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# HYDROLATES AND ESSENTIAL OILS AFFECT *Varroa destructor* MITES IN *in vitro* ASSAYS

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## ABSTRACT

**The beekeeping industry is affected by infestation of hives by *Varroa destructor* mites, reducing both honey yield and quality. The objective of this study was to evaluate the effects of hydrolates or essential oils on the survival of *V. destructor* mites using in vitro assays. Six hydrolates obtained from *Lavandula angustifolia*, *Tithonia diversifolia*, *Ruta graveolens*, *Dalbergia palo-escrito*, *Populus alba*, and *Retama sphaerocarpa*, as well as two essential oils from *Lavandula angustifolia* and *Origanum vulgare* were used. Behavioral changes and survival rates were evaluated to determine the effects of hydrolates or plant-based essential oils on *V. destructor* mites. Results indicate that essential oils of *L. angustifolia* and *O. vulgare* at low concentration (1.25 µL), as well as the hydrolate obtained from the flowers of *Retama sphaerocarpa* are effective against *V. destructor* without compromising bee survival. In conclusion, essential oils and hydrolates may serve as alternative treatments to reduce *V. destructor* mite infestations, particularly *R. sphaerocarpa* flower and *T. diversifolia* hydrolates, and 4 µL of *O. vulgare* essential oil.**

**Keywords:** *Dalbergia palo-escrito, Lavandula angustifolia, Populus alba, Ruta graveolens, Tithonia diversifolia.*

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## INTRODUCTION

Beekeeping is an important livestock activity because of its socioeconomic and ecological significance, contributing to foreign income (9.5% of global agricultural value) through the production of honey, pollen, propolis, and other beehive products (Schouten, 2021). In addition, this industry plays a fundamental role in maintaining the ecological balance due to pollination activity (Khalifa et al., 2021). Bees are often affected by varroasis, a disease caused by *Varroa destructor*, an ectoparasite that attacks both adult and young bees (Traynor et al., 2020). Noël et al. (2020) reviewed the impact of this ectoparasite at both individual and colony levels, reporting reduced body weight in young bees. The authors further noted that *Varroa* disrupts bee flight, neural processes, and immune responses, facilitating the spread of *Varroa* to other colonies and leading to honeybee losses.

The main acaricidal compounds used globally to control *Varroa* infestations are the pyrethroids fluvalinate and flumethrin (Yarsan et al., 2024). However, the inappropriate or indiscriminate use of these acaricides has caused mites to develop resistance, while their use can also contaminate honey and wax, potentially harming consumer health (Mitton et al., 2022). Bava et al. (2023) proposed an alternative approach for varroosis control by reviewing the use of essential oils as acaricides. Plant defense against pathogenic agents is based on the production of secondary metabolites with antimicrobial and antiparasitic activity (Fitch et al., 2022). Some techniques can be used to obtain these metabolites from plants, but one of the most commonly used is extraction by steam distillation of the leaves, flowers, stems, seeds, or roots of plants to obtain essential oils and produce an aqueous residue known as hydrolate (Tavares et al., 2022). Both extract types have antibacterial, antifungal, and antiparasitic properties related to the synthesis inhibition of certain essential components in microorganisms and can also prevent the fixation and colonization of viruses in cells (Sitarek et al., 2020). Many plants have biological activities, including oregano (*O. vulgare* Labiatae), an aromatic plant with antioxidant, antimicrobial, antiparasitic, estrogenic, insecticidal, and antigenotoxic activities (Arcila-Lozano et al., 2004). Tavares et al. (2022) reviewed the potential uses of hydrolates and indicated that they have several biological properties and potential uses and applications in different industries. However, more research is needed to elucidate the effects of hydrolates.

There are many plants from which hydrolates can be obtained, including acahual (*Tithonia diversifolia*), a robust herbaceous and shrubby plant that shows biological activity and is capable of bioaccumulation, as well as antimalarial, antibacterial and antifungal properties (Ajao and Moteetee, 2017). Furthermore, studies have shown that Rue (*Ruta graveolens*) has antimicrobial and antioxidant properties, attributable to its rich content in secondary metabolites, such as coumarins, alkaloids, volatile oils, flavonoids, and phenolic acids, which are responsible for various biological effects (França-Orlanda et al., 2015; Pushpa et al., 2015). In addition, English lavender (*Lavandula angustifolia*) has been reported to have insecticidal, antibacterial, and antiviral properties (Basch et al., 2014). Poplar (*Populus alba*) has demonstrated antimicrobial activity (Sosa Castañeda et al., 2022) and some species of *Dalbergia* have been reported to contain flavonoids and sesquiterpenes with antioxidant and antibacterial activities (Singh et al., 2017). There is extensive information on the use of essential oils obtained from different plants (Bava et al., 2023), but data on the utilization of hydrolates are limited. In this study, we compare plant extracts previously used against *Varroa*, while most of the hydrolates tested here have not been applied to control varroosis. This study aimed to evaluate the effect of hydrolates and essential oils obtained from several aromatic plants on *V. destructor* using *in vitro* tests to suggest alternative treatments to mitigate varroosis.

## MATERIALS AND METHODS

### Plant material collection and preparation of essential oils and hydrolates

*Origanum vulgare* (Labiatae) was collected from Mineral del Chico (20°12′53″ N, 98°43′50″ W), while *Ruta graveolens, Dalbergia palo-escrito, Tithonia diversifolia* and *Populus alba* were obtained from Tulancingo de Bravo (20°5′9″ N, 98°21′48″ W). *Lavandula angustifolia* was obtained from Mineral de la Reforma (20°2′44.68″ N, 98°43′15.88″ W) in Hidalgo, Mexico. To obtain essential oils and hydrolates from all of these plants, aerial parts of the plants were air-dried while protected from sunlight (environmental conditions: 23 °C, 35% relative humidity) during five days. Subsequently, samples were ground in a Swissmex SW610350 grinder (Swissmex-Rapid, Lagos de Moreno, Jalisco, Mexico); the resulting powder was not sieved. The powder was used for hydrodistillation with a Niang apparatus (Dongguan Niangge Machinery Equipment Co, Beijing, China). Each powder was placed in the apparatus (50 g $L^{-1}$) for 4 h at 90 °C to obtain both hydrolates and essential oils.

***In vitro* tests**

All experiments were performed in Nutrition Lab in the Instituto de Ciencias Agropecuarias, where the environmental conditions were 24 °C and 50% relative humidity. For each trial, approximately 500 live worker honeybees (*Apis mellifera*) and 5 combs with capped and uncapped brood were collected from 5 different hives in an apiary located in Tulancingo de Bravo, Hidalgo, Mexico (20°5′9″ N, 98°21′48″W). The level of V. destructor infestation was determined using the Jong test (De Jong et al., 1982) to estimate the parasite load in the bee population. The experiments were performed as described by Mahmood et al. (2014). Briefly, three adult bees (*A. mellifera*) and three *V. destructor* mites were placed in glass Petri dishes (90 × 15 mm) with their respective treatments (2 mL of each extract). Hydrolates, essential oils, or substances used in Trials 1–4 were previously embedded on plates. In Trial 5, the plates were conditioned with a feeder containing 1 mL of honey diluted 1:1 with distilled water and incubated at 35 °C for 72 h, with observations recorded at 24, 48, and 72 h to determine the survival rate of the bees and mites.

Five in vitro trials were conducted, including two qualitative assessment. For Trial 1, seven treatments were performed: without alcohol, 70° ethyl alcohol, 0.5 g $L^{-1}$ thymol, and 1.25, 2.5, 5, 10 μL $mL^{-1}$ of *O. vulgare* essential oil with three repetitions. For Trial 2 (qualitative), the following 12 treatments were tested: without alcohol, 70° ethyl alcohol, 0.5 g $mL^{-1}$ evaporated thymol, 0.5 g $L^{-1}$ thymol loaded in a sponge, 0.5, 1, 2, 4 μL of *O. vulgare* essential oil, 12.5, 25, 50 and 100 μL $mL^{-1}$ of *O. vulgare* hydrolate. For Trial 3 (quantitative), 18 treatments were tested: alcohol-free, 70° ethyl alcohol, 0.5 g $L^{-1}$ thymol, 1 mg fluvalinate, 0.675, 1.25 μL $mL^{-1}$ of *O. vulgare* essential oil, as well as 25, 50 and 100 μL $mL^{-1}$ of hydrolates from *O. vulgare, R. graveolens, D. palo-escrito,* and *T. diversifolia*. For Trial 4 (quantitative), 16 treatments were tested: alcohol-free, 70° ethyl alcohol, 1 mg fluvalinate, 0.5 g $L^{-1}$ thymol, 0.5, 1, 2 μL $mL^{-1}$ of *O. vulgare* essential oil, as well as 25, 50 and 100 μL $mL^{-1}$ hydrolates from *O. vulgare*, *P. alba*, *T. diversifolia*. Finally, for Trial 5 (quantitative), 15 treatments were tested: alcohol-free, 70° ethyl alcohol, 1 mg fluvalinate, 0.5 g $L^{-1}$ thymol, 0.312 μL $mL^{-1}$ of *O. vulgare* essential oil, 0.650 μL $mL^{-1}$ *Lavandula angustifolia* essential oil, 100 μL $mL^{-1}$ of hydrolates from *L. angustifolia*, *P. alba*, *O. vulgare*, *T. diversifolia*, *R. sphaerocarpa* (flowers), *R. sphaerocarpa* (leaves), *R. sphaerocarpa* (pods), *R. sphaerocarpa* (stems), and *R. graveolens*). All trials were performed in triplicate.

Bee behavior was assessed through visual observation, inspecting live or dead individuals, and noting whether the bee or mite was calm, aggressive, feeding, or exhibiting other behaviors. An individual was considered dead if, upon opening the Petri plate, it remained immobile and failed to respond to stimulation with a paintbrush. The bee and mite survival rate were determined as the number of live bees or mites divided by the total number of individuals, expressed per 100 bees or mites.

**Statistical analysis**

The first two trials were descriptive, using qualitative variables. The trial number three, four and five were performed using a completely randomized design. An analysis of variance was then carried out following a general linear model procedure, followed by Fisher´s test to identify significant differences ($P<0.05$) among the treatments. The following statistical model was used:

$$Yij = \mu + \beta i + \varepsilon ij,$$

where Yij = dependent variable, μ = mean of the variable, βi = the fixed effect of the ith bee or mite in the group, and εij = experimental error associated with the observation Yij.

All analyses were performed using Minitab version 20.3.

## RESULTS

Before performing the in vitro trials, a Jong test was conducted to confirm the presence of *Varroa* infestation. The results of Trial 1 are listed in Table 1. Exposure to 10 and 20 μL of *O. vulgare* essential oil had a detrimental effect on bees: they exhibited aggressive behavior immediately after exposure and died within minutes, accompanied by darkening of the body. After 24 h, similar effects were observed with 5 μL of *O. vulgare* essential oil. In contrast, bees treated with thymol, ethyl alcohol, or 2.5 μL of *O. vulgare* essential oil exhibited normal behavior, remaining calm and feeding.

In Trial 2, results showed that the inclusion of *O. vulgare* essential oil at concentrations of 0.5, 1, 2 and 4 μL allowed bees to remain alive until the 72-h time point, while *V. destructor* died within 24 h. In contrast, when *O. vulgare* hydrolate was applied at concentrations of 25, 50, 100, and 200 μL, bees also survived until 72 h, while *V. destructor* died after 72 h, indicating that this hydrolate has a less pronounced effect on *V. destructor* (Table 2).

In Trial 3, the treatment with 2.5 μL of *O. vulgare* essential oil had the greatest effect ($P<0.05$) on bee survival, followed by the treatments with

**Table 1. Behavior of *A. mellifera* in vitro following exposure to *Origanum vulgare* essential oil.**

| Treatment | Observation of bees | |
|---|---|---|
| | 0 h | 24 h |
| Without alcohol | Calm, feeding | Alive, normal behavior |
| 70° Alcohol | Calm, feeding | Alive, normal behavior |
| Evaporated thymol | Calm, feeding | Alive, normal behavior |
| 2.5 µL | Calm, feeding | Alive, normal behavior |
| 5 µL | Calm, feeding | All bees died |
| 10 µL | Black color, aggressive and dead | All bees died |
| 20 µL | Black color, aggressive and death | All bees died |

**Table 2. Behavior of *A. mellifera* and *V. destructor* in vitro following exposure to *Origanum vulgare* essential oil and hydrolate.**

| Treatment | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| | Bee | *Varroa* | Bee | *Varroa* | Bee | *Varroa* |
| Without alcohol | A | A | A | A | A | A |
| Evaporated thymol | A | A | A | A | A | A |
| Thymol in sponge | A | D | A | D | A | D |
| 70° Alcohol | A | A | A | A | A | A |
| *O. vulgare* essential oil, 0.5 µL | A | D | A | D | A | D |
| *O. vulgare* essential oil, 1 µL | A | D | A | D | A | D |
| *O. vulgare* essential oil, 2 µL | A | D | A | D | A | D |
| *O. vulgare* essential oil, 4 µL | A | D | A | D | A | D |
| *O. vulgare* hydrolate, 25 µL | A | A | A | A | A | D |
| *O. vulgare* hydrolate, 50 µL | A | A | A | A | A | D |
| *O. vulgare* hydrolate, 100 µL | A | A | A | A | A | D |
| *O. vulgare* hydrolate, 200 µL | A | A | A | A | A | D |

A=Alive; D=Dead.

fluvalinate, 200 µL of *D. palo-escrito* hydrolate, and thymol (Table 3). This former treatment produced the same survival rates (P>0.05) among the bees as with *O. vulgare* and *R. graveolens* hydrolates, presenting greater (P<0.05) survival rates than 1.25 µL O. vulgare essential oil and *T. diversifolia* hydrolate at concentrations of 50, 100 and 200 µL. Regarding *V. destructor*, its survival rate was lower with fluvalinate and *O. vulgare* essential oil, followed by thymol and 50 µL of hydrolates from aromatic plants, with the highest survival rates occurring with 200 µL of hydrolates from aromatic plants (*O. vulgare*, *T. diversifolia*, *D. palo-escrito* and *R. graveolens*).

The results of Trial 4 are shown in Table 4. At 72 h, fluvalinate significantly decreased (P<0.05) the survival rate of *V. destructor* by up to 11%, but also negatively affected the survival of *A. mellifera* by up to 22%. A similar pattern was observed with 2 or 4 µL of *O. vulgare* essential oil. In contrast, exposure to thymol and 200 µL of hydrolates from *O. vulgare*, *P. albus*, and *T. diversfolia* reduced *V. destructor* survival without affecting *A. mellifera*.

In Trial 5, results show that at 72 h, fluvalinate effectively eliminated *V. destructor*, while thymol, 1.25 µL of *O. vulgare* essential oil, and 200 µL of *R. sphaerocarpa* flower hydrolate were similarly effective against the mite, without affecting bees (Table 5).

## DISCUSSION

Controlling *V. destructor* is crucial because this pest is a major barrier to sustaining a profitable beekeeping industry. One promising strategy involves the use of natural extracts obtained from plants. Topal et al. (2020) demonstrated

**Table 3. In vitro survival rates for *A. mellifera* and *V. destructor* following exposure to different aromatic plants.**

| | Survival Rates % | |
|---|---|---|
| **Treatment** | **Bees** | ***Varroa destructor*** |
| Without alcohol | 100.00a | 100.00a |
| 70° Alcohol | 91.67a | 100.00a |
| Thymol | 86.67ab | 42.33b |
| Fluvalinate | 66.67bc | 0.00 d |
| *O. vulgare* essential oil, µL | | |
| 1.25 | 100.00a | 6.67d |
| 2.5 | 58.33d | 3.67d |
| *O. vulgare* hydrolate, µL | | |
| 50 | 83.33b | 46.67b |
| 100 | 85.00b | 33.33c |
| 200 | 85.00b | 26.67c |
| *T. diversifolia* hydrolate, µL | | |
| 50 | 100.00a | 60.00b |
| 100 | 100.00a | 33.33c |
| 200 | 100.00a | 26.67c |
| *D. palo-escrito* hydrolate, µL | | |
| 50 | 85.00b | 60.00b |
| 100 | 78.33bc | 33.33c |
| 200 | 73.33cd | 26.67c |
| *R. graveolens* hydrolate, µL | | |
| 50 | 93.33a | 53.33b |
| 100 | 78.33b | 53.33b |
| 200 | 64.00b | 40.00b |
| SEM | 12.19 | 13.41 |

that aromatic plants can be used to treat bee diseases; however, it is important to consider that even natural products may exert toxic effects on honeybees. Our observations corroborated this risk: exposure to *O. vulgare* essential oil at concentrations of 5, 10 and 20 µL resulted in 100% bee mortality (Table 1). This effect is likely attributable to bioactive compounds such as thymol, carvacrol, or other terpenoids, which may interfere with the central nervous system of A. mellifera as the antennae and sensory-olfactory organs contain nervous receptors (Blenau et al., 2012). Consistent with our findings, Pohorecka (2004) reported that the application of *O. vulgare* essential oil produced honeybee mortality exceeding 80%. Additionally, Glavan et al. (2020), using carvacrol and thymol, evidenced that 5% carvacrol and 1% thymol resulted in 100 and 45% bee mortality, respectively. Albo et al. (2003) reported acute oral toxicity in adult honeybees caused by essential oils in vitro, with the highest mortality rate occurring with 5, 3, 2 and 8 µg per bee for savory, thyme, lemongrass, and oregano, respectively. However, Lin et al. (2019) found that even at the maximum dosage, plant oil induced only approximately 20% mortality in honeybees.

In Trial 2, survival rates for *A. mellifera* were not affected when the dosages of *O. vulgare* essential oil were reduced, although the acaricidal effect was observed until the 24 h time point. Even though this trial did not measure percentage mortality, a study by Romo-Chacon et al. (2016) found that the effectiveness of pure oregano essential oil at doses of 1.16 and 1.5 µL ranged between 57 and 74%, suggesting that oregano essential oil can be used as a viable alternative to acaricides in an effort to control *V. destructor*. Therefore, the use of *O. vulgare* hydrolates warrants consideration, as no significant differences were observed ($p>0.05$) with the essential oil since *A. mellifera* survived for up to 72 h, while V. destructor survival was reduced. This shows that hydrolates may act more slowly. Supporting this, Iglesias et al. (2022) reported that *Humulus lupulus* hydrolates can affect *V. destructor*, likely due to their chemical composition, such as β-caryophyllene and

**Table 4. In vitro survival rates for *A. mellifera* and *V. destructor* following exposure to *O. vulgare* essential oil and *O. vulgare*, *P. albus* and *T. diversfolia* hydrolates.**

| | % Survival Rates | | | | | |
|---|---|---|---|---|---|---|
| | *A. mellifera* | | | *Varroa destructor* | | |
| **Treatment** | **24 h** | **48 h** | **72 h** | **24 h** | **48 h** | **72 h** |
| Without alcohol | 89.66a | 78.00a | 78.00 a | 77.66a | 66.66ab | 66.66ab |
| 70° Alcohol | 89.00a | 89.00a | 89.00 a | 78.00a | 67.00ab | 61.33ab |
| Thymol | 80.66a | 69.66a | 69.66a | 66.66a | 66.66ab | 66.66ab |
| Fluvalinate | 77.66b | 22.00b | 22.00b | 11.00b | 11.00b | 11.00b |
| *O. vulgare* essential oil, μL | | | | | | |
| 1 | 89.00a | 89.00a | 89.00a | 41.66a | 33.33b | 33.33b |
| 2 | 78.00a | 33.33b | 33.33b | 11.00b | 11.00b | 11.00b |
| 4 | 38.66b | 30.33b | 30.33b | 0.00b | 0.00b | 0.00b |
| *O. vulgare* hydrolate, μL | | | | | | |
| 50 | 100.00a | 100.00 a | 100.00a | 89.00a | 55.66ab | 55.66ab |
| 100 | 100.00a | 78.00 a | 78.00a | 89.00a | 22.33b | 11.00b |
| 200 | 78.00a | 77.00 a | 77.00a | 55.66a | 22.00b | 11.00b |
| *P. albus* hydrolate, μL | | | | | | |
| 50 | 100.00 a | 100.00a | 100.00a | 67.00a | 44.33ab | 44.33ab |
| 100 | 100.00 a | 100.00a | 100.00a | 27.66b | 27.66b | 27.66b |
| 200 | 89.66a | 89.00a | 89.00a | 22.00b | 22.00b | 22.00b |
| *T. diversifolia* hydrolate, μL | | | | | | |
| 50 | 100.00a | 100.00a | 100.00a | 44.33a | 44.33ab | 33.00b |
| 100 | 100.00a | 100.00a | 100.00a | 55.66a | 55.66ab | 44.33ab |
| 200 | 89.00a | 89.00a | 89.00a | 22.33b | 22.33b | 16.66b |
| SEM | 9.60 | 10.41 | 10.71 | 15.00 | 15.94 | 14.52 |

[ab] Different letters indicate significant differences between rows. A t test ($p<0.05$) was used for comparison.

linalool, which contribute to mite inactivation without causing honeybee toxicity.

Among the components that destroy arthropods are monoterpenes, which are a component of the volatile essence present in both essential oils and hydrolates. This ability to affect mites is linked to neurotoxic activity through the inhibition of acetylcholinesterase and by acting antagonistically with octopaminergic receptors (Gimenez-Martinez et al. 2022). Products obtained from the hydrodistillation of aromatic plants at different concentrations have a less pronounced effect on bee physiology (Bava et al. 2023).

In Trial 3, a 100% survival rate was recorded among bees treated with O. vulgare essential oil at a concentration of 1.25 μL, as well as with *T. diversifolia* hydrolates at concentrations of 50, 100 and 200 μL. These values were significantly higher ($p < 0.05$) than those recorded for fluvalinate and O. vulgare essential oils at a concentration of 2.5 μL. Although various studies have focused on the effects of essential oils on arthropods, according to Park and Tak (2016), the mode of action of essential oils on insect pests involves neurotoxic effects, blocking of growth hormones, or killing by lipophilic oils. Neural effects were observed in both honeybees and *V. destructor* mites. Toxicity may also affect the respiration process of *V. destructor* mites, and some monoterpenes inhibit acetylcholinesterase (Umpierrez et al. 2011). It is possible that the volatile compounds obtained from plants, such as formic acid, affect the cellular respiratory chain of *V. destructor* mites (Vilarem et al. 2021).

Volatile organic compounds are widely used in nature by diverse organisms because of their roles in attraction, repellence, toxicity, and disorientation. The olfactory system is a biological sensor (Conchou et al. 2019) with a critical influence on parasite behavior, enabling *V. destructor* mite to recognize different developmental stages of honeybees (Ruffinengo et al. 2014). This parasite can differentiate between foragers and nurse bees solely through smell; thus, disrupting *V. destructor*'s orientation alters its behavior, providing a potential avenue

**Table 5. In vitro survival rates for *A. mellifera* bees and *Varroa destructor* following exposure to *O. vulgare* and *L. angustifolia* essential oils, and *P. albus*, *T. diversifolia*, *R. sphaerocarpa*, *Ruta graveolens*, *L. angustifolia* and *O. vulgare* hydrolates.**

| | Survival Rates (%) | | | | | |
|---|---|---|---|---|---|---|
| | *A. mellifera* | | | *Varroa destructor* | | |
| **Treatment** | **24 h** | **48 h** | **72 h** | **24 h** | **48 h** | **72 h** |
| Without alcohol | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a |
| 70% Alcohol | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a |
| Thymol | 100.00a | 100.00a | 100.00a | 0.00e | 0.00c | 0.00c |
| Fluvalinate | 88.88a | 11.11d | 0.00c | 0.00e | 0.00c | 0.00c |
| Essential oil, 1.25 µL | | | | | | |
| *O. vulgare* | 100a | 88.88ab | 88.88a | 11.11de | 0.00c | 0.00c |
| *L. angustifolia* | 100a | 88.88ab | 66.66ab | 44.44bc | 0.00c | 0.00c |
| Hydrolate, 200 µL | | | | | | |
| *R. sphaerocarpa* flower | 100a | 100a | 100a | 0.00e | 0.00c | 0.00c |
| *R. sphaerocarpa* leaf | 88.88a | 88.88ab | 77.77ab | 44.44bc | 0.00c | 0.00c |
| *R. sphaerocarpa* stem | 100a | 100a | 66.66ab | 66.66b | 0.00c | 0.00c |
| *R. sphaerocarpa* pod | 100a | 88.88ab | 55.55b | 33.33cd | 0.00c | 0.00c |
| *Ruta graveolen*s | 100a | 77.77bc | 77.77ab | 66.66b | 0.00c | 0.00c |
| *O. vulgare* | 66.66b | 66.66c | 66.66ab | 33.33cd | 0.00c | 0.00c |
| *L. angustifolia* | 100a | 100a | 66.66ab | 66.66b | 0.00c | 0.00c |
| *Populus alba* | 88.88a | 88.88ab | 33.33bc | 55.55bc | 44.44b | 33.33b |
| *Tithonia diversifolia* | 100.00a | 100.00a | 33.33bc | 0.00e | 0.00c | 0.00c |

for direct control. *V. destructor* mites may detect the volatile compounds that constantly move from one bee to another within a colony, and thus disorientation would interfere with their infestation strategy (Reams and Rangel, 2022).

In Trial 4, essential oils and hydrolates were shown to affect *V. destructor* at concentrations that were safe for honeybees under in vitro conditions. Pătruică et al. (2023) evaluated the in vitro effects of manuka, mint, oregano, litsea and cinnamon extracts on mite and honeybee mortality rates and demonstrated that each active substance must be carefully evaluated, as the phytochemical compounds contained within these substances may affect honeybees. In this sense, Trial 5 identified suitable concentrations that killed *V. destructor* while not compromising bee survival. This coincides with the findings of Neira et al. (2004), who evaluated the use of lavender and bay essential oils and reported a 100% detachment of mites from their host *A. mellifera*, with a 25% decrease in survival rates for *V. destructor* without affecting bee survival rates. However, numerous factors can influence treatment efficiency, including concentration, application method, treatment duration, and colony environmental conditions. Reducing the use of chemical substances is essential, as increasing *V. destructor* resistance also threatens bee survival rates. In this context, low concentrations of *O. vulgare* and *L. angustifolia* essential oils, as well as *R. sphaerocarpa* flower hydrolate, appear to be the most effective options against *V. destructor* without adversely affect bees. This study represents an exploratory research, proving a foundation for identifying novel compounds in hydrolates of some of the tested plants and, ultimately, proposing new strategies for *V. destructor* control.

## CONCLUSIONS

The essential oils and hydrolates, particularly *R. sphaerocarpa* flower and *T. diversifolia* hydrolates, and 4 µL of *O. vulgare* essential oil, show promise as alternative treatments for reducing *V. destructor* infestations. However, further research is needed to optimize delivery methods for safe and effective application within beehives.

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**Author contributions**

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