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STUDY OF THE PHYTOCHEMICAL AND BIOLOGICAL VARIABILITY OF THE GENUS *ORIGANUM* IN ALGERIA

A thesis submitted by

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Defended publicly on the 16th of NOV 2024, in front of jury members:

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Abstract

This research aims to valorize Algeria's endemic medicinal and aromatic plants by exploring the phytochemical and biological variability of three *Origanum* populations: *O. floribundum* (from Blida and Guelma) and *O. glandulosum* (from Jijel). Essential oils were extracted using hydrodistillation, analyzed via GC-MS, and assessed for their acaricidal, antioxidant, and anti-inflammatory activities.

The essential oil yields were 2.9% (*O. floribundum* G), 1.5% (*O. glandulosum*), and 0.9% (*O. floribundum* B). Chemical analysis revealed a predominance of carvacrol in all three populations: 43.04% in *O. floribundum* B, 40% in *O. floribundum* G, and 29.93% in *O. glandulosum*. *O. glandulosum* also exhibited high levels of γ-terpinene (25.48%), thymol (12%), and *p*-cymene (15.14%). In contrast, *O. floribundum* showed significant concentrations of cis-sabinene hydrate (31.56% in Blida, 22.21% in Guelma) and cis-β-ocimene (24.44% in Guelma, 13.76% in Blida).

Among the three tested concentrations, preparations with 2% of *Origanum floribundum* essential oils demonstrated strong acaricidal activity: 76% for *O. floribundum* G, 75% for *O. floribundum* B, surpassing the chemical treatment Carvacrol at 73%. However, *O. glandulosum* was less effective at the same dose, showing 57%.

The antioxidant activity, measured using the DPPH assay, revealed IC50 values of 54.4 µg/mL (*O. floribundum* B), 66.7 µg/mL (*O. floribundum* G), and 60.4 µg/mL (*O. glandulosum*), demonstrating a significant ability to neutralize free radicals, though less effective than ascorbic acid (4.43 µg/mL).

Finally, the in vivo anti-inflammatory activity, evaluated using the carrageenan-induced paw edema method, showed maximum reductions of 78.25% (*O. floribundum* G at 100 mg/kg) and 64.05% (*O. floribundum* B at 100 mg/kg), while *O. glandulosum* achieved only 57.37% at the same dose.

These findings highlight the strong potential of *Origanum* essential oils, particularly *O. floribundum*, for various biological applications, contributing to the valorization of Algerian plant resources.

Keywords: *Origanum*, essential oils, acaricidal activity, antioxidant activity, anti-inflammatory activity.

Etude de la variabilité phytochimique et biologique du genre *Origanum* en Algérie.

Résumé

Cette recherche vise à valoriser les plantes médicinales et aromatiques endémiques de l'Algérie en explorant la variabilité phytochimique et biologique de trois populations du genre *Origanum* : *O. floribundum* (provenant de Blida et de Guelma) et *O. glandulosum* (provenant de Jijel). Les huiles essentielles ont été extraites par hydrodistillation, analysées par GC-MS, et évaluées pour leurs activités acaricide, antioxydante et anti-inflammatoire.

Les rendements en huile essentielle étaient de 2,9 % (*O. floribundum* G), 1,5 % (*O. glandulosum*), et 0,9 % (*O. floribundum* B). L'analyse chimique a révélé une prédominance de carvacrol dans les trois populations : 43,04 % chez *O. floribundum* B, 40 % chez *O. floribundum* G, et 29,93 % chez *O. glandulosum*. Par ailleurs, *O. glandulosum* présentait des teneurs élevées en γ-terpinène (25,48 %), thymol (12 %) et p-cymène (15,14 %). En revanche, *O. floribundum* montrait des concentrations significatives en cis-hydrate de sabinène (31,56 % à Blida, 22,21 % à Guelma) et en cis-β-ocimène (24,44 % à Guelma, 13,76 % à Blida).

Parmi les trois concentrations a testé, des préparations à 2 % des huiles essentielles d'*O. floribundum* ont démontré une forte activité acaricide (76,79 % pour *O. floribundum* G, 75,89 % pour *O. floribundum* B), surpassant le traitement chimique (Carvacrol) (73,69 %). Cependant, *O. glandulosum* était moins efficace à la même dose (57,37 %).

L'activité antioxydante, mesurée par le test au DPPH, a révélé des valeurs d'IC50 de 54,4 µg/mL (*O. floribundum* B), 66,7 µg/mL (*O. floribundum* G), et 60,4 µg/mL (*O. glandulosum*), démontrant une capacité significative à neutraliser les radicaux libres, bien qu'inférieure à celle de l'acide ascorbique (4,43 µg/mL).

Enfin, l'activité anti-inflammatoire in vivo, évaluée par la méthode de l'œdème induit par la carraghénine, a montré des réductions maximales de 78,25 % (*O. floribundum* G à 100 mg/kg) et 64,05 % (*O. floribundum* B à 100 mg/kg), tandis que *O. glandulosum* n'a atteint que 57,37 % à la même dose.

Ces résultats mettent en évidence le fort potentiel des huiles essentielles d'*Origanum*, en particulier de *O. floribundum*, pour diverses applications biologiques, contribuant ainsi à la valorisation des ressources végétales algériennes.

Mots-clés : *Origanum*, huiles essentielles, activité acaricide, activité antioxydante, activité anti-inflammatoire.

دراسة التباين الكيميائي والبيولوجي لنبات الأوريجانوم في الجزائر.

ملخص

تهدف هذه الدراسة إلى تثمين النباتات الطبية والعطرية المستوطنة في الجزائر من خلال دراسة التباين الفيتوكيميائي والبيولوجي لثلاثة مجموعات من جنس (*Origanum*) : (*O. floribundum*) من البليدة وقالمة و (*O. glandulosum*) من جيجل. تم استخلاص الزيوت الأساسية باستخدام تقنية التقطير المائي وتحليلها بواسطة (GC/MS) ، كما تم تقييم أنشطتها في مكافحة العث، بالإضافة إلى خصائصها المضادة للأكسدة والمضادة للالتهابات.

بلغ مردود الزيوت الأساسية نسبة 2.9% في (*O. floribundum*) قالمة ، 1,5% في (*O. glandulosum*)، و0.9% في (*O. floribundum*) البليدة. التحاليل الكيميائية اظهرت هيمنة المركب (carvacrol) في جميع المجموعات بنسبة 43.04% في (*O. floribundum*) البليدة، و40% في (*O. floribundum*) قالمة، و29,93% في (*O. glandulosum*). كما احتوى (*O. glandulosum*) على نسب مرتفعة من (γ-terpinène) 25,48% و (thymol) 12% و (p-cymène) 15,14%. أما (*O. floribundum*) فقد تميز بتراكيز عالية من (cis-sabinene hydrate) 31,56% في البليدة و 22,21%.في قالمة. و (cis-β-ocimene) 24,44% في قالمة ، و 13,76% في البليدة.

من بين التراكيز الثلاثة المختبرة، أظهرت المستحضرات بنسبة 2% من الزيوت الأساسية لنبات (*O. floribundum*) نشاطاً قوياً ضد العث (*Varroa destructor*)، حيث بلغ نسبة 76.79% ل (*O. floribundum*) قالمة و75.89% لـ (*O. floribundum*) البليدة، متفوقاً على العلاج الكيميائي (Bayvarol) الذي سجل 73.69%. بالمقابل، أظهر (*O. glandulosum*) فعالية أقل عند نفس التركيز بنسبة 57.37%.

بالنسبة للنشاط المضاد للأكسدة، والذي تم قياسه باستخدام اختبار تحييد الجذور الحرة (DPPH)، فقد أظهرت الزيوت الأساسية قيمًا لتركيز التثبيط عند 50% (IC50) بلغت 54.4 ميكروغرام/مل في (*O. floribundum*) البليدة، و66.7 ميكروغرام/مل في (*O. floribundum*) قالمة، و60.4 ميكروغرام/مل في (*O. glandulosum*)، مما يدل على قدرة جيدة على تحييد الجذور الحرة، لكنها أقل فعالية من حمض الأسكوربيك (4.43 ميكروغرام/مل).

أخيرًا، أظهر النشاط المضاد للالتهابات*، والذي تم تقييمه باستخدام طريقة الوذمة المحفزة بالكارجينين في قدم الجرذ، اعلى انخفاض سجل بنسبة 78.25% في (*O. floribundum*) عند جرعة 100 ملغ/كلغ، و بنسبة 64.05 % في (*O. floribundum*) البليدة عند نفس الجرعة، بينما سجل (*O. glandulosum*) انخفاضًا بنسبة 57.37%.

تظهر هذه النتائج الإمكانيات الكبيرة للزيوت الأساسية لجنس (*Origanum*) ، خاصةً (*O. floribundum*)، في التطبيقات البيولوجية المختلفة، مما يساهم في تثمين الموارد النباتية الجزائرية.

الكلمات المفتاحية : الأوريغانوم، الزيوت الأساسية، النشاط المبيد للقراد، النشاط المضاد للأوكسدة، النشاط المضاد للالتهابات.

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List of Abbreviations

APG: Angiosperm Phylogeny Group

COX: Cyclooxygenase

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

EO: Essential Oil

GC/MS: Gas Chromatography-Mass Spectrometry

GSH: Glutathione

GPx: Glutathione Peroxidase

HD: Hydrodistillation

ROS: Reactive Oxygen Species

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Introduction

Introduction

Alongside the development of the chemical sector, the manufacture of chemical drugs has become more easily achievable, leading to a decrease in interest in plant-derived components. However, the undesirable effects caused by these drugs have recently revived interest in botanical components (Karagöz, 2019). Nowadays, the demand for natural products derived from medicinal and aromatic plants, as substitutes for artificial additives and as pharmacologically active agents, has significantly increased (Atanasov *et al.*, 2015). This is attributed to the lower frequency of adverse effects observed in herbal medicines compared to chemical drugs.

Among various natural products, essential oils (EOs) have gained immense popularity in diverse industries, including food, cosmetics, and pharmaceuticals, due to their remarkable characteristics such as strong odor, unique colors, and high volatility (Carvalho *et al.*, 2016; Khan *et al.*, 2019). Particularly, essential oils play a significant role in the healthcare sector due to their remarkable biological activities directly associated with the biologically active components of their essential oils (Raut & Karuppayil, 2014; Khan *et al.*, 2019).

Many genera within the *Lamiaceae* family are well known for their applications in ethnobotanical practices (Ibadullayeva *et al.*, 2012). Within this family, *Origanum* comprises approximately 40 species, naturally distributed across different parts of the world, including the Mediterranean, Central Asia, the Arabian Peninsula, North Africa, and Europe.

In ancient times, *Origanum* species were used for various medicinal purposes, including their mental purifying properties, beneficial effects on vision, ability to alleviate digestive disorders, and their use in the treatment of venomous insect stings such as spiders and scorpions (Ozdemir *et al.*, 2018).

Essential oils from the family *Lamiaceae*, renowned for their biological activities and diverse applications in the food, cosmetic, and pharmaceutical industries, display significant variation in their chemical composition and essential oil polymorphisms for various reasons (Keefover-Ring *et al.* 2009). These reasons include ecological and environmental effects, as well as genetic variations (Vokou *et al.*, 1993; Moghaddam & Mehdizadeh, 2017). Furthermore, other factors, including available nutrients (nitrogen, water, and minerals), photoperiod, radiation, and temperature, also have a significant effect on the content and quality of essential oils (Kokkini *et al.*, 1994).

Therefore, a comparative study of *Origanum* species from different regions would be beneficial to explore their chemical diversities, influenced by species and spatiogeographical factors, and their impact on the variation of biological activities. This approach would help answer the central question: Do the essential oils of *Origanum* genus from Algeria show significant variation in their chemical composition? If so, does this variation impact their biological activities? In this context, the present study aimed to characterize the chemical composition of essential oils belonging to three Algerian populations of the *Origanum* genus, originating from different bioclimatic and geographical zones of Algeria: *Origanum floribundum Munby* from Blida, *Origanum floribundum Munby* from Guelma, and *Origanum glandulosum (Desf.*) from Jijel. Following the identified phytochemical profile of the essential oils, the evaluation of their biological activities was conducted, primarily the acaricidal activity against the *Varroa destructor* parasite of domestic honeybees (*Apis mellifera*), using the method of studying natural *Varroa* falls. Additionally, the antioxidant activity of the essential oil was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test, and finally, the in vivo evaluation of the anti-inflammatory activity of the essential oils was conducted using the carrageenan-induced edema method.

Chapter 1: Literature Review

1.1. Overview of the Genus *Origanum*.

1.1.1. Etymology and Taxonomic Classification

Origanum is a Latin term derived from the Greek word *origanon*, composed of *oros* ("mountain") and *ganos* ("radiance" or "shining beauty"). This etymology highlights the plant's natural adaptation to Mediterranean mountainous regions (Caillaud, 2013).

1.1.1.1. Taxonomic Position

The classification of species within the genus *Origanum* has been studied by several researchers. In this work, the most recent classification, proposed by the APG system (2009), is adopted (Table 1) (Tela Botanica, 2024).

Table 1. Systematic classification of the genus *Origanum* according to APG III (Tela Botanica, 2024).

Rank	**Scientific Name**
Clade	Angiosperms
Clade	Eudicotyledons
Clade	Core Eudicots
Clade	Asterids
Clade	Lamiids
Order	Lamiales
Family	Lamiaceae
Genus	*Origanum*

1.1.2. Botanical Description

The most recognized classification of the *Origanum* genus is that of Ietswaart (1980), after the genus was subjected to numerous complex classifications. Ietswaart based his classification on morphological characteristics such as stem length, arrangement, number and length of branches, as well as leaf shape. He divided the genus into 3 groups, 10 divisions, 38 species, 6 subspecies, and 16 hybrids. Since its publication, the genus has expanded with the addition of at least five new species and an additional hybrid (Kintzios, 2002).

The distinctive characteristics of the *Origanum* genus according to Ietswaart (1980) are :

Stems: the lower portions are generally woody and persistent. Several upright or ascending stems are present, bearing lateral branches on the upper quarter or half, with very variable length from 10 to 60 cm; most stems have hairs, at least at the base in all species; the hairs are simple (Figure 1 A).

Leaves: sessile, subsessile or petiolate especially at the lower nodes; the petiole reaches a quarter or half of the blade dimension, the hairs carried by the leaves and stems are identical. The leaves may also be more or less glabrous, in which case they are almost always glaucous because they are covered by a thin layer of wax (Figure 1 B). The leaves bear sessile or pedunculate secretory pouches. These secretory glands are also present on stems, bracts, calyces, and corollas.

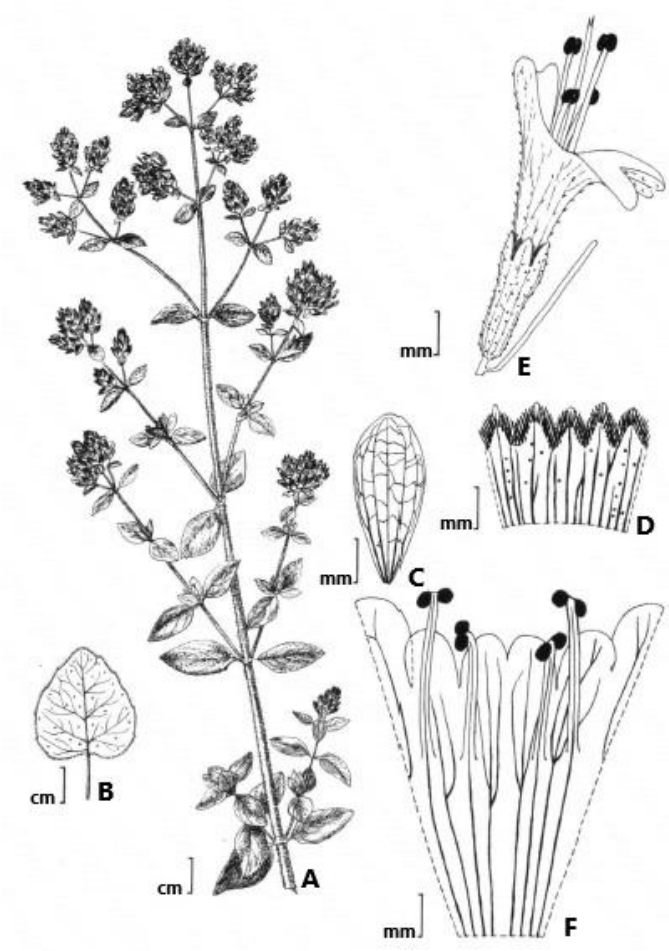
Inflorescences: carried by each stem and each branch; the panicle appearance will depend on the number of branches, the bracts are rounded, oval or lanceolate; the smallest ones resemble leaves, the largest are thin and membranous, often purple or yellow-green (Figure 1 C). Many variations are possible in the size of inflorescences and/or bracts; these variations allow, among other things, to differentiate the sections.

Calyx: the most variable part, in the *Origanum* genus, it has 5 teeth more or less fused or is formed by one or two more or less toothed lips (Figure 1 D). The classification into different sections also involves the distinctive characteristics of the calyx.

Generally, the corolla in the shape of a tube is upright with 2 lips from 3 to 14 mm, its color is white, pink, or purple.

The stamens can be of very different shape and size and are adapted to insect pollination.

The fruits are ovoid achenes, brown, measuring 1 to 5 mm long and 0.5 mm wide.



A. habit; **B.** leaf; **C.** bract; **D.** calyx cut through the lower lip; **E.** flower with bract in side view; **F.** corolla cut through the lower lip

Figure 1. Illustration of *O. vulgare ssp. vulgare*. (Ietswaart, 1980).

1.1.3. Geographical Distribution

The genus *Origanum* has a wide geographical distribution, ranging from the Canary Islands and the Azores to Northern Europe and East Asia (Figure 2). It can also be found in cultivation in Cuba and the Reunion Island. However, it is in the Mediterranean region where the most significant distribution area for the genus *Origanum* is observed.

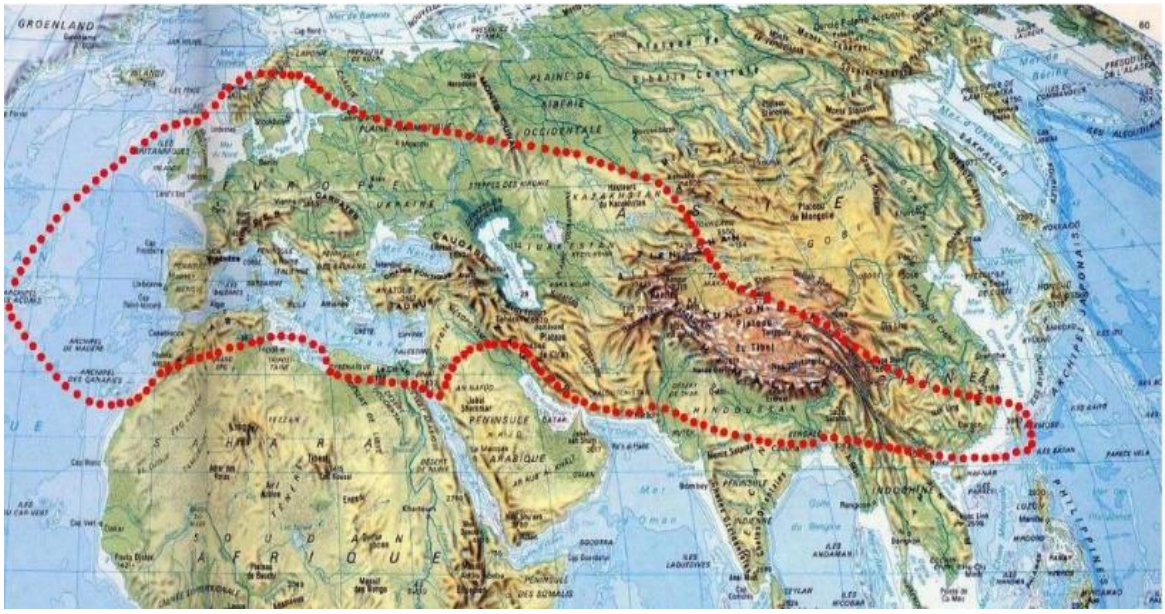


Figure 2. Distribution area of the genus *Origanum* (Ietswaart, 1980).

Some species are specific to certain countries, including Turkey, which is considered the genetic center of the *Origanum* genus with sixteen species present. Additionally, Turkey also plays a significant role as the genetic center of the *Lamiaceae* family (Figueredo, 2007).

1.1.4. *Origanum* Species in Algeria

In Algeria, the genus *Origanum* is commonly referred to by its vernacular name (**زعتر**) *zaatar*. However, this term can be ambiguous, as it is also used to designate the genus *Thymus*. In regions where both genera coexist, the terms *zaatar* and *zaaitra* are employed to distinguish them. Specifically, plants of the genus *Origanum*, characterized by larger leaves, are called *zaatar*, while those of the genus *Thymus*, with smaller leaves, are referred to as *zaaitra*. In areas where only one genus is present, the term *zaatar* is used without distinction between the two genera.

Algeria is home to two species of oregano: *Origanum vulgare* ssp. *glandulosum* and the endemic *Origanum floribundum Munby*. The latter, an endemic plant of Algeria, thrives in pastures and mountainous areas up to an altitude of 1500 meters (d'Alger & de Paris, 2012). In contrast, *Origanum vulgare* ssp. *glandulosum* is endemic to the Algiers-Tunisian region (Quezel *et al.,* 1962). These two species reflect the widespread presence of oregano in Algeria.

The geographic distribution of *Origanum* taxa across Mediterranean countries is shown in (Figure 3), which highlights their natural presence in the region (Padulosi, 1996).

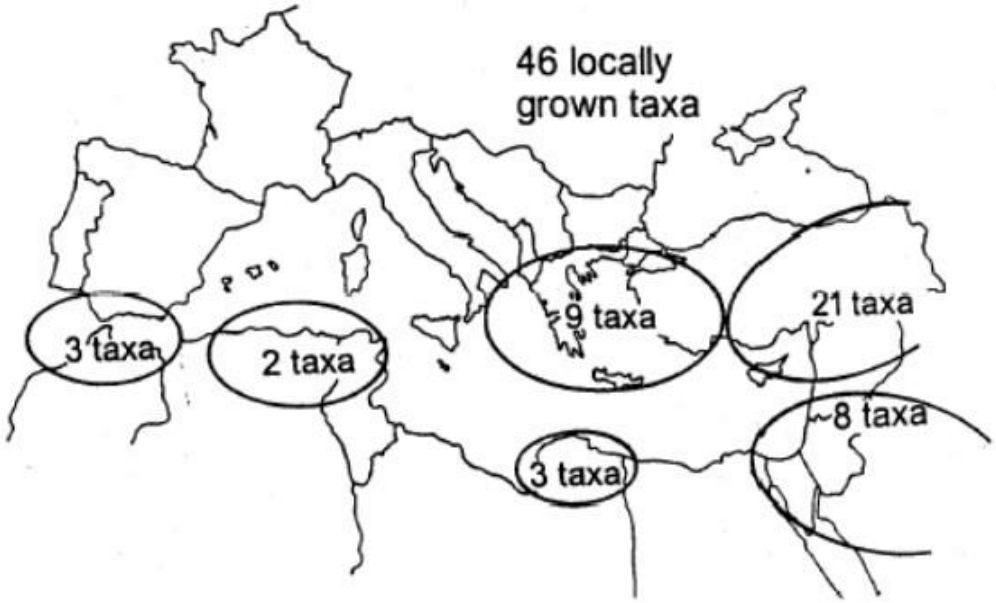


Figure 3. The natural presence of *Origanum* taxa in Mediterranean countries (Padulosi, 1996).

The genus *Origanum* is widely distributed in Algeria, as summarized in (Table 2). Regarding the species present, *Origanum glandulosum* occurs in Guelma (Nechmaya) (Bouhaddouda, 2016; Mahfouf *et al.,* 2018), Constantine, Jijel (Berrehal *et al.,* 2010), and Boumerdès, particularly in the Guerrouma mountain (Bendifallah *et al.,* 2015). Its presence has also been reported in Sétif (Aïn El Kebira, Aïn Abassa, Aïn Roua, Bougaâ, Bouandas, Babor, and Béni Mouhli) (Sari *et al.,* 2006; Ali *et al.,* 2020), Béjaïa (Kherrata, El K'Ser, Teskriout), Bordj Bou Arréridj (Bordj El Ghadir), Biskra, and M'Sila (Sari *et al.,* 2006). Additionally, it has been documented in Sebdou, within the Tlemcen region (Bendahou *et al.,* 2008), as well as in Blida (Ouled Slama, Souhane) and Béjaïa (Ighzer Amokrane) (Hazzit *et al.,* 2006).

Origanum floribundum is found in Blida, particularly in Chréa and Hammam Melouane (Hazzit *et al.,* 2006; Boulaghmen, 2012), in Bouira (Lakhdaria) (Mir *et al.*, 2022), and in Aïn Defla (Brada *et al.,* 2012). It is also mentioned in the Tizi-Ouzou region (Bouhaddouda, 2016).

Table 2. Geographic Distribution of *Origanum* Species in Algeria.

Species	Region(s)	References
Origanum glandulosum	Guelma (Nechmaya), Constantine, Jijel, Boumerdès (Guerrouma), Sétif (Ain El Kebira, Ain Abassa, Ain Roua, Bougâa, Bouandas, Babor, Beni Mouhli), Béjaïa (Kherrata, El K'Ser, Teskriout), Bordj Bou Arréridj (Bordj El Ghadir), Biskra, M'sila, Sebdou (Tlemcen), Blida (Ouled Slama, Souhane), Béjaïa (Ighzer Amokrane)	Sari *et al.,* 2006; Hazzit *et al.,* 2006; Bendahou *et al.,* 2008; Berrehal *et al.,* 2010; Bendifallah, 2015; Bouhadouda, 2016; Mahfouf, 2018; Ali, 2020.
Origanum floribundum	Blida (Chréa, Hammam Melouane), Bouira (Lakhdaria), Ain Defla, Tizi-Ouzou	Baser, 2000; Hazzit *et al.,* 2006; Boulaghnem, 2012; Brada, 2012; Bouhadouda, 2016; Mir, 2022.

1.1.4.1. Morphological Characteristics of *Origanum* Species in Algeria

The *Origanum* species found in Algeria exhibit a range of morphological traits, with upright or decumbent stems and an herbaceous to woody growth habit. These plants adapt to various altitudinal ranges and substrates, particularly favoring limestone soils. Their flowers are hermaphroditic and arranged in inflorescences, while the fruits are achenes or tetra-achenes. Notably, certain species, such as *Origanum* vulgare L. subsp. *glandulosum*, possess secretory glands that produce essential oils, contributing to their characteristic aroma (Quezel *et al.,* 1962; Gallouin & Arvy, 2003; Teuscher *et al.,* 2005).

1.1.4.1.1. Morphological Characteristics of *Origanum floribundum Munby*

Origanum floribundum Munby is distinguished by its prostrate stem at the base, with young decumbent and quadrangular shoots (Quezel *et al.,* 1962). Its branches are short, semi-persistent, green, and pubescent. The root system comprises a woody rhizome and adventitious roots, ensuring a strong anchorage in high-altitude regions (Daoudi & Dahmani, 2013). The flowers are loosely arranged in spikes, and the calyx has five short teeth, while the corolla features lips of approximately equal size. The fruit is a blackish, smooth tetra-achene (Quezel *et al.,* 1962; Daoudi & Dahmani, 2013).

1.1.4.1.2. Morphological Characteristics of *Origanum vulgare L. subsp. Glandulosum*

Origanum vulgare L. subsp. glandulosum is an herbaceous plant measuring 30 to 60 cm in height. Its stems are upright, reddish, and covered with white hairs. The leaves are oval, veined on the underside, glandular, and moderately petiolate. The plant is notable for its golden-yellow secretory glands on the bracts, calyx, and corolla, which produce essential oils, giving it a spicy, phenolic aroma (Teuscher *et al.*, 2005; Figueredo, 2007). The flowers are white or pink, bilabiate, and clustered in inflorescences. The calyx is cylindrical and persistent, with five small teeth, while the fruit consists of smooth achenes (Quezel *et al.*, 1962; Teuscher *et al.*, 2005; Caillaud, 2013).

1.1.5. Ecological and Growth Conditions for *Origanum*

Oregano is a plant that exhibits a certain tolerance to cold and drought, making it resilient and capable of withstanding frost while playing a crucial role in soil protection on sloping terrain. In the wild, it is predominantly found on the slopes of Mediterranean countries, at higher altitudes, in average-quality soils, and it prefers cooler summers. That's why it can be found even in mountainous regions. In harsher climates, the aboveground parts of the plant are destroyed during winter, but the root system withstands and regenerates from renewal buds. In milder regions, oregano maintains its vegetation throughout the winter (Kintzios, 2002; Rameau *et al.*, 2008).

Oregano is a heliophilous or semi-shade species, but it is also a plant that thrives in long-day conditions. Indeed, the photoperiod influences the plant's growth and floral differentiation. Thus, a plant growing under twelve to sixteen hours of light per day reaches its full floral differentiation stage within sixteen to nineteen days. These same plants are also more vigorous and have a larger leaf area. Similarly, the amount of sunlight received by the plant determines its pungency, which is proportional to the received sunlight (Padulosi, 1996; Gallouin & Arvy, 2003; Rameau *et al.*, 2008).

1.1.5.1. Soil Requirements

Oregano is a plant that thrives in dry, calcareous soils with ample sunlight, making it a mesoxerophytic species. However, it can be cultivated in various soil types. As a result, it is grown worldwide, including in countries such as India and Mexico. Additionally, *Oregano* was introduced in Finland as early as the 1600s and has become a popular decorative plant in Finnish gardens. (Gallouin & Arvy, 2003).

Oregano grows quite easily in the wild, with an optimal pH of 6.8. It is particularly found on sloping soils, where the plant serves as a protective element while also being resistant to cold and drought conditions. Thus, during winter, the above-ground parts of the plant are destroyed, unlike the roots that maintain their vitality to stabilize the soil and allow the plant to regenerate in spring (Kintzios, 2002; Gallouin & Arvy, 2003).

1.1.5.2. Climatic Characteristics of the Regions of Origin of the Studied Populations

The species studied in this work originate from three regions: Blida (Chréa), Jijel (Ghebala), and Guelma (Nechmaya). Detailed descriptions of these areas are provided in the Study Area section of the (Materials and Methods chapter). Below is an overview of the climatic conditions characterizing these regions.

Blida features a temperate warm climate, classified as Csa according to the Köppen and Geiger classification. The average annual temperature is 17.1 °C, with an average annual precipitation of approximately 641 mm (Climate Data, 2021). The mountainous region of Chréa, however, experiences a rigorous and humid climate. The wet season, which includes precipitation, snow, and cloudiness, typically extends from mid-September to April, interspersed with dry periods. While this area is Mediterranean, its humid character is strongly influenced by altitude and the presence of the Atlas Mountain range, making Chréa the rainiest region in Algeria (Dorleans, 1972).

Guelma is characterized by sub-humid microclimates in the central and northern regions, alongside a semi-arid climate in the southern part. This diversity arises from factors such as increased humidity levels, proximity to the sea (60 km), the presence of the Seybouse River, vast forested massifs, thermal springs, and dams (Medjelekh, 2006).

Nechmaya exhibits a warm and temperate climate, also classified as Csa according to the Köppen-Geiger classification. The average annual temperature is 16.7 °C, with an annual precipitation of 623 mm (Climate Data, 2021).

Ghebala, located southeast of the Jijel province and approximately 35 kilometers from the coast, also features a warm temperate climate. It experiences higher precipitation in winter compared to summer and is classified as Csa under the Köppen and Geiger system. The average annual temperature in Jijel is 18.1 °C, with an average annual precipitation of about 982 mm (Climate Data, 2021)

1.2. Essential oils

Essential oils are volatile and aromatic liquids extracted from plant materials such as flowers, roots, bark, leaves, seeds, fruits, or entire plants (Hyldgaard *et al.,* 2012). The extraction of these oils from aromatic plants dates back to antiquity. Historically, they have been employed in various domains, including medicine, perfumery, and religious rituals. Ancient Egyptians utilized essential oils for mummification and medicinal treatments, while Asian and Mediterranean civilizations developed sophisticated knowledge of perfumery and therapeutic applications (Sonwa, 2000). During the Middle Ages, essential oils gained prominence in Europe through the efforts of alchemists and monastic institutions, culminating in their extensive use in perfumery and cosmetics during the Renaissance (Sonwa, 2000).

In contemporary science, essential oils are rigorously defined and studied, emphasizing their composition and extraction methods. They are highly volatile substances primarily obtained through steam distillation or mechanical expression for citrus fruits. According to the French Pharmacopoeia, essential oils are characterized by their complex composition and plant origin. These oils naturally occur in plant cells as aromatic droplets within the cytoplasm (Sonwa, 2000; Figueredo, 2007). Their extraction must preserve their natural composition without significant alterations (Figueredo, 2007).

1.2.1. Distribution and Roles of Essential Oils in Plants:

Essential oils are predominantly found in higher plants, particularly in aromatic species belonging to families such as *Myrtaceae*, *Lauraceae*, *Rutaceae*, *Lamiaceae*, *Asteraceae*, and others (Lawrence, 1995; Bruneton, 1999). These oils are synthesized and stored in specific plant organs, including undifferentiated cells (*Lauraceae*) and specialized secretory structures such as glandular hairs (*Lamiaceae*, *Asteraceae*), secretory canals (*Myrtaceae*, *Rutaceae*), and cavities (Conifers) (Werker *et al.,* 1993). Their distribution spans vegetative and reproductive organs, with significant concentrations in floral parts (e.g., lavender, mint), leaves (e.g., *eucalyptus*, *laurus*), fruits (e.g., anise, star anise), seeds (e.g., nutmeg), bark (e.g., cinnamon), wood (e.g., *Santalum*), roots (e.g., vetiver), and rhizomes (e.g., *Zingiber*) (Belaiche, 1979).

In addition to their structural presence, essential oils fulfill multifaceted ecological and physiological roles. They facilitate pollination and chemical communication, acting as

attractants for pollinators or as defense mechanisms against predators such as microorganisms, fungi, and herbivores (Bruneton, 1999; Figueredo, 2007). Their antioxidant properties help protect plants from environmental stress by regulating oxidation reactions, while some constituents, such as 1,8-cineole and camphor, inhibit the germination of infected organs and pathogen growth (Nicholas, 1973). Furthermore, essential oils may serve as metabolic intermediates or reserve energy sources during periods of reduced chlorophyll assimilation (Lutz, 1940; Armand, 1972). These diverse roles underline the importance of essential oils in mediating plant-environment interactions.

1.2.2. Chemical composition and physical properties of essential oils

Essential oils are complex natural compounds that exhibit a wide range of chemical diversity, typically composed of 20 to 60 components present in varying concentrations. These oils are characterized by the dominance of two or three major components, occurring at relatively high levels (20-70%), while the remaining constituents are present in trace amounts (Croteau *et al.,* 2000; Betts, 2001; Bowles, 2003; Pichersky *et al.*, 2006).

The constituents of essential oils can be classified into two major groups based on their biosynthetic pathways: terpenoids, which encompass a diverse array of terpene compounds, and phenylpropanoids (Buchanan *et al.*, 2000).

- The group of terpenoids, also known as terpenes, constitutes a widely distributed family of compounds in the plant kingdom. These compounds are formed through the combination of a set of five carbon atoms (C5) known as isoprene. This group is subdivided into two distinct subgroups: monoterpenes and sesquiterpenes.
- The group of phenylpropanoids, also referred to as aromatic compounds, they are much less common than terpenoids. They encompass several chemical functions, including alcohols, phenols, methoxy derivatives, and methylenedioxy compounds.

Essential oils are liquids that emit a highly pronounced aromatic odor at room temperature. They have an oily but non-greasy consistency and are volatile, which distinguishes them from fixed oils. Generally, their density is lower than that of water, ranging from 0.850 to 0.950. However, cinnamon and clove oils have densities of 1.025-1.070 and 1.044-1.057, respectively. Their boiling points range from 160°C to 240°C. Although they are steam-distillable, essential oils are highly insoluble in water. Instead, they are soluble in alcohols, fixed oils, and most organic solvents. It is important to note that essential oils readily oxidize

in the presence of light and resinify upon oxygen absorption. Consequently, their odor changes, their boiling point increases, and their solubility decreases (Bruneton, 1999; Baser & Buchbauer, 2010).

1.2.2.1. Chemical Composition of *Origanum* Essential Oils

The essential oils of plants belonging to the *Origanum* genus are dominated by two main chemotypes. The first is rich in phenols, predominantly thymol and/or carvacrol, which are monoterpenic phenols with well-documented biological properties. The second chemotype is rich in monoterpene hydrocarbons, with p-cymene and/or γ-terpinene as the major constituents, often identified as precursors of phenols.

In addition to these primary components, *Origanum* essential oils contain other chemical compounds in varying proportions. Among the monoterpene hydrocarbons, camphene, limonene, myrcene, and cis- and trans-ocimene are frequently present. Oxygenated monoterpenes include, besides phenols (carvacrol and thymol), linalool, α-terpineol, trans-sabinene hydrate, terpinene-4-ol, and borneol. The oils also contain sesquiterpene hydrocarbons such as β-caryophyllene, germacrene-D, bicyclogermacrene, and β-bourbonene, along with oxygenated sesquiterpenes like spathulenol, elemol, and α-cadinol (Baser *et al.,* 2000; Sari *et al.,* 2006; Boulaghmen, 2012; Brada *et al.,* 2012; Bouhaddouda, 2016; Ali *et al.*, 2020).

1.2.2.2. Biological Properties of *Origanum* Essential Oils

The essential oils of *Origanum* species are widely recognized for their broad spectrum of biological activities, attributed to their diverse composition, including phenols such as thymol and carvacrol, as well as monoterpene hydrocarbons like p-cymene and γ-terpinene, and other constituents (Zielinska-Błajet & Feder-Kubis, 2020).

- **Antioxidant Activity**: *Origanum* essential oils exhibit significant antioxidant potential, widely documented in various studies conducted on Algerian and Mediterranean populations (Sari *et al.,* 2006; Boulaghmen, 2012; Lombrea *et al.,* 2020).
- **Antimicrobial and Antifungal Activities**: The antimicrobial properties of these oils have been demonstrated against numerous bacterial and fungal strains. They are particularly effective due to the predominance of phenolic monoterpenes (Bouhaddouda, 2016; Marrelli *et al.,* 2018).

- **Anti-inflammatory Activity**: Studies highlight the anti-inflammatory potential of *Origanum* oils, supported by the presence of key bioactive compounds (Lombrea *et al.,* 2020).
- **Antitumoral and Antiproliferative Activities**: Research has shown that *Origanum* essential oils possess anticancer properties, with several studies reporting their effects on various tumor cell lines (Ali *et al.,* 2020; Lombrea *et al.,* 2020; Sharifi-Rad *et al.,* 2021).
- **Other Activities**: In addition to their therapeutic effects, *Origanum* essential oils also display insecticidal, antiparasitic, and wound-healing properties, broadening their application potential (Karpouhtsis *et al.,* 1998; Boulaghmen, 2012; Lombrea *et al.,* 2020).

These biological activities underscore the therapeutic potential of *Origanum* essential oils and their versatility for applications in pharmaceuticals, agriculture, and other industries.

1.2.3. Extraction methods of essential oils.

The process of obtaining essential oils from diverse plant sources involves several extraction methods, each tailored to specific botanical materials and their states. This extraction process significantly impacts the overall quality of the essential oil. Therefore, careful consideration must be given to selecting appropriate extraction techniques to avoid any potential damage or alteration to the essential oil's chemical composition, which could result in diminished bioactivity and loss of its natural characteristics. In severe instances, this may lead to undesired effects like discoloration, off-odor/flavor, or changes in physical attributes, such as increased viscosity. Consequently, it is imperative to ensure that the extraction of essential oils is conducted with utmost care and precision, employing various suitable means to achieve optimal results (Tongnuanchan & Benjakul, 2014).

Several techniques are available to achieve this objective, including commonly employed methods like hydro-distillation (HD), steam distillation, cold pressing (CP), solvent extraction, and simultaneous distillation-extraction techniques, among others (Chemat, 2010).

1.2.3.1. Conventional Extraction Methods

1.2.3.1.1. Cold extraction

Cold pressing, one of the oldest methods for obtaining essential oils, is particularly suitable for citrus peels. This technique minimizes heat exposure, preserving the integrity of

thermally unstable compounds like aldehydes. However, it yields essential oils mixed with non-volatile substances, such as coumarins and pigments, requiring further purification processes for obtaining pure oils (Kubeczka, 2010; Van Doosselaere, 2013).

1.2.3.1.2. Distillation

Distillation is a traditional and widely used method for isolating essential oils from plant materials. It involves exposing plant material to boiling water or steam, causing volatile compounds to vaporize. These compounds are condensed into a liquid mixture, which naturally separates into two layers based on density: water and essential oil. Precise temperature control during this process prevents thermal degradation of sensitive compounds (Thoppil, 2004; Sell, 2006).

Three main types of distillation are distinguished based on the interaction between water and plant material: hydrodistillation, steam distillation, and water/steam distillation (Mendes *et al.*, 2007).

- a) **Hydrodistillation**: In hydrodistillation, plant material is fully immersed in boiling water, facilitating direct contact with the heat source. The vaporized mixture is condensed and separated, with the lighter essential oil collected. This method is cost-effective and preserves thermally sensitive components, making it a standard choice for aromatic plants (Kubeczka, 2010).
- b) **Steam distillation:** Steam distillation employs externally generated steam that passes through plant material placed on a perforated tray. This method avoids direct contact with boiling water, reducing the risk of overheating while ensuring effective extraction of essential oils (Baser & Buchbauer, 2010).
- c) **Water/steam distillation:** In this method, also known as hydrodiffusion, steam is generated within the same container as the plant material, rather than being supplied externally. This slight variation allows for efficient extraction while minimizing the energy required (Benjilali, 2004).

1.2.3.1.3. Solvent extraction.

This method employs organic solvents like hexane or ethanol to extract essential oils, especially from delicate floral materials. While effective in preserving aromatic profiles, it carries risks of contamination and the loss of volatile compounds during solvent evaporation. Additionally, its high cost and long processing time limit its application(Nakatsu *et al.*, 2000; Tongnuanchan & Benjakul, 2014).

1.2.3.2. Alternative Extraction Methods

To enhance efficiency and preserve volatile compounds, alternative extraction methods have been developed, including (Camel, 2001; Pereda *et al.*, 2007; Pingret *et al.*, 2013):

- **Supercritical Fluid Extraction (SFE)**: Utilizes supercritical CO_2 for selective and environmentally friendly extraction (Zizovic *et al.*, 2007).
- **Microwave-Assisted Extraction (MAE)**: Employs microwave radiation to reduce processing time and energy consumption (Vian *et al.*, 2008).
- **Ultrasound-Assisted Extraction (UAE)**: Uses ultrasonic waves to disrupt cell walls and improve solvent penetration (Wang & Weller, 2006).

While these advanced techniques offer specific advantages, they are less commonly employed due to their cost, complexity, and specialized requirements.

1.3. Biological activities

Essential oils are studied for their various biological properties. This work examines three key activities: acaricidal, antioxidant, and anti-inflammatory. These activities highlight the potential of *Origanum* essential oils in addressing specific challenges in health and agriculture.

1.3.1. acaricidal activity

1.3.1.1. Etymology and Systematic Position of the Mite *Varroa destructor*

1.3.1.1.1. Systematic Position (Anderson & Trueman, 2000)

A persistent ambiguity existed in the classification of *Varroa* species until the work of Anderson and Trueman (2000), who distinguished *Varroa destructor* based on morphological and genetic criteria. This systematic revision is now widely accepted (Rosenkranz *et al.*, 2010).

Phylum: Arthropoda

Subphylum: Chelicerata

Class: Arachnida

Order: Acari

Suborder: Mesostigmata

Family: Dermanycidae (Gamassidae)

Subfamily: Varroinae

Genus: *Varroa*

Species: *Varroa destructor*

1.3.1.1.2. Taxonomy

The emergence of the mite parasite dates back to the period following World War II, when several European honeybee varieties of *Apis mellifera* were imported to Indonesia to improve honey production. As these European bees interacted with local Asian bees, specifically *Apis cerana*, the parasite *Varroa jacobsoni* quickly found a new host. Interestingly, while *Varroa jacobsoni* had little impact on *Apis cerana* and even maintained

an ecological balance with this species (Rath, 1999), it became a significant parasite for *Apis mellifera* (Giovenazzo, 2011). Until 1999, there were three species listed in the genus *Varroa*:

- *Varroa Underwoodi* (described by (Delfinado-Baker & Aggarwal, 1987)).
- *Varroa Jacobsoni* (initially described by (Oudemans, 1904)).
- *Varroa Rindereri* (described by (de Guzman & Delfinado-Baker, 1996)).

It was believed that *Varroa jacobsoni* had successfully transitioned from one host, *Apis cerana*, to another, *Apis mellifera*, spreading to different continents. However, in 2000, Anderson and Trueman distinguished *Varroa destructor* from *Varroa jacobsoni* using morphological and genetic criteria (Rosenkranz *et al.*, 2010; Riva, 2017). The mite responsible for the clinical symptoms of Varroosis in *Apis mellifera* actually belongs to the species *Varroa destructor*, which was commonly referred to as *Varroa jacobsoni* until 2000 (Anderson & Trueman, 2000). Therefore, most writings from the previous century about *Varroa* referred to *Varroa jacobsoni*, while research was actually focused on *Varroa destructor* (Rosenkranz *et al.*, 2010).

1.3.1.2. Morphology and Feeding Behavior

The host-parasite relationship reflects an adaptation of *Varroa destructor* to its host. *Varroa*'s biology is fully adapted to that of the bee, whether in terms of morphology, feeding behavior, development cycle, or means of dispersion (Almecija, 2021).

Varroa mites exhibit distinct sexual dimorphism (Ifantidis, 1983). A common feature in both sexes is the division of the body into two well-defined parts: the idiosoma and the gnathosome. The idiosoma includes the larger part and a dorsal shield along with various ventral shields. Mature female mites, known as foundresses, have a flattened ellipsoidal idiosoma that is wider than it is long (1.6mm in width and 1.1mm in length) and is covered by a dark red cuticle (Mondet *et al.*, 2016). The female's legs are short and stout, featuring specialized structures called claws or apoteles for host adhesion. Both the dorsal and ventral shields are strongly sclerotized, with thin and flexible membranes between them allowing mite movement (Rosenkranz *et al.*, 2010; Mondet *et al.*, 2016). The young female, called a protonymph, is white and round. It develops into a deutonymph, which is more oval in shape. Over time, the cuticle of the deutonymph darkens until reaching sexual maturity (Figure 4) (Almecija, 2021).

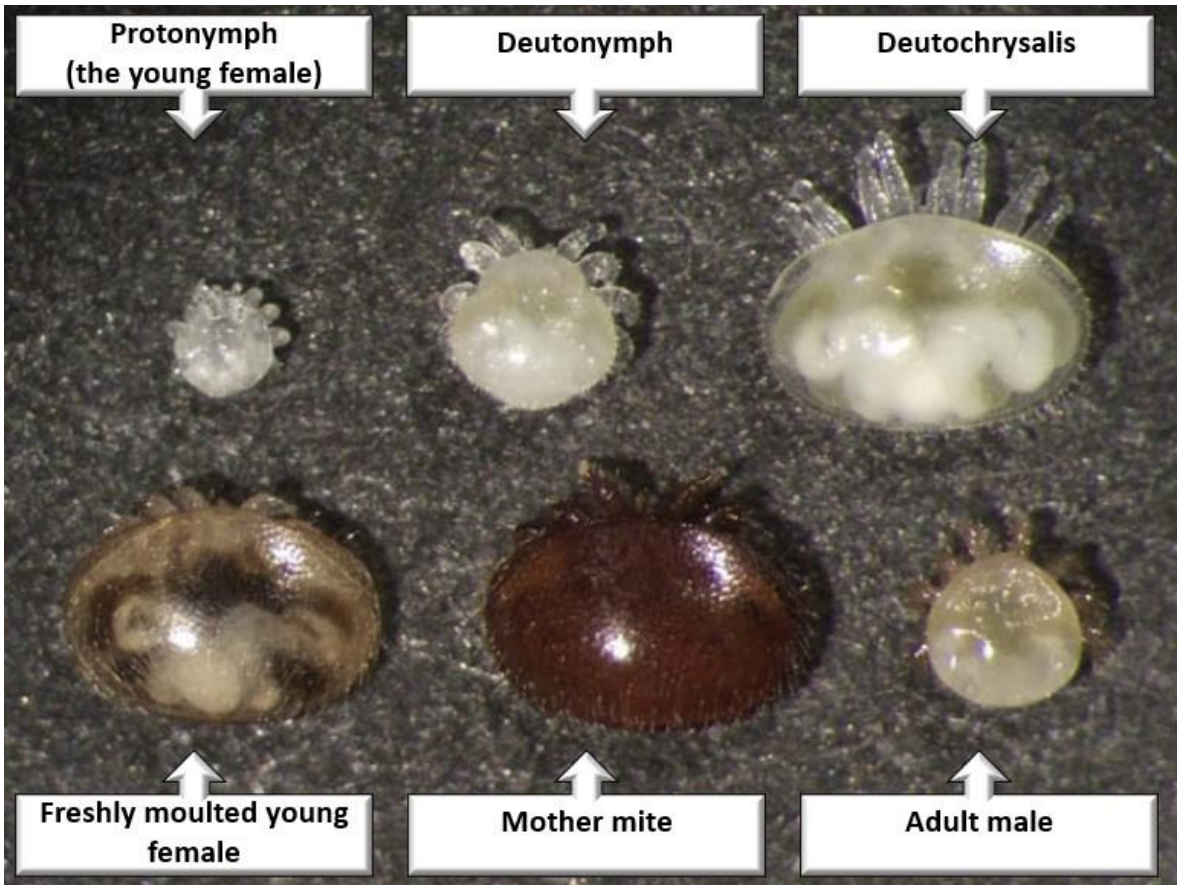


Figure 4. Composition of a *Varroa* family in a capped honey bee worker brood cell. approximately 11 days post-capping (Rosenkranz *et al.,* 2010).

1.3.1.3. *Varroa* development cycle

The phenology of *Varroa destructor* is closely linked to the developmental cycle of the honeybee *A. mellifera*, with females having a lifespan of up to two and a half months in summer (De Ruijter, 1987). The life cycle of *Varroa destructor* females is divided into two distinct phases: a phoretic phase, during which they move using bees as carriers, and a reproductive phase that takes place within the sealed brood cells, whether of workers or drones **(**Figure 5).

Phoresy involves one individual using another for mobility without causing harm. However, the term phoretic *Varroa* does not fully capture the complexity of this situation, and the term dispersion would be more appropriate (Traynor *et al.,* 2020).

During the phoretic phase, adult females of *Varroa destructor* regularly feed by extracting hemolymph and lipids from the host bee (Traynor *et al.,* 2020). *Varroa* preferentially initiates this phase by targeting nurse bees (80%) rather than foragers (20%) (Kuenen &

Calderone, 1997). This preference can be explained by the proximity of nurse bees to the brood, as well as the abundance of lipids in these bees (Ramsey *et al.*, 2019). In the presence of brood, the phoretic phase lasts for a period ranging from 4.5 to 11 days (Fries *et al.*, 1994).

The reproductive cycle of *Varroa destructor* and its ontogenesis occur exclusively inside the sealed brood cells (Rosenkranz *et al.*, 2010).

The process of brood infestation is characterized by the transition of the adult female *Varroa*, commonly referred to as the foundress, from a phoretic lifestyle associated with adult bees to a reproductive lifestyle inside a brood cell shortly before it is sealed. This transition occurs approximately 20 hours for a worker cell and 40 hours for a drone cell. It is worth noting that drone brood attracts between 8 and 11.5 times more *Varroa* mites than worker brood (Almecija, 2021). Some pheromones present in drone brood seem to have enhanced attraction capabilities (Almecija, 2021). In addition to olfactory cues, cell size is a significant parameter for *Varroa* attraction (Almecija, 2021). Before cell sealing, the female *Varroa* conceals itself, remaining immobile and hidden within a mixture of honey and pollen (Traynor *et al.*, 2020).

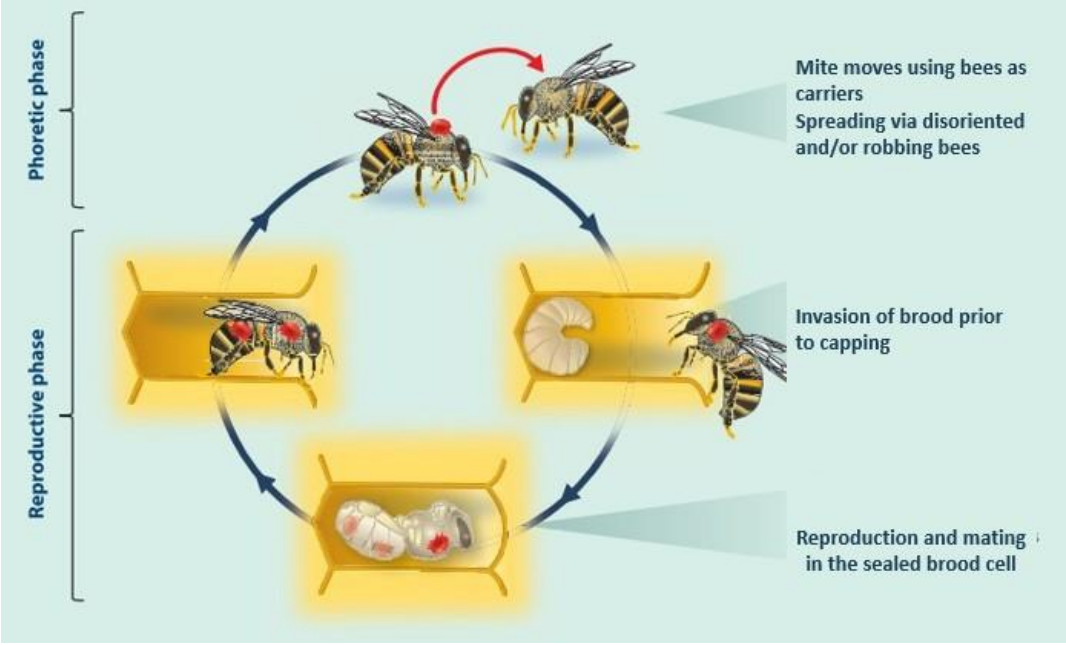


Figure 5. The life cycle of *Varroa destructor* involves phoretic periods on adult bees and reproductive periods in brood cells (Nazzi & Le Conte, 2016).

Ontogenesis takes place on the inner wall of the cell. Approximately 70 hours after cell sealing (Donzé & Guerin, 1994). The foundress lays the first egg, containing a haploid embryo that will develop into a male *Varroa*, all other offspring are young females (Martin,

1994), with an egg-laying rate of one egg every 30 hours (Donzé & Guerin, 1994). Young *Varroa* mites go through two immature stages: the protonymph, initially mobile, becomes immobile shortly before the first molt, and the deutonymph, initially mobile, becomes immobile before the imaginal molt that leads to sexually mature adulthood (Figure 6) (Riva, 2017). The male reaches maturity in approximately 6.6 days after egg-laying, while it takes about 5.8 days for a female *Varroa* to emerge as an adult (Rosenkranz *et al.*, 2010). Considering the egg-laying interval between the first two eggs, the male reaches adulthood about twenty hours before the first female. Consequently, the male waits for the first female to complete its imaginal molt before mating with her. The first mating occurs approximately 230 hours after cell sealing, whether in worker or drone brood (Riva, 2017). All matings take place inside sealed brood cells, with male *Varroa* mites completing their entire life cycle within the cell. *Varroa* reproduction is therefore consanguineous, except in cases of multiple foundress infestations of a brood cell. This co-infestation tendency increases as brood availability decreases toward the end of the season (Beaurepaire *et al.*, 2017). The reproductive phase is interrupted by the emergence of the bee. Only adult female *Varroa* mites (fertilized) survive in the hive, where they parasitize adult bees, while immature females at the time of emergence, as well as males, are destined to die quickly (Rosenkranz *et al.*, 2010). A new female *Varroa* is produced in each reproductive cycle that occurs within a worker bee cell, whereas a foundress performing its cycle in drone brood produces an average of 2.9 young female *Varroa* mites (Riva, 2017).

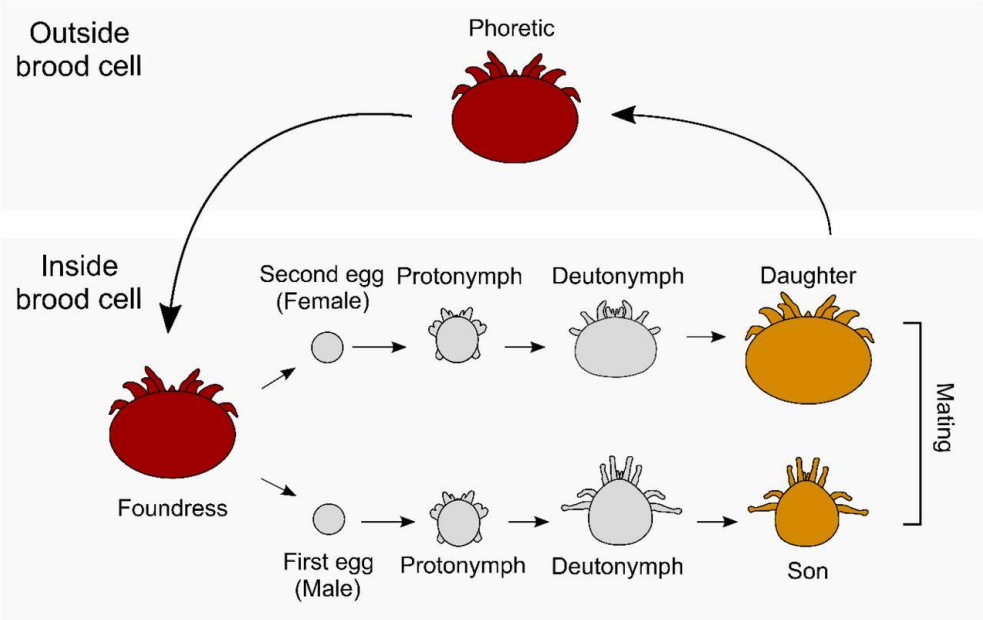


Figure 6. Developmental stages of *Varroa destructor*: egg, protonymph, deutonymph, and adult males and females (McAfee *et al.*, 2017).

1.3.1.4. Devastating Impacts of *Varroa destructor* on Bees and Their Colonies

The infestation of bee colonies by the *Varroa* parasite can have significant consequences, both for the colony as a whole and for each individual bee. The symptoms of varroosis vary depending on the level of infestation (Boecking & Genersch, 2008). Initially, the signs are inconspicuous and become evident only through specific examination. However, as the infestation level increases, more severe symptoms become apparent. For instance, towards the end of summer, when the pressure on the colony is at its peak, the queen's egg-laying decreases, leading to irregular patterns of larvae and high larval mortality. Even a relatively modest infestation, with only 2,000 to 3,600 *Varroa* mites in the autumn (Martin, 2001), can decimate a colony.

After the death of bee colonies, characteristic signs of a gradual depopulation of the hive become evident. These signs include empty hives, with very few dead bees found both inside and in front of the hives, often with small clusters of dead bees on top of the frames, and substantial stores of honey and bee bread. Varroosis thus represents, on a colony-wide scale, the consequences that the parasite can have on each of the individuals comprising the colony (Rosenkranz *et al.*, 2010).

At the individual level, parasitism by the *Varroa* mite has significant consequences. Firstly, it results in a reduced lifespan of bees, especially in the case of winter bees. This decrease in individual longevity can threaten the colony's survival, as a reduced population cannot effectively maintain hive temperature, thereby increasing the risk of colony mortality (Nazzi & Le Conte, 2016; Rosenkranz *et al.*, 2010).

Moreover, the *Varroa* mite impairs the cognitive abilities of bees, disrupting their learning aptitude and their capacity to return to the hive. Infested bees exhibit reduced efficiency in foraging and colony entrance, compromising their role as foragers (Kralj *et al.*, 2007).

Furthermore, the *Varroa* mite has an impact on the immune system of bees by reducing the number of hemocytes, which are immune cells, in infested individuals. This decrease in immunocompetence is accompanied by a reduction in the expression of genes related to immunity, thereby weakening the bees' ability to defend themselves against pathogens. Additionally, *Varroa* acts as a vector for several viruses that affect bees, contributing to the spread of viral diseases among colonies. It promotes the replication of these viruses by weakening the bees' immune system, which has serious implications for colony health (Nazzi & Le Conte, 2016).

1.3.1.5. *Varroa destructor* Control Methods

1.3.1.5.1. Infestation Rate Evaluation

The control of *Varroa destructor* is crucial for the preservation of bee colonies. Accurate assessment of the infestation rate by *Varroa* mites plays an essential role in the implementation of effective management strategies (Le Conte *et al.*, 2010; Rosenkranz *et al.*, 2010; Nazzi & Le Conte, 2016).

Quantitative counting of *Varroa* mites can be conducted through various approaches. The technique of sticky boards, known as natural mite drop, involves counting the *Varroa* mites that naturally fall to the bottom of the hive over a specific period. A greased board is placed beneath the hive to collect the mites. This method has the advantage of not disturbing the colony but requires observations spread over several days. It should be noted that the number of mites falling varies from day to day, justifying the monitoring over a minimum period of seven days. The *Varroa* mites are then quantified to determine a daily rate of natural mite drop. Generally, the threshold considered detrimental to the colony is set at 30 *Varroa* mites per day, although this number may be adjusted downwards depending on the season (Riva, 2017).

Another approach is to assess the infestation rate of capped drone brood. This method involves uncapping and inspecting at least 100 drone cells to determine the percentage of infested cells (Wilkinson *et al.*, 2002). Infestation exceeding 15% is generally considered the threshold indicating the need for treatment for the colony (Wilkinson & Smith, 2002).

It is also possible to estimate infestation by phoretic *Varroa* mites, i.e., the percentage of *Varroa* mites present on the bees. Three sampling methods for phoretic *Varroa* mites are available: the CO2 wash method, the powdered sugar wash method, and the detergent wash method. The latter method has a 100% efficiency, meaning it counts all phoretic *Varroa* mites, and the calculation of the number of phoretic *Varroa* mites does not require the application of a correction factor (Cc). The CO2 and powdered sugar methods have respective efficiencies of 73% and 92%. Therefore, correction factors Cc of 1.4 and 1.1 are used for the CO2 and powdered sugar methods. In 2014, ADAPI established critical thresholds not to exceed: 1 phoretic *Varroa* mite per 100 bees in the spring, and 2 *Varroa* mites in the fall after *Varroa* treatment (Riva, 2017).

1.3.1.6. The Struggle Against *Varroa destructor*

Strategies employed in the battle against *Varroa* can be categorized into two primary categories. The first and most commonly utilized approach involves the use of acaricides, which can be subdivided into two groups: synthetic acaricides and biological acaricides. The second category encompasses mechanical and population-based methods (Mondet *et al.,* 2016).

1.3.1.6.1. Chemical Method (Acaricide)

The fundamental principle behind the use of acaricides lies in the pursuit of optimizing outcomes while minimizing associated risks (Faucon, 1992). This becomes particularly crucial in the context of *Varroa* mite control, where the establishment of an efficacy threshold of at least 90% is imperative (Rosenkranz *et al.,* 2010). The objective of chemotherapy is to substantially reduce the infestation rate by *Varroa destructor* while extending colony vitality. However, this is contingent upon the chemical agents meeting strict criteria, including optimal tolerance by bees and brood, queen preservation, minimization of robbing risks, in addition to proven effectiveness against mites and ensuring the absence of honey contamination (Gregorc & Sampson, 2019).

1.3.1.6.2. Synthetic Acaricides

Among the commonly employed synthetic molecules are Amitraz, a volatile lipophilic molecule belonging to the formamidine family, pyrethroids such as Tau-fluvalinate, which has been used in *Varroa* control since 1988, as well as flumethrin and acrinathrin. Other options include Coumaphos, an organophosphate, Cymiazole from the formamidine family, and Bromopropylate (Riva, 2017). It is worth noting that prolonged or excessive use of these chemical products can lead to detrimental consequences, including irreversible damage to colonies (Chaimanee & Pettis, 2019), health risks for beekeepers, and the potential for hive product contamination, including honey (Rosenkranz *et al.,* 2010; Tette *et al.,* 2016). Furthermore, sublethal exposure of mites to residues of these chemical agents can promote the emergence of resistant strains (Rosenkranz *et al.,* 2010), underscoring the imperative of implementing integrated and sustainable strategies for the management of *Varroa destructor* within bee colonies.

1.3.1.6.3. Biological Acaricides

In the fight against *Varroa destructor*, biological molecules stand out due to their alternative approach, highlighting compounds such as organic acids and essential oils. In line with the fundamental principle of optimizing outcomes while minimizing risks (Faucon, 1992), these methods seek to significantly reduce mite infestation rates while preserving the vitality of bee colonies (Rosenkranz *et al.,* 2010). They are guided by rigorous criteria, including optimal tolerance by bees and brood, queen protection, minimizing robbing risks, as well as proven effectiveness against mites, all while ensuring the absence of honey contamination (Gregorc & Sampson, 2019).

Essential oils, obtained from plant extracts such as thyme, mint, rosemary, and eucalyptus, have garnered increasing interest as biological agents in the battle against *Varroa destructor*. These oils contain active compounds, notably terpenes, which disrupt the respiratory system and metabolism of mites, leading to their mortality (Bava *et al.,* 2023). The application of these essential oils is often in the form of vaporization or infusion in beehives, offering a promising alternative to synthetic acaricides.

Similarly, organic acids such as oxalic acid and formic acid are employed in biological methods (Riva, 2017). Formic acid is the only molecule currently recognized for its action on *Varroa* mites within capped brood cells (Calderon *et al.,* 2000).

1.3.1.6.4. Mechanical Methods

Various non-chemical strategies are employed to reduce infestations within a beehive, some of which complement chemical treatments, while others are applied during the season to lower infestation levels. One notable advantage of these approaches lies in their reduced or absence of chemical product usage within the colony. Several complementary approaches can be distinguished: biotechnical methods, thermal treatment, and the selection of bees that are tolerant or resistant to *Varroa* (Almecija, 2021).

1.3.1.6.4.1. Biotechnical Methods

Among the biomechanical methods, the *Varroa* trapping method using male brood, commonly known as removal of male brood, stands out. It is essential to note that male brood exhibits approximately eleven times greater attractiveness to *Varroa* compared to worker brood (Boot *et al.,* 1995). This method involves introducing frames of male brood during the spring season to capture *Varroa* mites early in the season within these brood cells.

Consequently, before the emergence of males and *Varroa* mites, the male brood is removed from the colonies. This procedure is repeated three times from April to June to maximize its effectiveness, leading to a reduction in infestation in the short term (Almecija, 2021).

Another biomechanical method, complementary to chemical treatment, is known as population-based control, which involves artificially placing colonies in a state without capped brood, facilitating the highly effective action of treatment molecules. This approach can be implemented in two ways: the removal of all brood frames or the caging of the queen. In both cases, the operation results in a broodless colony, enabling effective *Varroa* treatment using acaricides (Mondet *et al.,* 2016).

1.3.1.6.4.2. Thermal Method

The thermal method entails killing *Varroa* mites through the application of heat, Brood frames without bees are placed in a chamber and heated to 42°C for a few minutes. This temperature kills *Varroa* mites while sparing the brood. Subsequently, these frames are returned to the hive. It is worth noting that to eliminate *Varroa* mites in the phoretic phase, an acaricide treatment must be applied, making this method complementary (Almecija, 2021).

1.3.1.6.4.3. Selection of *Varroa*-Tolerant/Resistant Bees

The selection of *Varroa*-tolerant or resistant bees represents a promising and sustainable long-term approach for dealing with *Varroa* infestations (Mondet *et al.*, 2016). Breeding and selection techniques are common practices in beekeeping. Efforts to select *Varroa*-tolerant bees, initiated since the emergence of *Varroa* on *Apis mellifera*, have revealed various resistance strategies that reduce the pressure exerted by *Varroa* within colonies by slowing the growth of the parasite's population. The selection of *Varroa*-tolerant bees involves two distinct approaches: firstly, breeding from colonies that have survived for several years without intervention, and secondly, the selection of bees exhibiting specific behaviors against *Varroa* (Riva, 2017). Among these behaviors, the Suppressed Mite Reproduction (SMR) method limits the reproductive success of *Varroa* through various adaptations, such as reducing the attractiveness of larvae to *Varroa*, controlling *Varroa* fertility, or employing the *Varroa* Sensitive Hygiene (VSH) behavior. The latter involves the uncapping of cells and the removal of *Varroa*-infested brood, thereby slowing the growth of the *Varroa* population without significantly impacting the colony (Harbo & Harris, 2005).

1.3.2. anti-inflammatory activity

Natural barriers, such as the skin and mucous membranes, play a crucial role in protecting the body from external assaults. However, these barriers can be compromised in the event of injury, burns, microbial, viral, fungal infections, or cellular dysfunction. In response to such situations, our body mobilizes specialized agents to combat the invaders. Dormant immune cells automatically detect the intrusion and initiate a series of biochemical reactions aimed at preventing the spread of the aggressor while initiating a repair process. This process is commonly referred to as inflammation or an inflammatory response (Diallo, 2019).

Inflammation represents the predominant biological response to a variety of local stimuli and insults. Inflammatory reactions can be induced by physical or chemical trauma, the intrusion of pathogenic organisms, as well as reactions involving antigens and antibodies. Often, they are exacerbated by the consequent formation of tissue swellings or edema, the presence of pain, and even cellular damage (Sun *et al.,* 2016).

1.3.2.1. Inflammatory Triggers

The triggers for inflammatory processes can take on a variety of forms. They include physical factors, such as heat (causing burns) and cold (resulting in frostbite), as well as ionizing radiation, which leads to tissue damage and the release of degradation products such as collagen. Additionally, they encompass solid elements, whether exogenous or endogenous in origin, such as microbial pathogens, insect bites, or microcrystals (such as urate crystals), chemicals (including acids, bases, or toxins), biological products (notably toxins and tissue degradation products), as well as compounds arising from immune responses, such as immune complexes, cytotoxic antibodies, and cytokines. Regardless of the nature of the stimulus, the manifestations of the inflammatory response remain constant. It is the intensity and duration of these manifestations that vary and determine the beneficial or detrimental consequences of the inflammatory reaction (Weill & Batteux, 2003).

1.3.2.2. Different Forms of Inflammation (Acute and Chronic)

1.3.2.2.1. Acute Inflammation

It is characterized by four typical cardinal signs: tumor, dolor, calor, color, which are swelling (edema), pain, heat, and erythema (redness). This reaction may be accompanied by regional functional impairment (loss of function) depending on the severity of the aggression (Weill & Batteux, 2003; Cimino *et al.,* 2021; Zigterman & Dubois, 2022).

Acute inflammation can be subdivided into three distinct phases. Firstly, an immediate vascular phase, which occurs within minutes, is characterized by alterations in local microcirculation. The secretion of vasoactive inflammatory mediators such as histamine and serotonin by basophils and mast cells leads to vasodilation and increased blood flow, resulting in the observed redness and heat. This is followed by increased capillary permeability, facilitating the outflow of plasma into the inflammatory area, leading to edema formation. Simultaneously, the combination of vasodilation, molecular mediator secretion, and adhesion molecule expression promotes the recruitment of circulating phagocytes through diapedesis (Amroun, 2021).

The second phase, the cellular phase, occurs after the mobilization of various types of circulating phagocytes, such as neutrophils, monocytes, and macrophages. This cellular mobilization allows for the elimination of pathogens and damaged tissues (Weill & Batteux, 2003; Febvre-James, 2019). However, there are cases in which the innate immune response may not be sufficient to eliminate inflammatory stimuli. In such instances, an adaptive immune response is required. During this process, CD4 T lymphocytes are activated by antigen-presenting cells (APCs) like dendritic cells and macrophages and differentiate into cytokine-producing Th cells. Two types of responses can be deployed: cell-mediated response and humoral response, each aiming to eliminate threats in a specific manner. Activated Th1 cells produce cytokines such as IL 1 and 2 and interferon-gamma (IFN-γ) or directly interact with APCs to become IL-2-producing Th1 cells, which activate CD8 cytotoxic T lymphocytes to eliminate target cells through apoptosis. On the other hand, the humoral response involves the production of antigen-specific antibodies under the influence of interleukin 4 (IL-4) and other cytokines, neutralizing antigens, whether bacterial, viral, or toxic in origin (Febvre-James, 2019; Amroun, 2021).

Finally, the third phase of acute inflammation is the resolution and healing phase, which occurs over a few days and aims to restore damaged tissues. The resolution capacity depends on the severity of tissue damage. Under favorable conditions, neutrophils eliminate aggressors, while macrophages phagocytize degradation products and cellular debris. Macrophages then secrete cytokines and mediators that initiate the healing and tissue regeneration phase. In more severe cases, fibrocytes and fibroblasts come into play, producing the matrix proteins necessary for tissue reconstruction, such as collagen, fibronectin, and laminin. The angiogenesis system is also restored, and the inflammatory response ultimately subsides (Febvre-James, 2019; Amroun, 2021).

1.3.2.2.2. Chronic Inflammation

The initial signs of chronic inflammation are analogous to those of acute inflammation; however, the resulting tissue damage is more severe and induces profound functional alterations. It is characterized by the persistence of inflammation for a duration exceeding 6 weeks, with this temporal criterion often used to establish its chronic nature. In many cases, chronic inflammation appears to be present from the outset, and it is marked by simultaneous processes of connective tissue remodeling, destruction, and repair. The underlying mechanisms for this chronicity are not always clearly understood. In some situations, it results from the persistence of a pathogenic substance that the body proves incapable of eliminating. Consequently, inflammation fails in its primary function, which is to maintain the integrity of the self. In other cases, it can be self-sustaining, with intermediary mechanisms persisting even after the elimination of the pathogenic substance that originally triggered it (Weill & Batteux, 2003).

1.3.2.3. Inflammatory Medications

The most effective treatment is etiological therapy, which targets the cause of inflammation, for example, by administering antibiotics if the cause is an infection. If this is not sufficient or if the cause is unclear, traditional anti-inflammatory drugs are also used. They fall into two categories: non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Particularly in the treatment of chronic inflammation (Cimino *et al.*, 2021).

Anti-inflammatories are defined as substances that act on pain and swelling that result from an assault by a pathogenic agent. They inhibit the secretion or action of certain chemical mediators of inflammation (such as prostaglandins), thereby reducing the sensation of pain and inflammation (Diallo, 2019). Commercially approved and commonly used anti-inflammatory agents for relieving inflammation are classified into three categories: non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs), and natural origin anti-inflammatories (Romano *et al.*, 2015; Amroun, 2021).

1.3.2.3.1. Non-Steroidal Anti-Inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs), often referred to by their acronym NSAIDs, represent a widely prescribed category of medications globally due to their anti-inflammatory, analgesic, antipyretic, and, for some, antiplatelet properties (Vonkeman & van de Laar, 2010). These pharmaceutical agents are primarily used to mitigate the deleterious effects of inflammation by blocking the synthesis of prostaglandins.

Prostaglandins are lipid metabolites derived from arachidonic acid, a phospholipid component of cell membranes.

Arachidonic acid, released from cell membranes under the action of phospholipase A2 (PLA2), is metabolized by the enzyme cyclooxygenase (COX) to produce various types of prostanoids, including prostaglandins, prostacyclin, and thromboxane (Amroun, 2021). COX exists in three main isoforms: COX-1, COX-2, and COX-3. COX-1, termed constitutive, is present in most tissues and plays a vital role in the production of prostaglandins, which are crucial for normal physiological processes such as maintaining renal blood flow, preserving gastric mucosal integrity, and platelet aggregation. COX-2, on the other hand, is generally absent from tissues except in the brain, uterus, kidneys, and prostate, and its levels significantly increase during an inflammatory response. As the action of NSAIDs is primarily directed towards inhibiting COX-2, their use results in a reduction in the synthesis of this enzyme, thereby leading to a decrease in prostaglandin production, which are important mediators of inflammation (Diallo, 2019).

It is noteworthy that NSAIDs are associated with a potential adverse effect, namely an increased risk of gastric ulcers, due to their inhibitory action on the synthesis of other prostaglandins that contribute to gastric mucosal protection. Gastrointestinal symptoms such as stomach pain, gastroduodenal ulceration, and gastrointestinal bleeding are primarily attributed to the inhibition of cyclooxygenases of type I (Heymonet, 2013).

1.3.2.3.2. Corticosteroid Anti-inflammatories (CSA)

Corticosteroid anti-inflammatories, also known as corticosteroids (prednisone, prednisolone, betamethasone), are synthetic derivatives of cortisone. They are potent anti-inflammatories with immunomodulatory and anti-allergic properties, widely used to suppress the deleterious effects of inflammatory responses. They act at various levels by regulating (activating or inhibiting) the transcription of a large number of target genes. By preventing the activation of phospholipase A2, they block both the prostaglandin and leukotriene pathways (Janeway *et al.*, 2009; Heymonet, 2013). They freely cross membranes and bind to a specific receptor belonging to the steroid nuclear receptor superfamily, releasing chaperone molecules including Hsp90 (heat shock protein). The glucocorticoid-receptor complex forms in the cytoplasm and then migrates to the nucleus to regulate the transcription of target genes. Glucocorticoids inhibit the production of PLA2 and, consequently, the production of eicosanoids (prostaglandins and leukotrienes). They also inhibit many pro-inflammatory

cytokines (interleukins) by acting on certain nuclear transcription factors. However, they have numerous adverse effects such as skin alterations, bone fragility, the onset of diabetes, or hypertension. Corticosteroids improve the prognosis and functional outcomes of many diseases but do not address the root cause of the disease (Bony, 2010).

1.3.2.3.3. Natural Anti-inflammatories

Conventional treatment of inflammation primarily relies on the use of NSAIDs and CSA, which in the long term can lead to the development of severe side effects, not to mention the high costs of treatment. This is why there is a growing interest in the use of natural compounds as an alternative to these therapies. The significance of natural medicines in the treatment of various inflammatory conditions has been rooted since ancient times. Humans have long turned to the use of medicinal plants found in their environment for self-healing (Amroun, 2021; Cimino *et al.,* 2021). These plants synthesize a variety of biomolecules, among which stand out compounds with notable biological effects, including anti-inflammatory properties (Nunes *et al.*, 2020). For example, 'Queen of Hungary Water,' an infusion of rosemary essential oil, dates back to the 16th century for the treatment of rheumatism. Over time, this preparation spread as a remedy at the court of Louis XIV. Numerous historical evidence related to traditional medicine attests to the use of essential oils to treat various inflammatory conditions, such as rheumatoid arthritis, asthma, bronchitis (Cimino *et al.,* 2021). Natural products from plants with anti-inflammatory effects mainly include polyphenols, flavonoids, alkaloids, monoterpenes, diterpenes, triterpenes, phenylpropanoids, lignanoids, coumarins, and anthraquinones (Wang & Zeng, 2019).

1.3.3. Antioxidant activity

The inevitable generation of reactive oxygen species (ROS) is a vital process in aerobic life. Organisms require energy substrates from the environment to produce ATP, essential for repair, growth, and reproduction. Throughout life, a delicate balance persists between the oxidative process releasing energy from substrates and antioxidant defense minimizing damage. Oxidative stress occurs when the pro-oxidative process prevails over cellular antioxidant defense due to disruption in redox signaling (Ji & Yeo, 2021).

Oxidative stress is defined as the inability of antioxidant systems to neutralize and eliminate the overproduction of reactive oxygen species (ROS). The disruption of redox balance can induce oxidative damage to cellular components such as DNA, proteins, lipids, and sugars (Amroun, 2021).

1.3.3.1. Reactive Species

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are byproducts of the normal metabolic processes in all aerobic organisms. These reactive species can be classified into two groups of compounds:

- Free radicals: These are chemical species containing at least one unpaired electron located in the valence shell or outer orbit, rendering them unstable and highly reactive. To attain stability, they associate with electrons from other molecules, turning those molecules into free radicals themselves, initiating a cascade of chain reactions that ultimately damage the living cell (Del Río, 2015).
- Non-radical species: These do not possess unpaired electrons but are highly reactive and can serve as precursors to free radicals in living organisms (Phaniendra *et al.*, 2015).

Reactive oxygen species (ROS) are primarily generated in the form of superoxide through the reduction of an electron from oxygen by various oxidases, including Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, xanthine oxidases, cyclooxygenase, as well as the mitochondrial electron transport chain during oxidative phosphorylation, crucial for ATP production (Rahman *et al.*, 2012).

1.3.3.2. Sources of ROS

Reactive oxygen species (ROS), responsible for disrupting cellular homeostasis, can originate from both exogenous (external) and endogenous (intracellular) sources (Sies, 2018). Exogenous sources may include UV radiation (direct oxidation of cellular components), ultrasound, drugs (like narcotics, anaesthetizes, adreamicine, nitroglycerine and belomycinem), foods (containing oxidants such as transition metals, aldehydes, fatty acids, and peroxides), radiation, pollutants, xenobiotics, and toxic chemicals (alcohol, phosphine, mustard gas) (Ahmad *et al.*, 2017). At the intracellular level, ROS can be generated by various sources and mechanisms. While the mitochondrial electron transport chain represents the primary site of ROS formation, numerous enzymes found in various intracellular compartments (organelles) can also generate ROS (Hameister *et al.*, 2020).

1.3.3.3. Impacts of Oxidative Stress

Oxidative stress exhibits a duality closely linked to variations in the concentrations of reactive oxygen species (ROS) and the state of compensatory mechanisms aimed at regulating them (Di Meo *et al.*, 2016). Indeed, when their concentrations are low to

moderate, ROS are involved in numerous vital physiological processes, they play a role in various signaling cascades, such as the response to growth factor stimulation and the control of inflammatory responses. They participate in the regulation of many cellular processes, including differentiation, proliferation, growth, apoptosis, and cytoskeletal regulation, they also contribute to defense against infectious agents and the maintenance of redox homeostasis (Brieger *et al.,* 2012; Ahmad *et al.,* 2017; Hameister *et al.,* 2020).

Although the production of reactive species can be beneficial in certain circumstances, most ROS/RNS research focuses on their harmful effects (Hameister *et al.,* 2020). High concentrations of ROS/RNS lead to the onset of oxidative/nitrosative stress, which, in turn, could result in the damage of biomolecules such as nucleic acids, lipids, and proteins, inhibiting their normal functions. This could compromise cell viability or induce various cellular responses, ultimately leading to cell death through necrosis or apoptosis (Ahmad *et al.,* 2017).

ROS/RNS can damage nucleic acids through oxidation. The •OH radical is the primary ROS that directly interacts with all components of DNA, such as purine and pyrimidine bases and the deoxyribose sugar backbone. This interaction causes various alterations, including single and double strand breaks within the DNA strand. The •OH radical acts by extracting hydrogen atoms, producing modified purines, as well as by-products of pyrimidine bases and cross-links between DNA and proteins. Oxidative/nitrosative damage to DNA induces mutagenic lesions implicated in carcinogenesis and aging (Phaniendra *et al.,* 2015).

Protein oxidation can be induced by radical species such as O2 -, •OH, peroxyl, as well as non-radical species such as H2O2, O3, HOCl, singlet oxygen, OONO-. ROS induce the oxidation of various amino acids present in proteins, leading to the formation of protein-protein cross-links, resulting in denaturation and loss of functionality. This includes loss of enzymatic activity, impairment of receptor function, and disruption of transport proteins (Phaniendra *et al.,* 2015).

Membrane lipids, especially phospholipids containing unsaturated fatty acids, are highly sensitive to oxidation by reactive species. Oxidative/nitrosative stress-induced lipid membrane peroxidation can be very detrimental, as it results in the alteration of biological properties of the plasma membrane, such as the degree of fluidity. This can lead to the inactivation of transmembrane receptors or enzymes, which in turn may compromise normal cellular function and increase tissue permeability (Amroun, 2021).

1.3.3.4. Antioxidant System

An antioxidant is defined as a substance that, at a relatively low concentration, competes with other oxidizable substrates, significantly delaying or preventing the oxidation of biomolecules such as lipids, proteins, and DNA. In simple terms, it interacts with reactive oxygen species (ROS), ensuring effective protection against oxidative damage to oxidizable substrates (Berger, 2006; Mahfouf *et al.*, 2018).

To guard against damage associated with oxidative/nitrosative stress, the human body possesses an endogenous antioxidant defense system. However, this first line of defense can be quickly overwhelmed. In this context, the incorporation of exogenous antioxidants is essential to enhance the effectiveness of the endogenous antioxidant defense system (Amroun, 2021).

1.3.3.4.1. Endogenous Antioxidant System

The endogenous antioxidant defense system is primarily divided into two categories: (Amroun, 2021).

- Enzymatic antioxidants include Superoxide Dismutase (SOD), which remains the most potent endogenous antioxidant enzyme, Catalase (CAT), which catalyzes the conversion of hydrogen peroxide (H2O2) into water (H2O) and molecular oxygen (O2), and Glutathione Peroxidase (GPx), an intracellular enzyme (Aguilar *et al.*, 2016).
- Non-enzymatic antioxidants comprise compounds such as glutathione (GSH), proteins, and low-molecular-weight scavengers like coenzyme Q10 and uric acid, which are naturally present in the human body (Poljsak *et al.*, 2013; Amroun, 2021).

1.3.3.4.2. Exogenous Antioxidant System

Exogenous antioxidants originate from dietary sources and medicine. Antioxidants such as vitamin C (ascorbic acid), vitamin E, carotenoids, and phenolic compounds are considered the main components of the exogenous antioxidant system (Amroun, 2021).

In vitro, antiradical methods are commonly used to assess the antioxidant activity of molecules (Mahfouf *et al.*, 2018). the reaction equation of DPPH with a molecule RH can be written as shown in (Figure 7).

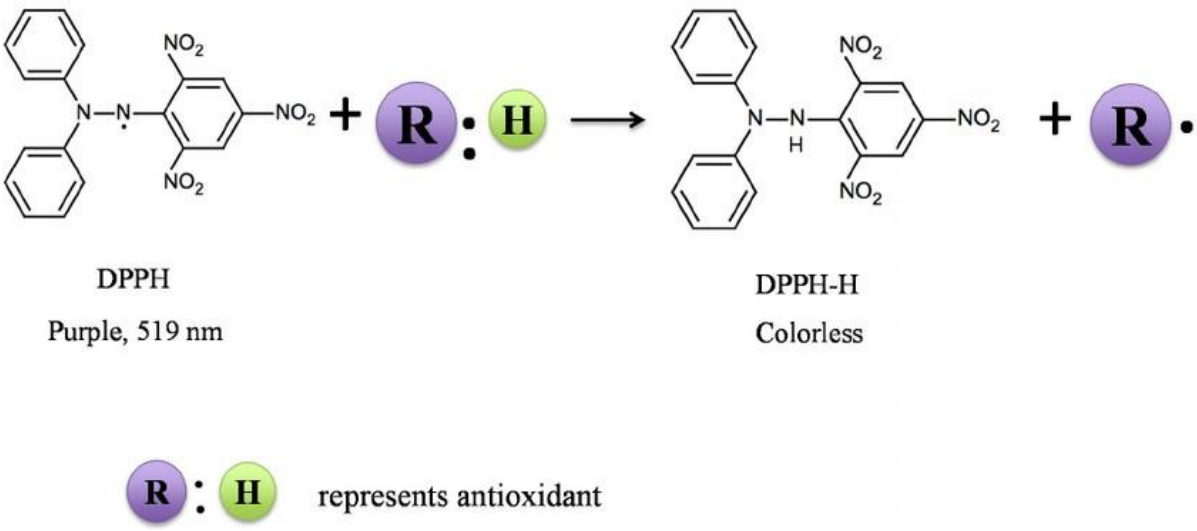


Figure 7. Reaction of DPPH with a proton-donating molecule (Liang NingJian & Kitts, 2014).

Chapter 2 : Materials and Methods

2.1. Study Area

The plant material used in this study comes from three populations of *Origanum*, each originating from a distinct region. The study area encompasses these regions: Chrea in the Blida province, Nechmaya in the Guelma province, and Ghebala in the Jijel province. The selection of these areas was based on a bibliographic research and informed recommendations from botanists and local traditional practitioners, who assured us of the abundant and specific presence of species from the *Origanum* genus in these regions.

2.1.1. Geographic location

The details regarding the geographical location of each region, including their coordinates, are presented in the table below (Table 3) and are also visible on the associated geographic map (Figure 8).

Table 3. Origin data of the samples.

Species	**The region of origin**	**Geographic coordinates**	**Elevation**	**Type of Habitat**
Origanum floribundum Munby	Chrea, Blida	36° 42′ 91″ N, 2° 88′ 07″ E	1550m	forest
Origanum floribundum Munby	Nechmaya, Guelma	36° 61′ 24″ N, 7° 51′ 24″ E	254 m	Maquis
***Origanum vulgare ssp glandulosum* (Desf.)**	Ghebala, Jijel	36° 63′ 15″ N, 6° 37′ 55″ E	492 m	Maquis

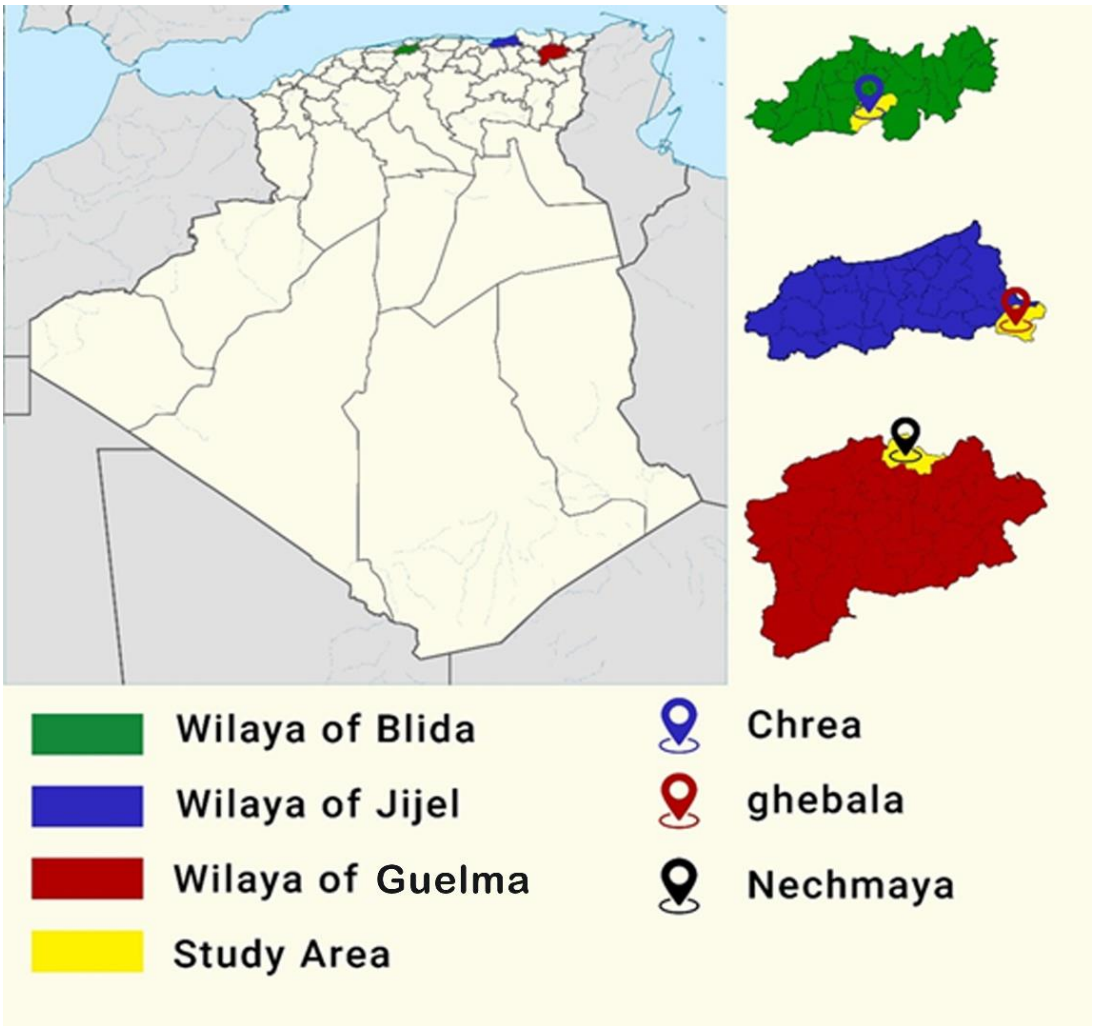


Figure 8. Geographical locations of the sampling sites.

2.1.2. Climates of the Study Areas

2.1.2.1. Precipitation

The lowest precipitation values in Blida are recorded in the month of July, totaling only 1.2 mm, whereas November registers the highest rainfall rate, reaching 266 mm. The annual precipitation in Blida stands at 514 mm (Figure 9). In Nechemaya, precipitation is more substantial in winter than in summer, with a minimum of 0.9 mm in July and a maximum of 111 mm in October, followed by 86 mm and 87 mm in the months of March and November, respectively. The annual precipitation stands at 647 mm (Figure 9). In Jijel, the month of July is the driest, displaying only 0.3 mm of precipitation. The month of November records the highest amount of precipitation, reaching up to 90 mm, followed by 66 mm in May and 41 mm in April, the annual precipitation in Jijel stands at 433 mm (NASA POWER, 2021).

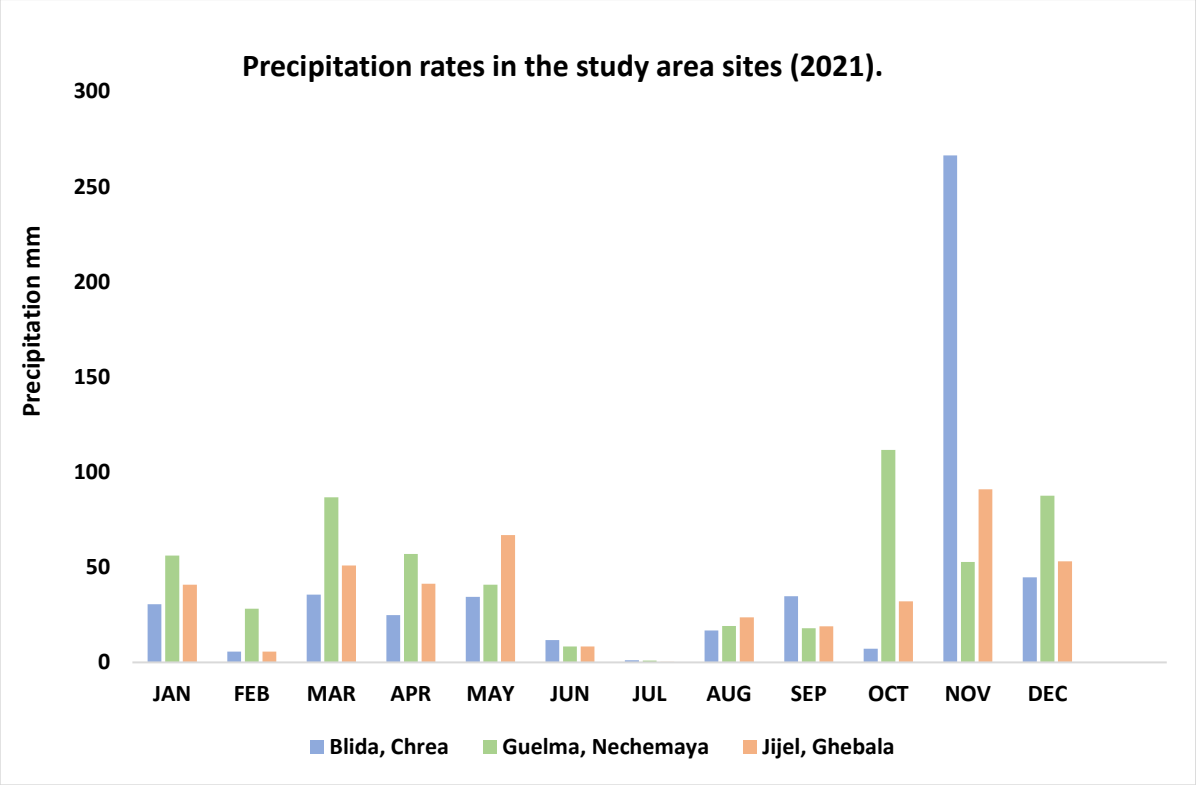


Figure 9. Precipitation data for three regions: Blida, Guelma and Jijel in 2021 (NASA POWER, 2021).

2.1.2.2. Temperature

The highest temperatures in Blida are recorded in the month of August, with an average of 29.5 °C, making this month the warmest of the year. In contrast, the month of December stands out for the coldest temperatures, with an average of 10.38 °C, marking the peak of the annual coolness (NASA POWER, 2021).

In the Guelma region, on a monthly scale, the average temperature experiences a notable increase during the dry period, spanning from June to September, reaching a maximum of approximately 29.13 °C recorded in the month of July. In contrast, the winter period, from December to February, is characterized by lower values ranging between 9 and 11 °C, with a minimum that can drop to 8.95 °C, observed in the month of January (NASA POWER, 2021) (Figure 10).

In Jijel, the highest average temperature, with an average of 29.77 °C, is recorded in the month of August, marking the peak of the annual heat. Conversely, the month of January displays the coolest temperatures of the year, with a low average of 8.19 °C, characterizing the peak of winter coolness (Figure 10) (NASA POWER, 2021).

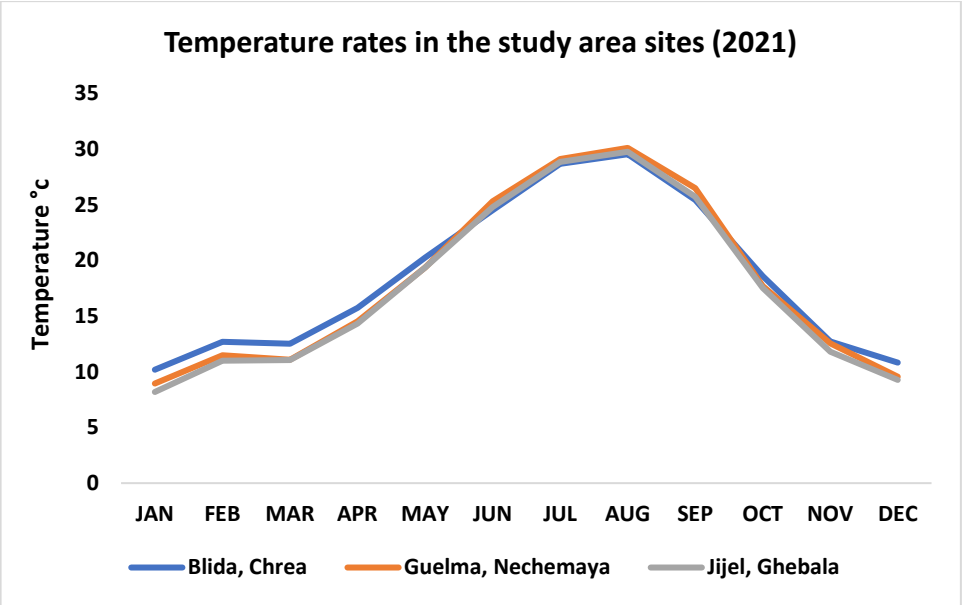


Figure 10. Temperature rates in three regions: Blida, Guelma, and Jijel in 2021 (NASA POWER, 2021).

2.2. Plant material and essential oil extraction

Oregano samples were collected in June 2021 at the flowering stage, when the content of secondary metabolites is at its maximum (Sellami *et al.*, 2009; Bouyahya *et al.*, 2017), Sample collection took place early in the day. An amount of the aerial parts of the plants was harvested, placed in Kraft paper bags, labeled with information regarding the region, species, and the date of collection. Subsequently, these samples were cleaned and then dried in the open air for a duration of 15 days, shielded from light, with the aim of preserving their high content of volatile compounds (Ozdemir *et al.*, 2018). Additionally, a complete botanical specimen of each plant was preserved for identification purposes. Dr. AIT HAMOU of IBN KHALDOUN University, Tiaret, conducted the botanical identification of three species.

The extraction of essential oils was conducted using the widely employed Hydrodistillation method. The distillation process persists as the primary approach for obtaining aromatic compounds owing to its capability to produce volatile substances easily amenable to analysis through gas chromatography (GC). This method offers the advantage of integrating a relatively straightforward technology, resulting in more moderate costs while ensuring easily manageable reproducibility (Benjilali, 2004).

2.2.1. Extraction procedure

Whith a Clevenger apparatus. In a 1000 ml flask filled two-thirds full with distilled water (600 ml), 50 g of plant material was directly immersed and boiled for one hour and twenty minutes (Figure 11) (Garnero, 1991).

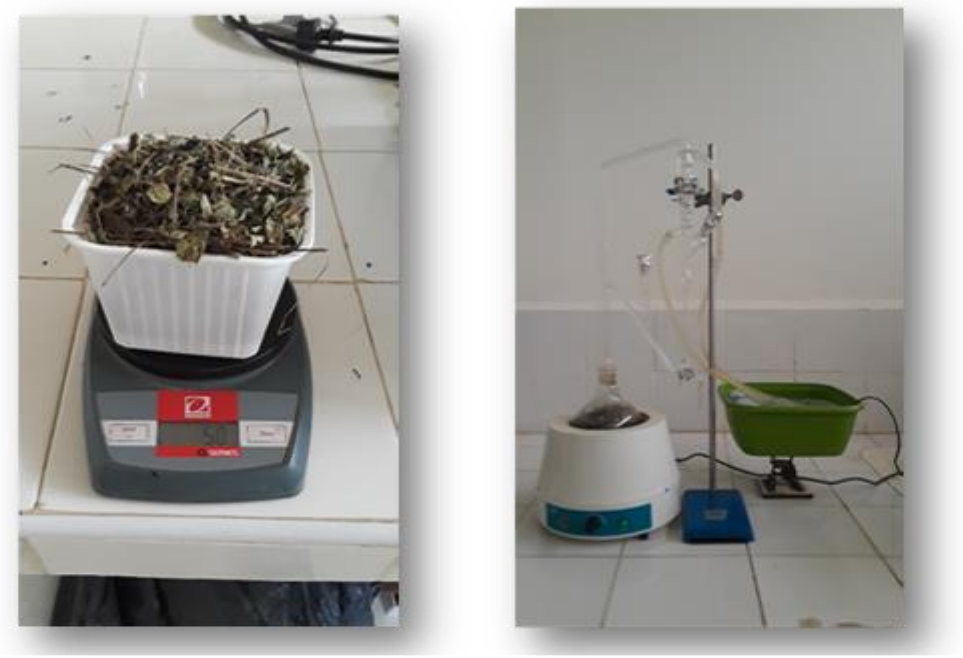


Figure 11. Preparation of plant material and setup of the hydrodistillation apparatus.

The yield is defined as the ratio between the quantity of recovered essential oil and the quantity of processed dry plant material, expressed as a percentage. The extraction process was repeated five times to calculate the precise yield of essential oils, employing the following formula (Akrout *et al.*, 2001; Bousbia, 2011):

$$Y = \frac{we}{wp} X\ 100$$

Where:

Y: Essential oil yield (in percentage).

We: Weight of the recovered essential oil in grams.

Wp: Weight of the dry plant material in grams.

2.3. Phytochemical analysis of essential oils by GC-MS

To determine the chemical composition of the essential oils from plants, a Gas Chromatograph combined with Mass Spectrometer (GC-MS) was utilized at the research center (CRAPC) in BOUSMAIL, Tipaza, Algeria

Advancements in separation techniques, notably Gas Chromatography (GC), are particularly well-suited for the analysis of volatile constituents present in aromatic extracts. GC can be coupled with spectral methods such as infrared or mass spectrometry (MS), with the latter being by far the most widely utilized (Figueredo, 2007).

2.3.1. Chromatography coupled with GC/MS mass spectrometry

2.3.1.1. Operating conditions

The GC-MS analysis was carried out using an Agilent 6890 Plus coupled to an Agilent 5973 mass spectrometer from Hewlett Packard (Figure 12). The column used was a HP-5MS capillary column (30 m long, diameter 0.25 mm, 0.25 µm film thickness) with a stationary phase consisting of 5% phenyl and 95% dimethylpolysiloxane. Helium (He) was used as the carrier gas, and the flow rate was 0.5 ml/min.

The column temperature was programmed to start at 60°C for 8 minutes, followed by a gradual increase to 250°C at a rate of 2°C/min, and then maintained isothermally for 10 min. Injection mode: split 1; 20, and a volume of 0.2 µl was injected at 250°C.

The identification of compounds was conducted by comparing their mass spectra and Kovats Indices (KI) with those in the Adams, NIST, and Wiley databases.

Figure 12. CG/MS apparatus (CRAPC, BOUSMAIL, Tipaza, Algeria).

2.4. Biological Activities

This study focused on three major biological activities of essential oils extracted from *Origanum* plants: acaricidal activity, aimed at combating *Varroa destructor* in experimental apiaries; anti-inflammatory activity, evaluated using animal models to measure their capacity to reduce edema; and antioxidant activity, analyzed using the DPPH method to assess their potential for neutralizing free radicals. These activities were selected due to their significance in the fields of apiculture, pharmacology, and food science.

2.4.1. Acaricidal activity

The acaricidal activity test of essential oils extracted from *Origanum* plants against the *Varroa* mite was conducted at the ITELV (Technical Institute of Livestock) experimental apiary in Algiers. The apiary, located in the BABA ALI region (36°39'12.6" N 3°03'25.1" E), consists of 33 hives populated with *Apis mellifera Intermissa*, infested by *Varroa destructor*. Covering an area of 4 hectares, the site is primarily covered with vegetation composed of Carob (*Ceratonia siliqua*) and Woody fleabane (*Dittrichia Viscosa*). Additionally, the colonies are strategically positioned for easy access.

2.4.1.1. Acaricidal activity test

The assessment of acaricidal activity through the natural mite fall method for *Varroa* females relies on estimating infestation levels in the hive before and after treatment, with quantification accomplished using greased sheets. The disparity between these two values indicates the efficacy of the product employed against mites (Moussaoui *et al.,* 2014).

2.4.1.1.1. Infestation levels estimation before treatment.

The fieldwork initiation involved applying a layer of Vaseline-coated metal at the bottom of the hive frame. Throughout the pre-treatment phase, quantification was conducted every 2 days for 28 days to determine the average daily natural fall of *Varroa* mites. This average was then multiplied by 90 days (the life cycle of the *Varroa* female) to estimate the total number of *Varroa* mites in a hive (Moussaoui *et al.,* 2014; Belguendouz *et al.,* 2018).

2.4.1.1.2. Formulation of acaricide treatments

To dilute the essential oil, an ointment was prepared based on beeswax, refined beef tallow, and sunflower oil in order to protect the essential oils from external factors and control evaporation during treatment (Original, 2024), with doses of 2%, 1.5%, and 1% for three essential oil treatments. Bayvarol, a commercially available product commonly used against *Varroa* in the experimental field, served as the positive control (C+). As a negative control (C-), Only the dilution ointment (without essential oils) was used.

2.4.1.1.3. The Application of Treatments

Each of nine treatments (three doses x three EOs) and controls (positive and negative) was applied four times over a period of four weeks to eleven batches of three hives each (three replicates) (Figure 13). The treatment was administered by placing the ointment onto a wooden applicator, which was then positioned directly on the hive frames. The number of fallen mites was calculated throughout the treatment period, which lasted for 28 days.

2.4.1.1.4. Data Processing and Statistical Analysis

The efficacy of the treatment was assessed by calculating the percentage reduction in the number of parasites using the following equation (Robaux, 1986).

$$\textbf{Efficiency}\% = \frac{\textbf{Infestation before treatment} - \textbf{Infestation after treatment}}{\textbf{Infestation level before treatment}} * \textbf{100}$$

Infestation level after treatment = Infestation level before treatment - *Varroa* dropped during treatment.

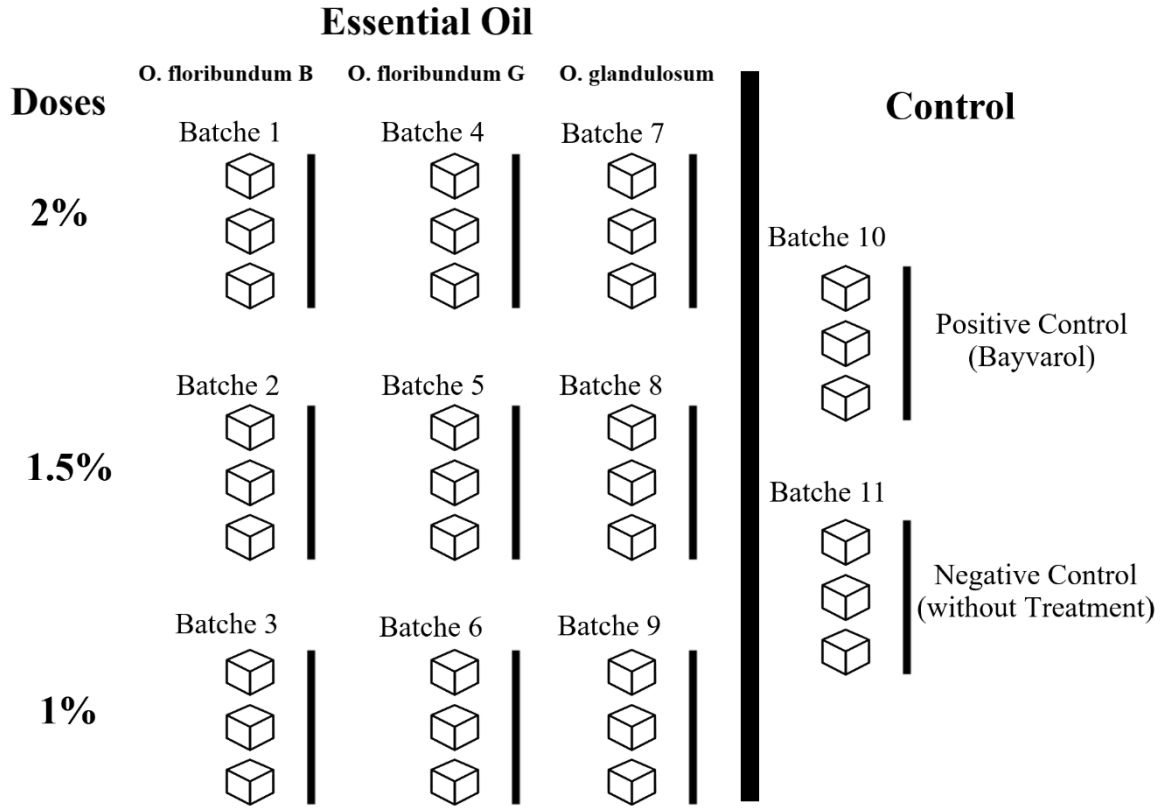


Figure 13. Experimental Setup for the Application of Essential Oil Treatments in Hives (Original, 2024).

2.4.1.1.5. Statistical analysis

The study utilized one-way analysis of variance (ANOVA) and Tukey's HSD test for multiple comparisons to evaluate the impact of various essential oils and their dosages on Acaricidal activity. A significance level of 5% was considered for all statistical tests. IBM SPSS Statistics 20 software was employed for conducting the statistical analysis.

2.4.2. Anti-inflammatory activity.

The study, conducted within the pharmacotoxicology laboratory of the pharmaceutical group SAIDAL to assess the anti-inflammatory activity of essential oils on a homogeneous group of albino mice (male IMRI mice weighing between 20 and 25 grams), involved meticulous control of housing conditions. This included maintaining an ambient temperature between 20 and 25 degrees Celsius, a humidity level of 50%, and an artificial lighting cycle of 12 hours of light and 12 hours of darkness. The tests followed the method of edema induced by carrageenan, and after a fasting period of 18 hours, the mice were sorted into homogeneous batches in accordance with international standards for animal care. The study also adhered to ethical guidelines, specifically, the 'Ethical Guidelines for investigations of Experimental Pain in Conscious Animals (Zimmermann, 1983)

2.4.2.1. Assessment of the Anti-Inflammatory Activity of *Origanum* Essential Oils

In accordance with the work of Levy (1969), the administration of carrageenan under the plantar aponeurosis of the mouse's paw triggers an inflammatory reaction that can be alleviated by the use of an anti-inflammatory agent. The objective of this study is to compare the volume of edema after the administration of various doses of the anti-inflammatory agent under evaluation with corresponding reference products.

The method employed entails assessing the anti-inflammatory effect of *Origanum* essential oils at different concentrations (100 mg/kg, 50 mg/kg et 30 mg/kg) on the edema of the hind paw induced by the injection of 1% carrageenan.

2.4.2.1.1. Mice Preparation

The mice were grouped into 11 batches, with each batch consisting of 5 mice. They underwent an 18 hour fast with access to water only, after which the administration of solutions took place.

2.4.2.1.2. Treatments Administration

At time T_0 (after 18 hours of fasting), the solutions (physiological saline, various concentrations of essential oils, and diclofenac solution) were orally administered using a gavage tube.

Batches 1 to 3: Each mouse received 0.5 ml of essential oil at respective doses of 100, 50, and 25 mg/kg of O. floribundum B.

Batches 4 to 6: Each mouse received 0.5 ml of essential oil at respective doses of 100, 50, and 25 mg/kg of O. floribundum G.

Batches 7 to 9: Each mouse received 0.5 ml of essential oil at respective doses of 100, 50, and 25 mg/kg of O. glandulosum.

Batches 10 (Negative control): Each mouse received 0.5 ml of physiological saline solution.

Batches 11 (Positive control): Each mouse received 0.5 ml of diclofenac solution.

2.4.2.1.3. Edema Induction

At time T_0+30 minutes, the carrageenan solution was administered by injection under the plantar aponeurosis of the left hind paw of all the mice.

2.4.2.1.4. Paw weighing

At time T_0+4 hours, following euthanasia of the mice, the limbs were severed at the joint and weighed.

2.4.2.1.5. Expression of results

The anti-inflammatory effect of essential oils was demonstrated by the reduction of edema in mice treated with anti-inflammatory agents, compared to the negative control group

- The means of the left paw and right paw weights were calculated for each batch.
- The percentage increase in weight (% edema) of the left paw was calculated in relation to the weight of the right paw using the following formula:

$$\textbf{\% edema} = \frac{\textbf{Mean weight of left paws - Mean weight of right paws}}{\textbf{Mean weight of right paws}} * \textbf{100}$$

- The percentage reduction of edema in treated mice was calculated in comparison to the negative control group using the following formula

$$\textbf{\% reduction in edema} = \frac{\textbf{\% edema control - \% edema test}}{\textbf{\% edema control}} * \textbf{100}$$

2.4.3. Antioxidant Activity

The assessment of the antioxidant activity of the essential oil was conducted using the method of free radical scavenging with 2,2-diphenyl-1-picrylhydrazyl (DPPH). This approach relies on the application of the stable radical DPPH, initially described by Blois in

1958 and subsequently extensively modified by numerous researchers. DPPH, exhibiting a violet color in solution, displays maximum absorption at 515 nm. It undergoes reduction to a yellow compound, with the intensity of the color being inversely proportional to the reducing capacity of antioxidants present in the medium. However, it should be noted that DPPH is susceptible to light, oxygen, pH, and the nature of the solvent used (Mahfouf *et al.*, 2018).

2.4.3.1. Experimental Procedure

The fundamental idea behind the method used to evaluate antioxidant activity with the DPPH test is that antioxidants act as providers of hydrogen. The DPPH radical, which appears violet and has a distinctive absorption band at 517 nm, takes in the hydrogen given by the antioxidant, leading to the creation of DPPHH. The effectiveness of the antioxidant corresponds directly to the decrease in the DPPH• radical and the change in color of the solution from violet to yellow (Sarr *et al.*, 2015).

The preparation of the DPPH solution involved dissolving DPPH in ethanol to achieve a concentration of 0.0768 mg/ml (0.195 µM). Subsequently, various concentrations of essential oil, ranging from 2.5% to 20%, were added at a volume of 100 µl to 2 ml of the DPPH solution. The mixture was then incubated in darkness for 30 minutes, followed by the measurement of absorbances at 517 nm against the blank (ethanol).

Under the same conditions, the inhibition of the DPPH free radical by ascorbic acid was conducted as positive controls. All tests were performed with three replicates for each concentration.

2.4.3.2. Results Presentation

The antiradical activity reflects the antioxidant's ability to neutralize free radicals, measured as a percentage of DPPH discoloration in a methanol solution. The absorbance measurement is then converted into a percentage of inhibition relative to the absorbance of the control solution, following the methodology outlined by Connan in 2004. The percentage of antioxidant activity is determined by the following equation.

$$\textbf{Percentage of Inhibition} = \frac{\textbf{Abs control - Abs sample}}{\textbf{Abs control}} * \textbf{100}$$

Where Abs control represents the absorbance of DPPH.

The IC50 (inhibitory concentration at 50%) is employed to calculate the concentration of the tested sample required to reduce DPPH radicals by 50%. This value is obtained graphically through linear regression of plots, correlating the inhibition percentages with different concentrations of the fractions used, following the methodology outlined by (Mahfouf *et al.*, 2018).

Chapter 3: Results and Discussion

3.1. Yield

The essential oils of *Origanum floribundum* and *Origanum glandulosum* were extracted through hydrodistillation, a widely employed method for obtaining volatile compounds from plant materials. The process yielded clear yellow oils characterized by a potent and persistent oregano fragrance, which is typical of species from the genus *Origanum*. The yields of essential oil exhibited significant variation depending on both the species and their geographical origin, reflecting the influence of environmental and genetic factors on secondary metabolite production.

Among the analyzed samples, the highest yield was observed in *Origanum floribundum* from the Guelma region (*O. floribundum* G), which reached an impressive value of 2.9 ± 0.23% (Figure 14).

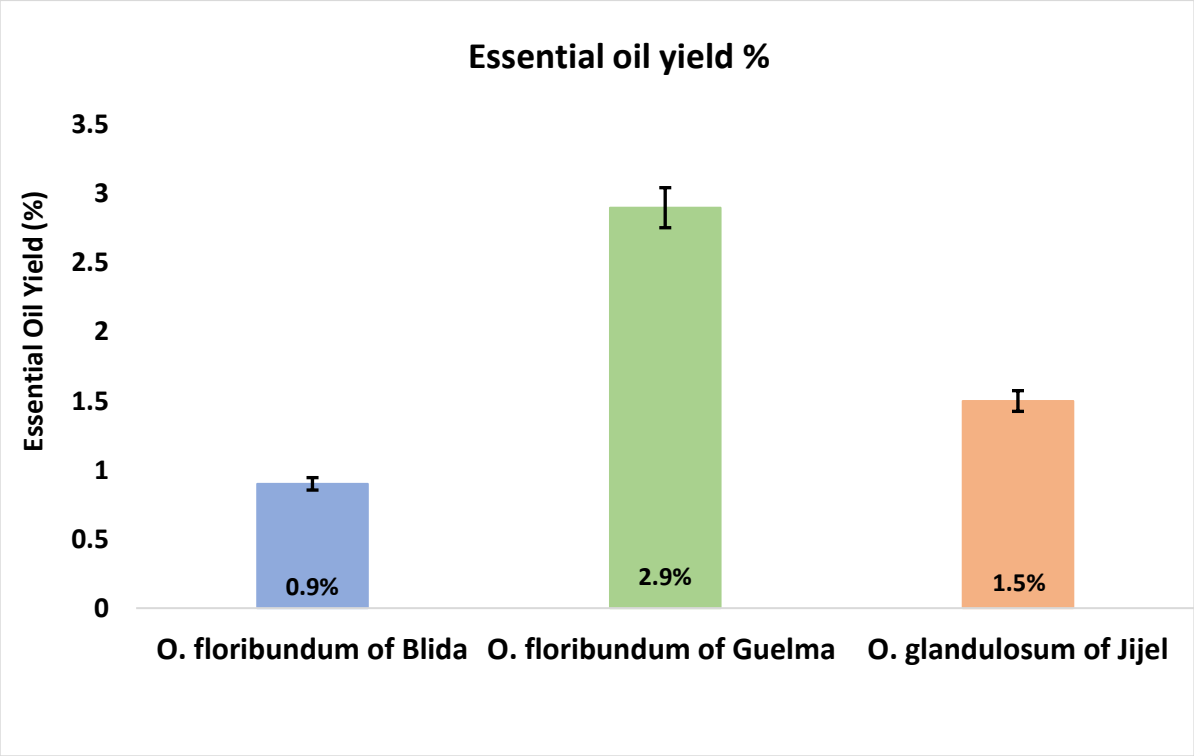


Figure 14. The essential oil yield of *Origanum* plants.

This yield surpasses values reported in subsequent studies on the essential oil of *O. floribundum*, emphasizing the notable productivity of this population under the specific ecological conditions of the Guelma region. It also compares favorably to the yield of 2.5% reported by Bouhaddouda (2016) for *O. glandulosum* from the same region. Similarly, it aligns with findings from (Sahraoui *et al.*, 2007), who observed an average yield of 2.5% for *O. glandulosum* in the Sétif region. A detailed summary of essential oil yields from various

Origanum species across different studies is presented in (Table 4), offering a broader perspective on the variability of oil yields within this genus.

Table 4. Comparative yields of essential oils from *Origanum* species reported in various studies.

Species	Origin	Yield (%)	Reference
O. floribundum	Guelma	2.9 ± 0.23	This study
O. floribundum	Blida	0.9 ± 0.1	This study
O. glandulosum	Jijel	1.5 ± 0.1	This study
O. glandulosum	Guelma	2.5	(Bouhaddouda, 2016)
O. glandulosum	Sétif	2.5	(Sahraoui *et al.*, 2007)
O. glandulosum	Guelma	1.15	(Mahfouf *et al.*, 2018)
O. glandulosum	Sétif	1.73	(Ali *et al.*, 2020)
O. floribundum	Blida	0.66	(Baser *et al.*, 2000)
O. floribundum	Ain-Defla	1.6	(Brada *et al.*, 2012)

In contrast, the yield of *O. glandulosum* essential oil from the Jijel region was notably lower, measured at 1.5 ± 0.1%. This value, while reduced compared to the Guelma population, aligns with previously observed yields for the same species. For instance, (Mahfouf *et al.*, 2018) reported a yield of 1.15% for *O. glandulosum* from the Guelma region, while (Ali *et al.*, 2020) recorded a slightly higher value of 1.73% for the same species in the Sétif region. These differences highlight the impact of geographical and environmental factors, as well as potential variations in harvest timing or plant developmental stages, on essential oil productivity.

Another interesting observation was the yield of *O. floribundum* from the Blida region (*O. floribundum B*), which was among the lowest in our study, at 0.9 ± 0.1%. This result is consistent with previous investigations on *O. floribundum* from nearby regions. For instance, studies conducted by Baser *et al.* (2000) and Brada *et al.* (2012) reported yields of 0.66% and 1.6%, respectively, for populations from the Blida and Ain-Defla regions. Such findings underscore the variability in essential oil yields even within the same species, reflecting the complex interplay of genetic and environmental influences.

It is worth noting that essential oil yields across the genus *Origanum* can exhibit remarkable diversity. For example, some species, such as *O. vulgare L.*, have been reported to produce yields as high as 8.0%, while others, in contrast, exhibit minimal productivity, with yields as low as 0.1% (Kokkini & Vokou, 1989). These wide-ranging values are attributed to multiple factors, including differences in growth conditions, plant origin, and developmental stages. Additionally, physiological responses to environmental variables and stressors, such as temperature, soil composition, and water availability, play a crucial role. Furthermore, harvest timing, drying techniques, and essential oil extraction methods are known to influence the final yield and quality of the oil (Arranz *et al.*, 2015; Moghaddam & Mehdizadeh, 2017).

Overall, the results highlight the importance of understanding the interplay between intrinsic and extrinsic factors in determining essential oil yield and quality. This variability also emphasizes the need for targeted approaches when optimizing cultivation and extraction protocols to maximize the therapeutic and commercial potential of *Origanum* essential oils.

3.2. Chemical composition

The chemical composition analysis of the essential oils extracted from the three populations (*O. floribundum* B, *O. floribundum* G, and *O. glandulosum*) provided a detailed overview of their respective phytochemical profiles. For the essential oil derived from *O. floribundum* B, a total of 20 distinct chemical compounds were identified, accounting for 97.07% of the total composition. Similarly, the essential oil of *O. floribundum* G revealed a slightly richer profile, with 24 identified compounds making up 95.25% of its overall composition. The essential oil of *O. glandulosum*, the most chemically diverse among the three, included 28 compounds, representing 96.02% of its total makeup. These results highlight the variability in the complexity of the chemical profiles across the three populations. The detailed findings, showcasing the specific compounds and their relative abundances, are presented comprehensively in (Table 5).

Table 5. GC-MS analysis of essential oils of *Origanum floribundum* from Blida (*O.f* B), *Origanum floribundum* from Guelma (*O.f* G), and *Origanum glandulosum* (*O.g*).

N	RI	RT	Compounds	% of compounds		
				O.f B	*O.f* G	*O.g*
1	927	7.82	α-Thujene	0.80	0.5	0.5
2	934	8.23	α-Pinene	0.53	0.45	0.42
3	951	9.17	Camphene	0.08	0.08	0.09
4	981	10.82	β-Pinene	0.15	0.09	0.10
5	996	11.66	β-Myrcene	1.03	1.02	1.18
6	1010	12.72	3-Carene	0.23	0.22	0.30
7	1020	13.48	α-Terpinene	2.32	1.76	2.07
8	1029	14.19	**P- cymene**	**-**	**-**	**15.14**
9	1032	14.43	Limonene	-	-	0.11
10	1034	14.52	β-Phellandrene	-	-	0.08
11	1038	14.85	**Cis-ß-Ocimene**	**13.76**	**24.44**	**0.11**
12	1047	15.6	Trans-ß-Ocimene	-	0.06	0.10
13	1063	16.79	**γ-Terpinene**	**-**	**-**	**25.48**
14	1072	17.53	**Cis-Sabinene hydrate**	**31.56**	**22.21**	**0.07**
15	1081	18.19	Terpinolene	-	0.08	0.10
16	1101	19.75	Linalool	0.28	0.03	0.73
17	1178	25.39	Terpin-4-ol	-	0.06	0.36
18	1204	27.17	ɣ-Terpineol	-	0.33	0.24
19	1229	28.72	Thymol, methyl ether	0.19	0.18	0.08
20	1240	29.37	Carvacrol, methyl ether	0.18	0.05	0.96
21	1318	34.2	**Thymol**	**0.33**	**0.1**	**12.00**
22	1331	35.1	**Carvacrol**	**43.04**	**40.94**	**29.93**
23	1422	40.95	Trans-Caryophyllene	0.88	0.96	2.46
24	1512	46.57	Cis-ɣ-Bisabolene	0.84	0.24	0.68
25	1520	47.07	δ-Cadinene	0.20	0.07	0.24
26	1530	47.61	β-Sesquiphellandrene	0.38	0.68	1.82
27	1545	48.49	Trans-α-Bisabolene	-	0.32	0.20
28	1587	50.95	Caryophyllene oxide	0.29	0.38	0.47
			Total	97.07	95.25	96.02

3.2.1. Main Compounds

The chemical analysis of the three essential oils revealed the following main compounds, emphasizing the diversity and specificity of their chemical profiles. Carvacrol emerged as the predominant chemotype across the essential oils of the three plant populations. This compound is widely recognized for its significant biological activities, which underscore its importance in medicinal and aromatic applications. Among the samples analyzed, *O. floribundum* B exhibited the highest concentration of carvacrol, reaching 43.04%, followed closely by *O. floribundum* G with 40%, and *O. glandulosum* with a slightly lower level of 29.93%.

Additionally, the essential oil of *O. glandulosum* displayed noteworthy levels of γ-Terpinene (25.48%) and p-cymene (15.14%), both of which are known precursors in the biosynthesis of carvacrol and thymol. These two compounds were, however, entirely absent in the essential oils derived from *O. floribundum* B and *O. floribundum* G, suggesting potential differences in the biosynthetic pathways influenced by genetic or environmental factors.

Cis-Sabinene hydrate, another major compound identified, was present in significant concentrations in the essential oils of *O. floribundum* B (31.56%) and *O. floribundum* G (22.21%), yet only found at a trace level (0.07%) in *O. glandulosum*. Similarly, cis-ß-Ocimene was abundant in *O. floribundum* G (24.44%) and *O. floribundum* B (13.76%), while its presence was minimal in *O. glandulosum*, with a concentration of just 0.1%.

Furthermore, thymol, a compound with well-documented biological properties, was detected at a concentration of 12% in *O. glandulosum*. Its presence in *O. floribundum* B and *O. floribundum* G was notably lower, measured at 0.33% and 0.1%, respectively. These observations, illustrated in Figures 15, 16, and 17, provide a clear depiction of the variability in chemical composition among the three populations. This variability not only highlights the influence of environmental and/or genetic factors, but also underlines the potential of these essential oils for various applications in the pharmaceutical and industrial fields.

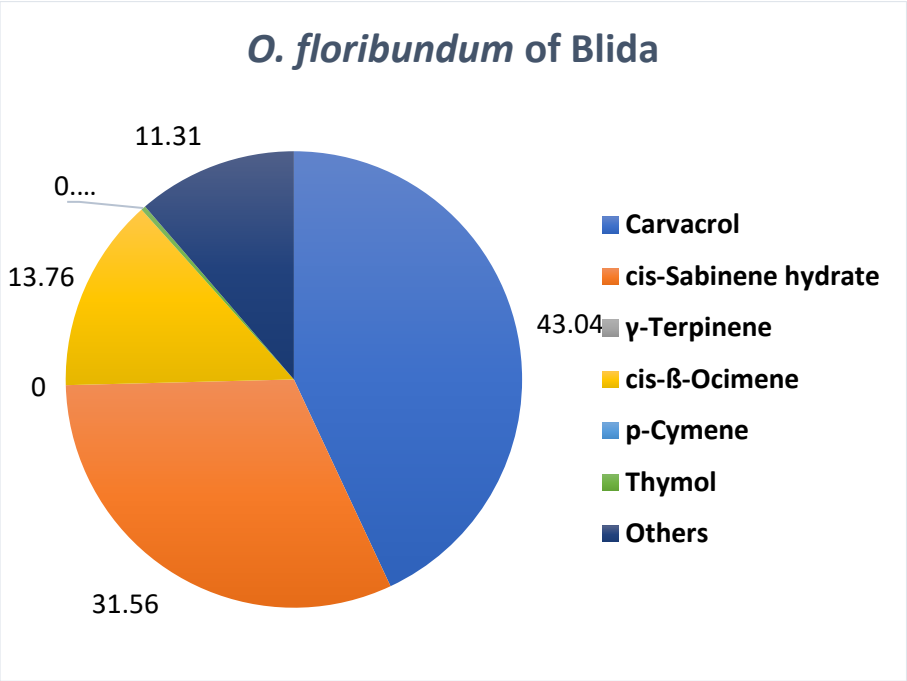


Figure 15. Percentages of major chemical compounds in *Origanum floribundum* Essential Oil from Blida.

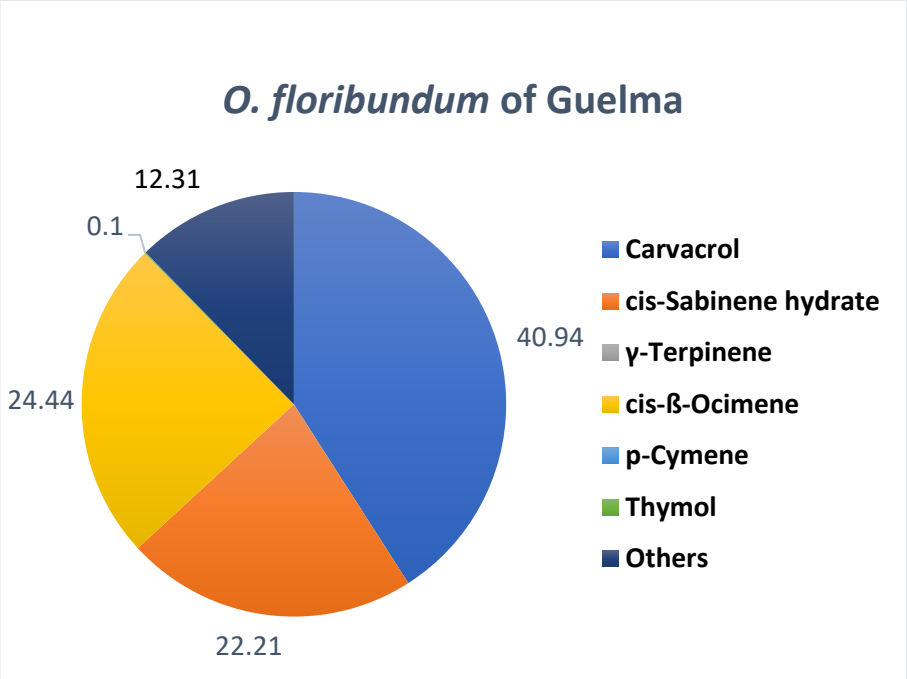


Figure 16. Percentages of major chemical compounds in *Origanum floribundum* Essential Oil from Guelma.

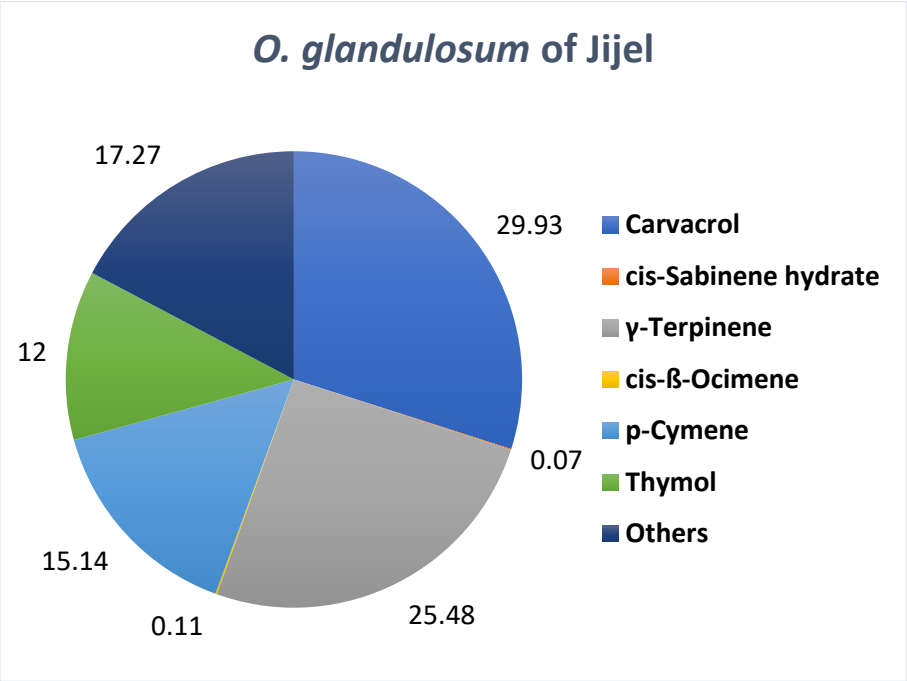


Figure 17. Percentages of major chemical compounds in *Origanum glandulosum* Essential Oil from Jijel.

The essential oils of *Origanum* species have been studied for their chemical compositions in different regions of Algeria. Comparing our results with previous studies, several observations can be highlighted, as illustrated in (Figure 18):

Carvacrol, the main compound in our samples, reached maximum concentrations of 43.04% in *O. floribundum* B and 40.94% in *O. floribundum* G. These results are slightly higher than those reported for *O. floribundum* from Blida (40%) by Baser *et al*. (2000) and for *O. glandulosum* from Sétif (36.29%) by Ali *et al.* (2020).

Cis-sabinene hydrate, abundant in our essential oils of *O. floribundum* B (31.56%) and *O. floribundum* G (22.21%), is almost absent in previous studies, except for a low concentration of 0.19% reported in *O. glandulosum* from Guelma (Bouhaddouda, 2016). This striking difference highlights the unique chemical profiles of our samples

γ-Terpinene, present at 25.48% in our essential oil of *O. glandulosum*, is also found in earlier studies, with variations ranging from 16.61% in *O. glandulosum* from Guelma (Bouhaddouda, 2016) to 23.43% in *O. glandulosum* from Sétif (Ali *et al.*, 2020).

Regarding cis-ß-ocimene, its concentration in our samples ranges from 24.44% for *O. floribundum* G to 13.76% for *O. floribundum* B, but it is practically absent in our *O.*

glandulosum samples (0.1%). This compound is even rarer in previous studies, where it was detected only at a low level of 0.07% in *O. glandulosum* from Guelma (Bouhaddouda, 2016).

P-cymene, detected only in the essential oil of *O. glandulosum* at a concentration of 15.14%, is also reported in several previous studies. It reached maximum levels of 27.6% in *O. floribundum* from Ain Defla (Brada *et al.*, 2012) and 25.61% in *O. glandulosum* from Guelma (Bouhaddouda, 2016).

Finally, thymol, identified at 12% in our essential oil of *O. glandulosum*, achieved high levels in some previous studies, notably 44.6% in *O. glandulosum* from Sétif (Sari *et al.*, 2006), showing significant variation depending on the region and species.

The variation in composition may be attributed to minor genetic and Epigenetic alterations that may not significantly affect morphology or anatomy, but can result in substantial differences in chemical phenotype (Keefover-Ring *et al.*, 2009).

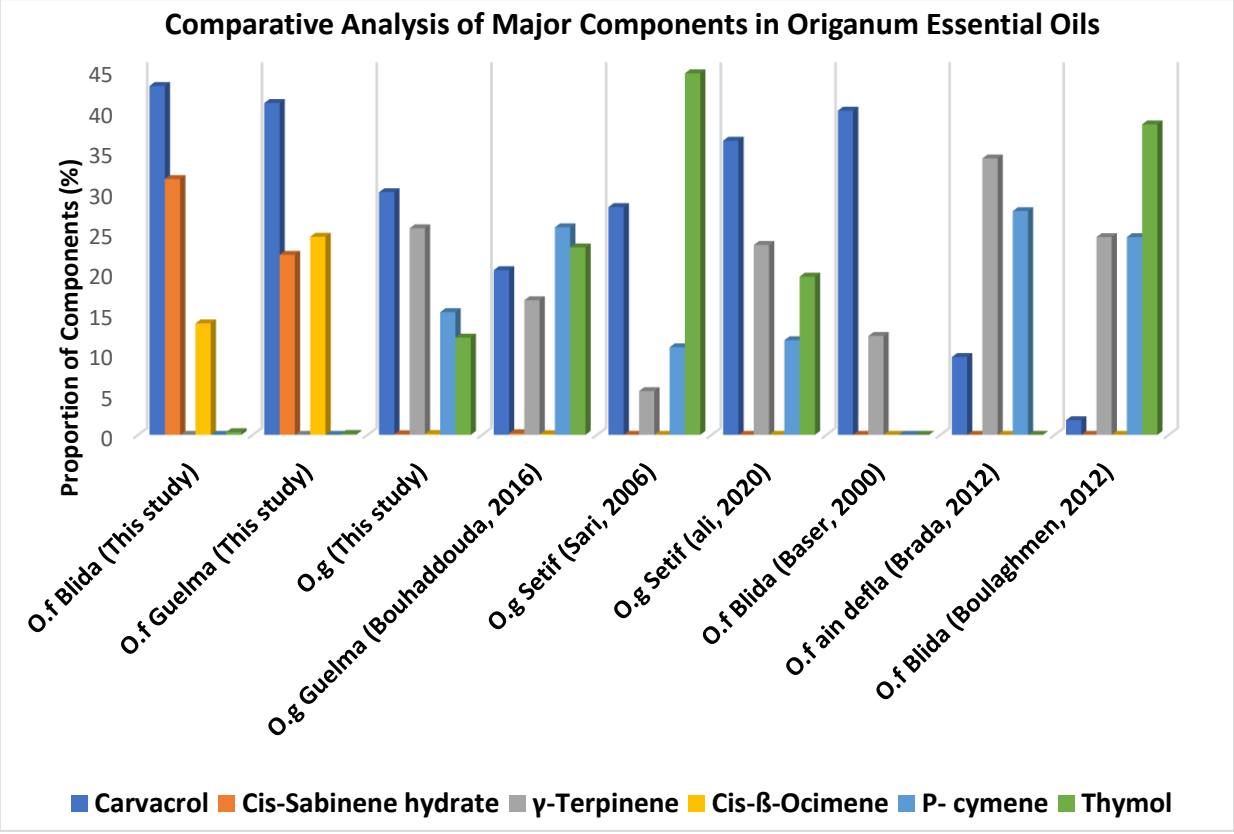


Figure 18. Major components in *Origanum* essential oils*:* comparison of this study and literature data *(O. f: O. floribundum* and *O.g: O. glandulosum).*

3.2.2. Chemical Compounds Families

The chemical study of the three essential oils revealed that their constituents could be classified into five distinct chemical families.

Phenols represented the major family in *O. floribundum* G and *O. floribundum* B, constituting 41.27% and 43.74% of their essential oils, respectively. In *O. glandulosum*, phenols were also abundant at 42.97%, but this family was the second most prominent after monoterpene hydrocarbons, which accounted for 45.75% of its composition. Monoterpene hydrocarbons were present in lower proportions in *O. floribundum* G and *O. floribundum* B, with percentages of 28.7% and 18.9%, respectively (Figure 19).

Oxygenated monoterpenes were found at relatively low concentrations in *O. glandulosum*, representing 1.38% of its essential oil. However, these compounds were more abundant in *O. floribundum* G and *O. floribundum* B, accounting for 22.63% and 31.84% of their respective oils.

Oxygenated sesquiterpenes were detected at minimal levels in all three populations. Their percentages were 0.29% in *O. floribundum* B, 0.38% in *O. floribundum* G, and 0.47% in *O. glandulosum*.

This classification into chemical families illustrates the variability in the chemical composition of the three populations and highlights notable differences between species.

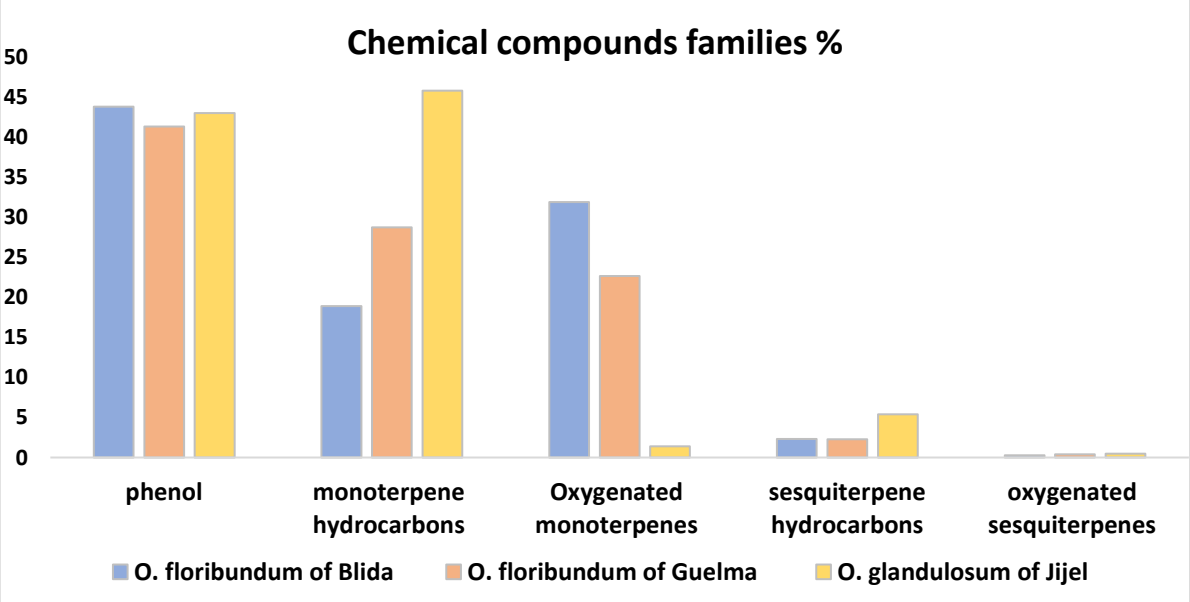


Figure 19. Chemical compound families of the three plants' essential oils.

3.3. Acaricidal activity

The evaluation of the acaricidal activity of essential oils was conducted in vivo on a domestic bee colony of the species *Apis mellifera*, naturally infested by the *Varroa destructor* mite. The study aimed to assess the effectiveness of different essential oil treatments in reducing mite infestations while comparing their performance to a standard chemical control. The acaricidal effectiveness of the oils was determined by calculating the ratio of infestation rates before and after treatment, using a standardized methodology for precise quantification.

Prior to the treatments, the infestation rate was estimated by calculating the number of *Varroa* mites present in the hive. This was achieved by recording the average daily mite drop over a specified period and multiplying it by 90, corresponding to the approximate lifespan of female *Varroa*. This method provided an accurate baseline for infestation levels, ensuring comparability between treatment groups. Following treatment, the infestation rate was reassessed by subtracting the total number of mites fallen post-treatment from the initial infestation rate. This approach allowed for an evaluation of the oils' acaricidal potential by accounting for the mites directly affected by the treatment.

Among the tested treatments, the 2% essential oil of *O. floribundum* sourced from Guelma exhibited the highest acaricidal activity, achieving a remarkable reduction in mite infestation with a mortality rate of 76.79% (Figure 20). Similarly, the 2% essential oil of *O. floribundum* from Blida demonstrated a comparable efficacy of 75.89%. Both treatments surpassed the acaricidal efficiency of the chemical control (C+), Bayvarol, which showed an efficacy of 73.69%. These findings underline the potential of *O. floribundum* essential oils as a promising alternative to chemical acaricides in integrated pest management strategies.

Conversely, the 2% dose of *O. glandulosum* essential oil exhibited a notably lower efficacy, not exceeding 57.37%. Even at reduced doses of 1.5%, the essential oils of *O. floribundum* from Guelma and Blida maintained moderate acaricidal effects, with mortality rates of 49.10% and 51.64%, respectively. In contrast, the impact of *O. glandulosum* essential oil at this concentration remained limited to 45.12%, further highlighting its comparatively weaker acaricidal potential. A similar trend was observed at the lowest tested dose (1%), where the efficacy of *O. glandulosum* remained significantly lower than that of the two *O. floribundum* essential oils.

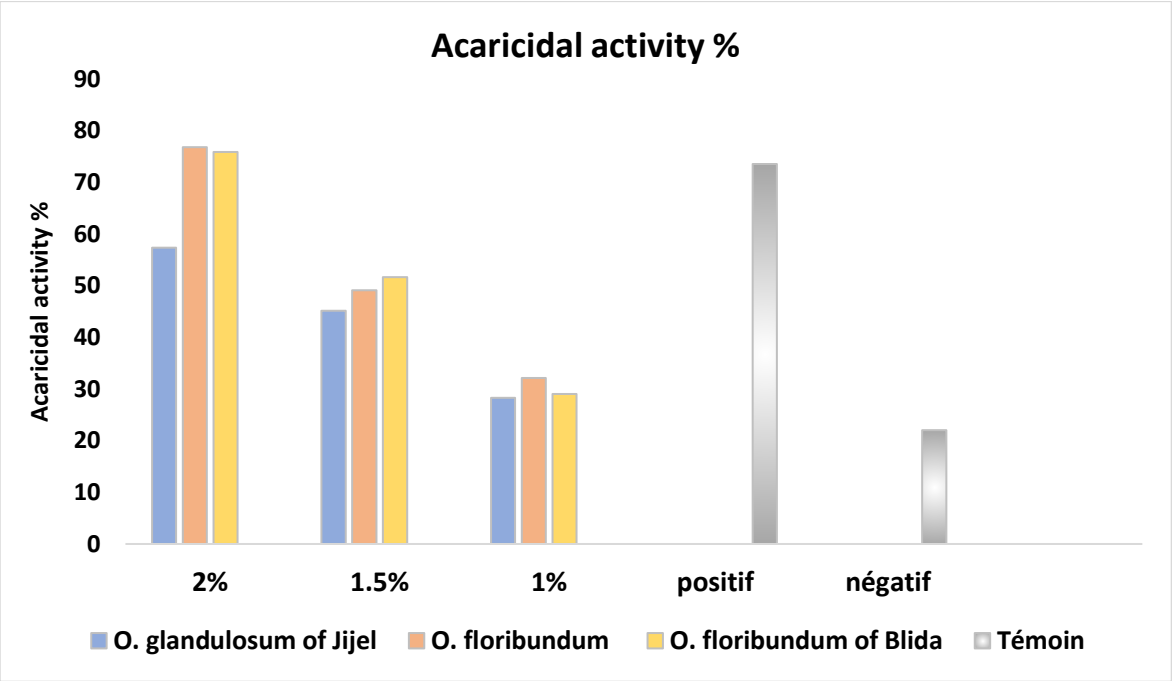


Figure 20. Acaricidal activity of essential oils from *O. floribundum* (Blida), *O. floribundum* (Guelma), and *O. glandulosum* (Jijel).

The ANOVA analysis revealed a highly significant difference (p=0.000) in the efficacy values among the different treatments. The subsequent Tukey HSD test demonstrated significant differences, ranging from very significant to extremely significant, between the various doses of the three oils. Notably, there was no significant difference in the efficacy between the 2% doses of *O. floribundum* G essential oils and the chemical control (C+/2% *O. floribundum* G, p=0.912, and C+/2% *O. floribundum* B, p=0.990). It is worth mentioning that the effectiveness of *O. floribundum* essential oils from Guelma and Blida at 2% was superior to that of the chemical treatment. On the other hand, the difference in efficacy between the chemical control and the 2% dose of *O. glandulosum* essential oil was highly significant (C+/2% *O. glandulosum*, p=0.000). Furthermore, the 2% dose of *O. glandulosum* essential oil differed significantly from the 2% doses of *O. floribundum* essential oils (2% *O. glandulosum*/2% *O. floribundum* G, p=0.000, and 2% *O. glandulosum*/2% *O. floribundum* B, p=0.000). However, there was no significant difference in efficacy between the 2% doses of *O. floribundum* essential oils from Guelma and Blida (p=1.000).

The difference in acaricidal efficacy between the chemical control and the 1.5% doses of the three oils was extremely significant, with no significant difference among the three oils at the 1.5% dose (*O. floribundum* G/*O. glandulosum*, p=0.737; *O. floribundum* B/*O.*

glandulosum, p=0.163; *O. floribundum* G/*O. floribundum* B, p=0.974). Similar results were observed with the 1% dose.

The essential oils of *O. floribundum* plants from Guelma and Blida, which are of the same species but originate from different provenances, exhibit a remarkably similar chemical composition and comparable efficacy against *Varroa destructor*. This similarity highlights the genetic stability of this species in terms of its ability to synthesize bioactive compounds. The differences between these provenances were predominantly observed in the yield of the essential oils, which varied depending on the growth conditions. This variability in yield underscores the critical role of external factors in shaping the productivity of medicinal and aromatic plants, even when their chemical profiles remain largely consistent.

In contrast, the essential oils of *O. glandulosum*, another species within the same genus as *O. floribundum*, displayed distinct differences in chemical composition and exhibited significantly lower acaricidal activity. This divergence can be attributed to species-specific metabolic pathways that result in the production of unique combinations of bioactive compounds. These findings suggest that, while species from the same genus share certain traits, their biological activity can vary greatly, reinforcing the importance of species-level characterization in evaluating their potential applications.

Notably, the essential oils of *O. floribundum* B and *O. floribundum* G, which contained carvacrol as the sole phenolic compound, demonstrated superior acaricidal activity when compared to the essential oil of *O. glandulosum*, which included both carvacrol and thymol. This finding suggests that the interaction between carvacrol and thymol within the essential oil matrix may negatively impact the overall efficacy. Supporting this hypothesis, Karpouhtsis *et al.,* (1998) reported that while carvacrol is inherently more toxic than thymol, its activity decreases in the presence of thymol, indicating potential antagonistic interactions between these two phenols. Such interactions could involve competitive binding at biological targets, reducing the toxic potential of carvacrol when combined with thymol.

The acaricidal activity observed for *O. floribundum* B and *O. floribundum* G essential oils at a 2% concentration aligns with the findings of Romo-Chacón *et al.,* (2016), who reported an efficiency of 74% using doses of 1.16 mL of oregano essential oils containing approximately 60% carvacrol. These results further reinforce the potential of high-carvacrol essential oils as a natural alternative for the control of *Varroa destructor*. Additionally, Melathopoulos (2001) emphasized that essential oils with more than 70% acaricidal

efficiency could serve as a viable alternative for pest control in apiculture. Importantly, the doses used in this study, as well as those reported by Romo-Chacón *et al.*, (2016), were shown to be safe for bees and posed no risk to the quality of hive products, ensuring the sustainability and applicability of these natural solutions in beekeeping practices.

Despite numerous studies highlighting the importance of phenolic compounds such as carvacrol and thymol in determining the biological activity of essential oils (Sacchetti *et al.*, 2005; Ait-Ouazzou *et al.*, 2011), the results of this study suggest that the specific chemical interactions between these compounds are of greater significance than their individual concentrations. In the case of *O. glandulosum*, the coexistence of carvacrol and thymol appears to reduce acaricidal efficacy compared to *O. floribundum*, where carvacrol is the predominant phenolic compound.

Additionally, the chemical composition of the essential oils revealed notable differences in other classes of compounds, such as monoterpene hydrocarbons and oxygenated monoterpenes. *O. glandulosum* essential oils exhibited significantly higher levels of monoterpene hydrocarbons compared to *O. floribundum*, suggesting a possible antagonistic effect on acaricidal activity. Conversely, oxygenated monoterpenes were more abundant in *O. floribundum*, potentially contributing to a synergistic enhancement of biological activity. These findings are consistent with the work of Karpouhtsis *et al.*, (1998), which highlights the critical role of complex interactions between phytoconstituents in modulating the efficacy of essential oils.

The biological activities of essential oils are thus the result of intricate interactions among their various chemical constituents, which can lead to either synergistic or antagonistic effects. Such interactions are not only influenced by major compounds like carvacrol and thymol but can also be significantly affected by the presence of minor constituents. As noted by Gutierrez *et al.*, (2008), even small quantities of certain compounds can have a profound impact on the overall biological activity of essential oils. This highlights the need for a comprehensive understanding of the chemical matrix within essential oils to fully elucidate their potential as natural bioactive agents.

3.4. Antioxidant Activity

The antioxidant activity of the essential oils was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, with results expressed as the percentage of inhibition (Figure 21 and 22). These figures depict the inhibition curves for the tested essential oils compared to the standard antioxidant, ascorbic acid. The DPPH assay is a reliable and widely used method for quantifying antioxidant activity by assessing the capacity of compounds to neutralize free radicals.

In this study, the essential oils demonstrated a notable antioxidant potential, as evidenced by their ability to stabilize DPPH radicals and prevent oxidative damage. Their effectiveness was compared to ascorbic acid, which served as a benchmark due to its well-documented antioxidant properties.

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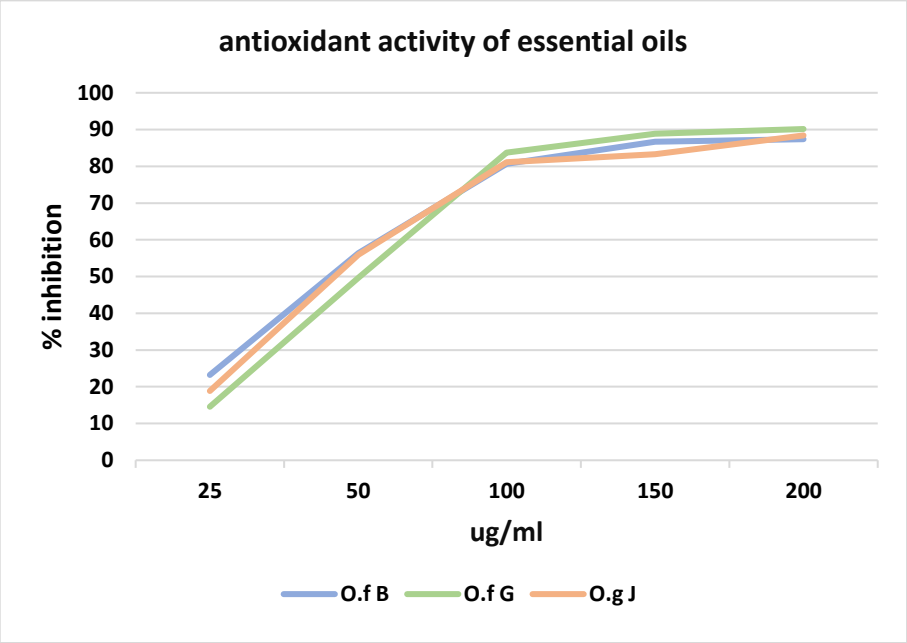

(O.f B: *O. floribundum* from Blida, O.f G: *O. floribundum* from Guelma and O.g J: *O. glandulosum* from Jijel)

Figure 21. DPPH Inhibition Percentage by Essential Oils.

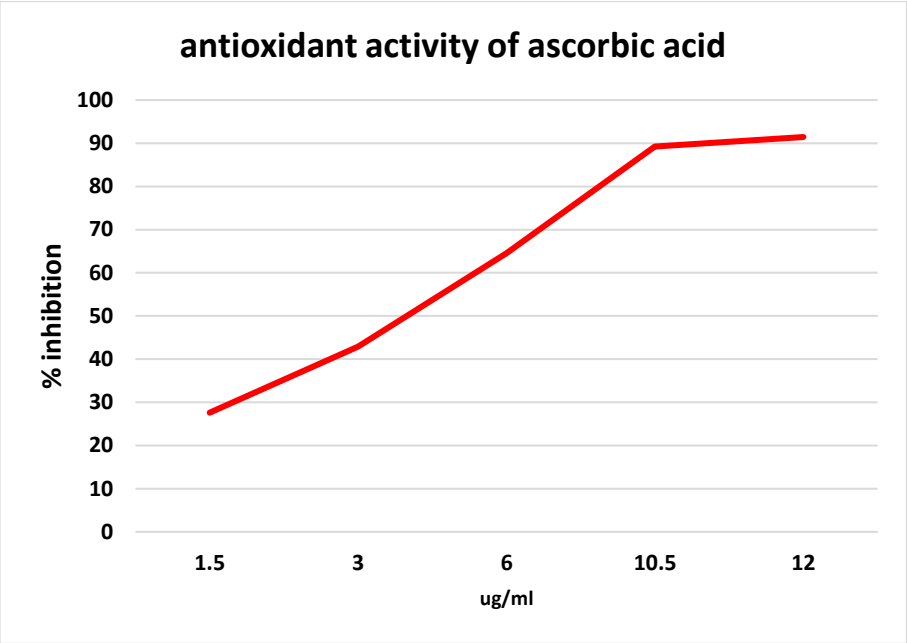

Figure 22. DPPH Inhibition Percentage by Ascorbic Acid.

According to the graphical curve (Figure 21), it is observed that the three essential oils exhibited nearly identical DPPH inhibition rates, reflecting their similar antioxidant properties. At a lower concentration of 25 µg/ml, the oils demonstrated a modest inhibition percentage, indicating limited activity at this dosage. However, as the concentration increased to 100 µg/ml, a marked improvement was observed, with inhibition rates reaching 80.68%, 83.71%, and 81.12% for *O. floribundum* B, *O. floribundum* G, and *O. glandulosum*, respectively. This significant increase in activity suggests that higher concentrations are essential to achieving optimal scavenging efficiency.

At even higher concentrations of 150 and 200 µg/ml, the inhibition percentages continued to rise slightly, peaking at 87.37%, 90.14%, and 88.39% for *O. floribundum* B, *O. floribundum* G, and *O. glandulosum*, respectively. This saturation effect suggests that the antioxidant compounds in the oils may reach a threshold beyond which further increases in concentration have diminishing returns in terms of DPPH scavenging activity.

Regarding (Figure 22), the control (ascorbic acid) displayed a notable antioxidant capacity even at much lower concentrations, ranging from 1.5 to 12 µg/ml. At the minimal concentration of 1.5 µg/ml, the inhibition percentage was relatively low at 27.60%. However, a dramatic increase was observed at 10.5 µg/ml, where the inhibition rate reached 89.26%, showcasing the potency of ascorbic acid as a benchmark antioxidant.

Both curves clearly illustrate the dose-dependent relationship between the tested samples and their DPPH scavenging activity. The proportionality observed with increasing concentrations highlights the importance of concentration as a determinant of antioxidant effectiveness. While the essential oils required higher concentrations to achieve significant inhibition rates, they still demonstrated promising activity, supporting their potential as natural alternatives for antioxidant applications.

3.4.1. Determination of the inhibitory concentration 50%

By exploiting the graphical curves of antioxidant activity and applying the regression line equation to each curve (Figure 23), the IC50 was calculated. This value represents the concentration required for the essential oil or ascorbic acid to inhibit 50% of the DPPH radical present in the medium. A lower IC50 indicates a more significant antioxidant activity of the compound.

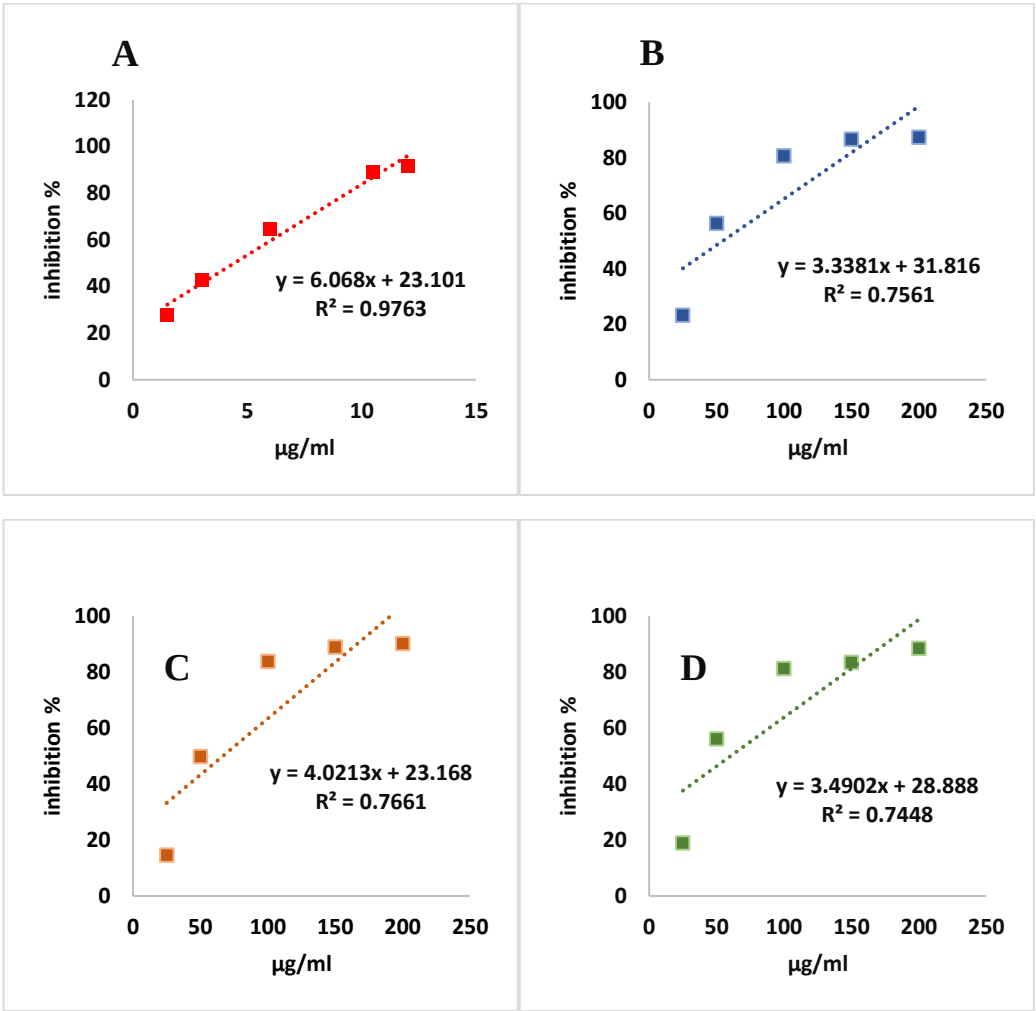

Figure 23. determination of Ic50 for: (A): ascorbic acid; (B): *O. floribundum* of Blida; (C): *O. floribundum* of Guelma and (D): *O. glandulosum* of Jijel.

The inhibitory concentrations 50% (IC50) of the essential oils were 54.4 µg/mL ± 0.9 for *O. floribundum* B, 66.7 µg/mL ± 3.9 for *O. floribundum* G, and 60.4 µg/mL ± 4 for *O. glandulosum*. Although these values indicate a significant capacity to neutralize free radicals, they are notably less effective compared to the IC50 of the control (ascorbic acid), which was established at 4.43 µg/mL ± 0.37.

Despite the observed variations in the chemical composition of the essential oils of *O. floribundum* and *O. glandulosum*, particularly in the major components, the percentage of carvacrol was high in *O. floribundum* B and *O. floribundum* G, reaching 43.04% and 40%, respectively, while it was relatively low in *O. glandulosum*, at 29.93%. Similarly, the presence of cis-sabinene hydrate was notable in both *O. floribundum* B and G, at 13.76% and 24.44%, respectively, while it was present in trace amounts in *O. glandulosum*. Conversely, the presence of thymol at 12%, γ-terpinene at 25.48%, and p-cymene at 15.14% was observed in *O. glandulosum*, while they were absent in both *O. floribundum* samples.

Interestingly, despite these differences in chemical composition, particularly in the major and minor constituents, no significant impact on the antioxidant activity of the oils was observed. This observation led us to investigate the chemical families in greater detail to identify potential similarities, particularly among the phenols and oxygenated sesquiterpenes. The analysis revealed that the three oils shared comparable proportions of phenols and nearly identical proportions of oxygenated sesquiterpenes. While some differences were evident in the groups of monoterpene hydrocarbons and oxygenated monoterpenes, these variations appeared to exert no notable influence on the antioxidant activity. These findings suggest that phenol groups might play a predominant role in driving the antioxidant properties of these essential oils, potentially serving as the main bioactive components responsible for their effectiveness.

The results of this experiment and the values obtained by the essential oils strengthens the argument in favor of their significant effectiveness as an antioxidant. A comparison with similar studies on oregano essential oils tested using the same DPPH model reveals parallel results. For instance, the outcomes align with those of (Sari *et al.,* 2006), who investigated various species of *O. glandulosum* and obtained IC50 values ranging from 16.2 to 26.7 µg/ml. Similarly, the findings of (Mahfouf *et al.,* 2018), studying *O. glandulosum* essential oils, disclosed an IC50 of 1.28 ± 0.07 mg/ml, while the standard's IC50 was 0.361 ± 0.04 mg/ml. Although the reducing power of essential oils is lower than that of the standard, they

demonstrate substantial antioxidant activity. Many other similar studies have shown the power of these essential oils as a good inhibitor of free radicals (Mahfouf *et al.,* 2018).

The essential oils of *Origanum* act as good hydrogen donors, capable of trapping free radicals and mitigating their harmful effects (Krimat *et al.,* 2019). This capability is largely attributed to the high content of thymol, as demonstrated in Sari's 2006 study, along with other compounds such as carvacrol, γ-terpinene, p-cymene, carvacrol methyl ether, and thymol methyl ether (Boulaghmen, 2012). Additionally, the antioxidant activity of *Origanum* essential oils is attributed to the ability of carvacrol, thymol, and p-cymene to form chemical complexes with metal ions and free radicals, thereby enhancing their effectiveness (Lombrea *et al.*, 2020). Furthermore, the potential presence of monoterpenes and/or oxygenated sesquiterpenes may also contribute to this antioxidant activity (Miladi *et al.*, 2013). The major components within *Origanum* essential oils, working synergistically with minor compounds, could thus yield a more substantial antioxidant activity (Mahfouf *et al.*, 2018).

3.5. Anti-inflammatory activity

In vivo evaluation of the anti-inflammatory activity of essential oils was conducted using the carrageenan-induced edema method. Carrageenan injection was administered 30 minutes after the essential oils were administered, with diclofenac serving as a positive control and untreated subjects acting as a negative control. Edema measurements were taken 4 hours after carrageenan injection. Edema was calculated by comparing the weight of the left hind paw with that of the right hind paw, and the results are presented in (Figure 24).

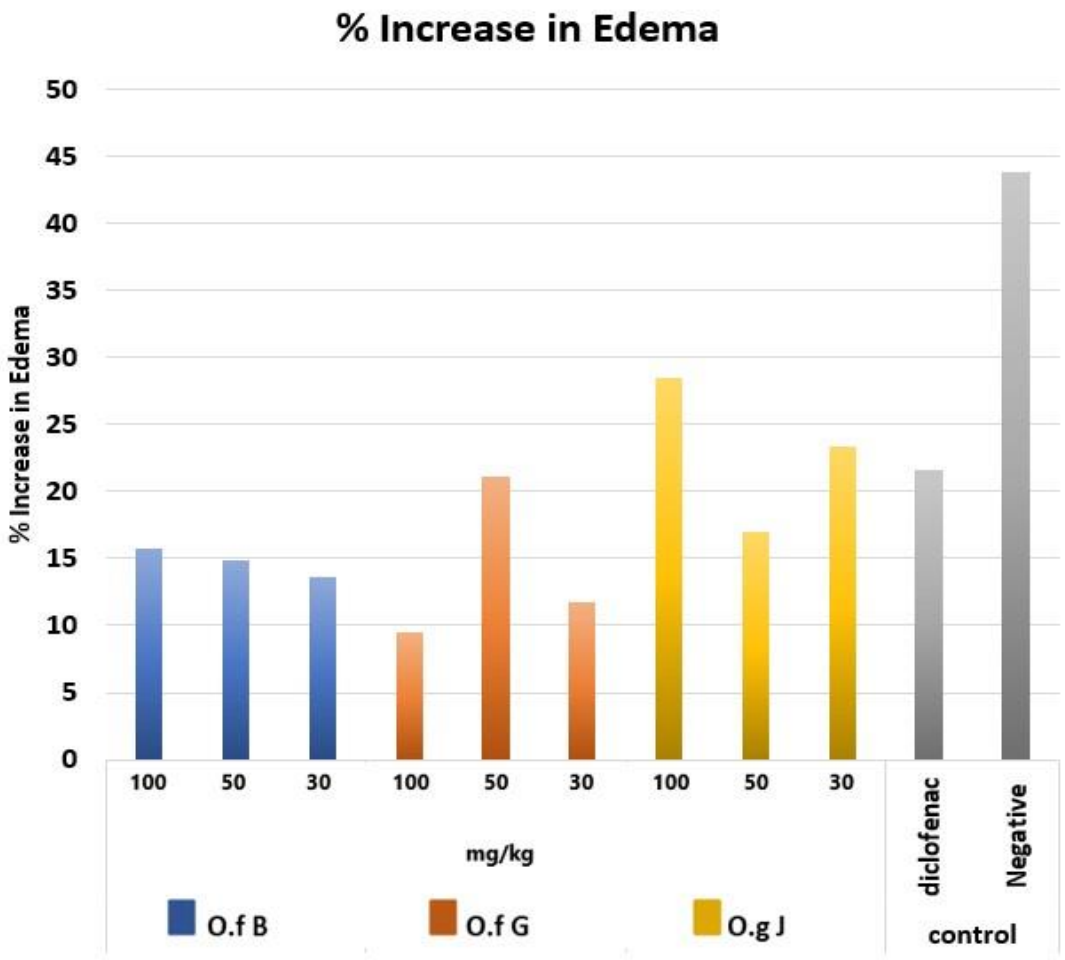

Figure 24. Percentage Increase in Edema.

It is evident from (Figure 24) that the most significant edema was recorded in the mice of the negative control group, reaching 44%. In contrast, the edema size in the positive control group (diclofenac) was approximately half of that recorded in the negative control group. The edema sizes in the treated mice varied depending on the essential oils and concentrations used. To determine the edema reduction rate for each treatment, we compared it to the negative control group, expressed as a percentage, representing the anti-inflammatory efficacy. The edema reduction percentages are illustrated in (Figure 25).

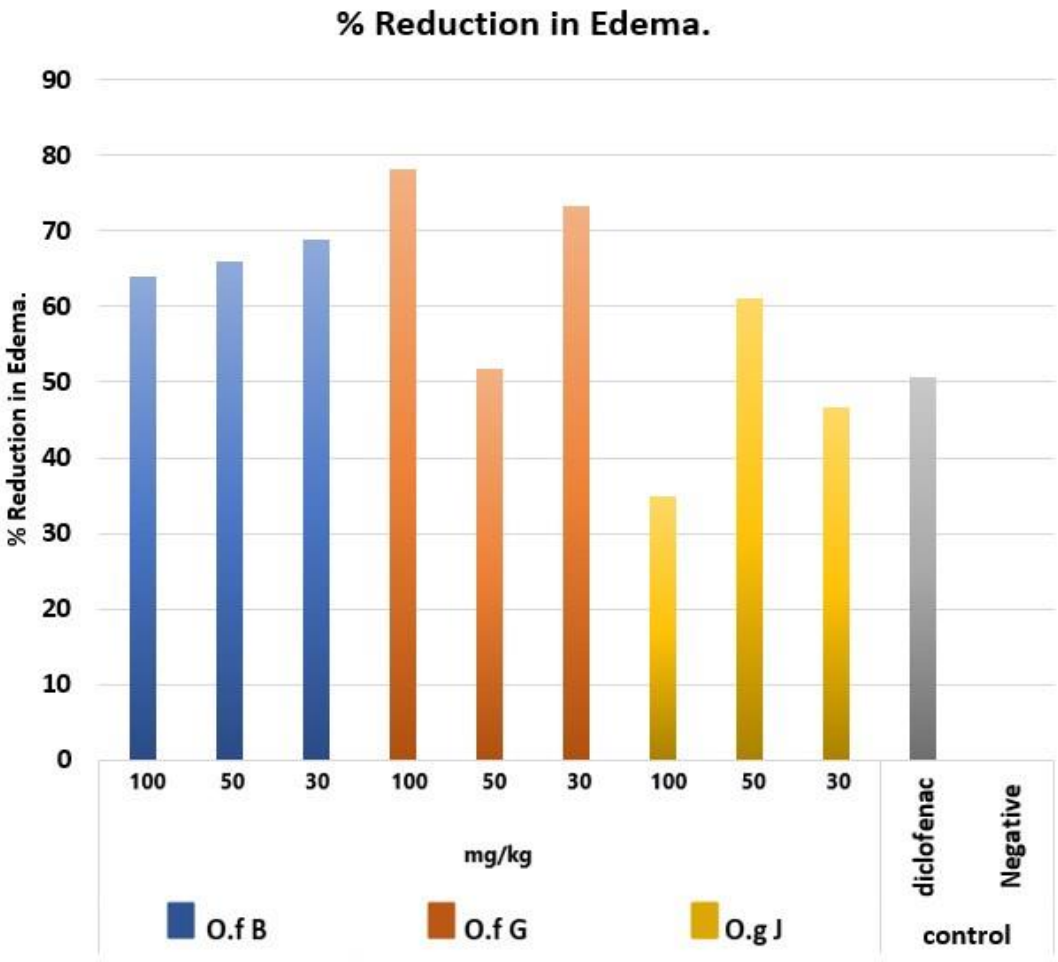

Figure 25. percentage of edema reduction.

The results from (Figure 25) highlight the impact of various doses of *Origanum* essential oils on edema reduction compared to the negative control group, considered to have a reduction of 0%. The positive control group (diclofenac) showed a significant edema reduction evaluated at 50.71%, serving as a reference.

Regarding the essential oil *O. floribundum* B, the three doses (100 mg/kg, 50 mg/kg, 30 mg/kg) demonstrate a gradual increase in anti-inflammatory efficacy, with respective reductions of 64.05%, 66.04%, and 68.87%. This trend suggests a negative dose-dependency of anti-inflammatory activity for *O. floribundum* B. The highest dose, 100 mg/kg, showed a 64.05% edema reduction, while the intermediate dose, 50 mg/kg, and the lowest dose, 30 mg/kg, exhibited more significant reductions, with values of 66.04% and 68.87%, respectively.

On the other hand, *O. floribundum* G oil exhibits a variation in its efficacy depending on the doses. Doses of 100 mg/kg and 30 mg/kg showed reductions of 78.25% and 73.25%,

respectively, indicating strong anti-inflammatory efficacy. However, the intermediate dose, 50 mg/kg, reveals a less pronounced anti-inflammatory response with a reduction of 51.81%.

The *O. glandulosum* oil also demonstrates a variation in its anti-inflammatory efficacy depending on the doses. Doses of 100 mg/kg (35.00%) and 30 mg/kg (50.71%) showed relatively lower reductions, while the intermediate dose of 50 mg/kg achieves an efficacy with a reduction of 61.10%. These results highlight the complexity of the inflammatory response induced by this oil.

The comparison of the three *Origanum* essential oils reveals significant anti-inflammatory properties. *O. floribundum* B and *O. floribundum* G demonstrate remarkable anti-inflammatory efficacy, even surpassing diclofenac at certain concentrations, while *O. glandulosum* oil exhibits a variable response.

The results of the anti-inflammatory activity test align with several previous studies, thereby enhancing the understanding of the effects of essential oils. An analysis of *Origanum* EOs, primarily rich in carvacrol, revealed significant anti-inflammatory action, as evidenced by Silva et al (2012) and Pérez *et al.* (2013). Additionally, previous research focusing on the carrageenan-induced edema model highlighted the notable effect of essential oils on edema reduction, as demonstrated by (Hamamouchi *et al.,* 2021). A separate study on thyme EO, rich in thymol, underscored its anti-inflammatory activity (Abdelli *et al.,* 2017).

The activity of essential oils can be attributed to various mechanisms, including the reduction of the synthesis of pro-inflammatory cytokines TNF-a, IL-1b, and IL-6, as highlighted by (Pérez *et al.,* 2013), and the inhibition of vascular permeability, as demonstrated by (Silva *et al.,* 2012). Furthermore, the inhibition of cyclooxygenase (COX) and potent antioxidant activity, may also contribute to this anti-inflammatory activity. Moreover, the anti-inflammatory activity of *Origanum* essential oils has been linked to the presence of carvacrol and γ-terpinene as major compounds, which likely play a central role in modulating inflammatory responses (Lombrea *et al.,* 2020). Additionally, minor compounds such as p-cymene and linalool could play a substantial role in this anti-inflammatory activity (Abdelli *et al.,* 2017).

Conclusion

Conclusion

This study focused on investigating the phytochemical and biological variability of plants from three different origins of the *Origanum* genus, namely *Origanum glandulosum* and *Origanum floribundum*, endemic to Algeria. Essential oils were extracted by hydrodistillation, followed by GC-MS chemical analysis of their composition. Subsequently, their biological properties, including acaricidal activity against *Varroa*, antioxidant activity, and anti-inflammatory activity, were evaluated to highlight variations between the two species, *Origanum glandulosum* and *Origanum floribundum*, as well as the effect of variability attributable to different origins and environments.

Initially, regarding essential oils yield, variations were observed according to species and origins. Among the three species studied, *Origanum floribundum* from Guelma exhibited the highest yield, *Origanum floribundum* from Blida showed the lowest yield, while *Origanum glandulosum* presented an intermediate yield. Growth conditions, plant origin, and even species genetics may account for these variations. It is noteworthy that all samples underwent a uniform drying and extraction process.

Regarding chemical composition, the analysis revealed the presence of different compounds, notably carvacrol, which was the predominant compound in the three essential oils. The concentrations of phenols in each oil were close. Similarities were observed between the two populations of *Origanum floribundum*. However, significant variations were observed in compound concentrations between different species. Additionally, the results obtained showed both similarities and differences compared to previous studies, thus emphasizing the importance of chemical variations attributable to genetic and environmental factors.

Concerning acaricidal activity, essential oils from *Origanum floribundum* demonstrated notable effectiveness against *Varroa destructor*, especially at high concentrations. However, it is worth noting that essential oils from *Origanum glandulosum* did not exhibit as significant acaricidal activity. This biological difference in the acaricidal properties of essential oils underscores the impact of interspecies variability. Nonetheless, these results remain promising and suggest that *Origanum* essential oils could be used as an alternative for controlling this parasite, with effects comparable to or even superior to conventional chemical treatments.

Regarding antioxidant activity, essential oils from the three populations of *Origanum* showed significant capacity to neutralize free radicals, with IC50 values relatively close to

those of ascorbic acid, used as a control. These results highlight the strong potential of *Origanum* essential oils as natural antioxidant agents.

Finally, concerning anti-inflammatory activity, *Origanum* essential oils demonstrated remarkable ability to reduce carrageenan-induced edema in mice, with variations depending on doses, species, and their origins. These results corroborate previous studies that have emphasized the anti-inflammatory effect of essential oils rich in carvacrol and thymol.

In conclusion, the results of this study highlight the potential of Algerian *Origanum* essential oils as sources of biologically active agents, particularly in controlling bee parasites, protecting against oxidative stress, and modulating inflammatory response. Differences were observed in essential oil yields based on species and origin. Furthermore, variations were noted in compound concentrations between the two species studied, as well as in their effectiveness as acaricidal agents. It is noteworthy that all three EO exhibited good anti-inflammatory activity and similar effects in their antioxidant activities. These results pave the way for further research into the use of *Origanum* essential oils in various fields, including veterinary medicine and phytotherapy, as well as the underlying mechanisms of their biological activities.

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