agriculture

MDPI

Article

GC-MS Analysis and Evaluation of Essential Oils as Volatile Biopesticides: Assessing Their Acaricidal Potential against *Varroa destructor*

Muhammad Fahad Raza [1,2], Moazam Hyder [3], Chonghui Zhao [1] and Wenfeng Li [1,*]

[1] Guangdong Key Laboratory of Animal Conservation and Resource Utilization, Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Institute of Zoology, Guangdong Academy of Sciences, Guangzhou 510260, China; fbiuaf@giz.gd.cn or fbiuaf91@gmail.com (M.F.R.); zhaochonghui1029@163.com (C.Z.)
[2] Department of Agriculture Entomology, University of Agriculture, Faisalabad 38000, Pakistan
[3] State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Key Laboratory of Biopesticide and Chemical Biology, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, China; 000b387972@fafu.edu.cn
* Correspondence: wfli.bees@giz.gd.cn; Tel.: +86-020-8419-1089

Abstract: Honey bees are crucial for ecosystem pollination and honey production, yet the *Varroa destructor* mite hinders their productivity and health. Efforts to manage *Varroa* mites with synthetic pesticides have had limited success, highlighting the need for naturally derived acaricides as a primary option. However, the acaricidal efficacy of essential oils from *Salvia officinalis* L. (sage), *Cannabis sativa* (hemp), and *Laurus nobilis* (laurel) remains to be fully understood. This study aims to investigate the acaricidal efficacy of these three essential oils at varying concentrations and their impact on honey production, focusing on the efficient reduction in *Varroa* mites. The sugar roll method was employed to assess *Varroa* mite infestation levels, while GC-MS analysis was utilized to verify the composition of the essential oils. Honey production measurements were also performed. The efficacy rates (%) at concentrations of 15%, 10%, and 5% for hemp oil were 95.4% ± 0.30%, 85.71% ± 0.85%, and 64.48% ± 0.26%, respectively; for sage oil, they were 81.08% ± 0.57%, 69.42% ± 1.72%, and 50.35% ± 0.70%; and for laurel oil, they were 68.96% ± 0.34%, 54.66% ± 0.37%, and 33.58% ± 0.30%, respectively. Key compounds identified include trans-caryophyllene, α-pinene, and viridiflorol in hemp oil; myrcene, limonene, and β-caryophyllene in sage oil; and phytol, β-myrcene, and n-heneicosane in laurel oil. The overall findings indicate that hemp oil is highly effective in controlling *Varroa* mites. However, further research is needed to evaluate its potential side effects on bees to ensure its sustainability and safety.

Keywords: pollination; essential oils; *Varroa destructor*; acaricidal effect; GC-MS analysis; honey bees; sugar roll method; honey production

Citation: Raza, M.F.; Hyder, M.; Zhao, C.; Li, W. GC-MS Analysis and Evaluation of Essential Oils as Volatile Biopesticides: Assessing Their Acaricidal Potential against *Varroa destructor*. *Agriculture* **2024**, *14*, 940. https://doi.org/10.3390/agriculture14060940

Academic Editor: Nikoletta G. Ntalli

Received: 8 May 2024
Revised: 6 June 2024
Accepted: 12 June 2024
Published: 16 June 2024

1. Introduction

Apis mellifera L. is a species of crucial environmental, agricultural, and economic importance [1] that exists worldwide except in Antarctica [2]. Honey bees represent a vital component of agricultural systems, primarily due to their pollination activities, encompassing fruits, vegetables, and cash crops. This pollination significantly contributes to approximately one-third of the American food supply, underlining the indispensable role of honey bees in food production and ecosystem health. Moreover, honey bees are prolific producers of various by-products such as honey, royal jelly, beeswax, and propolis [3,4]. Recent declines in global honey bee populations, accompanied by an uptick in colony losses reported by beekeepers, have sparked scientific concern [5]. With the precipitous decline of honey bee populations, specifically *A. mellifera*, the academic sphere has increasingly recognized this phenomenon, with numerous studies pinpointing various contributing

elements [6]. These encompass pathogens, parasites, environmental stressors, and the deployment of agricultural pesticides. Amongst the array of threats to global honey bee populations, such as habitat degradation and pesticide application, the *V. destructor* mite is identified as a predominant factor exacerbating colony collapses. *Varroa* mites cause stress to honey bees and can be a vector for viral pathogens [7,8]. Significantly, Rosenkranz et al., 2010 highlight the *Varroa* mite as a predominant factor exacerbating colony collapses (as reviewed in their article) [9].

The *Varroa* mite is an ectoparasite afflicting honey bee colonies, leading to significant health detriments. This parasitic mite damages the bees' immune systems, culminating in adverse effects such as reduced lifespan, impaired metabolic processes, and increased rates of viral infections [10–12]. Additionally, *Varroa* mites indirectly harm honey bees by lowering the number of bees in the colony, affecting their ability to forage and pollinate, reducing honey production as a result of fewer foraging trips and the poor health of worker bees, and making colonies weaker and more vulnerable to other environmental pressures. These mites multiply fast and can take over a bee colony in roughly half a year [13–15].

The challenge in controlling these mites is heightened as they have developed resistance to the acaricides traditionally used to combat them [16]. Mechanical and cultural *Varroa* management methods are widely used, but their efficacy is inconsistent. Global research has identified promising chemical compounds for mite toxicity, but prohibitive costs have hindered further experimentation. Despite this, chemical screenings are helpful, but their effects on *Varroa* mites and honey bees remain unexamined [17–19]. Predominantly, *Varroa* control methodologies employ synthetic chemical agents. These pyrethroid insecticides, acaricides like tau-fluvalinate and organophosphate insecticides like Coumaphos, are reported to be persistent and tend to accumulate in the hive products, like honey and beeswax, and this is likely to cause sublethal exposure of the *Varroa* mites for a long period. Such prolonged exposure to the acaricides is expected to contribute to the resistance buildup in the mites [20–22]. Additionally, research sheds light on the harmful effects of these substances on bee health and the broader environment, compelling the search for safer alternatives [23–25].

Conventional pesticides pose several challenges, including pest resistance, environmental pollution, public health threats, and food contamination, considering that new and safer products are lacking in the market [26,27]. Botanical pesticides, seen as low-risk and environmentally friendly, are drawing researchers' eyes. They promise a green twist on fighting pests, blending old wisdom with new science for a healthier farm future [28–30]. They are not just for managing pests anymore but are stepping stones towards greener, safer veterinary products. This shift toward sustainability is crucial for protecting our environment while ensuring the health of our valuable insects, crops, and livestock [31–34]. Botanicals are considered safe because they are low in toxicity to mammals, do not pollute, and steer clear of environmental toxins, making them a popular choice for both people and the planet [35]. Plant chemicals like pyrethrins, alkaloids, and terpenoids boast a range of biological activities. Their diverse biological actions make them versatile [36]; although the compounds have short-term persistence and low non-target toxicity, there are exceptions. Pyrethrin products like PyGanic can also have toxic effects on pollinators, so special care should be taken to protect the beneficial insects [37,38]. Essential oils can be used as an attractor or repellent for pests, which makes them suitable for use in a trap, and they repel queen bumble bees even if the results are non-lethal [39,40].

The interest in plant secondary metabolites can lead to sublethal effects like reduced hatching, shorter lifespans, or abnormal development. Meanwhile, the volatile compounds in honey do not harm bees. Recent studies show that organic acids and essential oils act effectively against *Varroa* mites in organic beekeeping [41–44]. In beekeeping, using natural compounds derived from plants is gaining attention. Terpenoids and other secondary metabolites [45] have acaricidal properties that stand out for their ability to fend off pests [46,47], and subsequently deter them from feeding, disrupt their growth, and affect their reproduction [48]. *Cannabis sativa* L produces essential oils that are noted for

their larvicidal and biopesticidal efficacy against pests like the peach–potato aphid and house fly [48–50]. Another research study focuses on various application techniques and assesses the efficiency of essential oils, advocating their incorporation within integrated pest management schemes [51]. The essential oil of *S. officinalis* L. has several constituents in the form of borneol, camphor, and pinene, and its activity on the human nervous system is interesting [52,53]. The findings revealed that sage oil has significant acaricidal properties against *Varroa* mites [54]. Laurel, an aromatic angiosperm native to the Mediterranean, is cultivated for its medicinal properties, including antifungal, antibacterial, antiviral, insecticidal, and acaricidal properties [55–60]. Bearing the above in mind, we evaluated the acaricidal properties of hemp, sage, and laurel essential oils (EOs) and their main constituents against *Varroa* mites. Significant increases in honey production and the number of fallen mites were also recorded.

2. Materials and Methods

The pictorial representation of the present research experimental protocol was conducted to assess the efficiency of essential oil, GC-MS analysis, sticky board method, sugar roll method, and yield production after treatment, as shown in Figure 1.

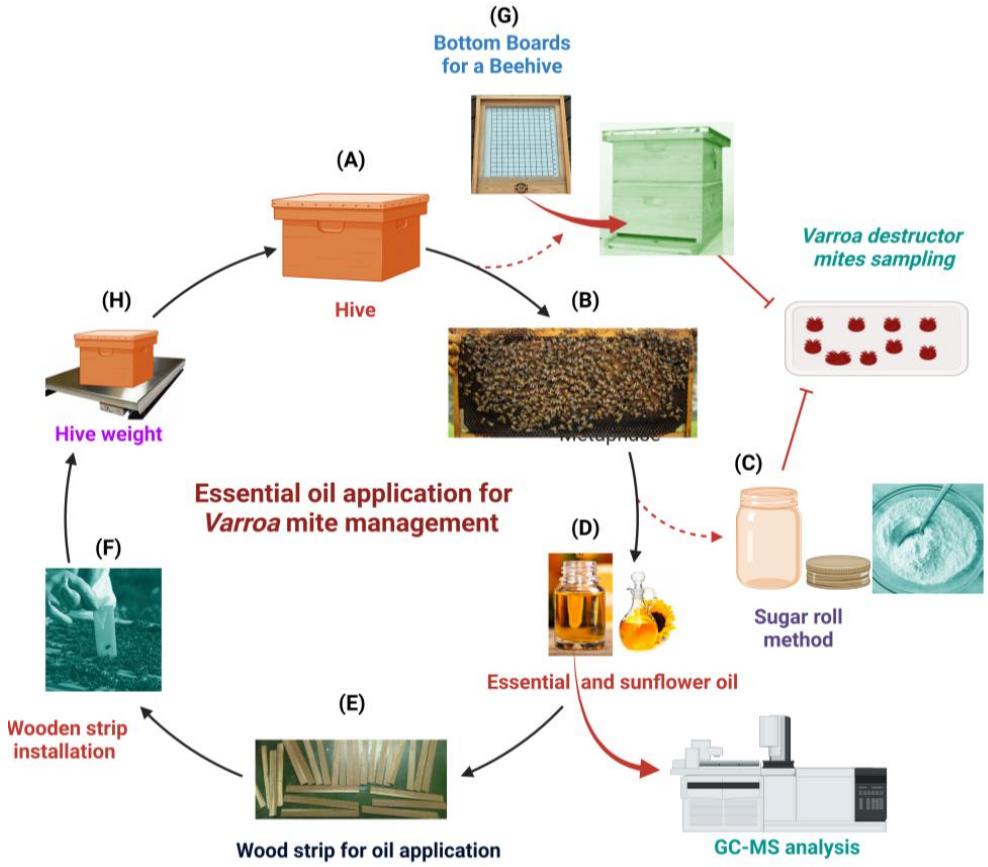

Figure 1. Pictorial representation of the experimental protocol, where (**A**) signifies the experimental colonies, (**B**) indicates brood comb for bee collection for sugar roll method, (**C**) is to check the infestation level, (**D**) describes the use of essential oil with sunflower oil and GC-MS analysis (**E**) indicates the wooden strip that is used for essential oil application, (**F**) represents the installation of wooden strips in experimental colonies, (**G**) is the sticky bottom board for *Varroa* mite monitoring, and (**H**) shows honey production after the treatment with essential oil.

2.1. Honey Bee Colonies

Prior to the commencement of the research, we meticulously put out 75 colonies of *A. mellifera* to document the natural drop of *Varroa* mites. The experimental colonies were

all accommodated within conventional Langstroth hives. Through a rigorous screening process, we narrowed our focus to 50 colonies. This selection was based on several critical criteria: the size of the honey bee population was comparable across the chosen colonies, they were queen right, and they exhibited a mite infestation level exceeding the economic threshold of 3 to >10 adult mites per 100 bees [61]. Moreover, the colonies designated for the experiments were in a mature stage of development and demonstrated high productivity levels. Before initiating the study, these colonies were observed to maintain an average of 8 ± 2 brood frames within their brood chambers [62,63]. In our experiment, we used 50 bee hives, kept at a minimum distance of ten meters, which were divided into ten treatment groups with five replications: three essential oils (hemp, sage, laurel) at three concentrations each (5%, 10%, 15%) and a control group (untreated). Those treated included fifteen colonies of each essential oil and five colonies of the control group; every concentration of each oil had five colonies treated, implementing a randomized block design for uniform environmental conditions across all treatments. The present research was conducted during the winter season, from December to January, at the apiary within the Department of Entomology at the University of Agriculture Faisalabad, Islamic Republic of Pakistan. During the study period, December's meteorological data indicated a maximum temperature of 20.4 °C and a minimum of 8.2 °C. Wind conditions were recorded at an average of 10.56 km/day, with precipitation levels reaching 13 mm and average relative humidity standing at 60%. In the subsequent month of January, the data reflected a slight decrease in thermal readings, with the maximum and minimum temperatures documented at 16.9 °C and 5.5 °C, respectively. The wind speed escalated to an average of 15.88 km/day, while the rainfall increased significantly to 19 mm, accompanied by a rise in average relative humidity.

2.2. Plant Material Collection and Extraction

Three botanicals, namely *L. nobilis*, *C. sativa*, and *S. officinalis*, were utilized in the study. The seeds and leaves of these three plants were purchased from a traditional medicine shop in Lahore, Pakistan. The extraction methodology, as outlined by Moazam Hyder et al. (2022a) and Moazam Hyder et al. (2022b), was employed with minor modifications [64,65]. Leaves of the botanicals were subjected to washing, followed by drying at 45 °C for 48 h. The dried material was then pulverized and sieved through a 40 µm mesh screen, with each botanical powder stored separately.

The powder was made by mixing one gram of initial powder with five milliliters of ethanol in bottles, which were kept at room temperature (18–25 °C) for seven days. The bottles were agitated twice daily to facilitate the dissolution of the powder in ethanol. Subsequently, the mixture was filtered through filter paper, and any residue was further dissolved using 2.5 mL of the same solvent per gram of powder. The combined first and second solvent fractions were concentrated using a rotary evaporator until the liquid evaporated, resulting in oil extraction, which was stored at 4 °C in brown collection containers. To achieve a final concentration of 10 mg/mL (10,000 ppm), we started with 0.01 g of extract dissolved in 0.3 mL of DMSO. This creates a stock solution with a concentration of 33.3 mg/mL. To dilute the stock to the desired 10 mg/mL, add 0.7 mL of double distilled water to 0.3 mL of the 33.3 mg/mL stock solution. A control treatment was prepared by mixing the same solution, without added oil, with 0.3 mL of DMSO, 1% Tween-20, and distilled water to achieve a volume of 5 mL.

2.3. Identification of the Chemical Components of the Essential Oil

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted on the obtained essential oil samples. The analysis was performed using a 50-GC/320-MS instrument (Varian, Inc., Walnut Creek, CA, USA) equipped with a flame ionization detector and an HP-5MS capillary column (film thickness: 30 m length, 0.25 mm inner diameter). For the gas chromatography phase, the injector oven temperature profile was as follows: initially held at 60 °C for 2.5 min, then ramped at a rate of 10 °C/min to 180 °C and maintained for

1 min, and finally ramped at a rate of 20 °C/min to 280 °C and held for 15 min. Sample injection was performed with one microliter of samples diluted in 1% hexane, using a split ratio 1:10. Helium gas was employed as the carrier gas at a flow rate of 1.0 mL/min, while the column pressure was maintained at 100 kPa. Chemical constituents present in the gas chromatogram were identified by comparing them with NIST 23 Gas Chromatography (GC) [66].

2.4. Sugar Roll Method

Before applying essential oils, the sugar roll method was used to quantify the prevalence of *V. destructor* infestation in 50 test colonies of *A. mellifera* [67,68]. For the *Varroa* mite collection, three brood frames from each colony ensured an accurate representation of the colony's bee population. The procedure entailed the meticulous transfer of bees from brood comb into a clean white tray through the gentle application of a soft brush, a measure taken to avoid injuring the bees and prevent contamination, excluding the queen to avoid any harm. An approximate tally of 300 bees were transferred into this jar. A glass canning jar with a fine mesh screen lid (8 per inch) was utilized. This setup was crucial as it allowed mites to move through the mesh while ensuring the bees were securely contained within the jar. Subsequently, the procedure involved the addition of three tablespoons of powdered sugar through the mesh into the jar. The bees within were then gently agitated by rolling the jar for 2 to 3 min, ensuring an even coating of powdered sugar on all bees. This gentle agitation was pivotal for the mite-removal process from the bee bodies. The jar was left undisturbed for another 2 to 3 min, allowing the bees sufficient time to self-clean, thereby dislodging the mites and the sugar coating from their bodies. After the time for self-cleaning, the jar was carefully inverted and lightly rolled again, facilitating the collection of the powdered sugar, now containing the dislodged mites, onto a white paper chart. The white paper chart was then scanned for the presence of mites that were counted. The bees, now sampled and free of mites, were gently returned to their hive either at the top of the colony or the entrance, ensuring minimal disruption to their natural state. The sugar roll method was applied immediately before and after a 30-day treatment with essential oils. The final step involved the calculation of the mite infestation rate, which was achieved by dividing the total number of mites by the total number of bees sampled. The research established a standard economic threshold for action at a 2% mite presence per 100 bees, translating to a critical limit of 6 *Varroa* mites per 300 adult bees during periods of low population (dearth periods) and a heightened threshold of 9 *Varroa* mites per 300 bees during peak population seasons, typically observed from February to March [61]. The acaricidal potential of the oils was quantified using the following formula for percentage reduction. The reduction (%) in mites was counted per 300 bees by using the sugar roll method before the initial count and post-treatment (final count).

$$\text{Change in mite population\%} = \frac{\text{Initial count} - \text{final count}}{\text{Initial count}} \times 100$$

2.5. Essential Oils Treatment

The essential oils used for the experiment were hemp, sage, and laurel. We experimented with three levels of concentration: 5%, 10%, and 15% for the 3 × 3 factorial block design. The preparation of the treatment solutions involved meticulous dilution of sage, hemp, and laurel oils to concentrations of 5%, 10%, and 15% in sunflower oil, ensuring precision in the formulation for application consistency. In this study, we utilized untreated wooden strips measuring 2 cm in width, 0.5 cm in thickness, and 27 cm in length, tailored to fit within standard Langstroth beehive frames. The wooden strips were immersed in oil solutions for 24 h to ensure deep saturation before being strategically placed within the hives. Precisely, in each colony, three such strips were carefully positioned between the brood combs. These strips were routinely exchanged every three days for optimal control and effectiveness. The experimental framework included a total of 50 hives, categorized into four distinct groups: 45 of these hives were allocated into separate clusters, each

receiving a unique concentration of essential oils, while the remaining 5 served as control groups, receiving no essential oil treatment. In our study, the effectiveness of essential oils was determined by counting the mites on approximately 300 bees from each colony before and after treatment.

2.6. Mite Collection

This investigation sought to elucidate the efficacy of evaporated essential oils in mitigating *Varroa* mite infestation levels within apian colonies. The screened bottom board technique was employed [69], incorporating a layer of petroleum jelly as a viscous trap beneath a modified bottom board precisely dimensioned to the specifications of the beehive. This innovative approach not only preserved the inherent behaviors of the bee populations but also maximized the efficiency of mite capture. The bottom board, tailored and balanced concerning the dimensions of the hive, was efficient in maintaining the required efficacy and was effective in trapping mites due to the uniform application of petroleum jelly. To systematically monitor the levels of mite infestation and evaluate the effectiveness of the applied mite treatments, collection trays, safeguarded by a wire mesh to deter the removal of bees, were strategically situated at the posterior section of the hives. These trays, serving the dual objective of monitoring and evaluation, enabled the quantification of mite infestation levels at predetermined intervals of 10, 20, and 30 days post-treatment, providing a comprehensive assessment of the treatment efficacy over time [63,70,71].

2.7. Quantification of Honey Production

Following the experimental phase's conclusion post-treatment and *Varroa* assessment through the sugar shake method, the honey extraction procedure was initiated utilizing a manually operated honey harvester. The objective was to ascertain the honey yield from the treated colonies, which was achieved by assessing the weight of each hive body designated for honey collection before and after the extraction process. The variance in weight was then quantified as the volume of honey eligible for harvest. This methodological approach ensured a precise measurement of the honey yield, contributing to the comprehensive analysis of the experiment's outcomes [63,72]. The total number of fallen mites was counted using three concentrations: 5%, 10%, and 15% of hemp, sage, and laurel oils and the control group. The experimental colonies (*n*-50) were divided into three treatment groups of colonies (*n*-15), each along with a control group of colonies (*n*-5). The time and dose-dependent effect on the number of fallen mites after the treatment with hemp, sage, and laurel oils at 5%, 10%, and 15% concentrations was evaluated over intervals of 10 days, 20 days, and 30 days.

2.8. Statistical Analyses

We used ANOVA analysis for comparative efficiency across the different treatment groups (hemp oil, sage oil, laurel oil, control), Levene's test to perform the equality of variances, and Tukey's HSD test to find out which treatment groups had significantly different effects on reducing mite populations. A two-factor ANOVA to evaluate the impacts of each treatment of hemp, sage, and laurel oils (3 groups) and their quantities/concentrations of 5%, 10%, and 15% (9 treatments) on *Varroa* mites was conducted using Graphpad prism Version 9 [73]. The heat maps were formed to visualize the number of fallen mites after 10, 20, and 30 days. In the case of honey yield, ANOVA and Tukey's HSD test were performed to evaluate the impact of different essentials and their concentrations on honey yield.

3. Results

3.1. Treatment Efficacy among Essential Oils

The study found that the efficacy of the three major treatments of sage $F(6) = 11.68$, $p < 0.001$), hemp $F(6) = 12.79$, $p < 0.001$), and laurel essential oils $F(6) = 10.59$, $p < 0.01$) to the control group significantly differed, as depicted in Figure 2. Hemp oil had the highest efficiency (81.89%), followed by sage and laurel oils at 66.95% and 52.40%. The

control group showed an 88.65% increase in the mite population, indicating significant mite proliferation without treatment. Mite counts were based immediately before the first application of the essential oils, and the post-treatment counts were carried out after 30 days post-treatment, which commenced with the sugar shake method.

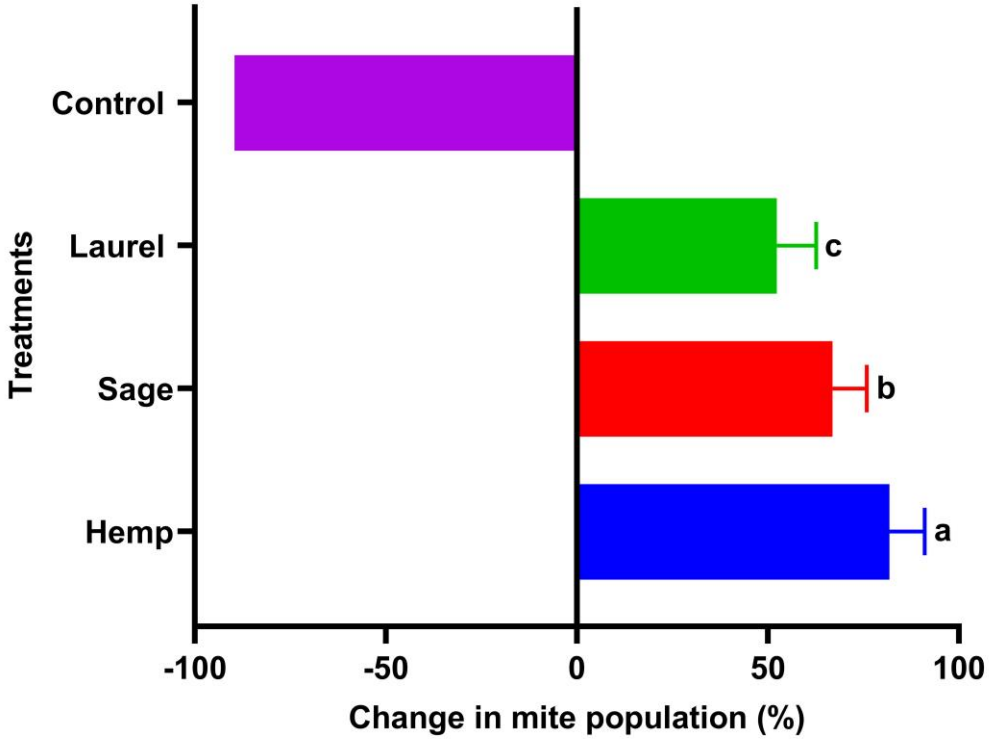

Figure 2. The percentage change in mite populations approximately per 300 bees, measured by the sugar roll method, after 30 days of treatment. Evaluation comparison of the effectiveness of different essential oil (hemp, sage, and laurel) groups with the control group showed significant differences. The experimental colonies (*n*-50) were divided into three treatment groups of colonies each (*n*-15), along with a control group of colonies (*n*-5). Bars with different letters a, b and c, showed significant differences ($p < 0.05$) in the efficiency of different essential oils.

Tukey's HSD test revealed significant differences in mite population reduction between treatments with hemp oil, laurel oil, and sage oil to the control group ($p > 0.001$). Additionally, comparing the essential oils themselves, hemp oil was more effective than both laurel and sage oils, while no significant difference in efficacy was observed between laurel and sage oils ($p = 0.391$).

3.2. Concentration Effectiveness

The quantitative analysis delineates a discernible increment in the efficacy of hemp oil as its concentration is augmented from 5% to 15%, as illustrated in Figure 3A. In a parallel vein, sage oil displays a similar trajectory of efficiency enhancement in alignment with concentration increments. However, it is imperative to note that the foundational efficiency of sage oil at a 5% concentration markedly trails behind that of hemp oil, indicating a variance in intrinsic efficacy as shown in Figure 3B. By the time the concentration reaches 15%, as shown in Figure 3C, laurel oil's efficiency aligns closely with sage oil's, suggesting a convergence of efficacy at higher concentrations.

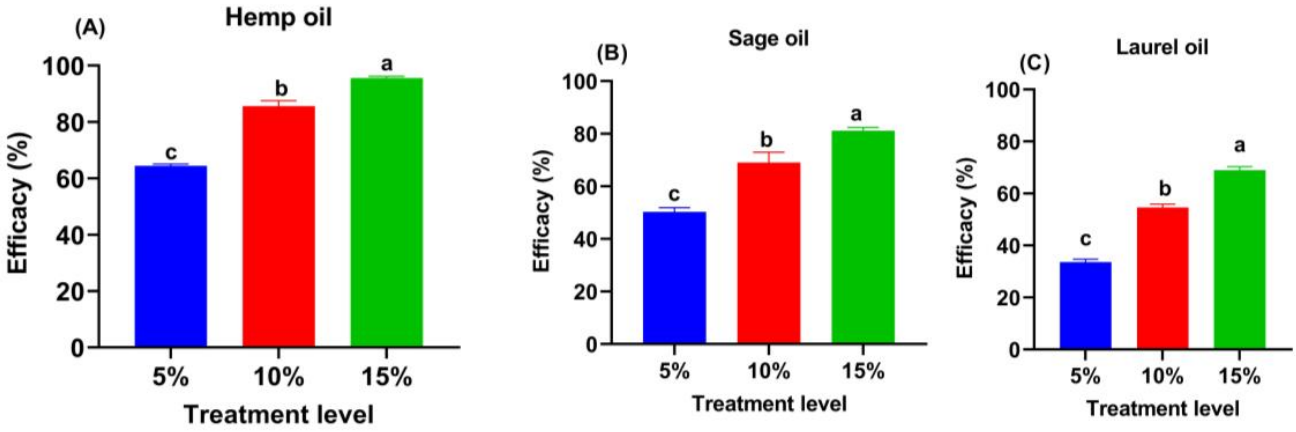

Figure 3. Efficacy ± SE of essential oils with different concentrations of 5%, 10%, and 15% against *V. destructor* mites, measured approximately per 300 bees through the sugar roll method. The colonies (*n*-5) were used for each concentration of each oil treatment. (**A**) Hemp oil: The efficacy of hemp oil at three different concentrations (5%, 10%, and 15%). The bars indicate significant differences with the letters c, b, and a representing $p < 0.05$. (**B**) Sage oil: The efficacy of sage oil at three different concentrations (5%, 10%, and 15%). The bars indicate significant differences with the letters c, b, and a representing $p < 0.05$. (**C**) Laurel oil: The efficacy of laurel oil at three different concentrations (5%, 10%, and 15%). Efficacy values were expressed as means ± SE. Bars with different letters, a, b, and c, showed significant differences ($p < 0.05$).

Results for the colonies treated with hemp oil at 15%, 10%, and 5% were 95.4% ± 0.30, 85.71% ± 0.85, and 64.48% ± 0.26, (95.49% ± 2.5%) respectively; for sage oil, they were 81.08% ± 0.57%, 69.42% ± 1.72%, and 50.35% ± 0.70%; and for laurel oil, they were 68.96% ± 0.34%, 54.66% ± 0.37%, and 33.58% ± 0.30%, respectively. The hemp oil showed higher efficacy at 15% and lowest efficacy with laurel oil at 5%. Hemp oil showed a significant difference with $F(2, 12) = 66.78$, $p < 0.0001$, from concentrations of 5% to 15%, followed by sage oil with $F(2, 12) = 190.2$, $p < 0.0001$ and laurel oil with $F(2, 12) = 326.8$, $p < 0.00013$.

3.3. Number of Fallen Mites

The level of effectiveness of different essential oils (hemp, sage, and laurel) after 10, 20, and 30 days of treatment regarding the number of fallen mites compared to the control through the application of heatmaps for enhanced visual interpretation is depicted in Figure 4A. The acaricidal efficiency of essential oils derived from hemp, sage, and laurel was evaluated using concentrations of 5%, 10%, and 15% over intervals of 10, 20, and 30 days as shown in Figure 4B–D. The results demonstrated notable differences in the rates of mite fall, which were dependent on the essential oil used, its concentration, and the length of exposure. Among the oils tested, hemp oil exhibited superior effectiveness across all tested concentrations and durations, particularly at lower concentrations and for brief exposure periods.

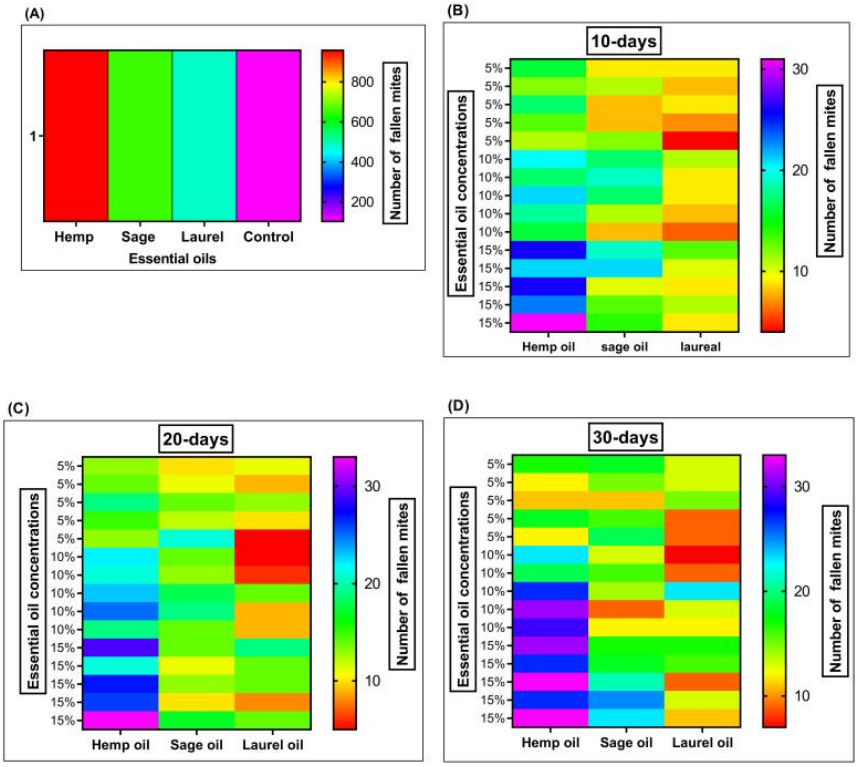

Figure 4. The heatmap presents data regarding the number of fallen mites after essential oil application. (**A**) shows the cumulative number of fallen mites after 30 days for each treatment group (hemp oil, sage oil, laurel oil) and the control group over the entire experimental period, with the colour bar indicating the total number of fallen mites. (**B**–**D**) display heatmaps of the number of fallen mites at different concentrations (5%, 10%, 15%) for each essential oil (hemp oil, sage oil, laurel oil) after 10, 20, and 30 days of treatment, respectively. The *x*-axis represents the type of essential oil, and the *y*-axis represents the concentration, with the colour bar on the right side indicating the number of fallen mites.

3.4. Honey Yield

Hemp oil reflected a higher yield than sage and laurel, and when compared to the control group, it shows that hemp oil has a significantly strong positive correlation with honey production; comparison was made between essential oils, $F(3, 6) = 28.68$, $p = 0.0006$, as illustrated in Figure 5A.

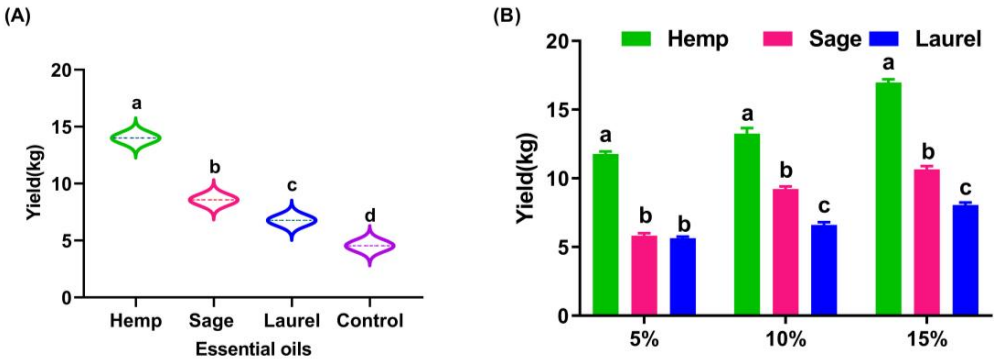

Figure 5. Comparative analysis of essential oil on honey yields at varying essential oil concentrations and with the control group at varying essential oil concentrations. (**A**) The violin plot represents the overall yield for all concentrations pooled in each essential oil and the control, the effectiveness of

essential oils on honey yield. Bars with different letters a, b, c, and d showed significant differences ($p < 0.05$). (**B**) A bar graph representation providing insights into the yield by the same concentration for each EO and different letters, a, b, and c, indicate statistically significant differences ($p < 0.05$), but in the case of 5% sage and laurel oil are not significantly different. Assessments were performed within each essential oil across different concentrations, meaning that letters indicate differences only within the same essential oil.

The highest average honey yield was recorded in treated honey bee colonies with hemp oil at different concentrations (5%, 10%, and 15%) at 11.778 kg, 13.26 kg, and 16.98 kg, respectively, as shown in Figure 5B. The lowest average honey yield was recorded and treated with laurel oil (5%, 10%, and 15%) at 5.64 kg, 6.6 kg, and 8.06 kg, respectively, as shown in Figure 5B. The results of Tukey's multiple comparisons tests were as follows: for 5% hemp vs. sage, $F(12) = 81.47$, $p < 0.0001$; for hemp vs. laurel, $F(12) = 83.93$, $p < 0.0001$; and for sage vs. laurel, $F(12) = 2.461$, $p = 0.2308$: for 10% hemp vs. sage, $F(8) = 30.75$, $p < 0.0001$; for hemp vs. laurel, $F(8) = 50.68$, $p < 0.0001$; and for sage vs. laurel, $F(8) = 19.94$, $p < 0.0001$. Lastly, in the case of 15% concentration, the results showed hemp vs. sage, $F(8) = 62.27$, $p < 0.0001$; hemp vs. laurel, $F(8) = 87.89$, $p < 0.0001$; and sage vs. laurel, $F(8) = 25.62$, $p < 0.0001$.

3.5. GC-MS Analysis

The components of hemp oil, sage oil, and laurel oil were analyzed using GC-MS analysis to study their acaricidal potential against *Varroa* mites. The components and their percentages in the essential oils are illustrated in Figures 6–8.

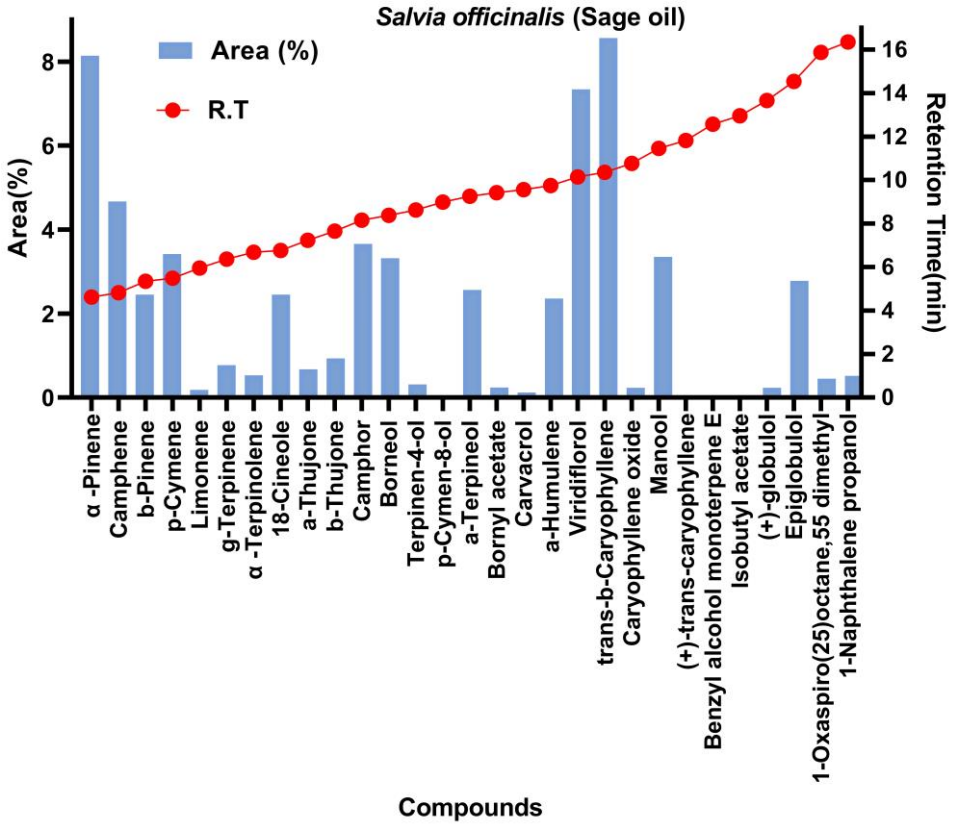

Figure 6. Composition of essential oil components from *S. officinalis* (sage oil) by Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The *x*-axis represents the identified compounds. At the same time, the left *y*-axis shows the area (%), and the right *y*-axis indicates the retention time (min). The blue bars represent the percentage area of each compound, and the red dotted line indicates their respective retention times.

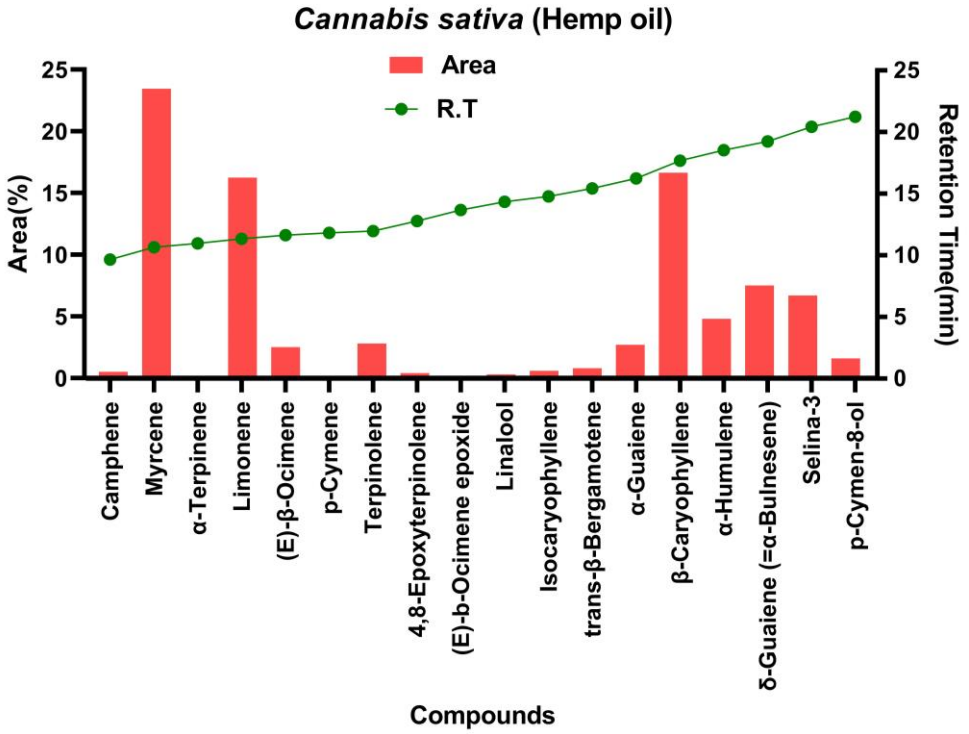

Figure 7. Composition of essential oil components from *C. sativa* (hemp oil) by Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The compounds identified are plotted on the *x*-axis. The area (%) of each compound is depicted by red bars (left *y*-axis), and the retention time (min) is indicated by a green line (right *y*-axis).

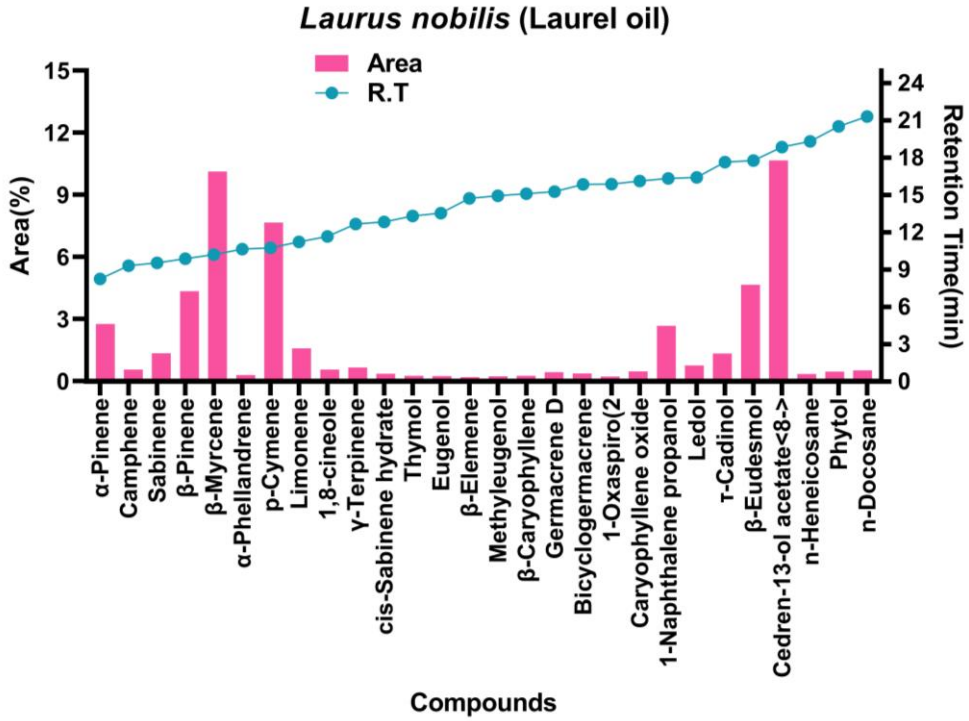

Figure 8. Composition of essential oil components from *L. nobilis* (laurel oil) by Chromatography–Mass Spectrometry (GC-MS) analysis. The *x*-axis enumerates the compounds identified. The area (%) occupied by each compound is represented by pink bars (left *y*-axis), and the retention time (min) is shown by a blue line (right *y*-axis).

In the GC-MS analysis of *S. officinalis*, three compounds stood out as the most abundant constituents: trans-b-caryophyllene (8.56%), α-pinene (8.14%), and viridiflorol (7.34%). These compounds represent the major components contributing significantly to the chemical profile of *S. officinalis*. On the other end of the spectrum, the three compounds with the lowest percentages were p-Cymen-8-ol (0.01%), (+)-trans-caryophyllene (0.05%), and isobutyl acetate (0.04%). While present in smaller quantities, these compounds add to the overall diversity of chemical constituents identified in *S. officinalis,* as depicted in Figure 6.

In the chemical analysis of *C. sativa* by GC-MS, the three compounds with the highest percentages were myrcene (23.45%), limonene (16.24%), and β-caryophyllene (16.64%). These constituents represent the major components contributing significantly to the volatile profile of *C. sativa*. Conversely, the three compounds with the lowest percentages were camphene (0.5%), (E)-β-ocimene epoxide (0.1%), and linalool (0.3%). While present in smaller quantities, these compounds still contribute to the overall chemical diversity of *C. sativa*, as shown in Figure 7.

In the GC-MS analysis of *L. nobilis* essential oil, phytol, β-myrcene, and n-heneicosane emerged as the compounds with the highest area percentages, at 10.65%, 10.12% and 4.65%, respectively. Conversely, the compounds with the lowest detected area percentages were eugenol, thymol, and β-caryophyllene, each constituting less than 0.3% of the oil's composition, with exact values of 0.24%, 0.26%, and 0.26%, respectively, as illustrated in Figure 8. These findings highlight the dominant and trace components contributing to the oil's unique chemical profile.

4. Discussion

Hemp plays a promising role in integrated pest management thanks to its widespread availability, affordability, and eco-friendliness. In 2018, Benelli and colleagues demonstrated the environmental safety of *C. sativa* essential oil residues, specifically their non-toxicity to non-target organisms [47]. Past research has validated the effectiveness of *C. sativa* against mites. Its traditional use in Pakistan for treating livestock ticks with its leaves underscores its acaricidal potential [74]. *C. sativa* L. is a useful and environmentally benign plant distinguished for its natural resilience against parasites and pests. The formation of bioactive secondary metabolites, such as terpenes and cannabinoids, ensures the presence of this feature. Hemp essential oil (EO), known for its terpenoid compounds with pesticide properties, was evaluated for its efficacy against mites *Dermanyssus gallinae* and ticks *Hyalomma dromedarii* [75]. Our analysis revealed that *C. sativa* (EO) demonstrated acaricidal activity, presenting a potential treatment method against the mite. Further, the study expanded to compare the effectiveness of various essential oils under winter conditions, including hemp, sage, and laurel, with hemp EO showing notable efficacy. These findings contribute to the growing body of evidence supporting essential oils as viable alternatives to chemical pesticides in managing mite infestations in beekeeping.

Essential oil derived from *S. officinalis*' aerial parts is rich in over 120 identified components, including borneol, camphor, caryophyllene, cineole, elemene, humulene, ledene, pinene, and thujone [57,76,77]. Significantly, this oil impacts the nervous system [78], with compounds like camphor, thujone, and terpene ketones noted for their potent toxicity. Research by Veličković et al. [79] highlights bornyl acetate, camphene, camphor, humulene, limonene, and thujone among the most prevalent phytochemicals in the leaves. However, environmental factors such as climate, water availability, and altitude influence *S. officinalis*'s chemical composition, echoing the variability observed in other herbs [80]. In our study on treating bee mites, we found that the essential oil of sage (*S. officinalis* L.) is effective against the *V. destructor* mite, a common parasite in honey bees (*A. mellifera* L.). The effectiveness of the treatment changes depending on how much oil is used and how long the mites are exposed to it. Specifically, we discovered that using a 5% oil concentration led to a 50.35% success rate, while a 15% concentration increased the success rate to 81.07%. These findings are significantly better than those of previous studies, like Bendifallah, Leila, et al., who reported a 21.07% effectiveness [54], and Ghomari et al. [81],

who saw a 48.7% success rate; our results even surpassed the effectiveness of Apivar. This commonly used mite treatment has a 3.13% success rate and was much more effective than the disappointing results seen with *Thymus vulgaris* oil, as noted by Giovenazzol et al. [82]. Additionally, Moussaoui et al. [83] have indicated that the eucalyptus bioproduct shows toxicity at an early stage. It is important to note that the chemical makeup of essential oils, and thus their biological activity, can vary based on the part of the plant used, the extraction method, and where the plant was grown [84].

Recent studies on the composition of laurel oil revealed a mix of key components like 1,8-cineole, α-terpinyl acetate, sabinene, linalool, and methyl-eugenol [85]. Linalool, in particular, stands out due to its abundance in *L. nobilis*, *L. officinalis*, and *L. hybrida* oils. Field research shows that its effectiveness varies significantly between bee colonies [86]. Moreover, laboratory tests have indicated that high doses of linalool can negatively affect brood survival and mite reproduction [87]. Our findings have shown that, at a 15% concentration, laurel oil is notably effective against *Varroa* mites, with a success rate of approximately 69%. This was in line with studies by Al-Ghzawi and his team in 2008, which tested the impact of various essential oils on *Varroa* mites. They found *L. nobilis* oil to be particularly potent [88]. Likewise, research by El-Zemity and colleagues in 2006 discovered similar efficacy with other oils. For example, *T. vulgaris* oil was capable of eliminating 50% of mites with the lowest dosage after just 24 h. However, the most effective results over longer exposures, 48 h and 72 h, were observed with *L. officinalis* oil, which showed the best balance of effectiveness and safety for bees [89]. In 1999, Colin et al. posited a hypothesis regarding long-term repellency's impact on female mites' fecundity. They suggested that minimal concentrations of specific natural compounds, notably monoterpenes and phenolic compounds, could significantly diminish mite fecundity, thereby mitigating the risk of severe parasitism within bee colonies [90]. Further investigations into the composition of essential oils and their acaricidal effects underscored the significance of 1,8-cineole, a major constituent of *L. nobilis* essential oil. Imdorf et al. (2006) and Ruffinengo et al. (2007) corroborated the high mortality rates among mites attributed to 1,8-cineole exposure [91,92]. Lee et al. (2008) expanded on these findings, associating the efficacy of various essential oils against pests with their molecular structures and emphasizing the role of functional groups over properties like hydrophobicity or vapor pressure in determining toxicity levels [93]. The comprehensive analysis by Calderone and Spivak (1995) illuminated the multitude of factors influencing the effectiveness of acaricide treatments. They delineated how the compound's concentration, treatment duration, application methodology, intra-colony conditions, apiary environment, and ambient temperature collectively determine a treatment's success [94]. The study conducted in 2014 showed that trans-β-caryophyllene showed acaricidal activity in two different species of dust mite [95]. The research conducted by Sarri in 2023 showed that essential oil was found to be rich in terpinolene, myristicin, myrcene, limonene, γ-terpinene, and (Z)-caryophyllene, which have acaricidal effects and anti-inflammatory potential [96]. α-pinene, the active component of essential oils of *Trachyspermum ammi* and *Thymus schimperi*, exerted toxicity against *Varroa* mites, as evidenced by antennectomized mites showing signs of restlessness and then moving very fast shortly before death [97]. Ghasemi et al. (2011, 2016) described a large research project in Iran to investigate the potential of essential oils from the medicinal plant flora of Iran to control the *Varroa* mite. To determine the oil yield percentage, in vitro mites were screened on seeds from many species from different plant families for the safety of the honey bee. The test results revealed a few oils, including *Thymus kotschyanus* Bioss & Hohen, (47.99% carvacrol, 30.61% thymol), *Mentha longifolia* L. (36.86% piperitenone, 27.53% piperitenone oxide, 22.21% cis-piperitone epoxide, 8.38% pulegone), and *Eucalyptus camaldulensis* Dehnh. However, in laboratory studies, these essential oils (74.7% 1,8-cineol and 8.35% α-pinene) effectively killed *Varroa* mites without causing high mortalities of honey bees [98,99].

Addressing the challenge of controlling *Varroa* mite populations in honey bee colonies necessitates treatments that exhibit potent acaricidal activity without adversely affecting the bees. Moreover, these treatments should ensure minimal residual presence in honey

and wax to safeguard consumer health. Adamczyk et al. (2005) concluded that residual components of essential oils in honey do not pose a health risk, albeit potentially altering its flavor [100]. Isman (2000) attributed the selective efficacy of essential oils as insecticides or acaricides to the absence of octopamine receptors in mammals, a feature exploited by these natural compounds [101]. This body of research collectively underscores the nuanced relationship between acaricide composition, application, and environmental factors and their collective impact on bee colony health. It also highlights the potential of essential oils as a mite control strategy, balancing efficacy with safety for both bees and consumers. But there is much we still need to learn. Critical areas for future studies include figuring out how these oils affect mite numbers and bee health over the long haul, their effectiveness when breeding bees, and how different weather conditions might change things. We also need to see how these oils impact bee behavior and how the colony gets along. Plus, it is essential to know how these treatments can fit into the bigger picture of pest management and whether they are cost-effective for beekeepers worldwide. Diving deeper into these questions will help create better, more sustainable mite control methods that beekeepers can rely on in the future.

5. Conclusions

Our study investigates the acaricidal activity of essential oils extracted from *S. officinalis*, *C. sativa*, and *L. nobilis* on *V. destructor* mites, a major threat to honey bee colonies. The most effective oil tested was hemp oil, showing rates of effectiveness of 95.4%, 85.71%, and 64.48% at concentrations of 15%, 10%, and 5%, respectively. Sage oil was effective to some extent, while laurel oil was the least effective. Essential oils are a promising and sustainable primary option for controlling *V. destructor* mites. Major compounds identified in the EOs responsible for their acaricidal properties were trans-caryophyllene, α-pinene, and viridiflorol in hemp oil; myrcene, limonene, and β-caryophyllene in sage oil; and phytol, β-myrcene, and n-heneicosane in laurel oil. Long-term research under field conditions is necessary to confirm the results, with a focus on the effects of these EOs on honey production and colony health. The integrated pest management strategy employing these EOs will allow beekeepers to control their colonies and avoid damage by *V. destructor* sustainably.

Author Contributions: Conceptualization, M.F.R., W.L. and M.H.; methodology, M.F.R.; software, M.F.R.; validation, M.F.R. and C.Z.; formal analysis, M.F.R.; investigation, M.F.R.; data curation, M.F.R.; writing—original draft preparation, M.F.R. and W.L.; writing—review and editing, M.F.R., W.L., C.Z. and M.H.; visualization, W.L. and M.F.R.; supervision, M.F.R.; project administration, W.L.; funding acquisition, W.L. All authors have read and agreed to the published version of the manuscript.

Funding: GDAS' Project of Science and Technology Development (Grant numbers 2022GDASZH-2022010102), the National Natural Science Foundation of China (Grant number 32000347), the Seed Industry Revitalization Action of the Department of Agriculture and Rural Affairs of Guangdong Province (Grant number 2022-440000-4301030211-9465), the Guangzhou Science and Technology Plan Project (Grand number 202103000009), Ecosystem and Biodiversity Monitoring in Nanling National Park (Phase 1) (LC-2021124), and the Talent Project of Guangdong Institute of Applied Biological Resources (Grant number GIABR-gjrc201702).

Institutional Review Board Statement: "Not applicable" for studies not involving humans or animals.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Papa, G.; Maier, R.; Durazzo, A.; Lucarini, M.; Karabagias, I.K.; Plutino, M.; Bianchetto, E.; Aromolo, R.; Pignatti, G.; Ambrogio, A.; et al. The honey bee *Apis mellifera*: An insect at the interface between human and ecosystem health. *Biology* **2022**, *11*, 233. [CrossRef] [PubMed]
2. De la Rúa, P.; Jaffé, R.; Dall'Olio, R.; Muñoz, I.; Serrano, J. Biodiversity, conservation and current threats to European honeybees. *Apidologie* **2009**, *40*, 263–284. [CrossRef]

3. FDA. *Helping Agriculture's Helpful Honey Bees*; FDA: Silver Spring, MD, USA, 2018.
4. Kurek-Górecka, A.; Górecki, M.; Rzepecka-Stojko, A.; Balwierz, R.; Stojko, J. Bee products in dermatology and skin care. *Molecules* **2020**, *25*, 556. [CrossRef] [PubMed]
5. Lakhran, H.; Bajiya, R.; Verma, H.P.; Todawat, A.; Gupta, A.; Kanwar, S.; Choudhary, M. Present scenario monitoring and management of environment pollution. In *Sustainable Development for Agriculture and Environment*, 1st ed.; ANU Publisher: Meerut, India, 2018; p. 69.
6. Neov, B.; Georgieva, A.; Shumkova, R.; Radoslavov, G.; Hristov, P. Biotic and abiotic factors associated with colonies mortalities of managed honey bee (*Apis mellifera*). *Diversity* **2019**, *11*, 237. [CrossRef]
7. Hristov, P.; Shumkova, R.; Palova, N.; Neov, B. Honey bee colony losses: Why are honey bees disappearing? *Sociobiology* **2021**, *68*, e5851. [CrossRef]
8. Brown, M.J.; Paxton, R.J. The conservation of bees: A global perspective. *Apidologie* **2009**, *40*, 410–416. [CrossRef]
9. Rosenkranz, P.; Aumeier, P.; Ziegelmann, B. Biology and control of *Varroa destructor*. *J. Invertebr. Pathol.* **2010**, *103*, 96–119. [CrossRef] [PubMed]
10. Traynor, K.S.; Mondet, F.; de Miranda, J.R.; Techer, M.; Kowallik, V.; Oddie, M.A.; Chantawannakul, P.; McAfee, A. *Varroa destructor*: A complex parasite, crippling honey bees worldwide. *Trends Parasitol.* **2020**, *36*, 592–606. [CrossRef] [PubMed]
11. Martin, S.J.; Highfield, A.C.; Brettell, L.; Villalobos, E.M.; Budge, G.E.; Powell, M.; Nikaido, S.; Schroeder, D.C. Global honey bee viral landscape altered by a parasitic mite. *Science* **2012**, *33*, 1304–1306. [CrossRef] [PubMed]
12. Moore, P.A.; Wilson, M.E.; Skinner, J.A. Honey bee viruses, the deadly *Varroa* Mite Associates. *Bee Health* **2015**, *19*, 2015.
13. Page, R. Blessing or curse? Varroa mite impacts Africanized bee spread and beekeeping. *Calif. Agric.* **1998**, *52*, 9–13.
14. Muijres, F.T.; Van Dooremalen, C.; Lankheet, M.; Lugt, H.; De Vries, L.J.; Van Langevelde, F. *Varroa destructor* infestation impairs the improvement of landing performance in foraging honeybees. *R. Soc. Open Sci.* **2020**, *7*, 201222. [CrossRef] [PubMed]
15. Duay, P.; De Jong, D.; Engels, W. Weight loss in drone pupae (*Apis mellifera*) multiply infested by *Varroa destructor* mites. *Apidologie* **2003**, *34*, 61–65. [CrossRef]
16. Underwood, R.; López-Uribe, M. Methods to Control Varroa Mites: An Integrated Pest Management Approach 2022. Available online: https://scholarsphere.psu.edu/resources/498200d1-389a-4221-987b-c8d9147f0d40 (accessed on 23 November 2023).
17. Fassbinder, C.; Grodnitzky, J.; Coats, J. Monoterpenoids as possible control agents for *Varroa destructor*. *J. Apic. Res.* **2002**, *41*, 83–88. [CrossRef]
18. Lin, Z.; Su, X.; Wang, S.; Ji, T.; Hu, F.L.; Zheng, H.Q. Fumigant toxicity of eleven Chinese herbal essential oils against an ectoparasitic mite (*Varroa destructor*) of the honey bee (*Apis mellifera*). *J. Apic. Res.* **2020**, *59*, 204–210. [CrossRef]
19. Jack, C.J.; Ellis, J.D. Integrated pest management control of *Varroa destructor* (Acari: Varroidae), the most damaging pest of (*Apis mellifera* L.(Hymenoptera: Apidae)) colonies. *J. Insect Sci.* **2021**, *21*, 6. [CrossRef] [PubMed]
20. Alissandrakis, E.; Ilias, A.; Tsagkarakou, A. Pyrethroid target site resistance in Greek populations of the honey bee parasite *Varroa destructor* (Acari: Varroidae). *J. Api. Res.* **2017**, *56*, 625–630. [CrossRef]
21. Hernández-Rodríguez, C.S.; Marín, Ó.; Calatayud, F.; Mahiques, M.J.; Mompó, A.; Segura, I.; Simó, E.; González-Cabrera, J. Large-scale monitoring of resistance to coumaphos, amitraz, and pyrethroids in *Varroa destructor*. *Insects* **2021**, *12*, 27. [CrossRef] [PubMed]
22. Morsy, A.R.; Nowar, E.E. Evaluation of acaricide toxicity to the parasitic mite (*Varroa destructor*), honeybee workers (*Apis mellifera* L.) and their residues in honey and beeswax. *Int. J. Sci.* **2022**, *5*, 29–50.
23. Wallner, K. Varroacides and their residues in bee products. *Apidologie* **1999**, *30*, 235–248. [CrossRef]
24. Hazam, S.; Touati, S.; Touati, L.; Saher, L.; Khedidji, H.; Ait Kaki, S.; Chemat, S. Promising Algerian essential oils as natural acaricides against the honey bee mite *Varroa destructor* (Acari: Varroidae). *Exp. Appl. Acarol.* **2024**, *92*, 87–107. [CrossRef] [PubMed]
25. Bava, R.; Castagna, F.; Palma, E.; Marrelli, M.; Conforti, F.; Musolino, V.; Carresi, C.; Lupia, C.; Ceniti, C.; Tilocca, B.; et al. Essential oils for a sustainable control of honeybee varroosis. *Vet. Sci.* **2023**, *10*, 308. [CrossRef] [PubMed]
26. Pathak, V.M.; Verma, V.K.; Rawat, B.S.; Kaur, B.; Babu, N.; Sharma, A.; Dewali, S.; Yadav, M.; Kumari, R.; Singh, S.; et al. Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. *Front. microbiol.* **2022**, *13*, 962619.
27. Pavela, R.; Benelli, G. Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends Plant Sci.* **2016**, *21*, 1000–1007. [CrossRef] [PubMed]
28. Kim, S.I.; Yi, J.H.; Tak, J.H.; Ahn, Y.J. Acaricidal activity of plant essential oils against *Dermanyssus gallinae* (Acari: Dermanyssidae). *Vet. Parasitol.* **2004**, *120*, 297–304. [CrossRef] [PubMed]
29. Tabari, M.A.; Youssefi, M.R.; Benelli, G. Eco-friendly control of the poultry red mite, *Dermanyssus gallinae* (Dermanyssidae), using the α-thujone-rich essential oil of *Artemisia sieberi* (Asteraceae): Toxic and repellent potential. *Parasitol. Res.* **2017**, *116*, 1545–1551. [CrossRef] [PubMed]
30. Tabari, M.A.; Youssefi, M.R.; Maggi, F.; Benelli, G. Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, *Ixodes ricinus* (Acari: Ixodidae). *Vet. Parasitol.* **2017**, *245*, 86–91. [CrossRef] [PubMed]
31. Chowański, S.; Adamski, Z.; Marciniak, P.; Rosiński, G.; Büyükgüzel, E.; Büyükgüzel, K.; Falabella, P.; Scrano, L.; Ventrella, E.; Lelario, F.; et al. A review of bioinsecticidal activity of Solanaceae alkaloids. *Toxins* **2016**, *8*, 60. [CrossRef] [PubMed]
32. Suteu, D.; Rusu, L.; Zaharia, C.; Badeanu, M.; Daraban, G.M. Challenge of utilization vegetal extracts as natural plant protection products. *Appl. Sci.* **2020**, *10*, 8913. [CrossRef]

33. Ntalli, N.; Koliopoulos, G.; Giatropoulos, A.; Menkissoglu-Spiroudi, U. Plant secondary metabolites against arthropods of medical importance. *Phytochem. Rev.* **2019**, *18*, 1255–1275. [CrossRef]
34. Dehsheikh, A.B.; Sourestani, M.M.; Dehsheikh, P.B.; Mottaghipisheh, J.; Vitalini, S.; Iriti, M. Monoterpenes: Essential oil components with valuable features. *Mini-Rev. Med. Chem.* **2020**, *20*, 958–974. [CrossRef] [PubMed]
35. Dolan, M.C.; Dietrich, G.; Panella, N.A.; Montenieri, J.A.; Karchesy, J.J. Biocidal activity of three wood essential oils against *Ixodes scapularis* (Acari: Ixodidae), *Xenopsylla cheopis* (Siphonaptera: Pulicidae), and *Aedes aegypti* (Diptera: Culicidae). *J. Econ. Entomol.* **2014**, *100*, 622–625. [CrossRef]
36. Spochacz, M.; Chowański, S.; Walkowiak-Nowicka, K.; Szymczak, M.; Adamski, Z. Plant-derived substances used against beetles–pests of stored crops and food–and their mode of action: A review. *CRFSFS* **2018**, *17*, 1339–1366. [CrossRef] [PubMed]
37. Senthil-Nathan, S. A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. *Front. Physiol.* **2020**, *10*, 473482. [CrossRef] [PubMed]
38. Pavela, R.; Maggi, F.; Petrelli, R.; Cappellacci, L.; Buccioni, M.; Palmieri, A.; Canale, A.; Benelli, G. Outstanding insecticidal activity and sublethal effects of Carlina acaulis root essential oil on the housefly, Musca domestica, with insights on its toxicity on human cells. *Food. Chem. Toxicol.* **2020**, *136*, 111037. [CrossRef] [PubMed]
39. Stuhl, C.J. The development of an attract-and-kill bait for controlling the small hive beetle (Coleoptera: Nitidulidae). *Apidologie* **2020**, *51*, 428–435. [CrossRef]
40. Peng, G.; Kashio, M.; Morimoto, T.; Li, T.; Zhu, J.; Tominaga, M.; Kadowaki, T. Plant-derived tick repellents activate the honey bee ectoparasitic mite TRPA1. *Cell Rep.* **2015**, *12*, 190–202. [CrossRef] [PubMed]
41. Soares, S.; Amaral, J.S.; Oliveira, M.B.P.; Mafra, I. A comprehensive review on the main honey authentication issues: Production and origin. *CRFSFS* **2017**, *16*, 1072–1100. [CrossRef] [PubMed]
42. Gregorc, A.; Sampson, B. Diagnosis of *Varroa* mite (*Varroa destructor*) and sustainable control in honey bee (*Apis mellifera*) colonies a review. *Diversity* **2019**, *11*, 243. [CrossRef]
43. Flamini, G. Acaricides of natural origin. Part 2. Review of the literature (2002–2006). *Nat. Prod. Commun.* **2006**, *1*, 1934578X0600101216. [CrossRef]
44. Umpiérrez, M.L.; Santos, E.; González, A.; Rossini, C. Plant essential oils as potential control agents of Varroatosis. *Phytochem. Rev.* **2011**, *10*, 227–244. [CrossRef]
45. Booth, J.K.; Page, J.E.; Bohlmann, J. Terpene synthases from Cannabis sativa. *PLoS ONE* **2017**, *12*, e0173911. [CrossRef] [PubMed]
46. George, D.R.; Smith, T.J.; Shiel, R.S.; Sparagano, O.; Guy, J.H. Mode of action and variability in efficacy of plant essential oils showing toxicity against the poultry red mite, *Dermanyssus gallinae*. *Vet. Parasitol.* **2009**, *161*, 276–282. [CrossRef] [PubMed]
47. Benelli, G.; Pavela, R.; Petrelli, R.; Cappellacci, L.; Santini, G.; Fiorini, D.; Sut, S.; Dall'Acqua, S.; Canale, A.; Maggi, F. The essential oil from industrial hemp (*Cannabis sativa* L.) by-products as an effective tool for insect pest management in organic crops. *Ind. Crops Prod.* **2018**, *122*, 308–315. [CrossRef]
48. Castilhos, R.V.; Grützmacher, A.D.; Coats, J.R. Acute toxicity and sublethal effects of terpenoids and essential oils on the predator *Chrysoperla externa* (Neuroptera: Chrysopidae). *Neotrop. Entomol.* **2018**, *47*, 311–317. [CrossRef] [PubMed]
49. Bonini, S.A.; Premoli, M.; Tambaro, S.; Kumar, A.; Maccarinelli, G.; Memo, M.; Mastinu, A. Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. *J. Ethnopharmacol.* **2018**, *227*, 300–315. [CrossRef] [PubMed]
50. Nissen, L.; Zatta, A.; Stefanini, I.; Grandi, S.; Sgorbati, B.; Biavati, B.; Monti, A. Characterization and antimicrobial activity of essential oils of industrial hemp varieties (*Cannabis sativa* L.). *Fitoterapia* **2010**, *81*, 413–419. [CrossRef] [PubMed]
51. Imdorf, A.; Bogdanov, S.; Ochoa, R.I.; Calderone, N.W. Use of essential oils for the control of *Varroa* jacobsoni Oud. in honey bee colonies. *Apidologie* **1999**, *30*, 209–228. [CrossRef]
52. Mills, S.Y.; Bone, K. *The Essential Guide to Herbal Safety*, 1st ed.; Elsevier Health Sciences, Churchill Livingstone: London, UK, 2004; pp. 51–52.
53. Badiee, P.; Nasirzadeh, A.R.; Motaffa, M. Comparison of *Salvia officinalis* L. essential oil and antifungal agents against candida species. *J. Pharm. Technol. Drug Res.* **2012**, *1*, 1–5.
54. Bendifallah, L.; Belguendouz, R.; Hamoudi, L.; Arab, K. Biological activity of the *Salvia officinalis* L.(Lamiaceae) essential oil on *Varroa destructor* infested honeybees. *Plants* **2018**, *7*, 44. [CrossRef] [PubMed]
55. Caputo, L.; Nazzaro, F.; Souza, L.F.; Aliberti, L.; De Martino, L.; Fratianni, F.; Coppola, R.; De Feo, V. Laurus nobilis: Composition of essential oil and its biological activities. *Molecules* **2017**, *22*, 930. [CrossRef] [PubMed]
56. Simić, A.; Soković, M.D.; Ristić, M.; Grujić-Jovanović, S.; Vukojević, J.; Marin, P.D. The chemical composition of some Lauraceae essential oils and their antifungal activities. *Phytother. Res. Int. J. Pharmacol. Toxicol. Eval. Nat. Prod. Deriv.* **2004**, *18*, 713–717. [CrossRef] [PubMed]
57. Sırıken, B.; Yavuz, C.; Güler, A. Antibacterial Activity of Laurus nobilis: A review of literature. *Med.Sci. Discov.* **2018**, *5*, 374–379. [CrossRef]
58. Loizzo, M.R.; Tundis, R.; Menichini, F.; Saab, A.M.; Statti, G.A.; Menichini, F. Cytotoxic activity of essential oils from Labiatae and Lauraceae families against in vitro human tumor models. *Anticancer Res.* **2007**, *27*, 3293–3299. [PubMed]
59. Jemâa, J.M.B.; Tersim, N.; Toudert, K.T.; Khouja, M.L. Insecticidal activities of essential oils from leaves of *Laurus nobilis* L. from Tunisia, Algeria and Morocco, and comparative chemical composition. *J. Stored Prod. Res.* **2012**, *48*, 97–104. [CrossRef]

60. Fernandez, C.M.M.; da Rosa, M.F.; Fernandez, A.C.A.M.; Bortolucci, W.D.C.; Ferreira, F.B.P.; Linde, G.A.; Colauto, N.B.; Simões, M.R.; Lobo, V.D.S.; Gazim, Z.C. Essential oil and fractions isolated of Laurel to control adults and larvae of cattle ticks. *Nat. Prod. Res.* **2020**, *34*, 731–735. [CrossRef] [PubMed]
61. Sajid, Z.N.; Aziz, M.A.; Bodlah, I.; Rana, R.M.; Ghramh, H.A.; Khan, K.A. Efficacy assessment of soft and hard acaricides against *Varroa destructor* mite infesting honey bee (*Apis mellifera*) colonies, through sugar roll method. *Saudi J. Biol. Sci.* **2020**, *27*, 53–59. [CrossRef] [PubMed]
62. Gregorc, A.; Adamczyk, J.; Kapun, S.; Planinc, I. Integrated *Varroa* control in honey bee (*Apis mellifera carnica*) colonies with or without brood. *J. Apic. Res.* **2016**, *55*, 253–258. [CrossRef]
63. Qadir, Z.A.; Idrees, A.; Mahmood, R.; Sarwar, G.; Bakar, M.A.; Ahmad, S.; Raza, M.M.; Li, J. Effectiveness of different soft acaricides against honey bee ectoparasitic mite *Varroa destructor* (Acari: Varroidae). *Insects* **2021**, *12*, 1032. [CrossRef] [PubMed]
64. Hyder, M.; Li, Y.; Wang, M.; Mao, J.; Mari, J.; Bukero, A.; Soomro, H.; Bukero, A.; Zhang, L. Insecticidal activity, Chemical Constituents of *Trachyspermum ammi*, *Withania coagulans* and *Murraya koenigii* ethanloic extracts against *Bemisia tabaci*. *Braz. J. Biol.* **2022**, *84*, e260298.
65. Hyder, M.; Li, Y.; Wang, M.; Mao, J.; Zhang, L.; Research, E. Insecticidal activity of ethanol and aqueous extracts of medicinal plants against green peach aphid (*Myzus persicae*). *Appl. Ecol. Environ. Res.* **2022**, *20*, 347. [CrossRef]
66. Wagan, T.A.; Wang, W.; Hua, H.; Cai, W. Chemical constituents and toxic, repellent, and oviposition-deterrent effects of ethanol-extracted *Myristica fragrans* (Myristicaceae) oil on *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Fla. Entomol.* **2017**, *1*, 594–601. [CrossRef]
67. Ellis, M.D.; Paula, A. Using the Sugar Roll Technique to Detect Varroa Mites in Honey Bee Colonies. *Hist. Mater. Univ. Neb.-Linc. Ext.* **2001**, *116*. Available online: https://digitalcommons.unl.edu/extensionhist/116 (accessed on 6 January 2024).
68. Gregorc, A.; Knight, P.R.; Adamczyk, J. Powdered sugar shake to monitor and oxalic acid treatments to control *Varroa* mites (*Varroa destructor* Anderson and Trueman) in honey bee (*Apis mellifera*) colonies. *J. Apic. Res.* **2017**, *56*, 71–75. [CrossRef]
69. Liu, F.; Xu, X.; Zhang, Y.; Zhao, H.; Huang, Z.Y. A Meta-Analysis shows that screen bottom boards can significantly reduce *Varroa destructor* population. *Insects* **2020**, *9*, 624.
70. Mahmood, R.; Asad, S.; Ahmad, W.; Sarwar, G.; Muhamamd, K.R.; Islam, N.; Qadir, Z.A.; Abiden, Z.U. Efficacy of screen bottom board tray with and without soft chemicals for controlling *Varroa destructor* in honeybee colonies. *Pak. J. Zool.* **2017**, *49*, 9–13. [CrossRef]
71. Mahmood, R.; Wagchoure, E.S.; Raja, S.; Sarwar, G.; Aslam, M. Effect of thymol and formic acid against ectoparasitic brood mite *Tropilaelaps clareae* in *Apis mellifera* colonies. *Pak. J. Zool.* **2011**, *43*, 91–95.
72. Rashid, M.; Wagchoure, E.S.; Mohsin, A.U.; Raja, S.; Sarwar, G. Control of ectoparasitic mite *Varroa destructor* in honeybee (*Apis mellifera* L.) colonies by using different concentrations of oxalic acid. *J. Anim. Plant Sci.* **2012**, *22*, 72–76.
73. Ott, R.L.; Longnecker, M. *An Introduction to Statistical Methods and Data Analysis*, 7th ed.; Cengage Learning: Andover, UK, 2016; pp. 23–25.
74. Pavela, R.; Canale, A.; Mehlhorn, H.; Benelli, G. Application of ethnobotanical repellents and acaricides in prevention, control and management of livestock ticks: A review. *Res. J. Vet. Sci.* **2016**, *109*, 1–9. [CrossRef]
75. Tabari, M.A.; Khodashenas, A.; Jafari, M.; Petrelli, R.; Cappellacci, L.; Nabissi, M.; Maggi, F.; Pavela, R.; Youssefi, M.R. Acaricidal properties of hemp (*Cannabis sativa* L.) essential oil against *Dermanyssus gallinae* and *Hyalomma dromedarii*. *Ind. Crops. Prod.* **2020**, *147*, 112238. [CrossRef]
76. Hayouni, E.A.; Chraief, I.; Abedrabba, M.; Bouix, M.; Leveau, J.Y.; Mohammed, H.; Hamdi, M. Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: Their chemical compositions and their preservative effects against Salmonella inoculated in minced beef meat. *Int. J. Food Microbiol.* **2008**, *125*, 242–251. [CrossRef] [PubMed]
77. Länger, R.; Mechtler, C.; Jurenitsch, J. Composition of the essential oils of commercial samples of *Salvia officinalis* L. and *S. fruticosa* Miller: A comparison of oils obtained by extraction and steam distillation. *Phytochem. Analys.* **1996**, *7*, 289–293. [CrossRef]
78. Blenau, W.; Rademacher, E.; Baumann, A. Plant essential oils and formamidines as insecticides/acaricides: What are the molecular targets? *Apidologie* **2012**, *43*, 334–347. [CrossRef]
79. Veličković, D.T.; Ranđelović, N.V.; Ristić, M.S.; Veličković, A.S.; Šmelcerović, A.A. Chemical constituents and antimicrobial activity of the ethanol extracts obtained from the flower, leaf and stem of *Salvia officinalis* L. *J. Serb. Chem. Soc.* **2003**, *68*, 17–24. [CrossRef]
80. Russo, A.; Formisano, C.; Rigano, D.; Senatore, F.; Delfine, S.; Cardile, V.; Rosselli, S.; Bruno, M. Chemical composition and anticancer activity of essential oils of Mediterranean sage (*Salvia officinalis* L.) grown in different environmental conditions. *Food Chem Toxicol.* **2013**, *55*, 42–47. [CrossRef] [PubMed]
81. Ghomari, F.N.; Kouache, B.; Arous, A.; Cherchali, S. Effet de traitement par fumigation du thym (*Thymus vulgaris*) sur le *Varroa destructor* agent de la Varroase des abeilles. *Nat. Technol.* **2014**, *10*, 34–38.
82. Giovenazzol, P.; Marceau, J.; Dube, S. Essais préliminaires sur le traitement de colonies d'abeilles *Apis mellifera* infestées par le parasite *Varroa* jacobsoni en chambre d'hivernage. *L'abeille* **1999**, *19*, 5–9.
83. Moussaoui, K.; Ahmed Hedjala, O.; Zitouni, G.; Djazouli, Z. Estimation de la toxicité des d'huiles essentielles formulées de thym et d'eucalyptus et d'un produit de synthèse sur le parasite de l'abeille tellienne *Varroa destructor* (arachnida, varroidae). *Agrobiologie* **2014**, *4*, 17–26.

84. Türkmen, M.; Kara, M.; Maral, H.; Soylu, S. Determination of chemical component of essential oil of Origanum dubium plants grown at different altitudes and antifungal activity against *Sclerotinia sclerotiorum*. *J. Food Process. Preser.* **2022**, *46*, e15787. [CrossRef]
85. Alimi, D.; Hajri, A.; Jallouli, S.; Sebai, H. In vitro acaricidal activity of essential oil and crude extracts of *Laurus nobilis*, (Lauraceae) grown in Tunisia, against arthropod ectoparasites of livestock and poultry: *Hyalomma scupense* and *Dermanyssus gallinae*. *Vet. Parasitol.* **2021**, *298*, 109507. [CrossRef] [PubMed]
86. Calderone, N.W.; Wilson, W.T.; Spivak, M. Plant extracts used for control of the parasitic mites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae). *J. Econ. Entomol.* **1997**, *90*, 1080–1086. [CrossRef]
87. Abo-Taka, S.M.; Sharaf El-Din, H.A. Studies on the control of parasitic honey bee mite *Varroa* jacobsoni Oud. *J. Insect Sci* **1992**, *65*, 72–75.
88. Al-Ghzawi, A.M.; Zaitoun, S.; Alkofahi, A. The effect of different plant extracts on honeybees *Apis mellifera* (Hymenoptera: Apidae) and its parasite, *Varroa destructor* (Acari: Varroidae). *Belgian J. Entomol.* **2008**, *10*, 57–65.
89. El-Zemity, S.R.; Rezk, H.A.; Farok, S.; Zaitoon, A.A. Acaricidal activity of some essential oils and their monoterpenoidal constituents against the house dust mite, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). In *Trends in Acarology*; Sabelis, M., Bruin, J., Eds.; Springer: Dordrecht, The Netherlands, 2010. [CrossRef]
90. Colin, M.E.; Ball, B.V. *Bee Disease Diagnosis*; CIHEAM: Paris, France, 1999; pp. 10–11.
91. Imdorf, A.; Bogdanov, S.; Kilchenmann, V.; Berger, T. Toxic effects of essential oils and some of their components on *Varroa destructor* Oud and *Apis mellifera* L. under laboratory conditions. *ALP Sci.* **2006**, *495*, 18.
92. Ruffinengo, S.; Maggi, M.; Faverin, C.; Rosa, S.B.; Bailac, P.; Principal, J.; Eguaras, M. Essential oils toxicity related to *Varroa destructor* and *Apis mellifera* under laboratory conditions. *Zootecnia Trop* **2007**, *25*, 63–69.
93. Lee, E.J.; Kim, J.R.; Choi, D.R.; Ahn, Y.J. Toxicity of cassia and cinnamon oil compounds and cinnamaldehyde-related compounds to *Sitophilus oryzae* (Coleoptera: Curculionidae). *J. Econ. Entomol.* **2008**, *101*, 1960–1966. [CrossRef] [PubMed]
94. Calderone, N.W.; Spivak, M. Plant extracts for control of the parasitic mite *Varroa jacobsoni* (Acari: Varroidae) in colonies of the western honey bee (Hymenoptera: Apidae). *J. Econ. Entomol.* **1995**, *88*, 1211–1215. [CrossRef]
95. Oh, M.S.; Yang, J.Y.; Kim, M.G.; Lee, H.S. Acaricidal activities of β-caryophyllene oxide and structural analogues derived from *Psidium cattleianum* oil against house dust mites. *Pest Manag. Sci.* **2014**, *70*, 757–762. [PubMed]
96. Sarri, M.; Fodil, H.; Hendel, N.; Sarri, D.; Bruno, M.; Maggi, F. Essential oil of *Apium nodiflorum* (L.) Lag. growing in Ksob River, Algeria. *Nat. Prod. Res.* **2023**, *20*, 1–4. [CrossRef] [PubMed]
97. Begna, T.; Ulziibayar, D.; Bisrat, D.; Jung, C. Acaricidal Toxicity of Four Essential Oils, Their Predominant Constituents, Their Mixtures against *Varroa* Mite, and Their Selectivity to Honey Bees (*Apis cerana* and *A. mellifera*). *Insects* **2023**, *14*, 735. [CrossRef]
98. Ghasemi, V.; Moharramipour, S.; Tahmasbi, G. Biological activity of some plant essential oils against *Varroa destructor* (Acari: Varroidae), an ectoparasitic mite of *Apis mellifera* (Hymenoptera: Apidae). *Exp. Appl. Acarol.* **2011**, *55*, 147–154. [CrossRef] [PubMed]
99. Ghasemi, V.; Moharramipour, S.; Tahmasbi, G.H. Laboratory cage studies on the efficacy of some medicinal plant essential oils for controlling varroosis in *Apis mellifera* (Hym.: Apidae). *Syst. Appl. Acarol.* **2016**, *21*, 1681–1692.
100. Adamczyk, S.; Lázaro, R.; Pérez-Arquillué, C.; Conchello, P.; Herrera, A. Evaluation of residues of essential oil components in honey after different anti-*Varroa* treatments. *J. Agric. Food Chem.* **2005**, *53*, 10085–10090. [CrossRef] [PubMed]
101. Isman, M.B. Plant essential oils for pest and disease management. *Crop Prot.* **2000**, *19*, 603–608. [CrossRef]