2)/R_1] \times 100$

where R_1 represents the colony radial growth of pathogen on the control plates, and R_2 is the radial growth of pathogen in test plates (Yang et al., 2020).

Statistical Analysis

All experiments were performed in triplicates, and statistical analysis was carried out using SAS/STAT® 9.2 software. Group comparisons were performed using the two-way ANOVA followed by Student-Newman-Keuls multip-rang test. Results are represented as mean ± standard deviation (SD), and significant effects of treatments were determined by F values ($P \leq 0.05$).

Results and Discussion

Isolation of Endophytic Fungi

After epiphytic sterilization, a total of 18 fungal isolates were isolated from 105 segments of the plant used, 7 isolates from the roots, 7 isolates from the stems, and 4 isolates from the young branches. All plant parts used during isolation were found colonized by endophytic fungi. The colonization percentages of roots (68.57%) and stems (71.43%) were very close to each other and higher than those observed for young branches (31,43). The same was observed with isolation rates, where roots and stems were more colonized with endophytic fungi compared to young branches (Figure 1).

These results are consistent with many other reports that have found differences in species abundance between plant tissues, such as bark, stem and leaves, between midrib and laminar tissues, between the vein, midrib and pseudostem, between bark and twigs, and between roots and leaves (Naik et al., 2009). The difference in the assembly of endophytic fungi in different types of tissues could be due to the ability to utilize the substrates as well as factors such as physiology and the difference in structure and chemical composition of different organs (Sharma et al., 2018).

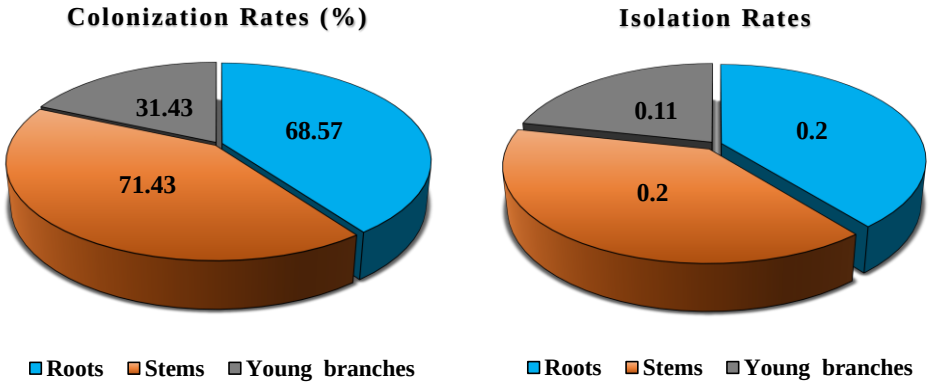

Figure 1. Colonization rates (%) and Isolation rates of endophytic fungi from *Salicornia arabica*

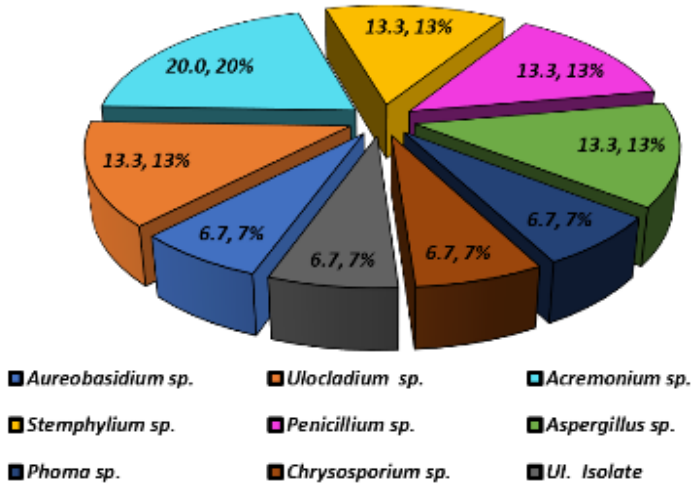

Figure 2. Relative frequencies (%) of different endophytic taxa isolated from Salicornia arabica.UI: Unidentified.

The results are also consistent with those obtained by Park et al.(2017) and Li et al. (2020) who also isolated more endophytic fungi from the roots of many plants than from their aerial parts and explained this by the diversity and richness of the soil in fungi, as well as by the abundance of nutrients contained in the roots compared to other organs. This might also be because roots are the interfaces that connect plants with the soil and the soil-associated microbiome, including some soil microbes that could potentially be plant endophytes (Xia et al., 2019). Thus, the distribution of extended root systems in the soil and nutrient status in a desert environment may lead to higher endophytic fungal diversity in the roots than in aerial parts tissues by providing more host and substrate for infection of these fungi (Zuo et al., 2022).

The larger surface area of the leaves and the presence of stomata may provide passage to the entry of fungal mycelia, harboring different endophytic fungi (Prasher and Kumar, 2021). In this study, we found that young branches harbor fewer endophytes than stems and roots. This may be due to the presence of a limited number of stomata on the surface of young branches and their narrower surface exposed to the environment.

The difference in the distribution of endophytic fungi in different plant parts is also influenced by tissue age. In our study, colonization rates were higher in older tissues (roots and stems) than younger tissues (young branches). This is consistent with a hypothesis of predominantly horizontal transmission of endophytes, as old plant tissues would have had more time to accumulate endophytes from the environment, as opposed to outgrowth from a few initial infection sites (Guo et al., 2008). The more the host tissue is older, the more it is subjected to repeated infections. Fungi spread by air and rainfall affords older trees the opportunity for repeat infection; on the other hand, changes in plant physiological status and tree tissue structure in aging trees create new access in the plant tissue, allowing fungal invasion (Zheng et al., 2016).

Identification of Endophytic Fungi

A total of 18 culturable fungal endophytes were isolated from different tissues (young branches, stems, and roots) of *Salicornia arabica*. The morphological characteristics revealed that these fungal endophytes belong to 8 different genera, namely *Aureobasidium* sp. (1 isolate, 6.7%), *Ulocladium* sp. (2 isolates, 13.3%), *Acremonium* sp. (3 isolates, 20%), *Stemphylium* sp. (2 isolates, 13.3%), *Penicillium* sp. (2 isolates, 13.3%), *Aspergillus sp.* (2 isolates, 13.3%), *Phoma* sp. (1 isolate, 6.7%), and *Chrysosporium* sp. (1 isolate, 6.7%) (Figure 2, 3) and (Table 1).

Table 1. Source and identity of endophytic fungi isolated from *Salicornia arabica*

Isolate codes	Source of isolates			Identification of isolates
	Roots	Stems	Young branches	
SR1	+	-	-	*Aureobasidium* sp.
SR2	+	-	-	*Ulocladium* sp.1
SR3	+	-	-	*Ulocladium* sp.2
SR4	+	-	-	*Acremonium* sp.1
SR5	+	-	-	UI. isolate
SR6	+	-	-	*Acremonium* sp.2
SR7	+	-	-	*Acremonium* sp. 3
SF1	-	+	-	*Stemphylium* sp.1
SF3	-	+	-	*Penicillium* sp.1
SF4	-	+	-	*Penicillium* sp.2
ST1	-	+	+	*Stemphylium* sp.2
ST3	-	-	+	*Aspergillus sp.1*
ST4	-	-	+	*Chrysosporium* sp.
ST5	-	-	+	*Aspergillus* sp.2
ST7	-	-	+	*Phoma* sp.

+: presence, -: absence. UI: Unidentified.

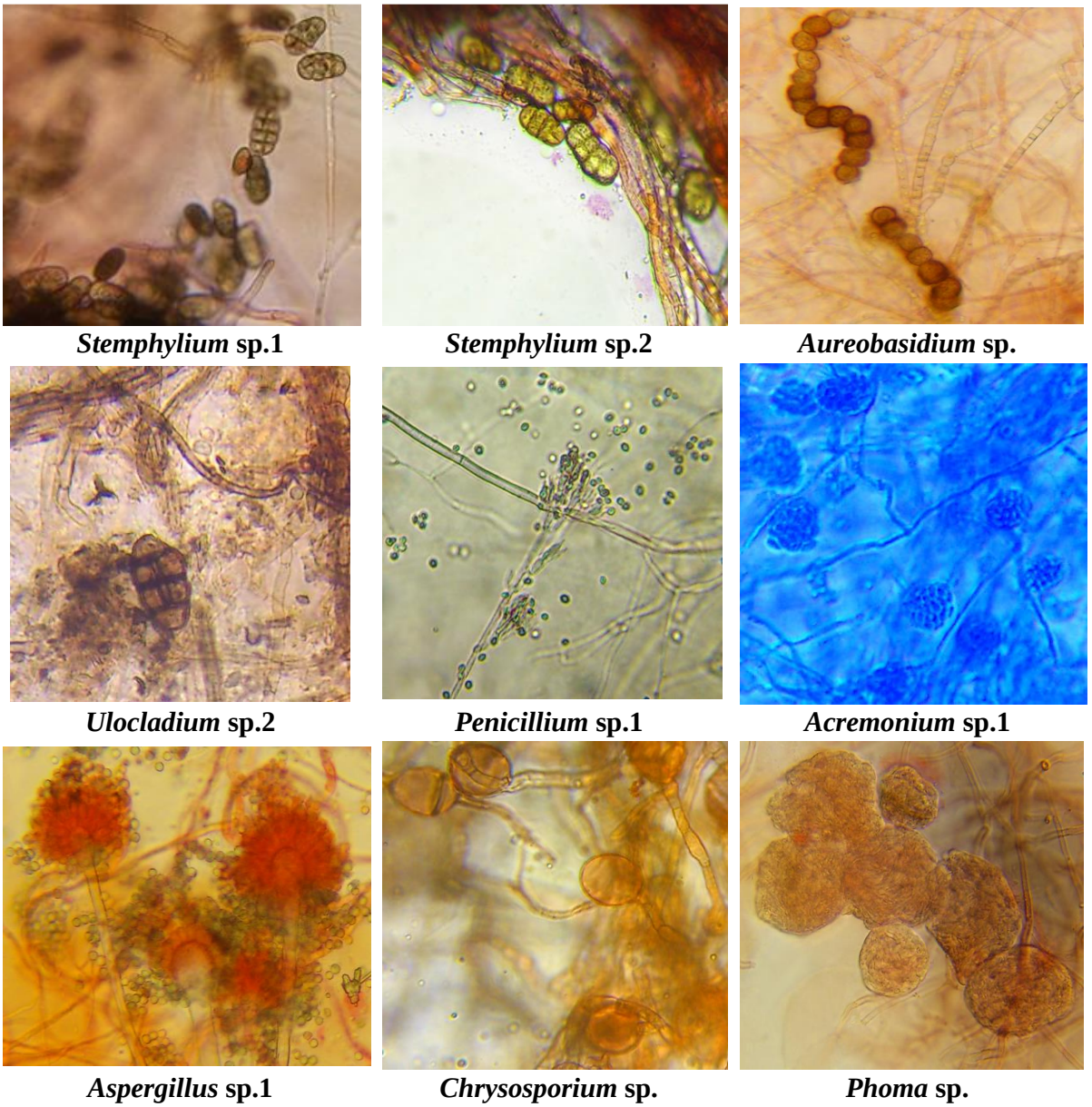

Figure 3. Microscopic characteristics of endophytic fungi isolated from Salicornia arabica

The percentage distribution of isolated fungi of each genera showed that the *Acremonium* sp., *Stemphylium* sp., *Ulocladium* sp., *Penicillium* sp., *Aspergillus sp.* were found to be the most common isolates. The most isolated ubiquitous fungal taxa in this study were reported as endophytes from previous studies on various halophytes and non-halophytes plants (El-Nagerabi et al., 2014 ; Li et al., 2020 ; Rim et al., 2021). In Japan, *Stemphylium* spp. was dominant endophyte of *Salicornia europaea*(Sun et al., 2011) . The genera *Acremonium, Aspergillus, Aureobasidium* and *Ulocladium* were isolated in similar study from stems and roots of many desert halophytes in northwest China (Li et al., 2020). *Ulocladium* sp., *Penicillium* sp., and *Phoma sp.* were also isolated as endophytes from tow halophytes species *Suaeda microphylla* and *Suaeda corniculate* (Sun et al., 2011). Some of these ubiquitous fungi including the genera of *Aspergillus, Penicillium, Phoma,* and *Ulocladium* were already reported to be isolated as endophytes in similar previous studies from many other plants such as *Pinus* Species in Korea (Rim et al., 2021).

Antibacterial activity

According to the results represented on figure 4; the five bacteria *Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa* and *Escherichia coli* were inhibited by at least one endophytic fungus, while the bacterium *Enterococcus faecalis* was completely resistant. The six (40% of isolates) endophytic isolates; *Ulocladium* sp.1, *Stemphylium* sp.2, *Aspergillus* sp.1, *Aspergillus* sp.2, *Phoma* sp. and the unidentified isolate showed no activity against all or most bacteria.

While the six endophyte isolates *Aureobasidium* sp., *Ulocladium* sp.2, *Stemphylium* sp.1, *Penicillium* sp.1, *Penicillium* sp.2, and *Chrysosporium* sp. showed an antibacterial power at least on a one pathogenic bacterium. On the other hand, the three isolates (20% of isolates) of the genus *Acremonium* (*Acremonium* sp.1, *Acremonium* sp.2, *Acremonium* sp.3) were highly active against all tested bacteria except *Enterococcus faecalis.*

Comparison of the means of inhibition zones of the active isolates showed that the three fungal isolates of the genus *Acremonium* were the most active, followed by *Chrysosporium* sp., *Penicillium* sp.1, *Aureobasidium* sp., *Stemphylium* sp.1, *Penicillium* sp. .2, and *Ulocladium* sp.2 respectively. The widest zones of inhibition were 22.33 and 20.33mm for *Acremonium* sp.3, 18.33 and 15.33mm for *Aureobasidium* sp., 19.33mm for *Penicillium* sp.1, and 19 and 15mm for *Stemphylium* sp.1 always obtained against *Bacillus cereus* and *Staphylococcus aureus* bacteria respectively (figure 5 and 6).

Endophytic fungi produce novel bioactive compounds possessing a variety of biological properties that include antibacterial, antiviral, antifungal, antiprotozoal, antiparasitic, antioxidant, immunosuppressant, and anticancer functions (Manganyi and Ateba, 2020). In several studies, a large number of antimicrobial compounds were purified from extracts of endophytic fungi, belonging to several structural classes like flavonoids, terpenoids, peptides, phenols, steroids, quinines and alkaloids (Pimentel et al., 2011 ; Gunasekaran et al., 2017).

Many species of *Acremonium* genus have been identified as producers of useful metabolites. Cephalosporins, which belong to the *β*-lactam class of antibiotics, were derived from *Acremonium* (Khan et al., 2021). In this study, the three isolates of the genus *Acremonium* were the most active against most bacteria, which is in agreement with several previous studies. Nineteen compounds from the fungal extract of endophytic fungus *Acremonium coenophialum* demonstrated antimicrobial activity against Gram-Positive Bacteria, Gram-Negative Bacteria and against yeasts with MIC values range between (25–100), (50-100) µg/ml, and (100) µg/ml respectively (Hateet, 2020). The extract obtained from the isolate Macof08 (*Acremonium* sp.) exhibited strong inhibition on test bacteria (Tijiang Shan, 2012).

Penicillium species also are known to produce a wide range of biologically active secondary metabolites, including several antibacterial and antifungal compounds. The first antibiotic substance in history as the most emblematic example of a drug of fungal origin is penicillin produced by *Penicillium* (Assaf et al., 2020). *Penicillium* sp.1 *Penicillium* sp.2 isolated in the present study showed strong antibacterial activity especially against *Bacillus cereus* and *Staphylococcus aureus* bacteria. This genus has also been reported in several studies on endophytic fungi to have high antimicrobial potency (Chirane et al., 2020).

Among the endophytes isolated from olive leaves, *Penicillium commune* and *Penicillium canescens* were the most active against Gram-positive and negative bacteria (up to 2.7-fold compared to 30 µg/ mL chloramphenicol) (up to 2.7-fold compared to 30 µg/ mL chloramphenicol) (Malhadas et al., 2017). The endophytic *Penicillium griseofulvum* isolated *Mentha pulegium* L. showed A high antibacterial activity against a wide range of pathogenic bacteria with MIC values ranging from 100 to 100 µg/ml (Zerroug et al., 2018). *Penicillium roqueforti* (CGF-1) isolated from *Solanum surattense* and had broad-spectrum antibacterial activity against plant pathogenic bacteria (Ikram et al., 2019).

During this study, the endophyte *Chrysosporium* sp. showed moderate activity against most bacteria. There are very few studies that have isolated the genus *Chrysosporium* as endophyte. However, several studies have been conducted on the isolation of this genus from other sources and which led to the discovery of groups of compounds that have different biological activities (Correa et al., 2019).

Naphthoquinone-type compounds isolated from other species *Chrysosporium queenslandicum* have shown to be active against the gram-positive bacteria *Micrococcus luteus* and *Bacillus subtilis* with MIC value 33 µg/mL; but have been inactive against Gram negative bacterium, yeast and filamentous fungi (Ivanova et al. 2002). Compound 5,6-dihydro-4-methoxy-6-(1-oxopentyl)-2H-pyran-2-one (4) purified from *Chrysosporium multifidum* extract isolated from *Hermetia illucens* gut showed moderate activity (MIC = 62.5 µg/mL) against methicillin-resistant *Staphylococcus aureus* (MRSA) (Correa et al., 2019).

The genus *Stemphylium* is also frequently found able to produce secondary metabolites which have antibacterial activity. Debbab et al. (2010) isolated six new bisanthraquinones, together with four known related compounds from *Stemphylium globuliferum*, an endophytic fungus obtained from *Mentha pulegium*.

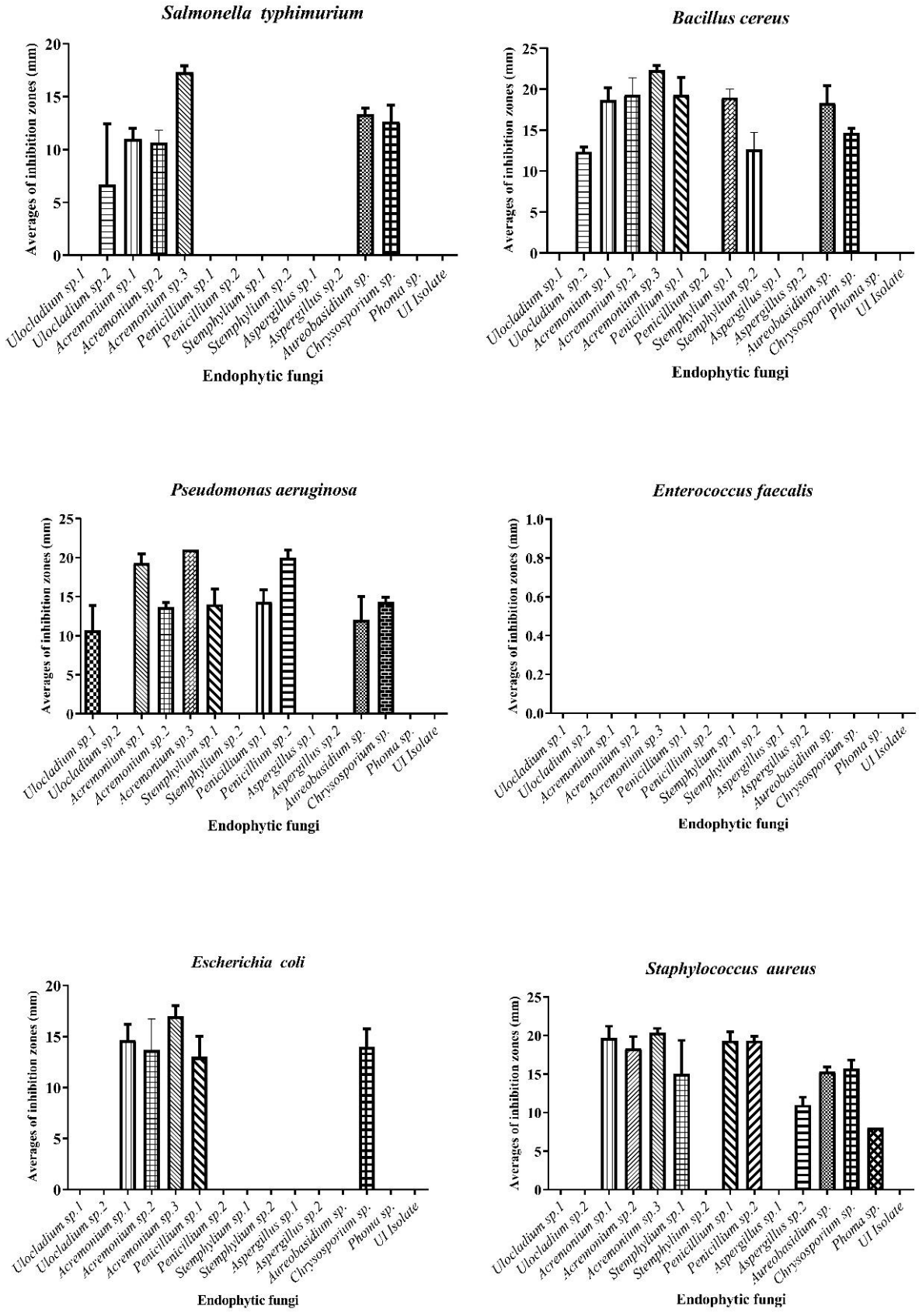

Figure 4. Antibacterial effect of endophytic fungi isolated from *Salicornia arabica*, (n=3, mean of inhibition zones ±SD), UI: Unidentified

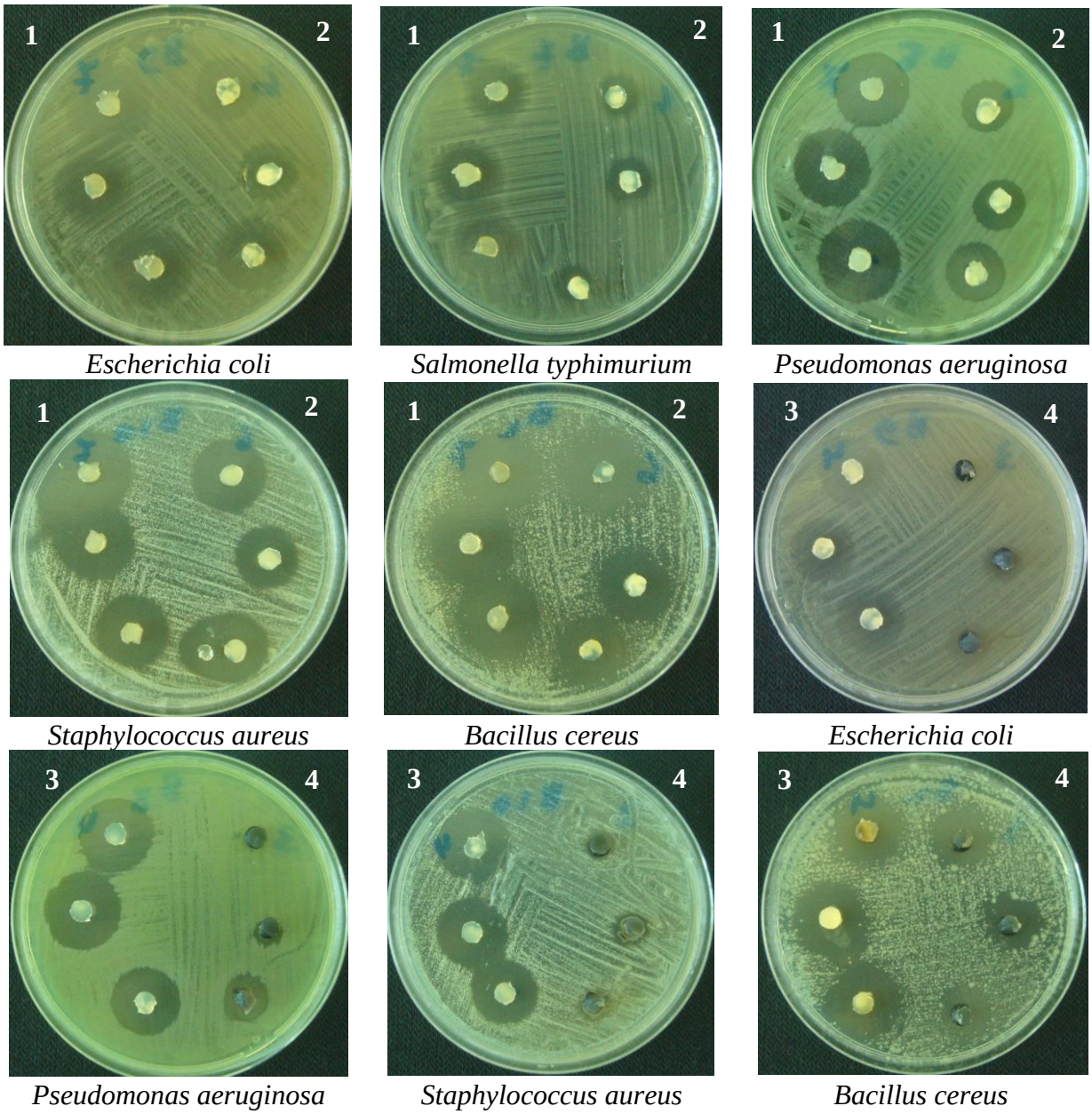

Figure 5. Antibacterial effect of endophytic fungi, 1: *Acremonium* sp.3, 2: *Acremonium* sp.2, 3: *Acremonium* sp.1, 4: *Ulocladium* sp.2.

Two new α-pyrone derivatives, infectopyrones A and B, have been obtained from the endophytic fungus *Stemphylium* sp. 33231 isolated from *Brguiera sexangula* var. *rhynchopetala*, these compounds had a broad spectrum of antibacterial activity against five bacterial terrestrial pathogens (Zhou et al., 2014). The strain *Stemphylium lycopersici* isolated from a traditional Chinese medicinal plant *Sophora tonkinensis* was found produce seven compounds; four of them exhibited inhibitory activities against pathogenic bacteria (Xu et al., 2020). An endophytic *Stemphylium* sp. 33231 isolate produced stemphol A, and stemphol B, which all exhibited antibacterial properties (Zhou et al., 2015).

Antifungal Activity

Most endophytic fungi exhibited weak antifungal activity. However, the two isolates *Aureobasidium* sp. and *Phoma* sp. showed moderate antifungal activity. The best inhibitory activity was 80 and 64.70% obtained with the isolate *Penicillium* sp.2 against *Fusarium oxysporum* f.sp. *ciccri* and *Fusarium oxysporum* f.sp. *albedinis*, respectively, and of 63.29 and 58% observed against *Phytophthora infestans* and *Fusarium oxysporum* f.sp. *ciccri*, respectively, obtained with the endophytic isolate *Aspergillus* sp.2 (Table 2).

The genera *Penicillium* and *Aspergillus* are among the largest groups of fungi that are most abundant in nature. Endophytic species of these genera have been isolated from various plant species, they conferred protection against various biotic stresses by different methods, such as the production of antagonist compounds (Toghueo and Boyom, 2020). Many studies have shown the antifungal potency of various endophytic isolates of *Penicillium*, we will cite that of Ma et al., (2017) who identified three molecules: 5-hydroxy-8-methoxy-4-phenylisoquinolin-1(2H)-one, 3-O methylviridicatin and viridicatol produced

by *Penicillium* sp. R22, an endophytic fungus of *Nerium indicum* with strong antifungal activity with MICs values reaching the 31.2 µg/mL. Another isolate *Penicillium chrysogenum* QEN-24S isolated from the marine red algae *Laurencia* sp. produced a novel penicisteroids A, which exhibited strong antifungal activity (El-Bondkly et al., 2021).

Fumiquinazo-line D, fumiquinazoline J, fumiquinazoline C, and fumiquinazoline H produced and isolated from the endophytic fungus *Aspergillus* sp. hosted in the roots of *Astragalus membranaceus* showed strong antifungal activity against the tested fungal strains with MIC values ranging from 0.5 to 16 mg/mL (El-Hawary et al., 2020). 12β-hydroxy-13α-methoxyverruculogen TR-2, fumitremorgin B, and verruculogen, isolated from *Aspergillus fumigatus* LN-4, an endophytic fungus associated with *Melia azedarach* showed broad-spectrum anti-phytopathogenic activities against eight fungi (*Botrytis cinerea*, *Alternaria solani*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Fusarium solani*, *Fusarium oxysporum* f. sp. *niveum*, *Fusarium oxysporum* f. sp. *vasinfectum*, and *Gibberella saubinettii*), with MIC values of 13.7-100 µM, which were comparable to the positive controls (Xu et al., 2021).

From the obtained results, the present study concludes that the isolated endophytic strains from *Salicornia arabica* especially *Acremonium* strains, *Chrysosporium* sp., *Penicillium* sp.1, *Aureobasidium* sp., *Stemphylium* sp.1 and *Aspergillus* sp.2 can be used to produce antimicrobial agents and can be of interest to pharmaceutical industries.

Aknowledgments

This work was supported by the General Directorate for Scientific Research and Technological Development of Algeria.

Table 2. Screening of antifungal activity by dual culture method of endophytic fungi, (n=3, mean of inhibition percentages ±SD).

Endophytic fungi	Inhibition percentages (%) ± SD			
	Fusarium oxysporum f.sp. *albedinis*	*Phytophthora infestans*	*Fusarium solani* var. *coeruleum*	*Fusarium oxysporum* f.sp. *ciccri*
Aureobasidium sp.	43.52±2.88	36.70±4.74	33.83±5.76	57±2.83
Ulocladium sp.1	28.23±3.33	31.64±6.20	13.82±5.76	32.99±1.41
Ulocladium sp.2	31.76±4.40	4.63±3.62	12.28±3.77	54±1.41
Acremonium sp.1	31.76±1.66	12.65±3.10	18.44±2.18	47.99±1.41
UI. isolate	-	15.18±3.58	-	-
Acremonium sp.2	25.87±0	22.78±1.79	12.28±3.77	37.99±2.83
Acremonium sp. 3	36.46±0	16.45±3.10	24.59±2.18	48.99±0
Stemphylium sp.1	30.58±4.40	-	-	28.99±3.74
Penicillium sp.1	28.23±7.25	-	19.98±9.49	40.99±5.10
Penicillium sp.2	64.70±0.00	54.42±3.10	59.99±4.35	80±1.41
Stemphylium sp.2	22.34±2.88	34.17±1.79	-	35.99±2.83
Aspergillus sp.1	-	-	-	14.99±2.83
Chrysosporium sp.	-	-	-	-
Aspergillus sp.2	51.76±7.25	63.29±1.79	38.44±2.18	58±6.48
Phoma sp.	48.23±1.66	48.09±1.79	38.44±2.18	66±3.74

UI : Unidentified.

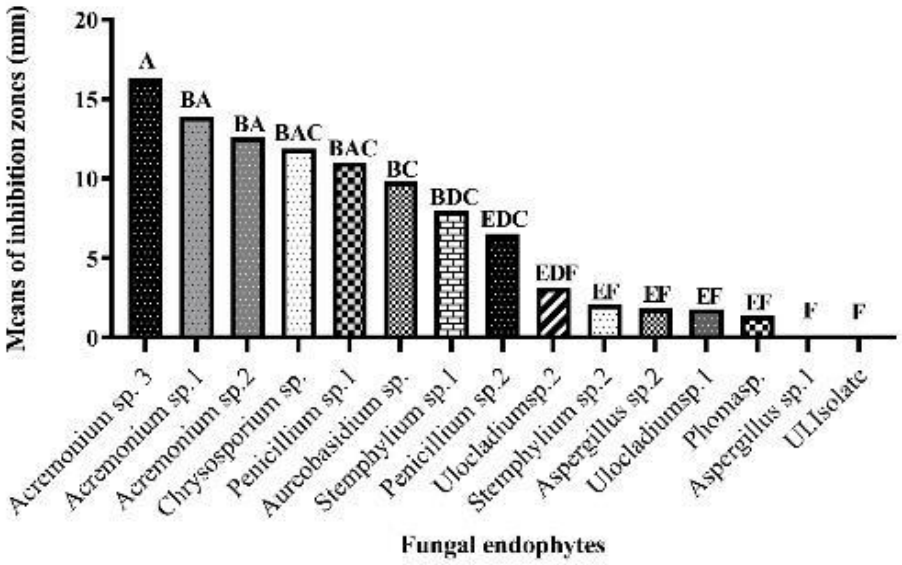

Figure 6. Comparison of inhibition zone averages of endophytic fungi and their effect on the growth of pathogenic bacteria, (Means with the same letters are not significantly different at (P<0.05).

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