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# Biological activity of some plant extracts on *Varroa* mite (*Varroa destructor*) and Iranian honeybee (*Apis mellifera meda*)

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## ABSTRACT

Research and development of new natural and safe veterinary medicaments to treat honeybee (*Apis mellifera*) varroosis caused by *Varroa destructor* is an essential step on the path to embarking on organic beekeeping. One of the feasible alternative treatments being used for their control is plant extracts. This experiment was conducted to evaluate the acaricidal and insecticidal activity of extracts of *Ferula assa-foetida*, *Euphorbia serrata*, *Achillea millefolium*, and *Salvia rosmarinus* plants on *Varroa* mite and its host in honeybee colonies in Kurdistan province, Iran during 2021 to 2023. In this research, all the different concentrations of the studied plant extract caused mite mortality without severe harmful effects on worker bees (adults and brood). Based on the results of statistical analysis, the concentration of 50% extract of *F. assa-foetida* plant showed the most significant effect on the mortality percentage of *Varroa* mite (88.1 ± 1.3 %) in the studied honey bee colonies which its difference was significant compared to other different concentrations of studied plants extract ($p<0.01$). An acceptable safety margin for bees (eggs, larvae, adult workers, and queen) were observed after employing the extracts over a 20 days treatment period in the current experiment. Therefore, it is possible to recommend the concentration of 50% *F. assa-foetida* extract as a suitable alternative to synthetic acaricides to control the *Varroa* mites in the honeybee colonies.


**Keywords:** Acaricidal activity, Honeybee, Insecticidal activity, Plant extract, Varroa mite.

## INTRODUCTION

The honeybee (*Apis mellifera* L.) is one of the most important social and pollinating insects in the world, which plays a prominent role in pollination and providing food security to the people of the world (Rahimi *et al.,* 2023). The infestation of honeybee colonies by *Varroa* mite (*Varroa destructor* Anderson & Trueman, 2000) is one of the most pressing challenges and problems in the beekeeping industry in the world. This pest is active on both female and male

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brood as well as on adult honeybees. It causes much economic damage to beekeepers every year due to feeding on fat bodies and hemolymph of adult and brood bees, the transmission of viral pathogens and finally destroying the honeybee colony (Ramsey *et al.,* 2019; Noor *et al.,* 2020). On the other hand, the control of *Varroa* mites is complicated in honeybee colonies due to the increase in the cost of chemical control, the use of synthetic acaricides along with the problems of its ecological residues, and the emergence of resistance in the populations of this parasite. Therefore, this mite destroys a considerable part of honeybee colonies worldwide, including Iran every year.

Many studies have been conducted to control the *Varroa* mite in the world. By using chemical acaricides such as Apistan, Amitraz, Fulbex, etc., they have been able to prevent the outbreak of the *Varroa* mite population to some extent. However, recent reports indicated that the populations of this mite have become resistant to the mentioned synthetic acaricides and gradually faded their effect in controlling this mite (Bahreini *et al.,* 2025). In addition, it has caused harmful effects on humans, bees, and the environment, and despite these cases, undesirable residues of these synthetic acaricides have been reported in colony products, especially honey and wax (Radakovic *et al.,* 2014). The use of different synthetic acaricides to control the *Varroa* mite, especially in Iran, is a common and well-known method. Also in some areas, beekeepers rely on formic acid and oxalic acid to treat of varroosis in honeybee colonies. Despite the acceptable efficacy, the need for research and development of new, safer alternatives to these synthetic acaricides and organic acids is stressed. Natural products such as plant extracts offer a highly desirable alternative to synthetic acaricides and organic acids. Recently, researchers have recommended the use of plant extracts as an organic compound and a suitable alternative to chemical acaricides along with other non-chemical control methods in *Varroa* mite control (Damiani *et al.,* 2022; Rahimi and Parichehreh, 2024).

To date, extensive effort has been put in globally to discover a possible application of plant derivatives including extracts for varroosis treatment (Islam *et al.,* 2016; Ramzia *et al.,* 2017). Most investigations suggest that they may be a useful alternative in maintaining lower mite infestation rates in colonies. For example, Damiani *et al.* (2022) tested the biological activity of the ethanol extract of *Baccharis flabellate* (Asteraceae) and *Minthostachys verticillate* (Lamiaceae*)* plants on honeybees and *Varroa* mites in honeybee colonies. Their study showed that both of these extracts had a high mortality and repellent effect on the *Varroa* mite and were almost safe for honeybees. Therefore, these researchers suggested that these extracts can be a suitable alternative to synthetic acaricides in controlling of *Varroa* mites. In another study, Islam and coauthors (2023) investigated the effect of the ethanolic extracts of basil, garlic, lemon, lemongrass, and thyme plants in three concentrations of 200, 400, and 500 ppm to reduce *Varroa* mite damage in honeybee colonies. The results showed that the concentration of 500 ppm compared to other concentrations showed a much better control effect, and all the extracts were almost safe on honeybees.

The plants of *F. assa-foetida* (Apiaceae), *E. serrata* (Juglandaceae), *A. millefolium* (Asteraceae), and *S. rosmarinus* (Lamiaceae) are native to the Kurdistan province of Iran, and local beekeepers use these plants in the form of smoke to control *Varroa* mites in their apiaries. This is one of the main reasons for choosing the mentioned plants among the wide range of medicinal plants of Kurdistan province in the present study. Considering the high prevalence of varroosis in Iranian apiaries, the economic importance of its damage, the side effects and adverse effects of using synthetic acaricides to control this parasite, and on the other hand, the richness of some provinces of Iran including Kurdistan province in terms of medicinal plants effective in control this pest, the present study aims to evaluate the acaricidal and insecticidal activity of plant extracts of *F. assa-foetida*, *E. serrata*, *A. millefolium*, and *S. rosmarinus* on *Varroa* mite and its host in honeybee colonies in Kurdistan province, Iran.

## MATERIALS AND METHODS

### Date and location of the experiment

The current study was carried out from March 2021 to Novembre 2023 in the research apiary of the Kurdistan Agricultural and Natural Resources Research and Education Center (located in Garezeh Station, 35°16'18.8"N - 47°01'44.9"E), Kurdistan province, Iran. The mean ambient temperature and the average percentages of relative humidity recorded during this period were 15.94 ± 3 °C and 44.5 ± 8.5 %, respectively. Also, the mean ambient temperature and the average percentages of relative humidity recorded in 2023/08/23 to 2023/08/22 (extract spraying period on colonies (the experimental period)) were 25.11 ± 3 °C and 41.7 ± 4.7 %, respectively.

### Plant species and plant extract isolation

The plants of *F. assa-foetida* (Apiaceae), *E. serrata* (Euphorbiaceae), *A. millefolium* (Asteraceae), and *S. rosmarinus* (Lamiaceae) were collected from April to June 2021 in different geographic regions from Kurdistan province, Iran in the flowering stage and then identified. The aerial parts of the studied plants were dried separately in the shade and at room temperature (28°C and 45% relative humidity) and then crushed and powdered using a grinder.

The extract plants were separately isolated from the Soxhlet device (manufactured by Royan Teb Company, Iran) for eight hours. An average of 20 gr of powdered aerial parts of each plant was used for extracting each plant. In this study, absolute ethanol was used as a solvent. After extraction, a rotary device (manufactured by Royan Tab Company, Iran) was used to remove the solvent. The stock solutions of the extracts were stored in screw-capped dark glass vials and kept in the refrigerator at 4°C until the time of field experiments and chemical analysis. Before each time the extract spraying on the colonies during the experiment period, 20, 35, and 50% concentrations of each plant extract were prepared from the stock solutions of the extracts via $C_1V_1= C_2V_2$ formula and used on the same day.

**Setting up the honeybee colonies and experimental design**

Seventy honeybee (*Apis mellifera meda*) colonies infested with *Varroa* mite were selected in the different apiaries of Kurdistan province, Iran and transferred to Garezah station of the Kurdistan agricultural and natural resources research and education center of Iran. These colonies were manually equipped with a mobile bottom sticky plastic plate. The plastic plate was divided into 10 x 10 cm dimensions for easier counting of mites. Also, the surface of the plastic plate was coated with odorless grease. Each colony comprised seven combs of worker bees naturally infected by *V. destructor* and a one-year-old queen. These colonies were not treated with any acaricide or other *Varroa* control methods at least nine months before the start of the experiments. The experimental honeybee colonies were equalized in terms of queen age, population (adults and brood), and honey storage according to the instructions of Delaplane et al. (2013) before beginning the experiment. In addition, the initial infection rate of colonies with *Varroa* mite was evaluated using the guidelines of Dietemann *et al.* (2013) for adult bees and based on the guidelines of Zemene *et al.* (2015) for brood. This study was conducted in the form of a factorial experiment based on a completely randomized design with 14 treatments and five replications (five colony per treatment). The experimental treatments consisted of 20, 35 and 50% concentrations of extract of *F. assa-foetida*, *E. serrata*, *A. millefolium*, and *S. rosmarinus* plants and two control treatments (one as a positive control and the other as a negative control).

**Acaricidal and insecticidal activity bioassays**

**Assessment of varroacidal activity**

In this study, seventy Langstroth colonies, manually equipped with movable bottom sticky white plastic plates, were used. The concentration and values of the plant extract were determined according to our initial field experiments in different apiaries of Kurdistan province during 2021 and 2022. Field experiments were carried out at the beginning of September 2022. Experimental colonies were treated with 20, 35 and 50% concentrations of plant extract at sunset when all bees were present in the colony. In this study, 15 ml of 20, 35, and 50% concentrations of the extract of each plant were sprayed on bees and mites on all frames of each colony. Apistan anti-*Varroa* mite strip was used (one strip inside each hive) in the positive control treatment and water in the negative control treatment. The plastic sheet from the bottom of the colonies was removed every five days at 10 am, and the number of dead bees and mites on them was counted. The experimental treatments were treated every five days with different concentrations of each plant extract. In total, 60 ml was applied over 20 days. In the positive control, the Apistan strip was replaced every five days, and the negative control treatment was sprayed with water every five days. To evaluate the number of remaining mites at the end of the experiment, two strips of Apistan were placed inside each colony for two weeks. The percentage mortality of the treatments was determined based on Adam and coauthors formula (Adam et al., 2013):

$$\%\ \text{Mortality} = \frac{\text{No.of fallen mites during treatment}}{\text{No.of fallen mites during treatment+No.of deat mite after treatment with Apistan}} \times 100$$

Due to considerable natural mite fall in the control colonies, the mortality of the treatments was corrected using Abbott's formula (Abbott, 1925):

$$\%\ \text{Corrected mortality} = \frac{Mo-Mc}{100-Mc} \times 100$$

Where, Mo= observed mortality (%), and Mc= control mortality (%).

**Assessment of insecticidal activity**

The effect of different concentrations of each plant extract on the population growth rate of bees was also evaluated in the current study.

**Effects on brood and adult bee population**

**Mortality effects on eggs, larvae, adult workers, and queen bees**

To carry out the procedure, 100 cells of each colony in the brood area containing eggs or 1- to 2-day-old larvae were marked by colored pins before starting the experiment. After seven days, the cells were checked. The sealed cells or those with larvae at last larval instar were considered alive, while in the case of the empty cells or ones replaced with a new egg, the brood was considered dead (Giusti *et al.,* 2017). In addition, the number of dead bees found in pollen traps or inside the colonies and the presence of an egg-laying queen in each colony were checked every five days over the entire treatment period. The average effect of different concentrations of extracts of each plant on the population of adults and brood was recorded as the effect of different concentrations of extracts of each plant for each colony. The percentage of bee mortality in each experimental treatment was evaluated based on the formula of Adam et al. (2013). Also, the bee mortality of the each treatments was corrected using Abbott's formula (Abbott, 1925).

## Identification of active ingredients of *F. assa-foetida* extract

GC analysis was performed on an Agilent Technologies model TRACEMS gas chromatograph equipped with an FID and a BP-5 (non-polar) capillary column (60 m × 0.25 mm × 0.25 µm film thickness). The oven temperature was kept at 60 °C for 4 min and planned to increase at 5 °C/min to 100 °C for 3 min and then 4 °C/min to 250 °C for 5 min. Other operating conditions were as follows: carrier gas He, at a flow rate of 1.1 mL/min; injector temperature 260 °C; detector temperature 245 °C; and split 50.1, column flow ratio, 1:8 mL/min. GC/MS analysis was performed on an Agilent Technologies model TRACEMS coupled with a mass spectroscopy model FI 5875C. The operating conditions of the mass system were the same as those described already. The mass spectra were obtained at 70 eV. The mass range was from m /z 50– 500 amu. Quantitative data were obtained from the electronic integration of the FID peak areas. The active ingredients of each plant extract were identified by comparison of their mass spectra and retention indices with those published in the literature (Adams, 2007), and presented in the MS computer library.

## Data analysis

In this study, the normality of the data distribution was tested by the Kolmogorov Smirnov test using the univariate procedure in SAS 9.4 M6 statistical software. After ensuring the normality of the data, the data were analyzed based on the factorial analysis based on a completely randomized design based on the GLM procedure embedded in the statistical software SAS V. 9.4. In addition, the means comparison was done by the least significant difference (LSD) method using SAS software ($p<0.01$).

## Varroacidal activity

## Variance analysis and means comparison

Variance analysis results of acaricidal activity of different concentrations of extract of *F. assa-foetida*, *E. serrata*, *A. millefolium*, and *S. rosmarinus* plants on the percentage of *Varroa* mite mortality in the studied honeybee colonies are presented in Table 1. The results showed the effect of the studied plants extract, different concentrations of the extract of each plant, the number of times of extract spraying, the interaction effect of plants extract × concentrations, the interaction effect of plants extract × the number of times extract spraying, the interaction effect of concentrations × the number of times of extract spraying × the interaction effect of plants extract, concentrations and the number of spraying times on the percentage of *Varroa* mite mortality in the studied bee colonies were significant ($p<0.01$). All different concentrations of plants extract especially *F. assa-foetida* extract indicated significantly higher levels of mortality than the negative control. The percentage of mortality significantly increased with increasing extract concentration in all treatments. Mean comparison results of the acaricidal activity of different concentrations of plants extract on the percentage of *Varroa* mite mortality in the honey bee colonies studied based on the LSD method are shown in Table 2. Based on the obtained results, the highest percentage of mortality on *Varroa* mite is related to the first time of spraying with a 50% concentration of *F assa-foetida* plant extract (88.1 ± 1.3%), and the lowest percentage of mortality on this parasite in the studied honey bee colonies is related to the negative control treatment (2%).

**Table 1.** Variance analysis of the acaricidal activity effect of different concentrations of the studied plants extract on the mortality percentage of *Varroa* mite in the studied honeybee colonies.

| Variations Sources | df | Sum of Squares | Mean of Squares | F | P-Value |
|---|---|---|---|---|---|
| Extract of plants | 3 | 56801.12 | 18241.41 | 213.30 | 0.0001 |
| Concentrations | 2 | 36210.12 | 17950.25 | 205.14 | 0.0001 |
| Plant extract × concentrations | 6 | 2511.35 | 410.21 | 5.1 | 0.0002 |
| Number of times of spraying | 3 | 263200.15 | 88001.14 | 1001.45 | 0.0001 |
| Plant extract ×number of times of spraying | 9 | 29002.37 | 3200.95 | 36.2 | 0.0001 |
| Concentrations ×number of times of spraying | 6 | 7520.11 | 1311.10 | 15.3 | 0.0391 |
| Plant extract× concentrations ×number of times of spraying | 18 | 7421.41 | 410.42 | 5.1 | 0.003 |

**Table 2.** Comparison of means of effect acaricidal activity of different concentrations of the studied plants extract on the percentage of *Varroa* mite mortality in studied bee colonies based on the LSD method (Mean ± SD).

| Extracts of plants | Concentrations | Number of times of spraying | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| *F. assa-foetida* | 20 % | 33.5 ± 1.48$^{cd}$ | 21.5 ± 0.82$^{ij}$ | 18.5 ± 1.26$^{pqrs}$ | 17 ± 1.11$^{stu}$ |
| | 35 % | 64.7 ± 0.77$^{a}$ | 31 ± 1.41$^{def}$ | 28.5 ± 0.82$^{lmno}$ | 25.4 ± 1.12$^{nopq}$ |
| | 50 % | 88.1 ± 1.3$^{a}$ | 37.5 ± 1.49$^{cd}$ | 33 ± 2.12$^{klmn}$ | 27.5 ± 1.17$^{mnopq}$ |
| *A. millefolium* | 20 % | 17.1 ± 1.30$^{ij}$ | 12.5 ±1.26$^{rstu}$ | 10.1 ± 1.30 $^{tu}$ | 8.5 ±0.12$^{vw}$ |
| | 35 % | 24.1 ± 1.18 $^{g}$ | 13.7 ± 1.18$^{nopq}$ | 11 ± 1.11$^{rstu}$ | 9.1 ± 1.17 $^{uv}$ |
| | 50 % | 28.4 ± 0.12$^{efg}$ | 14.5 ±1.46$^{mnopq}$ | 12.5 ± 1.22 $^{rstu}$ | 10.6 ±2.10$^{tu}$ |
| *S. rosmarinus* | 20 % | 31.1 ± 1.02$^{h}$ | 21.5 ±1.17$^{jk}$ | 15.5 ± 1.86$^{pqrs}$ | 11.5 ±0.86$^{tu}$ |
| | 35 % | 35.1 ±1.01$^{fg}$ | 24.5 ± 1.48$^{hi}$ | 17 ± 1.04 $^{jklm}$ | 13 ±0.4$^{lmnop}$ |
| | 50 % | 42 ± 0.4$^{fg}$ | 27.1 ± 1.25$^{de}$ | 20.5 ±2.18$^{jkim}$ | 15.1 ±2.13 $^{lmnop}$ |
| *E. serrata* | 20 % | 40.4 ±1.42$^{h}$ | 29.1 ± 0.52 $^{nopq}$ | 18 ± 1.01 $^{rstu}$ | 15.4 ± 1.43 $^{uv}$ |
| | 35 % | 47.5 ± 0.82$^{bc}$ | 33.1 ± 1.04$^{jklm}$ | 21.5 ±1.46 $^{opqr}$ | 17.4 ± 1.13 $^{rst}$ |
| | 50 % | 51.1 ± 2.01 $^{bc}$ | 36.5 ± 2.43$^{jkl}$ | 23.5 ± 1.47 $^{qrs}$ | 19.6 ± 2.3$^{pqrs}$ |
| Control (positive) | | 91.1 ± 8.18$^{a}$ | 41.8 ± 4.89$^{h}$ | 35.5 ± 5.19 $^{rst}$ | 21.2 ± 2.19 $^{w}$ |
| Control (negative) | | 2 ± 0.14$^{x}$ | 2 ± 0.4 $^{x}$ | 2.1 ± 0.28$^{x}$ | 2 ± 0.54 $^{x}$ |

* Means with at least one letter in common have no significant difference at the 5% probability level based on the LSD test.

## Insecticidal activity

### Variance analysis and means comparison

In this research, no queen losses were observed in any of the experimental treatment replicates during project implementation. Variance analysis results of the insecticidal activity of different concentrations of extract of *F. assa-foetida*, *E. serrata*, *A. millefolium*, and *S. rosmarinus* plants on the percentage of bees' mortality in the studied honeybee colonies are presented in Table 3. The results showed the effect of the studied plants extract, different concentrations of the extract of each plant, the number of times of extract spraying and the interaction effect of plants extract × concentrations at the 1% probability level and the interaction effects plants extract × concentrations × the number of times of extract spraying at the 5% probability level was significant. Nevertheless, there was no significant difference between the interaction effects of different concentrations of plants extract × the number of spraying times on the percentage of bee mortality in this study. The results showed that with the increase in the concentration of plants extract in the experimental treatments, the percentage of bee mortality increased in the experiment treatments. However, bee mortality was not significant in contrast with the previous experiment *Varroa* mortality. Means comparison results of the insecticidal activity effect of different concentrations of plants extract on the percentage of bee mortality in the studied honeybee colonies based on the LSD method are given in Table 4. Based on the results, the highest percentage of mortality in bees was related to the fourth time of spraying of 50% concentration of *E. serrata* plant extract (8.5 ± 0.27 %), and the lowest percentage of mortality in bees in the studied honeybee colonies was observed in the negative control treatment (0.2%).

**Table 3.** Variance analysis of the insecticidal activity effect of different concentrations of the studied plants extracts on the mortality percentage of bees in the studied honeybee colonies

| Variations Sources | df | Sum of Squares | Mean of Squares | F | P-Value |
|---|---|---|---|---|---|
| Extract of plants | 3 | 246.8 | 82.7 | 37.53 | 0.0001 |
| Concentrations | 2 | 1375.25 | 687.65 | 311.50 | 0.0001 |
| Plant extract × concentrations | 6 | 65.1 | 11.5 | 5.7 | 0.0001 |
| Number of times of spraying | 3 | 69.51 | 23.75 | 11.30 | 0.0001 |
| Plant extract ×number of times of spraying | 9 | 135.5 | 15.75 | 7.1 | 0.0001 |
| Concentrations ×number of times of spraying | 6 | 31.17 | 6.01 | 3.5 | 0.0401 |
| Plant extract× concentrations ×number of times of spraying | 18 | 84.4 | 5.71 | 2.18 | 0.003 |

**Table 4.** Comparison of means of the insecticidal activity effect of different concentrations of the studied plants extract on the percentage of bee mortality in studied bee colonies based on the LSD method (Mean ± SD).

| Extracts of plants | Concentrations | Number of times of spraying | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| | 20 % | 3.1 ± 0.27$^{lmnop}$ | 1.3 ± 0.30$^{pqrs}$ | 2.9 ± 0.40$^{lmnopq}$ | 0.9 ± 0.51$^{rs}$ |
| *F. assa-foetida* | 35 % | 6.1 ± 0.22$^{efgh}$ | 5.1 ± 0.20$^{hjk}$ | 3.1 ± 0.28 $^{lmnop}$ | 3 ± 0.50$^{jkm}$ |
| | 50 % | 7.1 ± 0.30$^{abc}$ | 7 ± 0.16$^{defg}$ | 7 ± 0.573$^{defg}$ | 7.1 ± 0.27$^{a}$ |
| | 20 % | 0.5 ± 0.14$^{s}$ | 0.4 ± 0.05$^{s}$ | 0.4 ± 0.14$^{s}$ | 0.5 ± 0.27$^{rs}$ |
| *A. millefolium* | 35 % | 2.8 ± 0.54$^{lmnopq}$ | 1.1 ± 0.27$^{qrs}$ | 0.9 ± 0.11$^{rs}$ | 1.5 ± 0.07$^{nopqrs}$ |
| | 50 % | 5.2 ± 0.88$^{efghi}$ | 4.4 ± 0.27$^{ijkl}$ | 3.4 ± 0.20$^{jklm}$ | 4.1 ± 0.26$^{hijkl}$ |
| | 20 % | 0.6 ± 0.64$^{s}$ | 0.7 ± 0.07$^{rs}$ | 0.4 ± 0.34$^{s}$ | 0.9 ± 0.21$^{rs}$ |
| *S. rosmarinus* | 35 % | 1 ± 0.41$^{rs}$ | 2.4 ± 0.27$^{mnopqrs}$ | 1.4 ± 0.50$^{opqrs}$ | 1.2 ± 0.24$^{pqrs}$ |
| | 50 % | 6.5 ± 1.42$^{cdef}$ | 8.1 ± 1.29$^{abcd}$ | 7.1 ± 0.11$^{cdef}$ | 5 ± 0.14$^{fghi}$ |
| | 20 % | 3.3 ± 0.22$^{jklmn}$ | 1.1 ± 0.24$^{pqrs}$ | 0.4 ± 0.20$^{rs}$ | 0.9 ± 0.4$^{rs}$ |
| *E. serrata* | 35 % | 7 ± 1.55 $^{bcde}$ | 0.7 ± 0.24$^{s}$ | 0.7 ± 0.34$^{s}$ | 1.2 ± 0.34$^{pqrs}$ |
| | 50 % | 8.5 ± 0.27$^{ab}$ | 1.3 ± 1.49$^{pqrs}$ | 1.2 ± 1.29$^{pqrs}$ | 4.5 ± 1.2$^{ghij}$ |
| Control (positive) | | 2.5 ± 0.01$^{mnopqr}$ | 1.2 ± 0.84$^{pqrs}$ | 1.2 ± 0.30$^{pqrs}$ | 1.2 ± 0.50$^{opqrs}$ |
| Control (negative | | 0.3 ± 0.34$^{s}$ | 1.1 ± 0.17$^{qrs}$ | 1.4 ± 0.21$^{opqrs}$ | 0.2 ± 0.24$^{s}$ |

*Means with at least one common letter in common have no significant difference at the 5% probability level based on the LSD test.

## The number of times of spraying on mortality

The effect of the number of times of spraying the extract of the studied plants on the mortality percentage of *Varroa* mite is presented in Figure 1. Based on the obtained results, the percentage of *Varroa* mite mortality in the present study decreases with the increase in the number of spraying times.

## Active ingredients of the *F. assa-foetida*

The extract of *F. assa-foetida* plant caused the highest mortality percentage on *Varroa* mite in the present study, and its difference with the extracts of other plants was significant. Therefore, only its active ingredients are mentioned here. The results of the chemical analysis of the *F. assa-foetida* extract are presented in Table 5. The results showed that E-1-propenyl sec-butyl disulfide (25.38 %), Z-1-propenyl sec-butyl disulfide (17.37 %), n-propenyl sec butyl disulfide (15.54 %), Guaiol (11.99%), and β-pinene (10.69 %) were the major active ingredients of *F. assa-foetida* extract in present study.

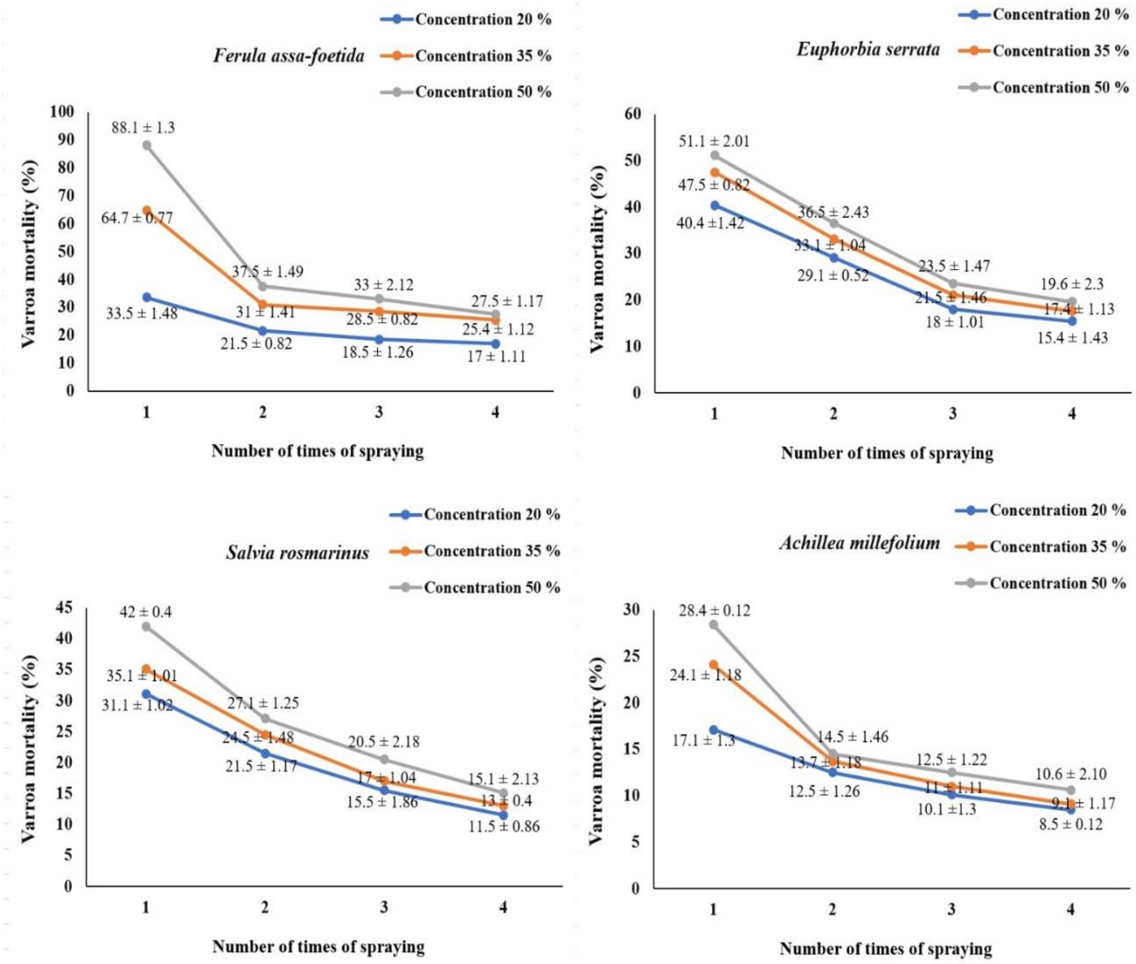


**Figure 1:** The effect of the number of times of spraying the extract of the studied plants on the mortality percentage of *Varroa* mite (Mean ± SD).

**Table 5.** Active ingredients of *F. assa-foetida* plant extract in the present study.

| no | Component | Retention indices | Percentage |
|---|---|---|---|
| 1 | 1,1,3-Triethoxypropane | 668 | 0.11 |
| 2 | 1.2,3-Dihydroxypropanal | 702 | 0.13 |
| 3 | α-pinene | 761 | 1.69 |
| 4 | 3,4-Dimethylthiophene | 777 | 0.13 |
| 5 | 5,5-Dimethylpyrazolidin3-one | 803 | 0.07 |
| 6 | 3-methyl Nonane | 833 | 0.11 |
| 7 | (5E)-4,4-Dimethyl-5-octenal | 861 | 0.99 |
| 8 | 2-Dimethyl(prop-2-enyl) silyloxydodecane | 917 | 1.57 |
| **9** | **β-pinene** | **925** | **10.69** |
| 10 | Tricyclene | 928 | 0.59 |
| 11 | Hexadecane | 959 | 0.76 |
| 12 | Myrcene | 983 | 1.86 |
| 13 | â-copaene | 989 | 0.58 |
| 14 | á-acorenol | 995 | 0.61 |
| 15 | Decane | 999 | 0.12 |
| 16 | α-terpinolene | 1002 | 0.67 |
| 17 | α-ohellandrene | 1003 | 0.95 |
| 18 | Limonene | 1029 | 1.54 |
| 19 | Z-β-Ocimene | 1042 | 1.6 |
| 20 | E-β-Ocimene | 1052 | 0.52 |
| 21 | Epicubebol | 1067 | 1.06 |
| 22 | a-terpinene | 1071 | 0.1 |
| 23 | 3-methyl Nonane | 1112 | 0.58 |
| 24 | 2-methylene | 1113 | 1.58 |
| 25 | Di-sec-butyl-disulfide | 1211 | 0.59 |
| 26 | Thymol-methyl-ether | 1232 | 0.21 |
| **27** | **n-propenyl sec-butyl disulfide** | **1244** | **15.54** |
| **28** | **Z-1-propenyl sec-butyl disulfide** | **1249** | **17.37** |
| **29** | **E-1-propenyl sec-butyl disulfide** | **1251** | **25.38** |
| 30 | Carvacrol | 1260 | 1.45 |
| 31 | €-3-Tetradecene | 1392 | 0.19 |
| 32 | Selin-4, 7(11)-diene | 1452 | 0.94 |
| 33 | α-Humulene | 1454 | 0.63 |
| 34 | Cadina-4-diene | 1520 | 0.57 |
| 35 | β-Himachalene | 1537 | 0.44 |
| 36 | δ-Cadinene | 1558 | 0.58 |
| 37 | Spathulenol | 1579 | 0.12 |
| 38 | Hexadecane | 1603 | 0.29 |
| **39** | **Guaiol** | **1642** | **11.99** |

## DISCUSSION

Considering the high prevalence of Varroosis in apiaries of Iran, the economic importance of its damage, the adverse effects of using chemical acaricides to control *Varroa* mite, and helping beekeepers select safe *Varroa* mite control alternatives, we evaluated the acaricidal and insecticidal activity of native plants extract of Kurdistan province on *Varroa* mite and its host in honeybee colonies in the climatic conditions of Kurdistan province, Iran. In general, the results showed that all the different concentrations of plants extract in the present study had favorable acaricidal activities and a significant effect on mite mortality as compared to the negative control ($p<0.01$). Based on the obtained results, the concentration of 50% of *F. assa-foetida* extract at the first time of spraying showed the most significant effect on the percentage of *Varroa* mite mortality in the studied bee colonies, whose difference was significant compared to other treatments ($p< 0.01$). The percentage of bee mortality in none of the experimental treatments was more than eight percent. Therefore, a 50% concentration of *F. assa-foetida* extract can be recommended to beekeepers as a suitable alternative to synthetic acaricides for *Varroa* mite control in honey bee colonies. Shaddel Tilly *et al*. (2008) investigated the extract of tobacco, pecan and mountain thyme plants to *Varroa* mite control in honeybee colonies in field conditions. Their study showed that thymus extract had the greatest effect on *Varroa* mite control and the least negative effect on bees. These researchers in line with the result of the present study recommended the use of plants extract, especially thymus plant extract, as a suitable alternative to chemical acaricides to control *Varroa* mites in honey bee colonies. Ramzanpour and Ykhchali, (2020) investigated the effect of *F. assa-foetida* (collected *F. assa-foetida* from eastern Iran) extract in controlling *Varroa* mite in honey bee colonies by injection method between frames. Based on the obtained results, the extract of *F. assa-foetida* showed high mortality in *Varroa* mite compared to the control group. In line with the results of the present research, these researchers reported that the extract of *F. assa-foetida* can play an important role in controlling *Varroa* mite in honeybee colonies.

The GC and GC/MS results proved that five components including E-1-propenyl sec-butyl disulfide, Z-1-propenyl sec-butyl disulfide, n-propenyl sec-butyl disulfide, Guaiol and β-pinene are the main components in *F. assa-foetida* extract. These components comprise 80.97% of the components involved in the test *F. assafoetida* extract. These results were in accordance with those reported by Ramzanpour and Ykhchali (2020), and Farhadi *et al*. (2020). In a study, Ramzanpour and Ykhchali (2020) reviewed the efficacy and safety of *F. assa-foetida* extract components for treating honey bee varroosis. 1E-1-propenyl sec-butyl disulfide, the most abundant active ingredient of the *F. assafoetida* extract, or plant extract rich in this component has already been proven effective against *Varroa* mite. Also, the extract of this plant has been tested on agricultural pests such as *Acythopeus curvirostris persicus*, mites, *Spectrobates ceratoniae* and whitefliesand has effectively controlled them (Tavassoli *et al.,* 2021). Therefore, the high efficacy of our test *F. assa-foetida* could be attributed to the high potential of its major components.

Control of *V. destructor* population in honeybee colonies requires that treatments show an acceptable acaricidal activity without side effects on honey bees, and leave no or minimal residues in honey and wax with a margin of safety for the consumer. Adamczyk *et al.* (2005) reported that the presence of residues of plant extract components in honey samples does not represent a hygienic risk or a risk for human health, it only may change the taste of the honey. The plant extracts used in the present study, especially *F. assa-foetida* extract, did not show adverse effects on bees and colony products despite having high acaricidal activities. Therefore, *F. assa-foetida* extract can be considered as a suitable alternative to chemical acaricides due to its high acaricidal activities.

The researchers reported that several factors contribute to the overall efficacy of any acaricide. The concentration of the compounds used, the length of the treatment, the method of application, the colony environment, the apiary environment and the ambient temperature can all affect the effectiveness of the treatment (Calderone and Spivak, 1995). In this research, the acaricidal and insecticidal activity of the studied plant extract was investigated at the end of summer, by spray method and three different concentrations. Therefore, it is suggested to conduct more studies on *F. assa-foetida* extract to achieve more accurate results.

## CONCLUSIONS

In this research, the acaricidal and insecticidal activity of concentrations of 25, 35 and 50% of native plants extract (*F. assa-foetida*, *E. serrata*, *A. millefolium*, and *S. Rosmarinus* plants) of Kurdistan province, Iran on *Varroa* mite and its host in bee colonies were evaluated by spray method. Based on our findings, the concentration of 50% *F. assa-foetida* extract showed the most significant effect on the percentage of *Varroa* mite mortality (88.1%) in bee colonies ($p<0.01$). The mortality percentage of different concentrations of studied plant extracts on bees was not more than eight percent in any experimental treatments. Therefore, it is possible to recommend the concentration of 50% *F. assa-foetida* extract as a suitable alternative to synthetic acaricides to control the *Varroa* mites in the honeybee colonies.

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## REFERENCES

1. Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18 (2):** 265-267.
2. Adams, R.P. 2007. Identification of essential oil components by gas chromatography/mass spectrometry. 4th edition, Allured Publishing Corporation, Carol Stream, USA.

3. Adamczyk, S., Lázaro, R., Pérez-Arquillué, C., Conchello, P. and Herrera, A. 2005. Evaluation of residues of essential oil components in honey after different anti-Varroa treatments. *J. Agric. Food Chem*., **53 (26):** 10085–10090.
4. Damiani, N., Liesel, B., Matías, D., Marcangeli, A. and Eguaras, J. 2022. Repellent and acaricidal effects of botanical extracts on *Varroa destructor*. *Parasitol. Res.*, **108 (1):** 79–86.
5. Delaplane, K.S., Van der Steen, J. and Guzmán-Novoa, E. 2013) Standard methods for estimating strength parameters of *Apis mellifera* colonies. *J. Apic. Sci.,* **52 (1):** 1-12.
6. Dietemann, V., Nazz, F., Martin, S.J., Anderson, D.L., Locke, B., Delaplane, K.S., Wauquiez, Q., Tannahill, C., Frey, E., Ziegelmann, B., Rosenkranz, P. and Ellis, J.D. 2013. Standard methods for *Varroa* research. In: Dietemann V, Ellis JD, Neumann P (Eds) The COLOSS BEEBOOK, volume II: standard methods for *Apis mellifera* pest and pathogen research. *J. Apic. Res.*, **52 (1):** 1-54.
7. Farid, P., Ghasemi, Y., Gholami, A., Mehregan, I. and Mohagheghzadeh, A. 2008. Antimicrobial essential oil from Smyrniopsis aucheri. *Chem Nat Compd*, **44 (1):** 116-118.
8. Giusti, M., Sabelli, C., Di, Donato, A., Lamberti, D., Paturzo, C.E., Polignano, V., Lazzari, R. and Felicioli, A. 2017. Efficacy and safety of Var terminator, a new formic acid medicine against the *Varroa* mite. *J. Apic. Res.*, **56 (13):** 162-167.
9. Islam, N., Amjad, M., Haq, E. and Naz, F. 2023. Comparative field efficiency of the extracts of plant materials for controlling *Varroa destructor* about brood development in honey bee (*Apis mellifera*) colonies. *IJB*, **16 (1):** 126-138.
10. Islam, N., Amjad, M., Ehsan-ul-Haq, M., Stephen, E. and Naz, N. (2016). Management of *Varroa destructor* by essential oils and formic acid in *Apis mellifera* Linn. colonies. *J. Entomol. Zool. Stud.,* **4 (8):** 97-104.
11. Noor, I., Muhammad, A., Ehsan, U. and Falak, N. 2020. Comparative field efficiency of the extracts of plant materials for controlling *Varroa destructor* about brood development in honey bee (*Apis mellifera*) colonies. *IJB*, **16 (1):** 126-138.
12. Radakovic, M., Stevanovic, J., Djelic, N., Lakic, N., Knezevic-Vukcevic, J., Vukovic-Gacic, B. and Stanimirovic, Z. 2013. Evaluation of the DNA damaging effects of amitraz on human lymphocytes in the Comet assay. *J. Biosci.*, **38(1):** 53-62.

13. Rahimi, A. and Pariichehreh, S. 2024. Evaluation and introduction of a new plant-based formulation to control Varroa mite (*Varroa destructor*) in honey bee colonies (*Apis mellifera*). *J Entomol Soc Iran*, **44 (4):** 417-428.
14. Rahimi, A., Kahrizi, D., Mirmoayedi, A., Zarei, L. and Jamali, S. 2023. Genetic Characterizations of the Iranian Honey Bee (*Apis mellifera meda* Skorikov 1929) Populations Using the Microsatellite DNA Markers. *Biochem Genet,* **61(6):** 2293-2317.
15. Rahimi, A., Mirmoayedi, A., Kahriz, D., Zarei, L. and Jamali, J. 2022. Molecular genetic diversity and population structure of Iranian honey bee (*Apis mellifera meda*) populations: Implications for breeding and conservation. *J Plant Dis Prot*, **129:** 1331–1342.
16. Ramsey, S.D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J.D., Mowery, A., Lim, D., Joklik, J., Cicero, J.M., Ellis, J.D., Hawthorne, D. and vanEngelsdorp, D. 2019. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proc Natl Acad Sci U S A,* **16 (5):** 1792-1801.
17. Ramzanpour, A. and Ykhchali, M. 2020. Study on the effect of *Ferula pseudalliacea* (Family: Apiacea) extract on *Varroa destructor* (Acari: Varroidae) infestation in honeybee (Hymenoptera: Apidae, *Apis melifera*) by injection method. *Iran. J. Vet. Res.,* **15 (4):** 85-92.
18. Sammataro, D., Degrandi-Hofman, G., Needham, G. and Wardell, G. 1998. Some volatile plant oils as potential control agents for *Varroa* mites (Acari: Varroidae) in honey bee colonies (Hymenoptera: Apidae). *American bee journal,* **138(9):** 681–685.
19. Sanda, K., Koba, K., Akpagana, K. and Tchepan, T. 2001. Teneur et compostion chimique de l´huile essentielle de Ocimum basilicum L. et Ocimum gratissicum L. a diferentes periodes de recolte apres semis. *Rivista italiana EPPOS*, **31 (2):** 3–7.
20. Shaddel Tilly, A., Mahri Sis, N., Aghajanzadeh Golshani, A., Asadi Disney, A. and Ahmadzadeh, A. 2008. Investigating the effect of using tobacco, pecan and mountain thyme plants in controlling honey bee *Varroa* mite. *J. Agric. Sci.*, **4 (2):** 67-72.
21. Tavassoli, M., Ghanbarpoor, L. and Shamsi, L. 2021. Study of pesticide effects of *Matricaria chamomilla* extract on *Argas persicus* Ticks. *J. Vet. Res.,* **73 (2):** 135-139.
22. Zemene, M., Bogale, B., Derso, S., Belete, S., Melaku, S. and Hailu, H. 2015. A review on *Varroa* mites of honey bees. *AJE,* **8 (3):** 150-159.

# بررسی فعالیت بیولوژیکی تعدادی از عصاره گیاهان روی کنه‌واروآ (*Varroa destructor*) و زنبورعسل ایرانی (*Apis mellifera meda*)

**عطااله رحيمي، روخوش راشد، و بناز عبداله**

**چکیده**

تحقیق و توسعه داروهای دامپزشکی طبیعی و امن برای درمان بیماری واروآزیس زنبورعسل (*Apis mellifera*) ناشی از کنه *Varroa destructor*، گامی اساسی در مسیر شروع به کار زنبورداری ارگانیک است**.** یکی از درمان‌های جایگزین ممکن برای کنترل کنه واروآ، عصاره‌های گیاهی است. این آزمایش به منظور بررسی فعالیت کنه‌کشی و حشره‌کشی عصاره‌های گیاه *Ferula assa-foetida*، *Euphorbia serrata*، *Achillea millefolium* و *Salvia rosmarinus* روی کنه‌واروآ و میزبان آن در کلنی‌های زنبورعسل در استان کردستان طی سال‌های 1401تا 1402 انجام شد. در این تحقیق تمام غلظت‌های مختلف عصاره گیاهان مورد مطالعه باعث مرگ کنه‌ها بدون اثرات مضر شدید بر زنبورهای عسل (بالغ و نوزادان) شد. بر اساس نتایج تجزیه و تحلیل آماری، غلظت 50 درصد عصاره گیاه *Ferula assa-foetida* بیشترین تأثیر را بر درصد تلفات کنه واروآ (1/3 ± 88/1 %) در کلنی‌های زنبور عسل نشان داد که تفاوت آن در مقایسه با سایر غلظت‌های مختلف عصاره گیاهان مورد مطالعه معنی‌دار بود (p <0/01). حاشیه ایمنی قابل قبولی برای زنبورها (تخم‌ها، لاروها، کارگران بالغ و ملکه) پس از استفاده از عصاره‌ها در یک دوره درمان 20 روزه در آزمایش فعلی مشاهده شد. بنابراین می‌توان غلظت 50 درصد عصاره *Ferula assa-foetida* را به عنوان جایگزین مناسب برای کنه کش‌های مصنوعی برای کنترل کنه‌های واروآ در کلنی های زنبور عسل توصیه کرد.