الجمهورية الجزائرية الديمقراطية الشعبية

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

Université Ibn Khaldoun–Tiaret

Faculté des Sciences de la Nature et de la Vie

Département des Sciences de la Nature et de la Vie

Spécialité : Génétique Moléculaire et Amélioration des Plantes

Mémoire de fin d'études en vue de l'obtention du diplôme de Master académique

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Thème

Isolation and characterization of new strains of arbuscular mycorrhizal fungi and evaluation of their effect on secondary metabolites contents in wild jujube (*Ziziphus lotus* L.)

Soutenu publiquement le 22/09/2021

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Année universitaire : 2020 -2021

Acknowledgements

First of all, we want to thank the Mighty Lord« **ALLAH** » for watching over us, for guiding us and giving us the wisdom and the strength in every step of the way to finish our work.

To our dear promoter, Mrs. Fatima Dahlia for agreeing to lead this work, her support, skills and foresight have been invaluable to us.

We address Our sincere thanks to the jury members

Furthermore, we would like to show our deepest gratitude to all the professors, laboratory chefs, every person that helped in the achievement of our work by their words, their advice and their criticisms, guided our reflection and answered our questions during my research

We thank our dear parents, who have always been there for us. We thank our sisters and brothers for their encouragement.

Finally, we thank our friends, who have always been there for us. Their unconditional support and encouragement have been a great help.

Dedications

I dedicate this memoire

To my father **Sahraoui Zghida** and my mother **Khaira Allouba** for their unconditional love, their sacrifices, their confidence, their support.

To my grandmother **Oum Elbakht Allouba**, for all the affection she has given me and for her precious encouragement.

In the memory of the loving son, brother, father, friend **ALLOUBA MILOUD** you may rest in peace knowing that you're remembered and loved more than ever.

To my sister and my brothers, **Aya**, **Fathi Fouad, Farouk, Attef**, for their tenderness, their awkward sense of humor that helped overcome this challenge.

To my aunts and uncles of the **Allouba** family as well as **Khaira Djninat**, especially for their words of encouragement and kindness.

To my friends **Racha, Nadjat, Fatima, Mohammed, Wahid, Kika**, **Omar, Gaya,** and my soul sister **Chaima** the greatest sources of my happiness, as well as to my second Family the **C-ART** club.

To all my comrades from the **IBN KHALDOUN** University of Tiaret.

Imen

Dedications

I am dedicating this thesis to the person who have meant and continue to mean so much to me. Although he is no longer of this world, his memories continue to regulate my life my father Hadj whose love for me knew no bounds and who taught me the value of hard work, I will never forget you.

I also dedicate this work to my mother Belhadj Malika who has encouraged me by all the way and whose encouragement has made sure that I give it all it takes to finish that which I have started.

To my sister's Fatima, Asma, Djahida, Chahinez, Hanaà, Youssra and Chahrazed who have supported me throughout the process. Without forgetting my nephews Sami, Adam, Ayman and my beautiful niece Nihal.

To both my families Kendricha and Belhadj.

To Araria Ahmed and his family

To Madjdoub Abdelkader and his family

To all my friends

To my team-work Tayeb and Imen

To all my colleagues specially Ilyes, Djamel, Samar and Nawal.

Abdelkader

Dedications

I am dedicating this thesis to my beloved favorite person who have meant and continue to mean so much to me. Although she is no longer of this world, her memories continue to regulate my life. First and foremost, to my paternal grandmother Boussmaha Reguia whose love for me knew no bounds and, who taught me the value of hard work and supported me throughout every step possible. Thank you so much "ma", I will never forget you.

I also dedicate this thesis to my parents Badaoui Fatiha and Riah Noureddine who have supported me throughout the process. I will always appreciate all they have done, for their love and support throughout my life. Thank you both for giving me strength to do my best and chase my dreams.

Thank you to my lovely brother and sister Aziz and Reguia for being my amazing support system and being there for me always.

To my grandparents Tayeb and Tayeb and Ramlia.

To Professor Fatima Dahlia, my supervisor. I am grateful for the trust deposited in my work and for the motivation demonstrated along this arduous course. Her support was without a doubt crucial in my dedication this work.

To both families I am proud to be part of "Riah" and "Badaoui" and my uncles and aunts from both sides and especially in memory of my aunt we lost this year Riah Salwa.

To all my cousins and friends and colleagues that I am grateful knowing throughout my journey.

Last but not least to my work-team Kendricha Abdelkader and Zghida Zineb for their hard work and determination.

Tayeb

List of abbreviations

AM: Arbuscular mycorrhizal

AMF: Arbuscular mycorrhizal fungi

dof: Degree of freedom

EcM: Ectomycorrhizal

ErM: Ericoid mycorrhizal

F: *Fisher* test

MS: Mean squares

P: Probability

SS: Sum of squares

VAM: Vesicular arbuscular mycorrhiza

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Introduction

The Mediterranean climate favors the growth of numerous plant species, some of which have various ecological, nutritional, and medicinal potential properties. Unfortunately, the biological and ecological knowledge on native plants in arid regions constitutes a big handicap for the management programs mainly for the endangered species (Boussaid *et al.,* 2018).

Several plant genera are recognized for their contribution to both traditional and modern medicine, one of which is the genus Ziziphus of the Rhamnaceae family (El Maaiden *et al*., 2020), that includes evergreen or deciduous trees or shrubs which are usually armed with unequal stipular spines, is composed of approximately 170 species. In Algeria, Ziziphus is represented by five species: *Z. mauritiana* Lam., *Z. spina-christi*(L.) Willd, *Z. vulgaris* Lam., *Z. saharae* Batt. &Trab. and *Z. lotus* (L.) Desf. (**Zerrouk *et al.,* 2020**). *Ziziphus lotus (Z. Lotus)*, also known as jujube which is a tropical and subtropical plant, *Z. Lotus* grows generally in arid and semiarid countries and is widely distributed in China, Iran,Africa, South Korea, and Europe like Cyprus, Spain, Greece, and Sicily. In Africa, *Z. Lotus* is widely distributed in Mediterranean region, like Algeria, Morocco, Tunisia, and Libya (Abdoul-Azize, 2016).

Ziziphus lotus (L.), is a deciduous shrub. It reaches 2 m, with intricately branched stems and smaller flowers and fruits which known as "N'beg". This specie which is indigenous to Algeria and called "Sedra" has a wide ecological and geographical distribution in the different climatic stage of Algeria and grows under a variety of environmental conditions (Dahlia *et al.,* 2020).

One of the promising approaches that can be used in the domestication of indigenous jujube tree is the arbuscular mycorrhizal(AM) inoculation, since it is established that this particular tree benefit in terms of growth and mineral nutrition from this symbiotic association. The arbuscular mycorrhizal fungi receive plant-synthesized carbon and increase capacity of plants for nutrient capture through its network of external hyphae, and are promoted as bio fertilizers for sustainable agriculture (Thioye *et al*., 2018). The jujube tree is highly dependent on arbuscular mycorrhizal symbiosis and it has been suggested that arbuscular mycorrhizal fungal root colonization of jujube seedlings in a nursery was an essential prerequisite to limit the mortality of out planted jujube trees in the field. AM fungi are known for their ability to improve plant growth and notably to efficiently scavenge for soil phosphorus (P) resources (Thioye *et al.,* 2019).

Arbuscular mycorrhizae are the most widespread plant symbiosis that occur in nature (in about 80% of plant species) and mycorrhizal fungi are key components of natural ecosystems. They

are considered as essential for ecosystem functioning because they play a fundamental role in soil fertility and in the maintenance of stability and biodiversity within plant communities. The success of any reforestation program depends on colonization of the new woodland stands by mycorrhizae (Abbas, 2006).

The inoculation of plants with arbuscular mycorrhizal fungi (AMF) can also alter the production of secondary metabolites. Whereas individual primary metabolites are present throughout the plant kingdom, secondary metabolites have a more limited distribution and specialized function. Thus, while they may not be essential for life, they can be important for plant survival and reproductive success (Pedone-Bonfim *et al.,* 2015). Plants secondary metabolites exhibit both ecological and physiological significance and they include phenols, flavonoids, sterols, tannins, terpenes and lectins. It is essential to understand their nature, biosynthesis, their key regulatory enzymes, storage in cells, etc. (Venkat *et al.,* 2015).

However, relatively little is still known about the potential of this AM symbiosis to affect plant secondary metabolic pathways. And since in Algeria, this specie *Ziziphus lotus* L. (Desf.) has not been subjected to detailed study concerning their mycorrhizal associations and their secondary metabolites. Thus, the present study aimed to identify and isolate new strains of *Mycorrhizae* and the study of their effect on secondary metabolites of *Ziziphus* lot*us "L. Desf".*

Bibliographic synthesis

1. Mycorrhizae

1.1. Generalities

Plant life in nature is by definition communal, sometimes plants depend upon other plants (parasites, epiphytes, climbing plants); other times those unions are symbiotic (rhizobacteria and mycorrhizal fungi) (Rainer and West, 2016). The difference between the mycorrhizal symbiosis and those symbioses caused by parasites which lead to disease is that the mycorrhizal condition is the normal state for most plants under most ecological conditions (Smith and Read, 1996).

In 1885 Albert Bernard Frank, in his study of soil microbial-plant relationships, introduced the Greek term 'mycorrhiza', which literally means 'fungus roots' (Ramakrishnan and Bhuvaneswari, 2015). The mycorrhizal status of many plants is unknown, but for the 6,507 species that have been examined, only 18 percent do not form mycorrhizal associations (Vellinga, 2011).

Mycorrhizal fungi are one of the commonly occurring living organism in soil facilitating plants in growth, development, stress tolerance, soil pollutants remediation, C-sequestration, food security and agricultural sustainability (Ortas and Rafique, 2018). These benefits of mycorrhizal symbioses, both agronomically by increased growth and yield as well as ecologically by improved fitness, indicate that mycorrhizal plants are often more competitive and better able to tolerate environmental stress (Thangadurai *et al.,* 2010).

The symbiotic nature of the interaction between plant roots and mycorrhizal fungi is based on nutritional exchanges. The extra radical mycorrhizal mycelium, which grows out from the roots in soil, has access to mineral nutrients that are delivered to the host plants in exchange for carbon compounds (Balestrini and Lumini, 2018).

Mycorrhizal fungi form a mutualistic symbiosis with plants and infect roots without causing root disease. These fungi can be found in the rhizosphere of most plants and form associations with all gymnosperms and more than 83% of dicotyledonous and 79% of monocotyledonous plants. Mycorrhizal fungi can form structures either on the outside (ectomychorrhizae) or inside (endomycorrhizae) of plant roots (Kennedy and De luna, 2005).

1.2. Different types of mycorrhizae

The most widely used classification recognizes five broad mycorrhizal groups. They are based solely on the position of fungal mycelium in relation to root structure; the categories are

purely descriptive and imply no functional significance. Although these subdivisions may serve useful purposes in promoting mycorrhizal research, their significance is not completely understood (Fig. 1). They are:

ECTO - ectotrophic; ectocellular; sheathing; hartigian

ENDO - endotrophic; phycomycetous; vesicular-arbuscular; arbuscular

ENDO - endotrophic; ericaceous; ericoid

ENDO - endotrophic, orchidaceous

ECTENDO - ect-endotrophic; ericaceous; arbutoid

Ecto- and orchidaceous groups may also sometimes have common fungi since some orchids are connected by rhizomorphs and hyphae to ectomycorrhizae (Varma, 1998).

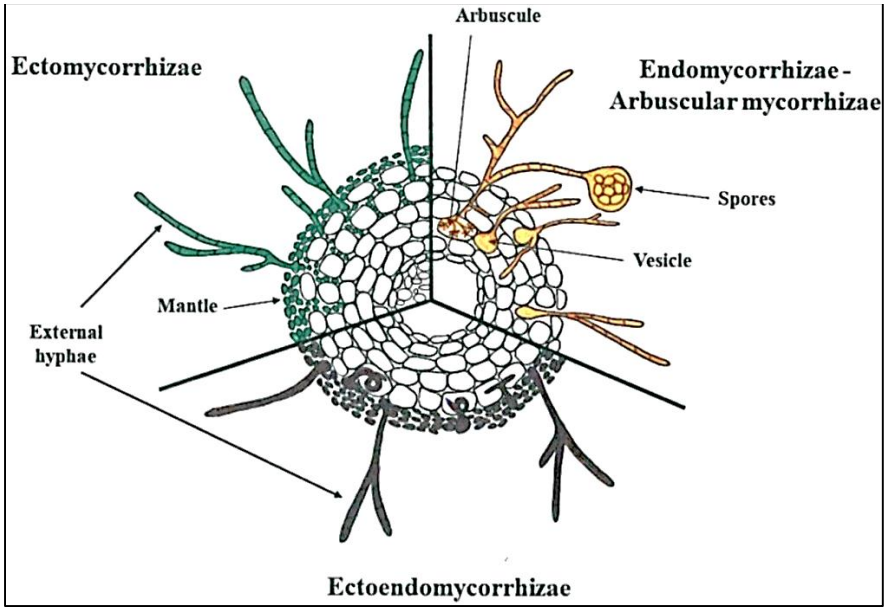

Figure 1: Different associations between a mycorrhizal fungus and plant roots (Ganugi *et al.*, 2019).

Arbuscular mycorrhizal fungi (on the right) penetrate the cortical cells of the root, forming structures such as arbuscules and vesicles. Ectomycorrhizal fungi (on the left) completely cover the plant root system with a mantle of fungal tissue, and the hyphae surround the plant cells within the root cortex. With ectoendo mycorrhizal fungi (bottom), the fungus mantle is formed but the hyphae may also penetrate the plant cells (Ganugi*et al.*, 2019).

The gross morphology and structural characteristics of mycorrhizae are sufficiently characteristic for them to be grouped into classes (Marks and Foster, 1973). Despite the fact that types of mycorrhizas are classified into morphological categories using criteria designed by humans, these categories also seem to have biological relevance as they are highly consistent within plant and fungal lineages and each has characteristic physiological attributes. Seven or more types of mycorrhizas have been recognized, but some are very similar. Early morphological classifications separated mycorrhizas into endomycorrhizal, ectomycorrhizal and ectendomycorrhizal associations based on the relative location of fungi in roots (Brundrett, 2004). Four general types (Table 1) are often recognized based upon the identities of the plant and fungal partners (Smith and Read, 2008; van der Heijden *et al.*,2015; Johnson *et al.*, 2017).

Table 1: Four Major Types of Mycorrhizas Distinguished by the Taxa of Host Plants, Fungal Symbionts, and the Biomes Where the Mycorrhizas Are Most Common (Smith and Read, 2008; van der Heijden *et al.*, 2015; Johnson *et al.*, 2017).

Mycorrhizal Type	Host Plants	Main Fungal Symbionts	Predominant Biomes
Arbuscularmycorrhizal (AM)	~200,000 Species of angiosperms,gymnosperms, bryophytes,and pteridophytes	~300–1600 species of Glomeromycota	Tropical and temperate forests, grasslands, savannas, shrublands, deserts, and most agricultural crops including fruit trees
Ectomycorrhizal (EcM)	~6000 Species of angiosperms and gymnosperms	~20,000 species of Basidiomycota and Ascomycota	Boreal (taiga), temperate, and tropical forests; tundra; and agroforestry
Ericoidmycorrhizal (ErM)	Members of the Ericaceae, Epacridaceae, and Empetraceae families, and some bryophytes	>150 species of Ascomycota (primarily) and some Basidiomycota	Tundra, boreal, and temperate forests
Orchidmycorrhizal	All Orchidaceae	~25,000 species of Basidiomycota	Tropical and temperate biomes

1.2.1. Ectomycorrhizae

Ectomycorrhizae (EcM) represents one of the commonest forms of mycorrhizal associations encountered and are the dominant forms in most forest trees (Marks and Foster, 1973). The number of EcM fungal species is estimated between 20,000 - 25000, and the number of plants, mainly trees and woody shrubs of tropical and temperate forests are estimated to 6000 species. In different forest ecosystems. EcM fungi have been reported to play an important role in seedling survival, establishment and growth. EcM symbiosis differ from other mutualistic plant fungi interactions by the presence of a mantle, formed by fungal colonization of short feeder roots, and a Hartig net representing an intercellular hyphal penetration between epidermal or cortical cells. Hartig net is the place of massive bidirectional exchanges of nutrients between the fungus and host plant (Fig. 2). EcM symbionts are normally reported to colonies soils where nutrients are bound in organic compound (Kumar and Atri, 2018).

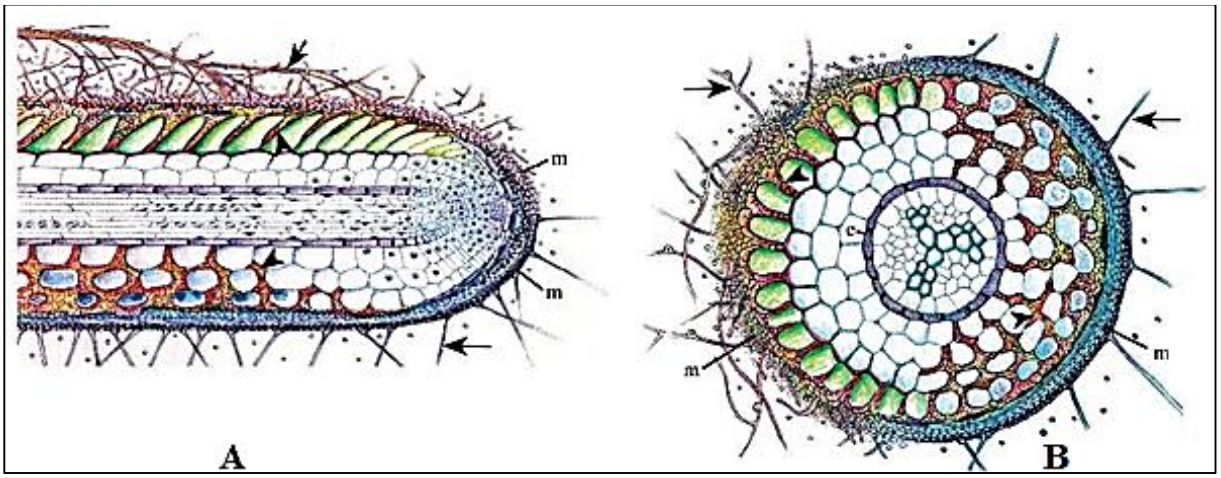

Figure 2: Longitudinal and transverse sections of a root with a schematic representation of ectomycorrhiza (Lakhanpal, 2000 ;Peterson *et al.,* 2003).

A: Diagram of ectomycorrhiza in longitudinal section illustrating the major features that occur in angiosperms (top half) and conifers (bottom half). Both have a mantle (m), Hartig net (arrowheads), and extra radical mycelium (arrows). The main difference between these two systems is that the Hartig net in angiosperms is usually confined to the epidermis whereas in conifers it forms around both epidermal and cortical cells (Peterson *et al.*, 2003).

B: Diagram of ectomycorrhiza in transverse section illustrating the features of angiosperms (left portion) and conifers (right portion). Extra radical mycelium (arrows), mantle (m), and Hartig net

(arrowheads) are indicated. In conifers, Hartig net hyphae are blocked from entering the vascular cylinder by the endodermis (e) (Peterson *et al.*, 2003).

EcM particularly improves the uptake of nutrients present with low mobility in the soil, e.g., phosphorus. EcM fungi assist plants through augmenting hydraulic conductivity, tolerance to drought, and resistance to soil-borne pathogens. Extrametrical hyphae of EcM fungi form an extensive hyphal network, which liberates various chemicals in the soil. This holds the soil particles together and helps to improve the quality of soil (Charya and Garg, 2019).

1.2.2. Ectendomycorrhizae

One type of putative mycorrhizal association, ectendomycorrhiza, is confined to *Pinus ssp.*and *Larix spp.* and is common in conifer nurseries and in disturbed habitats (Yu *et al.*, 2001). Initially the fungi forming ectendomycorrhizae were grouped as E-strain fungi mainly because sexual stages were not identified; only general morphological characteristics of hyphae and chlamydospores were used to characterize the isolates. With the discovery of sexual stages and with the use of molecular methods, a limited number of ascomycetes have been identified as fungal partners in ectendomycorrhizas (Peterson *et al.*, 2003).

Mycorrhizal associations that exhibit morphological characteristics of both ecto- and endomycorrhiza (Fig. 3), in that hyphae form a mantle around the root and may form a distinct Hartig net, while also growing intracellularly (Lewis, 2016).

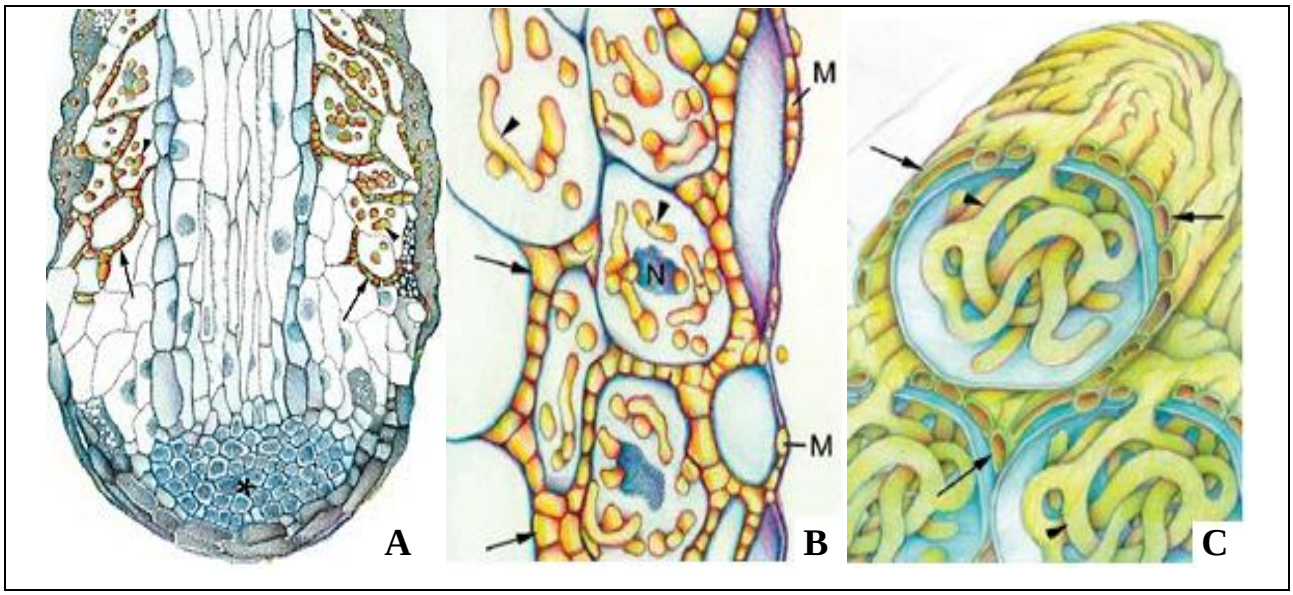

Figure 3: Diagrammatic representations of Ectendomycorrhizae (Peterson *et al.*, 2003).

A: Longitudinal section of root tip showing apical meristem (*), Hartig net (arrows), and intracellular hyphae (arrowheads).
B: Transverse section showing thin mantle (M), Hartig net hyphae (arrows), and intracellular hyphae (arrowheads). Cortical cell nuclei (N) occur surrounded by intracellular hyphae.
C: Diagram showing the relationship between Hartig net hyphae (arrows), and intracellular hyphae (arrowheads).

1.2.3. Endomycorrhizae

Mycorrhizal symbiotic interaction that involves fungal penetration inside living root epidermal and cortical cells (Balestrini and Lumini, 2018) is called Endomycorrhiza. The fungi form structures within the cortical cells and also grow intracellularly. Hence, at the fungus–plant interface, the membranes of the fungus and the plant are in direct contact with each other. There are several types of endomycorrhiza, the best known being arbuscular mycorrhiza (AM), formerly called vesicular-arbuscular mycorrhiza (VAM), ericoid and orchid mycorrhiza (Marschner, 2012).

A characteristic of endomycorrhizae is penetration of the root cell by the mycorrhizal fungus. Ectendo-, arbutoid, and monotropoid mycorrhizae are similar to ectomycorrhizae in that they form a sheath and Hartig net, but there is also penetration of the root cells. Ericoid and orchid mycorrhizae do not form a sheath or Hartig net. The arbuscular mycorrhizal fungi (AMF) lack a fungal sheath and Hartig net, but produce characteristic arbuscules and also may produce characteristic vesicles (Duffy and Cassells, 2003).

Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi are characterized by the formation of arbuscules, or tree-shaped structures, by the fungal hyphae after penetration of the cell wall of host plant root cells (Fig. 4). These arbuscules increase the surface area of the fungus–plant interface, increasing the efficiency of resource exchange (Lewis, 2016).

The majority of Arbuscular mycorrhizal fungi (AMF) species have been described and named accordingly with the morphology of their spores, but this structure is not always distinguishable among species, genus, families or even orders. Thus, recent genetic studies of mycorrhizal fungi have the potential to improve AMF taxonomical classification. Morphological studies of the spores revealed that they are multinucleated and, depending on the species, they may contain until one million of nuclei. They also vary color, size and shape (Souza, 2015).

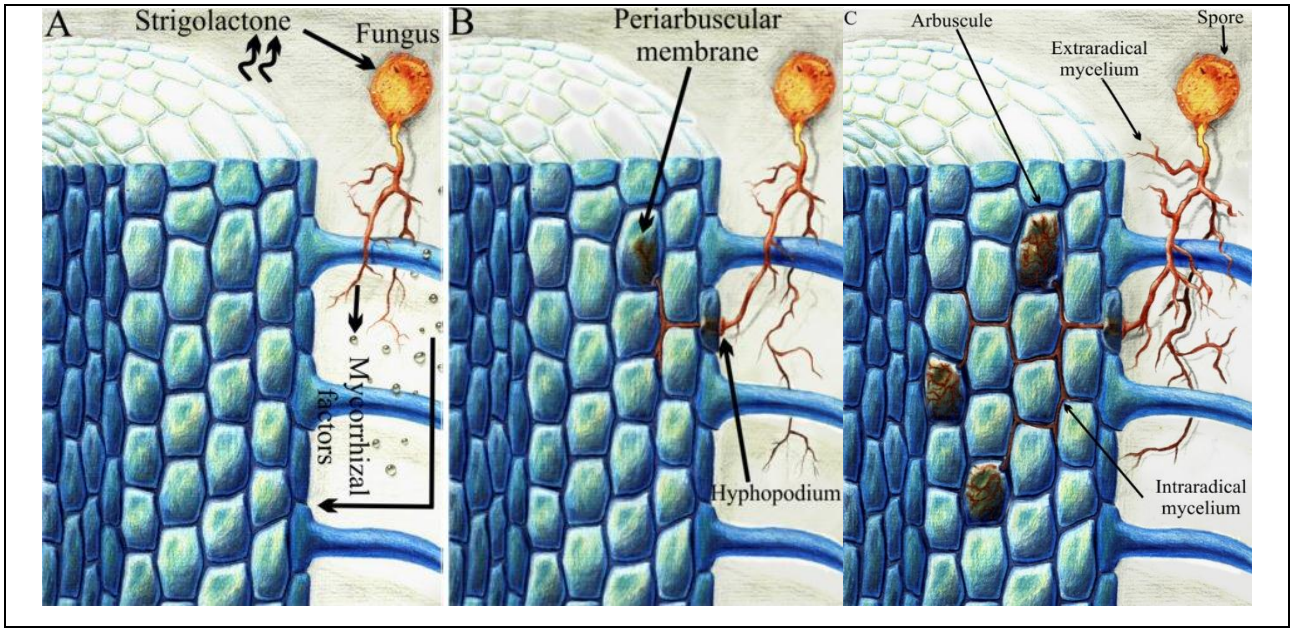

Figure 4 : Hypothetical root colonized by an arbuscular mycorrhizal fungus (Vergara *et al.*, 2019).

A: the root of the host plant signals that it is apt to establish arbuscular mycorrhizal symbiosis, by releasing strigolactone, which promotes the germination of the fungal spores and stimulates the branching of the extra radical mycelium of the AMF, and the fungus responds with the production of the mycorrhizal factors (lipo-chitooligosaccharides and chitooligosaccharides) that are recognized by the host plant.

B and C: the physical contact between the root surface and the AMF allows of the formation of the hyphopodium, which leads to the intercellular proliferation of the intraradical mycelium in the cortex cells and to the intracellular development of the arbuscules surrounded by the periarbuscular membrane derived from the plasma membrane of the plant cell.

About 70 percent of all terrestrial plants from the same arbuscular mycorrhizal associations found in fossils from the Devonian period, about 420 million years ago. All arbuscular mycorrhizal are members of the phylum glomeromycetes and form the dominant of mycorrhizae, although there are only about 230 species of glomeromycetes, they form mycorrhizae with more than 400,000 different plants. These fungi are not very host-specific and form other associations in nature, including those with many liverworts and mosses. They are not discriminating (Lowenfels, 2017).

1.3. Mycorrhizae taxonomy

The early phase of taxonomy of mycorrhiza fungi solely relied on few morphological parameters such as sporocarp. Later, when free and single spores have been reported, they were utilized in the identification and naming of AM fungi. Since the method of wet-sieving and decanting, the AM fungal taxonomy gained a momentum. Later, "wall layers" and other morphological parameters were adapted for the AM fungal species identification. Ontogeny of AM fungal spore is also established as one of the important parameters to distinguish the AM fungal species. Development in the techniques of molecular biology has opened a new dimension (Fig. 5) in AM fungal taxonomy (Kehri *et al.,* 2018).

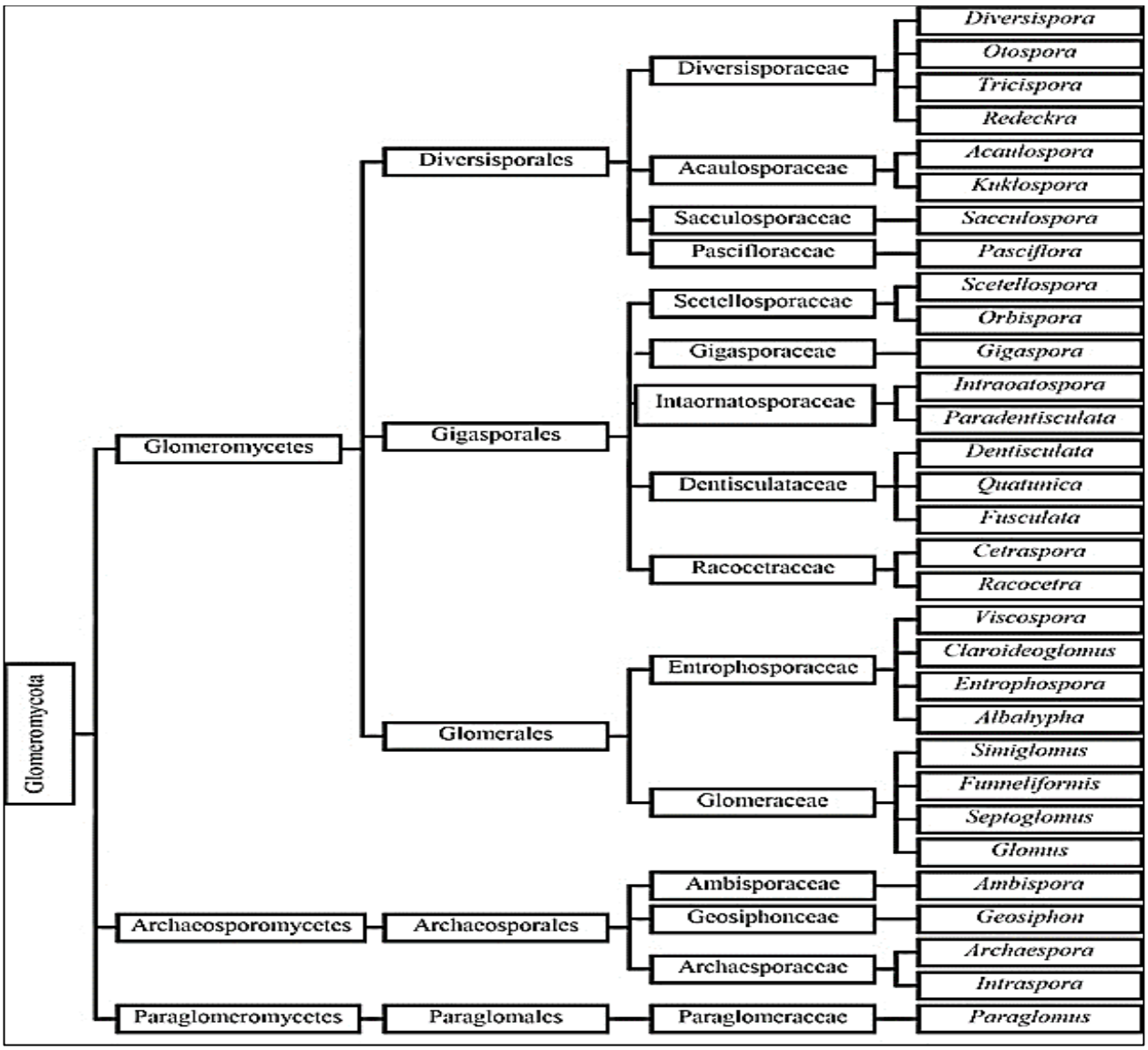

Figure 5: Classification of AM fungi (Kehri *et al.*, 2018).

1.4. Mycorrhizae function

Symbiotic interactions of AM fungi with roots of land plants are widespread. They occur in both natural and agricultural ecosystems and probably involve ~80% of land plants. Conventionally, the symbiosis is considered to be a mutualism, based upon the reciprocal exchange of nutrients. The fungi are obligate symbionts, relying on plants as sole sources of carbon (C). In return, plants receive nutrients such as phosphorus (P) and nitrogen, which are taken up from the soil by external hyphae of the fungi. The evolutionary conservation and widespread occurrence of AM symbioses are testaments to the importance of AM in plant function (Grace *et al.*, 2009). Results of experiments suggest that AM fungi absorb N, P, K, Ca, S, Cu, and Zn from the soil and translocate them to associated plants. However, the most prominent and consistent nutritional effect of AM fungi is in the improved uptake of immobile nutrients, particularly P, Cu, and Zn. The fungi enhance immobile nutrient uptake by increasing the absorptive surfaces of the root (Habte, 2000).

Indeed, AM symbioses have been demonstrated to improve disease tolerance increase drought resistance and decrease the accumulation of heavy metals (Grace *et al*., 2009). Owing to their ability to tolerate heavy metals and maintain their growth in contaminated soils, AM fungi play a critical role in the phytoremediation of heavy metals (Nadeem *et al.*, 2017).

Mycorrhizal association is also helpful to introduce new plant species in an area. This association enables the plant to survive in a new environment. It has been observed that the survival of plants in the absence of this association was not significant in a new environment (Nadeem *et al.*, 2017).

In general, mycorrhizal fungi form symbiotic association with almost all types of plants. This association is common in both normal and stress environments. This symbiotic association provides a number of benefits to the plant including availability of essential nutrients, enhancing water uptake, and promoting growth. This growth promotion takes place not only in normal but also in adverse soil environment. (Mahendra and Ajit, 2011; Nadeem *et al.*, 2017).

1.5. Mycorrhizae mechanism

In all types of mycorrhizae, hyphae extend from the root into the surrounding soil, greatly increasing the surface area for absorption of nutrients, particularly phosphate, nitrogen, and potassium. In return for shunting some of these nutrients into the plant, the fungus receives some sugars from plant photosynthesis. Thus, both organisms' benefit (Volk, 2013).

The hyphal network of arbuscular mycorrhizal fungi (AMF) extends beyond the depletion zone, accessing a greater area of soil for nutrient uptake (Fig. 6). A mycorrhizal-nutrient depletion zone will also eventually form around AM hyphae. Benefits from colonization include tolerances to many abiotic and biotic stresses through induction of systemic acquired resistance (SAR) (Jacott *et al.*, 2017).

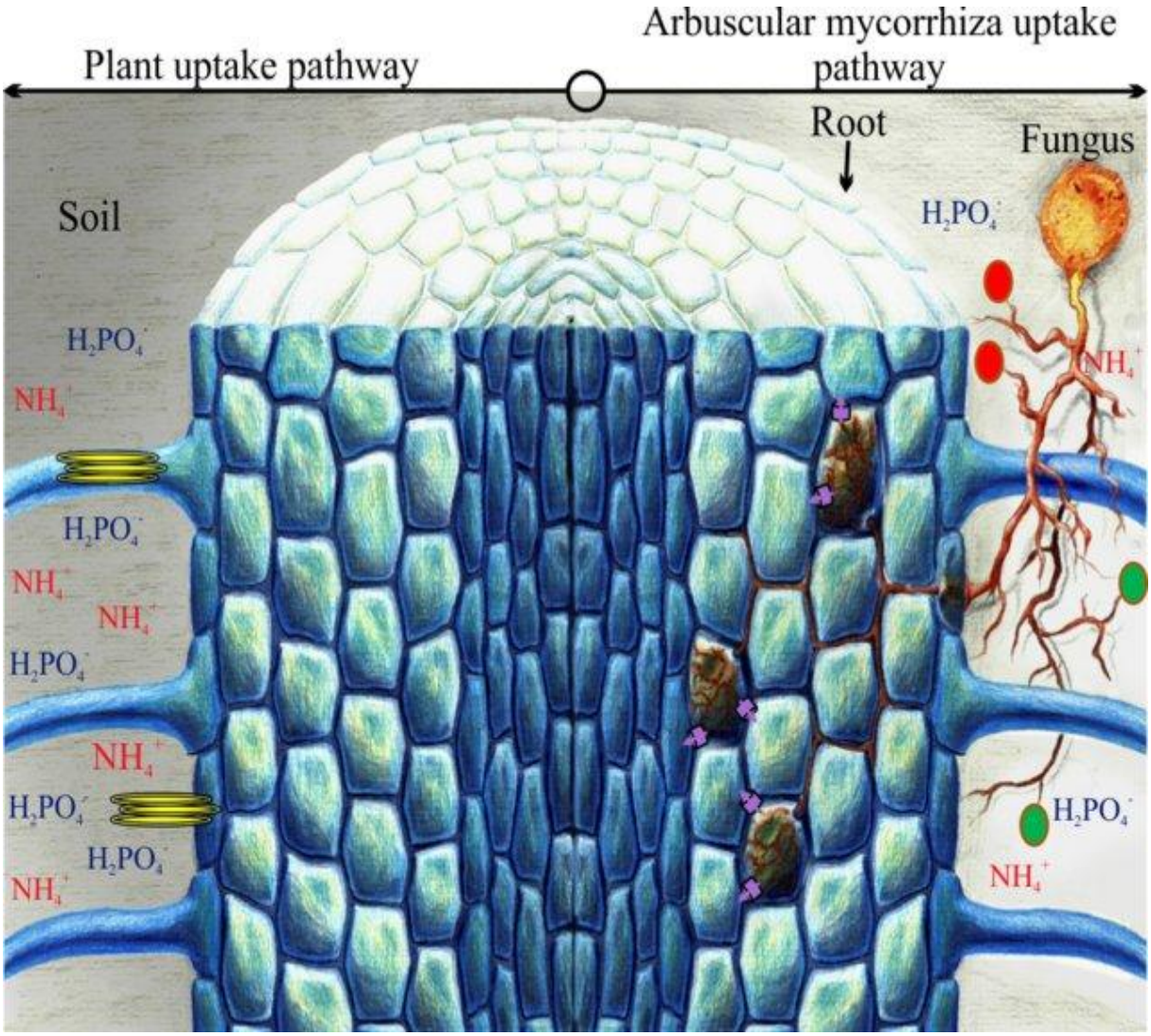

Figure 6: Plant and arbuscular mycorrhiza uptake pathways (Vergara *et al.*, 2019).

Yellow symbols represent the transporters located in the epidermis and in the root hairs; red or green symbols, fungi transporters located in the extra radical mycelium; and purple symbols, plant transporters induced by the arbuscular mycorrhiza and that are located in the periarbuscular membrane.

2. Wild jujube (*Ziziphus lotus* L. Desf.)

2.1. Generalities

Ziziphus lotus is known as ‘Sedra’. The fruit is the edible part of the plant by local population. *Ziziphus lotus L. (Desf.)* is abundantly present in the Mediterranean region and southern European countries (Benammar*et al.*,2010).

Species of the genus Ziziphus are known in North Africa under the vernacular names sedra, addhal, roubaidh, dhouachaouk, sedrealberri and sedrenabga (Borgi and Chouchane, 2009). It is also called "anneb"; it gave its name to the city of Annaba because it was very abundant in the vicinity of this city in eastern Algeria. In Kabyle, it is called "azzouggart" or "tazoura". Thazouggwarth means "red": a reference to the color of the fruit (Hammiche, 2014). The fruit is also known as "nbag".

Several parts of Ziziphus have been used by traditional and ancestral medicine, both in North Africa and Middle East, for the treatment of several pathologies including digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, fever, diarrhoea and insomnia (Benammar *et al.*,2010).

Ziziphus species are *sclerophyllous* evergreen trees or shrubs with a high degree of drought tolerance. Several reports have investigated their physiological and morphological adaptations to water deficit stress. The ability of Ziziphus species to survive drought has been attributed to a combination of avoidance and tolerance mechanisms, including osmotic adjustment and stomatal control (Maraghni *et al.,* 2013).

2.2. Repartition of *Ziziphus lotus*

2.2.1. In the world

Ziziphus lotus grows generally in arid and semiarid countries and is widely distributed in China, Iran, Africa, South Korea, and Europe like Cyprus, Spain, Greece, and Sicily. In Africa, *Ziziphus lotus* is widely distributed in Mediterranean region, like Algeria, Morocco, Tunisia, and Libya (Abdoul-Azize, 2016).

2.2.2. In Algeria

Ziziphus lotus has a low penetration in the northern Sahara of Algeria (Ghedira, 2013). It is encountered mainly in the arid regions of the South (Ain Oussera and Messad, Wilaya of Djelfa)

with arid climate and Taghit (Wilaya of Béchar) with Saharan climate (Fig. 7). The plant is widespread throughout all Algeria except the Algerian-Constantinian Tell (Djemai, 2009).

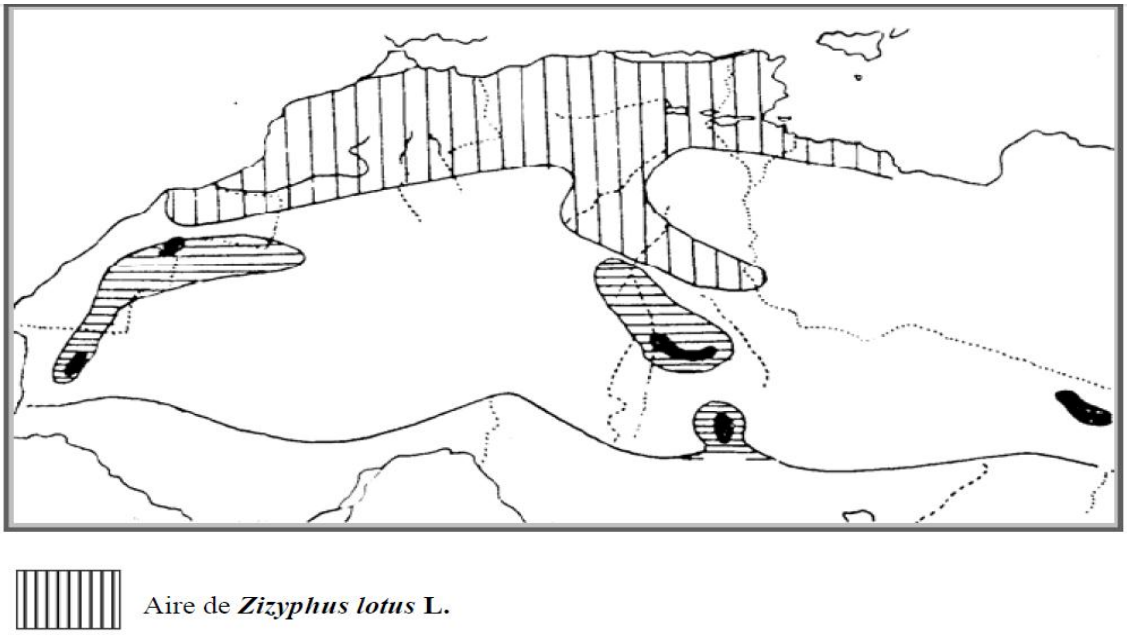

Figure *7* **:**Repartition of *Ziziphus lotus* (Djemai, 2009).

2.3. Botanical classificationof *Ziziphus lotus*

Wild jujube (*Ziziphus lotus*) belongs to the Rhamnaceae family, which has 58 genera and over 900 species. They are trees, shrubs, vines or herbaceous plants (Punt *et al.*, 2003; Kaleem *et al.*, 2014). The genus Ziziphus has about 170 species, such as *Ziziphus lotus*, *Ziziphus spina-christi*, *Ziziphus jujuba*, *Ziziphus mucronata*, *Ziziphus nummularia*, *Ziziphus mauritiana*, *Ziziphus spinosa*, *Ziziphus vulgaris*, *Ziziphus oenoplia* Mill. etc., (Memon *et al.*, 2013; Asatryan and Tel-Zur, 2014).

Along with genera other than *Ziziphus*, does not include many economic species except for some wild species with edible fruits or of interest for medicinal products or dyestuffs (Azam-Ali*etal.*,2001). According to APG IV (2016), the classification of wild jujube is as follows:

Reign: Planta

Branch: *Magnoliophyta* (Phanerogams)

Sub-branch: *Magnoliophytina* (Angiosperms)

Class: *Magnoliopsida* (Dicotyledons)

Sub-class: *Rosidae*

Order: *Rhamnales*

Family: *Rhamnaceae*

Tribe: *Ziziphae*

Genre: *Ziziphus*

Species: *Ziziphus lotus* (L.) Desf.

2.4. Botanical description of *Ziziphus lotus*

Wild jujube is a shrub (Fig. 8) in the form of a thorny bush not exceeding 2.5 m (Rabaa, 2007). It forms tufts of a few meters in diameter, with deciduous, bright green leaves, zigzag branches, very thorny, very hardy (Tardío *et al.*, 2016). This tree suckers a lot. Older specimens have a cracked trunk. The foliage and habit (Fig. 9) of this tree contribute to its exotic appearance (Brosse, 2000).

According to Ghedira (2013), the branches (Fig. 10) are curved downward, flexuous, grayish-white with straight or curved spines in pairs (Fig. 11).

Leaves (Fig. 12) are small, alternate, short, oval, crenate, short-stalked, more or less elliptical, 1-2 cm long and 7 mm wide (Azam-Ali *et al*., 2006; Ghedira, 2013). They are glabrous with entire margins and have three prominent longitudinal veins extending from the petiole. Each leaf bears two stipules at its base that are transformed into unequal and vulnerable spines (Tardío*et al*., 2016).

The flowers (Fig. 13) are conspicuous pentameric yellow (Rsaissi and Bouchache, 2002) with open star-shaped sepals, small petals and a bisexual superect ovary (Rabaa, 2007). They are small (Maraghni *et al.,* 2011), solitary or clustered with a single short pedicel (Azam-Ali *et al.,* 2006), with funnel-shaped calyx (Ghedira, 2013; Tardío *et al.*, 2016).

The fruits (Fig. 14) are spherical drupes, 1-2 cm in diameter (Azam-Ali *et al.*, 2006), reddish-brown in color and bearing very hard, small, round, binocular bony stones that are covered by a semi-starchy, very quickly drying pulp (Ghedira, 2013; Hammiche, 2014; Tardío *et al.*, 2016). The thick pulp can be greenish-white and sweet-tart tasting or yellowish-brown, somewhat glutinous, sweet-tasting and bland (Bayer *et al.*, 2009).


Figure 8: Shrub of wild jujube **Figure 9:** Port of wild jujube


Figure 10: Branches of wild jujube **Figure 11:** Spines of wild jujube


Figure 12: Leaves of wild jujube **Figure 13:** Flowers of wild jujube

Figure 14: Fruits of wild jujube

Sources:

Figures 2, 3and 5: Original photos taken on July 14th, 2018 at Hessiane Eth-Thibe (Laghouat).

Figures 4, 6, 7 and 8: Original photos taken on August 04th, 2018 à Essadra Al Arida (Tissemsilt).

2.5. General compound content of *Ziziphus lotus*

Ziziphus lotus fruit contains substantial amounts of glutamic acid, mineral matter, sterols, vitamins, tocopherols, fibres, amino acids, triacylglycerol, fatty acid, carbohydrate, and antioxidant compounds (phenols, flavonoids, etc.). *Ziziphus lotus* leaves contain different carbohydrates and dammarane saponins notably jujube side B, three jujube genin glycosides and jujube saponine IV (Abdeddaim*et al*., 2014; Abdoul-Azize, 2016).

2.6. *Ziziphus lotus* uses

2.6.1. *Ziziphus lotus* in ancestral medicine

Several parts of *Ziziphus lotus* have been used in traditional medicine for the treatment of bronchitis, and abscess. IN addition, the powder of dried leaves and fruit mixed with water or milk is used for the treatment of boils and the root bark for the treatment of diabetes. The juice from *Ziziphus lotus* root would be efficient in the treatment of eye leucomas. The fruits and the leaves of *Ziziphus lotus* are used as emollient and in the treatment of diarrhoea and intestinal diseases (Abdoul-Azize, 2016).

According to historical usage in China, one of the main functions of jujube was considered to benefit our brain by calming down the mind and improving quality of sleep. In modern science, benefiting our brain is usually related to neuro beneficial effects, for example, neuroprotection effect and neurotrophic action. In neurological disorders, for example, neurodegenerative diseases, insomnia, and depression, several common pathological conditions among them are found, that is, neurogenesis impairment, neurotrophic factor deficiency, and oxidative stress. Hence, the traditional function of jujube in benefiting the brain may be closely related to its neuro beneficial effects (Chen *et al.*, 2017).

2.6.2. *Ziziphus lotus* in nutrition

Jujube fruits are spherical drupes with a size of a pupil, and are eaten at full maturity in October. Their taste evokes candied apple and their texture is similar to dates. They are marketed for human consumption as a fermented drink by mixing crushed fruits with water, and as flour after drying it (El Cadi *et al.*, 2020).

Ziziphus lotus fruits would still be consumed by local population in North Africa. The fruits are dried and processed into flour to make pancakes with very pleasant flavor. The nutritional virtue

of *Ziziphus lotus* is mainly based on its composition rich in vitamin E, vitamin C, fibres, fatty acids, amino acids, calcium, magnesium and considerable amounts of sugars (Abdeddaim *et al*., 2014). *Ziziphus lotus* oil is of high quality, because of its content in unsaturated fatty acids and other bioactive compounds (Abdoul-Azize, 2016).

2.6.3. *Ziziphus lotus* in ecology

It is found in very low rainfall stations (100 mm) and supports high temperatures (20 to 35°C) and long dry periods (sometimes 6 to 12 months). Its ecological plasticity gives it increased interest especially in arid areas in a context of desertification and decline in agricultural production. The jujube tree is autochthonous, rustic species of great ecological plasticity. They survive well in arid environments thanks to their physiological and morphological adaptation mechanisms. They play a very important role in soil conservation thanks to their deep and vigorous root systems that stabilize the substrates and protect them from erosion. Tufts of jujube grow on all types of soils and can withstand temporary flooding. They constitute a place of sand accumulation and alluvial deposits forming a biotope for reptiles, hedgehogs, foxes, rodents (hares, jerboas, birds, rats ...) and even arachnids (scorpions and spiders) (Amara and Benabdeli, 2017).

3. Secondary metabolites

3.1. Generalities

Plants produce an enormous variety of natural products with highly diverse structures. These products are commonly termed "secondary metabolites" in contrast to the "primary metabolites" (Springob and Kutchan, 2009). Plant primary metabolites are organic compounds, common to all or most plant species. Their functions are essential for plant growth, development, and reproduction. They are intermediates and products of metabolism involved in photosynthesis and other biosynthetic processes (Deborde and Jacob, 2014).

Secondary metabolites (SM) are compounds that are not necessary for a cell (organism) to live, but play a role in the interaction of the cell (organism) with its environment. These compounds are often involved in plants protection against biotic or abiotic stresses. Secondary metabolites are from different metabolites families that can be highly inducible in response to stresses (Pagare *et al.*, 2015).

3.2. Role of secondary metabolites

For many years' secondary metabolites have been considered as more or less waste products, with no apparent use for the plant. Still our knowledge about the role of the secondary metabolites is limited, but now it is generally accepted that secondary metabolism is involved in the relationship of the organism with its environment, e.g. in resistance against pests and diseases, as attractant of pollinators, or as signal compound (Verpoorte and Alfermann, 2000).

Secondary metabolites play a major role in the adaptation of plants to the environment and in overcoming stress conditions. Environmental factors viz. temperature, humidity, light intensity, the supply of water, minerals, and CO2 influence the growth of a plant and secondary metabolite production. Drought, high salinity, and freezing temperatures are environmental conditions that cause adverse effects on the growth of plants and the productivity of crops (Akula and Ravishankar, 2011).

Plant secondary metabolites represent an enormous value from economical point of view. Quite a few are used as specialty chemicals, such as drugs, flavors, fragrances, insecticides and dyes (Verpoorte and Alfermann, 2000).

The major functions of the secondary metabolites are: competitive weapons against other livings such as animals, plants, insects, and microorganisms, metal transporting agents, agents for symbiotic relation with other organisms, reproductive agent and differentiation effectors and agents of communication between organisms (Demain and Fang, 2000).

3.3. Classification of secondary metabolites

Over 2,140,000 secondary metabolites are known and are commonly classified according to their vast diversity in structure, function, and biosynthesis (McMurry, 2015). Secondary plant metabolites are classified according to their chemical structures into several classes. The classes of secondary plant metabolites include (Hussein and El Anssary, 2019):

- ✓ Phenolic compounds;
- ✓ Alkaloids;
- ✓ Terpenes;
- ✓ Saponins.

We will only deal, in this part, with the phenolic compounds which will be evaluated, thereafter, in our study.

Phenolics are present in high concentrations in the epidermis of leaves and the skin of fruits and have important and varied roles as secondary metabolites. They are characterized by having at least one aromatic ring with one or more hydroxyl groups attached (Crozier *et al.*, 2006).

Phenolic are valued pharmacologically for their anti-inflammatory activities (e.g., Quercetin) or anti hepatotoxic properties such as (e.g., Silybin) (Husseinand El-Anssary, 2019).

They are characterized by their structure, which includes a minimum of one phenol ring. They are highly structurally diverse, containing simple molecules (e.g., vanillin, gallic acid, and caffeic acid) and polyphenols (e.g., stilbenes, flavonoids, and polymers). Phenolic compounds are usually present in plants in soluble or bound forms but they can also be categorized into subgroups according to their chemical structures. Soluble phenolic compounds are commonly synthesized in the endoplasmic reticulum and preserved in vacuoles, whereas bound phenolic compounds are produced by the transformation of soluble phenolic compounds to the cell wall, where they conjugate with the molecules of the cell wall through glyosidic and ester bonds (Gan *et al.*, 2019).

According to their structures, phenolics can be classified into simple phenolics, tannins, flavonoids, coumarins, lignans, stilbenes, chromones and xanthones (Husseinand El-Anssary, 2019).

In this section, we will develop only three metabolites of phenolic compounds that will be the subject of our study.

3.3.1. Simple phenolics

Phenolic acids are a major class of phenolic compounds, widely occurring in the plant kingdom (Cai *et al.*, 2004). Most phenolics acids have antioxidant capacity, and the radical scavenging ability of phenolics acids depends on the number and position of hydroxyl groups and methoxy substituents in the molecules. In addition, phenolics acids and analogs can inhibit tumor cells and induce apoptosis by inducing cell cycle arrest; regulating signal transduction pathways; inducing or inhibiting some enzymes, and enhancing detoxification (Huang *et al.*, 2009). Several spices and their compounds, such as polyphenols, ascorbic acid and capsaicinoids, have been found to inhibit the inflammation process as well as tumorigenesis in experimental animals, and are

considered to be potential drug candidates against the inflammation-related pathological processes (Spiller *et al.,* 2008).

3.3.2. Tannins

Tannins are natural, water-soluble, polyphenolic compounds, usually classified into 2 classes: hydrolysable tannins (gallo- and ellagi-tannins) and condensed tannins (proanthocyanidins). They are a large class of polyphenolics in dietary plants and medicinal herbs. Oligomeric proanthocyanidins, which are widely distributed in grape seed and skin and pine bark, are considered to be the most potent antioxidants and frequently used in health care and cancer treatment (Krzyzowska *et al*., 2017).

3.3.3. Flavonoids

Flavonoids are a class of phenolic compounds widely distributed in plants. Quercetin and rutin are among the most largely found flavonoids in a great variety of fruits and vegetables, including tea, coffee, and other grains. As it has been observed with other biological active no nutrient components, flavonoids may promote desirable and non-desirable physiological effects in humans (Trugo *et al.,* 2003). They are considered as health promoting and disease preventing dietary supplements. Epidemiological, clinical and animal studies reveal that flavonoids may exert protective effects against various disease conditions including cardiovascular disease and cancer (Babu and Liu, 2009).

Flavonoids also inhibit bio-molecular damage by peroxynitrite*in vitro*, prevent carcinogen metabolic activation, induce apoptosis by arresting cell cycle, promote differentiation, modulate multidrug resistance, and inhibit proliferation and angiogenic process. These activities of flavonoids are related to their structures. Flavonols containing more hydroxyl groups exhibit very high radical scavenging activity, for example, myricetin, quercetin, rutin, and quercitrin are wellknown potent antioxidants. Flavanols with additional catechol structure (3-galloyl group) have significantly enhanced antiradical activity. Moreover, glycosylation of hydroxyl groups and substitution of other substituents (e.g., methoxy groups) also affects the antioxidant activity of flavonoids (Trugo *et al.,* 2003).

3.4. Factors influencing the biosynthesis of secondary metabolites

The synthesis and accumulation of secondary metabolites are very complex, which are affected by many factors including internal developmental genetic circuits (regulated gene,

enzyme) and by external environment factors (light, temperature, water, salinity, etc.) or the association of other plants and other organisms (Mohiuddin, 2019). Symbiotic fungi usually perform compatible and friendly interactions with host plants, which contribute to growth promotion and secondary metabolites accumulation simultaneously, such as alkaloids and terpenoid (Zhi-Lin *et al.,* 2007). Secondary metabolites may be affected by arbuscular mycorrhizal fungi (AMF), which are beneficial symbionts associated with the roots of most plant species (Pistelli *et al.,* 2017).

Environmental factors significantly affect plant growth and biosynthesis of secondary metabolites. Plant growth and productivity is negatively affected by temperature extremes, salinity, and drought stress (Mohiuddin, 2019). Secondary metabolites accumulation is strongly dependent on a variety of environmental factors such as light, temperature, soil water, soil fertility and salinity, and for most plants, a change in an individual factor may alter the content of SMs even if other factors remain constant (Li et *al.,* 2018). Salt stress often creates both ionic as well as osmotic stress in plants, resulting in accumulation or decrease of specific secondary metabolites in plants (Mahajan and Tuteja, 2005).

3.5. Secondary metabolites of wild jujube

Ziziphus lotus is known for its content in biologically active molecules such as polyphenols (flavonoids, tannins), triterpenes, anthraquinones, alkaloids (cyclopeptides and isoquinolides), saponosides (Borgi and Chouchane, 2006). *Z. lotus* is rich in many antioxidant compounds such as phenolic acids, flavonoids, alkaloids, and saponins. These components have been shown to prevent oxidative stress and inflammation by reducing reactive oxygen species (ROS) (Mothana, 2011). According to Nag and Chouhan (2009), plant growing in the xeric and harsh environment of desert produces various types of secondary metabolites which not only play a part in defense against drought, salinity and pathogens but may also serve as an excellent source of bioactive metabolites such as flavonoids, alkaloids, steroids etc.

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Experimental part

Chapter 1:Material and methods

1. Materials

In the field, Sterile container, Rope, Plastic bags and GPS, Nitrile gloves, Mattock and spade, were used. In the laboratory we used Laboratory glassware, equipment, chemicals and culture media that are listed and mentioned in table 2.

Table 2: Equipment, glassware, chemicals and culture media used for the different experiments

Instrument	Glassware	Chemical Product	Culture medium
• Autoclave (WOLF) • Analytical Balance (KERN) • Bunsen burner • Deep Freezer • Magnetic Stirrer (IKARCT BASIC. VELP) • Optical Microscope (B-350 OPTIKA) • Laboratory Oven (NUVE) • Vortex Mixture • Spectrophoto meter	• Beakers • Conical flasks • Glass syringe • Graduated pipettes • Marker pens and stickers • Measuring cylinders • Measuring flasks • Pasteur pipettes • Petri dishes • Pipettes • Plastic syringe • Platinum wire • Reagent bottles • Slides and cover slip • Spatula • Sterile scalpel • Test tubes • Test tube baskets • Test tube racks • Thermometer • Tweezers • Wash bottles • Watch glasses	• Methylene Blue • Hydrogen peroxide • Ethanol 96% • Oil immersion • Tween 80 • Folin–Cicalteu • sodium carbonate (Na2CO3) • fresh aluminum chloride solution (AlCl3, 2%) • ferrous sulfate solution • Butanol • HCl	• Agar agar 2% • PDA

2. Methods

2.1. Experimental Protocol

The different steps of this study are summarized in figure 15.

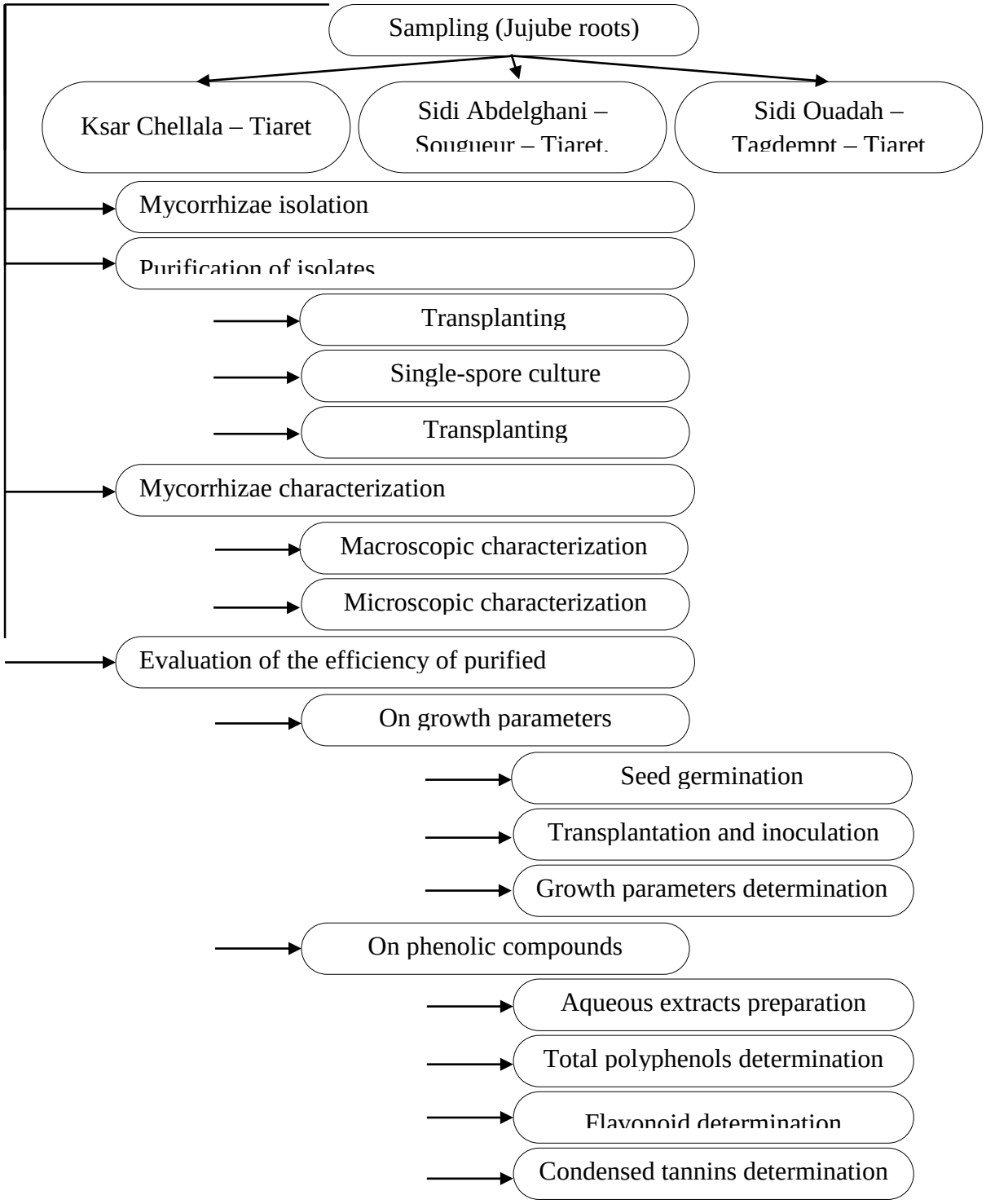


Figure *15***:** Experimental Protocol used for the realization of the different experiments.

2.2 Place and period of work

Our work has been made within the laboratories of microbiology and sol sciences of the Faculty of Natural and Life Sciences, Ibn Khaldoun University -Tiaret, during the period from April to July 2021.

2.3 Plant material and sampling

The roots and the Rhizospheric soil samples, that has been used for isolation of mycorrhizae, were collected from spontaneous jujube tree (*Ziziphus lotus*) using a subjective sampling. They were collected during April 2021 from three districts of Tiaret (table 3). The areas mentioned are Ksar Chellala, Si Abdelghani (Sougueur) and Sidi Ouadhah (Tagdempt). Roots were sampled under sterile conditions (Fig. 16) using nitrile gloves and sterile scissors, taken and transported in well-closed sterile containers and stored at −4 °Cin the laboratory.

Table3: Geographical location of the regions of Tiaret during sampling roots of jujube tree.

	Ksar Chellala (18-19h) 10 April	Si Abdelghani (12-13h) 10 April	Sidi Ouadah (11-12h) 10 April
Longitude	35.2616371 N	35.2124692 N	35.2955607 N
Latitude	2,34119343 E	1,6170998 E	1,2738387 E
Altitude	1.047m	813m	918 m

A BC

Figure 16: The three different sampling sites. **A:** soil and root sampling from Ksar Chellala; **B:** soil and root sampling from Si Abdelghani; **C:** soil and root sampling from Sidi Ouadah.

2.4 Mycorrhizae isolation

Collected roots were washed thoroughly under tap water to remove the attached soil particles. Roots were disinfected by treating them with oxygenated water solution in a beaker till the segments are bleached and clear for 5 minutes and then rinsing them with sterile distilled water. This operation has been repeated several times. and after washing the roots, they were then immersed in sterile distilled water containing 2 drop of Tween 80 for 3 minutes and washed thoroughly with distilled water (Gryndler *et al.,* 1997).

Disinfected roots were transferred and cut, with the help of a sterile expanded polystyrene foam and a sterile blade, into segments of 1-2 mm length. They were then placed on 15 ml of agar (2%) in Petri dishes at a rate of 3 root fragments per Petri dish. The dishes were incubated at 25-30 ℃. After 48-72h of incubation and being able to observe mycelium growth from our selected roots, few drops of PDA were added on the surface, in order to allow the development of fungus. After 48h of incubated at 25 ℃, when colonies diameters were about 1 to 2 cm, small discs of Mycelium were transplanted into new dishes containing each 15 ml of PDA culture medium and then incubated for 5 to 7 days at 25°C (Gryndler *et al.,* 1997).

2.5 Purification of isolates

2.5.1 Transplanting

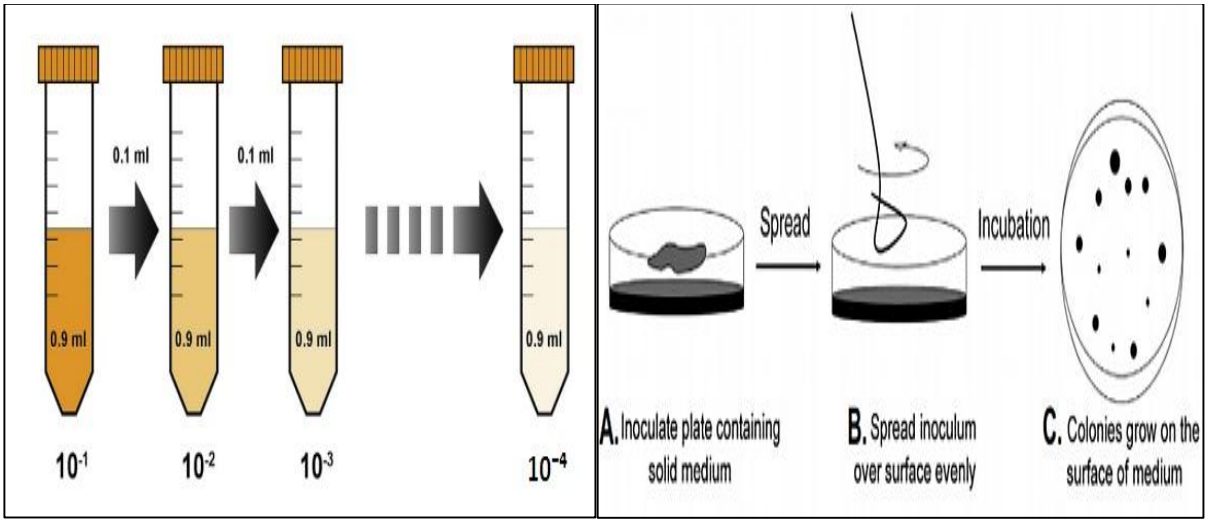
Using sterile platinum lance, a fungal disc was placed in the center of new Petri dishes containing 15 ml of PDA culture medium. The dishes were then incubated for 5 to 7 days at 25°C.

2.5.2 Single-spore culture

The main goal of this technique is to obtain pure cultures of individual fungi. Purification was performed by single-spore culture following the method of Henni *et al.* (1994) with few modifications.

Transportation of a fragment of mycelium from the PDA dish by scraping with a sterile loop, from the outer part of the culture, because the culture is younger in that part and actively growing, was placed immediately in a tube. A 10-fold dilution series was performed. Under aseptic conditions, 0.1 ml of the sample was added to a 0.9 ml sterilized distilled water. After thorough mixing with a vortex, 0.1 ml of the mixture (10^{-1}) was added to a tube containing 0.9 ml of sterilized distilled water and mixed again. Using this method, 10^{-4} fold dilutions are made in series (Fig. 17A). A volume of 0.1ml of this suspension from the final dilution 10^{-4} was transferred to the center of an agar (2%) plate and spread evenly over the surface with a sterile L-shaped bent glass

rod, while the petri dish was spun (Fig. 17B), the agar plate was incubated at 25-29 C° for 48-72h. After this incubation period, a binocular lamp was used to visualize the colonies.



A **B**

Figure 17: Single-spore culture essay (Zhou and Li, 2015). **A**:sample dilution (10-fold serial dilution); **B**: Spread method protocol

2.5.3 Transplanting

Using sterile platinum lance, a colony was placed in the center of new Petri dishes containing 15 ml of PDA culture medium. The dishes were then incubated for 5 to 7 days at a temperature of 25°C.

2.6 Characterization of mycorrhizae

2.6.1 Macroscopic characterization

For macroscopic observation of purified fungi, the cultural appearances (pigmentation of the face and back of the colonies and the outline of the colonies) were observed on potato dextrose agar (PDA) medium based on naked eye observations according to the modifies the method described by Nelson *et al.* (1981) and Booth (1984).

2.6.2 Microscopic characterization (adhesive tape technique)

For a good observation of the microscopic characters of fungi, we used the adhesive tape modified technique described by Hughes (2004) which allows direct examination of a mycelial culture on a glass slide which can be preserved afterwards, and an excellent technique for the rapid mounting of sporulating fungi because it keeps more of the reproductive structures intact. This technique works by gently pressing the sticky side of the adhesive onto the surface of the culture

and then placing the adhesive onto a small drop of methylene blue on a clean glass slide, afterwards observing by microscope using the x40 objective.

2.7 Evaluation of the efficiency of purified mycorrhizal strains

2.7.1 On the growth parameters

» **Seed germination**

The Jujube (*Ziziphus lotus*) seeds extracted from the almonds were disinfected with bleach for 5 minutes then soaked in distilled water for 5 minutes for 5 times. They were then distributed in Petri dishes at a rate of 30 seeds in each, on filter paper soaked in 10 to 20 ml of distilled water (Fig. 18). The seeds were incubated in laboratory oven at 35 °C.

Figure 18: Seed germination of wild jujube (*Ziziphus lotus*) in the laboratory.

» **Transplantation and inoculation**

The seeds were then planted in moistened sterile phosphorus deficient soil in 10 cm plastic pots. The lots were divided into six groups; the first five were inoculated with a different strain of Mycorrhiza ((A"1), (D"1), (F"'1), (G"2) or (H "1)), while the sixth lot served as a negative control with no inoculums (T). This essay was repeated tree times.

The batches were then incubated in a culture chamber at 35°C for 16 hours per day. They were watered on a daily basis. A recall on the inoculated plants took place twice during the 15 days after the seeds. Each pot was watered with a volume of 10 ml from each strain of Mycorrhiza suspensions (Fig. 19).

A **B**

Figure 19: Inoculation with different strains of mycorrhizae into pots containing plants of the wild jujube (*Ziziphus lotus*). **A**: tubes filled with mycorrhizae strains each with a letter similar to the one in the pot. **B**: inoculation.

» **Growth parameters determination**

The entire length from the root initiation point to the cap, as well as the entire length from the stem initiation point to the top of the seedling, were measured using a ruler (Fig. 20). The number of leaves was calculated.

By cutting at the crown, the roots were separated from the aerial parts. To determine fresh aerial and root biomass, each portion was weighed separately. Their fresh weighs were noted. Then they were dried at room temperature at 30°C for several days and their dry weighs were also noted.

Figure 20: Measures of growth parameters of wild jujube (*Ziziphus lotus*) plants.

2.7.2 On phenolic compounds contents

» **Aqueous extracts preparation**

In order to preserve the totality of the molecules and pigments, the leaves and roots of *Ziziphus lotus* were stored at room temperature, protected from heat and light. The extraction was done according to the modified maceration method described by Slimani *et al.* (2017). To prepare

an aqueous extract, the dried roots and leaves of *Ziziphus lotus* were ground with a porcelain mortar to obtain a fine powder. The powder was put in a desiccator and then stored in the dark in a dry place. Aqueous maceration was used to obtain the aqueous extracts. It consists in putting 1 g of crushed material of each sample with 10 ml of sterile distilled water in sterile test tubes under horizontal shaking at ambient temperature of the laboratory during 24h. After, filtration was performed. After the recovery of the extract, a first filtration was carried out using fine stockings and a second time by filter paper (Fig. 21). Afterwards, the filtrate is suitable for assays and to be put in tubes covered in aluminum.

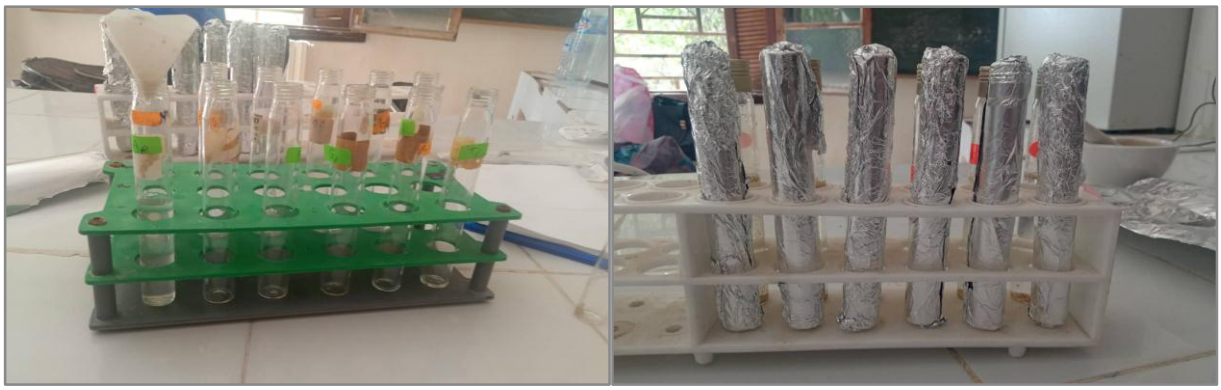


Figure 21: Aqueous extraction of leaves and roots of the jujube plants *Ziziphus lotus.* **A**: filtration of the sample using filter paper. ***B*** and **C**: tubes containing the extraction samples covered in aluminum.

» **Total polyphenols determination**

Folin–Cicalteu reagent was used to determine the total phenolic compound concentrations as described by Singleton and Rossi. (1965). The reagent is formed by phosphomolybdic acid $H_3PMo_{12}O_4$ and phosphotungstic acid $H_3PWO_{12}O_{40}$ and is reduced by the oxidation of phenols to blue oxides of tungsten W_8O_{23} and molybdenum Mo_8O_3 (Ollivier*et al.*, 2004).

In test tubes, 0.5 ml of each aqueous extract (of the leaves or roots) was added to 2.5 ml of 10% Folin-Ciocalteu. After incubation for 3 minutes, 1 ml of 20% sodium carbonate (Na_2CO_3) was added. The resulting mixture was incubated again for 15 minutes at room temperature and in the dark (Singleton and Rossi., 1965). Absorbance readings were taken at wave length λ= 760 nm against a blank without extract.

» **Flavonoid determination**

According to the method described by Elfalleh *et al.* (2019), the colorimetric assay was used to assess the total flavonoid content in all extracts. One ml of a fresh aluminum chloride

solution ($AlCl_3$, 2%) was added to 1,5 ml of samples of each extract and the mixture was shaken vigorously. The absorbance was determined at 460 nm, after incubation for 10 min at room temperature (25 ˚C) and being protected from light.

» **Condensed tannins determination**

The vanillin method described by Deshpande *et al.* (1986) is used for the determination of condensed tannins. This method is based on the ability of vanillin to react with condensed tannin units in an acidic medium to produce a red complex measured at 500 nm.

0.5 of each extract and 2.5 ml of ferrous sulfate solution (77 mg of ferric ammonium sulfate $Fe_2(SO_4)_3$ dissolved in 500 ml of 3v:2v butanol: HCl) were mixed. After incubation at 95 °C in a water bath for 50 min, the absorbance was measured at 530 nm.

3. Statistical analysis

The statistical study of the results obtained is expressed as mean ± standard deviation. The comparison between the different mycorrhizal strains is carried out for the growth parameters (seedling weight and stem and root dimensions) as well as for the phenolic compound contents, by the ANOVA test (analysis of variance). These analyses were carried out using the software (SPSS V. 21) Type III. Homogeneous groups of each measured trait are separated by *Tukey's* test of comparison of means.

Chapter 2: Results and discussions

1. **Isolation of mycorrhizae from roots of spontaneous jujube tree**

Cultivation of disinfected roots of spontaneous jujube (*Ziziphus lotus*), from three regions, on 2%agar water led to the formation of mycelia of different sizes (Fig. 22). The mycelia developed, from the roots of the spontaneous jujube tree from Ksar Chellala, had a rapid growth rate and were the largest. While the one developed from the roots of the spontaneous jujube tree, from Si Abdelghani, had a medium growth rate and therefore were medium in size. While, the mycelia developed from Sidi Ouadhah had a slow growth rate and therefore, they were the smallest.

A **B** **C**

Figure 22: Morphological appearance of mycorrhizal mycelia on 2% agar after 48h of culture. (A) : Ksar Chellala strain, (B) : Si Abdelghani strain, (C) : Sidi Ouadhah strain.

After 72 h of PDA addition, there was the appearance of mycelium germination which was characterized by the appearance of white pigmentation at the root tips of the three regions (Fig. 23). The growth rate as well as the size of the colonies varied from one root to another and from one region to another.

A **B** **C**

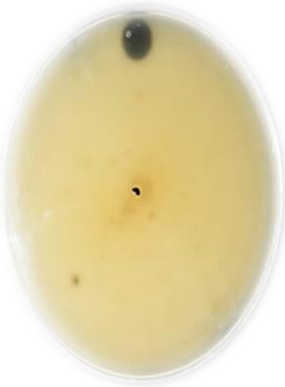
Figure 23: Morphological aspect of mycorrhizal mycelia on 2% agar at 72h after the addition of PDA. (A) : Ksar Chellala strain, (B) : Si Abdelghani strain (C) : Sidi Ouadhah strain.

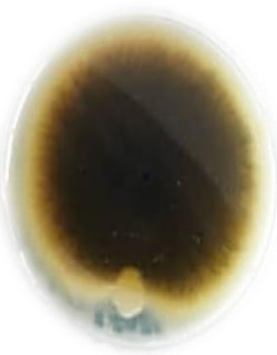
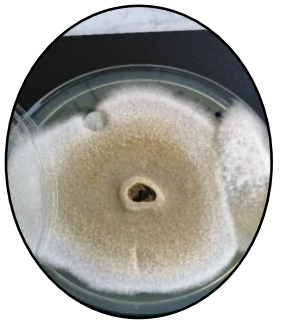
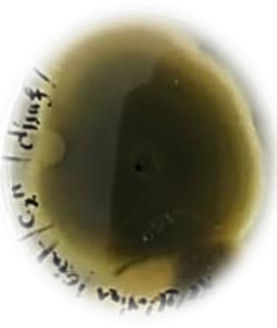
2. Characterization of mycorrhizal fungi

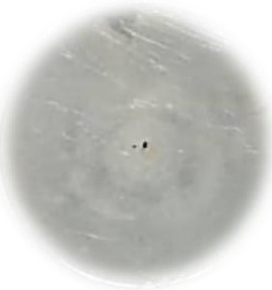
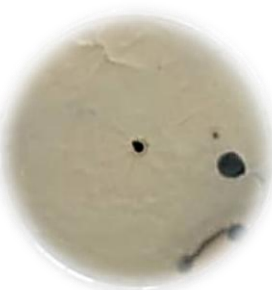
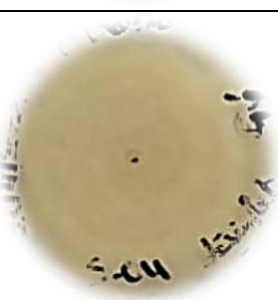
2.1. Macroscopic characterization

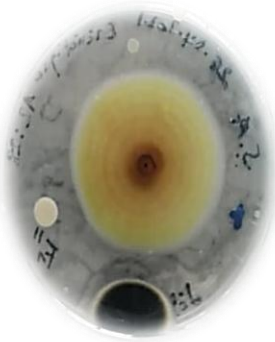
After seven days of transplanting the fungal discs onto PDA culture medium, 17 different phenotypes were separated (table 4). 8 phenotypes from the roots of the spontaneous jujube tree of Ksar Chellala, 6 phenotypes from the roots of the spontaneous jujube tree of Sidi Ouadhah and 3 phenotypes from the roots of the spontaneous jujube tree of Si Abdelghani.

Table4: Description of the pigmentation of the face, back and the outline of the colonies.

Root's provenance	Front of the colony	Back of the colony	Description
Ksar Chellala			**Form**: Circular. **Colony pigmentation:** • **Front:** White-Brown. • **Back:** Orange. **Colony outline:** Filamentous orange-brown.
			**Form:** Circular**.** **Colony pigmentation:** • **Front:** White. • **Back:** Cream**.** **Colony outline:** Filamentous white.
			**Form:** Circular**.** **Colony pigmentation:** • **Front:** Whitish- Light brown. • **Back:** Red-Whitish. **Colony outline:** Filamentous light brown.

Ksar Chellala			**Form:** Irregular. **Colony pigmentation:** • **Front:** Brown-Creamy white. • **Back:** Brown-Yellowish orange**.** **Colony outline:** Filamentous white.
			**Form:** Irregular **Colony pigmentation:** • **Front:** Brown-Off-White-Yellow**.** • **Back:** Yellow-Orange**.** **Colony outline**: Irregular corrugated white**.**
			**Form:** Circular. **Colony pigmentation:** • **Front:** Light Brown-Greyish-White. • **Back:** Dark Brown-Light yellow **Colony outline**: Filamentous white.
			**Form:** Circular. **Colony pigmentation:** • **Front:** Greyish-Orange**.** • **Back:** Blackish-Orange. **Colony outline:** Filamentous white.
			**Form:** Irregular **Colony pigmentation:** • **Front:** Brown-White. • **Back:** Blackish-Brown- Cream**.** **Colony outline:** Filamentous white.

Sidi Ouadhah			**Form:** Circular. **Colony pigmentation:** • **Front:** White. • **Back:** Cream. **Colony outline:** Filamentous white.
			**Form:** Circular. **Colony pigmentation:** • **Front:** White. • **Back:** White. **Colony outline:** Filamentous white.
			**Form:** Circular. **Colony pigmentation:** • **Front:** Brown-White. • **Back:** Cream. **Colony outline:** Filamentous white.
			**Form:** Irregular **Colony pigmentation:** • **Front:** Yellow-White. • **Back:** Light orange. **Colony outline:** Filamentous white.
			**Form:** Irregular **Colony pigmentation:** • **Front:** White-Brown. • **Back:** Cream-Light orange. **Colony outline:** Filamentous white.
			**Form:** Irregular **Colony pigmentation:** • **Front:** White and transparent. • **Back:** Light orange. **Colony outline:** Serrated white.

Sidi Abdelghani			**Form:** Circular **Colony pigmentation:** • **Front:** Orange-Whitish-Yellow • **Back:** Brown-Orange-Yellow-White **Colony outline:** Filamentous white**.**
			**Form:** Circular **Colony pigmentation:** • **Front:** White • **Back:** Off white **Colony outline:** Filamentous white.
			**Form:** Circular **Colony pigmentation:** • **Front:** Off white • **Back:** Cream **Colony outline:** Filamentous white.

2.2. Microscopic characterization

From the 17 selected phenotypes, purification of the strains was performed using single-spore culture (Fig. 24). This technique, followed by subculturing of a single colony from a single spore, allows to have a pure fungal strain.

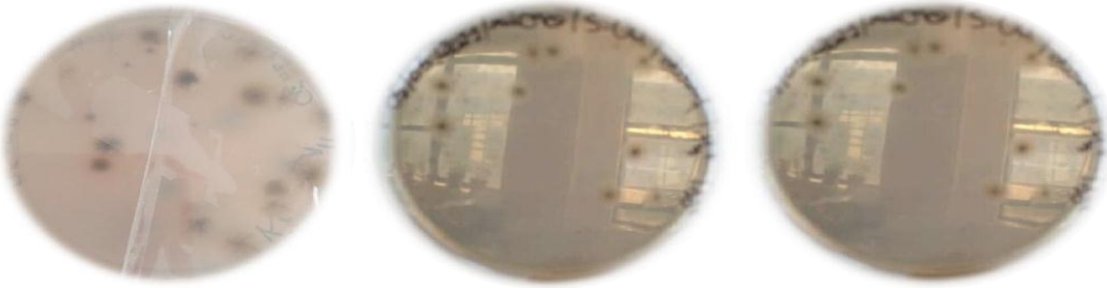


Figure 24: Observation of fungal colonies from spores spread on 2% agar.

The subculture of these colonies on PDA medium should lead to the appearance of the 17 pure strains of the selected phenotypes. However, at this stage, most of the Petri dishes were

contaminated by *Rhizopus sp*. In the end, only five mycorrhizal strains were purified, which are shown in table 6. Macroscopic observation was carried out to confirm the identity of the pigmentation (front and back and the contour of the colonies) with the selected phenotypes. And a microscopic observation was performed using the flag technique.

From the pictures, illustrated in table 6 we notice a good identity, for macroscopic characters, between the five previously selected phenotypes and the five purified mycorrhizal strains. The microscopic aspect reveals the presence of arthrospores resulting from the fragmentation of thick-walled transparent hyphae.

3. Evaluation of mycorrhizal isolates on a young crop of wild jujube (*Ziziphus lotus* L. Desf.)

3.1. Evaluation of mycorrhizal isolates on growth parameters

3.1.1. Stems High

The result of the Analysis of variance, grouped in a table5, shows a highly significant difference ($P<0.01$) between mycorrhizal strains treatments. This indicates that the presence or absence of mycorrhizal strains had different effects on stem high and also that jujube plants responded differently to the different mycorrhizal strains.

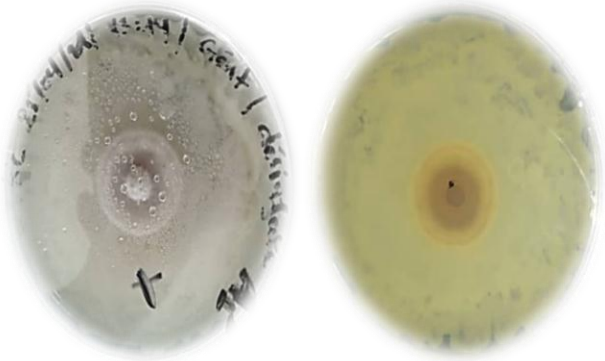
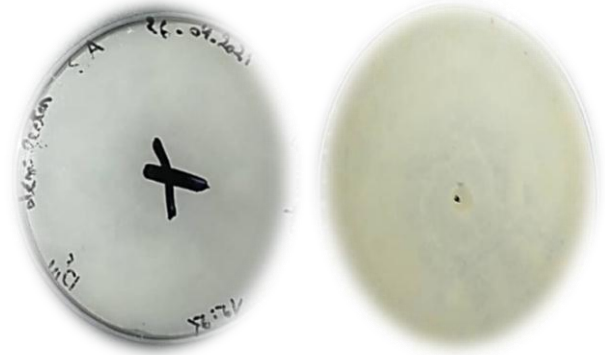
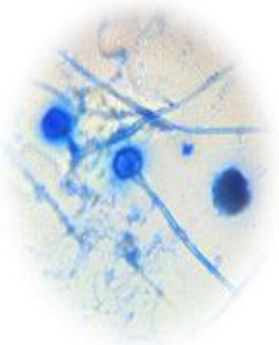
Table 5: Anova test of stem height

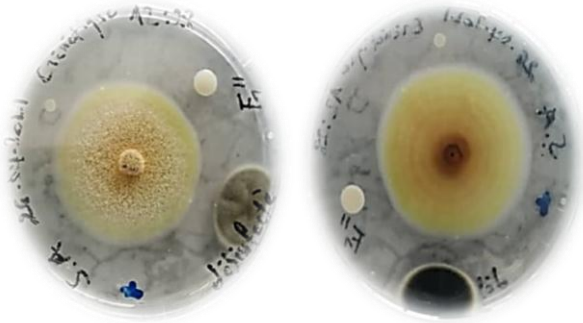
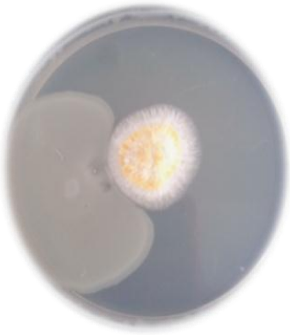
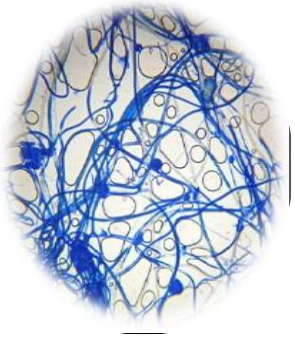
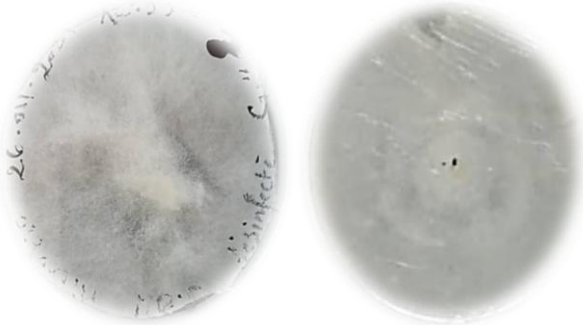
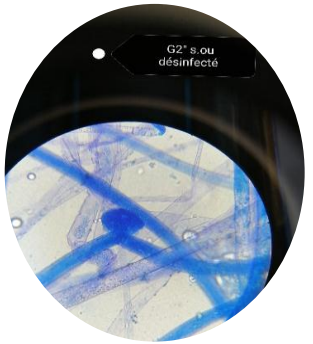
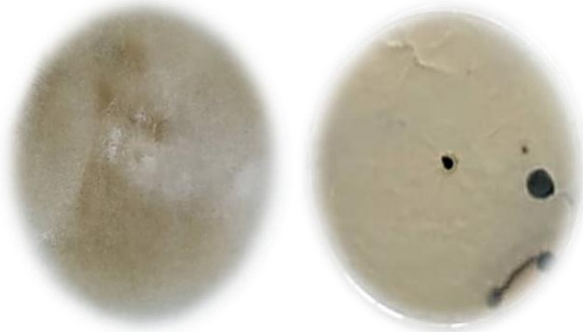
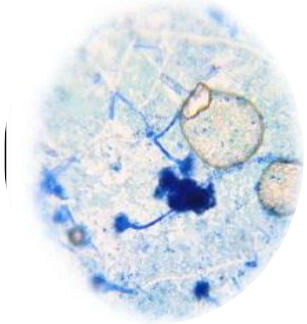
Variation's sources	SS	dof	MS	F	P.
Mycorrhizalstrains	60.378	5	12.076	4.452	0.001**
Residual	306.471	113	2.712		
Total	366.849	118			

The histogram (Fig. 25) illustrates the variation in stem heights of jujube plants according to the strain of mycorrhiza used.

Different effects of mycorrhizal strains on the stem height of jujube plants are observed. The control plants, without mycorrhizal strains, showed the lowest stem heights with an average of 5.333±1.57 cm. This average is individualized in the homogeneous group (a) according to *Tukey's* test of comparison of means, while the means of the strains (A"1) from Ksar Chellala, (D"1) from Si Abdelghani and (H"1) from Sidi Ouadhah which manifest higher stems are classified in the homogeneous group (b). The means of the strains (F"'1) from Si Abdelghani and (G"2) from Sidi Ouadhah were closed to both of group (a) and group (b) so they are classified in the heterogeneous group (ab).

Table6**:**Morphological and microscopic appearance of fungi after purification.

region	strain	Selected phenotype	Pure strains obtained		Description
			Macroscopic aspect	Macroscopic aspect	
Ksar Chellala	**A2"**				**Colony pigmentation:** Cream in the center then white. **Colony outline:** Filamentous white.
Si Abdelghani	**D1"**				**Colony pigmentation:** off white **Colony outline**: Filamentous white

Si Abdelghani	**F1"**				**Colony pigmentation:** Orange and off-white. **Colony outline:** Filamentous white.
Sidi Ouadhah	**G2"'**				**Colony pigmentation:** White. **Colony outline:** Filamentous white.
	H1"				**Colony pigmentation:** Brown and white. **Colony outline:** Filamentous White.

The improvements in the growth of the aerial part were the higher for the strain (D1") with 51.44%, they were medium for the strains (A"1) and (H"1) with 33.84% and 34.66% respectively and they were the lowest for the strains (F"'1) and (G"2) with 26.81% and 23.66 % respectively according to the control (Fig. 25).

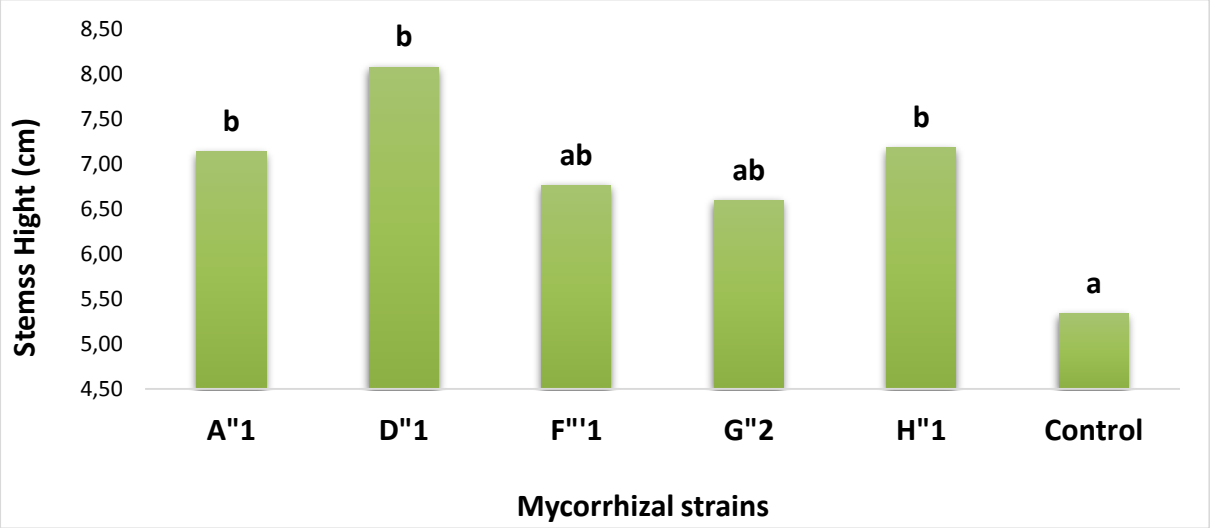


Figure 25: Variation of stem heights of jujube plants according to the mycorrhizal strains used.

3.1.2. Roots length

The results of the analysis of the variance grouped below show that there is a very highly significant difference (P<0.001) between the mycorrhizal strains and the control (table 7). This indicates that the presence or absence of mycorrhizal strains has different effects on root length, and the response of jujube plants to different mycorrhizal strains is also different.

Table7: Anova test of root length

Variation's sources	SS	dof	SM	F	P.
Mycorrhizal strains	211.102	5	42.22	5.218	0***
Residual	914.315	113	8.091		
Total	1125.417	118			

The histogram of the figure 26 illustrates the change in the length of roots of the jujube tree according to the different strains used.

Different effects of mycorrhizal strains on roots length of jujube plants were observed. The averages of roots length are individualized in 3 different groups according to *Tukey's* test of comparison of means. The control plants without mycorrhizal strains, the strains (A"1) from Ksar Chellala and the strains (D"1) from Si Abdelghani showed the lowest roots length with 4.8±2.29 cm, 5.115±1.502 cm and 5,914±1.852 cm respectively. Those averages were individualized in the homogeneous group (a). While the means of the strains (H"1) from Sidi Ouadhah manifest the longest roots and they are classified in the homogeneous group (b). The means of the strains (F"'1) from Si Abdelghani and (G"2) from Sidi Ouadhah showed a medium root length so they were classified in the heterogeneous group (ab).

The strain (D"1) seems to be the least efficient compared to the other strains used. While the strain (H "1) had the greatest effect on root length, it improved root development by 85.606% compared to the control (Fig. 26).

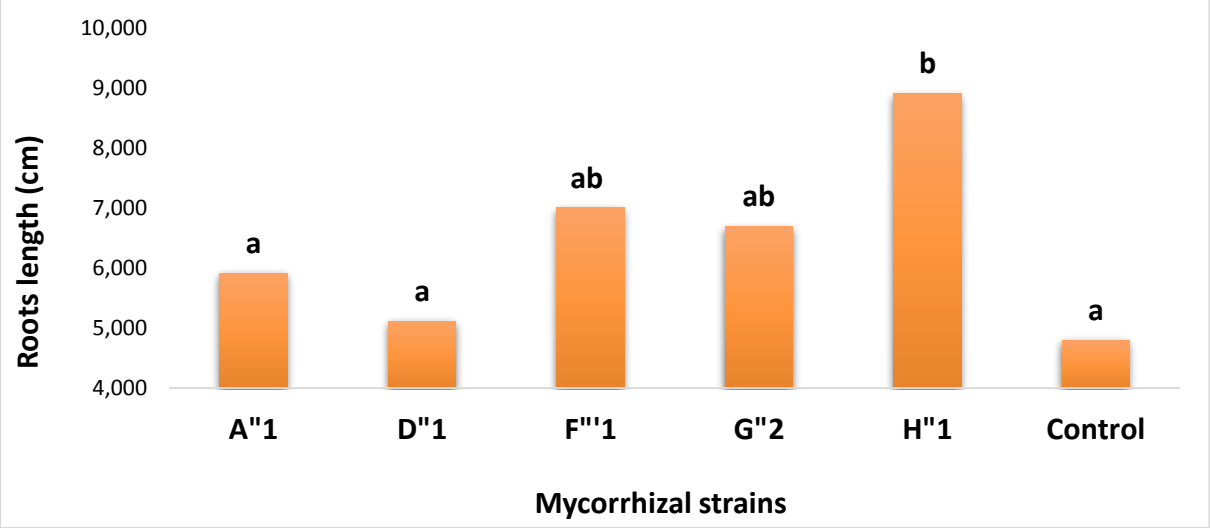


Figure 26: Variation of roots length of jujube plants according to the mycorrhizal strains used.

3.1.3. Fresh shoots weight

The results of the analysis of the variance grouped in the table 8show that there is a significant difference between the mycorrhizal strains and the control ($P<0.05$). This indicates that the presence or absence of mycorrhizal strains has different effects on the fresh weight of leaves, and the response of jujube plants to different mycorrhizal strains is also different.

Table 8: Anova test of fresh shoots weight.

Variation's sources	SS	dof	SM	F	P.
Mycorrhizal strains	0.016	5	0.003	1.583	0.049*
Residual	0.222	113	0.002		
Total	0.238	118			

The histogram of the figure 27 illustrates the variation in the fresh weight of shoots of the jujube tree according to the different strains used.

The different mycorrhizal strains show different effects on the fresh leaves weight of jujube plants. The control plants without mycorrhizal strains had the smallest fresh leaves weight with only 0.138±0.037 g. *Tukey's* test of comparison of means is used to individualize this mean in the homogeneous group (a). While plants with mycorrhizal inoculation had higher fresh leaves weight comparing to the control. Those means were classed in the homogeneous group (b).

The improvements in leaves development increased from 22.569% by the strain (H"1) from Sidi Ouadhah to 27.668% by the strain (D"1) from Si Abdelghani. The strain (G"2) isolated from roots of spontaneous jujube of Sidi Ouadhah had low effect on the fresh leaves weight with 9.551% compared to the control (Fig. 27).

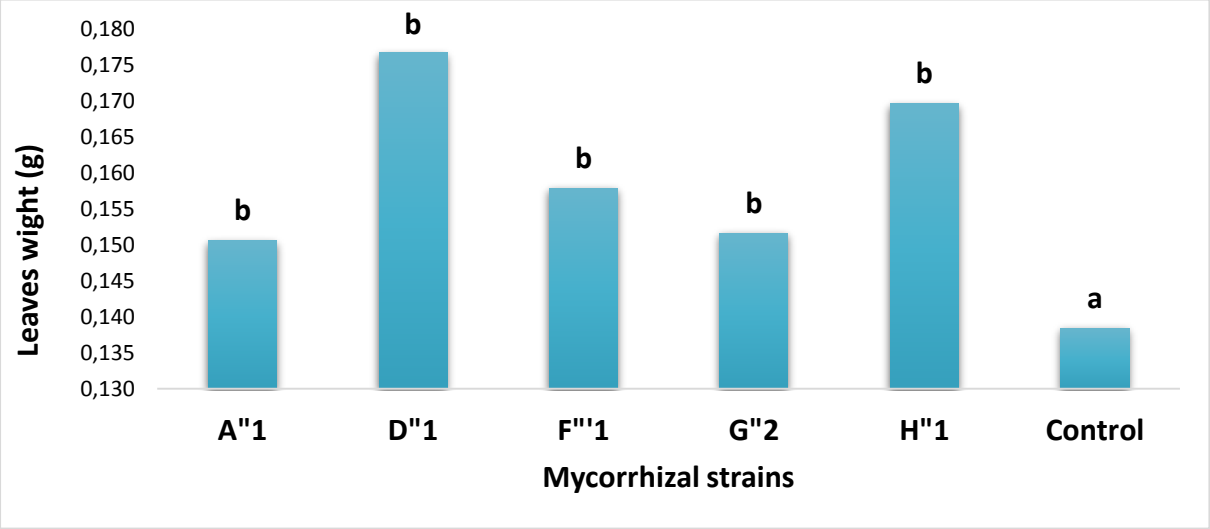


Figure 27: Variation of the shoots weight of jujube plants according to the mycorrhizal strains used.

3.1.4. Dry shoots weight

The results of the analysis of the variance grouped below show that there is a very highly significant difference (P<0.001) between the mycorrhizal strains and the control (table 9). This indicates that the presence or absence of mycorrhizal strains has different effects on dry leaves weight, and the response of jujube plants to different mycorrhizal strains is also different.

Table 9: Anova test of dry shoots weight.

Variation's sources	SS	dof	MS	F	P.
Strains	0.001	5	0.0002	11.699	0 ***
Residual	0.002	113	0.00001827		
Total	0.003	118			

The histogram of the figure 28 shows the variation in the dry shoots weight of wild jujube tree based on the different strains used.

The different mycorrhizal strains have different effects on the dry aerial weight of jujube plants. The plants that did not have mycorrhizal strains (control) had the smallest dry aerial weight, weighing only 0.0118±0.0032 g. *Tukey's* test of comparison of means is used to personalize this mean in the homogeneous group (a).

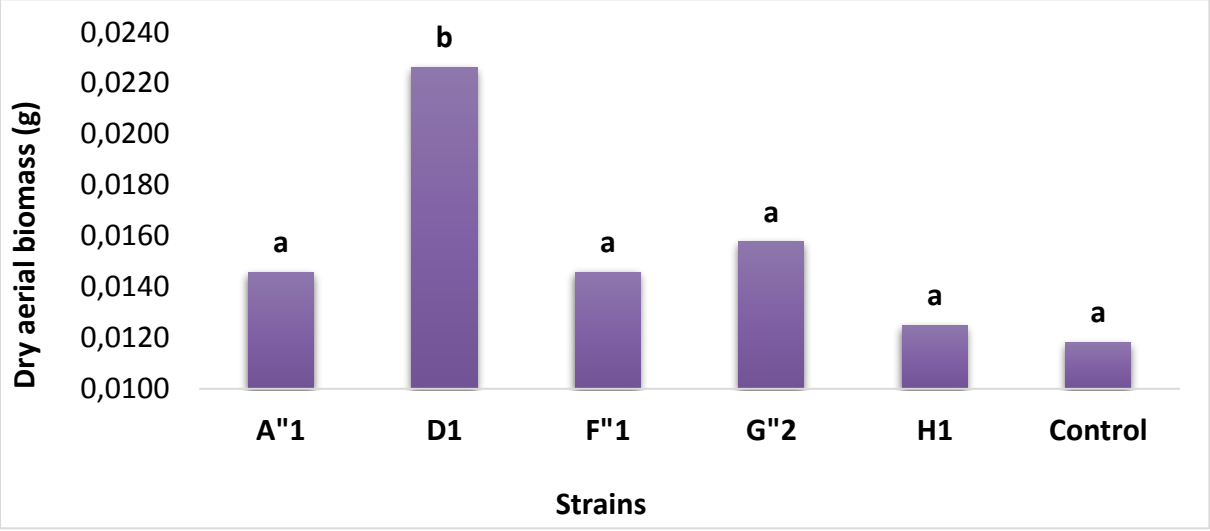


Figure 28: Variation of the dry shoots weight of jujube plants according to the mycorrhizal strains used.

The means of the strains (A"1) from Ksar Chellala, (F'"1) from Si Abdelghani and (H"1) and (G"2) from Sidi Ouadhah had also low dry leaves weight and were classed also in the

homogeneous group (a). The average of the strain (D"1) from Si Abdelghani has the highest dry leaves weight with 0.0226±0.0063 g and was classed in group (b).

The strain (H"1) seems to be the least efficient compared to the other strains used with only 5,70339%. While the strain (D"1) had the greatest effect on dry leaves weight, it improved leaves development by 91,6525 % compared to the control.

3.1.5. Fresh roots weight

The results of the analysis of the variance grouped in the table 10 show that there is a highly significant difference (P<0.01) between the mycorrhizal strains and the control. This suggests that the presence or absence of mycorrhizal strains has distinct impacts on fresh root weight and that jujube plants respond differently to different mycorrhizal strains.

Table 10: Anova test of fresh roots weight.

Variation's sources	SS	dof	SM	F	P.
Mycorrhizal strains	0.011	5	0.002	3.885	0.003**
Residual	0.063	113	0.001		
Total	0.074	118			

The histogram of figure 29 shows the variation in the fresh weight of jujube tree roots based on the different strains used.

Different mycorrhizal strains have different effects on the root weight of jujube plants. The plants that did not have mycorrhizal strains (control) had the smallest fresh roots, weighing only 0.036±0.033 g. *Tukey's* test of comparison of means classified this mean in the homogeneous group (a). While the means of the strains (A"1) from Ksar Chellala, (D"1) from Si Abdelghani and (H"1) and (G"2) from Sidi Ouadhah had the biggest roots fresh weight and were classed in the homogeneous group (b). The mean of the strains (F"'1) from Si Abdelghani has a medium roots weight with 0,052±0.021 g was classed in heterogeneous group (ab).

Fresh root development was improved from 80,211% by the strain (G"2) from Sidi Ouadhah to 84,617% by the strain (D "1) from Si Abdelghani compared to the control, but it was only improved by 43,99% when using the strain (F"'1) from Abdelghani (Fig. 29).

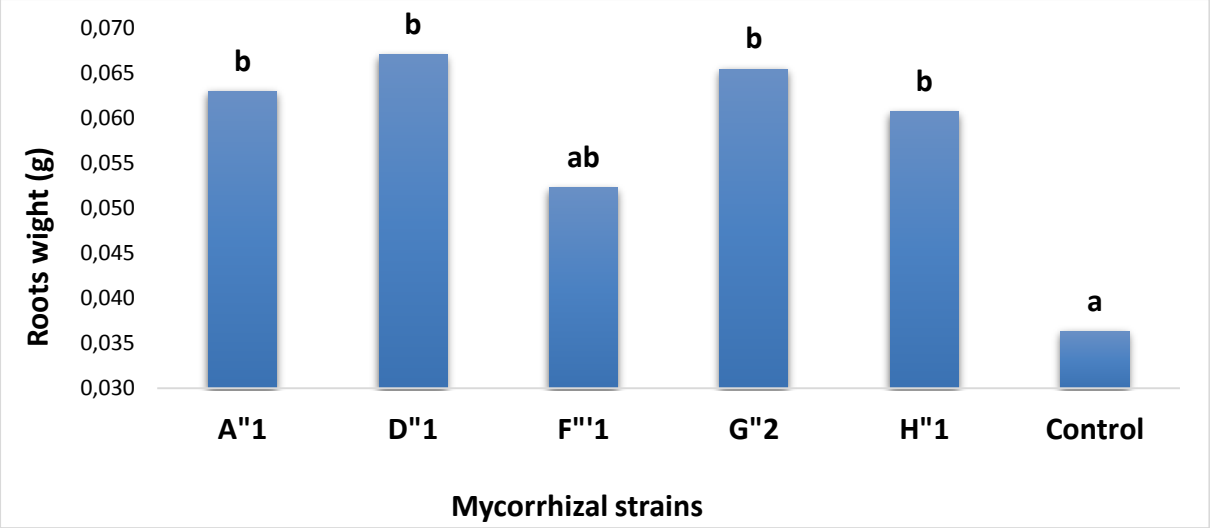


Figure 29**:** Variation of fresh roots weight of jujube plants according to the mycorrhizal strains used.

3.1.6. Dry root weight

The results of the analysis of the variance grouped below show that there is a very highly significant difference (P<0.001) between the mycorrhizal strains and the control (table 11). This indicates that the presence or absence of mycorrhizal strains has different effects on root length, and the response of jujube plants to different mycorrhizal strains is also different.

Table 11: Anova test of dry roots weight.

Variation's sources	SS	dof	MS	F	Sig.
Strains	0.000209	5	0.0000419	27.563	0 ***
Residual	0.000171	113	0.00000152		
Total	0.0003805	118			

The histogram of the figure 30 shows the variation in the dry weight of jujube tree roots based on the different strains used.

Different mycorrhizal strains have different effects on the dry root weight of jujube plants. The control plants that did not have mycorrhizal inoculation had the smallest weight roots, weighing only 0.0021±0.0019 g. *Tukey's* test of comparison of means classified this mean in the homogeneous group (a). The means of the strains (A"1) from Ksar Chellala (F"'1) from Si Abdelghani, (H "1) and (G"2) from Sidi Ouadhah had also low dry roots weight and were also classed in the homogeneous group (a). The average of the strains (D"1) from Si Abdelghani has

the best dry roots weight with 0.00684±0.0015 g and was individualized in homogeneous group (b).

The improvements in leaves development increased from 40,7354% by the strain (H"1) to 48.4761% by the strain (A"1) from Ksar Chellala. The strain (D"1) from Si Abdelghani had high effect on the fresh leaves weight with 231.205 % compared to the control.

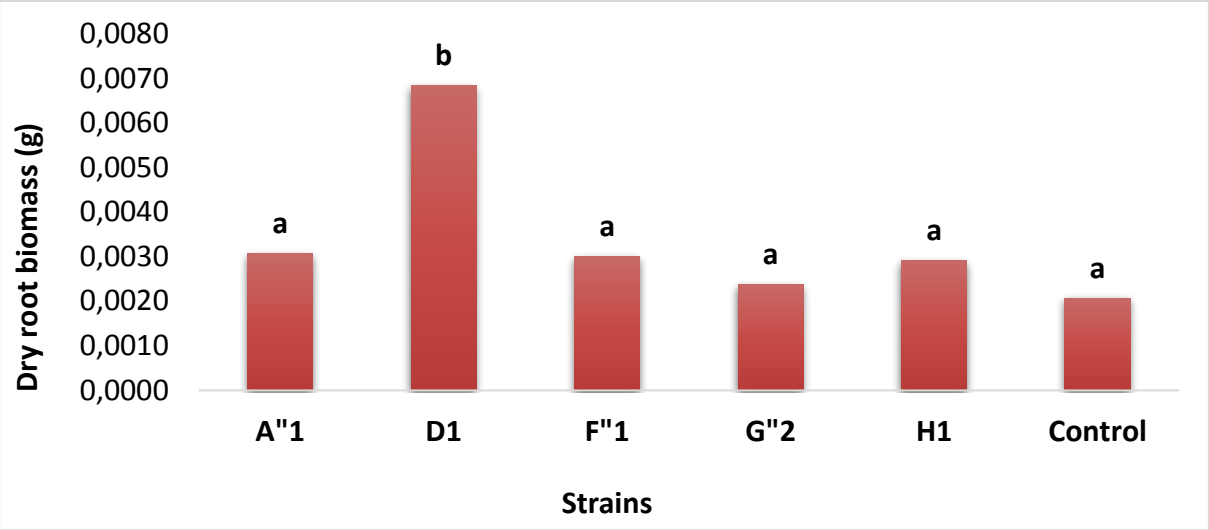


Figure 30: Variation of the weight of the dry roots of jujube plants according to the mycorrhizal strains used.

3.2. Evaluation of mycorrhizal isolates on secondary metabolite biosynthesis

The analysis of variance (table 12) shows that there is a highly significant difference ($P<0.001$) between the aqueous extracts of leaves and roots of inoculated plants with mycorrhizal strains and the control for polyphenol, flavonoids and condensed tannins content. This indicates that the mycorrhizal strains have a significant effect on the polyphenol, flavonoids and condensed tannins contents in leaves and roots. These results indicate also that the polyphenol, flavonoids and condensed tannins contents in leaves and roots varied considerably between inoculated and non-inoculated plants.

Table12: Anova test of polyphenol, flavonoids and condensed tannins contents in leaves and roots.

Variation's sources			SS	dof	MS	F.	P.
Polyphenol content	**Leaves**	**Mycorrhizal strains**	3813623	5	762724.7	50.645	0 ***
	Roots	**Mycorrhizal strains**	1355463	5	271092.6	60.467	0 ***
Flavonoid content	**Leaves**	**Mycorrhizal strains**	14800.22	5	2960.043	1026.765	0 ***
	Roots	**Mycorrhizal strains**	50595.18	5	10119.04	3063.33	0 ***
Tannins content	**Leaves**	**Mycorrhizal strains**	51501.96	5	10300.39	401.983	0 ***
	Roots	**Mycorrhizal strains**	226434	5	45286.8	2643.773	0 ***

3.2.1. Total polyphenol content

The aqueous extracts of the leaves of uninoculated plants of *Ziziphus lotus* had the lowest content in polyphenol with an average of 717.938±65.881 mg GAE.100 g^{-1} DM. The aqueous extracts of the leaves of *Ziziphus lotus* plants inoculated with the strains (F"'1) from Si Abdelghani and (G"2) and (H"1) from Sidi Ouadhah had close averages to those of control plant which indicates that these strains had low efficiency to stimulate the biosynthesis of polyphenol in *Ziziphus lotus* plant (Fig. 31). However, the aqueous extracts of the leaves of *Ziziphus lotus* plants inoculated with the strains (A"1) from Ksar Chellala and (D"1) from Si Abdelghani show the higher contents of total polyphenols. These strains stimulate the biosynthesis of polyphenol in *Ziziphus lotus* plant by 138.524% and 177.913%respectively compared to the control.

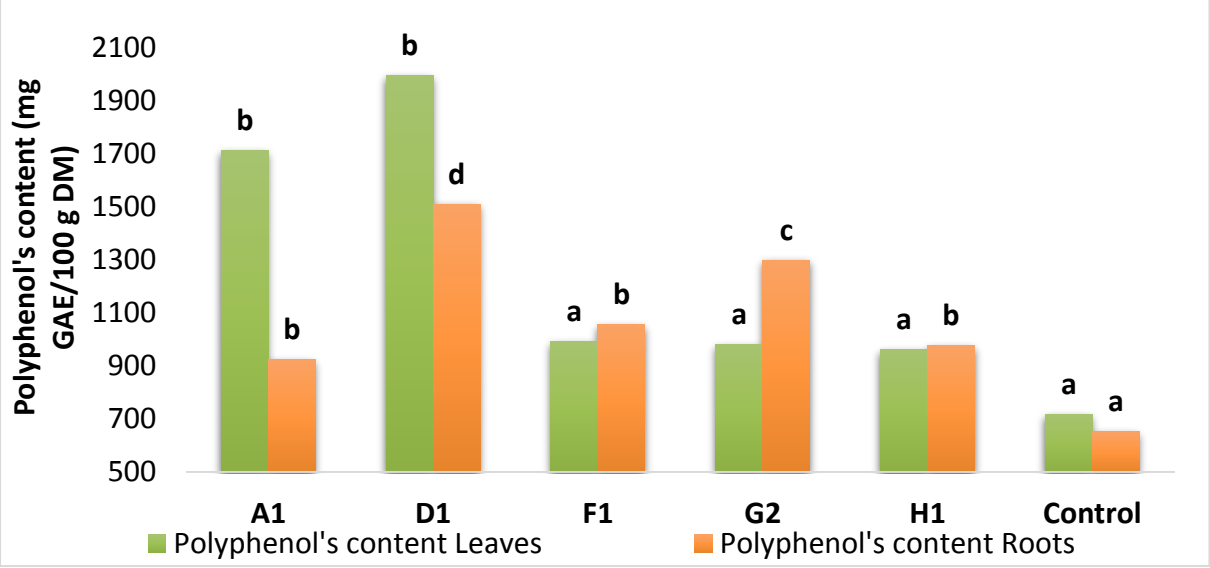


Figure 31: Variation of polyphenols content in aqueous extracts of leaves and roots of *Ziziphus lotus* plants according to the mycorrhizal strains used.

The aqueous extracts of the roots of uninoculated plants of *Ziziphus lotus* had the lowest content in polyphenol with an average of 652.533±60.326 mg GAE.100 g^{-1} DM. The aqueous extracts of the roots of *Ziziphus lotus* plants inoculated with the strains (A"1) from Ksar Chellala,

(H "1) from Sidi Ouadhah, (F"'1) from Si Abdelghani, (G"2) from Sidi Ouadhah and (D"1) from Si Abdelghani, showed higher values of polyphenol content (Fig. 31). These strains stimulate the biosynthesis of polyphenol, respectively, by 41.88%, 49.572%, 61.713%, 99.261% and 131.594% compared to the control.

3.2.2. Flavonoids content

The aqueous extracts of the leaves of uninoculated plants of *Ziziphus lotus* had the lowest content in flavonoid with an average of104.205± 0.338422 mg QE.100 g^{-1} DM. The aqueous extracts of the leaves of *Ziziphus lotus* plants inoculated with the strains (A"1) from Ksar Chellala, (H "1) from Sidi Ouadhah, (F"'1) from Si Abdelghani, (G"2) from Sidi Ouadhah and (D"1) from Si Abdelghani, showed higher values of flavonoid content (Fig. 32). These strains stimulate the biosynthesis of flavonoid, respectively, by 47.765%, 61.864%, 62.609%, 66.523%, and 90.048% compared to the control which has the lowest flavonoid content.

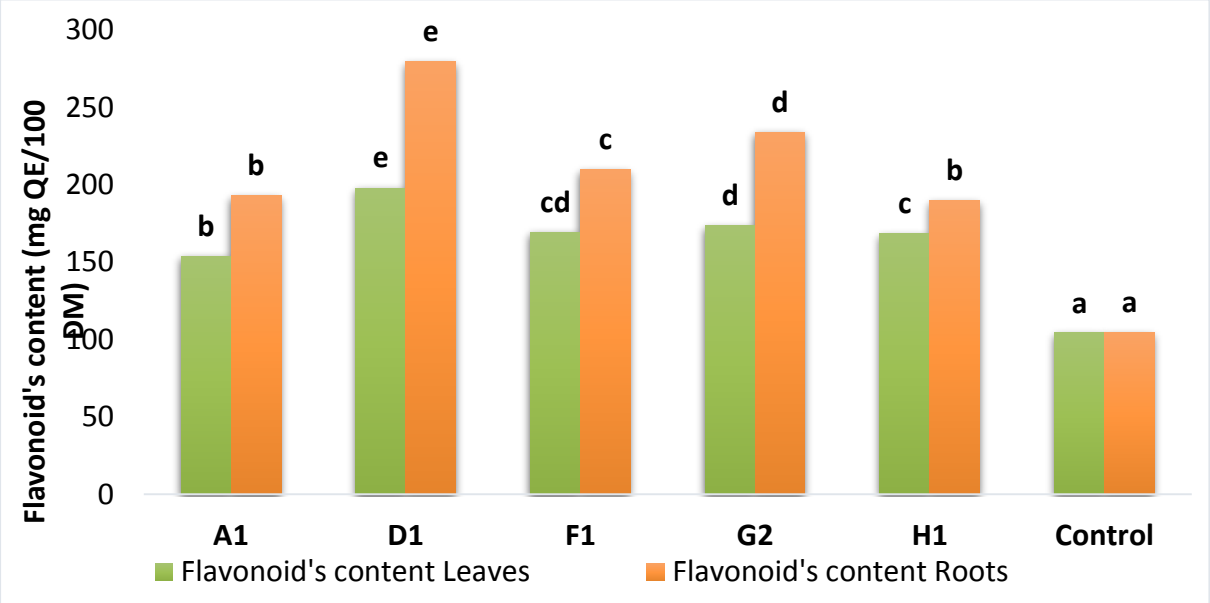


Figure 32: Variation of flavonoids content in aqueous extracts of leaves and roots of *Ziziphus lotus* plants according to the mycorrhizal strains used.

The aqueous extracts of the roots of uninoculated plants of *Ziziphus lotus* had the lowest content in flavonoid with an average of104.424± 01056mg QE.100 g^{-1} DM. The aqueous extracts of the roots of *Ziziphus lotus* plants inoculated with the strains (H "1) from Sidi Ouadhah, (A"1) from Ksar Chellala, (F"'1) from Si Abdelghani, (G"2) from Sidi Ouadhah and (D"1) from Si Abdelghani, showed higher values of flavonoid content (Fig. 32). These strains stimulate the

biosynthesis of flavonoid, respectively, by 81.650%, 84.934%, 101.163%, 124.220%, and 167.716% compared to the control.

3.2.3. Tannin content

The aqueous extracts of the leaves of uninoculated plants of *Ziziphus lotus* had the lowest content in tannin with an average of 133.906±1.8877 mg GAE.100 g^{-1} DM. The aqueous extracts of the leaves of *Ziziphus lotus* plants inoculated with the strains (F'"1) from Si Abdelghani, (H "1) from Sidi Ouadhah, (G"2) from Sidi Ouadhah, (A"1) from Ksar Chellala and (D"1) from Si Abdelghani, showed higher values of tannin content (Fig. 33). These strains stimulate the biosynthesis of tannin, respectively, by 55.395%, 57.316%, 62.098%, 114.769%, and 117.548% compared to the control which has the lowest tannin content.

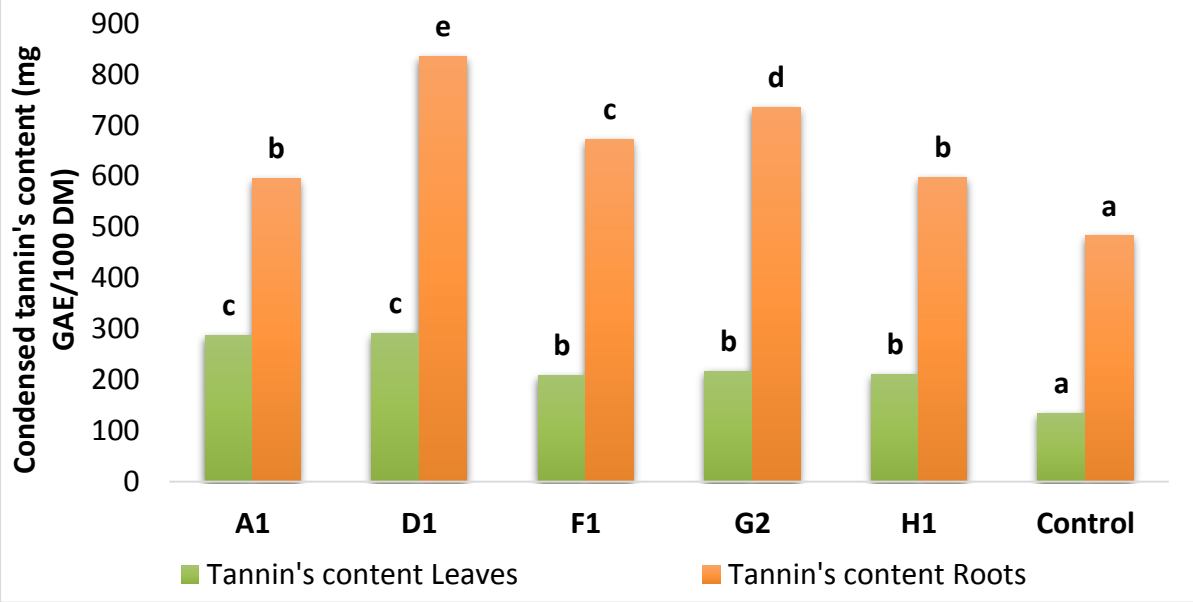


Figure 33: Variation of tannin content in aqueous extracts of leaves and roots of *Ziziphus lotus* plants according to the mycorrhizal strains used.

The aqueous extracts of the roots of uninoculated plants of *Ziziphus lotus* had the lowest content in tannin with an average of 482.581±1.5543 mg GAE.100 g^{-1} DM. The aqueous extracts of the leaves of *Ziziphus lotus* plants inoculated with the strains (A"1) from Ksar Chellala and (H"1) from Sidi Ouadhah had close averages to those of control plant which indicates that these strains had low efficiency to stimulate the biosynthesis of tannin in *Ziziphus lotus* plant (Fig. 33). However, the aqueous extracts of the leaves of *Ziziphus lotus* plants inoculated with the strains (F'"1) from Si Abdelghani, (G"2) from Sidi Ouadhah and (D"1) from Si Abdelghani show the

higher contents of tannin. These strains stimulate the biosynthesis of tannin in *Ziziphus lotus* plant by 39.117%, 52.114% and 72.951% respectively compared to the control.

4. Discussions

Mycorrhiza is a mutually beneficial symbiosis formed between soil fungi (AMFs) and plant roots. The association of roots with microorganisms relies on intricate molecular crosstalk which results from long-term co-evolution between plant hosts and microbial partners (Lambers *et al.*, 2009).

The countless interactions that occur in the rhizosphere between plants and its AMF symbionts are mediated through the plant and fungal metabolites that ensure partner recognition, colonization, and establishment of the symbiotic association (Kaur andSuseela, 2020).

In plant and mycorrhizal symbiosis, fungal partners may increase competitive abilities of host plant species through differential benefits (Callaway *et al.*, 2001).The main benefit for the plants is enhanced access to and uptake of nutrients (Cardoso and Kuyper, 2006). AMFs help plants capture water and mineral nutrients (especially P) from the soil, and in return, approximately 20% of plant-fixed carbon was transferred to fungi (Smith *et al.*, 2003). Phosphorus (P) is an essential plant nutrient that limits agricultural production on many soils (Vance, 2001). A recent study has showed that partitioning of soil P among different mycorrhizal trees contributed to species coexistence in subtropical natural forests (Liu *et al.*, 2018).

In this study, mycorrhizal strains have been isolated from natural populations of spontaneous jujube (*Ziziphus lotus*) to enhance our genetic heritage and use mycorrhizal strains adapted to our pedo-climatic changes.

Based on the spontaneous jujube roots growing in three different regions (Ksar Chellala, Sidi Ouadhah and Si Abdelghani) of Tiaret's Wilaya, five mycorrhizal strains were isolated, cleaned up, used for the formulation of liquid inoculation and tested for *Z.lotus* cultivated on deficient phosphorus soil in order to accelerate interaction between mycorrhizal strains and jujube's roots.

In this study, we determined the effects of five native AMFs colonization on growth parameters and secondary metabolite production of *Z.lotus* leaves and roots.

To investigate the effect of mycorrhizal isolates on *Z.lotus* growth and secondary metabolite levels, it was necessary to first characterize them. Classical morphological and biochemical methods were used in this context to characterize the five isolated strains, which were named (A"1) from Ksar Chellala, (D"1) and (F"'1) from Si Abdelghani, and (G"2) and (H "1) from Sidi Ouadhah.

In our study, the five Mycorrhizal strains(A"1), (D"1), (H"1), (G"2) and (F"'1), caused significant improvements in morphological parameters of *Ziziphus lotus*. Growth and morphological characteristics of *Ziziphus lotus* plants were significantly affected by plant mycorrhizal status. In the presence of mycorrhizal inoculation, roots were longer, with more fresh and dry mass as compared to those of non-inoculated plants. Mycorrhizal inoculation also promoted development of aerial parts, evident as an increase in plant height, fresh and dry mass compared to non-inoculated plants. The positive feedback mediated by mycorrhizal fungi tends to result in local dominance through promotion of their host performance (Peh*et al.,* 2011).Similar to the results of the present study, inoculation of *Acacia seyal* by *Glomus aggregatum* stimulated the development of fresh biomass of the aerial and root parts in the situation of stress (Manga *et al.*, 2018). Also, arbuscular mycorrhiza had a positive impact on improvement of tolerance to water stress in *Phaseolus vulgaris*, resulting in an increase in dry root and aerial biomass in stressed inoculated plants compared to non-inoculated stressed plants (Ganjeali *et al*., 2017). Other studies also reported that plants inoculated with AMF produced more dry weight than control plants (Thakur and Shinde 2020).

It has been already demonstrated that mycorrhizae improves plant. Several studies showed that inoculation with AMF increased plant height, biomass and dry weight in *Solanum lycopersicum* (Ruiz-Lozano *et al.,* 2015; Chitarra *et al.,* 2016); increased plant height, biomass, shoot and root dry matter, root length, shoot length in *Lycopersicon esculentum* (Subramanian *et al.,* 2006; Padmavathi *et al.,* 2016); increased biomass in *Lavandula spica* (Marulanda*et al.,* 2007); increased fresh and dry weight in Allium cepa (Nelsen, Safir 1982); and increased shoot and root weight and flavonoid concentration in *Pistacia vera* (Abbaspour*et al.,* 2012).

Mycorrhizal association is beneficial for production of new roots, their proliferation (increased root volume), their elongation (increase in length) and their maintenance under abiotic stress (Zou *et al.,* 2017; Wu and Zou, 2017; He *et al.,* 2019).

Special sources of pharmaceuticals, fragrances, flavors, food additives and other industrially relevant compounds include secondary plant metabolites. Secondary plant metabolites play their principal role in protecting plants from attack by plagues, herbivores and plant pathogens or in helping plants with other biotic and abiotic stresses.

Besides increase in plant growth, mycorrhizal inoculation increased concentration of phytochemical constituents (total phenolics, tannins, and flavonoids). Our findings show that inoculating *Z.lotus* with mycorrhizal significantly increases the levels of total polyphenols, flavonoids, and condensed tannins in the plant's leaves as well as the roots. The mycorrhizal strains (F"'1), (G"2), (D"1) gave the best results for the biosynthesis of tannin in *Ziziphus lotus* plant by 39,117%, 52,114% and 72,951%, respectively. The strains (A"1) from Ksar Chellala and (D"1) from Si Abdelghani show the higher contents of total polyphenols. These strains stimulate the biosynthesis of polyphenols in *Ziziphus lotus* by 138.524% and 177.913% respectively. The strains (A"1) from Ksar Chellala, (F"'1) from Si Abdelghani, (H "1) and (G"2) from Sidi Ouadhah and (D"1) from Si Abdelghani, stimulated the biosynthesis of flavonoids, by 47,765%, 61,864%, 62,609%, 66,523%, and 90,048% respectively. The strains (F"'1) from Si Abdelghani, (G"2)and(H "1) from Sidi Ouadhah, (A"1) from Ksar Chellala and (D"1) from Si Abdelghani, showed higher values of tannin content stimulated the biosynthesis of tannin, respectively, by 55,395%, 57,316%, 62,098%, 114,769%, and 117,548%.

It has been reported that the phenolic concentration of the aerial and root parts significantly increased in stressed and mycorrhizal plants, compared to non-mycorrhizal plants (Benjelloun*et al.*, 2014; Konéet *al.*, 2019). The study on the association of AMF with a cultivar of lettuce (Batavia Rubia Munguia) revealed that accumulation of phenolics in leaves increased significantly under optimal irrigation (Baslam and Goicoechea, 2012). Mycorrhizal inoculation of *L. albus*, concentration of phenolic compounds increased significantly in leaf and root extracts, as compared to non-inoculated plants (Abaid *et al.*, 2021). Plants of *Cynara cardunculus* L. var. *scolymus* F. accumulated more phenolic compounds and showed a higher antioxidant activity when inoculated with *R. intraradices* compared to those associated with *Funneliformismosseae* (=Glomus mosseae) (Pedone-Bonfim*et al.*, 2015). The higher levels of phenolics and tannins in *V. jatamansi*, following AMF treatment, correspond well with the report of increased production of phytochemicals due to infection of AMF (Toussaint *et al*.,2008). Seedlings of cebil (*Anadenanthera colubrina* (Vell.) Brenan) had an increase in the concentration of phenols, flavonoids, and tannins due to the

inoculation of mix (*G. albida* and *A. longula*) in relation to the non-inoculated control (Pedone-Bonfim *et al.*, 2013). It has been observed that the accumulation of flavonoids in roots of *Medicago sativa* L. is induced before the colonization and that it is dependent on the developing stage of the symbiosis and the AMF that are colonizing the plant (Pedone-Bonfim *et al.*, 2015).

The most likely mechanism involved in increasing concentration of certain phytochemicals is the improved nutrition that AMF can provide to plants (Crişan *et al.*, 2018). High production of phenolic compounds may be related to an increased activity of enzymes such as chalcone synthase and chalcone isomerase, which are involved in the synthesis of flavonoids, and phenylalanine ammonia lyase, responsible for catalyzing the deamination of phenylalanine, which is an important regulating stage in the formation of phenolic compounds, and which may have its activity increased by environmental factors and by biotic factors, such as colonization by fungi (Pedone-Bonfim *et al.*, 2015).

Conclusion

Soil microorganisms have vital roles in nutrient cycling, maintenance of soil fertility, sequestration of soil carbon and dynamics of vegetation.

The arbuscular mycorrhizal fungi may form mutual associations in a wide variety of ecosystems of around 80% of vascular plants and are one of the main microbial groups involved in sustainable agriculture development.

The host plant received return water, inorganic nutrients absorbed by the soil, as well as benefits such as increased root volume and longevity and pathogens resistance in the fungus obtaining carbohydrates and other elements that are important for its development and sporulation. The plant community needs arbuscular mycorrhizal fungi because they are linked to plant mineral nutrition, in particular soil low-mobility nutrients that are required in large quantities.

This study was conducted to isolate, purify and characterize the new native mycorrhizal strains from the roots of the wild jujube (*Ziziphus lotus*), which grow in the in three areas of the Wilaya of Tiaret.

Several of 17 mycorrhizal fungi of various phenotypes were isolated from the disinfected roots of wild jujube,8 of them were isolated from Ksar Chellala, 6 were isolated from Sidi Ouadhah and 3 were isolated from Si Abdelghani. The only strains maintained for the formulation of the liquid inoculation were: A2" from Ksar Chellala, H1" and G2" from Sidi Ouadhah and D"1 and F1'" of Si Abdelghani.

In comparison to controls (lack of mycorrhizal strains), the formulation was found to be effective. The effect of mycorrhizal liquid inoculation were different between strains, the strain (D1") from Sidi Abdelghani had the highest effect on the most of observed parameters in young crop of spontaneous jujube (*Ziziphus lotus L. Desf.*), it improved stems high, fresh and dry leaves weight, Fresh and dry roots weight, Total polyphenol content, Flavonoid content and Tannin content. While the strain (H "1) from Sidi Ouadhah had the greatest effect on root length development.

The collected results can be interpreted as a potential for the promotion of native micro-organisms. Our study does not show mycorrhizal strains representative of Algeria's agro ecological situation. Surveying must therefore be conducted in areas that have different pedoclimatic conditions.

Our findings can be supplemented by a careful description and microscopic or molecular identification of mycorrhizal arbuscular layers of indigenous strains in order to better structure their genetic diversity.

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Abstract

Plants interact with a large number of soil microbes to support them in acquiring nutritional resources, protecting them against pathogenic agents, and facing difficult and constantly shifting external conditions. These include arbuscular fungi whose an obligate biotrophic fungi that depend exclusively on carbon from their host plants to preserve their growth, development, and function.

The focus of this research is to isolate and purify new strains of Mycorrhiza in order to observe and test their impact on secondary metabolites synthesis in the *Z. lotus* plant.

The mycorrhizal strains were isolated from disinfected roots of wild jujube trees growing in three different locations of the Tiaret. Monospore culture was used to purify the strains. Five strains were chosen and used for the inoculation of *Z. lotus* plants.

The findings revealed that the presence of Mycorrhiza in the soil has a significant impact on the studied parameters. The mycorrhizal strain (H "1) from Sidi Ouadhah had the greatest effect on root length (85.606%), the growth of the aerial part was the higher for the strain (D1") with 51.44%, mycorrhizal strain(D"1) from Sidi Abdelghani stimulated the production of fresh leaves and root biomass by 27.668% and 84,617%, respectively. Results showed differences in quantity and quality for secondary metabolism products of jujube, the mycorrhizal strain (D"1) from Sidi Abdelghani show the higher contents phenolic compound. These strains stimulate the biosynthesis of polyphenols, flavonoids and tannin in *Ziziphus lotus* by 177.913%, 90.048% and 117,548%, respectively.

Key words: Arbuscular mycorrhizal fungi, efficiency, Growth parameters, isolation, secondary metabolites synthesis, *Ziziphus lotus*.

Résumés

Les plantes interagissent avec un grand nombre de microbes du sol pour les aider à acquérir des ressources nutritionnelles, les protéger contre les agents pathogènes et faire face à des conditions externes difficiles et changeantes. Il s'agit notamment des champignons arbuscular (AM) dont un champignon biotrophique obligatoire qui dépend exclusivement du carbone de leurs plantes hôtes pour préserver leur croissance, leur développement et leur fonction.

L'objectif de cette recherche est d'identifier et de purifier de nouvelles souches de Mycorhizes afin d'observer et de tester leurs impacts sur la synthèse de métabolites secondaires chez le *Z. lotus*.

Les souches Mycorhiziens ont été isolées à partir des racines désinfectées de jujubier spontané poussant dans trois endroits différents de la wilaya de Tiaret. La culture de monospores a été utilisée pour séparer les souches. Cinq souches ont été choisies et utilisées pour inoculer les plantes de *Z. lotus.*

Les résultats ont révélé que la présence de Mycorhizes dans le sol a un impact significatif sur les paramètres étudiés. La souche Mycorhizien (H "1) de Sidi Ouadhah a eu le plus grand effet sur la longueur des racines (85,606%), la croissance de la partie aérienne était la plus élevée pour la souche (D1") avec 51,44%, la souche Mycorhizienes (D"1) de Sidi Abdelghani a stimulé la production de biomasse aériennes et racinaires fraîches avec 27,668%, 84,617% respectivement. Les résultats ont montré des différences dans la quantité et la qualité des produits du métabolisme secondaire du jujubier. La souche Mycorhizien (D"1) de Sidi Abdelghani montrent les teneurs les plus élevées en composés phénoliques. Cette souche a stimulé la biosynthèse des polyphénols, flavonoïdes et tanins dans le *Ziziphus lotus* par 177,913% 90,048 et 117,548% respectivement.

Mots clé : Champignons mycorhizien à arbuscules, efficacité, paramètres de croissance, isolement, synthèse de métabolites secondaires, *Ziziphus lotus*.