الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria
وزارة التعليم العالي و البحث العلمي
Ministry of Higher Education and Scientific Research
جامعة زيان عاشور-الجلفة
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Faculty of Natural and Life Sciences

Final project for the Master degree

Field of study: Biological Sciences of Biology Department
Speciality: Plant Ecology and Environment

Theme

Contribution to the characterization of mycorrhizae of the olive tree in the steppe (study area Khatala). Commune of Messaad-Wilaya of Djelfa

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In front of the jury, composed of :

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Academic year 2022/2023

ABSTRACT

Because of its mycotrophic caracter, and its capability to live in the steppic environment, the olive tree is the host in this symbiosis. In this work, the variation of mycorrhization parameters : mycorrhizal frequency in the root system (F%), Intensity of mycorrhizal colonisation in the root system (M%), Intensity of mycorrhizal colonisation in root fragments (m%), Abundance of arbuscules in the mycorrhizal parts of root fragments (a%), Abundance of arbuscules in the root system (A%) are studied in two different age groups, in order to understand the differences in this case for the establishment of the symbiosis in this species. The physico-chemical properties of soils (pH, electric conductivity, organic matter,total limestone amount and active limestone) were determined for the two age groups.The study shows The frequency of mycorrhization is equal in the two groups unlike the other parameters, the adult group had the highest amount of the M%, m%, a%, A%. parameters.
Key words : Olive tree, Mycorrhizae, Steppe, Mycorrhization parameters.

RÉSUMÉ

En raison de son caractère mycotrophe et de sa capacité à vivre dans un environnement steppique, l'olivier est l'hôte de cette symbiose. Dans ce travail, la variation des paramètres de mycorhization : Fréquence des mycorhizes dans le système racinaire (F%), Intensité de la colonisation mycorhizienne dans le système racinaire (M%), Intensité de la colonisation mycorhizienne dans les fragments de racines (m%), Abondance des arbuscules dans les parties mycorhiziennes des fragments de racines (a%), Abondance des arbuscules dans le système racinaire (A%). sont étudiés dans deux groupes d'âge différents, afin de comprendre les différences dans ce cas pour l'établissement de la symbiose chez cette espèce. Les propriétés physico-chimiques des sols (pH, conductivité électrique, matière organique, calcaire totale et calcaire actif) ont été déterminées pour les deux groupes d'âge. L'étude montre que la fréquence de mycorhization F (%) est égale dans les deux groupes contrairement aux autres paramètres. Le groupe adulte a la quantité la plus élevée des paramètres M (%), m (%), a (%), et A (%).
Mots clés : Olivier, Mycorhizes, steppe, paramètres de mycorhization.

ملخص

بسبب طابعها الفطري، وقدرتها على العيش في البيئة السهوب، شجرة الزيتون هي المضيفة في هذا التعايش. في هذا العمل، تمت دراسة تباين معاملات التكاثر الفطري تواتر الجذور الفطرية في نظام الجذر (F%)، شدة استعمار الميكوريزا في نظام الجذر (M%)، شدة استعمار الميكوريزا في أجزاء الجذر(m%)، وفرة الشجيرات في الأجزاء الجذرية من شظايا الجذر (a%)، وفرة الشجيرات في نظام الجذر (A%). في فئتين عمريتين مختلفتين، من أجل فهم الاختلافات في هذه الحالة لتأسيس التعايش في هذا النوع. تم تحديد الخواص الفيزيائية والكيميائية للتربة (حموضة التربة ، التوصيل الكهربائي، المادة العضوية، كمية الحجر الجيري الكلي والحجر الجيري النشط) للفئتين العمريتين. أظهرت الدراسة أن تكرار الفطريات الفطرية متساوي في المجموعتين على عكس المعلمات الأخرى، فقد حصلت مجموعة البالغين على أعلى كمية من المعلمات A%, a%, m%, M%.
كلمات مفتاحية : شجرة الزيتون ، فطور جذرية ، السهب ، معلمات الفطور الجذرية .

Acknowledgements

This work would not have been possible without the support of the FSNV Djelfa lab, and the HCDS lab, who have been supportive of our goals and who worked actively to provide us with the protected academic time to pursue those goals.

We are grateful to all of those whom we had the pleasure to work with during this study, each of the members of the jury; Mme BELHADJ S and Mme NAAS O. We would especially like to thank Mme GHAZI M and Mme BOUABDELLI Z. As our teachers and mentors, provided us extensive personal and professional guidance and taught us a great deal about scientific research, they taught us more than we could ever give them credit for. They have shown us, by their example, what a good scientist (and person) should be.

We would like to thank Mr HASSANI and Mr AISSA for their guidance in the lab work, also great thanks to Mr GEUINI Mohammed who was very generous by hosting us in his farm and for his gratefulness for the enrichment of this research.

Dedication

We dedicate this memo to

Our very dear parents; no words could truly express your true value, and the depth of our respect and love. Thank you for your unwavering support and patience. May Allah bless you with health, happiness and a long life,

Our brothers and sisters; Thank you for continually encouraging us,

Our dear friends: Dida, Lina, Mima, Ikram, Souhila .Thank you for your kindness, your encouragement and the good times we spent together.

Thanks to Mr Bouragba and everyone who helped us directly or indirectly.

Figures list

Tables list

Abbreviation list

A :	Arbuscule.
A (%) :	Abundance of arbuscules in the root system.
a (%) :	Abundance of arbuscules in the mycorrhizal parts of root fragments.
AMF :	Arbuscular Mycorrhizal Fungus.
C :	Carbon.
CC :	Central cylinder.
°C :	Degree Celsius
$CaCO_3$:	Calcium carbonate.
EC :	Empty Cell.
F (%) :	mycorrhizal frequency in the root system
h :	The difference in altitude.
H :	Hyphae.
H_2O_2 :	Hydrogen peroxide.
H.C.D.S:	High Commission for the Development of the Steppe.
HCl :	Hydrochloric acid.
H.U :	Useful volume humidity.
KOH :	Potassium hydroxide.
LR :	Lateral ramification.
M :	Maximum temperature.
m :	Minimum temperature.
M (%) :	Intensity of mycorrhizal colonisation in the root system.

m (%) : Intensity of mycorrhizal colonisation in root fragments.

MO : Organic matter.

Mg : Magnesium.

n : Quantity dosage of the titration.

Na : Sodium.

P : Plot.

P(mm) : Precipitation in millimetres.

Pe : Sample weight in g.

PF : Final weight in g.

pH : Degree of acidity.

PI : Initial weight in g.

PMA : Average annual precipitation.

PV : Weight of empty container in g.

Q : The Emberger rainfall quotient.

QE : Quantity of samples in g.

QT : Quantity of sulfuric acid in the titration.

RH : Root hair.

T : Temperatur.

TA : Average temperature.

V : Vesicle.

% : Percentage.

α : Significance level.

Table of contents

Introduction

The study of ecosystem ecology has always largely underestimated the importance of below-ground biotic factors such as interactions between plants and microorganisms. Among microorganisms, mycorrhizal fungi are important members in terrestrial ecosystems, due to the symbiosis ecosystems through the symbiosis they form with plant roots (Carteron, 2020). This mycorrhizal symbiosis created with plants is "[...] unquestionably the most common and most important mutualistic symbiosis in terrestrial ecosystems". Mycorrhizal symbiosis is determined by the close and prolonged interaction between the fungus (mycobiont) and the plant (phytobiont) and a specific environment (Figure 01). The importance of this symbiosis is illustrated by its prevalence, since the mycorrhizal way of life from tropical environments to the tundra, and is thought to involve up to 95% of the world's plants on Earth. Mycorrhizal symbiosis has several types, the most widespread of which are the arbuscular mycorrhizae, ectomycorrhizae, ericoid mycorrhizae, and orchid mycorrhizae (Carteron, 2020).

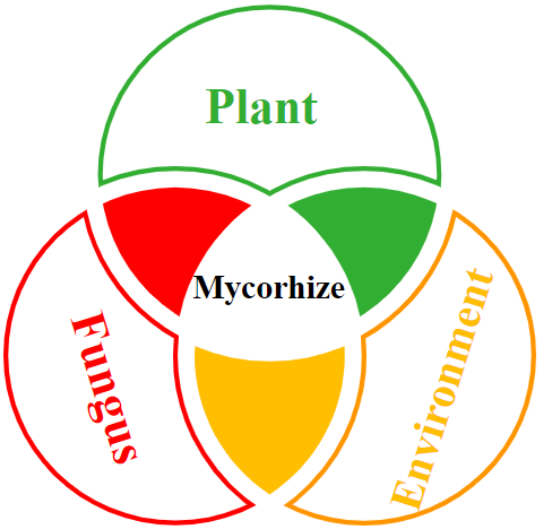

Figure 01. The mycorrhizal association is the result of a tripartite interaction between the mycobiont (fungus) and the phytobiont (plant) in a given environment (Carteron, 2020).

A mycorrhizae is a symbiotic association essential for one or both partners, between a fungus (specialised for life in soils and plants) and a root (or other substrate-contacting organ) of a living plant, that is primarily responsible for nutrient transfer. Mycorrhizas occur in a specialised plant organ where intimate contact results from synchronised plant-fungus development (Brundrett, 2004).

Introduction

In Algeria, the study of the biodiversity of arbuscular mycorrhizal fungi remains the poor relation of microbial ecology. The olive tree, among many other herbaceous and woody plants, naturally contracts the most widespread root symbioses in nature, such as vesicular and arbuscular endomycorrhizal symbioses (Mezghrani, 2015) and it has been shown that the olive tree is a particularly mycotrophic plant (Meddad & al., 2010).

According to Mezghrani (2015), the ecological conditions for the mycorrhizal symbiosis are: the optimal temperature between 12 and 20° C, the light and the minimum moisture are desirable, pH values of 4 and 5 intensify the formation of this association,organic material, when it is not toxic, is certainly a favourable place for the permanence of mycorrhizae in the soil, this association is not established in the presence of fertilisers and pesticides.

Mycorrhizae are durable unions based on mutual exchanges of metabolites between plant roots and some fungi present in the soil. The new mixed organ is formed from the tissues of the host plant and the mycorrhizal fungus (or fungal symbiont), and each partner optimises its development through this symbiosis (Châtaigner & Duponnois, 2017) and they are heterotrophic filamentous eukaryotic organisms. Their basic cellular unit is called a hyphae. They contain all the constituents of a typical cell, they multiply at their tips forming a tangled mass called mycelium. The ancient parts of the hyphae contain numerous vacuoles and can be separated from the young regions by transverse partitions called septum (Mezghrani, 2015).

Mycorrhizal fungi, as a part of the rhizosphere population of microorganisms, are unable of photosynthesis and are completely dependent on the plant they colonise for carbonated substances. Regardless of the type of mycorrhizae, various functions are generally modified by the presence of mycorrhizae: water and mineral uptake, hormonal activities and protection against pathogenic organisms (Bouabdelli, 2019).

Numerous research on olives have proven the advantages of symbiosis with mycorrhizal fungus, and it has been verified how the plantlet establishment improves significantly after its micropropagation since the symbiosis increases the acquisition of nutrients by the plant, its growth and development, and improves the biochemical profile of olives and oil. Faced with abiotic stresses, the olive-mycorrhizal fungal symbiosis has been reported as increasing plant tolerance under situations of salinity or drought and it has the ability to completely modify

the plant-related microbiota, as a consequence of establishing the symbiosis and modifying the nutritional profile of the rhizosphere (Poveda & Baptista, 2021).

To this end, it leads us to the following problematic :

Are there any mycorrhizae on the olive trees in this area? And if so, is this presence affected by the age of the tree?

Chapter I: Material and methods

1 Presentation of the study area

1.1 Geographical location of olive orchard

This present work is carried out at the level of the olive plantation of Khatala (Massaad, Djelfa province). This study area is found within the central portion of the nation. It is found 300 km south of the capital, at an average elevation of 1180 m above sea level. The climate of the study area regions is semi-arid, and arid. Messaad is located at 76 km from the South-East of Djelfa city, between 34° 8′ 31″ - 34° 10′ 52″ of latitude North and 3° 27′ 58″ - 3° 31′ 48″ of longitude East, its covers approximately 7.90 km^2 (Dib & al., 2022).

The olive grove selected for this study is found at 34°17′68″N366 and 3°47′61″E376, in Messaad, 4,4 km West-Northwest of Messaad centre. The study area is surrounded by plantations and it has an altitude of 792 m above sea level (Figure 03). According to the keeper of the grove, it has an olive-growing area of 1 ha of diverse ages (between 7 and 16 years old), of which 2/3 of them are grown-up productive trees and 1/3 are young non-productive trees.

1.2 Description of the selected experimental trees

These criterias considered the optimal for our study, the selected experimental trees are splitted in two categories (Figure 03).

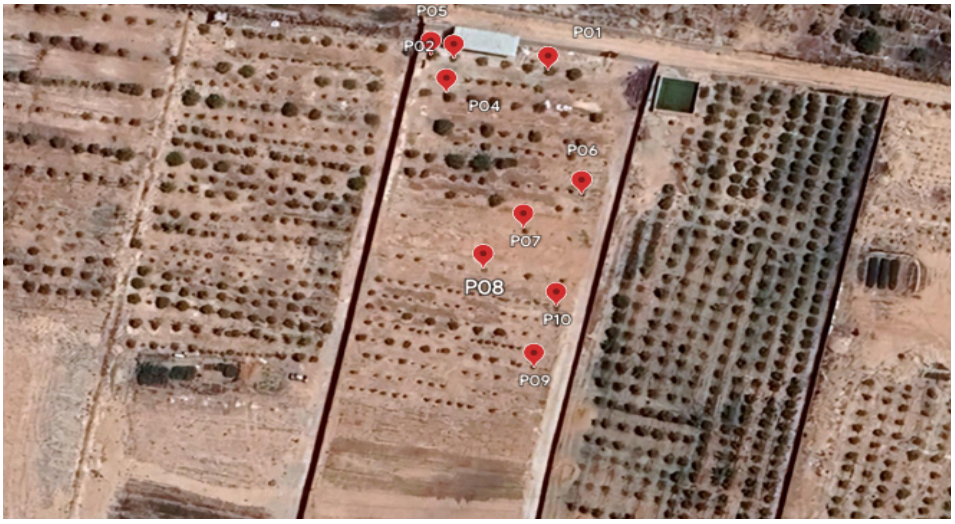

Figure 02. Study area and the selected experimental trees for the study (Photo taken by Google Earth, 2023).

1. The first 5 plants named (P1 to P5) are old productive olive trees.
2. The second 5 plants named (P6 to P10) are young non productive olive trees.

in each plant we selected 3 samples (referred to letter S) in different directions :

- S1 in the North-northeast direction according to the tree.
- S2 in the South-southeast direction according to the tree.
- S3 in the West direction according to the tree.

2. Climatic synthesis

2.1 Climatic factors

The foremost critical climatic factors are temperature and precipitation.

Due to the absence of climate stations within the study location, we used climatic data from the Djelfa weather station to characterise the climate of the Messaad station. For this site, we extrapolated the data obtained at the Djelfa weather station spanning 30 years (from 1990 to 2020), noting that we choose a random point in the study area with an altitude of 792 m. What determines whether there is a correction of the data or not is the geographic location of the monitoring station to the study area.

The correction calculations :

Calculations of the difference in altitude

h Djelfa - h Station = … m

If the difference in altitude is greater than 100 m in this case we apply the climatic corrections, with the corrections we will calculate the average monthly and annual precipitation of the study area in (mm) for the period (1990-2020) and the average monthly temperatures in C° of the study area (1990-2020). To determine the climate classification, we use the Bagnouls and Gaussen's ombro-thermal diagram and the emberger precipitation climatogram. These two systems summarise the bioclimate of a given station by three fundamental climate elements: precipitation (mm) - maximum and minimum temperatures (°C).

2.2 Classification of climate

2.2.1 Gaussen and Bagnoul's ombro-thermal diagram

Gaussen and Bagnoul's ombro-thermal diagram is a graphical method used to define the dry and wet periods of the year, where the months are plotted on the x-axis and the rainfall (P) and temperature (T) on the y-axis, with P=2T.

2.2.2 Climate Variability Analysis (Emberger Method)

A method designed by Emberger is widely used for climate classification in climatology, hydrology, ecology, and bioclimatic analysis. The method considers the two important climate parameters, precipitation and temperature

$$Q_3 = 3,43*P/(M-m)$$

- Q_3: Emberger's rainfall quotient.

- P: Average annual precipitation in mm.

- M: Maximum average of the hottest month.

- m: Minimum average of the coldest month.

After applying the formula, we will obtain the value of Q_3, and will find the climatic stage of the study area.

3. Soil analysis protocol

3.1 Humidity

1g of fresh soil was measured then put in the oven at 90°C for 24h and then it was measured again.The difference between the weight before and after the oven is the data for the humidity calculations .

3.2 pH and conductivity

The soil was dried at room temperature for 15 days and sieved with a 2 mm sieve.

For the measurement it need to prepare ⅕ diluted extract : First, 50 g of soil weighted and transferred to a 250 ml stirring falcon, then added 250 ml of distilled water and then agitated for 2 hours and put in a centrifuge for 4 minutes.

Each sample was measured with an Electronic Conductivity metre device and then a pH metre device.

3.3 Total limestone

1 gram of finely textured soil was measured out and deposited into the flask. Then proceeded to fill approximately three-fourths of the auxiliary chamber of the flask with hydrochloric acid (HCl), then establish a connection between the flask and BERNARD's calcimeter device, ensuring that the water levels in both the vertical column and the bulb of the

calcimeter are calibrated to zero. Gently the acid dispensed onto the soil, and using the bulb component, repositioned the water levels to their initial positions. then recorded the resulting volume, denoted as V, corresponding to the produced CO_2.

3.4 Active limestone

The Drouineau-Galet method was used: 10 grams of finely textured soil were placed into a flask and 250 millilitres of ammonium oxalate solution ($(NH_4)2C_2O_4$ H_2O)(0.2 M) were introduced to the flask.

Allowing the mixture to undergo agitation for a duration of 2 hours using a mechanical stirrer. Subsequently, filtered the resultant solution, discarding the first droplets of the filtrate, and subsequently subjecting them to re-filtration.

Then 10 millilitres of this solution were transferred into a beaker, followed by an additional 10 millilitres of H_2SO_4 (0.1 M). Then apply heat until the temperature reaches 60°C.

Proceeded to titrate the solution with potassium permanganate solution ($KMnO_4$) until a consistent pink hue becomes evident, then the volume of the potassium permanganate left was recorded.

3.5 Organic material

Organic material was measured by burning the carbon from the soil, then weighing 10 grams of soil in the porcelain dish and placing it in the Muffle furnace at 300 degrees for 24 hours. After that the samples were re-measured again, the difference between the weight before the Muffle furnace and after is the measurement that needed.

3.6 Granulometry

The granulometric analysis involves classifying the individual mineral particles according to their size, this separation allows for defining the nature of the soil texture. We used the Robinson pipette method with Mr.Hassani at the HCDS.

For granulometric analysis, 10 g of air-dried soil is sieved through 2 mm, treated with 25 ml of hydrogen peroxide, and heated until effervescence ceases. After filtration, the soil is recovered in a flask, mixed with 400 ml of distilled water, 40 ml of sodium hexametaphosphate, and 1 ml of pure ammonia, stirred for 2 hours, and adjusted to 1 litre. Using the Robinson pipette method, clay and silt are fractionated by shaking the cylinder, resuspending the deposit, and taking a 20 ml aliquot after specific settling times at 20°C. Sands are recovered by sieving through 50-micron, oven-dried at 105°C, weighed, and fine sands are separated from coarse sands using a 200-micron sieve, with coarse silts determined by difference.

4. Coloration protocol

4.1 Plant material

The plant material used in this study consists of olive tree roots collected by the following method:

15 Samples of olive roots were collected from 5 young non-productive trees taken at random. In the same way, we collected 15 samples of olive roots from 5 adult productive trees randomly chosen. All litter and herbaceous plants were removed from around each tree by the grove servient.

Then we dug to a depth of 20 to 30 cm using a pickaxe. We followed the large roots down to the finest, and took the clods of soil containing the roots around 300g for each sample. The samples were then transported to the laboratory in plastic bags labelled from P1_S1 to P10_S3 (30 samples). We rinsed the roots of each sample thoroughly with tap water and then saved them in a Sampling bottle filled with 70% ethanol until use.

4.2 Roots coloration

We used Phillips and Hayman technic (1970) :

1. We selected the suitable roots for AMF's staining from the highest quality and least fibrous ones, and then thorough and cautious rinsing with tap water to eliminate any remnants of the ethanol. Subsequently,trimmed them into small fragments approximately 1 cm long.
2. We placed the roots in a 10% KOH (potassium hydroxide) solution, (the roots from P04 to P10 were treated with 5%KOH) and then placed in a water bath at 90°C for about 30 min at least. We let them for a long time if they are pigmented and fibrous, we replaced the KOH solution as soon as it became dark (the purpose of KOH is to empty cells for their cytoplasm).
3. We rinsed the roots several times with tap water and then moved them to a solution of 10% H_2O_2 (hydrogen peroxide) and placed them in a water bath at 90°C for 15 to 20 minutes, ensuring the roots were fully bleached(we shorten the time for the roots from P7 to P10 to 7 min).
4. Then we rinsed the roots several times with water and neutralised them in a 10% lactic acid bath for 3 to 4 minutes.
5. We transferred the roots into 0.5% methylene blue solution then placed them in a water bath at 90°C for 1 hour (the roots from P04 to P10 were treated with 0.05% methylene blue and shortened the period to 30 min, in general).

6. Once the coloration period concluded, we rinsed the roots from the methylene blue solutions with water and placed them in a Petri dish filled with glycerol (glycerin) for a couple of days before mounting them for microscopic observation.

4.3 Observation

We removed the roots from the glycerin and placed 10 roots gently in the slide, each side of the slide has 5 roots, and then covered with the coverslip, then we gently pushed on the top of the coverslip to crush the root and isolated the air by applying a small layer of transparent nail polish on four sides of each coverslip.

Once the nail polish is dried, the roots are observed under a light microscope at magnifications x 100 and x 400. The estimation of the mycorrhization of the root system was carried out according to the method of Trouvelot (Dodd & al., 2001). The rate of endomycorrhizal colonisation is estimated according to an evaluation grid completed according to 2 scales:

-A 1st scale to assess the intensity of colonisation of the root cortex and comprising 5 classes rated from 0 to 5. Each class reflects the degree of colonisation intensity of the root cortex of each fragment observed;

-The 2nd scale allows the evaluation of the presence of arbuscules and vesicles. It is composed of 4 classes ranging from A0 to A3 indicating their frequency.

according to Mekahilia (2014), the estimated infection parameters are :

1. The frequency of mycorrhizal in the root system F (%): It expresses the percentage of the number of endomycorrhizal root fragments, which reflects the importance of the infection of the root system;
2. Intensity of the mycorrhizal colonisation in the root system M (%): It reflects the proportion of the estimated colonised cortex compared to the entire root system.
3. Intensity of the mycorrhizal colonisation m (%) in the root fragments.
4. Arbuscule abundance a (%) in mycorrhizal parts of root fragments.
5. The arbuscular abundance A (%) in the root system compared to the entire root system. It is the arbuscular proportion of the root system.

These parameters are calculated by entering the results of each grid into a computer program "Mycocalc" developed by Trouvelot (Dodd & al., 2001).

5. Statistical analysis

The Student's t test is used to compare the means between the two groups and the principal component analysis (PCA) was carried out to highlight the relationships between the different studied variables. In addition, a Pearson correlation test was performed to complete the analysis. STATISTICA software is used to treat the data of the mycorrhizal analysis and the soil analysis and the correlations between them .

Chapter II: Result and discussion

1.Climatic synthesis

1.1 Climatic factors

What determines whether there is a correction of the data or not is the geographic location of the monitoring station to the study area.

The correction calculations : Calculations of the difference in altitude

h Djelfa - h Station = … m

1180 - 792 = 388 m there for H = 388 m

Because the difference in altitude is greater than 100 m in this case we applied the climatic corrections, with the corrections we calculated the average monthly and annual precipitation of the study area in (mm) for the period (1990-2020) and the average monthly temperatures in C° of the study area (1990-2020)

1.1.1 precipitation

Table 01. Average monthly and annual precipitation of the study area in (mm) for the period (1990-2020). Source: Djelfa weather station (1990-2020)

Months	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	annual precipitation
P(mm)	24,7	18,8	21,8	24,2	23,3	11,6	6,9	16,1	25,2	19,8	17,7	18,1	228,2

1.1.2 temperature

According to the Djelfa station's monthly average temperatures, JANUARY is the coldest month with a temperature of (5,2°C). The month of JULY had the highest average temperature ever measured (26,9°C). The study area's station records JULY as having the highest monthly average temperature at an average of (29°C), and the lowest in the month of JANUARY (7,3°C) (Table 02).

Table 02. Average monthly temperatures in C° of the study area (1990-2020)(M: monthly maximum average; m: monthly minimum average; avg: monthly average).

Months	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
M (°C)	12,6	14,3	17,8	20,8	26,8	31,9	37,1	36	30	24,4	17,2	13,4
M (°C)	2,1	3	5,3	8	12,6	17,2	20,8	20,2	16,3	14,8	6,4	4
T Moy (°C)	7,3	8,6	11,5	14,4	19,7	24,5	29	28,1	23,15	19,6	11,8	8,7

Chapter II: Results and Discussion

1.2 Classification of climate

1.2.1 Gaussen and Bagnoul's ombro-thermal diagram

The dry and wet seasons of the year are represented graphically in an gaussen and bagnoul's ombro-thermal diagram. The months are plotted on the abscissa, and the ordinates indicate temperature (T) and rainfall (P), with P=2T, to identify the dry and wet seasons of the year (Haghighi and al.,2020).

The ombro-thermal diagram for the research region is shown in Figure 04 and is based on temperature and rainfall information. from 30-year-long calculations of the average monthly rainfall and temperature.

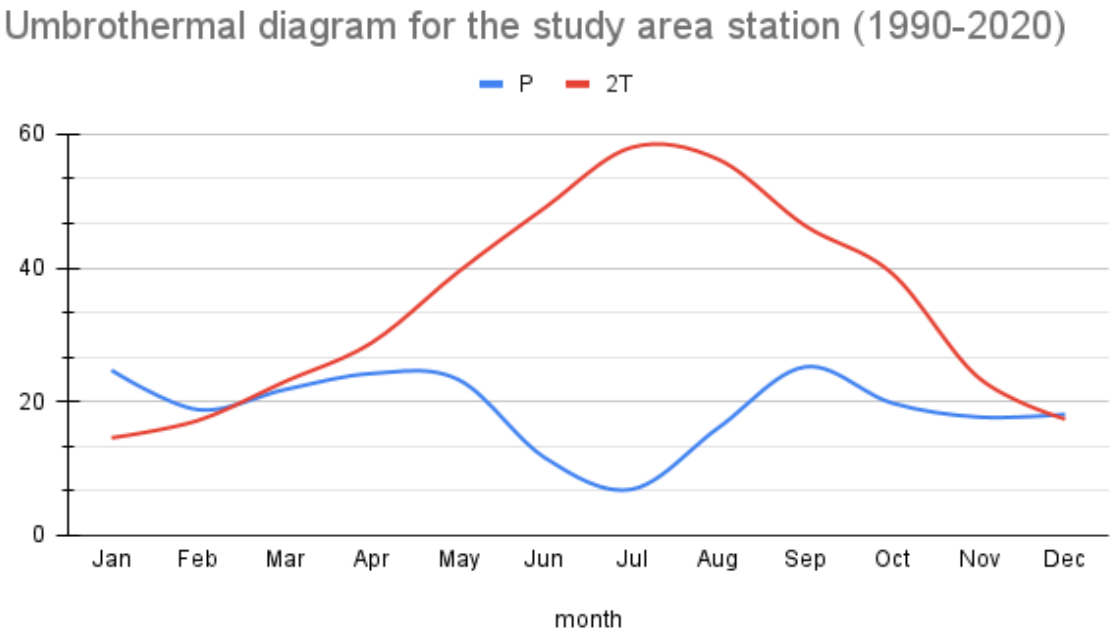

Figure 03. Ombro-thermal diagram for the study area station (1990-2020)

According to this diagram, the dry season extends over a period of 09 months during the year which is from february to december.

1.2.2 Climate Variability Analysis (Emberger Method)

A method designed by Emberger is widely used for climate classification in climatology, hydrology, ecology, and bioclimatic analysis. The method considers the two important climate parameters, precipitation and temperature

$$Q_3 = 3,43*P/(M-m)$$

- Q_3: Emberger's rainfall quotient.

- P: Average annual precipitation in mm (308,38 mm)

- M: Maximum average of the hottest month (37.1°C)

- m: Minimum average of the coldest month (2.1 °C)

After applying the formula, we obtain the value of Q_3 equal to 30.22.

The study area is located in the ARID stage (Winter variant: Cold) (Figure 05).

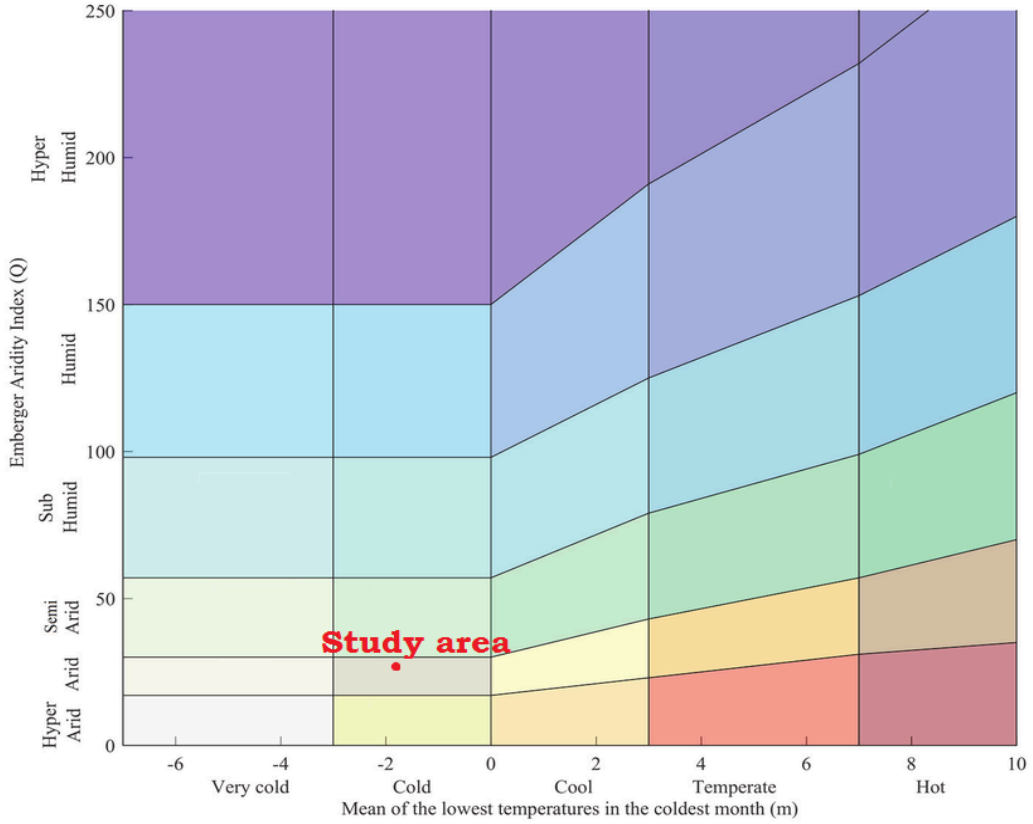

Figure 04. Emberger precipitation climatogram for the study area (1990-2020).

2. Soil analysis

2.1 Humidity

We can see that the highest humidity value is 20.30 %, which has been recorded in Tree 1, and the lowest humidity value is 0.64 % and it has been recorded in Tree 7.

Also we can see that for 9/10 from the trees, the humidity values are below 10%.

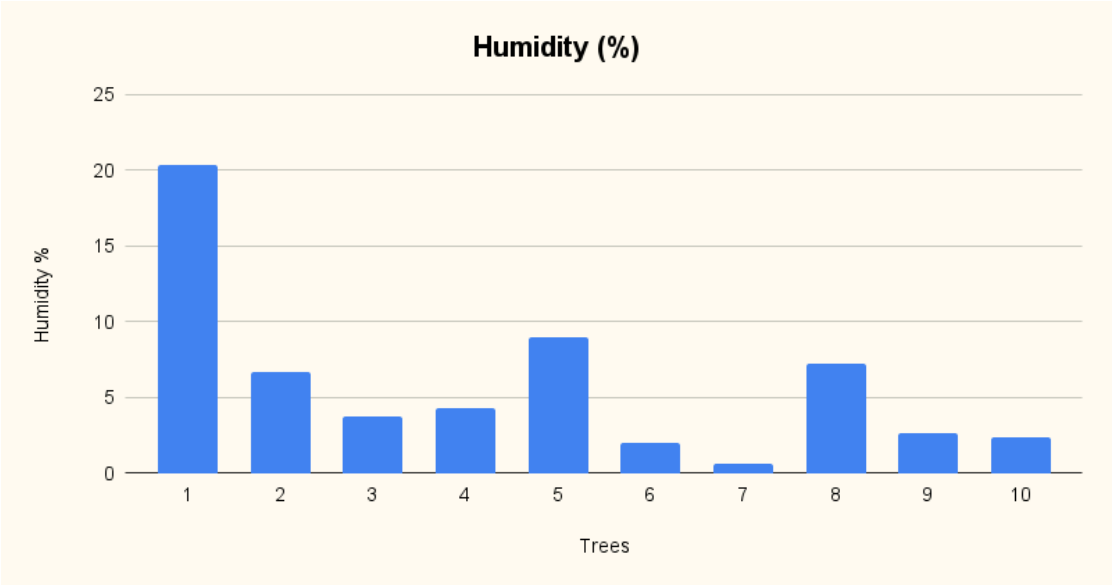

Figure 05. Percentage of Humidity per tree.

2.2 Conductivity

The highest conductivity value is 1272 µs/cm, which has been recorded in Tree 2, and the lowest conductivity value is 1024.666667 µs/cm recorded in Tree 3.

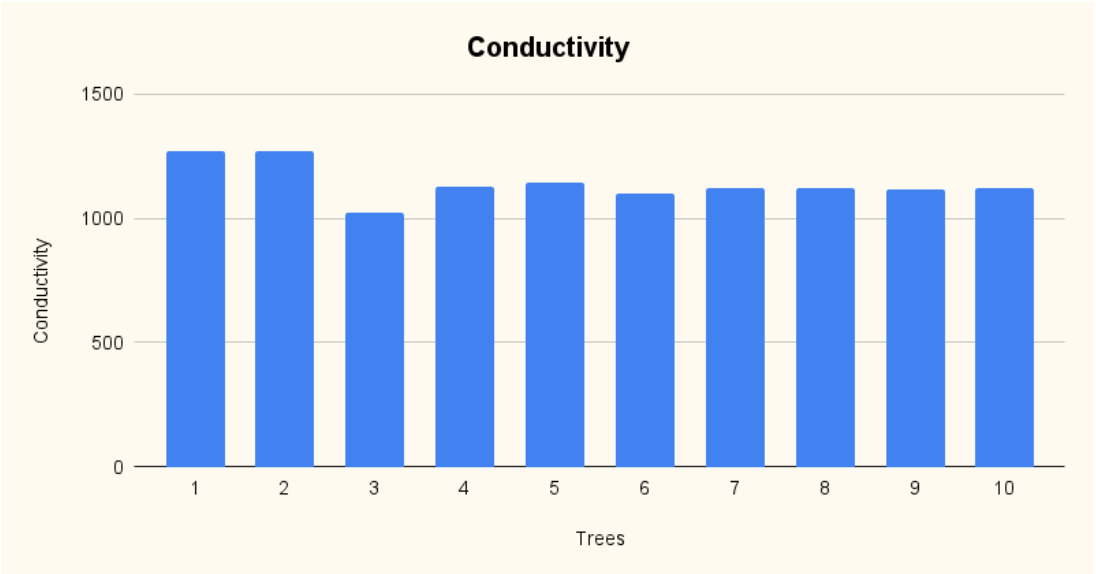

Figure 06. Conductivity per tree.

2.3 The pH

The highest pH value is 7.74, recorded in Tree 9, and the lowest is 6.79 recorded in Tree 1. Also for 7/10 from the trees, the pH values are more than 7.

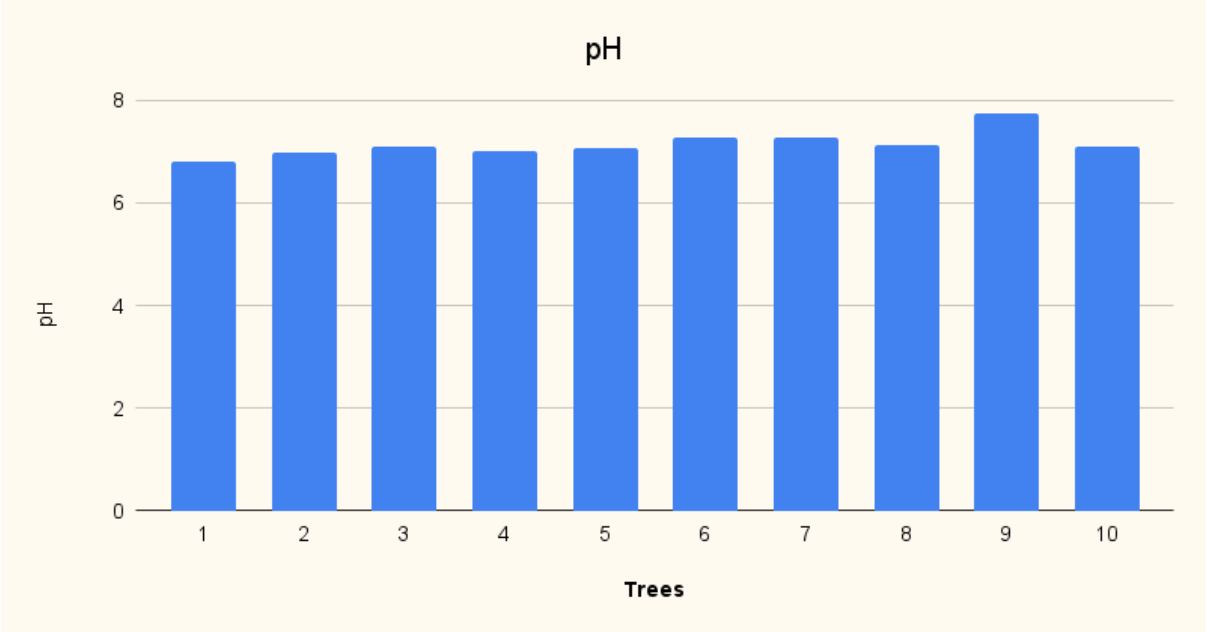

Figure 07. pH per tree.

2.4 Total limestone

The highest total limestone value is 42.10 %, recorded in Tree 4, and the lowest is 10.85 % recorded in Tree 9.

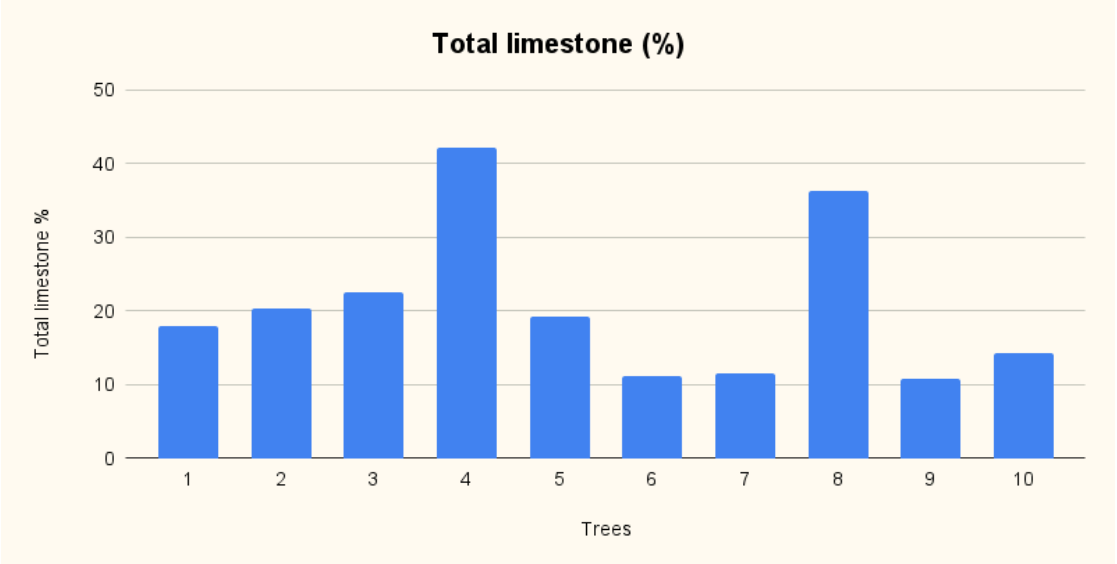

Figure 08. Total limestone per tree.

2.5 Active limestone

The highest active limestone value is 21.75, recorded in Tree 1, and the lowest is 13.62 recorded in Tree 9.

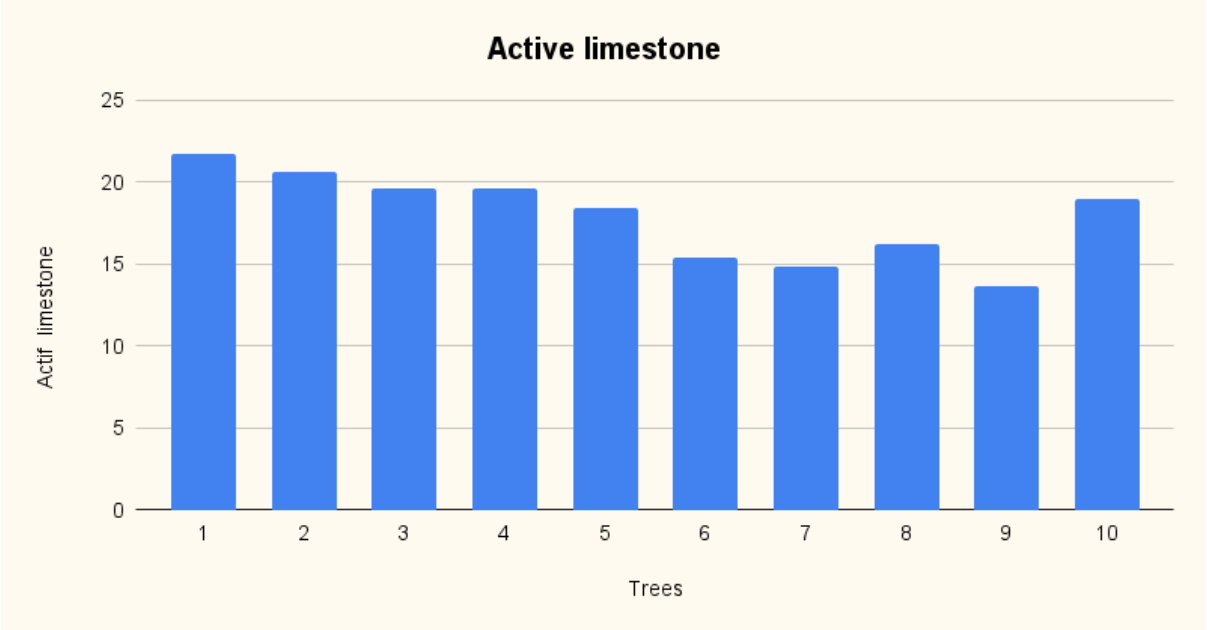

Figure 09. Active limestone per tree.

2.6 Organic material

The highest organic material value is 19.53 %, recorded in Tree1, and the lowest is 2.06 % recorded in Tree 7.

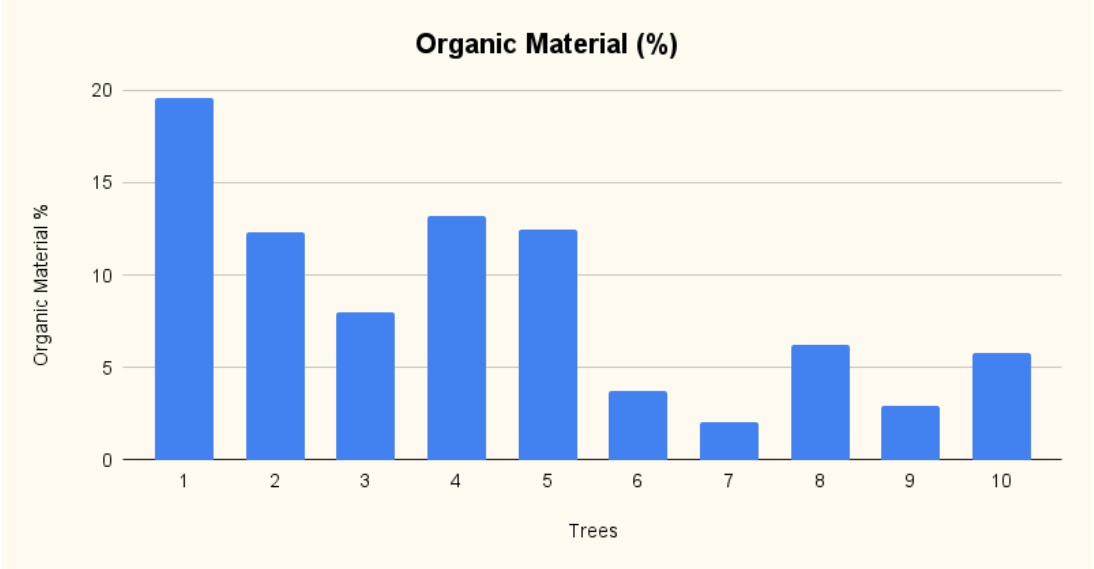

Figure 10. Organic material per tree.

Chapter II: Results and Discussion

2.7 Granulometry

The soil of the young trees site is sandy loam , and the soil of the old trees site is silt loam.

Figure 11. Granulometry per age group.

2.8 Comparison of soil parameters between young and adult trees

Looking at the figure below we noticed that the highest average humidity value was in adult trees, the lowest values are recorded for the young trees, almost half of the value in comparison to the adult trees.

The conductivity shows that the higher average value was recorded in adult trees.

Concerning the average pH value, shows that the highest average pH recorded in the young trees in comparison to the adult trees, but the values are almost equal with a difference of 0.3.

For total limestone, the lowest average value is in young trees, and the adult trees have the highest average.

The highest value of active limestone was in adult trees in comparison to the young trees.

For organic material, the highest value was in adult trees in comparison to the young trees, with a great difference between the two ages.

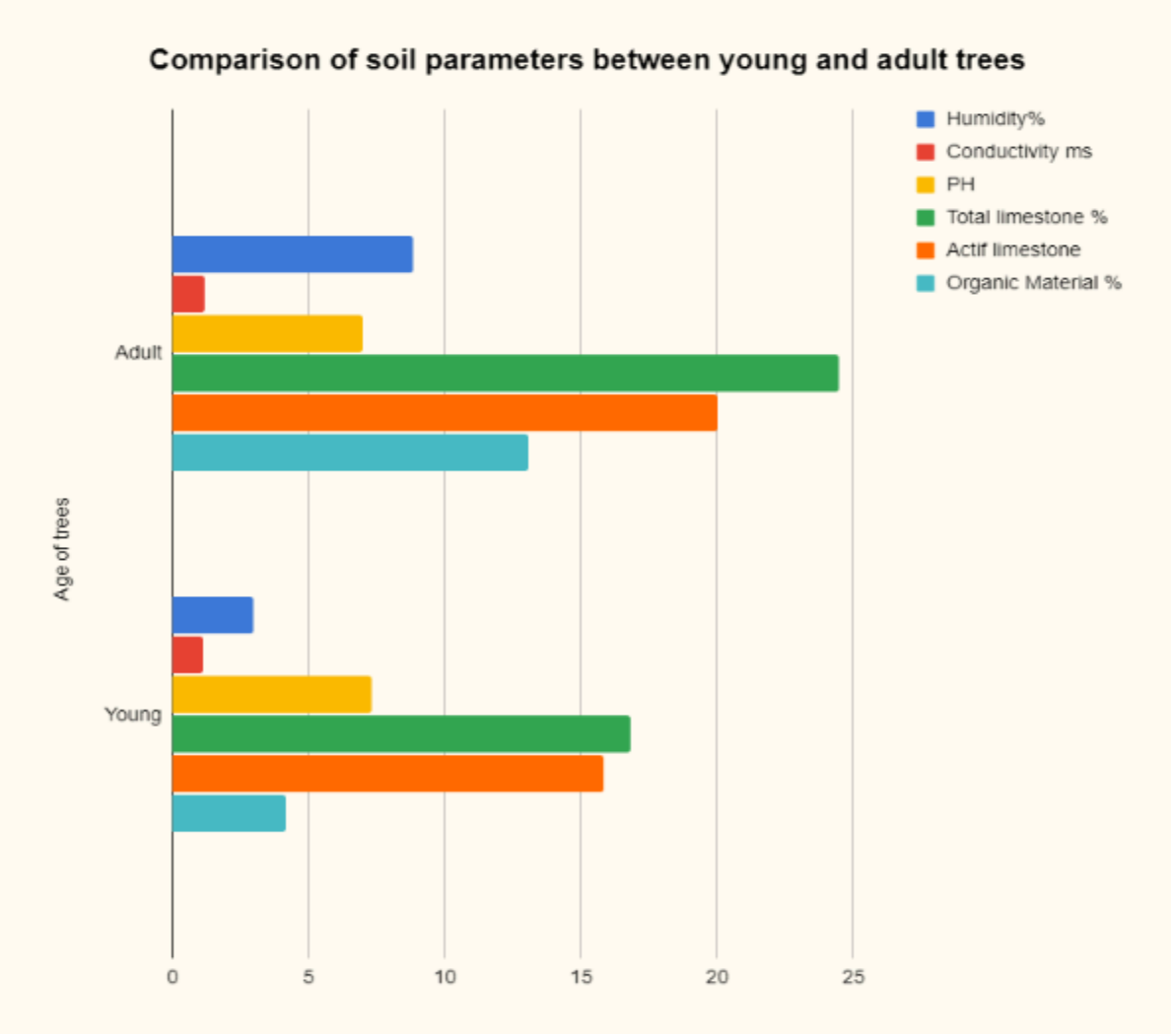

Figure 12. Comparison of soil parameters between young and adult trees.

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2.9 Comparison of soil parameters (average values)

Humidity is 5.88 % on average.

The soil is neutral (average pH 7.14).

Electrical conductivity is 1.14 mS/cm on average. Therefore the soil is non-saline.

Total limestone averaged 20.63 %, making the soil moderately calcareous.

The soil has a high active limestone content (the average content is 17.91%).

Organic material averaged 8.616 %, a soil that is very low in organic material.

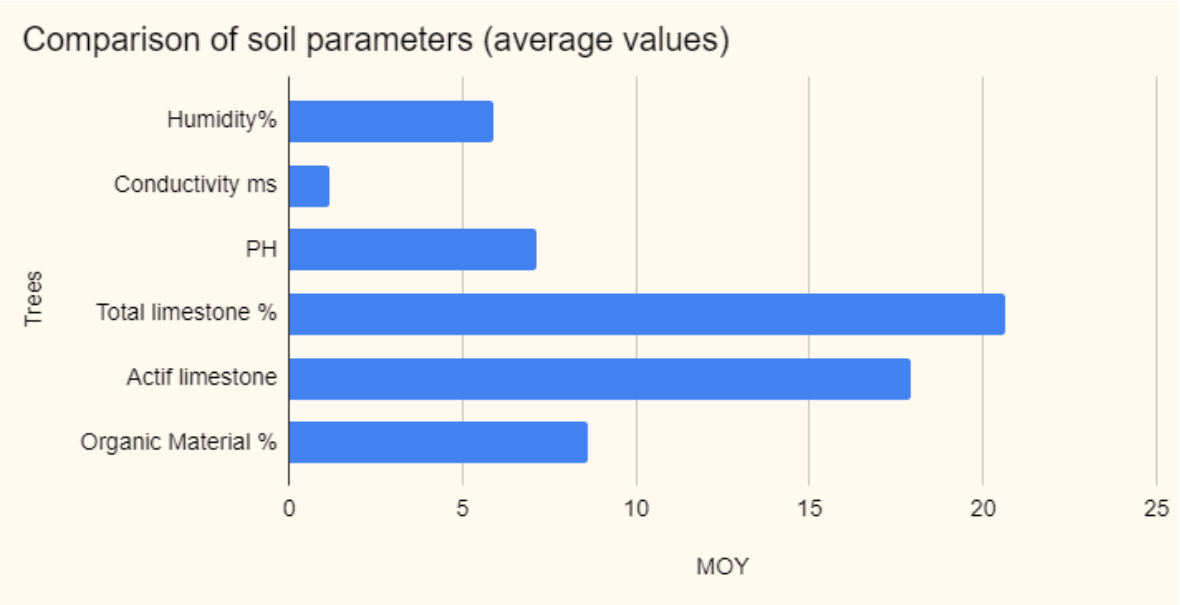

Figure 13. Comparison of soil parameters (average values).

Table 03. Soil analysis parameters (averages).

Average	Humidity (%)	pH	Conductivity (µš)	Organic material (%)	Total limestone (%)	Active limestone (%)
Adult	8.80	6.98	1167.31	13.08	24.43	20
Young	2.97	7.30	1115.32	4.14	16.82	15.82
Total	5.88	7.14	1141.32	8.61	20.63	17.91

3 Mycorrhization parameters

3.1 The typical structures

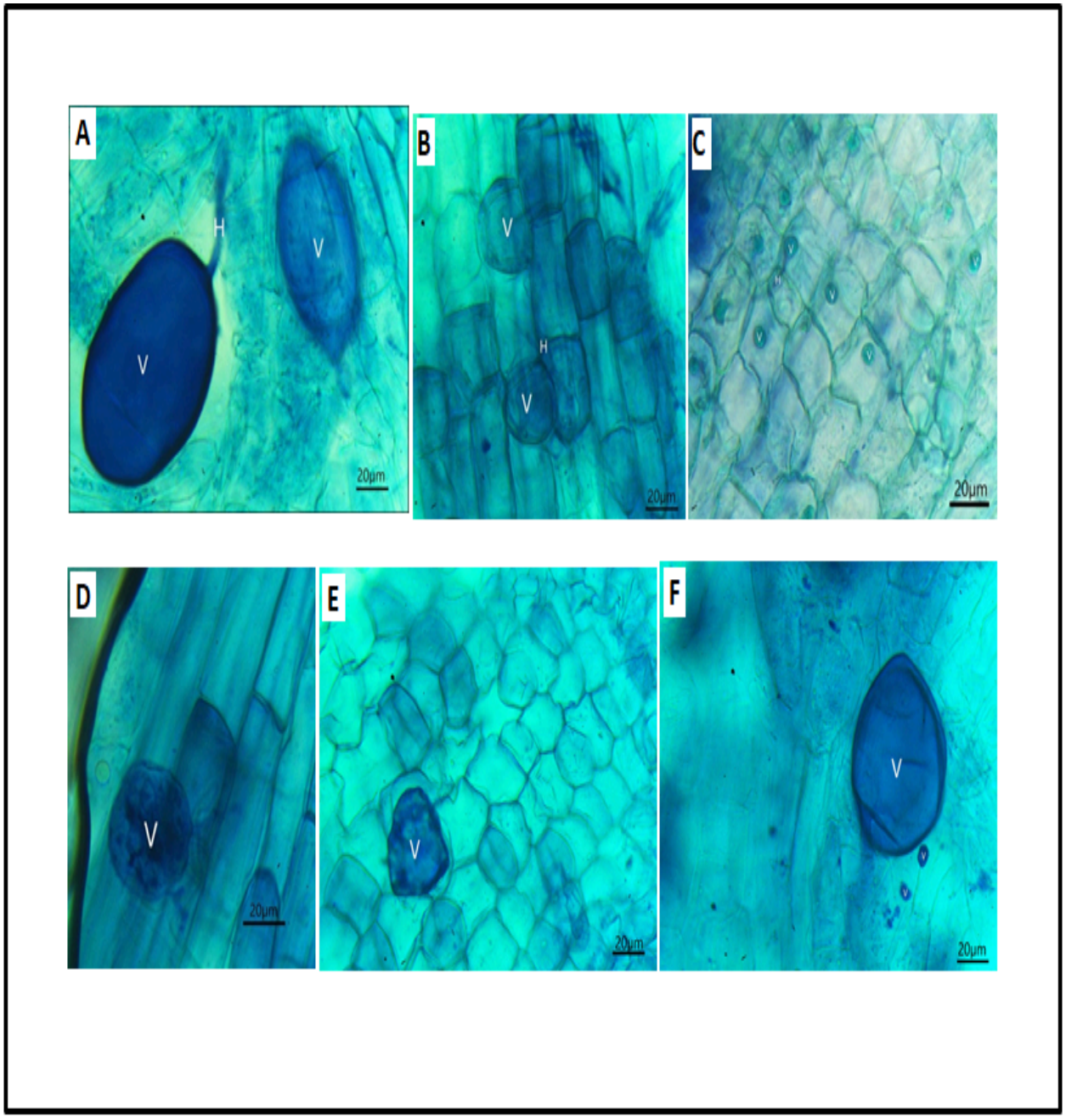

Figure 14. Typical structures of vesicle mycorrhizal fungi colonising the roots of olive trees. (A and F : Big vesicles. B, D and E : Medium vesicles. C : Small vesicles. V: vesicle. H: Hyphae). (Original photos, 2023).

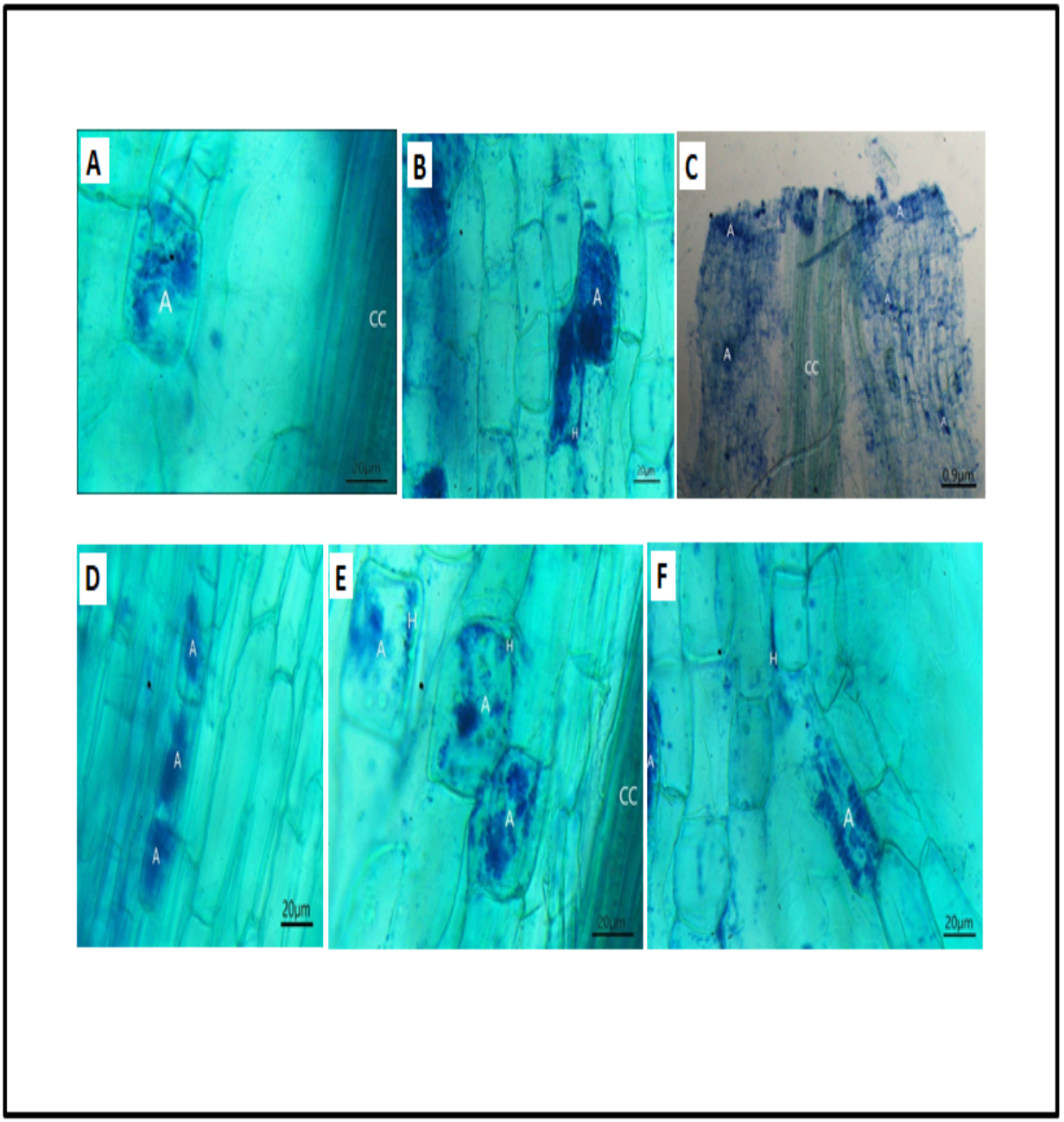

Figure 15. Typical structures of arbuscular mycorrhizal fungi colonising the roots of olive trees. (A: Arbuscule. H: Hyphae. CC: Central cylinder). (Original photos, 2023).

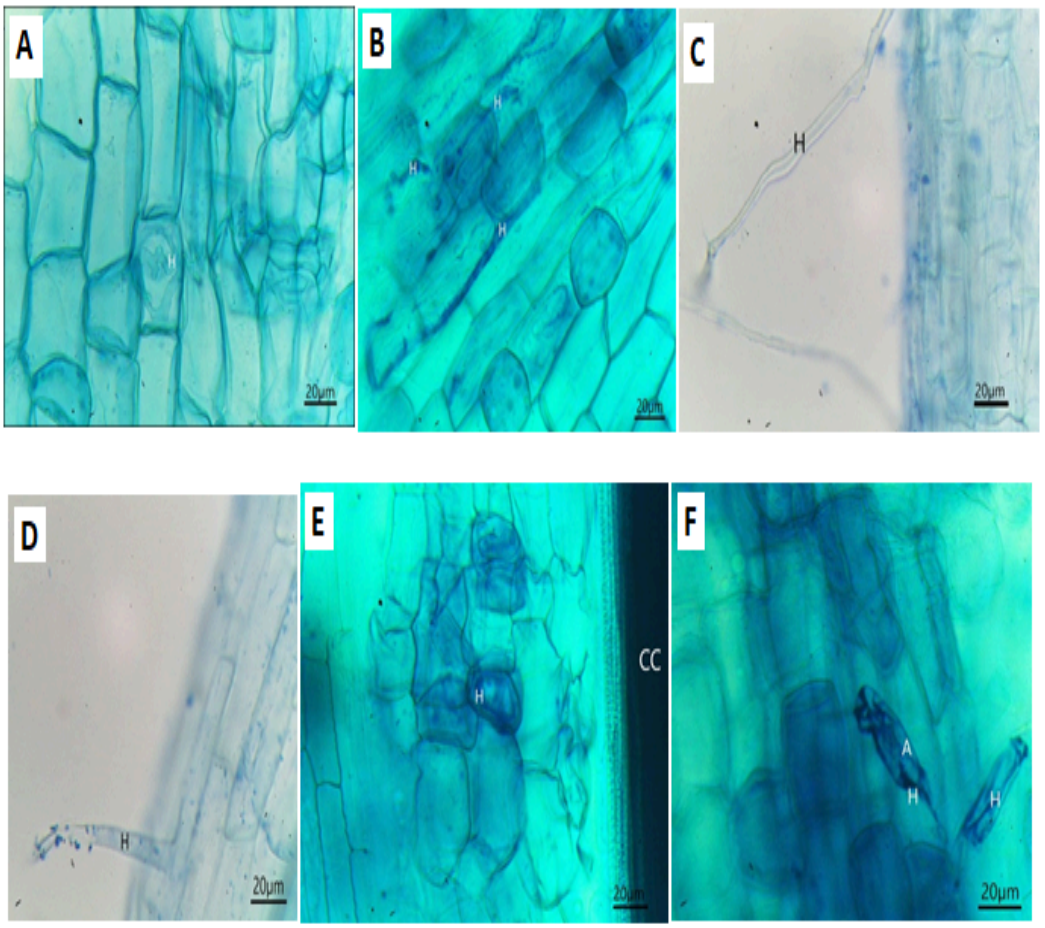

Figure 16. Typical structures of hyphae mycorrhizal fungi colonising the roots of olive trees. (A: Arbuscule. H: Hyphae. CC: Central cylinder). (Original photos, 2023).

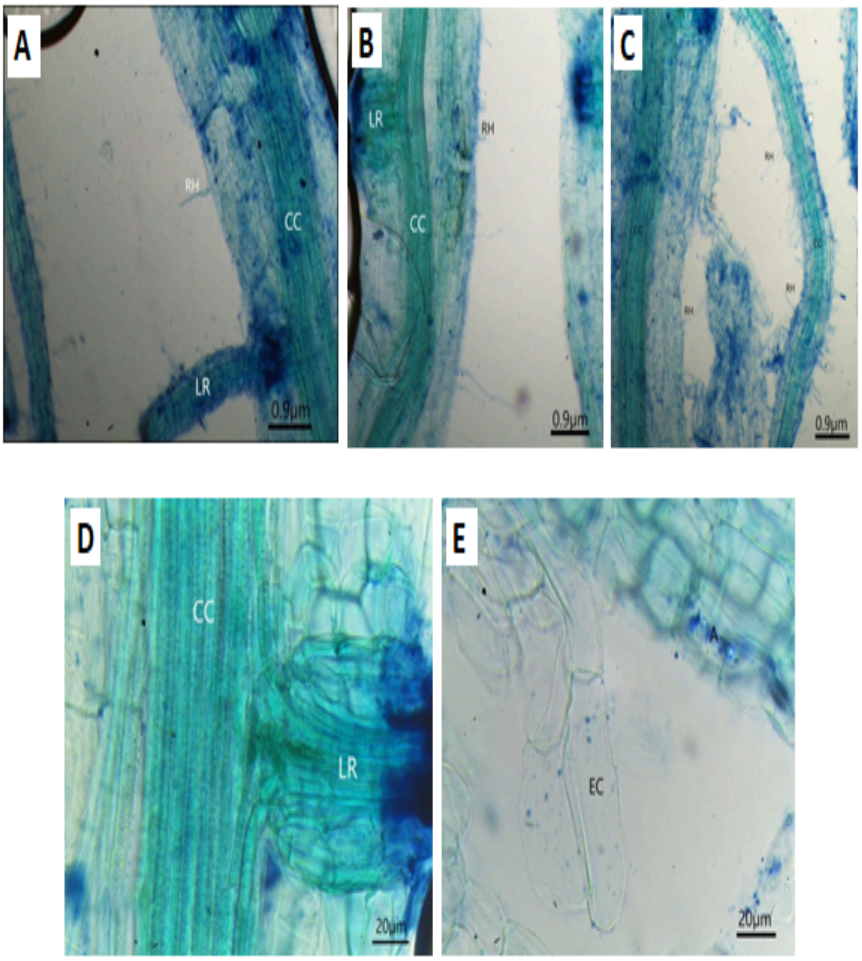

Figure 17. Typical root hair and typical lateral ramification of roots (A: Arbuscule. RH: Root hair. CC: Central cylinder. LR: Lateral ramification. EC: Empty Cell). (Original photos, 2023).

3.2 Mycorrhiza rate

In table 04, we noticed that the frequency of mycorrhizal in the root system (F%) was very high 100 %, followed by intensity of the mycorrhizal colonisation in the root system (M%) 16,886 % which is equal to intensity of the mycorrhizal colonisation in the root fragments (m%): 16,88 %, and arbuscule abundance in mycorrhizal parts of root fragments (a%): 38,514%. Finally, The arbuscular abundance in the root system (A%) reached 7,445% .

Table 04. The averages of the parameters calculated for each age group

parameter	Age of tree	average %	total average %
F%	Adult	100	100
	Young	100	
M%	Adult	22,57	16,88
	Young	11,19	
m%	Adult	22,57	16,88
	Young	11,19	
a%	Adult	46,03	38,51
	Young	30,99	
A%	Adult	11,55	7,44
	Young	3,33	

3.2.1 Mycorrhizal frequency in the root system (F%)

Both adult and young trees recorded the highest percentage of the frequency of mycorrhizal in the root system F (%) with an average of (100 %).

3.2.2 Intensity of the mycorrhizal colonisation in the root system (M%)

The Intensity of the mycorrhizal colonisation in the root system M (%) is higher in adult trees (22.57%) than the young trees (11.19%).

3.2.3 Intensity of the mycorrhizal colonisation in the root fragments (m %)

Same as the (M%), the intensity of the mycorrhizal colonisation in the root fragments m (%) is higher in adult trees (22.57%) than the young trees (11.19%).

3.2.4 Arbuscular abundance in mycorrhizal parts of root fragments (a %)

The arbuscule abundance in the mycorrhizal parts of root fragments a (%) is higher in adult trees (46,03%) then the young trees (30,99%).

3.2.5 Arbuscular abundance in the root system (A%)

The arbuscular abundance in the root system (A%) is higher in adult trees (11,55%) than the young trees (3,33%).

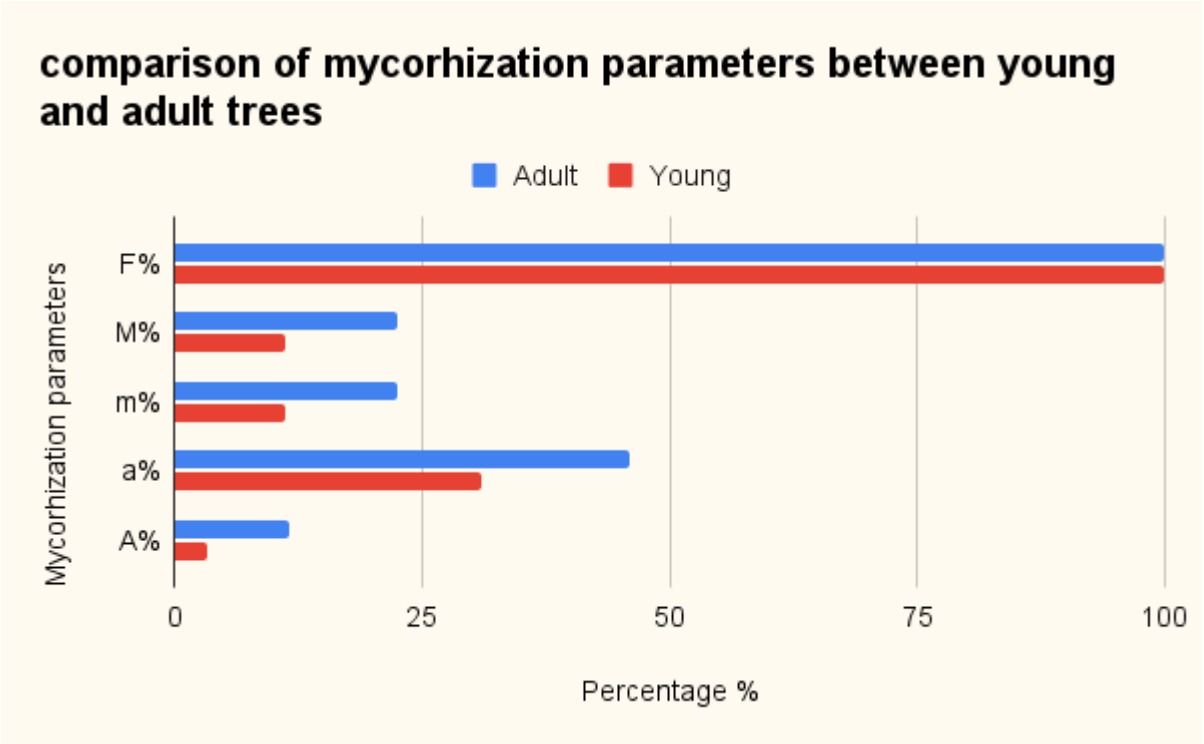

Figure 18. Comparison of mycorrhization parameters between young and adult trees.

Chapter II: Results and Discussion

4. Statistical analysis

4.1. Student Test

Table 05. Student test

	Mean AT	Mean YT	t-value	df	p	Valid number AT	Valid number YT	Std.Dev. AT	Std.Dev. YT	F-ratio	p
F%	**99.980**	**100.000**	**-1.00000**	**8**	**0.346593**	**5**	**5**	**0.0447**	**0.00000**	**0.0000**	**1.000000**
M%	**22.578**	**11.194**	**2.12645**	**8**	**0.066158**	**5**	**5**	**11.2165**	**4.18219**	**7.1930**	**0.082112**
m%	**22.578**	**11.194**	**2.12645**	**8**	**0.066158**	**5**	**5**	**11.2165**	**4.18219**	**7.1930**	**0.082112**
a%	**46.030**	**30.998**	**1.68684**	**8**	**0.130117**	**5**	**5**	**17.4097**	**9.69340**	**3.2258**	**0.282994**
A%	**11.556**	**3.334**	**2.03154**	**8**	**0.076674**	**5**	**5**	**8.9752**	**1.15950**	**59.9167**	**0.001599**
H	**8.801**	**2.978**	**1.80578**	**8**	**0.108589**	**5**	**5**	**6.7648**	**2.49526**	**7.3499**	**0.079186**
pH	**6.984**	**7.303**	**-2.51563**	**8**	**0.036053**	**5**	**5**	**0.1158**	**0.25848**	**4.9791**	**0.149123**
EC	**1167.311**	**1115.329**	**1.10627**	**8**	**0.300764**	**5**	**5**	**104.5967**	**9.95681**	**110.3559**	**0.000481**
TL	**24.434**	**16.827**	**1.14362**	**8**	**0.285844**	**5**	**5**	**10.0217**	**10.98979**	**1.2025**	**0.862460**
AL	**20.000**	**15.825**	**3.92914**	**8**	**0.004362**	**5**	**5**	**1.2624**	**2.01285**	**2.5422**	**0.388204**
OM	**13.086**	**4.146**	**4.42170**	**8**	**0.002221**	**5**	**5**	**4.1495**	**1.79478**	**5.3452**	**0.133368**

- F% : Mycorrhizal frequency in the root system.
- M% : Intensity of the mycorrhizal colonisation in the root system.
- m% : Intensity of the mycorrhizal colonisation in the root fragments.
- a% : Arbuscular abundance in mycorrhizal parts of root fragments.
- A% : Arbuscular abundance in the root system.
- H : Humidity.
- EC : Conductivity.
- TL : Total Limestone.
- AL : Active Limestone.
- OM : Organic material.
- AT : Adult trees group.
- YT : Young trees group.
- Std.Dev : Standard deviation.

4.2. Multivariate Analysis

A principal component analysis (PCA) was carried out to highlight the relationships between the different studied variables. In addition, a Pearson correlation test was performed to complete the analysis.The correlation matrix shows correlations between

-Soil parameters, with one negative and two positive correlations between Organic Matter and the four parameters Active limestone (AL) and pH and Electrical conductivity (EC) and Moisture (H) (r = 0.86 and r = -0.79 and r = 0.66 and r = 0.85 respectively), and between Moisture (H) and the two pH and Electrical conductivity (EC) parameters (r = -0.62 and r = 0.68 respectively).

-Mycorrhizae parameters between them; with two positive correlations. The first correlations between Arbuscular abundance in mycorrhizal parts of root fragments (a%) and the two parameters Intensity of the mycorrhizal colonisation in the root system (M%) and Intensity of the mycorrhizal colonisation in the root fragments (m%) (r =0.68 and r = 0.68 respectively). And the second correlations between Arbuscular abundance in the root system (A%) and the parameters Intensity of the mycorrhizal colonisation in the root system (M%), Intensity of the mycorrhizal colonisation in the root fragments (m%) and Arbuscular abundance in mycorrhizal parts of root fragments (a%) (r = 0.94 and r = 0.94 and r = 0.85 respectively)

-Mycorrhization and soil parameters show negative and significant correlations between Mycorrhizal frequency in the root system (F%) and the two parameters Moisture (H) and Organic Matter (OM) (r = -0.81 and r = -0.69, respectively) (Table 06).

Table 06. Pearson correlation matrix (p <0.05) for all mycorrhization and soil variables.

	F%	M%	m%	a%	A%	H	pH	EC	TL	AL	OM
F%	1.00	0.17	0.17	0.43	0.24	-0.89	0.49	-0.60	0.09	-0.50	-0.69
M%		1.00	1.00	0.68	0.94	-0.14	-0.32	-0.20	0.33	0.50	0.23
m%			1.00	0.68	0.94	-0.14	-0.32	-0.20	0.33	0.50	0.23
a%				1.00	0.85	-0.27	-0.04	-0.32	0.15	0.33	0.07
A%					1.00	-0.17	-0.23	-0.31	0.27	0.45	0.15
H						1.00	-0.62	0.68	0.14	0.62	0.85
pH							1.00	-0.47	-0.43	-0.88	-0.79
EC								1.00	-0.03	0.50	0.66
TL									1.00	0.34	0.38
AL										1.00	0.86
OM											1.00

The correlation circle shows that the majority of the variables are correlated with the two axes, with these two axes explaining 79.17 % of the total inertia. For axis 2, the soil variables (H, MO and EC) and mycorrhizae (M%, m% , a% A%) are positively correlated, while the two variables (F% and pH) are negatively correlated.

On axis 1, all mycorrhiza parameters with pH and TL are positively correlated and the three soil parameters AL, OM, H and EC are negatively correlated (Figure 20).

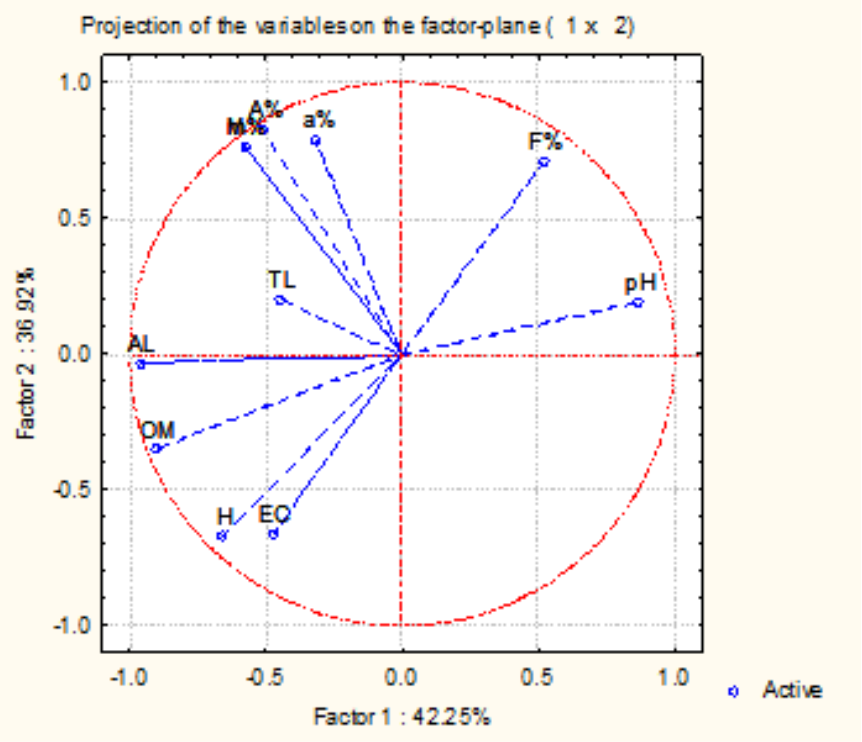

Figure 19. Correlation circle for variables; F: frequency of mycorrhization, M: intensity of colonisation of the root cortex,m: Intensity of mycorrhizal colonisation in root fragments, A: arbuscular content,a: Abundance of arbuscules in the mycorrhizal parts of root fragments, H: humidity, pH, EC: electrical conductivity, TL: total limestone, AL: active limestone, MO: organic material.

5. Discussion

The rates of the different mycorrhizal parameters recorded during this study vary in the two age groups, except the frequency of mycorrhizal in the root system F (%) where it reaches the maximum rate (100%) in both groups. Considering the time of the samples collection at the end of spring our results are close to those obtained by Bouadelli & al (2018) in the stations Sidi- Naaman (Temperate sub-humid), Guetia (Cold semi-arid),Ain Ouassara (Cool arid), Messaad (Cool arid). The rate of F% could differ depending on the climatic stage and the ligation of the study area like the results of Benzergua & Mammeri (2022) reached (85%) in Ghayaza (Cool arid). Also in the study of Kachkouch & al. (2012) the F% is (96%) in Khnichat, Morocco (sub humid). Considering the Intensity of the mycorrhizal colonisation in the root system M (%) we recorded (16,88%) which is lower than rates that recorded in the studies of Bouadelli & al (2018), Benzergua & Mammeri (2022) and Kachkouch & al. (2012). We recorded the same rate of m% as the M% which is also considered lower than the results recorded by Benzergua & Mammeri (2022).

For the a%, we recorded (38,51%) and it's also lower than the previous study.

As for the A%, we recorded (7,44%) and that is close to the result of Bouadelli & al. (2018) in the same area as our study (Messaad), and lower than results recorded by Benzergua & Mammeri (2022).

Considering the soil analysis, the humidity is 5.88 % on average which is considered favourable for the mycorrhization symbiosis, and the pH 7.14 which it accedes the preferable interval for the mycorrhizae (between 4 and 5).

Organic material (8.61%) is favourable at any rate. The conductivity shows that the soil is non salin (1.14 mS/cm), that could be because of the presence of mycorrhizae in this soil. Total limestone averaged 20.63%, making the soil moderately calcareous and that is accurate because our study area is steppic.

Conclusion

In conclusion, after studying the root fungal symbiosis in the olive tree *Olea europaea* on two different age groups in the steppe, mycorrhizal fungi are observed in both age groups. Identical structures were detected in the treated root fragments through the use of optical microscopy. Our study showed that the frequency (F%) of fungal infection was same between the two age groups. Otherwise, the mycorrhizal colonisation in the root system (M%) and in the root fragments (m%) was different, same observation about the arbuscule abundance in mycorrhizal parts of root system (A%) and in the root fragments (a%). This variation could be due to the age of the tree. All mycorrhization parameters of adult trees are almost twice as high compared to young plants.

At the end, it becomes evident that there is potential for further exploration of this aspect. Certainly the importance of transferring these resilient species rather than relying on synthetic chemicals and nutrients is crucial.

Algeria has strongly encouraged the development of olive growing throughout the national territory. However, the presence of mycorrhizal symbiosis on the olive tree and particularly in the steppe is essential because it is an indicator of resilience to the climatic and edaphic conditions of this zone. Better yet, the older the plant gets, the more mycorrhiza it has in its root system. Hence the interest of this study which should contribute to helping the research community and farmers in this region.

References

Benzergua A, Mammeri S, 2022. Contribution à l'estimation du taux de mycorhizes de l'olivier en steppe (zone de Sguiâa). Commune de Bouirat Lahdab- Wilaya de Djelfa. Master thesis . univ Djelfa .

Bouabdelli Z, Belhadj S, Smail Saadoun N, Philippe J, Notonnier R, Tonetto A, Ortas I, Gauquelin T, 2018. Influence de l'aridité sur la variation de la colonisation mycorhizienne arbusculaire chez cinq populations naturelles algériennes du pistachier de l'atlas (Pistacia atlantica Desf.) . *Revue d'Ecologie (Terre et Vie)*, Vol. 73 (3), 2018 : 330-344. https://hal.science/hal-03533275/document

Bouabdelli Z, 2019. Etude des mycorhizes des especes du genre pistacia, en fonction des conditions edapho-climatiques en algerie. Doctoral thesis . univ Djelfa P 08:10. https://www.researchgate.net/profile/Bouabdelli-Zahra/publication/334031318_ETUDE_DES_MYCORHIZES_DES_ESPECES_DU_GENRE_PISTACIA_EN_FONCTION_DES_CONDITIONS_EDAPHO-CLIMATIQUES_EN_ALGERIE/links/5d1b926e92851cf440600165/ETUDE-DES-MYCORHIZES-DES-ESPECES-DU-GENRE-PISTACIA-EN-FONCTION-DES-CONDITIONS-EDAPHO-CLIMATIQUES-EN-ALGERIE.pdf

Brundrett M, 2004. Diversity and classification of mycorrhizal associations. *Biological reviews of the Cambridge Philosophical Society* vol 79. p 473–495. https://sci-hub.se/10.1017/S1464793103006316

Carteron A, 2020. La dominance mycorhizienne en tant que facteur local déterminant des processus écologiques forestiers. Doctoral thesis. Univ Montréal. https://orcid.org/0000-0003-4162-9254

Châtaigner JM and Duponnois R, 2017. Les microorganismes du sol : des outils biologiques pour satisfaire les objectifs du développement durable (odd).P95. https://www.cairn.info/revue-realites-industrielles-2017-1-page-94.htm

Dib S , Nouari S , Benguesmia D, 2022. Extraction of urban areas using spectral indices combination and google earth engine in Algerian highlands (Case Study: Cities of Djelfa, Messaad, Ain Oussera). AGEO. vol. 45, 1-17p

References

https://www.redalyc.org/journal/6953/695375056030/695375056030.pdf

Dodd JC,Clapp JP and Zhao B, 2001. Arbuscular mycorrhizal fungi in plant production systems: detection, taxonomy, conservation and ecophysiology (Workshop).Mycorrhiza Manual, Huazhong Agricultural University, Wuhan, P.R. China.
https://www2.dijon.inrae.fr/mychintec/Protocole/Workshop_Procedures.html#ANchor-47857

Haghighi AT, Abou Zaki N, Rossi PM, Noori R, Hemmatzadeh AA, Saremi H and Kløve B, 2020. Unsustainability syndrome from meteorological to agricultural drought in arid and semi-arid regions. Water Vol.12 (3),1-27p.

Kachkouch W, Ouazzani Touhami A, Filali-Maltouf A, El Modafar C, Moukhli A, Oukabli A, Benkirane R, Douira A, 2012. Arbuscular mycorrhizal fungi species associated with rhizosphere of *Olea europaea L.* in Morocco. *Journal of Animal & Plant Sciences* .Vol.15 (3).
https://www.m.elewa.org/JAPS/2012/15.3/6.pdf

Meddad HA, Beddiar A, Gollotte A, Lemoine MC, Kuszala C and Gianinazzi S, 2010. Arbuscular mycorrhizal fungi improve the growth of olive trees and their resistance to transplantation stress. African Journal of Biotechnology . Vol. 9(8), P: 1159.
https://hal.inrae.fr/hal-02666039

Mekahilia M N, 2014. Dépendance mycorhizienne de l'olivier (olea europaea L.) dans la l'est algérien et mycorhization contrôlée de la variété Ferkeni. Doctoral thesis .univ Badji Mokhtar Annaba . p 43. http://www.secheresse.info/spip.php?article48176
https://biblio.univ-annaba.dz/wp-content/uploads/2015/11/these-Mekahlia-Mohamed-Nacer-.pdf

Mezghrani K, 2015. Essai de mycorhization des plants d'olivier a la pepiniere « SAFA ZACCAR » de HADJOUT. magister thesis.
https://di.univ-blida.dz/xmlui/bitstream/handle/123456789/4993/32-630-290-.pdf?sequence=1&isAllowed=y

References

Phillips JM & Hayman DS, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55(1), 158—IN18. https://www.sciencedirect.com/sdfe/pdf/download/eid/1-s2.0-S0007153670801103/first-page-pdf

Poveda J and Baptista P, 2021. Filamentous fungi as biocontrol agents in olive (*Olea europaea L.*) diseases: Mycorrhizal and endophytic fungi., Crop Protection 146. https://doi.org/10.1016/j.cropro.2021.105672

Annexes

Annex 01. Table for calculating the soil humidity

Tree N°	empty weighing dishes	humid soil	dry soil	Mw : water mass	Ms : soil particles mass	Mw/ Ms	Mw/ Ms %
1	1,5626	20,0189	16,9036	3,1153	15,341	0,203070204	20,3070204
2	1,5595	20,0113	18,852	1,1593	17,2925	0,06704062455	6,704062455
3	1,5242	20,0095	19,3455	0,664	17,8213	0,03725878583	3,725878583
4	1,5485	20,0007	19,244	0,7567	17,6955	0,0427622842	4,27622842
5	1,5348	20,012	18,488	1,524	16,9532	0,08989453319	8,989453319
6	1,4881	20,0025	19,6383	0,3642	18,1502	0,02006589459	2,006589459
7	1,4939	20,0086	19,8909	0,1177	18,397	0,006397782247	0,6397782247
8	1,4876	20,0067	18,7593	1,2474	17,2717	0,07222219006	7,222219006
9	1,5093	20,0159	19,5371	0,4788	18,0278	0,02655898113	2,655898113
10	1,5516	20,012	19,5857	0,4263	18,0341	0,02363855141	2,363855141

Annexes

Annex 02. Table of the soil pH.

Trees N°	**pH**
1	6.793333333
2	6.983333333
3	7.093333333
4	6.993333333
5	7.056666667
6	7.283333333
7	7.256666667
8	7.123333333
9	7.743333333
10	7.106666667

Annex 03. Table of the soil conductivity.

Trees N°	**Conductivity µš**
1	1269
2	1272
3	1024.666667
4	1129
5	1141.888889
6	1098.518519
7	1123.135802
8	1121.18107
9	1114.278464
10	1119.531779

Annexes

Annex 04. Table for calculating the percentage of total limestone in the soil.

Trees N°	V0	V1	V1*0,2	/29,5	*100
1	4	26,5	5,3	0,1796610169	17,96610169
2	4	30	6	0,2033898305	20,33898305
3	4	33,3	6,66	0,2257627119	22,57627119
4	4	62,1	12,42	0,4210169492	42,10169492
5	4	28,3	5,66	0,1918644068	19,18644068
6	4	16,5	3,3	0,1118644068	11,18644068
7	4	17	3,4	0,1152542373	11,52542373
8	4	53,6	10,72	0,3633898305	36,33898305
9	4	16	3,2	0,1084745763	10,84745763
10	4	21	4,2	0,1423728814	14,23728814

Annex 05. Calculation table for % active limestone in soil.

Trees N°	V0	V1	V1-V0	N-n(Vt-Ve)	*1,25
1	13	13,6	0,6	17,4	21,75
2	13,6	15,1	1,5	16,5	20,625
3	15,1	17,4	2,3	15,7	19,625
4	17,4	19,7	2,3	15,7	19,625
5	19,7	23	3,3	14,7	18,375
6	23	28,7	5,7	12,3	15,375
7	28,7	34,8	6,1	11,9	14,875
8	34,8	39,8	5	13	16,25
9	38,8	45,9	7,1	10,9	13,625
10	45,9	48,7	2,8	15,2	19

Annexes

Annex 06. Table for calculating the percentage of organic matter in soil.

Trees N°	Pv	Pe	Pi=Pe+Pv	Pf	Pi-Pf	*100
1	26,2321	3,0286	29,2607	29,0654	0,1953	19,53
2	28,4748	3,0333	31,5081	31,3848	0,1233	12,33
3	18,8215	3,0211	21,8426	21,7631	0,0795	7,95
4	26,745	3,0127	29,7577	29,6261	0,1316	13,16
5	26,148	3,016	29,164	29,0394	0,1246	12,46
6	28,7186	3,0101	31,7287	31,6914	0,0373	3,73
7	28,619	3,0246	31,6436	31,623	0,0206	2,06
8	1,4887	3,0157	4,5044	4,4423	0,0621	6,21
9	1,48	3,0141	4,4941	4,4646	0,0295	2,95
10	1,495	3,02	4,515	4,4572	0,0578	5,78

Annex 07. Calculation table of mycorrhizae rate in olive tree roots.

Tree N°	F%	M%	m%	a%	A%
1	100	11,93	11,93	19,58	2,34
2	100	26,27	26,27	49,45	12,99
3	100	37,5	37,5	68,13	25,55
4	100	26,4	26,4	44,02	11,62
5	100	10,79	10,79	48,97	5,28
6	100	18,23	18,23	20,49	3,74
7	100	8,93	8,93	27,92	2,49
8	100	8,88	8,88	24,37	2,17
9	100	8,1	8,1	39,14	3,17
10	100	11,83	11,83	43,07	5,1