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Varroacidal efficacies of essential oils extracted from *Lavandula officinalis*, *Foeniculum vulgare*, and *Laurus nobilis* in naturally infested honeybee (*Apis mellifera* L.) colonies

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Abstract: This study was performed to determine the efficacies of *Lavandula officinalis*, *Foeniculum vulgare*, and *Laurus nobilis* essential oils on the control of *Varroa destructor* (Acari: Varroidae), the most common parasite of honeybees (*Apis mellifera* L.). Experimental colonies from the province of Bursa, Turkey, were divided into 5 homogeneous groups of 8 hives each. The 3 essential oils plus thymol (Thymovar®, an essential oil-based drug with known efficacy that was used for comparison) were applied to 1 group each and 1 group was left untreated as a control. Each group was treated during 3 seasons, 2 consecutive autumns and 1 spring. Efficacies of the essential oils were determined by the Henderson-Tilton formula and the significance between the oils was determined by Tukey's multiple comparison test. In the first autumn, the highest efficacy was detected in Thymovar (79.4%) followed by *L. nobilis* (76.7%), *L. officinalis* (76.4%), and *F. vulgare* (74.5%) essential oils. In spring, the efficacy rates were 83.8% in *L. officinalis*, 81.8% in Thymovar, 78.8% in *F. vulgare*, and 70.8% in *L. nobilis*. In the second autumn, the efficacy rates were 78.4% in Thymovar, 76.6% in *L. officinalis*, 71.9% in *F. vulgare*, and 65.2% in *L. nobilis* respectively. No abnormal deaths were seen in adult bees during the treatment period.

Key words: Efficacy, essential oils, honeybee, *Varroa destructor*, Turkey

Introduction

The ectoparasitic mite *Varroa destructor* is a pest of the honeybee *Apis mellifera*. Colonies infested with *V. destructor* have significantly reduced worker bee populations and bees eventually die if left untreated (1,2).

V. destructor came to Turkey from the Thrace region in 1977 and was spread across the country by package bees in a short time, causing 600,000 colony deaths (3). As in other parts of world, the agent was initially named *Varroa jacobsoni*, but it was later identified genetically (4) and morphologically (5) as *V. destructor* in Turkey.

Several synthetic acaricides have been applied to hives in order to fight varroosis but, due to the widespread misuse of chemical treatments, several cases of resistance and contamination of hive products have been reported (6,7). Use of synthetic acaricides causes accumulation in apiary products such as beeswax, propolis, royal jelly, and honey (8,9).

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Natural products such as essential oils offer a highly desirable alternative to synthetic products. Products of botanical origin have shown a wide range of biological activities including toxicity, repellence, and antifeedant effects in parasites and growth regulatory properties (10).

Bursa Province of Turkey is in the Marmara Region, which has a quite humid (73% annual average) climate. Average minimum and maximum temperature ranges in September, October, March, and April are 13.5-27.0, 9.9-21.6, 3.5-13.6, and 7.2-18.7 °C, respectively (11).

The objective of this work was to determine the efficacies of the essential oils of *Lavandula officinalis* (lavender), *Foeniculum vulgare* (fennel), and *Laurus nobilis* (bay laurel) against *Varroa destructor* in the province of Bursa, Turkey. Thymol (Thymovar®), a drug with known efficacy, was used as a control to compare these essential oils.

Materials and methods

Characterization and preparation of essential oils

Oils were purchased from Mecit Efendi Ltd. Şti. (İzmir, Turkey), derived from the leaves of each plant. The characterization of the 3 essential oils was performed at the Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey, in August 2006.

The compositions of the oils were analyzed by gas chromatography-mass spectrometry (GC/MS), using an Agilent 5975 chromatograph equipped with an HP-Innovax fused silica capillary column (60 m × 0.25 mm, film thickness of 0.25 μm). The temperature was programmed to hold at 60 °C for 10 min and then to rise to 220 °C at 4 °C/min, and the final temperature was held for 10 min; injector and detector temperatures were 250 °C, the detector was a flame ionized detector (FID), and the carrier gas was helium at a flow of 0.8 mL/min. The identification of components was based on comparison of their mass spectra with those reported in literature (12) and by a computer search of their 70 eV mass spectra with those stored in the library of the GC/MS data system, as well as by retention indices.

Lavandula officinalis, *Foeniculum vulgare*, and *Laurus nobilis* essential oils were diluted with sunflower oil to obtain a 25% homogeneous mixture of each. Impregnated onto the 5 × 5 cm felts (a texture made of compressed matted animal fibers) was 10 cm^3 of each essential oil (7.5 cm^3 sunflower oil + 2.5 cm^3 essential oil). Each felt was covered with plastic nets to prevent bees from coming into direct contact with and eating the felt, and afterwards they were covered with zipper bags and put into a freezer to prevent evaporation before application.

Experimental colonies

There were 40 *Apis mellifera* colonies used, which were highly infested with *Varroa destructor*. The Langstroth-type hives, with 6-7 combs and large-size pollen drawers, were placed in İkizce village in the Nilüfer district of Bursa, Turkey. All colonies had been left untreated for *Varroa* during the preceding 12 months. The colonies were divided into 5 homogeneous groups with 8 hives in each group.

Treatment and assessment procedures

The colonies were treated in 3 seasons: September to October 2006 (first season), March to April 2007 (second season) and September to October 2007 (third season). The average temperatures at the application times were 21.1 °C in September 2006, 17.4 °C in October 2006, 13.5 °C in March 2007, 15.9 °C in April 2007, 22.0 °C in September 2007, and 18.2 °C in October 2007.

Doses consisting of 25% of *Lavandula officinalis*, *Foeniculum vulgare*, and *Laurus nobilis* essential oils and thymol (Thymovar®) were applied to each group; 1 group served as the untreated control. The control group was divided into 2 subgroups; 1 subgroup had only sunflower oil in the felt and the other subgroup was left blank to understand whether sunflower oil had an effect on the treatment.

Approximately 200 adult worker bees from outer frames of each hive were collected into jars containing cotton with diethyl ether to determine the rate of *Varroa* infectivity before and after treatment (13). Aliquoted adult bees and mites were counted. Additionally, the bottoms of the drawers were cleaned before the trial and were covered with white paper to count dead mites that dropped into the drawer after each drug application.

The chosen colonies were treated with essential oil-impregnated felts on days 1, 14, and 28 (once

every 2 weeks, totaling 3 applications). Only thymol (Thymovar®) was applied twice, as instructed, on days 1 and 21.

In each season, the dead mites that had dropped into the pollen drawers were counted on days 1, 3, 5, 7, 14, 21, 28, 35, and 42. Mites on bees were counted on day 42 after treatment.

Statistical analysis

The efficacies of essential oils were measured with the Henderson-Tilton formula. Significance between the essential oils and significance between the counts of dead mites per hive were determined via Tukey's multiple comparison test at the level of 0.05 (14,15).

$$\text{Corrected \%} = \left(1 - \frac{\text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}}\right) \times 100$$

Where n = mite population, T = treated, Co = control.

Results

The main chemical components of the essential oils evaluated by GC/MS are presented in Table 1. For *L. officinalis* the main compounds were α-pinene and linalool; for *F. vulgare* they were (E)-anethole and limonene; and for *L. nobilis* they were 1,8-cineole (eucalyptol) and α-terpinyl acetate (Table 1).

Table 1. Composition of the 3 principal essential oils of *Lavandula officinalis, Foeniculum vulgare*, and *Laurus nobilis.*

Essential oil	Compounds	Composition %*
Lavandula officinalis	α-pinene	49.4
	Linalool	16.3
	Linalyl acetate	13.0
Foeniculum vulgare	(E)-anethole	75.3
	Limonene	8.7
	Carvone	6.1
Laurus nobilis	1,8-cineole (eucalyptol)	54.6
	α-terpinyl acetate	8.9
	Sabinene	7.0

*Relative percentage obtained from GC peak area.

The efficacies of essential oils are presented in Table 2. The average efficacies of all 4 products were at similar levels, but *L. nobilis* essential oil had a lower efficacy compared to the others. The commercial thymol extract on average showed the best efficacy at the end of the 3 seasons (Table 2). Although an aggressive behavior was observed during the first minutes of applications, no abnormal bee mortality was observed during treatment.

Table 2. Efficacy of essential oils and thymol preparation on varroosis by Henderson-Tilton formula in 3 seasons.

Treatment	Season	Efficacy %
Lavandula officinalis	1	76.4
	2	83.8
	3	76.6
	Average total	78.9
Foeniculum vulgare	1	74.5
	2	78.8
	3	71.9
	Average total	75.0
Laurus nobilis	1	76.7
	2	70.8
	3	65.2
	Average total	70.9
Thymol (Thymovar®)	1	79.4
	2	81.8
	3	78.4
	Average total	79.8

The mean numbers of dead mites on pollen drawers in each treatment in each season are presented in Table 3.

According to the statistical data, all essential oils were efficient, but *L. officinalis* and thymol were similarly and significantly more efficient against *Varroa* in the first season. However, in the second and third seasons only the control group was significantly different from the treatment groups. All essential oils were thus found to be similarly efficient against *Varroa* in those seasons.

Table 3. Average number of dead mites dropped on pollen drawers per hive for 42 days in each essential oil.

Season	Essential oil	Average number of dead mites per hive	Standard error of mean
1	*Lavandula officinalis*	125.8[a]	6.7
	Foeniculum vulgare	75.1[b]	5.1
	Laurus nobilis	45.0[c]	3.4
	Thymol	115.0[a]	7.4
	Control	14.2[d]	1.1
2	*Lavandula officinalis*	97.5[a]	5.7
	Foeniculum vulgare	82.0[a]	5.5
	Laurus nobilis	54.5[a]	4.3
	Thymol	105.5[a]	5.7
	Control	10.1[b]	0.9
3	*Lavandula officinalis*	123.1[a]	6.9
	Foeniculum vulgare	77.8[a]	5.0
	Laurus nobilis	44.5[a]	3.9
	Thymol	110.7[a]	6.4
	Control	15.6[b]	1.3

[a, b, c, d]: values with different letters in each category are significantly different.

There were 2 subgroups of control and no difference was observed between the subgroups. They were therefore treated as one control group in the statistical analysis.

Discussion

Botanical extracts obtained from different plant species have been shown to have a broad spectrum of acaricidal activity against varroosis and also against other organisms such as insects, mites, bacteria, fungi, and nematodes (10,16,17).

Because of the possibility of resistance development of mites and harmful residues in bee products, usage of organic compounds has become widespread throughout the world. At present, mite resistance to essential oils used to control *Varroa* or hazardous residues (except in misuses) have not been detected (10). There can be an increase in thymol residue levels in honey due to the Thymovar® treatment. However, there is no maximum residual level for thymol and the average thymol residue level in the honey from honeybee colonies that were treated with Thymovar® is below the taste threshold (18).

The composition of the essential oils tested in this work was obtained with GC/MS and was in accordance with those reported by Adams (12). Several factors can influence the yield and the chemical composition of essential oils. Therefore, it is possible that different compositions and different percentages were obtained by different researchers (17).

In temperate climates, the best time to treat varroosis is late autumn, and treatment should be performed prior to the bees overwintering (19). Accordingly, 2 autumn treatments were performed in this research to better determine the efficacies of the essential oils.

Satisfactory results have been reported from the application of homemade essential oils such as thymol, *Baccharis flabellata* (Eastern baccharis), *Minthostachys verticillata* (peperina), *Lavandula* spp. (lavender), *Tagetes minuta* (wild chamomile), etc. (10,17,20,21). However, in some rare cases, only partial mite toxicity has been observed. When applied as fumigants, the effectiveness of essential oils against *Varroa* mites depends greatly on temperature, time of the year, and colony strength (16,21).

The percentage of efficacy and control of the mite presented in this work seems to be low in comparison to those obtained from synthetic acaricides commonly used to control *Varroa*, but the results are still promising. Although the development of miticides from natural substances, like essential oils and organic acids (22), is not likely to replace the use of conventional products in the control of the mite, it can still reduce the use of these chemicals to control *Varroa*.

According to Mondet et al., treatment using thymol as an active substance can have some adverse effects on forager bees, who initially appeared to be repelled by the treatment but can become habituated to it (23). This was supported in our work, where striking effects were observed in the use of essential oils and thymol with forager bees being repelled in the first minutes of treatment. Similar results were observed in previous research based on using the gel formulation of thymol in the treatment of varroosis (24).

Our results showed that use of these 3 essential oils and thymol has a moderate effect on varroosis. However, they can be used as a supplementary treatment with other organic compounds instead of the use of synthetic acaricides, particularly because they do not represent a resistance or sanitary risk. No distinct adverse effects are expected on broods or adult bees when the products are used properly.

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