Exploring the chemical and acaricidal diversity of algerian origanum essential oils against varroa destructor: a comparative study.

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Abstract

Several studies have shown that oregano (Origanum) essential oil has Acaricidal activity against Varroa Destructor, a parasitic pest of the honey bee Apis mellifera. While many researchers have demonstrated that essential oils of this genus are highly variable due to genetic factors and climatic condition, this raises the question of whether this variance affects their Acaricidal activity. If there are differences in effectiveness, which chemical composition exhibits the greatest bio-Acaricidal activity against Varroa Destructor? This comparative study aimed to analyze the chemical composition of essential oils (EOs) extracted by Hydrodistillation from three populations of Algerian Origanum [Origanum floribundum Munby from Blida (O.f B), Origanum floribundum Munby from Guelma (O.f G), and Origanum vulgare L. subsp. glandulosum (Desf.) from Jijel (O.g)], and evaluate their Acaricidal activity against Varroa Destructor. The results revealed significant variations in phenolic contents among the different oregano oils. Oregano oils, specifically Origanum floribundum Munby from Guelma and Origanum floribundum Munby from Blida, both rich in carvacrol, exhibited acaricidal efficacy comparable to the chemical acaricide Bayvarol. Conversely, Origanum glandulosum (Desf.) oil from Jijel displayed lower efficacy. These findings pave the way for future studies aimed at developing and optimizing natural acaricides, promoting a more environmentally and bee-friendly approach in apiculture.

Keywords: *Origanum, floribundum, glandulosum, essential oil, GC-MS, Varroa Destructor, Acaricidal activity.*

1. Introduction

The ectoparasitic mite *Varroa Destructor* is a significant pest in apiaries [1]. It poses the greatest threat to bees in the history of beekeeping due to its status as a relatively new pest with an unbalanced host-parasite relationship, Beekeepers often lack the knowledge and experience to effectively manage it, and without regular Acaricidal treatments, bee colonies can be destroyed within two to three years [2], This parasite impacts the life of the insect by affecting the Hemolymph [3,4]. And causing a decrease in bee defenses [5,6]. As a consequence, a cascade of ecosystem imbalances ensues, starting with the decline of this vital pollinator [7].

There are various methods employed for *Varroa* control, with the most common method being the use of synthetic acaricides, particularly Pyrethroids and Organophosphorus compounds like Flumethrin (Bayvarol) [8,9]. However, despite their effectiveness against mites, chemical methods have limitations. They can be expensive, leave residues, and contaminate hive materials such as honey, wax, and Propolis, posing

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a threat to the health of bees and humans [2,10]. Additionally, they can lead to the emergence and spread of new populations of resistant parasites [11].

Researchers have carried out experiments to test the potential of natural substances as Acaricidal against *Varroa Destructor* [12,13]. Indeed, many essential oils (EOs) based products have become alternatives for mite control [14,15]. These essential oils can cause toxicity, repellent effects, or inhibit pest reproduction [16,17]. The use of these natural essential oils as an agent against bee pests poses no risk to humans [18]. However, improper use of these oils can be toxic to bees, leave large amounts of residues [19], and impair the quality of hive production [18].

Origanum essential oils, known for their biological activities, and various applications in food , cosmetics, and pharmaceutical industries [20], have shown Acaricidal activity against *V. Destructor* [21]. Plants of the genus *Origanum* belong to the *Lamiaceae* family and are distributed throughout the Mediterranean. Of which *O. glandulosum* and *O. floribundum* are native to Algeria and Tunisia (two Afro-Mediterranean nations) [22]. Notably, the chemical components of oregano species' volatile oils vary due to a variety of variables, including the geographical origin [23,24].

The activity of essential oil is typically attributed to its main compounds [23,25,26]. However, it is likely the minority components act synergistically. Therefore, the value of essential oil is associated with its "totum," meaning all of its components and not just the majority compounds [16,27].

Several investigations have been carried out to assess the acaricidal properties of EOs extracted from oregano. According to the current information, no comparative study has been conducted to determine the efficacy of these EOs against *Varroa* mites or to investigate the potential variability in the chemical composition of Algerien *Origanum* essential oils and their impact on their effectiveness against *Varroa*. The present study was conducted to characterize the chemical compositions of essential oils from three Algerian oregano populations (*Origanum floribundum Munby* from Blida, *Origanum floribundum Munby* from Guelma, and *Origanum glandulosum (Desf.)* from Jijel) which originate from different bioclimatic, and geographical zones in Algeria, and to test their Acaricidal activity against *Varroa Destructor*. Essential oils were isolated by Hydrodistillation, and GC-MS analysis was performed to characterize their chemical composition. The EOs were then applied at the same doses in a homogenous apiary contaminated by *Varroa*, in order to determine if the chemical composition and Acaricidal activity of EOs from the genus *Origanum* are influenced by growing conditions and geographical origins. The present research aimed to identify the optimal alternative treatment against the *Varroa Destructor* parasite of *Apis mellifera* using safe doses.

2. Materials and methods

Tests on the Acaricidal activity of the EO were conducted at the apiary of the Technical Institute of Elevators BABA ALI (Algeria), Algeria, between September and December 2021. The essential oil was extracted, physically characterized, and formulated as a Bioacaricide at the Research Laboratory for Aromatic and Medicinal Plants (PAM) of Saad Dahleb University in Blida. The chemical analysis of the EO was

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performed at the Research and Technical Physical and Chemical Center (CRAPC) in Bou Ismaïl, Tipaza, Algeria.

2.1.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 [28,29], The origin and geographic coordinates of the tree population are presented in (**Table 1**).

Species	The region of origin	Geographic coordinates	Elevation
Origanum floribundum Munby	Chrea, Blida	36° 25′ 32″ N, 2° 52′ 36″ E	1927 m
Origanum floribundum Munby	Nechmaya, Guelma	36° 36′ 41″ N, 7° 30′ 48″ E	254 m
Origanum glandulosum (Desf.)	Ghebala, Jijel	36° 37′ 39″ N, 6° 23′ 23″ E	492 m

Table 1. Origins of the plants used in this study.

Samples were cleaned, air-dried, and protected from light for 15 days to maintain high volatile compound content [30]. Dr. AIT HAMOU of IBN KHALDOUN University, Tiaret, conducted the botanical identification of three species.

The extraction of essential oils was performed using the most common method, Hydrodistillation, with 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 three hours [31].

The extraction was repeated five times in order to calculate the precise yield of EOs, using the following formula [32,33]:

$$\text{Yield} = \frac{\text{weight of essential oil recovered in grams}}{\text{weight of dry plant matter in grams}} \text{ X } 100$$

2.2.Phytochemical analysis of essential oils by GC-MS

To determine the chemical composition of the EOs from plants, a Gas Chromatograph combined with Mass Spectrometer (GC-MS) was used in the research center (CRAPC) in BOUSMAIL Tipasa, Algeria. The GC-MS analysis conditions are as follows:

The GC-MS analysis was carried out using an Agilent 6890 Plus coupled to an Agilent 5973 mass spectrometer from Hewlett Packard. 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 be at 60 °C for 8 min and increasing to 250 °C at 2 °C/min. Injection mode: split 1; 20, and a volume of 0.2 µl was injected at 250 °C.

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2.3.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 EO from external factors and control evaporation during treatment. As a negative control, only diluted ointment (without essential oils) was used, 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+)

2.4.Acaricidal activity

The principle of the experiment is to estimate the infestation rate in the hive before and after treatment using the greasy layer method for quantification. The difference between these values will determine the effectiveness of the product used in the treatment [34].

2.4.1. Animal material

The Acaricidal activity of EOs extracted from Origanum plants on *Varroa* mites was evaluated at the ITELV (Technical Institute of Livestock) apiary in Algiers. This apiary, located in the Algerian region of BABA ALI (36°39'12.6 "N 3°03'25.1 "E), consists of 33 hives populated with the species *Apis mellifera Intermissa*, which were infested with *Varroa Destructors*. The apiary spans an area of 4 hectares and is primarily covered with vegetation composed of Carob (*Ceratonia siliqua*) and Woody fleabane (*Dittrichia Viscosa*).

2.4.2. Acaricide activity test

The principle of the experiment was to estimate the infestation levels in the hive before and after treatment by quantifying it using the greasy layer method. The difference between the two values indicates the effectiveness of the product used against mites [34].

2.4.2.1.The estimation of infestation levels before treatment.

The field survey began with applying a layer of vaseline-coated metal to the bottom of the hive frame. During the pre-treatment phase, quantification was performed every 2 days for 28 days to determine the average daily drop of natural *Varroa* mites. This average was then multiplied by 90 days (the life cycle of the female *Varroa*), to estimate the total number of *Varroa* in a hive [35].

2.4.2.2.The Application of Treatments

Each of nine treatments (three doses x three EOs) was applied four times over a period of four weeks to nine batches of three hives each (three replicates). The number of fallen mites was calculated throughout the treatment period, which lasted for 28 days.

2.4.2.3.Treatment efficacy

The efficacy of the treatment was assessed by calculating the percentage reduction in the number of parasites using the following equation [36].

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

Infestation level after treatment = Infestation level before treatment - Varroa dropping during treatment.

2.5.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. IBM SPSS Statistics 20 software was employed for conducting the statistical analysis.

3. Results and discussion

3.1.Yield

Origanum EOs yielded variedly according to species and origin. *O.f* G had the highest essential oil yield, reaching 2.9 ± 0.23 %, which was higher than the values reported by Brada et al. [37] and Baser et al. [38] , which were 1.6 % and 0.66 % respectively. The EO yield of O.g was 1.5 ± 0.1 %, lower than the values reported by Ali et al. [39] and Naima et al. [40] which were 2.5 % and 1.7 % respectively. The lowest essential oil yield was 0.9 ± 0.1 % in *O.f* B (**Figure 1**). The essential oil yield of some *Origanum* species, such as *O. Vulgare* L reached up to 8.0 %, while for other species it dropped to 0.1 % [23,41,42]. Such variations in yield are due to differences in growing conditions, plant origin, development stages, physiological changes in response to different environmental variables and stress, harvesting time, drying techniques, essential oil extraction methods, or solvents used for GC-MS [43,44].

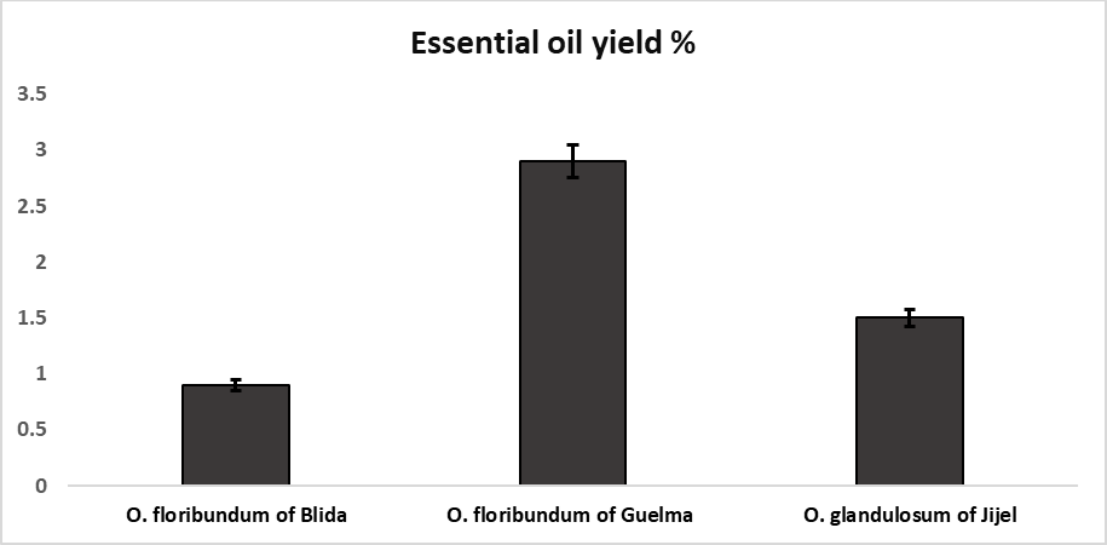

Figure 1. The essential oil yield of *Origanum* plants.

3.2.Chemical composition

The analysis of the chemical composition of the EOs of three plants *O.f* B, *O.f* G, and *O.g* allowed the identification of 20 compounds for specie *O.f* B, which representing 97.07 % of all the constituents of its essential oil, 24 compounds for specie

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O.f G, representing 95.25 %, and 28 compounds for specie *O.g*, representing 96.02 % of all the compounds. (**Table 2**).

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

Table 2. GC-MS analysis of essential oils of *Origanum floribundum* from Blida, *Origanum floribundum* from Guelma, and *Origanum glandulosum*.

The chemical analysis of the three EOs revealed the following main compounds: Carvacrol, which was the predominant Chemotype in the EOs of three plants, The highest level of Carvacrol was found to be 43.04 % in *O.f* B, 40 % in *O.f* G, and 29.93 % in *O.g*. Furthermore, the essential oils of *O.g* contained 25.48 % γ-Terpinene and 15.14 % P-cymene, which are precursors of Carvacrol and thymol. However, γ-Terpinene and P-cymene were completely absent in the EOs of *O.f* G and *O.f* B.

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Additionally, cis-Sabinene hydrated was present at concentrations of 31.56 % in EO *O.f* B, 22.21 % in EO *O.f* G, and 0.07 % in EO *O.g*. Cis-ß-Ocimene was found in EO *O.f* G and *O.f* B at concentrations of 24.44 % and 13.76 %, respectively, and at low levels of 0.1 % in EO *O.g*. Thymol was detected at 12 % in EO *O.g*, and in very small amounts of 0.1 % in EO *O.f* G and 0.33 % in EO *O.f* B (**Figure 2**).

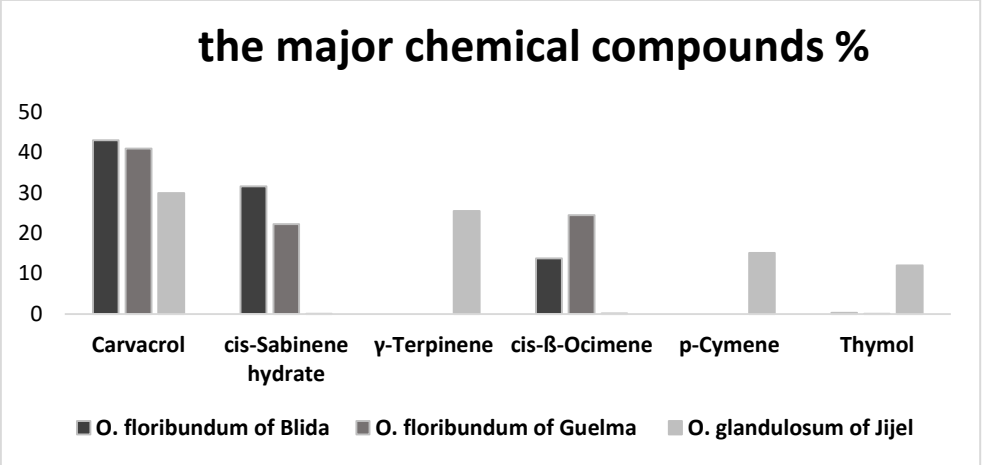

Figure 2. Percentages of major chemical compounds in three plants' essential oils (*O. floribundum* of Blida, *O. floribundum* of Guelma, and *O. glandulosum* of Jijel).

The chemical study of the three essential oils revealed that their constituents were classified into five chemical families. The major family of EO *O.f* G and EO *O.f* B was phenol, comprising 41.27 % and 43.74 % of the essential oils, respectively. Although EO *O.g* had a phenol rate of 42.97 %, it was the second most abundant family after Monoterpene hydrocarbons, which were present at 45.75 %. The percentages of Monoterpene hydrocarbons in EO *O.f* G and EO *O.f* B were 28.7 % and 18.9 %, respectively. Oxygenated Monoterpenes were found in low concentrations in EO *O.g* at 1.38 %, but were relatively abundant in EO *O.f* G and EO *O.f* B, constituting 22.63 % and 31.84 % of the essential oils, respectively. The presence of the Oxygenated Sesquiterpenes family was detected in EO *O.f* B, EO *O.f* G, and EO *O.g* with percentages of 0.29 %, 0.38 %, and 0.47 %, respectively (**Figure 3**). The analysis of Origanum glandulosum EO from the Sétif region by Ali et al. [39] resulted in the following chemical composition: Carvacrol 26.29 %, γ-Terpinene 23.43 %, Thymol 19.52 %, P-cymene 11.67 %, and α-Terpinene 3.02 %, These findings are similar to the chemical composition of EO *O.g* [39].

In a study conducted by Baser et al. [38], an analysis was performed on the essential oils of Origanum floribundum from Blida. The results revealed a chemical polymorphism, with the following composition: 40 % Carvacrol, 16.1 % linalool, 12.4 % P-cymene, 12.2 % γ-Terpinene, and 1.1 % thymol, these results differ from the EO *O.f* G and EO *O.f* B of this study, although the Carvacrol content was the same. 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 [24].

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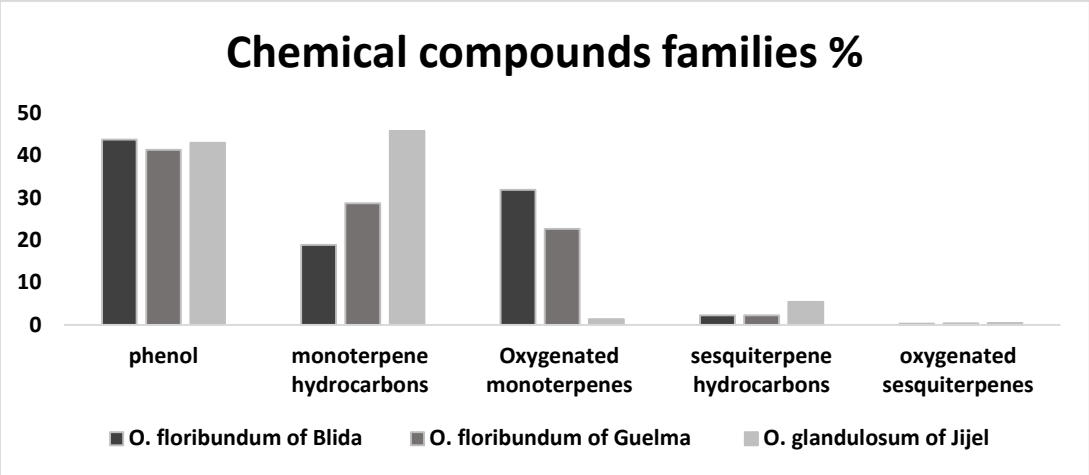

Figure 3. Chemical compound families of the three plants' essential oils.

3.3.Acaricidal activity

The greatest mortality rate was observed in hives treated with D1 (02 %) essential oil of *O.f* G (76.79 %), followed by D1 essential oil of *O.f* B (75.89 %). The efficiency of *O. floribundum* D1 doses was higher compared with the chemical control (C+) (Bayvarol) used, which was 73.69 %. However, the same D1 dose of *O.g* essential oil exhibited lower efficiency, not exceeding 57.37 %. Even at D2 doses, the impact of EOs *O.f* G and EO *O.f* B was around 49.10 % and 51.64 %, respectively, while the impact of EO *O.g* remained lower at 45.12 % in comparison with the other two *O. floribundum* essential oils (**Figure 4**).

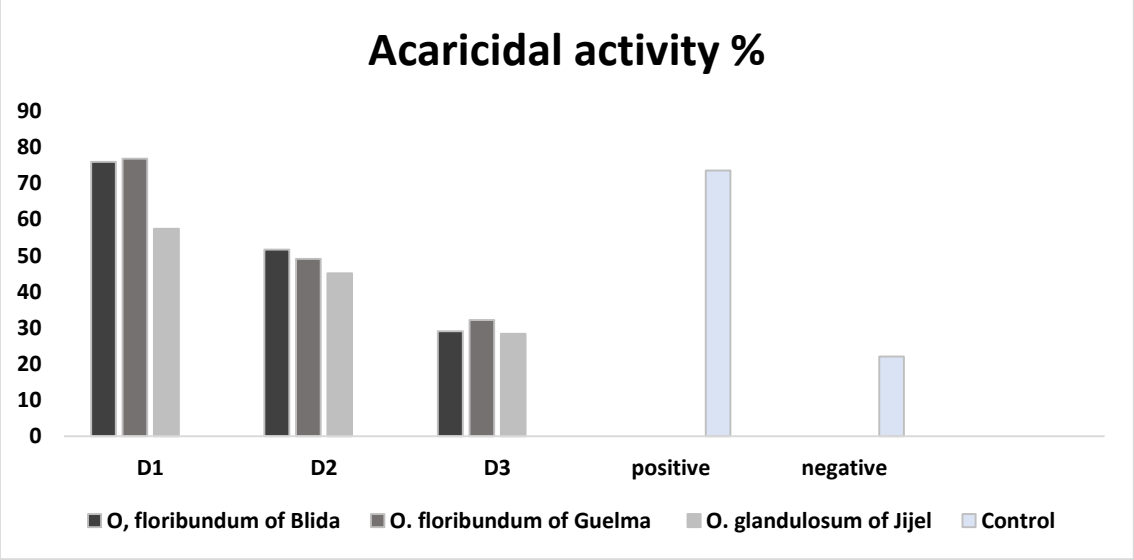

Figure 4. Acaricidal activity of three plants' essential oils (*O. floribundum* of Blida, *O. floribundum* of Guelma, and *O. glandulosum* of Jijel).

The ANOVA analysis of variance 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 three oils. Notably, there was no significant difference in the efficacy between *O. floribundum* essential oils at dose D1 and the chemical control (C+/EO *O.f* G p=0.912 and C+/EO *O.f* B p=0.990), It is worth

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mentioning that the effectiveness of EOs *O.f* G and *O.f* B was superior to that of the chemical treatment. On the other hand, the difference in the efficacy between the C+ and the D1 dose of EO *O.g* was highly significant (C+/EO *O.g* p=0.000), and even the efficacy of D1 *O.g* differed from that of *O. floribundum* (*O.g*/*O.f* G p=0.000 et *O.g*/*O.f* B p=0.000). However, there was no significant difference in the efficacy between D1 of EOs *O.f* G and *O.f* B (*O.f* G/*O.f* B p=1.000). The difference in the Acaricidal efficacy between control and the D2 doses of three oils was extremely significant, with no significant difference among the three oils at dose D2 (*O.f* G /*O.g* p=0.737), (*O.f* B/*O.g* p=0.163), and (*O.f* G / *O.f* B p=0.974), Similar results were observed with D3 doses.

The essential oils of *O. floribundum* plants from Guelma and Blida, which are of the same species, but from different provenances, exhibit a similar chemical composition and efficacy against *Varroa*. The effects of various growth factors and different origins on *O. floribundum* essential oils only influenced the yield, which varied. In contrast, the EO of *O. glandulosum*, a species from the same genus as *O. floribundum*, showed distinct differences in its chemical composition, and its Acaricidal activity was lower than that of *O. floribundum* essential oils.

The results of Oregano essential oils from *O. floribundum* (*O.f* G and *O.f* B) at dose D1 (2 %) are comparable to the findings of [21], who reported an Acaricidal activity efficiency of 74 % using doses of 1.16 ml of Oregano EO (60 % Carvacrol), Furthermore, based on research by Melathopoulos [45], essential oils that exhibit more than 70 % efficiency in Acaricidal activity could serve as a viable alternative for controlling V. Destructor. Note that the dose used in this study, as well as in Romo's work, does not pose any risks and does not affect the quality of hive products [21]. The phenol concentration in three essential oils was the same, but in *O.f* B and *O.f* G, phenol was only present in the form of Carvacrol, while in *O.g*, it was present in the form of both Carvacrol and thymol, Numerous scientific studies have consistently reported that essential oils which contain phenols or Aldehydes, including major components like thymol, Eugenol, Carvacrol, and Cinnamaldehyde, demonstrate significantly higher activity levels when compared to essential oils that contain terpenes or alcohol [46,47]. In this study, where the quantity of phenol groups was equal, but their Acaricidal activity varied, it is not the quantity of phenols that matters. Other differences between EOs *O.f* and *O.g* include the levels of Monoterpene hydrocarbons, which were significantly higher in *O.g* and lower in *O.f*, suggesting a possible antagonistic effect. Additionally, there were differences in the levels of Oxygenic Monoterpenes, which were much lower in *O.g* and higher in *O.f*, indicating a possible synergistic effect. The activities of EOs are attributed to the complex interactions among various classes of Phytoconstituents, these interactions may result in antagonistic or synergistic effects, which can significantly influence the overall activity of the essential oils. Even small constituents found in essential oils can have a significant impact on these effects [48].

4. Conclusions

Undoubtedly, the prevalent reliance on chemical-based Varroa control methods among beekeepers poses a significant risk to human health and exacerbates the host-parasite imbalance, potentially giving rise to highly resistant parasites. This scenario could lead to the extinction of bees and disrupt ecosystems. Without effective treatment, bees may face a grim future. Therefore, the quest for a safe and efficient alternative is imperative.

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Our study has unveiled variations in the chemical composition of Algerian oregano essential oils and their influence on Acaricidal activity. Notably, it has underscored the potential of Origanum floribundum essential oil as a reliable alternative acaricide, demonstrating its effectiveness across regions with varying growing conditions. Although yields may fluctuate, the fundamental chemical composition and Acaricidal activity of these essential oils remain remarkably consistent. The chemical analysis has shed light on the intricate interactions between different classes of phytoconstituents within Origanum essential oils, underscoring their collective role in combating Varroa.

This research sets the stage for further exploration of natural acaricides, offering a promising avenue for more environmentally friendly and bee-safe approaches in apiculture. The value of these findings lies in their potential to guide future developments and optimizations, ultimately contributing to the preservation of honey bee populations and the ecosystems they support.

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