NATURAL HISTORY
2025 126(3)
ISSN: 0028-0712

Vol.126, Issue.3, Part.1, March 2025, PP.1-40

WOS Indexing page: https://www.webofscience.com/wos/alldb/full-record/RC:143184019_S24

DOI Link: https://doi.org/10.59879/3WUjL

Generic mycodiversity of *Punica granatum* L. in a reassigned arid steppe area (Djelfa, Algeria).

Kahina Bourenine [*,1], Malika Boudiaf-Nait Kaci[1], Noria Saadoun[1].

[1]Natural Resources Laboratory, Faculty of Biological Sciences and Agricultural Sciences, Mouloud Mammeri University, Tizi-Ouzou, Algeria.
*Corresponding author: Kahina.bourenine@ummto.dz

Received Jan 2025 Accepted Feb 2025 Published March 2025

Abstract

The high Algerian steppe plains are currently experiencing a strong tendency toward soil degradation, disrupting ecological and socioeconomic balances. The reassignment of these soils in woody agrosystems with a local plant component undeniably offers positive externalities for these rural regions. Most studies concerning this action focus on the visible part of biodiversity, mainly on plant species, and few have been conducted on the microbiome associated with them and its structuring at the rhizosphere and the phyllosphere level after reassignment. In this study, we compare the diversity, richness, and abundance of fungal genera identified at the rhizosphere and phyllosphere level of a pomegranate cultivar from the Messaad region (Djelfa, Algeria) to understand their dynamics. Sampling was conducted on twelve vigorous and healthy trees during the summer season (July 2018). After isolation and microscopic identification, we noted a fungal diversity belonging to two phyla: *Ascomycota* and *Mucoromycota*, with a dominance of the first phylum in the two compartments. Fungal strains indicate a high abundance of the genus *Alternaria* in epiphytes and endophytes. The genera *Aspergillus* and *Rhizopus* are the most dominant in the rhizosphere. The dominant fungal genera of the two compartments are melanised. The Shannon-Wiener diversity and uniformity indices show higher values in the phyllospheric compartment compared to the rhizospheric one. However, the similarity is higher between the diversity of the rhizosphere and the diversity of the two micro-niches of the phyllosphere than that displayed between the two microniches of the latter. The principal component analysis differentiated along axis 1, the rhizosphere from the phyllosphere, each with its procession of fungi. Axis 2 distinguished the endosphere from the episphere, with a fungal procession specific to each micro- niche.

Keywords: Agroecology, soil fungi, mycoendophytes, epiphytic fungi, pomegranate, steppe.

ISSN: 0028-0712

Introduction

The pressure on the natural resources of Algeria's arid environments, particularly on soils, is becoming increasingly intense. This constraint leads to degradation, raising concerns about the reversibility and resilience of these ecosystems. Reassigning these environments to arboreal agroecosystems with local cultivars is a promising solution. It constitutes one of the best alternatives to revive the ecosystem capital of soils, especially if we take into account the fact that the management of these agrosystems is often carried out according to agroecological principles (installation of the agrosystem without excavation, absence of soil work, absence of application of synthetic chemical inputs, application of organic manure of local origin, permanent soil cover by spontaneous vegetation, etc.).In this context, the cultivation of the pomegranate tree (*Punica granatum* L.) is among the most prized crops for its robustness and ability to enhance marginal lands, especially for the Djelfa region. This culture displays a most interesting production for the Mediterranean basin (Lahouel and Belhadj, 2022). From this, it emerges that the ecological and socio-economic issues involved in such an approach are of considerable importance for these rural regions and join the proactive approach of the state, which is carrying out sectoral actions in a program to combat drought and desertification (Boukerker *et al*., 2021). This approach often involves monitoring and evaluation on several levels, and one of the strategies undertaken for this is the study of the microbiota associated with these cultivars. Moreover, if the plant is considered as a holobiont comprising the plant and its associated microbiota (Vandenkoornhuyse *et al*., 2015), the biological context of these agrosystems seems to offer a certain relevance in terms of identification of mycodiversity. Due to fungi's active role in agrosystems, understanding the foliar and terrestrial microbiota as providers of ecosystem services is based on continuous efforts to inventory them. The introduction of pomegranate cultivars in this context implies an adjustment dynamic between the microorganisms of the different compartments.

Indeed, the variability of the interactions linked to the characteristics of the place of growth and the genotype, both of the microbiota and of the host plant, and the organ concerned leads to a compartmentalization of the microbiome. In addition, the closeness of the links and the mutual influence of the microbiome of the plant and that of the soil associated with it in developing ecosystems has been highlighted by Van der Wal *et al*. (2006) and Bauer *et al*. (2015). This has resulted in the selectivity recruitment of an efficient microbiome, which offers resilience to the host plant, especially in the face of hostile conditions. The potential importance of this component associated with soil and growing plant organs has been characterized (Compant *et al*., 2011; Martins *et al*., 2013), particularly regarding plant growth and health (Wagner *et al*., 2016; Bergelson *et al*., 2019). The present study focused on the mycodiversity of the phyllosphere, with its two micro-niches (epiphyte and endophyte) and that of the rhizosphere associated with a cultivar of pomegranate tree (*Punica granatum* L.) cultivated in the Messaad region (Djelfa, Algeria) to analyze their structures and interactions for a better understanding of resilience mechanisms.

Material and methods

Study area

Our study was conducted in the wilaya of Djelfa, the commune of Messaad, in the so-called Melaga region (Algeria) (Figures 1 and 2). It is located between 34°13'N and 3°25'E. This region is situated at an altitude of 824.5 m. The choice of our study area is motivated by highlighting the adaptive potentialities of this pomegranate cultivar in the face of the ecological constraints of the arid environment. The selection of the summer season (which corresponds, from the phenological point of

view to the fruiting period) was motivated by the probable high interactivity between the host and the fungi of the two compartments: the rhizosphere and the phyllosphere (episphere and endosphere).

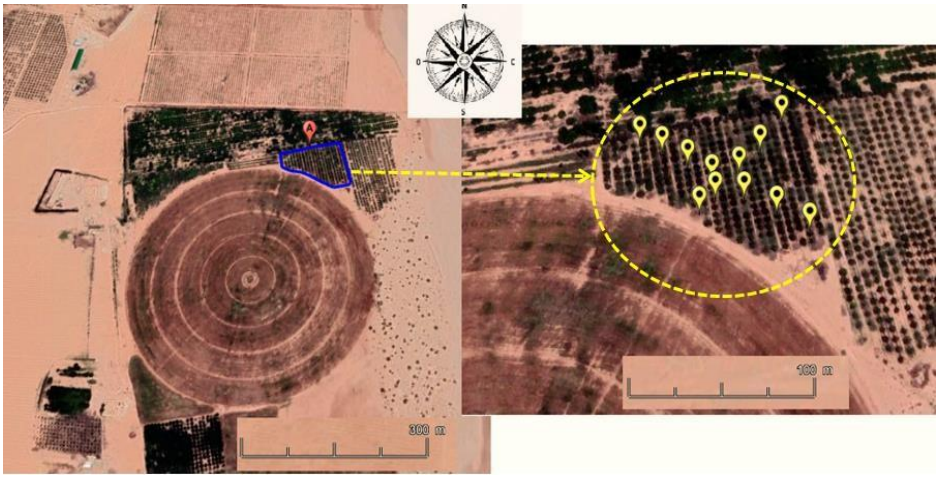

Figure 1. Location of the sampling site Melaga region (Messaad) (Google Earth 2022). *Left: sampled orchard (Kechida Ahmed property); right: sampling method (Sampled subjects).*

A 16-year climate study mentioned by Lahouel and Belhadj (2022) shows a dry season that extends over 8 months (from mid-March to mid-November). July is the hottest and driest month (Figure 2). The same study places the station in the arid stage in cool winter.

ISSN: 0028-0712

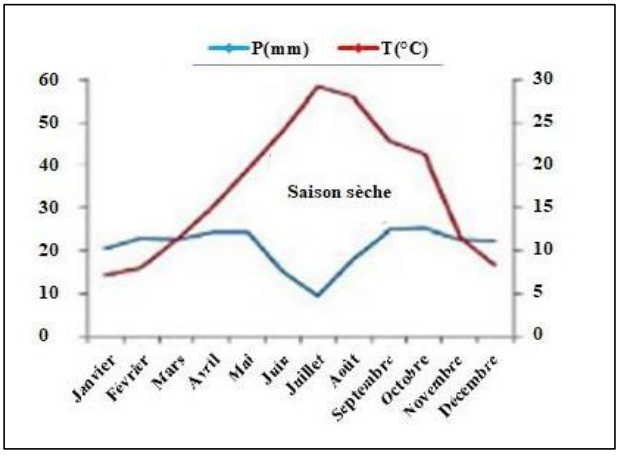

Figure 2. Ombrothermique diagram of the Messaad region (Lahouel and Belhadj 2022) *(P: average monthly precipitation in mm, T: average monthly temperature in °C)*

Sampling

The selected orchard is managed according to the principles of agroecology (Co-occurrence of spontaneous plants under the trees, absence of tillage, maintenance of part of the fruit production on foot, passage of livestock for brush clearing). It includes cultivars of the Messaad pomegranate tree. The trees are planted with a spacing of 5 × 5 m. Leaves of the pomegranate tree free of any visible damage were collected at 1.70 m and around the entire perimeter of the 12 trees chosen. Trees near the edges have been excluded. The leaves were collected with a row spacing between each sampling and on two crossed diagonals at the orchard level. After cleaning the soil surface of the plants present, the soil adjacent to the roots was harvested under each tree to a depth of 20 cm. The equipment used for this purpose has been sterilized before. Drying and sieving (sieve diameter 2 mm) were carried out. They were packaged in sterilized glass jars.

ISSN: 0028-0712

Isolation and culture of epiphytes

The epiphytic fungi were isolated according to the principle adopted in the protocol of Pusz *et al*. (2015), consisting of a sampling for each subject of five leaves on the parts of the branches out of the shade, apparently unharmed (orientation is not taken into account). The leaves are packaged in pre-sterilized paper bags and transported in a cooler to the laboratory. 10 ml of sterilized distilled water was added to the centrifuge cups. The buckets are centrifuged at 4250 revolutions per minute for 10 minutes beneath a hood and between two bunse burners. A sample of 1 ml of the supernatant obtained after centrifugation was poured onto potato dextrose agar (PDA), in the number of three repetitions for each subject. Chloramphenicol was added to the culture medium (concentration 100 mg/L) to reduce any bacterial proliferation. The incubation was conducted at ambient temperature and under real conditions (light during the day, darkness at night) for two months, with boxes sealed with parafilm.

Isolation and culture of endophytic fungi

A sampling of ten healthy leaves from each subject was carried out for the inoculation of the endophytes. The leaves were sterilized according to the principle of the protocol of Schulz *et al*. (1993). To do this, a washing with running water was applied for the removal of debris and dust as a pretreatment of these leaves. Triple sterilization is applied to the latter, rinsing with sterilized distilled water at each stage. This step was done in the following order: 95% ethanol for 2 minutes, bleach (99.99%) for 3 minutes, and 95% ethanol for 30 seconds. Four fragments of

ISSN: 0028-0712

5 to 7 millimeters are removed from each leaf (at the rate of 40 pieces per subject) and are seeded on PDA, to which chloramphenicol has been added (concentration 100 mg/L). The incubation was conducted at ambient temperature and under real conditions (light during the day, darkness at night) for 2 months, with boxes sealed with parafilm.

Isolation of fungi from the soil

The so-called direct suspension-dilution technique proposed by Rapilly (1968) has been adopted to isolate soil mycodiversity. Three doses of 1 gram of soil from each subject were subjected to different dilutions: 10^{-1}, 10^{-2}, and 10^{-3}. The suspensions were seeded on the PDA culture medium containing chloramphenicol. The incubation was conducted at ambient temperature for 2 months, with boxes sealed with parafilm.

Identification of fungal strains

The fungal colonies were identified by macroscopic and microscopic approaches, namely the morphology of the colonies (color, texture, contour, relief, etc) according to the Atlas of Mushrooms by Carmen and Sciortino (2017). The morphology of the fungal structures was observed with an OPTIKA B-192 binocular microscope and the taking of photos by a SONY DSC-W320 ISO 400 camera, the characterization (types of conidiophores, their structure, and mode of grouping, the conidia with their types, shape, partitioning, pigmentation, ornamentation, and arrangement relative to the conidiophore, etc…) referred to the identification keys Kiffer and Morelet (1997), Nagamani *et al*. ((2006), Boersma *et al*. (2004) and Emory (1967).

ISSN: 0028-0712

Data analysis and processing

Calculation of ecological indices

To characterize the rhizosphere and phyllosphere mycodiversity, the abundances of the different fungal genera recorded were calculated according to the formula: A (%) = Ng / (Nt), with "A" abundance of the genera; "Ng" the number of times the genus is recorded in a subject and "Nt" set of repetitions of a subject having fructified compared to all the petri dish considered.

The Shannon-Weaver index and the Piélou equitability index were calculated concomitantly to interpret the results better. The Shannon-Weaver index was calculated according to the following equation : $H' = -\Sigma$ pi ln (pi), where "pi" is the relative abundance and "ln" is the natural logarithm (Shannon and Weaver 1949). The Piélou equitability index was obtained by the following equation : $E = \frac{H'}{H'max}$; H'max = log S (S= total number of species).

The Sorensen similarity index: $S = \frac{2C}{a+b} \times 100$ is used to compare the rhizospheric/epiphytic, rhizospheric/endospheric, and epiphytic /endophytic mycodiversity (with "a" the number of species present in part considered, "b" the number of species present in the second part considered and "c" the number of species common to both parts) (Sorensen 1948).

ISSN: 0028-0712

Statistical analyses

An analysis of variance (ANOVA) was carried out for the mycodiversity of the episphere, the endosphere, and the rhizosphere to extract the statistically significant differences for the calculated means. The data are independent. The normality test shows that the abundances of the Fungi in the different compartments have a normal distribution since more than 50% of the mean values are included in the interval [x ± σ]. A principal component analysis (PCA) and a correlation matrix (Pearson) were carried out between the mycodiversities of the episphere, the endosphere, and the rhizosphere for the sampled subjects to highlight the different correlations. All these analyses were carried out using the STAT BOX 6.40 software.

Results

Composition of the mycodiversity of the phyllosphere and the rhizosphere

The fungal epiphytes and the fungi of the cultivable rhizosphere belong to two phyla: *Ascomycota* and *Mucoromycota*, with a remarkable dominance of the *Ascomycota* phylum for both compartments. The abundance of the *Mucoromycota* phylum seems higher in the soil compartment. The isolated mycoendophytic strains all belong to the *Ascomycota* (Figure 4).

ISSN: 0028-0712

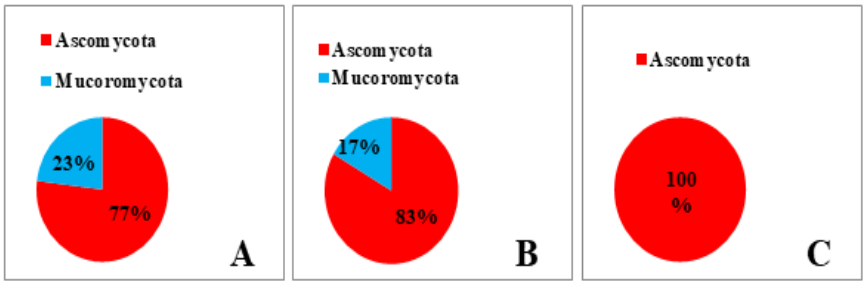

Figure 3. Graphical representation of the phyla of the two compartments (A: *rhizospheric fungi*, B: *epiphytic fungi*, C: *endophytic fungi*).

Twenty-five fungal genera have been identified for the two compartments. Differences in abundance are noted (Table 1). The mycodiversity obtained in the soil shows a dominance of the genus *Aspergillus*, followed by *Rhizopus*, *Ulocladium*, and *Alternaria*. However, that of the epiphytes displays a dominance of the genus *Alternaria*, followed by *Aureobasidium*, *Ulocladium*, and *Phoma*. The *Alternaria* genus is also dominant in the endosphere; it is followed by *Penicillium* and *Cladosporium*. The rest of the genres show a low percentage (for the majority less than 6%) for the two compartments combined (Figure 5).

Table 1. Abundance of fungal diversity at the level of the phyllosphere and the rhizosphere of *Punica granatum*.

		Abondance ± ES		
Genres	**Phylum**	**Rhizosphere**	**Epiphyte**	**Endophyte**
Absidia	*Mucoromycota*	00±00^{A}	0,46±036^{A}	00±00^{A}
Acremonium	*Ascomycota*	00±00^{A}	00±00^{A}	2,27±2,27^{A}
Alternaria	*Ascomycota*	7,99±1,48^{A}	21,33±1,43^{A}	45,38±3,72
Aspergillus	*Ascomycota*	44,35±3,24	3,02±1,25^{A}	0,42±0,41^{A}
Aureobasidium	*Ascomycota*	6,48±1,36^{B}	12,52±1,96	00±00^{A}
Bipolaris	*Ascomycota*	0,13±0,11^{B}	3,05±1,12^{B}	00±00^{A}
Candida	*Ascomycota*	00±00^{A}	00±00^{A}	2,08±1,68^{A}
Circinella	*Mucoromycota*	0,31±0,27^{A}	00±00^{A}	00±00^{A}
Chaetomium	*Ascomycota*	00±00^{A}	1,88±0,83^{A}	1,04±1,04^{A}
Chrysosporium	*Ascomycota*	00±00^{A}	1,16±0,62^{A}	3,13±3,12^{A}
Cladosporium	*Ascomycota*	0,23±0,20^{A}	4,51±1,64^{AB}	12,61±4,68^{B}
Exophiala	*Ascomycota*	00±00^{A}	3,29±1,59^{A}	00±00^{A}
Géotricum	*Ascomycota*	00±00^{A}	1,68±0,90^{A}	00±00^{A}
Memnoniela	*Ascomycota*	0,16±0,14^{A}	00±00^{A}	00±00^{A}
Monodictys	*Ascomycota*	00±00^{A}	0,52±0,41^{A}	00±00^{A}
Mucor	*Mucoromycota*	0,90±0,29^{A}	1,19±0,94^{A}	00±00^{A}
Oidium	*Ascomycota*	00±00^{A}	1,74±0,94^{A}	00±00^{A}
Penicillium	*Ascomycota*	0,75±0,55^{A}	4,07±1,27^{A}	13,23±6,86^{A}
Phoma	*Ascomycota*	1,55±0,73^{A}	10,86±1,15	00±00^{A}
Rhizopus	*Mucoromycota*	23,53±1,74	1,15±094^{A}	00±00^{A}
Scytalidium	*Ascomycota*	00±00^{A}	6,25±1,68	00±00^{A}
SNI	-	2,05±0,52^{A}	9,73±1,64	3,35±1,88^{A}
Trichocladium	*Ascomycota*	00±00^{A}	00±00^{A}	3,75±1,51^{A}
Trichophyton	*Ascomycota*	00±00^{A}	00±00^{A}	1,52±1,51^{A}
Torula	*Ascomycota*	0,16±014^{A}	00±00^{A}	00±00^{A}
Ulocladium	*Ascomycota*	10,85±1,84^{B}	11,52±1,74^{B}	1,22±0,73^{A}

ES: standard error; different capital letters for the same line correspond to different groups according to the Newman and Keuls test. **SNI**: Unidentified strain.

ISSN: 0028-0712

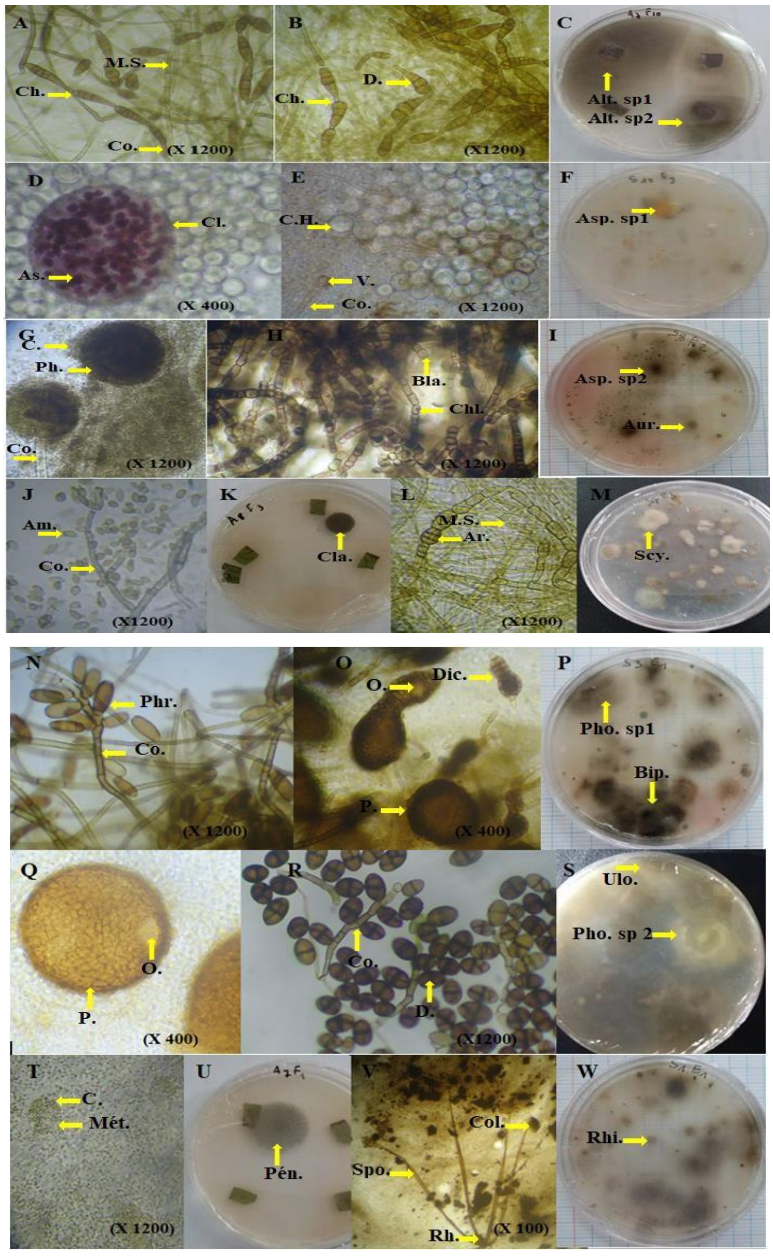

Figure 4. Microscopic and macroscopic aspects of the different dominant genera identified in the two compartments.

A: *Alternaria sp1* (Ch. Chainette, Co. Conidiophore, M.S. Mycelium Septate); **B**: *Alternaria sp2* (Ch. Chainette, D. Dictyospore); **C**: Macroscopic aspect of the colonies of *Alternaria sp1* and *sp2*; **D** and **E**: *Aspergillus nidulans* (teleomorph *Emericella nidulans*) (As. Ascospores, Cl. Cleistothecium, C.H. Hülle Cell, V. Vesicle, Co. Conidiophore); **G**: *Aspergillus niger* (C. Conidia, Co. Conidiophorus, Ph. Phialides); **H**: *Aureobasidium sp* (Bla. Blastoconidia, Chl. Chlamydoconidia); **I:** Macroscopic aspect of the colony of *Aspergillus niger* (Asp. sp2) and *Aureobasidium* (Aur.); **J**: *Cladosporium sp* (Am. Smooth amerospore with Hilum, Co. Conidiophore); **K**: Macroscopic aspect of the *Cladosporium sp* colony (Cla.); **L**: Scytalidium sp (Ar. Barrel-shaped arthroconidia, M.S. Septate Mycelium); **M**: Macroscopic appearance of the colony of Scytalidium sp (Scy.). **N**: Bipolaris sp (Phr. Phragmospores in clusters, Co. Conidiophore); **O**: Phoma *glomerata (teleomorph Peyronellaea) (Dic. Dictyochlamydospores, O. Ostiole, P.* Pycnide); **P**: Macroscopic aspect of the colony of Phoma glomerata (Pho. sp1) and Bipolarisp (Bip.); **Q** : Phoma herbarum (O. Ostiole, P. pseudoparenchymatous, hairless, thin-walled Pycnid); **R**: Ulocladium sp (Co. Conidiophore, D. Dictyospore in a cluster); **S** : Macroscopic aspect of the colony of Phoma herbarum (Pho. sp2) and of Ulocladium sp (Ulo.); **T**: Penicillium sp (C. Conidia, Met. Metules); **U** : Macroscopic aspect of the colony of Penicillium sp (Pen.); **V**: Rhizopus sp (Col. Broken columella, Rh. Rhyzoids, Pho. Sporocystophore); **W**: Macroscopic aspect of the colony of Rhizopus sp (Rhi.).

Figure 4. Microscopic and macroscopic aspects of the different dominant genera identified in the two compartments.

The differences between the compartments considered appear significant for the following genera: *Alternaria* (p=0.00), *Aspergillus* (p=0.00), *Aureobasidium* (p=0.00), *Bipolaris* (p=0.02), *Cladosporium* (p=0.02), *Phoma* (p=0.00), *Rhizopus* (p=0.00), *Scytalidium* (p=0.00) and *Ulocladium* (p=0.00). The ANOVA test carried out on diversity made it possible to distinguish three homogeneous groups based on the relative abundance of fungi; groups A and B show a statistically different diversity which may reveal a specific adaptation or a specific functional role of the fungi related to each group. Only the genus *Cladosporium* belongs to group AB, which suggests a non-differential presence in the two compartments studied. The most abundant fungi in the compartments studied isolate themselves outside these groups due to their remarkable preponderances.

The confrontation between the mycodiversity of the epiphytes and that of the rhizosphere highlighted ten genera in common. These are the genera *Alternaria*, *Aspergillus*, *Aureobasidium*, *Bipolaris*, *Cladosporium*, *Mucor*, *Penicillium*, *Phoma*, *Rhizopus*, and *Ulocladium*. Three genera are exclusive to the sol compartment, namely: *Circinella*, *Memnoniela*, and *Torula*. Seven genera are common between the episphere and the endosphere, namely: *Alternaria*, *Aspergillus*, *Chaetomium*, *Chrysosporium*, *Cladosporium*, *Penicillium*, and *Ulocladium*. Only four genera seem to be exclusive to the endosphere, namely: *Acremonium*, *Candida*, *Trichocladium* and *Trichophyton*. The genera exclusive to the phylloplan are: *Absidia*, *Scytalidium*, *Exophiala*, *Geotrichum*, *Monodictys*, and *Oidium*. The comparison of the mycodiversity of the soil and that of the endosphere revealed five genera in common, which are: *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Ulocladium*. The genera common between the soil and the endosphere are also the ubiquitous genera in the three parts studied. The exclusive involvement of the dominant genera in the positive correlations (Figure 4) for the endosphere and the rhizosphere supposes the efficiency in terms of selection (high selectivity), which seems less marked for the episphere.

ISSN: 0028-0712

Fungal diversity index of the phyllosphere and the rhizosphere

The highest values for the Shannon-Weaver index are recorded at the phyllospheric compartment (episphere and endosphere). The values displayed for the rhizosphere show an opposition: the Shannon-Weaver index shows the lowest value, and the highest value is displayed for the Piélou index. The global indices show that the phyllosphere is more diverse than the soil (Table 2).

Table 2. Calculated values for the Shannon-Weaver and Piélou indices for the compartments considered.

Compartments	Rhizosphere	Episphère	Endophyte
Shannon-Weaver index	2,13	3,17	2,50
Piélou's index	0,85	0,67	0,64

The results for the Sorensen index show that the similarity is higher in the soil compared to the two microniches of the phyllosphere, and very similar within the two micro-niches of the same compartment (Table 3).

Table 3. Calculated values for the Sorensen index for the parts considered.

Microniches	Rhizosphere/epiphyte	Rhizosphere/endophyte	Epiphyte/endophyte
Sorensen index	0,53	0,45	0,43

Interaction fungal diversity of the phyllosphere and the rhizosphere

The balance of the interactions obtained from the Pearson correlation matrix shows seven highly significant positive correlations between *Alternaria* and *Cladosporium* (1); the presence of *Aspergillus* is positively correlated with that of

ISSN: 0028-0712

Aureobasdium (1) and *Rhizopus* (1); that of *Aureobasdium* is positively correlated with that of *Rhizopus* (1); that of *Bipolaris* is positively correlated with that of *Scytalidium* (1) and that of *Phoma* (1) (Table 4).

Table 4. Significant correlations between phyllospheric fungi and soil fungi of *Punica granatum*

	Alternaria	***Aspergillus***	***Aureobasidium***	***Bipolaris***	***Scytalidium***	***Phoma***
Aureobasidium	ns	**1,00**	/	ns	ns	ns
Cladosporium	**1,00**	ns	ns	ns	ns	ns
Scytalidium	ns	ns	ns	**1,00**	/	ns
Phoma	ns	ns	ns	**1,00**	ns	/
Rhizopus	ns	**1,00**	**1,00**	ns	ns	ns
SNI	ns	ns	ns	ns	**1,00**	ns

In bold*, significant values (excluding diagonal) at the alpha threshold=0.05 (bilateral test). ns: not significant. SNI: Unidentified strain.*

The ½ plane of the PCA shows 100% of the total inertia, with 53% for axis 1 and 47% for axis 2. The presence of two distinct groups, A and B (Fig. 4), should be noted. According to axis 1, the group brought together the dominant fungal genera in the rhizosphere. According to axis 2, group B which brings together the phyllospheric fungal genera is subdivided into two subgroups; this corresponds to the two microniches of the phyllosphere (episphere and endosphere). The first subgroup, the epiphytes, shows correlations between the fungal genera *Bipolaris* and *Phoma*. The second subgroup represents the endophytes, they show a close correlation between *Alternaria* and *Cladosporium*.

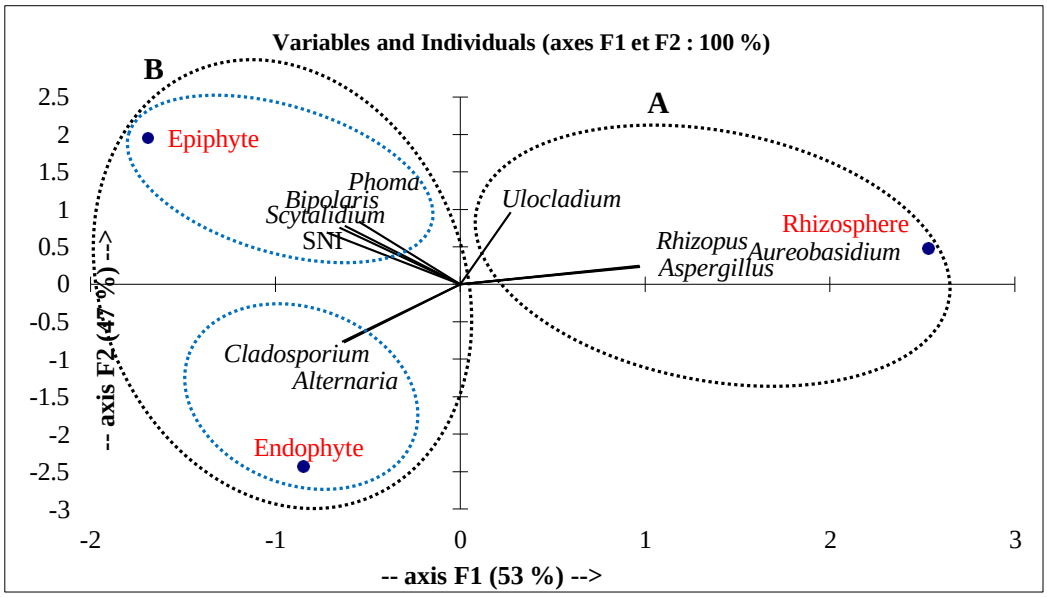

Figure 5. PCA of the distribution of phyllospheric and rhizospheric fungi of *Punica granatum.*

Discussion

Our results show the presence of two phyla, *Ascomycota* and *Mycoromycota* with a dominance of the *Ascomycota* phylum at the level of the two phyllospheric and telluric compartments. *Ascomycota* is considered one of the predominant groups, especially in agricultural soils (Viebahn *et al*., 2005). The dominance of this phylum has often been argued by its extreme diversification (Schoch *et al*., 2009) and the presence at the level of its representatives of numerous mechanisms of adaptability to stresses, in particular in hot desert environments (Buée *et al*., 2009; Massimo *et al*., 2015). This phylum is commonly found in soils with fresh organic matter (Seeliger *et al*., 2024). The management mode of our agrosystem can explain this

ISSN: 0028-0712

presence (soil with permanent cover of spontaneous plants from the steppe environment, recurring passages of sheep and goat herds). In the context of our study (ecological reallocation), our results suggest that the distribution of phyla at the level of the studied parts is adequate to the general pattern mentioned in the study by Egidi *et al*. (2019).

Considering the climatic data cited by the study by Lahouel and Belhadj (2022), in which it is mentioned that July is the hottest and driest month, the dominance of the genera observed in our soils seems to correspond to an environmental selection. The dominance of the genus *Aspergillus* is explained by this atmospheric and edaphic drought. Indeed, Dommergues and Mangenot (1970) mention that a certain number of *Aspergillus* have been attributed the xerothermophilic character so that the relative dominance of this genus in a population is an indication of a dry climate or microclimate. Castegnaro and Pfohl-Leszkowicz (2002) point out that *Aspergillus* is a fungal genus with a wide geographical distribution and is more often associated with regions with a warm climate. The same is true for the genus *Rhizopus*, to which the work attributes high thermal requirements, in addition, the latter adapts better to cultivated soils (Abdel-Hafez 1989). This is also the case for the genus *Ulocladium*, of which many species are recorded in regions with a hot climate, in particular thanks to their ability to resist solar radiation (Zaidan, 2011). Indeed, most of the identified genres are melanized, which may seem like a response to the conditions of the environment. Cockell and Knowland (1999) mention that a high temperature increases the melanin content of microorganisms. Grishkan and Nevo (2012) observed a positive correlation between the abundance of melanized species and temperature. Nevertheless, the same authors have proposed that the prevalence of a non-melanized mesophilic genus (Penicillium) in the soil under shrub cover is due to the microclimate of the station; which does not coincide with our results. Indeed, the alignment of the trees of our agrosystem can to a certain

ISSN: 0028-0712

extent offer characteristics of an open canopy, the shading offered by the wind breezes, as well as that of the tree crowns and the herbaceous stratum, undeniably modifies the amount of heat received and that lost by the soil, as well as the spectral composition of the solar radiation, certainly offering a mild microclimate compared to the ground outside the canopy. Nevertheless, considering the study by Kaisermann *et al*. (2017) which emphasizes that drought has lasting effects on underground communities, with consequences on plant-soil feedbacks,

we assume that the selection of this melanized mycodiversity was rather by habitat than by the station's microclimate. Therefore, the latter is most likely biotic inheritance of the soil favored/or maintained by the absence of soil modification.

This native mycodiversity adapted to arid conditions certainly contributes to the vigor of our agrosystem. Indeed, the article by det Gholizadeh *et al*. (2024) indicates that the adaptive response of plants to water stress was largely governed by the responses of underground microbial communities. In addition, it has been demonstrated that the adaptation of soil microbial communities to recurrent droughts improves plant health and their ability to withstand subsequent droughts. However, we cannot hide the probable intervention of our cultivar for the selection of the fungal taxa present, in particular, if we consider that the latter integrates the notion of the living which evolves (Demeulenaere and Bonneuil., 2010). Our cultivar is the product of a mass selection of local farmers; it has evolved in an environment with a low input supply, which has given it greater resistance to local stresses. The characteristics developed by our cultivar during its evolution in this environment may be at the origin of the selection of the fungal strains identified in our study. Indeed, different poplar cultivars have shown a selectivity of the root microbiome (Van Overbeek and Van Elsas, 2008). Moreover, it has been established that certain plant species select different fungal communities during drought (Compant *et al*., 2010), in particular, through modified carbon inputs (via litter or root exudates). This selectivity helps maintain the ecosystem's functioning in stressful environments (Griffiths *et al*., 2000).

ISSN: 0028-0712

The epiphytic fungal community turned out to be more diverse than the endophytic community. Our results seem consistent with those observed in the phyllosphere of the olive tree in the study by Gomes *et al*. (2018). We observe that among the four genera of fungi most abundant for the two microniches, three are recurrent contaminants of cultivated species, such as cereals, namely *Alternaria*, *Phoma*, and *Ulocladium* (Frisvard and Samson, 1991; Guiraud, 1998). The phylloplan is an outward-facing part of the foliar ecosystem. The dispersion by the wind of microorganisms is more important in open spaces, such as that represented by the

Algerian steppe regions. Moreover, this dispersion is more important for the foliar microbial communities of deciduous plants (Inácio *et al*., 2002). Indeed, Kembel *et al*. (2014) mentions that the conditions on the leaves of fast-growing trees (resource absorption strategy) act as a stronger ecological filter on potential colonists of the phyllosphere.

Seven genera are common between the two microniches. Even if the variability of the status of microorganisms in the epiphytic phyllosphere exists (Suslow, 2002; Zak, 2002), the endophytic association often has as an initial phase the epiphytic mode (Rodriguez *et al*., 2009; Porras-Alfaro and Bayman, 2011); which may partly explain our results. Currently, it is accepted that the host species impacts the abundance and diversity of the phyllospheric microbiome through its physiology (Shiraishi *et al*., 2015), the genotype, and the defense system (Leff *et al*., 2015; Copeland *et al*., 2015). However, environmental factors are as impactful as the host species for structuring, because they directly influence the passage of microorganisms to the phyllosphere (Lighthart, 1997). Moreover, the low rainfall for the sampling month can be advanced as an explanatory element of the low endophyte diversity, since this parameter has already been identified as a key factor for the endophyte colonization model (Martinez-Alvarez *et al*., 2012). On the other hand, the study by Materatski *et al*. (2019) cites that the olive tree "Elvas" cultivar presents a low value of diversity of phyllospheric endophyte due to reduced rainfall,

which seems to correspond to our results. The results note *Alternaria* as the dominant genus for the two microniches. The wide range of metabolites characteristic of this genus is probably at the origin of its ability to interact with the host (El Gobashy *et al*., 2018; Ramires *et al*., 2018; Patriarca *et al*., 2019). The abundance of the genus *Alternaria* is more marked in the endosphere, which seems to correspond to the observation established by Rashmi *et al*. (2019), where they give the checklist of the mycosphere the second place for the genus *Alternaria*, after the genus *Penicillium*.

As for the rhizosphere, the genera observed in the two micro-niches show a strong melanization which seems normal, since the melanized fungal bodies are more widespread and viable in the communities of aerial fungi and many fungi living on the leaves (Mirchink *et al*., 1968). It would seem that fungal melanin confers advantages in areas exposed to high levels of radiation and extreme temperatures, such as the Mediterranean regions (Kembel and Mueller, 2014). Moreover, the five fungal genera in common for the two compartments are dematiates. The latter characterizes specialized xero-thermophilic and photophilous populations (Dommergues and Mangenot, 1970).

The global diversity indices revealed an interesting diversity at the level of the studied agrosystem. The high values of H' and E for the phyllospheric compartment (the two microniches combined) suggest a certain stability of the fungal microbiome for this compartment if we consider the work Grall and Coïc (2006). Nevertheless, this observed stability is not necessarily an indication of the species-level stability within these genera, as highlighted by the study by Li *et al*. (2020). A question then arises: how can this stability be translated into a dynamic environment such as the phyllosphere, in particular the episphere? The entanglement encompassed by the dynamics of epiphytes (Hirano and Upper 1991; Unterseher *et al*., 2010), the identification of the status of endophytes at the

phyllosphere level and the presence of the multilayer model for microbiota/host interaction proposed by Shapira (2016) shows the complexity of phyllospheric interactions and make interpretations extremely difficult. Nevertheless, we assume that this stability is the result of the interactive lifestyle established by the microorganisms of the phyllosphere, which induces the establishment of a special niche (Agler *et al*., 2016), where the constant maintenance of the latter is admitted (San Roman and Wagner, 2018). The rhizospheric compartment shows opposite values between H' and E. This may mean the presence of a transition phase for the fungal microbiome present in the soil under *Punica granatum*, if we consider the work Grall and Coïc (2006). The history of our station, namely the installation of an engineer species on severely degraded soils with agroecological management, can be put forward as an argument for the results obtained.

The similarity for fungal communities is higher between the rhizosphere and the phyllosphere, compared to that of the two microniches of the phyllosphere. The similarity between the mycodiversity of the rhizosphere and that of the episphere can be explained by the fact that the soil has been proposed as a carrier material. Indeed, Manceau and Kasempour (2002) describe the soil and other components of the agrosystem as being a source of microorganisms for the phyllosphere. Moreover, the coarse texture described by Lahouel (2014) and Boudiaf *et al*. (2018) for our station combined with the wind (sirocco) which rages during July (Pouget, 1980; Lahouel and Belhadj, 2022) can explain the similarity observed, in particular, if the duration of these winds is taken into account. To another extent, the mode of management of our agrosystem, particularly the passage of local herds, can be a source of contamination for the phyllosphere. Indeed, Monk and Samuels (1990) affirm that spores and hyphal fragments can be released passively by the excreta of herbivores. It is also necessary to pay attention to the turbulence at ground level caused by their passage. Nevertheless, it must be borne in mind that tropospheric

ISSN: 0028-0712

microorganisms (aerosols) and the contribution of fungal mycodiversity associated with aerial tissues returning to the ground can contribute in part to this similarity. For the similarity noted between the mycodiversity of the rhizosphere and the endophytes, Zarraonaindia *et al*. (2015) proposed that the soil could serve as a potential inoculum reserve for the endophytes of the aerial organs, which may explain the similarity of the mycodiversity of the rhizosphere and the endosphere. The horizontal transmission of non-clavicipitaceous "type II" endophytes that colonize the leaves, can be an explanatory element (Rodriguez *et al*., 2009). The low similarity of the mycodiversity between the two phyllospheric microniches compared to that observed for the soil may suggest the intervention of a non-common inoculation source.

The positive correlation observed in our ACP (Figure 4) for the fungal genera *Aspergillus* and *Rhizopus* for the rhizosphere, can be explained as an adaptive strategy vis-à-vis certain edaphic characteristics of our station. Indeed, the soil of our resort is classified as xeric, calcimagnesic soil, with a calcareous crust (Lahouel, 2014). Boudiaf *et al*. (2012) and Boudiaf *et al*. (2018) mentioned low phosphorus levels in our station. In addition, this type of soil presents a problem as to the availability and absorption of phosphorus. Indeed, Morel (1996) mentioned that in calcareous soils there is a severe loss of phosphorus solubility. It would seem that the fungi most often cited for the activity of solubilization of soil phosphorus are *Aspergillus* and *Rhizopus* (Sindhu *et al*., 2014). An interesting feature is highlighted by Narsian and Patel (2000) and Srinivasan *et al*. (2012) for the genus *Aspergillus*, as to its ability to solubilize phosphorus at different levels of salinity. The permanence of the vegetation cover can also explain this interaction. Some species of the genus *Aspergillus* and *Rhizopus* have been described as having aptitudes for the decomposition of cellulose (Roussos and Raimbault, 1982). The Abdullah and

ISSN: 0028-0712

Al-Bader study (1990) confirms the capacity of degradation of cellulose compounds by thermotolerant and thermophilic fungi originating from the soil of Iraq, among which *Aspergillus* is cited. In addition, Dommergues and Mangenot (1970) have observed an association between cellulolytic fungi and phosphate- solubilizing bacteria in soils poor in assimilable phosphorus and without manure; this supports our suggestion cited above.

The positive correlation between *Aureobasidium* and *Aspergillus* may seem paradoxical, especially because of the antagonistic effects between these two genera. The work of Dimakopoulou *et al*. (2008) demonstrated that certain species of the genus *Aureobasidium* have antifungal power against *Aspergillus* species and that they could also degrade and detoxify ochratoxin A (mycotoxin produced by certain melanized *Aspergillus* species) (De Curtis, 2012). Nevertheless, the production of pectolytic enzymes by the genus *Aureobasidium* (Manachini *et al*., 1988) is inducible under conditions of carbon deficiency (Biely *et al*., 1996). This may explain the positive correlation observed for our study, especially if we consider the low carbon levels mentioned by Lahouel (2014) and Boudiaf *et al*. (2018). Moreover, Bencheqroun *et al*. (2007) cite that the level of protection of the genus *Aureobasidium* is reduced when there is competition for nutrients, which probably explains the observed inertia. We cannot confirm our assumptions as to the probable syntrophy and/or synergy as to these positive correlations. Nevertheless, a study under controlled conditions can clarify the knowledge about the interactions of these microorganisms in the case of our soils.

For the phyllosphere, the segregation of two subgroups of group B is certainly due to the distinct selective pressures between the two micro-niches (Santamaria and Bayman, 2005; Rastogi *et al*., 2013). The epiphytes seem to show correlations between fungal genera considered as potentially phytopathogenic at the level of the

ISSN: 0028-0712

episphere. The epiphytic lifestyle represents the initial phase of foliar colonization by many phytopathogens (Porras-Alfaro and Bayman, 2011). For epiphytes, a positive interaction is noted between the genera *Phoma*, *Scytalidium* and *Bipolaris*. We assume that this positive interaction reveals a correlated spatial and/or nutritional disposition, particularly if the spatial heterogeneity (Koskella, 2013) and the oligotrophy (Schlechter et al., 2019) of the phyllosphere are considered. This is all the more relevant since the disposition of epiphytic microorganisms takes place in colonial form, which offers protection to the latter against this difficult micro-habitat (Remus-Emsermann *et al*., 2012). A positive correlation is noted between *Alternaria* and *Cladosporium* for the endosphere. Both genera are capable of both endophytism and at least one other lifestyle; pathogenicity for *Alternaria* (DeMers, 2022) and saprophytism for *Cladosporium* (Salvatore *et al*., 2021). The observed interaction can be explained in part by the fact that for non-clavicipitaceous endophytes, the status of their interaction with the host can be transient; moreover, we are talking about cryptic occupation of plant tissues (Schulz and Boyle, 2005). Moreover, by a competitive effect and to establish themselves, endophytes can take advantage of the niche created by a virulent pathogen (Rodriguez *et al*., 2009; Latz *et al*., 2018; Yan *et al*., 2019). Nevertheless, it should be clarified that the assemblages of endophytes vary according to the habitat and that the most powerful filter for foliar endophyte communities is the host species (David *et al*., 2016). This positive interaction between *Alternaria* and *Cladosporium* is indicative of their importance for adapting to the context in which our cultivar is forced.

ISSN: 0028-0712

Conclusion

Our work is a preliminary contribution to studying the phyllospheric and rhizospheric fungal microbiome of *Punica granatum* variety Messaad in an arid zone of Algeria (Djelfa). Considering the fungal mycodiversity of the two compartments allowed us to highlight certain information explaining the resilience of our agrosystem. The fungal rhizobiome and phyllobiome of *Punica granatum* show a clear distinction. The phyllobiome seems to correspond to environmental constraints. The rhizobiome displays a diversity that seems not to be under the influence of the microclimate of the station but rather under the influence of the arid environment. The calculated genus-wide fungal community diversity indices show stability for the phyllospheric compartment compared to those of the rhizosphere compartment. The results obtained lead to new questions, and to answer them, we propose to extend this study by adopting the most suitable metagenomic sequencing method in collaboration with an equipped laboratory. In addition, for greater relevance, a comparison of similar studies with other stations in the region will make it possible to draw more significant conclusions for the promotion of arboriculture in arid environments by the Messaad pomegranate tree.

Acknowledgments

We thank the Directorate General for Scientific Research and Technological Development (DGRST) for its financial support. I also send my gratitude to the Engineer of the Natural Resources laboratory, Mrs. Sekhi Liela. I would also like to thank the firefighters' adjutant, Mr. Souaf Omar, and the owner of the orchard, Mr. Kechida Ahmed, for their invaluable help and support.

Références

Abdel-Hafez S. I. (1984). Composition of the fungal flora of four cereal grains in Saudi Arabia. *Mycopathologia*, *85*(1-2), 53–57. https://doi.org/10.1007/BF00436702.

Abdullah, S.K. & Al-Bader, S.M. (1990). On the thermophilic and thermotolerant mycoflora of Iraqi soils. *Sydowia*, 42,1-7. https://www.researchgate.net/publication/267996024.

Agler, M.T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.T., Weigel, D. & al. (2016). Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLOS Biology*, 14, e1002352. https://doi.org/10.1371/journal.pbio.1002352.

Bauer, J.T., Mack, K.M.L. & Bever, J.D. (2015). Plant-soil feedbacks as drivers of succession: evidence from remnant and restored tallgrass prairies. Ecosphere 6, 158. https://doi.org/10.1890/ES14-00480.1.

Bencheqroun, S.K., Bajji, M., Massart, S., Labhilili, M., El Jaafari, S. & Jijakli, M.H. (2007). In Vitro and in Situ Study of Postharvest Apple Blue Mold Biocontrol by *Aureobasidium pullulans*: Evidence for the Involvement of Competition for Nutrients. *Postharvest Biology and Technology*, 46, 128-135. https://doi.org/10.1016/j.postharvbio.2007.05.005.

Bergelson, J., Mittelstrass, J., & Horton, M. W. (2019). Characterizing both bacteria and fungi improves understanding of the Arabidopsis root microbiome. *Scientific reports*, *9* (1), 24. https://doi.org/10.1038/s41598-018- 37208-z.

Bernal, P., Eberl, L., de Jonge, R., Lepek, V. C., & Malone, J. G. (2021). Understanding plant-microorganism interactions to envision a future of sustainable agriculture. *Environmental microbiology*, *23*(4), 1809–1811. https://doi.org/10.1111/1462-2920.15479.

Biely, P., Heinrichová, K. & Kru, M. (1996). Induction and Inducers of the Pectolytic System in *Aureobasidium pullulans*. *Current Microbiology*, 33, 6-10. https://doi.org/10.1007/s002849900065.

Boerema, G. H., Gruyter, J. de., Noordeloos, M. E. & Hamers, M. E. C. (2004). *Phoma* identification manual. Differentiation of specific and infra-specific taxa in culture. Cabi publishing. 470 p.

Boudiaf-Nait Kaci, M., Hedde, M., Mouas-Bourbia, S., Siad, D. & Derridj, A. (2012). Biodisponibilité du phosphore dans la rhizosphère du grenadier (*Punica granatum* L.) sous climat aride : cas des vergers de Messaad Wilaya de Djelfa. 11èmes journées d'Etude des Sols. https://colloque4.inra.fr/journees_etude_sols_2012.

Boudiaf-Nait Kaci, M., Ouslimani, O., Mallek, T., Hadim, A., Hocine, F. & Lahoual, M. (2018). Impact du grenadier (*Punica granatum* L.) variété MESSAAD dans la mise en défens d'une steppe dégradée : cas des vergers de Melaga ALGERIE. https://dspace.univ-ouargla.dz/jspui/handle/123456789/16668.

Boukerker, H., Boumedjene, M.R., Doughbege, A.E., Belhouadjeb, F.A., Kherifi, W., Hecini, L. & Bekiri, F. (2021). State of pastoral resources in the Algerian steppe regions: main factors of degradation and definition of preservation and rehabilitation actions. *Livestock Research for Rural Development*, 33, 140. http://www.lrrd.org/lrrd33/12/33140hbouk.html.

Buée, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S. & al. (2009). 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist*, 184, 449-456. https://doi.org/10.1111/j.1469-8137.2009.03003.x.

Compant, S., Mitter, B., Colli-Mull, J. G., Gangl, H., & Sessitsch, A. (2011). Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microbial ecology*, *62*(1), 188–197. https://doi.org/10.1007/s00248-011-9883-y.

Castegnaro, M. & Pfohl-Leszkowicz, A. (2002). Les mycotoxines : contaminants omniprésents dans l'alimentation animale et humaine, dans La sécurité alimentaire du consommateur, Lavoisier, Tec&Doc.

Carmen, V. & Sciortino, Jr. (2017). Atlas of Clinically Important Fungi. Hoboken, New Jersey, John Wiley & Sons, Inc., 490 p.

Cockell, C.S. & Knowland, J. (1999). Ultraviolet radiation screening compounds. *Biological Reviews*, 74(3), 311-345. https://doi.org/10.1017/ S0006323199005356.

Compant, S., van der Heijden, M.G.A. & Sessitsch, A. (2010). Climate change effects on beneficial plant-microorganism interactions. *FEMS Microbiology Ecology*, 73, 197-214. https://doi.org/10.1111/j.1574-6941.2010.00900.x.

Copeland, J.K., Yuan, L., Layeghifard, M., Wang, P.W. & Guttman, D.S. (2015). Seasonal community succession of the phyllosphere microbiome. *Molecular Plant-Microbe Interactions*, 28(3), 274-285. https://doi.org/10.1094/MPMI-10-14-0331-FI.

David, A. S., Seabloom, E. W., & May, G. (2016). Plant Host Species and Geographic Distance Affect the Structure of Aboveground Fungal Symbiont Communities, and Environmental Filtering Affects Belowground Communities in a Coastal Dune Ecosystem. *Microbial ecology*, *71*(4), 912–926. https://doi.org/10.1007/s00248-015-0712-6.

De Curtis, F., De Cicco, V. & Lima, G. (2012). Efficacy of Biocontrol Yeasts Combined with Calcium Silicate or Sulphur for Controlling Durum Wheat Powdery Mildew and Increasing Grain Yield Components. *Field Crops Research*, 134, 36-46. https://doi.org/10.1016/j.fcr.2012.04.014.

DeMers, M. (2022). *Alternaria alternata* as endophyte and pathogen. *Microbiology*,168(3). 001153. https://doi.org/10.1099/mic.0.001153.

Demeulenaere, E. & Bonneuil, C. (2010). Cultiver la biodiversité. Semences et identité paysanne. Hervieu B., Mayer N., Müller P. Purseigle F., & J. Rémy. Les mondes agricoles en politique. De la fin des paysans au retour de la question agricole, Presses de Sciences Po, pp.73-92. ⟨hal-00528010⟩.

Dimakopoulou, M., Tjamos, S.E., Antoniou, P.P., Pietri, A., Battilani, P., Avramidis, N., Markakis, E.A. & Tjamos, E.C. (2008). Phyllosphere Grapevine Yeast *Aureobasidium pullulans* Reduces *Aspergillus Carbonarius* (Sour Rot) Incidence in Wine-Producing Vineyards in Greece. *Bioogical Control*, 46, 158-165. https://doi.org/10.1016/j.biocontrol.2008.04.015.

Dommergues, Y. & Mangenot, F. (1970). Ecologie microbienne du sol, Masson et C^{ie}, éditeurs Paris. 803 p. https://horizon.documentation.ird.fr/exl-doc/pleins_textes/divers20-09/08110.pdf.

Egidi, E., Delgado-Baquerizo, M., Plett, J. M., Wang, J., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., & Singh, B. K. (2019). A few Ascomycota taxa dominate soil fungal communities worldwide. *Nature communications*, *10*(1), 2369. https://doi.org/10.1038/s41467-019-10373-z.

El Gobashy, S.F., Mikhail, W.Z.A., Ismail, A.M., Zekry, A. & Moretti, A. (2018). Phylogenetic, toxigenic and virulence profiles of *Alternaria* species causing leaf blight of tomato in Egypt. *Mycological Progress*,17, 1269-1282. https://doi.org/10.1007/s11557-018-1442-1.

Emory, G. S. (1967). Typification of *Alternaria*, *Stemphylium*, and *Ulocladium*. Mycologia, 59(1), 67-92. http://www.jstor.org/stable/3756943.

Frisvard, J.C. & Samson, R.A. (1991). Filamentous fungi in foods and feeds: ecology, spoilage and mycotoxin production. Handbook of applied mycology, 3, foods and feeds, Dekker, M. New York: Arora, D.K., Mukerji, K. G et Marth. E.H., pp. 31-68.

Grall, J. & Coïc, N. (2006). Synthèse des méthodes d'évaluation de la qualité du benthos en milieu côtier. Institut Universitaire Européen de la Mer – Université de Bretagne Occidentale Laboratoire des sciences de l'Environnement marin. 90 p. REF. Ifremer DYNECO/VIGIES/06-13/REBENT.

Gholizadeh, S., Nemati, I., Vestergård, M., Barnes, C. J., Kudjordjie, E. N., & Nicolaisen, M. (2024). Harnessing root-soil-microbiota interactions for drought-resilient cereals. *Microbiological research*, *283*, 127698. https://doi.org/10.1016/j.micres.2024.127698 **Gomes, T., Pereira, J. A., Benhadi, J., Lino-Neto, T., & Baptista, P. (2018).** Endophytic and Epiphytic Phyllosphere Fungal Communities Are Shaped by Different Environmental Factors in a Mediterranean Ecosystem. *Microbial ecology*, *76*(3), 668–679. https://doi.org/10.1007/s00248-018-1161-9.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S.J., Bååth, E., Bloem, J., De Ruiter, P.C., Dolfing, J. & Nicolardot, B. (2000). Réponse écosystémique des communautés des sols de pâturage aux réductions de la diversité microbienne induites par la fumigation : un examen de la relation entre la fonction biodiversité et l'écosystème. *Oikos*, 90, 279-294**.** https://doi.org/10.1034/j.1600-0706.2000.900208.x.

Grishkan, I. & Nevo, E. (2012). Spatiotemporal dynamics of culturable microfungi in soil of Mount Hermon, Israel. Plant Biosystems. An International Journal Dealing with all Aspects of Plant Biology. *Official Journal of the Societa Botanica Italiana*, 146, 150-163. https://doi.org/10.1080/11263504.2012.687405.

Guiraud, J.P. (1998). Microbiologie alimentaire. Dunod. Paris, ISBN: 210 0036661, 696 p.

Hirano, S.S., Upper, C.D. (1991). Bacterial community dynamics. In: Andrews JH, Hirano SS (eds) Microbial Ecology on Leaves. Springer-Verlag: New York, 271-294. https://doi.org/10.1007/978-1-4612-3168-4_14.

Inácio, J., Pereira, P., de Carvalho, M., Fonseca, A., Amaral-Collaço, MT. & Spencer-Martins, I. (2002). Estimation and diversity of phylloplane mycobiota on selected plants in a Mediterranean-type ecosystem in Portugal. *Microbial Ecology*, 44(4), 344-53. https://doi.org/10.1007/s00248-002-2022-z.

Kaisermann, A., de Vries, F.T., Griffiths, R.I. & Bardgett, R.D. (2017). Legacy effects of drought on plant-soil feedbacks and plant-plant interactions. *New Phytologist*, 215, 1413-1424. https://doi.org/10.1111/nph.14661.

Kembel, S.W. & Mueller, R.C. (2014). Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. *Botany*, 92, 303-311. https://doi.org/10.1139/cjb-2013-0194.

Kiffer, E. & Morelet, M. (1997). Les Deutéromycètes; classification et clés d'identification générique. INRA, Paris, 306 p.

Koskella, B. (2013). Phage-mediated selection on microbiota of a long-lived host. *Current Biology*, 23,1256-1260. https://doi.org/10.1016/j.cub.2013.05.038.

Lahouel, M. (2014). Caractérisation de l'environnement racinaire du pistachier de l'Atlas, de l'olivier et du grenadier dans la région de Messaad. Mémoire de Magister. Faculté des Sciences De La Nature et de La Vie. Département des Sciences Agronomiques. Université Ziane Achour Djelfa. https://dspace.univ-djelfa.dz:8080/xmlui/handle/123456789/927.

Lahouel, M. & Belhadj, M. (2022). Morphological variability of the fruits of seven pomegranate (*Punica granatum* L.) cultivars grown in Messaad region in central Algeria. *BASE*, 26 (1), 16-29. https://popups.uliege.be/1780-4507/index.php?id=19447.

Latz, M.A.C., Jensen, B., Collinge, D.B. & Jorgensen, H.J.L. (2018). Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression**.** *Plant Ecology & Diversity*,11(5-6),555-567. https://doi.org/10.1080/17550874.2018.1534146.

Leff, J.W., Del Tredici, P., Friedman, W.E. & Fierer, N. (2015). Spatial structuring of bacterial communities within individual *Ginkgo biloba* trees. *Environmental Microbiology*, 17, 2352-2356. https://doi.org/10.1111/1462-2920.12695.

Li, M., Hong, L., Ye, W., Wang, Z., & Shen, H. (2022). Phyllosphere bacterial and fungal communities vary with host species identity, plant traits and seasonality in a subtropical forest. *Environmental microbiome*, *17*(1), 29. https://doi.org/10.1186/s40793-022-00423-3.

Lighthart, B. (1997). The ecology of bacteria in the alfresco atmosphere. *FEMS Microbiology Ecology*, 23, 263-274. https://doi.org/10.1111/j.1574-6941.1997.tb00408.x.

Manachini, P.L., Parini, C. & Fortina, M.G. (1988). Pectic Enzymes from *Aureobasidium pullulans* LV 10. *Enzyme and Microbial Technology*, 10, 682-685.

Manceau, C.R. & Kasempour, M.N. (2002). In Endophytic versus epiphytic colonization of plants: what comes first? In: Lindow SE, Hecht-Poinar EI, Elliott VJ (eds) *Phyllosphere microbiology*. APS Press, St Paul, USA, pp. 115-123.

Martinez-Alvarez, P., Martin-Garcia, J., Rodriguez-Ceinos, S. & Diez, J.J. (2012). Monitoring endophyte populations in pine plantations and native oak forests in Northern Spain. *Forest Systems*, 21, 373-382. https://doi.org/10.5424/fs/2012213-02254.

Martins, G., Lauga, B., Miot-Sertier, C., Mercier, A., Lonvaud, A., Soulas, M. L., Soulas, G., & Masneuf-Pomarède, I. (2013). Characterization of epiphytic bacterial communities from grapes, leaves, bark and soil of grapevine plants grown, and their relations. *PloS one*, *8*(8), e73013. https://doi.org/10.1371/journal.pone.0073013.

Marulanda, A., Barea, J.M. & Azcon, R. (2009). Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments: mechanisms related to bacterial effectiveness. *Journal of Plant Growth Regulation*, 28, 115-124. https://doi.org/10.1007/s00344-009-9079-6.

Massimo, N.C., Devan, M.M.N., Arendt, K.R., Wilch MH, Riddle, J.M., Furr, S.H., Steen, C., U'Ren, J.M., Sandberg, D.C. & Arnold, A.E. (2015). Fungal endophytes in above-ground tissues of desert plants: infrequent in culture, but highly diverse and distinctive symbionts. *Microbial Ecology*, 70, 61-76. https://doi.org/10.1007/s00248-014-0563-6.

Materatski, P., Varanda, C., Carvalho, T., Dias, A. B, Campos, M. D., Rei, F., do Rosario Felix, M. (2019). Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities, Botany 92: 303–311. https://doi.org//10.1139/cjb-2013-0194.

Mirchink, T.G., Kashinka, G.B. & Abaturov, Y.D. (1968). Resistance of the dark-coloured fungi *Stemphylium botryosum* WALLR and *Cladosporium cladosporoides* (FRIES) de Vries to gamma irradiation. *Mikrobiologia*, 37, 865-869.

Monk, K.A. & Samuels, G.J. (1990). Mycophagy in grasshoppers (Orthoptera, Acrididae) in Indo-Malayan rain forest. *Biotropica*, 22, 16-21.

Morel, R. (1996). Les sols cultivés. Lavoisier (deuxième édition). pp. 378.

Narsian, V. & Patel, H. (2000). *Aspergillus aculeatus* as a rock phosphate solubilizer. *Soil Biol Biochemistry*., 32, 559-565. https://doi.org/10.1016/S0038-0717(99)00184-4.

Nagamani, A., Kunwar, I.K. & Manoharachary, C. (2006). Handbook of Soil Fungi. International Pvt Ldt, New Delhi (India). 474p.

Oses, R., Valenzuela, S., Freer, J., Sanfuentes, E. & Rodriguez, J., (2008). Fungal endophytes in xylem of healthy chilean trees and their possible role in early wood decay. *Fungal Diversity*, 33, 77-86. https://www.fungaldiversity.org/fdp/sfdp/33-4.

Patriarca, A., da Cruz Cabral, L., Pavicich, M.A., Nielsen, K.F. & Andersen, B. (2019). Secondary metabolite profiles of small-spored *Alternaria* support the new phylogenetic organization of the genus. *International Journal of Food Microbiology*, 291,135-143. https://doi.org/10.1016/j.ijfoodmicro.2018.11.022.

Petrini, O. (1991). Fungal endophytes of tree leaves. In: Andrews J H, Hirano S S(eds), *Microbial Ecology of Leaves*. Springer-Verlag, New York, pp 179-197.

Porras-Alfaro, A. & Bayman, P. (2011). Hidden fungi, emergent properties: endophytes and microbiomes. *Phytopathology*, 49, 291-315. https://doi.org/10.1146/annurev-phyto-080508-081831.

Pouget, M. (1980). Les relations sol-végétation dans les steppes sud-algéroises. ORSTOM. Paris, 569 p.

Pusz, W., Pląskowska, E., Yildirim, I.& Weber, R. (2015). Fungi occurring on the plants of the genus *Amaranthus* L. *Turkish Journal of Botany*, 39, 147-161. https://doi.org/10.3906/bot-1403-106.

Ramires, F.A., Masiello, M., Somma, S., Villani, A., Susca, A., & al. (2018). Phylogeny and Mycotoxin Characterization of *Alternaria* Species Isolated from Wheat Grown in Tuscany, Italy. *Toxins* (Basel),10, E472. https://doi.org/10.3390/toxins10110472.

Rapilly, F. (1968). Les techniques de mycologie en pathologie végétale. *Annales des épiphytes*,19. Edition INRA. Paris. 102 p.

Rashmi, M., Kushveer, J.S. & Sarma, V.V. (2019). A worldwide list of endophytic fungi with notes on ecology and diversity. *Mycosphere*, 10 (1), 798-1079. https://doi.org/0.5943/mycosphere/10/1/19.

Rastogi, G., Coaker, G.L.& Leveau, J.H. (2013). New insights into the structure and function of phyllosphere microbiota through highthroughput molecular approaches. *FEMS Microbiology Letters*, 348, 1-10. https://doi.org/10.1111/1574-6968.12225.

Remus-Emsermann, M.N.P., Tecon, R., Kowalchuk, G.A. & Leveau, J.H.J. (2012). Variation in local carrying capacity and the individual fate of bacterial colonizers in the phyllosphere. *ISME Journal*, 6, 756-765. https://doi.org/10.1038/ismej.2011.209.

Rodriguez, R.J., White, J.F. Jr., Arnold, A.E. & Redman, R.S. (2009). Fungal endophytes: diversity and functional roles. *New Phytologist*, 182 (2), 314-330. https://doi.org/10.1111/j.1469-8137.2009.02773.x.

Roussos, S., & Raimbault, M., (1982). Hydrolyse de la cellulose par les moisissures : 1. Screening des souches cellulolytiques. *Annales de Microbiologie* (*Istitut Pasteur*), 133(b), 455-464.

Salvatore, M.M., Andolfi, A. & Nicoletti R (2021). The Genus *Cladosporium*: A Rich Source of Diverse and Bioactive Natural Compounds. *Molecules*, 26 (13), 3959. https://doi.org/10.3390/molecules26133959.

San Roman, M. & Wagner, A. (2018). An enormous potential for niche construction through bacterial cross-feeding in a homogeneous environment. *PLOS Computational Biology*, 14 (7), e1006340. 018. https://doi.org/10.1371/journal.pcbi.1006340.

Santamaria, J. & Bayman, P. (2005). Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). *Microbial Ecology*, 50,1-8. https://doi.org/10.1007/s00248-004-0002-1.

Schlechter, R.O., Mitja, M.M. & Remus-Emsermann, N.P. (2019). Driving factors of epiphytic bacterial communities: A review. *Journal of Advanced Research*, 19, 57-65. https://doi.org/10.1016/j.jare.2019.03.003.

Schoch, C.L., Sung, G.H., López-Giráldez, F., Townsend, J.P., Miadlikowska, J., Hofstetter, V. & Spatafora, J.W. (2009). The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits, *Systematic Biology*, 58, 224-239. https://doi.org/10.1093/sysbio/syp020.

Schulz, B. & Boyle, C. (2005). The endophytic continuum. *Mycological Research*,109 (6): 661-686. https://doi.org/10.1017/s095375620500273x.

Schulz, B., Wanke, S., Draeger, S. & Aust, H.J. (1993). Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological Research*, 97, 1447-1450. https://doi.org/10.1016/S0953-7562(09)80215-3.

Seeliger, M., Hilton, S., Muscatt, G., Walker, C., Bass, D., Albornoz, F., Standish, R. J., Gray, N. D., Mercy, L., Rempelos, L., Schneider, C., Ryan, M. H., Bilsborrow, P. E., & Bending, G. D. (2024). New fungal primers reveal the diversity of Mucoromycotinian arbuscular mycorrhizal fungi and their response to nitrogen application. *Environmental microbiome*, *19*(1), 71. https://doi.org/10.1186/s40793-024-00617-x.

Shannon, C.E. & Weaver, W. (1949). The Mathematical Theory of Communication. University of Illinois Press. Urbana. IL. USA. 131 p.

Shapira, M. (2016). Gut microbiotas and host evolution: scaling up symbiosis. Trends in *Ecology and Evolution*, 31, 539-549. https://doi.org/10.1016/j.tree.2016.03.006.

Shiraishi, K., Oku, M., Kawaguchi, K., Uchida, D., Yurimoto, H. & Sakai, Y. (2015). Yeast nitrogen utilization in the phyllosphere during plant lifespan under regulation of autophagy. *Scientific Reports*, 5, 09719. https://doi.org/10.1038/srep09719.

Sindhu, S.S., Phour, M., Choudhary, S.R. & Chaudhary, D. (2014). Phosphorus cycling: prospects of using rhizosphere microorganisms for improving phosphorus nutrition of plants. In: *Geomicrobiology and Biogeochemistry*, *Soil Biology*, 39, 199-237. https://doi.org/10.1007/978-3-642-41837-2_11.

Sorenson, T. (1948). A method of establishing groups of equal amplitudes in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. *Kongelige Danske Videnskabernes Selskab. Biologiske Skrifter*, 5, 1-34.

Srinivasan, R., Yandigeri, M.S., Kashyap, S. & Alagawadi, A.R. (2012). Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. *Saudi Journal of Biological Sciences*, 19, 427-434. https://doi.org/10.1016/j.sjbs.2012.05.004.

Suslow, T.V. (2002). Production practices affecting the potential for persistent contamination of plants by microbial foodborne pathogens. In: Lindow SE, Hecht-Poinar EI, ElliottVJ (eds) *Phyllosphere microbiology*. APS Press, St Paul, USA, pp, 241-256.

Unterseher, M., Jumpponen, A., Öpik, M., Tedersoo, L., Moora, M., Dormann, C.F. & al. (2010). Species abundance distributions and richness estimations in fungal metagenomics-lessons learned from community ecology. *Molecular Ecology*, 20, 275-285. https://doi.org/10.1111/j.1365-294X.2010.04948.x.

Van der Wal, A. & al. (2006). Fungal biomass development in a chronosequence of land abandonment. Soil Biol, Biochem 38, 51-60. https://doi.org/10.1016/j.soilbio.2005.04.017.

Van Overbeek, L. & Van Elsas, J.D. (2008). Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (*Solanum tuberosum* L.). *FEMS Microbiology Ecology*, 64, 283-296. https://doi.org/10.1111/j.1574-6941.2008.00469.x.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A. & Dufresne, A. (2015). L'importance du microbiome de la plante holobionte. *Nouveau Phytologue*, 206, 1196-1206. https://doi.org/10.1111/nph.13312.

Viebahn, M., Doornbos, R., Wernars, K., Van Loon, L.C., Smit, E. & Bakker, P.A.H.M. (2005). Ascomycete communities in the rhizosphere of field-grown wheat are not affected by introductions of genetically modified *Pseudomonas putida* WCS358r. *Environmental Microbiology*, 7, 1775-1785. https://doi.org/10.1111/j.1462-2920.2005.00783.x.

Wagner, M. R., Lundberg, D. S., Del Rio, T. G., Tringe, S. G., Dangl, J. L., & Mitchell-Olds, T. (2016). Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nature communications*, *7*, 12151. https://doi.org/10.1038/ncomms12151.

Yan, L., Zhu, J., Zhao, X., Shi, J., Jiang, C., & Shao, D. (2019). Beneficial effects of endophytic fungi colonization on plants. *Applied microbiology and biotechnology*, *103*(8), 3327–3340. https://doi.org/10.1007/s00253-019-09713-2.

Zaidan, K.I. (2011). Isolement et identification des espèces d'*Ulocladium preuss* de différentes régions en Irak. *Bassorah Journal of Agricultural Sciences*, 24, 2. https://www.researchgate.net/publication/277891358_Isolation_and_identification_species_of_Ulocladium_Preuss_from_different_regions_in_Iraq.

Zak, J.C. (2002). Implications of a leaf surface habitat for fungal community structure and function. In: Lindow SE, Hecht-Poinar EI, Elliott VJ (eds) *Phyllosphere Microbiology*. APS Press, St Paul, USA, pp, 299-315.

Zarraonaindia, I., Owens, S.M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N.A., Mills, D.A., Martin, G., Taghavi, S. & al. (2015). The soil microbiome influences grapevine-associate. *MBio*, 6, e02527–e02514. https://doi.org/10.1128/mBio.02527-14.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this research paper.