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Identification and Characterization of *Origanum vulgaris* in the wilaya of Tlemcen and Sidi Bel Abbes

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Abstract:

Algeria boasts a wealth of aromatic plants, including *Origanum vulgaris*, which belongs to the Lamiaceae family. Essential oils and extracts from oregano species are widely used in the pharmaceutical, cosmetic, and food industries. The present study involves a comparative analysis between spontaneously occurring and cultivated accessions in Tlemcen and Sidi Bel Abbes region (Northwest Algeria). Our study aims to conduct morphometric characterization as well as morpho-anatomical examination of the plant using five morphological traits. The obtained data underwent statistical analysis using the SPSS software. Additionally, we tested the antibacterial activity of essential oils extracted from both types of accessions. The results from multiple correspondence analysis (MCA) and hierarchical clustering analysis (HCA), along with microscopic examination, revealed a distinction between the two types of accessions. Regarding the macroscopic study, it exhibited a significant difference between the cultivated accession and the spontaneous accession. The tested bacterial strains proved to be sensitive to the essential oil extracted from the studied accessions, exhibiting antibacterial activity with an inhibition zone ranging between 9mm and 23mm.

Keywords: *Origanum vulgaris*, morphometric study, essential oil, antibacterial activity, Tlemcen and Sidi Belabes.

ملخص

تفتخر الجزائر بثروة من النباتات العطرية، بما في ذلك Origanum vulgaris، الذي ينتمي إلى عائلة Lamiaceae. تستخدم الزيوت الأساسية ومستخلصات أنواع الأوريجانو على نطاق واسع في الصناعات الدوائية ومستحضرات التجميل والأغذية. تتضمن الدراسة الحالية تحليلا مقارنا بين المدخلات التي تحدث تلقائيا والمزروعة في منطقة تلمسان وسيدي بلعباس (شمال غرب الجزائر). تهدف دراستنا إلى إجراء التوصيف المورفومترى بالإضافة إلى الفحص المورفوتشريحى للنبات باستخدام خمس صفات مورفولوجية. خضعت البيانات التي تم الحصول عليها للتحليل الإحصائي باستخدام برنامج SPSS. بالإضافة إلى ذلك، قمنا باختبار النشاط المضاد للبكتيريا للزيوت الأساسية المستخرجة من كلا النوعين من المدخلات. كشفت نتائج تحليل (MCA) وتحليل المجموعات الهرمية (HCA)، إلى جانب الفحص المجهري، عن وجود تمييز بين نوعي المدخلات. وفيما يتعلق بالدراسة العيانية، فقد أظهرت فرقا كبيرا بين المدخل المزروع والمدخل التلقائي. أثبتت السلالات البكتيرية التي تم اختبارها أنها حساسة للزيت العطري المستخرج من المدخلات المدروسة، وأظهرت نشاطًا مضادًا للبكتيريا مع منطقة تثبيط تتراوح بين 9 ملم و 23 ملم.

الكلمات المفتاحية: نبات الأورجانوم ، دراسة مورفومترية، الزيت العطري، النشاط المضاد للبكتيريا، تلمسان وسيدي بلعباس.

Introduction

Some species possess pharmacological properties that grant them medicinal interest. Natural remedies, particularly medicinal plants, have long been the primary, if not the sole, recourse of oral tradition in treating pathologies, concurrently serving as raw materials for modern medicine (Jean and Jiri, 1983).

The Algerian flora is characterized by its diversity: Mediterranean, Saharan, and a paleotropical flora, estimated to consist of over 3000 species belonging to several botanical families, of which 15% are endemic (Quezel & Santa, 1963).

The northwestern part of Algeria, particularly the Tlemcen region, harbors a rich heritage of beneficial plants. There are 275 species of medicinal plants belonging to various families, among which the most common.

The aim of our work is to characterise the morphometry of the *Origanum vulgaris* species at three different stations: Attar, the Tlemcen National Park (wilaya of Tlemcen) and Ain El Attouche (wilaya of Sidi Bel Abbes). We found two accessions after the field survey in the regions studied, one growing spontaneously and the other cultivated. Our survey of these two accessions gave us an overview of the distribution of oregano across the two wilayas, as well as its medicinal value, and enabled us to describe the accessions studied. We then moved on to the anatomical study of the plant and the biological study of the essential oil extracted from its leaves.ly used and sold by herbalists include thyme, mugwort, and verbena (Loukkas, 2006).

Experimental Protocol

Presentation of the study area

This work was carried out in the wilaya of Tlemcen and the wilaya of Sidi Bel Abbes in three different sites: Attar and the National Park of Tlemcen in the commune of Mansourah. And El Attouche in Sidi Bel Abbes, in the commune of Tessala.

Geographical location

Wilaya of Tlemcen

The wilaya of Tlemcen lies between 34° and 35° 40' north latitude and 0° 30' and 2° 30' west longitude. Geographically, it lies at the north-western end of the country and occupies western Oranie, stretching from the coastline in the north to the steppe in the south. It is bounded:

- To the north, by the Mediterranean ;
- To the west, by Morocco ;
- To the south, by the wilaya of Naâma ;
- To the east, by the wilayas of Sidi Bel Abbes and Ain Témouchent (Web 6, 2019)

It covers an area of 9017 km², and comprises 20 daïras subdivided into 53 communes, including our study area, the commune of Mansourah, where our two harvesting sites, Attar and the Tlemcen National Park, are located (Web 6, 2019) (Figure 1).

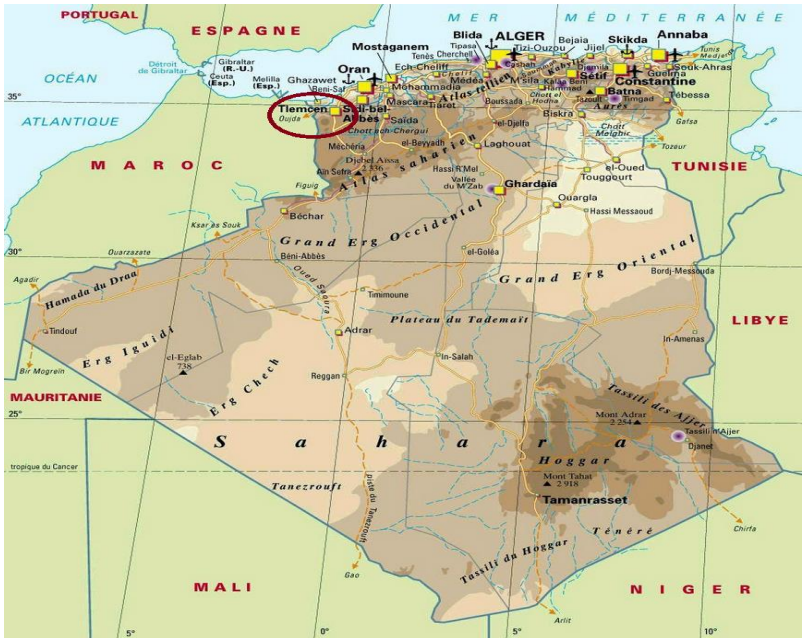


Figure 1: Geographical location of the wilaya of Tlemcen (Web 4, 2022).

Climate study

From a climatic point of view, the Tlemcen region is characterised by a Mediterranean-type climate, with relatively mild, short winters lasting from October to March and irregular rainfall; hot, dry summers with average rainfall and high temperatures lasting from 6 to 8 months in this region. Two bioclimatic stages dominate: semi-arid and sub-humid (Web 9, 2022) (Figure 2).

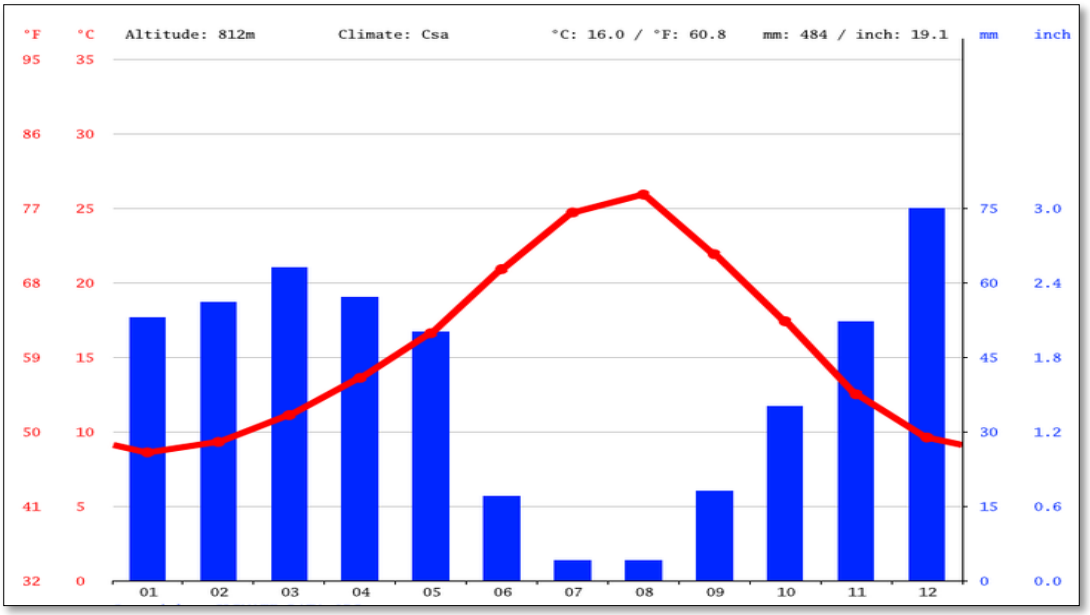


Figure 2 : Umbrothermal diagram for the wilaya of Tlemcen (Web 9, 2022).

Precipitation

The season with the most precipitation lasts 8.4 months, from 10 September to 23 May, with a daily precipitation probability of over 12%. The probability of precipitation peaks at 22% on 19 November. The driest season lasts 3.6 months, from 23 May to 10 September. The lowest probability of precipitation is 1% on 17 July.

For precipitation days, we distinguish between days with rain only, snow only or a mixture of the two. Based on this classification, the most common form of precipitation during the year is rain only, with a probability peaking at 22% on 19 November (Web 6, 2022).

Temperature

The very hot season lasts 2.8 months, from 19 June to 12 September, with an average daily maximum temperature of over 28°C. The hottest day of the year is 2 August, with an average maximum temperature of 32°C and a minimum of 18°C.

The cool season lasts 3.9 months, from 18 November to 15 March, with an average daily maximum temperature below 16°C. The coldest day of the year is 14 January, with an average minimum temperature of 2°C and a maximum of 13°C (Web 6, 2022) (Figure 3).

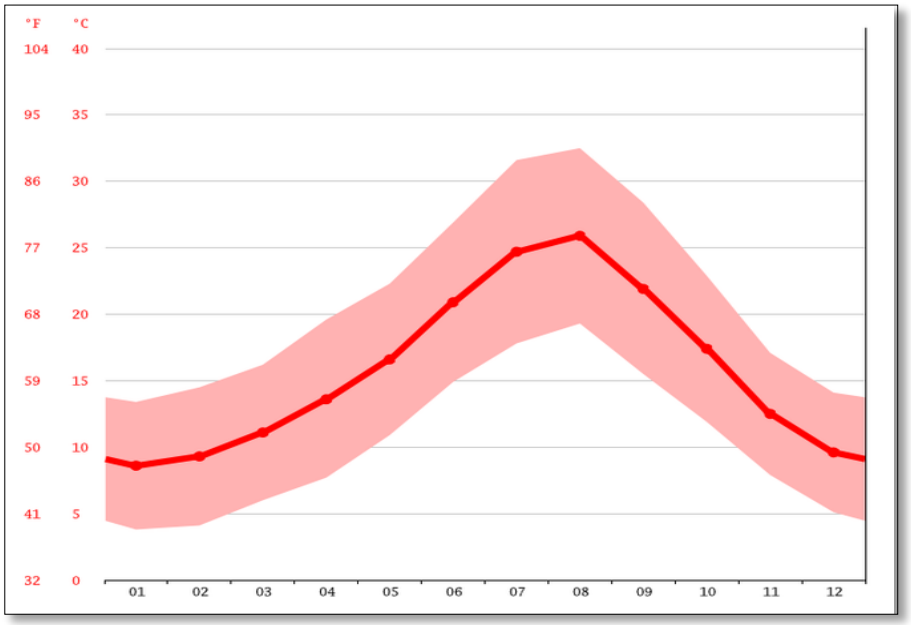


Figure 3 : Temperature curve for the wilaya of Tlemcen (Web 6, 2022).

The wilaya of Sidi Bel Abbes :

The wilaya of Sidi Bel Abbes lies between latitude 35° 11' 38"N and longitude 0° 38' 29"W. Geographically, it is situated in the north-west of Algeria and is bounded as follows:

-To the north by the wilaya of Oran ;
-To the north-west by the wilaya of Ain Temouchent;
-To the north-east by the wilaya of Mascara;
-To the west by the wilaya of Tlemcen;
To the east by the wilayas of Mascara and Saida;
-To the south by the wilayas of Nâama and El-Bayad and
-To the south-east by the wilaya of Saida.

It covers an area of 9017 km², and includes 15 daïras and 52 communes, including our study area, the commune of Tessala, which includes our El Attouche harvesting site (Web 10, 2022) (Figure 4).

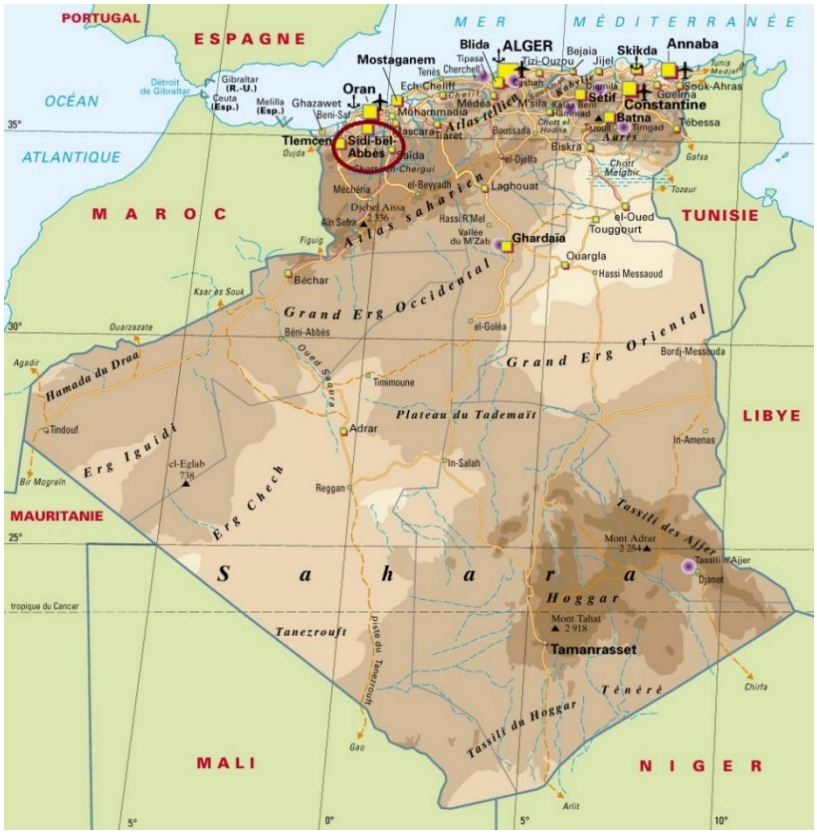


Figure 4 : Geographical location of the wilaya of Sidi Bel Abbes (Web 4, 2022).

Climate study

Sidi-Bel-Abbes has a warm Mediterranean climate with dry summers according to the Köppen-Geiger classification. Over the year, the average temperature in Sidi-Bel-Abbès is 18.9°C and rainfall averages 337.4 mm (Web 6, 2022) (Figure 5).

Precipitation

The season with the most precipitation lasts 8.5 months, from 12 September to 27 May, with a daily precipitation probability of over 12%. The month with the highest number of days with precipitation in Sidi-Bel-Abbès is February, with an average of 6.0 days with at least 1 millimetre of precipitation...
he driest season lasts 3.5 months, from 27 May to 12 September. The month with the fewest days of precipitation in Sidi-Bel-Abbès is July, with an average of 0.8 days with at least 1 millimetre of precipitation (Web 6, 2022).

Temperature

The very hot season lasts 2.8 months, from 19 June to 12 September, with an average daily maximum temperature of over 30°C. The hottest month of the year in Sidi-Bel-Abbès is August, with an average maximum temperature of 33°C and a minimum of 19°C.

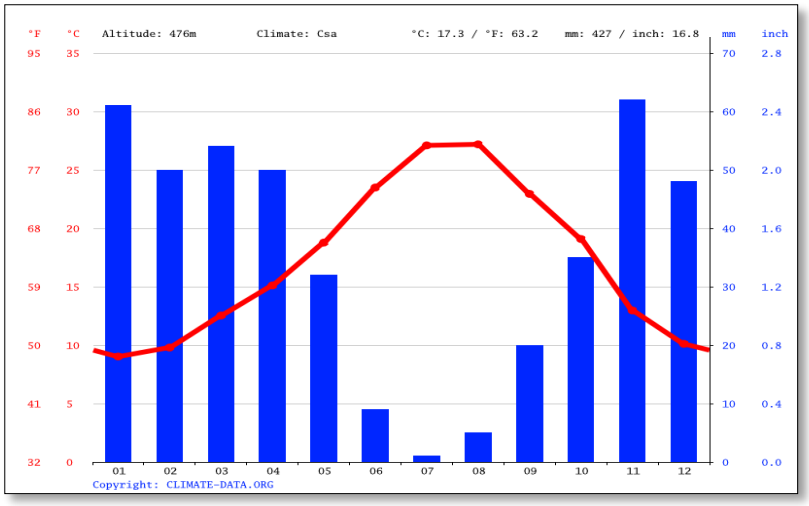


Figure 5 : Umbrothermal diagram for the wilaya of Sidi Bel Abbes (Web 6, 2022).

The cool season lasts 4.0 months, from 18 November to 17 March, with an average daily maximum temperature below 18°C. The coldest month of the year in Sidi-Bel-Abbès is January, with an average minimum temperature of 4°C and a maximum of 14°C (Web 6, 2022) (Figure 6).

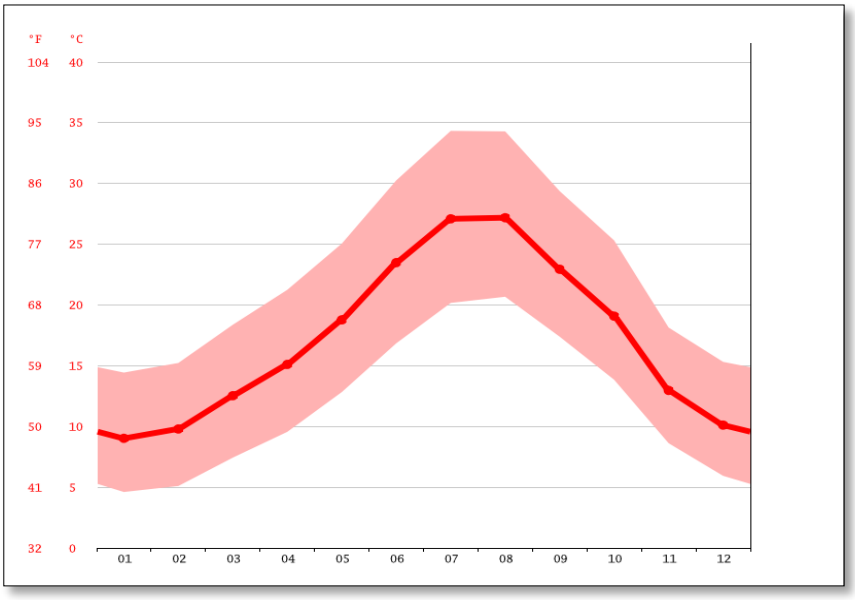


Figure 6 : Temperature curve for the wilaya of Sidi Bel Abbes (Web 6, 2022).

Pedology of the study area

Duchauffour (1977) points out that the majority of soils in Mediterranean regions, at least those with a Mediterranean-type climate, are characterised by so-called "fersialitic" soils. In Tlemcen and Sidi Bel Abbes region, as in the rest of northern Algeria, soils are undergoing severe degradation, particularly in mountainous areas. This degradation has become even more pronounced in recent decades. These mountain areas represent a major socio-economic challenge, and are still very much affected by the phenomenon of degradation. The balance between vegetation, soil and water is being disturbed Duchauffour (1977).

Materials and methods

Our work consiste of carrying out a field survey at three different sampling stations between the years of 2019-2022 (table 01), in order to compare two plants of the same species, one cultivated and the other spontaneous. The second part of our experiment consisted of morphometric measurements, morpho-anatomical studies of the plant, extraction of its essential oil and testing of its antibacterial activity.

Table 1 : Sampling locations and parameters (2019-2022).

The region	Sampling	Parameters
Attar	30 plants	- Sheet surface - Sheet length - Stem length - Node spacing length - Number of nodes
Tlemcen National Park	30 plants	
El- Attouche	30 plants	

Morphometric measurements

We took 30 samples from each station. 5 quantitative parameters were studied (International Plant Genetic Resources Institute IPGRI 1985):

Leaf area: We took mature leaves from the base of the plant and photographed them to measure their surface area using Image-J software. An average of three leaves were taken at random from each plant.

Leaf length: We took mature leaves from the base of the plant and photographed them to measure their length using Image-J software. For each plant we took three leaves and calculated the average.

Stem length: This involves measuring the length of the stem from the collar to the tip using a tape measure.

The length of the internodes: This involves measuring the length of the internodes using a tape measure. For each plant we took three knots from the base of the stem and calculated the average.

The number of internodes: we counted the number of internodes for each plant.

Statistical analysis

After transferring the data collected onto a matrix, several statistical tests were carried out using SPSS software (version 20).

A descriptive analysis of the quantitative variables was carried out, looking at the mean, median, minimum, maximum and standard deviation.

A principal component analysis (PCA) was carried out to describe the similarities between individuals for which several quantitative characteristics were measured on the basis of morpho-metric measurements, to define a classification of the plants and to construct a typology that consists of identifying individuals that are fairly similar to each other.

An ascending hierarchical classification (AHC) was carried out to obtain the optimum number of groups. These tests were obtained using SPSS software.

Finally, to obtain a comparison of quantitative and qualitative parameters, an ANOVA test was also carried out using SPSS software.

Morpho-anatomical study

Macroscopic study

The various organs of *Origanum vulgare* were described and illustrated using digital photos, with some details observed using a binocular microscope.

Microscopic study

Anatomical study by microscopic observation of cross-sections and drug sections of the leaf of Origanum vulgare reveals a number of tissues and structures of great importance in identifying species, and which make it possible to correlate the anatomy of the organ with environmental conditions.

The cross-sections were observed in the following stages:

- The leaves are placed in a mixture of distilled water and glycerine to keep them in a fresh, softened state and prevent them from drying out (Figure 7).

Figure 7 : Origanum vulgaris leaves immersed in a mixture of distilled water and glycerine (Senouci brixi, 2020)

- Cutting the leaf under study: Using a blade, the leaf was cut parallel to its axis to obtain thin cuts perpendicular to the axis of the organ (figure 8).

Figure 8: Leaf section of Origanum vulgaris (Senouci brixi, 2020).

- Staining the walls: the sections were immersed in eight successive baths:

1. A bath in dilute bleach for 30 minutes, the aim being to empty the cells of their cytoplasmic content, leaving only the skeletal walls.

- A distilled water bath to rinse off excess bleach.

2. An acetic acid bath (fixative) for 05 minutes, the aim being to increase the affinity of the different membranes and cell walls to the two dyes.
3. A distilled water bath to rinse off the excess acetic acid.
4. A methyl blue bath for 01 minute stains the lignified and sclerified structures blue.
5. A distilled water bath to rinse off excess dye.
6. A Congo Red bath for 05 minutes colours the cellulose structures pink.
7. A bath in distilled water to rinse off excess dye.

- Sections between slide and coverslip ready for observation.

Leaf drug observation was carried out in the following stages:

1. Dry the oregano leaves separately in an airy, dark place.
2. Grind the dried leaves into a powder using a mortar and pestle with circular movements (figure 9).
3. The powder is taken with a spatula and placed on a slide with two drops of Gazet's reagent.

All the elements in the plant powder become transparent or take on a particular colour under the action of the Gazet Le Chatelier reagent:

- The lignified elements (wood vessels, fibres, sclerotic cells and certain hairs) turn a very light yellow-green;

- The suberized elements are coloured red-brown

- Lipids, essential oils, resins and latex are coloured orange-red.

Figure 9 : Reduction of dried Origanum vulgaris leaves to a powder (Senouci brixi 2020)

Extraction of the essential oil (EO) by hydro distillation

❖ Protocol

The EO was extracted by hydrodistillation (Figure 10) for two and a half hours using a Clevenger-type apparatus in the plant biology laboratory of the biology department (Faculty of Natural and Life Sciences, Abou Bekr Belkaid University, Tlemcen). To do this, 38 g of leaves, previously dried in the shade, were crumbled and then placed in a flask with a quantity of distilled water. The flask was then brought to the boil on a flask heater.

Figure 10 : Clevenger extraction assembly (Senouci brixi 2022)

The yield is defined as the ratio between the quantity of HE recovered and the quantity of dry plant matter treated (Akrout, 2001), and is expressed as a percentage according to the following formula **R=Pb/Pax100**

Knowing that

R: yield of essential oil
Pb: weight of essential oil recovered in grams
Pa: weight of dry plant matter in grams

Antibacterial activity

The antibacterial activity of *O. vulgare* essential oil was evaluated using the solid-state diffusion method (Table 2). This study was carried out in the Microbiology Laboratory of the Tlemcen Faculty of Natural and Life Sciences.

Table 2 : Bacterial strains tested.

Gram	Family	Type	Species	Numbers of trains
Gram -	Enterobacteriaceae	Klebsiella	*Klebseilla pneumoniae*	1
	Pseudomonaceae	Pseudomonas	*Pseudomonas aeruginosa*	1
Gram +	Enterococcaceae	Enterococcus	*Enterococcus faecalis*	1
	Micrococcaceae	Staphylococcus	*Staphylococcus aureus*	1

The tests were carried out on 4 strains (Table 02), including strains of clinical origin. These strains were supplied to us by the microbiology research laboratory (Faculty of Natural and Life Sciences, Abou Bekr Belkaid University. Tlemcen).

Culture media

Depending on the methods employed, we used the following culture media:

- **Nutrient agar:** Non-selective isolation and preservation medium.
- **Mueller Hinton agar:** Microbiological growth medium commonly used for antibiotic susceptibility testing.
- **Strain storage:** Bacterial strains were stored at 5°C in sterile tubes containing 10ml of nutrient agar slants.

Qualitative assessment of antibacterial activity (aromatogram)

- **Principle**

This examination is carried out in the same way as an antibiogram, where the antibiotics are replaced by aromatic essences, selected and recognised beforehand. The aromatogram or agar diffusion method or disc method is a qualitative technique used to determine the sensitivity of micro-organisms to a substance reputed to be antibacterial. This method is based on the migratory capacity of HEs inside a Petri dish, in a solid nutrient medium (Mueller Hinton). The basic aromatogram protocol adopted is that proposed by Duraffourd (1990), to which a few modifications have been made.

- **Reactivation of bacterial strains**

To prepare bacterial suspensions according to CLSI (Clinical Laboratory Standards Institute) standards, the bacterial strains are reactivated by subculturing from the storage medium onto solid culture medium, previously melted, poured into Petri dishes 4 mm thick (the dishes must be cooled and dried before inoculation) (figure 11). After 24 h incubation in an oven (37°C), the dishes are removed and the pure, from which pure and young cultures are used to prepare the bacterial suspensions. The inoculation preparation method is that recommended by the CLSI, which consists of preparing a suspension in saline solution (0.9% NaCl) equivalent to the McFarland 0.5 standard (10^6CFU/ml) from an 18 to 24hour of the culture of bacteria studied on the agar medium. This suspension can be obtained by measuring the optical density (OD) ranging from 0.08 to 0.1 read at 625 nm.

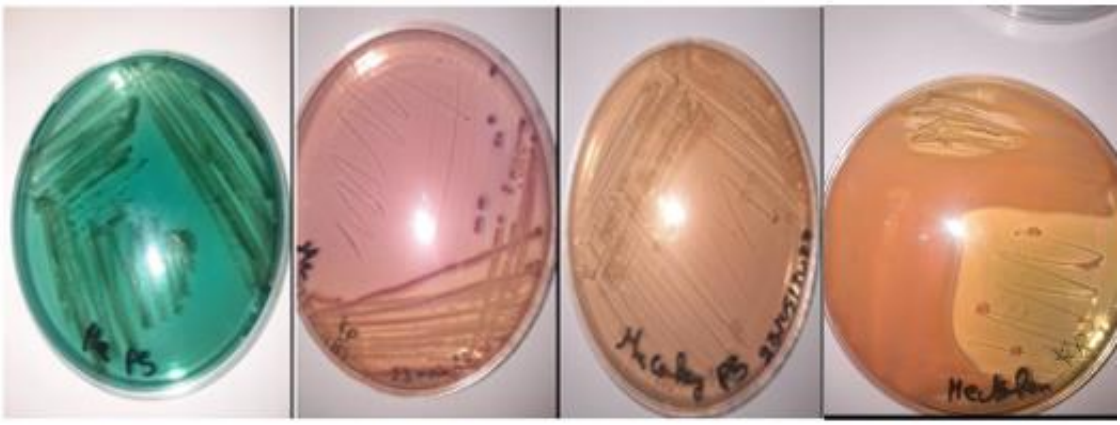


Figure 11 : Different conservation media for bacterial strains (Senouci brixi, 2022).

- **Seeding**

Pour out the culture media, Muller-Hinton agar for all the bacterial strains chosen, into the Petri dishes at a thickness of 4mm near the Benzene nozzle and leave the medium to solidify. The dried dishes are inoculated with the bacterial suspensions using sterile swabs. The swab is soaked in the bacterial suspension and squeezed against the inside wall of the test tube. Inoculation is carried out by making tight striations from top to bottom. The operation is repeated two or three times, turning the box 60° each time. The swab should be passed around the periphery of the agar.

- **Distrubition of discs and antibiotics**

Sterile Whatman paper discs, 6 mm in diameter, were grasped with sterile forceps and impregnated with 10 µl of essential oil. The discs were applied to the surface of culture media previously inoculated with a control antibiotic. Incubation in the incubator was at 37°C for 24 hours.

- **Reading**

After incubation for 24 h, the absence of bacterial growth expressing antimicrobial activity is indicated by a translucent halo around the disc, the same colour as the sterile agar, the diameter of which is measured using a calliper (expressed in mm).

Results and discussion

Statistical analyses were carried out to describe the difference between accessions *of Origanum vulgaris* growing spontaneously and those cultivated in the Tlemcen and Sidi Bel Abbes regions.

Morpho-metric measurements
Descriptive analysis

The averages, standard deviations, minima, maxima and coefficients of variation of the measurements taken are reported in (**Table 03).**

Table 3 : Descriptive analysis of measurements for the species studied.

	N	Minimum	Maximum	Average	Standard error	Standard deviation	Vriance
LT	90	14,000	73,500	42,38363	1,681853	15,955461	254,577
L EN	90	1,330	7,640	3,65713	0,134430	1,275313	1,626
Nbr EN	90	3	12	7,80	0,204	1,938	3,757
SF	90	3,592	48,432	15,29201	1,152964	10,937976	119,639
LF	90	0,305	3,379	1,69174	0,061943	0,587640	0,345

Leaf length (LF), leaf surface area (SF), number of internodes (EN), internode length (L EN), stem length (LT).

The length of the stem (LT) shows average values ranging from 14cm for the Attar region to 73.5cm for the population of the Tlemcen National Park with an average of 42.38cm;

The length of the internodes (LEN) shows average values ranging from 1.33cm for the Attar region to 7.64cm at the Tlemcen national park, with an average of 3.65cm;

The number of entres noeud (EN) shows average values ranging from 3 for the Attar region to 12 for the population of the Tlemcen national park with an average of 7.80;

The average leaf area (LA) ranges from 3.59cm2 in the Attar region to 48.43 cm2 in the Tlemcen national park, with an average of 15.29cm2

Leaf length (LF) shows average values ranging from 0.3cm for the Attar region to 3.37cm for the Tlemcen National Park population, with an average of 1.69cm.

Individual variation

Principal component analysis (PCA) was performed on the variables studied. The result of this analysis showed that the first two axes represented 77.81% of the total inertia, which is highly representative (Table 04).

Table 4 : Description of the PCA axes.

Component	Initial eigenvalues			Extraction Sum of squares of selected factors		
	Total	% of the variance	% cumulative	Total	% of the variance	% cumulative
1	2,76	55.25	55.25	2.76	55.25	55.25
2	1,12	22.55	77.81	1.12	22.55	77.81

Analysis of the parameters studied shows that the two axes account for 55.25% and 22.55% of the total inertia respectively. The information to be noted from the correlation circle shown in Fig. 12 is as follows:

- No negative correlation was observed,
- The variables LF, SF and LT are closest to the periphery of the circle, which is significant,

- The variables LF, SF, LT and L EN are well correlated with each other; and they are well correlated with axis 1 and form a group,
- The LEN and EN variables correlate very weakly with each other and weakly with the first group,
- The EN variable shows a low correlation with all the variables, but it should be noted that it shows high correlation with axis 2.
- The traits (LF, SF, LT and L EN) are positively correlated with each other. The correlation of these traits can be explained by the influence of genes, i.e. these traits are probably controlled by a number of genes in common.

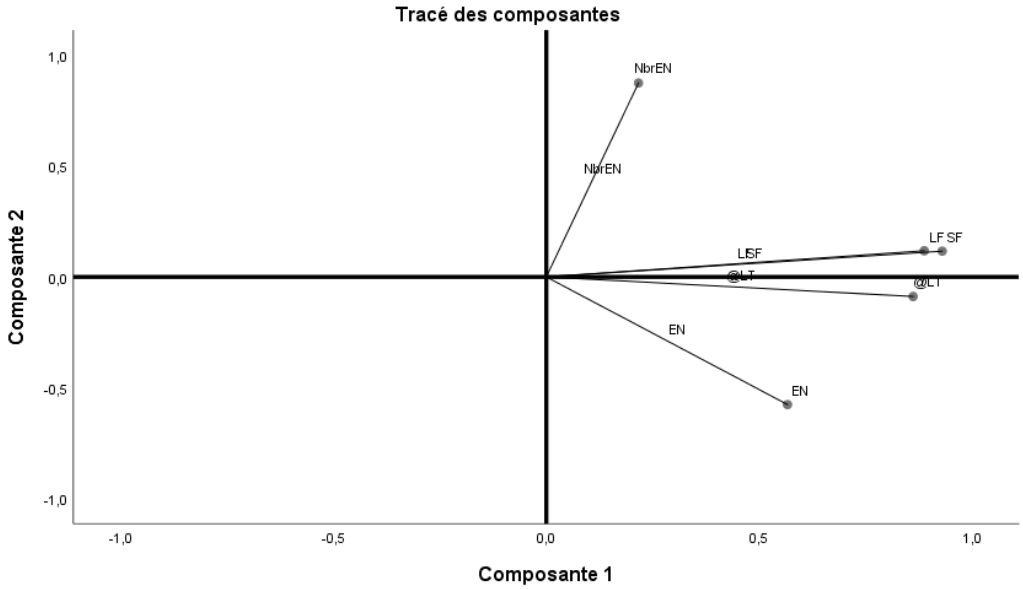


Figure 12 : Correlation circle for morphometric measurements using PCA.

The PCA on individuals shows the relationship between the different genotypes studied, spontaneous accession and cultivated accession, and divides the latter into two large groups :

The first group is made up of the genotype of the spontaneous *O. vulgaris* accession from the two regions, and the second group contains the genotype of the cultivated *O. vulgaris* accession (Figure 13).

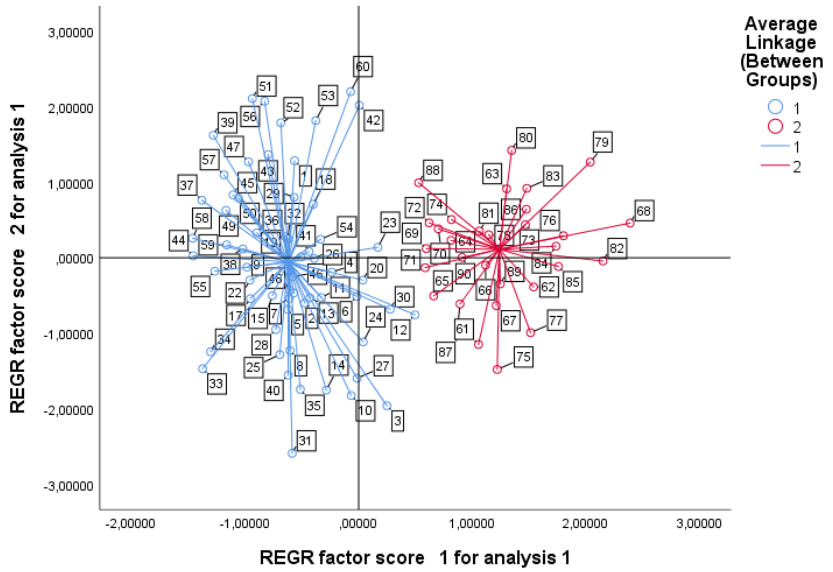


Figure 13 : Presentation of *O. vulgaris* individuals by PCA.

Analysis of variance (ANOVA)

Comparison of the quantitative parameters leaf length, leaf area, stem length, number of internodes and internode length with environmental factors (the region) revealed that all the characteristics studied

showed highly significant differences (the values recorded are below the significance threshold (0.05)) (Table 5).

Table 5 : Comparative ANOVA between quantitative characteristics and environmental factors. (the region)

Region	El Atouche	Attar	National Parc	P
N	30	30	30	
LT	38,100±3,8497	27,766±8,732	61,283±8,983	***
EN	3,982±1,383	2,692±1,151	4,296±0,507	***
Nbr EN	7,000±1,203	8,033±2,606	8,366±1,496	***
SF	9,134±2,956	7,934±4,0671	28,806±7,598	***
LF	1,476±0,362	1,306±0,383	2,292±0,454	***

Leaf length (LF), leaf surface area (SF), number of internodes (EN), internode length (L EN), stem length (LT). α=5% IC=95%

When the two accessions are compared, LT, EN, Nbr EN, and SF show a significant difference and highly significant differences. For LF, however, there was no significant difference (Table 6).

Table 6 : Comparative ANOVA between quantitative characteristics and environmental factors. (spontaneous/cultivated)

Culture	Spontaneous	Cultivated	P
N	60	30	
LT	32,933±8,480	61,283±8,983	***
EN	3,337±1,419	4,296±0,507	***
Nbr EN	7,520±2,079	8,370±1,497	**
SF	8,534±3,576	28,806±7,598	***
LF	1,391±0,379	2,292±0,454	Ns

Leaf length (LF), leaf surface area (SF), number of internodes (EN), internode length (L EN), stem length (LT). α=5% IC=95%.

Hierarchical ascending classification (HAC)

The CAH of our sample reflects the same results of the principal component analysis of the individuals, and has enabled us to distinguish two classes based on the various measurements made during our study (Figure 14).

PEARSON correlation

This correlation allows us to study the links between the different quantitative parameters. After processing the statistical data using a Pearson correlation test, we found that there were mainly strong links between the parameters LT, LF, nbr EN and SF. These results were confirmed by the multivariate PCA analysis (Figure 12). The labeled variables are those best represented on the plane). The P values < α, so the correlation observed between the parameters is not due to chance (Table 7).

Strong correlations are observed for :

86% between LF and SF

74% between SF and LT

61% between LT and LF

46% between LT and EN

On the other hand, the percentages of correlation of the characters nbr EN and the length of the EN with the other characters were average and weak : 35% between EN and LF

34% between EN and SF

20% between nbr EN and SF

19% between nbr EN and LF

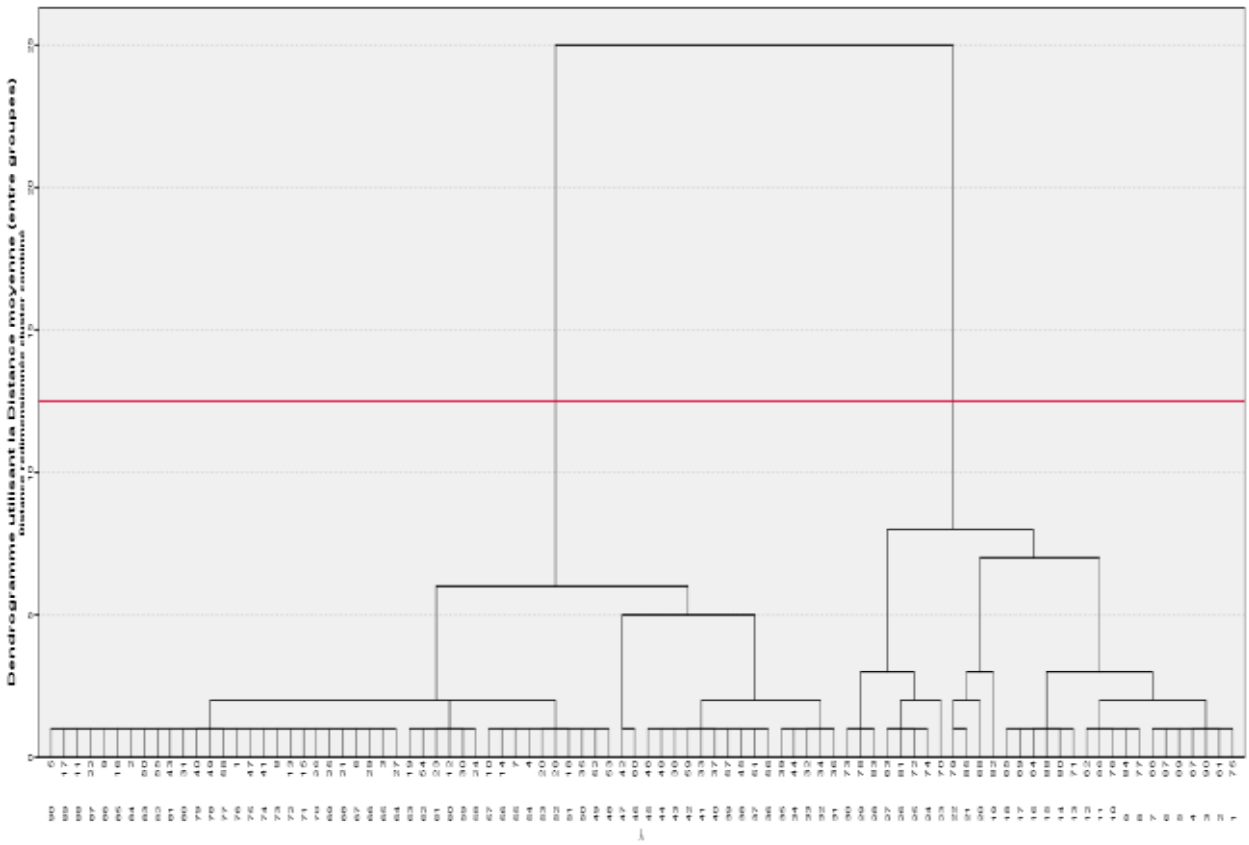


Figure 14 : Hierarchical ascending classification of spontaneous and cultivated *O. vulgaris*.

Table 7 : Correlation values between the parameters studied.

	LT	EN	Nbr EN	SF	LF
LT	1				
EN	0,46 ***	1			
Nbr EN	0,11 Ns	-0,13 Ns	1		
SF	0,74 ***	0,34 **	0,20*	1	
LF	0,61 ***	0,35 **	0,19 *	0,86 ***	1

(ns) not significant, (*) significant, (**) highly significant, (***) very highly significant

Morpho-anatomical study of the O.vulgaris species

Macroscopic study

The flowers are grouped together in dense inflorescences. It can be seen that the cultivated accession has a denser inflorescence than the spontaneous accession (Figure 15)

Figure 15 : Inflorescence of the species O.vulgare (Senouci brixi, 2020)
On the right is cultivated accession and on the left spontaneous accession.

The calyx of the flower of the cultivated accession is wider and shorter than that of the spontaneous accession (Figure 16).

On the right is cultivated accession, on the left spontaneous accession.It can be seen that the leaf of the cultivated accession contains fewer secretory hairs than the spontaneous accession (Figure 17). This may influence the yield of essential oil

Figure 16 : The flower of O. vulgare viewed under binoculars. (Senouci brixi , 2020)

Figure 17 : O. vulgare leaf viewed under binocular (Soulimane et al, 2020)
On the right is cultivated accession, on the left spontaneous accession.

It can be seen that the cultivated accession contains fewer secretory hairs than the volunteer accession at stem level, and that the stem in the cultivated accession can be up to three times the size of the volunteer accession (Figure 18).

Figure 18 : Stem of *O. vulgare* viewed under binocular. (Senouci brixi, 2020).
Right cultivated left spontaneous.

Microscopic study of the *O.vulgaris* species

Microscopic study of a cross-section of the leaf of O. vulgaris

Microscopic observation of transverse sections of the O. vulgaris leaf reveals the bifacial structure typical of Dicotyledons. The upper surface has a monostrate upper epidermis covered by a cuticle. It is dotted with tectorial hairs, secretory hairs and stomata. The lower surface has a lower epidermis that differs from the upper epidermis in having. a weakly cutinised wall. The mesophyll is heterogeneous and comprises the palisade parenchyma under the upper epidermis and the lacunar parenchyma under the lower epidermis. The cells of the lower epidermis appear smaller than those of the upper epidermis (Figure 19).

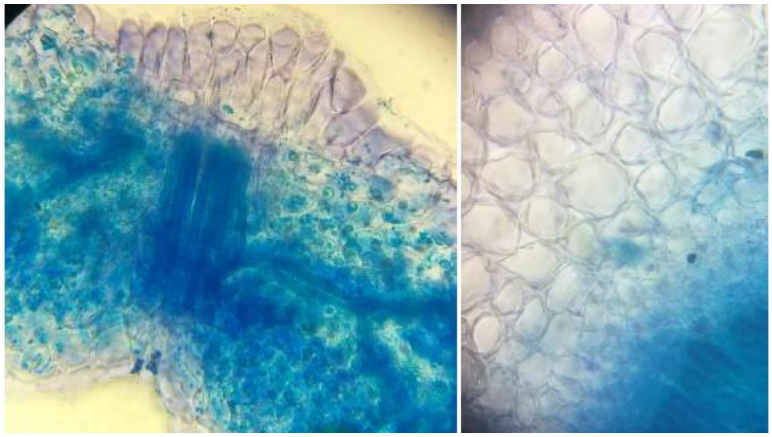


Figure 19 : Cross-section of the leaf of *O. vulgaris*
View under a light microscope (magnification x 40).

Stomata are found on both sides (figure 20). The stomata found in Lamiaceae are generally of the diacytic type.

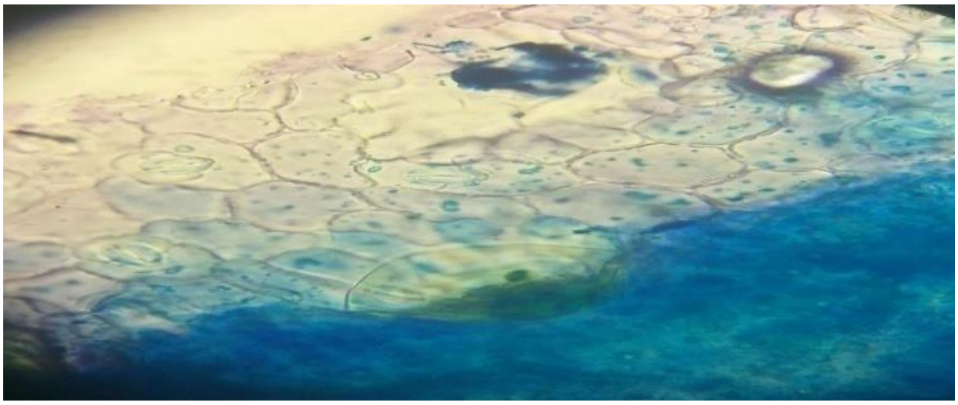


Figure 20 : Detail of stomata on the upper surface of an *O. vulgaris* leaf (M.O.: magnification x40) (Senouci brixi et, 2020).

The leaf is covered with several types of unicellular, bicellular and multicellular tector hairs (Figure 21).

Conical tector hair 2. Octacellular heads of secretory hairs 3 unicellular heads of secretory hairs

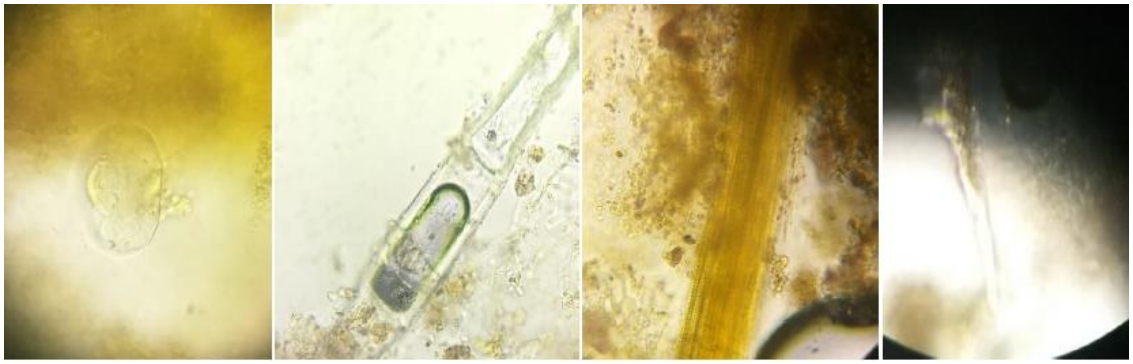


Figure 21 : Different hairs in the cross-section of the leaf of O. vulgaris (magnification 40×10). (Senouci brixi et, 2020)

Microscopic study of *O. vulgaris* leaf powder

Observation of the oregano drug powder under a microscope at magnification (10X40) showed the presence of the following elements (Figure 22) ;

- Uniseriunicellular conical tectorial hairs with a warty wall (4).

- Multi-cellular uniseriate tectorial hairs (2).

- Octacellular heads of secretory hair (1).

- Debris from spiral vessels (3).

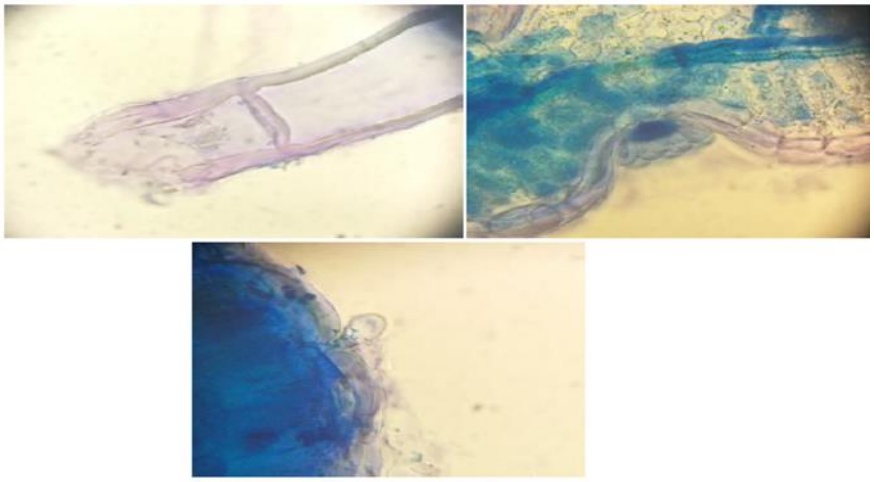


Figure 21 : Elements of O. vulgari leaf powder (40×10 magnification) (Senouci brixi 2020).

Extraction and calculation of essential oil yield

Our investigation focused on the EO of *O.vulgare* growing in the wilaya of Tlemcen and the wilaya of Sidi Bel Abbes.

Distillation was carried out for 2 h and 30 min on dried oregano leaves, using a "Clevenger" type hydrodistiller, the operating conditions of which are described in the chapter on materials and methods.

The EO obtained by hydrodistillation was yellow in colour (Table 08), with a strongly pungent flavour and a strong odour characteristic of aromatic plants. It was stored at low temperature (t<6°C) in airtight bottles. Table 08 shows the yields calculated for the EO studied in this work.

Table 8 : Organoleptic characteristics of EO of *O.vulgare*.

Aspect	Liquid
Coleur	yellow
Taste	Spicy
Odour	Thyme

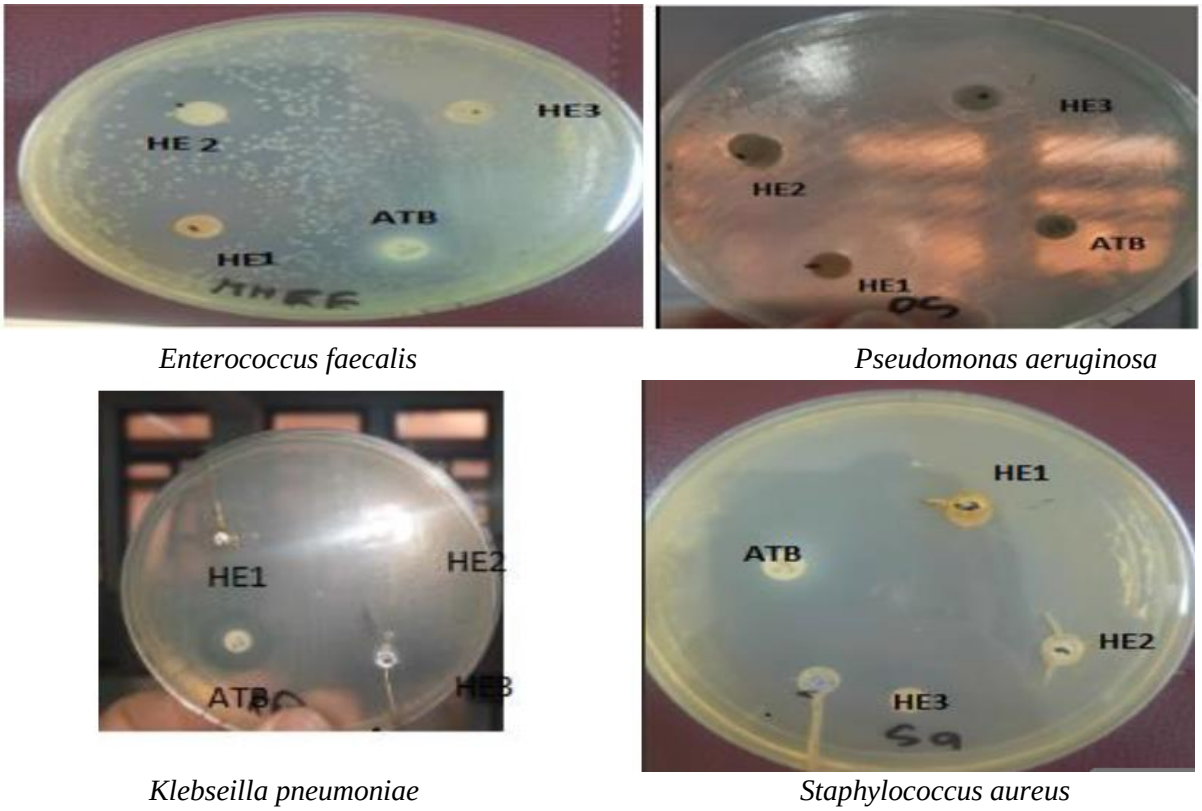
Spontaneous *O.vulgare* in the two wilayas studied has a relatively good yield of (1.72%) and (1.70%), compared with cultivated O.vulgare, which has a yield of (1.54%).

The yield is slightly higher in the spontaneous accession from Sidi Bel Abbes than in the spontaneous accession from Tlemcen. The difference between the extraction yields obtained is probably linked to the following factors: drying time and the water/plant matter ratio. It may also be linked to climatic factors (heat, cold, water stress), geographical factors (altitude, soil type, sun exposure) and genetic factors (natural crosses) (Veres et al., 2003).

The lowest yield is from the Tlemcen cultivated accession, which may be due to the use of pesticides, but also to the reduced number of secretory hairs in the latter.

Antibacterial activity

The antibacterial activity of EO (from the three regions) was evaluated on a total of 4 bacterial strains, on which a study was carried out including the determination of the diameters of the zones of inhibition (figure 23)



Enterococcus faecalis *Pseudomonas aeruginosa*

Klebseilla pneumoniae *Staphylococcus aureus*

Figure 22 : Antibiotic susceptibility test results (Senouci brixi, 2022)

To interpret our results, we used Duraffourd's (1990) interpretation scale. According to the latter, a bacterial strain is considered resistant (-) to a plant extract if its inhibition diameter is equal to 6 mm or less than 8 mm, it would be of limited sensitivity (+) if its inhibition diameter is between 8 and 14 mm, of average sensitivity (++) if its inhibition diameter is between 14 mm and 20 mm and finally very sensitive (+++) if its inhibition diameter is greater than 20 mm. Figure 24 summarises the results obtained by the three EO on the 04 bacterial strains tested.

Gram-negative bacteria
Pseudomonas aeruginosa

The Pseudomonas strain showed limited sensitivity (+) towards the three *O.vulgare* EOs with an average diameter of the inhibition zones of 10 mm for the Sidi Bel Abbes EO, 11mm for the spontaneous O.vulgare EO from Tlemcen and finally 9mm for the cultivated O.vulgare EO from Tlemcen.

P. aeruginosa is an opportunistic pathogen associated with a large number of nosocomial and community-acquired infections. The pathogenicity of this bacterium is due to the production of several intra- and extracellular virulence factors (Rumbaugh et al., 1999). In addition, several authors have reported that HEs from various oregano species have little or no activity against Ps.aeruginosa strains (Janssen et al., 1986; Ruberto & Baratta, 2000; Karaman et al., 2001; Pintore et al., 2001; Hersch-Martinez et al., 2005). In addition, Walsh et al (2003) report that thymol is not active against P. aeruginosa.

The P.aeruginosa species is known for its intrinsic resistance to antimicrobial agents, particularly antibiotics, due to the structure of their outer membranes, which are particularly impermeable to hydrophobic molecules (Abi-Ayad et al., 2011). The resistance of P.aerugiona strains may also be due to their ability to form a biofilm (Abi-Ayad et al., 2011).

- ***Klebsiella pneumoniae***

The average diameter was within a narrow range [16mm to 23mm], reflecting the greater specificity of action of oregano EO on these bacteria.

The klebsiella strain was moderately sensitive (++) to both cultivated and spontaneous O.vulgare EOs from Tlemcen, with inhibition zone diameters of 16mm and 20mm respectively, and highly sensitive (+++) to O.vulgare EO from Sidi Bel Abbes.

Bekhechi (2009) determined the antimicrobial power of Thymol-rich (51.3%) O. vulgare subsp. glandulosum EO from the Tlemcen region on several strains, in particular Klebsiella pneumonia.

Gram-positive bacteria

- ***Staphylococcus aureus***

The Gram-positive test showed total resistance of Staphylococcus aureus to all three EOs.

- ***Enterococcus fecalis***

The Enterococcus strain showed average sensitivity (++) towards the three O.vulgare EOs with respective inhibition diameters of 19mm for O.vulgare EO from Sidi Bel Abbes, 15mm for cultivated O.vulgare EO from Tlemcen, and 16mm for spontaneous O.vulgare EO from Tlemcen.

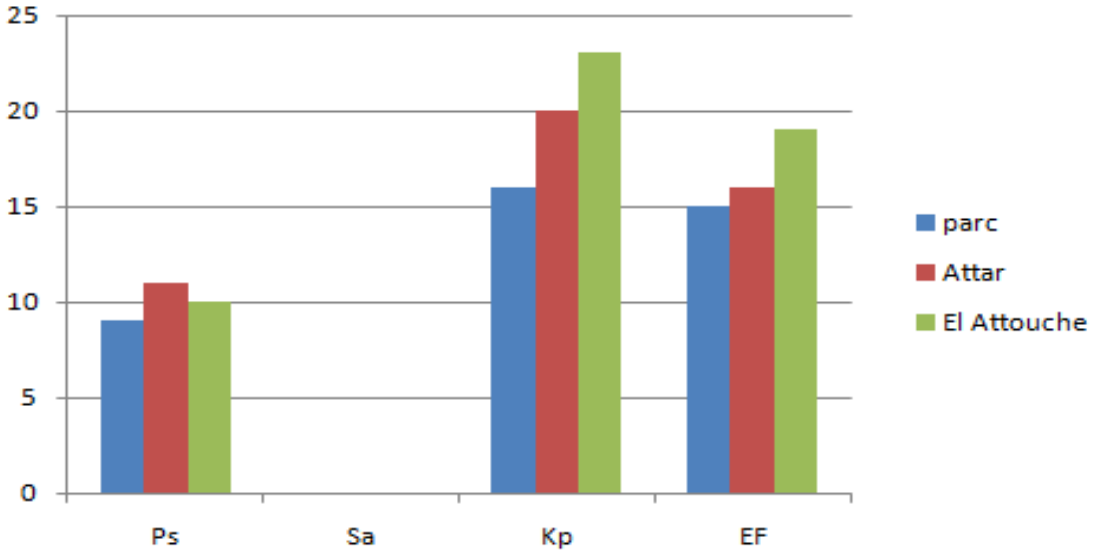


Figure 23 : Diameters of zones of inhibition (mm) of O. vulgare EO tested on Enterococcus fecalis (Ef), Staphylococcus aureus (Sa), Klebsiella pneumoniae (Kp), Pseudomonas aeruginosa (Pa) strains.

Conclusion

Algeria's flora is one of the richest in the world, containing many endemic species. Western Algeria is home to many of these species, most of which are used for medicinal purposes.

Our work is based on morpho-metric characterization and a comparative study of two *O. vulgaris* accessions, one that grows spontaneously and the other that is cultivated. The aim of the study is to analyze the phenotypic diversity based on the morphological characteristics of the species collected in the wilaya of Tlemcen, and showed that there is a very large unexploited diversity in the region.

The results showed that there is a high level of diversity in the characteristics studied, namely leaf area, leaf length, stem length, length of internodes and number of internodes.

The results of the multiple correspondence analysis (PCA) and the hierarchical classification (HCA) showed a clear difference between the spontaneous and cultivated accessions, but these differences are probably due to the fact that the characters chosen for this study are very much influenced by the environment. The results of the morpho-anatomical study of the species showed no difference between the two types of accession. The latter showed only that the same cellular components as well as the same cytological and histological organisation existed on both sides. However, the number of secretory stalks was much higher in the spontaneous accession. Extraction of the essential oil from *O. vulgare* leaves gave a yield of 1.7% for the spontaneous accession and 1.5% for the cultivated accession. This allowed us to deduce that the number of secretory hairs may have influenced the yield of essential oils.

The antibacterial activity of the EO on 4 strains was highly effective on the majority of bacterial strains.

These results add new elements to our knowledge of this species and confirm some of its medicinal properties, and it would be very interesting to study the aspects investigated in this work in greater depth.

The next logical step would be to increase the number of samples in order to study intra-population variations. The physico-chemical analysis of the essential oil needs to be more in-depth, the biological

study needs to be extended, and finally a molecular characterisation of the plant is essential to get a clear idea of the genetic diversity of this species.

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