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Research Article

Study of *Eucalyptus pauciflora* L. characteristics from Algeria and its impact on the mite affecting honeybees *Apis mellifera*

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Abstract

Essential oils play a crucial role in biological control against parasites threatening the beekeeping stock, both in Algeria and globally. Their appropriate use helps reduce the adverse effects of chemical products on human health and the environment. The purpose of this study is to analyze the components of *Eucalyptus pauciflora* essential oil grown in Algeria and to evaluate its impact on the marine shrimp *Artemia salina* and the mite *Varroa destructor*, while also comparing it to other species of Eucalyptus from the same region namely, *Eucalyptus robusta, globulus, sideroxylon,* and *amygdalina*, which have been examine in previous research. Steam distillation of fresh leaves from *Eucalyptus pauciflora* yielded 0.73% essential oils. This plant contains a total of thirty-nine chemical compounds, among which the most significant are 1,8-cineole (57.45%), β-cymene (5.44%), spathulenol (5.38%), trans-verbenol (4.31%), and α-pinene (3.11%). A lethality test on brine shrimp (BSL) revealed that *Eucalyptus pauciflora* exhibits toxicity with a median lethal concentration (LC50) of 53.51 ppm. Field trials conducted on hives infested with *Varroa destructor* showed that the acaricidal effect of *Eucalyptus pauciflora* is superior to that of *amygdalina* and *sideroxylon* species, but inferior to that of *globulus* and *robusta* species, all sourced from the same herbarium (Draa Naga) in northeastern Algeria. The results of this research highlight the benefits of Eucalyptus essential oils to be integrated into the formulation of natural acaricides that are environmentally friendly to combat *Varroa destructor* infestation.

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1. Introduction

Originating from Australia, Eucalyptus is now present worldwide, with over 800 species. Its ability to withstand various climatic conditions, including cold, and to grow in different types of soils (dry, siliceous, calcareous, wet, clayey, salty or not, near or far from the sea) makes it a highly adaptable plant (Bayle, 2017). Essential oils extracted from this group, which belongs to the Myrtaceae family, offer numerous benefits, including antiseptic properties, effects on the respiratory system, expectorant actions, and analgesic virtues (Shao et al., 2020; Sharifi-Rad et al., 2017). Furthermore, several studies have highlighted their anti-inflammatory, antimicrobial, antioxidant, and antiparasitic activities (Atmani-Merabet et al., 2018; Chaves et al., 2018; Harkat-Madouri et al., 2015; Limam et al., 2020; Chandorkar et al., 2021).

The medicinal properties of eucalyptus are primarily attributed to eucalyptol (1,8-cineole) found in its leaves. This compound has been extensively studied for its numerous biological actions, including anti-inflammatory, antioxidant, antiseptic, chemotherapeutic, respiratory and gastrointestinal disorder treatment (Cai et al., 2020; Dhakad et al., 2017). Furthermore, these essential oils act as broad-spectrum antimicrobial agents. (Aldoghaim et al., 2018) and possess insecticidal properties (Abdelkhalek et al., 2020; Russo et al., 2018), making them useful as bio pesticides in biological pest control. Bio pesticides derived from essential oils are characterized by their low persistence, low toxicity to humans, and targeted action against pests (Hussein et al., 2021; Khedhri et al., 2020; Maris et al., 2022). Among the most concerning pests is *Varroa destructor*, a mite that parasitizes the honeybee *Apis mellifera.* This parasite causes significant economic losses in the beekeeping industry and contributes to the decline of bee populations both in Algeria and worldwide (Adjlane et al., 2017; Ahumada et al., 2022; Atmani-Merabet, 2018; Bakar et al., 2017; Bakar et al., 2019).

Algerian beekeepers are trying to combat this scourge by using various commercial acaricides, but complete eradication of the mite remains difficult due to the emergence of resistance. A traditional beekeeping practice in Algeria involves combining conventional treatments with aromatic plants, which enhances their effectiveness and prolongs their action. For instance, Eucalyptus leaves are often used for fumigating the hives. Despite the numerous researchs conducted on the acaricidal properties of eucalyptus essential oils, there are still gaps in understanding their effectiveness and the mechanisms by which they act (Ahumada et al., 2022; Bakar et al., 2019).

It is in this context that our study is situated. The aim of this study is to conduct both qualitative and quantitative analysis of the components of *Eucalyptus pauciflora*. We also aim to highlight the acaricidal properties of Eucalyptus species cultivated in the Draa Naga herbarium, located in north eastern Algeria. Furthermore, this study will highlight the importance of eucalyptus essential oil as a natural solution to control this dangerous mite.

This work represents a preliminary step in a series of research efforts aimed at developing new, more effective products to combat the growing resistance of Varroa to existing treatments. It is crucial to note that all beekeepers face a major challenge: current treatments only target phoretic varroa, while this parasite also develops inside the cells alongside bee nymphs. The ultimate goal is to find a way to combat varroa within the cells while preserving the natural growth of bee nymphs.

2. Equipment and techniques

2.1 Botanical resources

The leaves of *Eucalyptus pauciflora* (Ep) were collected from trees growing in "Draa Naga" herbarium located in Djebel El Ouahch forest in Constantine region of northeastern Algeria. The species was identified the taxonomist by Dr Bouzid Mosbah.

2.2 Extraction of essential oil

Using a Clevenger device, steam distillation was used for six hours to extract plant oil from fresh leaves. Anhydrous sodium sulfate was then used to rapidly dehydrate the oil, and it was kept at 3°C until it was time for analysis.

2.3 GC/MS Analysis

Gas chromatography combined with mass spectrometry analysis (GC-MS) was carried out using an Agilent HP-5MS system, as detailed by the work of Atmani-Merabe et al (2018). Compounds of interest were identified by comparison with mass data. These compounds were confirmed against mass spectra obtained from the NIST-Wiley-MS library and validated by the Kovats index on the HP-5MS column.

2.4 Toxicity of E. pauciflora

The toxicity of *E. pauciflora* essential oil was assessed utilizing the brine shrimp lethality test (BSL) (Mac Laughlin et al., 1998). The *A. salina* eggs were incubated in a hatching chamber injected with seawater and continuously exposed to artificial light and room temperature (average 27 °C). Using a Pasteur pipette, the larvae were removed and counted after 48 hours. In a 100 mL volumetric flask, 100 mg of essential oil was diluted in 1.0 mL of DMSO to create a standard solution of 1000 µg/mL. The capacity was then filled with saltwater. Using the standard solution, concentrations of 1000, 100, 10, and 1 µg/mL were created. Ten brine shrimp larvae were utilized for each concentration, and they were put in flasks with a total capacity of five milliliters of seawater. Each concentration's tests were conducted in triplicate. Ten brine shrimp larvae, 4.9 mL of sea water, and 100 µL of DMSO were used to create a solution for the control group. The dead larvae were counted and documented after a 24-hour period. To find the oil's LC50, the acquired data were subjected to Finney's Probit analysis.

2.5 Acaricidal properties

The apiary where this experiment was carried out is situated in north eastern Algeria. Randomly chosen were twelve Langsthroth-style hives infested with *Varroa destructor* (*V. destructor*). Four lots, each with three hives, were created from these hives. According to the work of Atmani-Merabet et al (2018) and Goswami (2014) treatments based on *Eucalyptus pauciflora* essential oil (EpEO), thymol and the combination of EpEO+ thymol (v/v) at a rate of 1 ml/hive each week were administered to batches 1 to 3. The control was batch 4, or untreated hives. The biological technique known as "raised diapers" or "covers background" is used. The purpose of this technique is to locate and count the fallen mites. To find the dead varroa, diapers lubricated with vaseline are first put within the hive, then taken out and closely inspected using a hand lens. Every two days for 21 days, counting is done using this manner. The mortality means ± standard deviation is used to express the findings.

3. Result and discussion

3.1 Yield of E. pauciflora

In this study, 0.73% of *Eucalyptus* is extracted. It is higher than that observed in Tunisian species, which is 0.25% (Ghazghazi et al., 2019). This yield is also very close to those of other species collected in the same herbarium, namely *E. amygdalina* (0.77%), *E. robusta* (0.86%), *E. globulus*

(0.96%), and *E. sideroxylon* (1.09%) (Atmani-Merabet, 2018; Danna et al., 2024) (Table 3). Furthermore, it is comparable to the yields observed for various Eucalyptus species in Algeria, which range from 0.22% to 2.53% (Boukhatem et al., 2014; Harkat-Madouri et al., 2015).

However, according to the results from Filomeno et al (2016), the yields of Eucalyptus species can vary from 0.26% to 6.07%. In this context, it is significant to remark in this regard that, in comparison to these maximum values, the yields of *E. pauciflora* and the other species under study appear to be comparatively low. This raises questions about the factors that could influence these yields. Indeed, it is well established that the yield and quality of essential oils are affected by a multitude of factors, both endogenous (such as the plant's genetics) and exogenous (such as environmental conditions and extraction methods) (Bruneton, 1999 in Launay, 2017).

Thus, while the yield of *E. pauciflora* is higher than that of some Tunisian species and comparable to other Eucalyptus species, it remains essential to further explore the specific conditions that could optimize the extraction of essential oil. This analysis could not only enrich our understanding of yield variations but also contribute to more effective and sustainable extraction practice

3.2 Chemical composition

Thirty nine (39) compounds were found in *Eucalyptus pauciflora* by GC/MS analysis, emphasizing the presence of monoterpenes. (Figure 1, Table 1). Among these compounds, 1,8-cineole stands out as the primary component, representing 57.45%, Subsequently, β-cymene (5.44%), α-pinene (3.11%), trans-verbenol (4.31%), spathulenol (5.38%), and terpineol (2.84%) were observed. These results corroborate previous studies that emphasize the richness of bioactive compounds in this plant, with 1,8-cineole acknowledged globally as a distinct indicator of eucalyptus essential oils (Benabdesslem et al., 2020; Filomeno et al., 2016); Russo & Palacios, 2015; Sebei et al., 2015; Yones et al., 2016). On the other hand, Filomeno et al (2016) found that the composition of Brazilian *Eucalyptus pauciflora* essential oil differed, with a predominance of spathulenol (22.6%), p-cymene (19.4%) and globulol (10.4%). The biological and pharmacological characteristics of the essential oil may be significantly impacted by this difference, suggesting that environmental and geographical conditions influence the chemical composition of essential oils (Khayyat et al., 2018).

Comparatively speaking, *E. pauciflora* contains less 1,8-cineole than other species like *E. robusta* (65.97%) and *E. globulus* (78.45%), but higher than that of *E. sideroxylon* (40.24%) and *E. amygdalina* (35.78%), as indicated by data collected from the same herbarium (Table 3).

Finally, it is important to highlight that the Eucalyptus species from the Draa Naga herbarium studied are classified as 1,8-cineole chemotypes. In compliance with the British Pharmacopoeia's recommendations (Jordan, 2011), they can be used as insecticidal agents in the industry even if they are not appropriate for use in therapeutic purposes. This analysis highlights not only the chemical richness of *Eucalyptus pauciflora* but also its potential for application in various fields, while emphasizing the importance of a thorough understanding of the variations in chemical composition according to geographical origins.

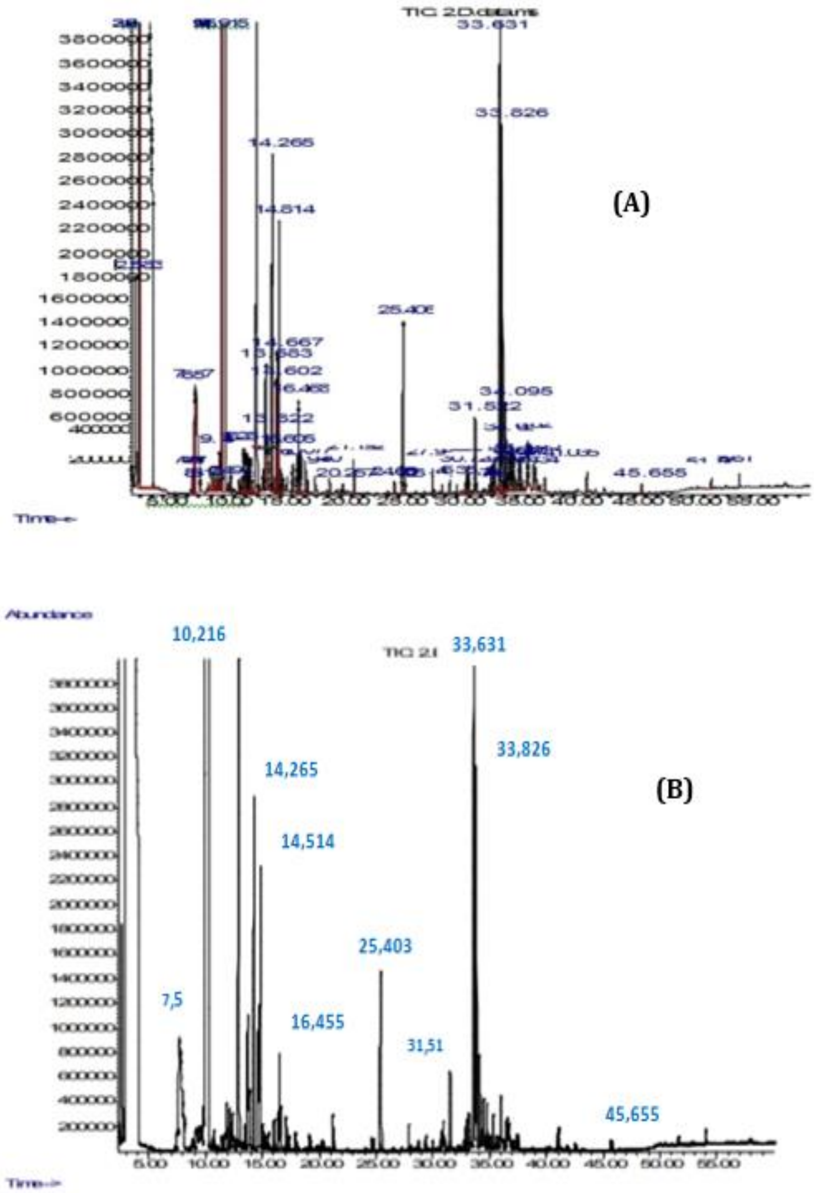


Figure 1. E. pauciflora leaf essential oil chromatogram (A-B)

Table 1. E. pauciflora's chemical composition

Components	% composition	Kovats index
α-thujene	0.14	930
α-pinene	3.11	939
β-pinene	0.12	979
β-myrcene	0.35	991
1-phellandrene	0.33	1003
p-cymene	0.14	1025
m-cymene	5.44	1031
1,8-cineole	57.45	1030
δ-terpinene	0.14	1047
Durol	0.14	1124
3-methyl Butylester	0.43	2388

Continued Table 1

Components	% composition	Kovats index
D-fenchylalcool	0.35	1139
α-campholenal	0.12	1126
Trans-verbenol	4.31	1144
Pinocarvone	1.02	1165
Borneole	0.55	1166
4-terpineole	2.84	1177
Trans-p-mentha-1(7), 8-dien-2-ol	1.04	1185
Terpineol	1.92	1189
Myrtenol	0.22	1327
Phellandrene epoxyde	0.16	1190
Cis-piperitol	0.12	1208
Carveol	0.23	1217
Cis-p-mentha-1(7),8-dien-2-ol	0.74	1235
2-methyl-3-phenyl propanal	0.29	1244
Piperitone	0.14	1259
Phellandral	0.16	1273
Carvacrol	0.35	1317
Camphene	1.98	966
Tetradecene	0.14	1400
2,4-di-ter-butylphenol	0.39	1513
Epiglobulol	0.18	1569
Aromadendrene	0.39	1441
Spathulenol	5.38	1578
Globulol	2.76	1578
Viridiflorol	0.82	1593
Isospathulenol	0.29	1644
Octadecene	0.16	1795

KI: The Kovats index on the HP-5MS column.
(%) composition: The proportion of concentrations determined by integrating the peak area.

3.3 Essential oil toxicity (BSL)

The result of the lethality test of *E.pauciflora* essential oil (EpEO) on brine shrimp is presented in Table 2. This test (BSL) revealed a CL50 value of 53.51 ppm which, according to Clarkson et al (2004), indicates that *E. pauciflora* is highly toxic. This toxicity is similar to that reported in other eucalyptus species in Algeria (Table 3) (Atmani-Merabet, 2018). It is also higher than that of *E. amygdalina* (Table 3) (Atmani-Merabet et al., 2020) and *E. citriodora,* which is considered non-toxic in Peru (Bussmann, 2011). It is significant to remember that in bioassays conducted on brine shrimp to determine the toxicity of plant extracts, an LC50 value less than 1000 μg/mL is typically regarded as bioactive (Clarkson et al., 2004).

Our results corroborate those of previous studies on various Eucalyptus species from the Draa Naga herbarium, all of which recorded a CL50 below 1000 (Table 3). In conclusion, these results highlight the significance of *Eucalyptus pauciflora* essential oil (EpEO) as a highly toxic agent for brine shrimp, which could have significant implications for its use and management in aquatic ecosystems. A comprehensive comprehension of this toxicity is necessary in order to evaluate the possible hazards linked to the application of EpEO in diverse environmental contexts.

Table 2. E. pauciflora essential oil lethality assay using brine shrimp

Dose [µg/ml]	Number of tested shrimps	Number of dead shrimps	LC50 [µg /mL]
1	40	13	53.51
10	40	18	
100	40	24	
1000	40	30	

Table 3. Eucalyptus species yields, main components, toxicity, and acaricidal activity

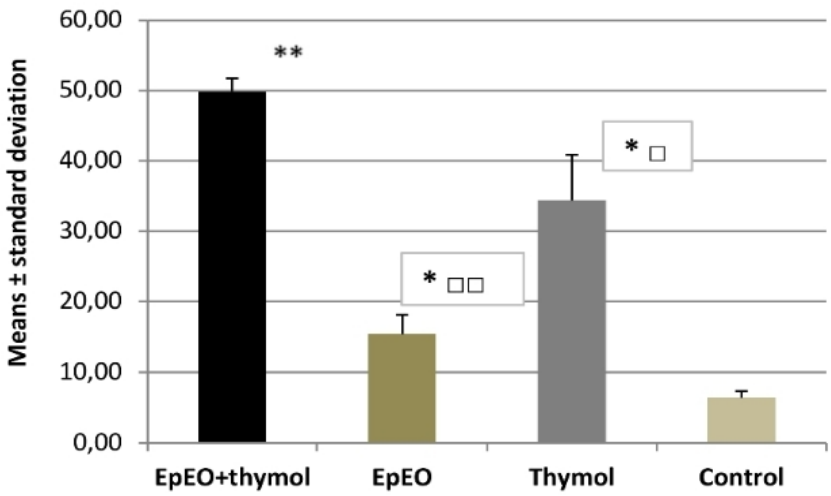
Species	Yields (%)	Components of EOs	Toxicity (*Artemia salina*)	Acaricidal activity (*Varroa destructor*)
E.robusta	0.83	1,8-cineole (65.97%), *p*-cymene (7.83%), α-pinene (1.89%), *o*-cymene (4.75%)	Highly toxic (LC50=9.4 µg/mL)	Very high effect
E.sideroxylon	1.09	1,8-cineole (40.24%), α-terpineol (2.07%) *p*-cymene (2.64%), α-pinene (2.19%), spathulenol (2.85%)	Highly toxic LC50= 34.80 µg/mL)	High effect
E. pauciflora	0.73	1,8-cineole (57.45%), α-pinene (3.11%) spathulenol (5.38%), trans verbenol 4.31%),	Toxic (LC50= 53.51 µg/mL)	High effect
E. globulus	0.93	β-cymene (5.44%), 1,8-cineole (78.45%) o-cymene (2.18%) isopinocarveole (1.74%) α-pinene (1.69%) pinocarvone (1.34%)	Toxic (LC50= 65.55 µg/mL)	High effect
E.amygdalina	0.77	1,8-cineole (35.78%), spathulenol (12.58%) camphene (7.73%) α-pinene (4.38%) valencene (2.64%)	Moderate (LC50= 116.06 µg/mL)	Moderate effect

3.4 Acaricidal activity

Figure 2 shows the outcomes of the field acaricide efficacy testing conducted on *Eucalyptus pauciflora* essential oil (EpEO). When compared to the control group, all infected hives that underwent treatment had a notable decrease in the *Varroa destructor* population. The most effective treatment to get rid of this ectoparasite was the combination of (*Eucalyptus pauciflora* essential oil + thymol), in fact a significant difference was recorded between treatment with this combination and treatment with thymol ($p = 0.04$). In addition, a highly significant difference was noted between treatment with this combination and treatment with *Eucalyptus pauciflora* essential oil ($p = 0.003$). Nevertheless, there was no discernible difference between the EpEO and thymol treaments. While thymol had a higher fallen Varroa value (34.37 ± 6.56) than EpEO (15.41 ± 2.74), the difference between the two is not statistically significant ($p = 0.06$), which may be attributed to the considerable variability observed in the apiary. Our results corroborate those of other studies (Abu Bakar et al., 2017; Adjlane et al., 2016; Conti et al., 2020; Elsadi et al., 2024) that have also highlighted the acaricidal potential of Eucalyptus essential oils against *Varroa destructor.*

While our study confirms previous findings, It highlights that thymol by itself is not as effective at reducing the parasite population in apiaries as *E.pauciflora* essential oil (EpEO) and thymol combined. It is noteworthy that there are currently three kinds of acaricidal compounds used to fight infestations, each with a different mechanism of action: formamidine amitraze, organophosphate coumaphos, and pyrethroid insecticides (fluvalinate and flumethrin) (Mitton et al., 2022). Regretfully, these chemicals are frequently present in honey and wax, endangering the health of bees and tainting apiculture products meant for human use. Furthermore, excessive use of these synthetic substances promotes the development of resistance in mites (Higes et al., 2020; Mitton et al., 2022).

Our suggested method, which uses thymol and essential oils together, attempts to deal with this resistance problem. The complexity of the composition of essential oils and their structural diversity present a challenge for the parasite, making the development of resistance more difficult (Khan et al., 2023). Furthermore, the majority of varroa therapies used in Algeria only include one active ingredient, indicating that essential oils may be able to lessen the phenomena of resistance due to their complex structure. Ultimately, the information gathered from this study opens the door to additional investigation into the elements of eucalyptus essential oils as botanical acaricides, providing a viable substitute for fighting *Varroa destructor* infestations. This strategy could guarantee the quality of apiculture goods while also enhancing bee health.



Value of dead *Varroa* expressed as means ± Standard deviation (n=3);
(p**< 0.01, p* < 0.05) value *vs* control;
(p□□ < 0.01, p□ < 0.05) value *vs* association of (Ep EO+ thymol) treatment

Figure 2. Means and standard deviations of dead varroas

4. Conclusion

Eucalyptus species investigated showed a significant efficacy threshold in BSL and acaricidal tests, and have a high 1,8-cineole content in its chemical composition. Eucalyptus species from the same region also recorded a richness in 1,8-cineole as well as a variable acaricidal effect on Varroa.

Understanding the structure-activity relationship of compounds is crucial to combining and enhancing the synergistic effects of many compounds operating on the same target or mechanism of action, and to fully comprehend the acaricidal activity. Therefore, it would be interesting to develop this work and examine the effect of essential oil associations on *Varroa destructor*, which could represent a promising alternative to synthetic acaricides. The broader implications of this study for pest management practices are significant. By using natural essential oils, we could reduce reliance on synthetic chemicals, thereby decreasing risks to the environment and human health. Future research should focus on optimizing essential oil combinations and evaluating their long-term effectiveness for sustainable pest management.

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Authors' declaration and contributions

There is no actual or potential conflict of interest in relation to this article. Ghania Atmani-Merabet (PhD in phytochimestry) was designed and conducted all of the experiments and wrote the manuscript. Hichem Hazmoune (Phd student in process engineering) was performed the essential oil extraction. Sihem Fellah (PhD in ecology and environment) was advised about the laboratory technique and conducted manuscript proofreading before submission. Abdelmalik Belkhiri (Professor of photochemistry) analyzed the results. All authors have read and approved the final version of the manuscript.

References

Abdelkhalek, A., Salem, M. Z. M., Kordy, A. M., Salem, A. Z. M., & Behiry, S. I. (2020). Antiviral, antifungal, and insecticidal activities of Eucalyptus bark extract: HPLC analysis of polyphenolic compounds. *Microbial Pathogenesis, 147,* 104383. CrossRef

Adjlane, N. (2017). Evaluation of the resistance of the mite *Varroa destructor* to the amitrazin in colonies of honey bees (*Apis mellifera*) in Algeria. *Uludağ Arıcılık Dergisi, 17*(1), 1–6. CrossRef

Ahumada, M. F., Marcos, J. L., Cadavid, A., Bañares, G. V., Silva, C. M., Olivares, Y. A., & Müller, H. Y. (2022). Evaluation of the efficacy of essential oils of *Lavandula angustifolia* and *Eucalyptus globulus* for the control of *Varroa destructor* in *Apis mellifera*: A randomised field study. *Australia Journal of Veterinary Sciences, 54*(2), 83–87. CrossRef

Aldoghaim, F. S., Flematti, G. R., & Hammer, K. A. (2018). Antimicrobial activity of several cineole-Rich western Australian Eucalyptus essential oils. *Microorganisms*, *6*(4), 122. CrossRef

Atmani- Merabet, G. (2018). *Huiles essentielles de trois espèces Algériennes d'Eucalyptus : composition chimique et activité acaricide* (*Varroa destructor).* [*Essential oils of three Algerian Eucalyptus species: chemical composition and acaricidal activity (Varroa destructor)*]. (Doctoral Thesis), Constantine1 University, Algeria.

Atmani-Merabet, G., Belkhiri, A., Dems, M. A., Lalaouna, A., Khalfaoui, Z., & Mosbah, B. (2018). Chemical composition, toxicity, and acaricidal activity of *Eucalyptus globulus* essential oil from Algeria. *Current Issues in Pharmacy and Medical Sciences, 31*(2), 89–93. CrossRef

Atmani-Merabet, G., Fellah, S., & Belkhiri, A. (2020). Comparative study of two Eucalyptus species from Algeria: chemical composition, toxicity and acaricidal effect on *Varroa destructor*. *Current Issues in Pharmacy and Medical Sciences, 33(*3), 144–148. CrossRef

Bakar, M. A., Aqueel, M. A., Raza, A. B. M., Mahmood, R., Qadir, Z. A., Arshad, M., & Sohail, M. (2019). Evaluation of essential oils for the management of parasitic bee mites, *Varroa* *destructor* (Acari: Varroidae) in vitro. *Pakistan Journal of Agricultural Research, 32*(4). CrossRef

Bakar, M. A., Aqueel, M. A., Raza, A. B. M., Ullah, M. I., Arshad, M., Sohail, M., & Molina-Ochoa, J. (2017). Evaluation of few essential oils for the management of parasitic bee mites, *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* L. colonies. *Pakistan Journal of Zoology*. CrossRef

Barbosa, L., Filomeno, C., Teixeira, R. (2016). Chemical variability and biological activities of Eucalyptus spp. essential oils. *Molecules, 21*(12), 1671. CrossRef

Bayle, G. (2019). Ecological and social impacts of eucalyptus tree plantation on the environment. *Journal of Biodiversity Conservation and Bioresource Management*, *5*(1), 93–104. CrossRef

Benabdesslem, Y., Hachem, K., & Mébarki, M. (2020). Chemical composition of the essential oil from the leaves of *Eucalyptus globulus* Labill. growing in south west Algeria. *Journal of Essential Oil Bearing Plants, 23*(5), 1154–1160. CrossRef

Boukhatem, M. N., Ferhat, M. A., Kameli, A., Saidi, F., Walid, K., & Mohamed, S. B. (2014). Quality assessment of the essential oil from *Eucalyptus globulus.* Labill of Blida (Algeria) Origin. *International Letters of Chemistry, Physics and Astronomy, 36*, 303–315. CrossRef

Bussmann, R. W., Malca, G., Glenn, A., Sharon, D., Nilsen, B., Parris, B., Dubose, D., Ruiz, D., Saleda, J., Martinez, M., Carillo, L., Walker, K., Kuhlman, A., & Townesmith, A. (2011). Toxicity of medicinal plants used in traditional medicine in Northern Peru. *Journal of Ethnopharmacology, 137*(1), 121–140. CrossRef

Cai, Z. M., Peng, J-Q., Chen, Y., Tao, L., Zhang, Y-Y., Fu, L-Y., Long, Q-D., & Shen, X-C. (2020). 1,8-Cineole: a review of source, biological activities, and application. *Journal of Asian Natural Products Research*, *23*(10), 938–954. CrossRef

Chandorkar, N., Tambe, S., Amin, P., Madankar, C. (2021). A systematic and comprehensive review on current understanding of the pharmacological actions, molecular mechanisms, and clinical implications of the genus *Eucalyptus. Phytomedicine Plus,* 100089 *1*(4). CrossRef

Chaves, T. P., Pinheiro, R. E. E., Melo, E. S., Soares, M. J. dos S., Souza, J. S. N., Andrade, T. B. de, Lemos, T. L. G. de, & Coutinho, H. D. M. (2018). Essential oil of *Eucalyptus camaldulensis* Dehn potentiates β-lactam activity against *Staphylococcus aureus* and *Escherichia coli* resistant strains. *Industrial Crops and Products, 112,* 70–74. CrossRef

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J., & Folb, P. I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology, 92*(2–3), 177–191. CrossRef

Conti, B., Bocchino, R., Cosci, F., Ascrizzi, R., Flamini, G., & Bedini, S. (2020). Essential oils against *Varroa destructor*: a soft way to fight the parasitic mite of *Apis mellifera*. *Journal of Apicultural Research, 59(*5), 774–782. CrossRef

Danna, C., Malaspina, P., Cornara, L., Smeriglio, A., Trombetta, D., De Feo, V., & Vanin, S. (2024). Eucalyptus essential oils in pest control: a review of chemical composition and applications against insects and mites. *Crop Protection*, *176,* 106319. CrossRef

Dhakad, A. K., Pandey, V. V., Beg, S., Rawat, J. M., & Singh, A. (2017). Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. *Journal of the Science of Food and Agriculture*, *98*(3), 833–848. Portico. CrossRef

Elsadi, M., Keshlaf, M., & Mirwan, H. (2024). Some essential oils as potential control agents for varroa mite (*Varroa destructor*) in infected honey bees (*Apis mellifera*). *Open Veterinary Journal*, *14*(2), 692. CrossRef

Ghazghazi, H., Essghaier, B., Riguene, H., Rigane, G., EL Aloui, M., Oueslati, M. A., Ben Salem, R., Sadfi Zouaoui, N., Naser, Z., & Laarbi Khouja, M. (2020). Phytochemical analysis, antioxidant and antimicrobial activities of Eucalyptus essential oil: a comparative study between *Eucalyptus marginata* L. and *Eucalyptus paucilora* L. *Revue Roumaine de Chimie, 64*(12), 1055–1062. CrossRef

Goswami, V., Srivastava, P., & Khan, M. S. (2014). Efficacy of essential oils against *Varroa Destructor* infesting *Apis mellifera* Linn. colonies and their impact on brood development. *Journal of Applied and Natural Science, 6*(1), 27–30. CrossRef

Harkat-Madouri, L., Asma, B., Madani, K., Bey-Ould Si Said, Z., Rigou, P., Grenier, D., Allalou, H., Remini, H., Adjaoud, A., & Boulekbache-Makhlouf, L. (2015). Chemical composition, antibacterial and antioxidant activities of essential oil of *Eucalyptus globulus* from Algeria. *Industrial Crops and Products, 78*, 148–153. CrossRef

Higes, M., Martín-Hernández, R., Hernández-Rodríguez, C. S., & González-Cabrera, J. (2020). Assessing the resistance to acaricides in *Varroa destructor* from several Spanish locations. *Parasitology Research, 119*(11), 3595–3601. CrossRef

Hussein, H. S., Tawfeek, M. E., & Abdelgaleil, S. A. M. (2021). Chemical composition, aphicidal and antiacetyl cholinesterase activities of essential oils against *Aphis nerii Boyer* de Fonscolombe (Hemiptera: Aphididae). Journal of Asia-Pacific Entomology, 24(2), 259–265. CrossRef

Jordan, S. (2011). British pharmacopoeia (BP) 2011The Stationery Office, Six-volume printed edition/CD-ROM/online edition British pharmacopoeia (BP) 2011 £830 978 0 1132 2849 2 011322849X. Nursing Standard, *25*(46), 31–31. CrossRef

Khan, S., Sahar, A., Tariq, T., Sameen, A., & Tariq, F. (2023). Essential oils in plants: Plant physiology, the chemical composition of the oil, and natural variation of the oils (chemotaxonomy and environmental effects, etc.). *Essential Oils,* 1–36. Academic Press. CrossRef

Khayyat, S. A., & Roselin, L. S. (2018). Recent progress in photochemical reaction on main components of some essential oils. *Journal of Saudi Chemical Society*, *22*(7), 855–875. CrossRef

Khedhri, S., Khammassi, M., Boukhris Bouhachem, S., Pieracci, Y., Flamini, G., Gargouri, S., Amri, I., & Hamrouni, L. (2024). Tunisian Eucalyptus essential oils: exploring their potential for biological applications. *Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology, 158*(1), 40–50. CrossRef

Launay, A. (2017). Pharmacognosie, phytochimie, plantes médicinales — cinquième édition J. Bruneton, Éditions Lavoisier Tec & Doc, 2016, 1 488 p, 195,00 €. *Phytothérapie, 15*(5), 316–316. CrossRef

Limam, H., Ben Jemaa, M., Tammar, S., Ksibi, N., Khammassi, S., Jallouli, S., Del Re, G., & Msaada, K. (2020). Variation in chemical profile of leaves essential oils from thirteen Tunisian Eucalyptus species and evaluation of their antioxidant and antibacterial properties. *Industrial Crops and Products, 158*, 112964. CrossRef

Maris, P., Utami, D. S., Marwoto, O., & Tarigan, N. (2022). Why *Eucalyptus citriodora* potentialas biopesticide? *Jurnal Ilmiah Agrineca*, *22*(2), 16–25. CrossRef

Mc Laughlin, J. L., Rogers, L. L., & Anderson, J. E. (1998). The use of biological assays to evaluatebotanicals. *Drug Information Journal, 32*(2), 513–524. CrossRef

Mitton, G. A., Meroi Arcerito, F., Cooley, H., Fernández de Landa, G., Eguaras, M. J., Ruffinengo, S. R., & Maggi, M. D. (2022). More than sixty years living with *Varroa destructor:* A review of acaricide resistance*. International Journal of Pest Management*, 1–18. CrossRef

Rossi, Y. E., & Palacios, S. M. (2015). Insecticidal toxicity of *Eucalyptus cinerea* essential oil and 1,8-cineole against *Musca domestica* and possible uses according to the metabolic response of flies. *Industrial Crops and Products, 63,* 133–137. CrossRef

Russo, S., Yaber Grass, M. A., Fontana, H. C., & Leonelli, E. (2018). Insecticidal activity of essential oil from *Eucalyptus globulus* against *Aphis nerii* (Boyer) and *Gynaikothrips ficorum* (Marchal). *AgriScientia*, *1*(35), 63. CrossRef

Sebei, K., Sakouhi, F., Herchi, W., Khouja, M., & Boukhchina, S. (2015). Chemical composition and antibacterial activities of seven Eucalyptus species essential oils leaves. *Biological Research*, *48*(1), 7. CrossRef

Shao, J., Yin, Z., Wang, Y., Yang, Y., Tang, Q., Zhang, M., Jiao, J., Liu, C., Yang, M., Zhen, L., Hassouna, A., White, W. L., & Lu, J. (2020). Effects of different doses of Eucalyptus oil From *Eucalyptus* *globulus* Labill. on respiratory tract immunity and immune function in healthy rats. *Frontiers in Pharmacology, 11*. CrossRef

Sharifi-Rad, J., Sureda, A., Tenore, G., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M., Ademiluyi, A., Sharifi-Rad, R., Ayatollahi, S., Iriti. (2017). Biological activities of essential oils: from plant chemoecology to traditional healing systems. *Molecules.*, *22*(1),70. CrossRef

Yones, D. A., Bakir, H. Y., & Bayoumi, S. A. L. (2016). Chemical composition and efficacy of some selected plant oils against *Pediculus humanus capitis* in vitro. *Parasitology Research, 115*(8), 3209–3218. CrossRef