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Research Article

Evaluation of Efficiency of Thyme Oil, *Cinnamomum verum, Melaleuca viridiflora, Syzygium aromaticum* Essential Oils, and Amitraz for *Varroa* Mite (Acari: Varroidae) Control in Honey Bee (Hymenoptera: Apidae) Colonies Under Field Conditions

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Abstract

Varroosis is a disease that can be observed in all life stages of honey bees and causes serious clinical findings in infested hives. This study aimed to investigate and compare the efficiency of thyme oil, *Cinnamomum verum*, *Melaleuca viridiflora, Syzygium aromaticum* essential oils, and amitraz against Varroosis in the field. After the essential oils were dissolved in glycerin, they were impregnated on strips and applied by placing them between frames. Amitraz was applied by fumigation. Positive and phoretic *Varroa* negative control groups were also included. Treatments were applied once a week for four weeks during the autumn season. The rates of acaricide efficacy and weekly mite count per bottom frame were determined for each of the treatments. According to the results, the highest effectiveness against Varroosis was detected in the amitraz treatment group at 81.3%. The *C. verum* treatment group had the highest efficacy of all the essential oil treatment groups at 73.5%. The efficacies in thyme oil, *M. viridiflora*, and *S. aromaticum* essential oils treatment groups were 71.9%, 71.3%, and 67.4%, respectively. According to the findings of the study, natural extracts can be used instead of chemical drugs in the fight against Varroosis. No toxicity or unnatural death was observed in honey bees throughout the study.

Keywords: Honey bee, Varroosis, Thyme oil, *Cinnamomum verum*, *Melaleuca viridiflora*, *Syzygium aromaticum, Varroa destructor*

Introduction

Honey bees serve a significant ecological function by pollinating the majority of closed-seeded plants [1]. This pollen-emitting insect is also highly desired since its cultivation provides crops with significant nutraceutical benefits [2]. Bee colonies are disappearing all across the world for a variety of reasons [3]. Although there are various reasons for these losses, the principal ones are caused by the *Varroa* agent and the diseases that it causes [4].

Varroa destructor, a parasitic mite, causes significant economic losses by damaging honey bee colonies [5]. The life cycle consists of 4 stages: egg, protonymph, deutonymph and adult [6]. *V. destructor* agents primarily feed on fat body, they also feed on a small amount of hemolymph. *V. destructor* causes weight loss, shortening of lifespan, learning, memory, behavioral disorders, carrying viruses, and immunosuppression in infected individuals [7]. If untreated, colonies of honey bees often do not survive for longer than two years [8]. Colonies infected with *V. destructor* and untreated colonies have lower yield characteristics, especially honey, compared to treated colonies [9]. Acaricides developed from past to present for the control of *V. destructor* are divided into two groups: hard and soft acaricides. The first group includes synthetic drugs such as taufluvalinate, flumethrin (pyrethroid), coumaphos (organophosphate), and amitraz (formamidine). Organic compounds, such as oxalic acid, formic acid, and essential oils, are most typically utilized in the second group [10]. Although traditional pest management options based on synthetic pesticides are popular due to their ease of administration

and superior capacity to decrease insect impact, excessive use endangers honey bee health. Some acaricides can accumulate in wax due to their lipophilic properties. Since hive products can retain residual chemicals, adult and juvenile honey bees can be exposed to these chemicals over long periods of time [11]. Even very low doses or concentrations of these chemicals have been shown to cause non-lethal changes in the neurological, metabolic, physiological and/or behavioural characteristics of honey bees [12]. The colony could face non-fatal effects. The hive may gradually become less populous. Furthermore, the resistance phenomenon has made these acaricides less effective [13].

Efforts are being made to eliminate the residue problems caused by pesticides, the resistance problems that develop against them and to reveal environmentally friendly compounds. As a result, essential oils and their monoterpenes are being extensively researched as potential pest management alternatives. In comparison to hard acaricides, essential oils (EOs) were carefully tested and proven to be long-term effective as miticides against *V. destructor* [14].

Essential oils generally work maximum against *Varroa* agents at temperatures between 20-25°C. However, in honey bee farming, the use of essential oils is ignored at low temperatures, especially in October and November before the winter season, and products such as oxalic acid are used. With our study, we tried to reveal whether essential oils can be used at low temperatures. The current study evaluated alternative techniques for controlling Varroosis by examining the acaridial activity of essential oils (Thyme oil, *Cinnamomum verum*, *Melaleuca viridiflora,* and *Syzygium aromaticum* essential oils) and amitraz in the field.

Material and Methods

Ethical Statement

This study does not require ethics committee approval.

Study Area

The study was conducted in October 2023 at the Balikesir Queen Bee and Bee Products Production Center. The study took place in an apiary with 950 hives. Balikesir is located in the Marmara Region of Türkiye.

Honey Bee Information

Studies were carried out on the *Apis mellifera anatoliaca* breed. The queen bees in each hive were one year old. The honey bee colonies used in the research had a population of approximately 15.000 to 20.000 individuals. Five-frame hives were included in the study. There are 2-3 brood frames and 1-2 frames with eggs in each of them (although no eggs are observed in all of them, there are at least a handful of eggs in each). It was stated that no chemicals or plant extracts were used in the treatment of diseases and pests in the colonies used in the research.

Field Experiment

The powdered sugar method was used to determine whether 150 hives were positive or negative for Varroosis [15]. In order to be placed in the Varroa test apparatus, 300-350 worker honey bees were taken from the combs, brushed and took in the 900 ml apparatus. Fifteen g of powdered sugar was filtered through a sieve. The jars were rolled to distribute the sugar evenly among the bees. After one minute, the jar and honey bees were shaken rapidly on a white paper plate for approximately four minutes, and the *Varroa* displaced during this time were counted [16]. To show field work Varroa tester apparatus and *Varroa* macroscopic appearance are included in *(Fig. 1, Fig. 2)*, respectively. Before each trial, the white paper placed in the pollen traps was renewed in order to count the *Varroa* mites.

General Information About Essential Oils and Amitraz

The essential oils used in the study were obtained from NU-KA DEFNE Essencia company. The essential

Fig 1. *Varroa* tester apparatus

Fig 2. *Varroa* macroscopic appearance *(red arrow)*

oils used were obtained through steam distillation. The information about thyme oil; 100% purity, active ingredients: thymol (48.50%), γ-terpinene (31.35%), and p-cymene (8.25%), linear formula: 2-[$(CH_3)2CH]C_6H_3$-5-$(CH_3)OH$, molecular weight: 150.20 g/mol. *C. verum* oil; 100% purity, active ingredients: linalool (36.0%), methyl eugenol (12.8%), limonene (8.3%), α-terpineol (7.8%) and terpinen-4-ol (6.4%), linear formula: $C_{11}H_{12}O_2$, molecular weight: 132.16 g/mol. *M. viridiflora* oil; 100% purity, active ingredients: 1,8-cineole (31.5%), viridiflorol (21.7%), α-pinene (17.9%), α-terpineol (6.5%), terpinene-4-ol (2.6%), γ-terpinene (2.3%), β-pinene (1.9%), and ledol (1.3%), linear formula: $C_{10}H_{16}$, molecular weight: 136.234 g/mol. *S. aromaticum* oil: eugenol (76.8%), beta-caryophyllene (17.4%), alpha-humulene (2.1%), and eugenyl acetate (1.2%), linear formula: $C_7H_{12}ClN_3O_2$, molecular weight: 205.642 g/mol. Vamitrat VA® is a commercial product containing amitraz (N-methyl-bis(2,4-xylyliminomethyl). Each strip contains 20 mg amitraz.

Preparation of Essential Oils

A total of 180 mL of solution was prepared by mixing 45 mL of essential oil with 135 mL of glycerin. Forty-two strips measuring 2x10 cm were placed inside and waited for five minutes [17]. All essential oils applied in the study were prepared according to the following method. Each strip contains 1.07 mL of essential oil and 3.21 mL of glycerin solution. A total of 84 strips were prepared for each essential oil.

Amitraz Treatment Group

The amitraz treatment group consisted of 7 Langstroth-type hives (each hive consists of 5-6 frames), as in the essential oils treatment and control groups. Amitraz application is shown in *Fig. 3*. After the amitraz strip was placed in the bee smoker, it was burned and applied to each hive through the hive entrance hole with 10 bee smoker movements. Amitraz was used four times in total, on days 0, 7, 14 and 21. Amitraz was applied close to the evening hours, when the bees returned to the hive.

Essential Oils Treatment and Control Groups

Fourty-two honey bee colonies in Langstroth type hives with five or six frames each were divided into six homogeneous groups, four essential oil treatment groups, one phoretic *Varroa* negative control group and one positive control group. There were 7 hives in each group. The essential oils treatments were administered weekly during for four weeks in autumn. Essential oil application was performed on days 0, 7, 14 and 21. Three strips of essential oil were applied to each hive and the strips were

Fig 3. Amitraz application

renewed every week. Count the number of mites in the powdered sugar were applied on days 0 (before treatment) and 28 (post-treatment) to determine *Varroa* loads in honey bee colonies. Similar to the treatment groups, count the number of mites in the powdered sugar were performed on days 0 and 28 in both the negative and the positive control groups, and *Varroa* agents that fell into the pollen trap were counted on days 7, 14, 21, and 28 [18]. Essential oils were applied close to the evening hours, as was the case with amitraz.

While creating the groups, attention was given to distribute the phoretic *Varroa* loads numerically homogeneous in all 7 hives on day 0.

Determination of Treatment Efficacy

The Henderson-Tilton formula was applied to evaluate the therapeutic efficacy of the essential oils and amitraz [18].

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

(where n=mite population, T=treated, Co=control).

Statistical Analysis

The statistical analyses were performed utilizing the IBM SPSS20 software. To determine the effects of the agents used in this study on *Varroa*, the One-Way ANOVA test analyzed the Henderson-Tilton treatment efficacy. Comparisons between agents were made with the Tukey-HSD post-hoc test. *Varroa* agents that fall into the pollen trap on different days and the interactions between the factors were evaluated with the Repeated Measures ANOVA test. Determination of statistical significance and whether the test assumptions were met were evaluated according to Mauchly's test results. Since the result of Mauchly's test was less than 0.05 and the epsilon value was less than 0.75, Greenhouse-Geisser correction was applied. Pairwise comparisons between days were determined by applying Bonferroni correction. In comparisons between groups, the Tukey multiple comparison test was used since the homogeneity of variances test assumptions were met. Differences between groups were evaluated as $P<0.05$ and data were presented as mean and standard error [19].

Results

As a result of field studies of 150 honey bee colonies using powdered sugar method, 108 positive (72%) and 42 negative (28%) colonies were detected in terms of *Varroa*. Beyond a threshold of three phoretic *Varroa* mites per 100 bees, the decrease in performance is correlated with the *Varroa* load. Colonies with a load of ten *Varroa* or more on live bees were treated [20]. Hives below ten *Varroa* load were not included in the positive control and treatment groups. Of the 42 *Varroa* positive hives, 28 were included in the four essential oil treatment groups, seven in the amitraz treatment group and the remaining seven in the positive control group. Seven out of the 108 *Varroa*-negative hives (phoretic *Varroa* was not detected these hives) were selected as the negative group in the study, considering their clinical status (clinically healthy hives where *Varroa* is not seen in adult bees, where there are no paralyzed bees or bee deaths in front of the hive). Of mites number of *Varroa* in the powdered sugar shake were applied on day 0 (before treatment) and day 28 (post-treatment) to determine the *Varroa* loads in colonies of honey bees, and the results are given in *Table 1*. On days 0

Table 1. *Day 0 (before treatment) and Day 28 (after treatment) powder sugar count results and treatment efficacies*

Hive No	Amitraz		Thyme oil		*C. verum*		*M. viridiflora*		*S. aromaticum*		Positive Control Group		Negative Control Group	
	D.0 V.L.	D.28 V.L.	D.0 V.L.	D.28 V.L.	D.0 V.L.	D.28 V.L.	D.0 V.L.	D.28 V.L.	D.0 V.L.	D.28 V.L.	D.0 V.L.	D.28 V.L.	D.0 V.L.	D.28 V.L.
1	40	11	43	15	40	17	44	20	46	40	45	58	0	13
2	40	15	34	21	33	16	36	18	38	18	32	40	0	23
3	45	10	48	18	45	20	51	20	53	20	44	48	0	26
4	20	8	21	10	19	10	23	10	25	20	17	36	0	25
5	30	10	19	10	17	9	20	10	22	12	16	48	0	21
6	50	15	58	20	56	15	60	23	62	17	55	69	0	22
7	50	6	51	18	49	13	53	19	53	15	45	70	0	19
Total	**275**	**75**	**274**	**112**	**259**	**100**	**287**	**120**	**299**	**142**	**254**	**369**	**0**	**149**
Days	*Varroa* percentage reductions in treatment groups compared to days 0 and 28													
0-28	Amitraz VCR: 72.72			Thyme oil VCR: 59.12			*C. verum* VCR: 61.38			*M. viridiflora*: 58.18			*S. aromaticum*: 52.50	

D.0: Day 0, D.28: Day 28, V.L:. Varroa Load, VCR: Varroa Count Reduction

Table 2. *Treatment groups pollen trap count results*

Hive No	Amitraz				Thyme oil				*C. verum*				*M. viridiflora*				*S. aromaticum*			
	D.7	D.14	D.21	D.28	D.7	D.14	D.21	D.28	D.7	D.14	D.21	D.28	D.7	D.14	D.21	D.28	D.7	D.14	D.21	D.28
1	40	38	35	32	25	20	18	17	23	18	15	14	27	22	20	18	30	28	25	23
2	53	50	45	40	30	25	20	19	27	22	18	16	36	30	25	23	40	35	30	28
3	68	65	55	50	39	35	30	27	35	30	25	23	45	40	35	33	50	45	40	38
4	30	28	25	22	13	10	9	8	12	9	8	7	15	13	12	10	18	15	13	12
5	28	25	23	20	17	15	13	11	15	12	11	10	18	15	14	12	20	17	16	15
6	65	60	55	50	45	41	40	35	40	37	30	25	50	43	40	34	60	55	50	45
7	55	49	45	40	38	32	30	23	35	30	26	21	40	34	30	25	50	43	40	35
Total	**339**	**315**	**283**	**254**	**207**	**178**	**160**	**140**	**187**	**158**	**133**	**116**	**231**	**197**	**176**	**155**	**268**	**238**	**214**	**196**

and 28, the reduction in *Varroa* numbers was compared. According to *Table 1*, the greatest reduction in phoretic *Varroa* load on days 0-28 was calculated as the amitraz treatment group (72.72%), and the least reduction was calculated as the *S. aromaticum* oil treatment group (52.50%). The number of phoretic *Varroa* increased in both the positive control group and the phoretic *Varroa* negative control group from the beginning to the end of the field studies *(Table 1)*. Additionally, all hives in the phoretic *Varroa* negative control group were *Varroa*-positive two weeks after the beginning of the study. The treatment efficacies of amitraz, thyme oil, *C. verum*, *M. viridiflora*, and *S. aromaticum* oils were calculated using the Henderson-Tilton formula to the counts on days 0 and 28, and the results were 81.3%, 71.9%, 73.5%, 71.3%, and 67.4%, respectively. Among the treatment groups, the highest number of *Varroa* falling into the pollen trap was detected in the amitraz treatment group, while the lowest number was detected in the *C. verum* treatment group. In all treatment groups, the number of *Varroa* falling into the pollen trap decreased from the day 7 to the day 28 *(Table 2)*. In addition, in the positive control group, *Varroa* agents were detected in the pollen traps on all counting days, while in the phoretic *Varroa* negative control group, *Varroa* agents were not observed in the pollen traps on day 7 and day 14, while *Varroa* agents were detected on day 21 and day 28 *(Table 3)*. According to Henderson-Tilton efficacy findings for the agents amitraz group has the highest treatment effectiveness (82.32±2.24), while the lowest treatment effectiveness (66.37±6.54) was calculated as the *S. aromaticum* treatment group. Also, treatment effectiveness were found as (73.65±3.99) in *M. viridiflora* treatment group, (72.42±3.72) in *C. verum* treatment group, (71.34±3.98) in thyme oil treatment group, respectively. The highest number of phoretic *Varroa* in pollen traps was detected in the amitraz group (1st count=48.42±6.08, 2nd count=45.00±5.79, 3rd count = 40.42±4.98, 4th count=36.28±4.61) while the least was observed in the *C. verum* (1st count = 26.71±4.03, 2nd count = 22.57±3.89, 3rd count = 19.00±3.12, 4th count = 16.57±2.55) treatment group. Also, number of phoretic *Varroa* in pollen traps were found in the *S. aromaticum* treatment group (1st count = 38.28±6.11, 2nd count = 34.00±5.63, 3rd count = 30.57±5.14, 4th count = 28.00±4.60), in the *M. viridiflora* treatment group (1st count = 33.00±5.07, 2nd count=28.14±4.47, 3rd count = 25.14±3.98, 4th count=22.14±3.57), in the thyme oil treatment group (1st count=29.57±4.51, 2nd count=25.42±4.24, 3rd count = 22.85±4.14, 4th count = 20.00±3.51), respectively. In the study, the difference between the factors in terms of

Table 3. *Phoretic Varroa negative control group and positive control group pollen trap count results*

Item	Hive No	Day 7	Day 14	Day 21	Day 28
Phoretic *Varroa* negative control group pollen trap count results	1	0	0	1	1
	2	0	0	2	1
	3	0	0	2	1
	4	0	0	2	1
	5	0	0	2	2
	6	0	0	2	2
	7	0	0	2	3
Total		**0**	**0**	**13**	**11**
Positive control group pollen trap count results	1	5	4	5	3
	2	3	6	3	4
	3	4	3	2	5
	4	4	5	4	2
	5	3	5	3	1
	6	3	3	1	3
	7	5	6	3	4
Total		**27**	**32**	**21**	**22**

Henderson-Tilton values is not statistically significant (P>0.05). The difference in pollen trap counts in terms of both factors and counting times was found to be statistically significant (P<0.001).

At each application stage of essential oils, it was checked whether there was a problem with brood cells in the previous application. Dead larvae were not observed in brood cells. Additionally, the inside and surroundings of the hive were carefully examined, and no dead adult honey bees were detected.

Discussion

This study was conducted to find an alternative treatment approach with four essential oils against *V. destructor* in honey bee colonies in Balikesir province, Marmara region of Türkiye. In field studies, thyme oil, *C. verum*, *M. viridiflora*, and *S. aromaticum* oil were impregnated on the strips, they were applied between the frames, three to each hive, four times a week apart, and amitraz was applied to the flight hole by fumigation four times a week apart. Forty-nine hives were divided into seven groups thyme oil, *C. verum*, *M. viridiflora*, *S. aromaticum* essential oil treatment groups, amitraz treatment group, and positive and phoretic *Varroa* negative control group.

The need to minimize or replace synthetic pesticides with natural alternatives has prompted the current seek for environmentally safe treatment options. The plant world has proven to be extremely beneficial, with a wealth of medical resources available to cure a wide range of human and animal ailments. EOs and related monoterpenes have been extensively researched for use in pest management programs [14]. These chemicals have been shown to effectively cure *Ascosphaera apis*, *Paenibacillus larvae*, *Nosema ceranae*, and other honey bee illnesses [21]. When compared to hard acaricides, EOs have been thoroughly tested and have proven effective as miticides against *V. destructor* over time. Extracts obtained from specific botanical species are useful, whereas others are unsuccessful. Presently available for purchase on a global basis are neem products, ryanodine, nicotine, sabadilla, pyrethrins, and rotenone fight against Varroosis [22]. In beekeeping, essential oil-based treatments for controlling *V. destructor* parasites have been licensed for commercialization. For example, Thymovar® (Andermatt BioVet, Grossdietwil, Switzerland) thymol cellulose sponge strips, in addition to ApiLife Var® (Chemicals Laif SPA; Vigonza, Italy) vermiculite tablets that contain eucalyptus oil, camphor, thymol, and, levomenthol, were introduced in Italy. Apiguard® products (Vita Europe Ltd., Basingstoke, England) are patented gels whose unique formulation allows for the gradual release of thymol. Very few herbal-based *Varroa* control products are utilized in beekeeping. Therefore, researchers are continuing studies under both laboratory and field conditions to increase the amount of plant-based products in the fight against *Varroa* in the global market. In one of these studies, one strip of the absorbent paper pad (2x10 cm^2) was dipped in pure rosemary essential oil and placed directly on the outer frames of each hive, with treatments applied at five different rates. The efficacy of rosemary essential oil was found as 93% [23]. Under the same conditions, oils derived from *Thymus satureioides* and *Origanum elongatum* showed comparable efficacy against *V. destructor*. As a result, these oils and their blends, when applied directly to bee hives, have been successfully tested against *Varroa* mites [24]. Camphor has previously been shown to have exceptional bioactivity against *Varroa* mites without being hazardous to bees [25]. In another trial conducted in 5-15 g, 50-150 g, and 20-60 g per liter of air, respectively, thymol, camphor, and menthol killed virtually entirely of the mites without appreciably reducing the bee colony [26]. Also, thymol was absorbed into powder and strips, tested in two different ways. Treatment efficiencies were determined as 96.6% and 92.4%, respectively [17].

S. aromaticum was applied to hives as a fumigant in two different studies. The efficacy was found to be 87% [27] in the first study and 54% [28] in the second study. In another study with *S. aromaticum*, water-soluble protein concentration reduced considerably after the treatments, showing that the mites' metabolism was impaired. Glutathione-S-transferase (GST) bioactivity increased at a low dosage (0.1 µL) but reduced with a greater dosage (1.0 µL), while superoxide dismutase (SOD) and Ca^{2+}-Mg^{2+}-ATPase activities were significantly boosted following treatments. These observations imply that the protective enzyme SOD and detoxifying enzymes Ca^{2+}-Mg^{2+}-ATPase and GST caused the stress response of *V. destructor* to the essential oils and that the detoxifying capacity of *V. destructor* by GST was reduced at higher dosages [29]. *C. verum* [27] and *Laurus nobilis* [28] essential oils were also applied to the hives by fumigation and their treatment efficacy was found to be 52.50% and 75%, respectively.

The effectiveness of nine different essential oils against *Varroa* was evaluated in vitro. According to the results, the activities of bitter melon oil 70%, garlic oil 70%, basil oil 66.6%, thyme oil 61.7%, mustard oil 61.7%, cloves oil 58.3%, eucalyptus oil 55.5%, rosemary oil 53.3%, and mint oil 53.3% were determined [30]. In a study conducted for the effectiveness of thymol, spearmint, tea tree oil and mixture of spearmint and lemongrass essential oils against *Varroa*, the activities were determined as 78.1%, 85.9%, 80.2%, and 84.3%, respectively [31]. The effectiveness against *Varroa* in the *M. viridiflora* treatment group used in our study was determined as 71.3%, and the effectiveness against *Varroa* in the *Melaleuca alternifolia* treatment group mentioned in this study was determined as 80.2%. The reason for this

difference may be the different *Melaleuca* species used, the application method, and geographical and regional differences.

M. viridiflora has previously been used against the American foulbrood agent *Paenibacillus larvae* at a level of 320 mg L^{-1} in laboratory environments and positive results were obtained [29]. In our study, *M. viridiflora* was tested for the first time against Varroosis, and its treatment efficacy was found to be 71.3%. Also, amitraz was used against Varrosis. As a result of this study efficacy was found to be 94.6% [32]. In a study, Apivar and Supatraz, which contain the active ingredient amitraz, were used against *Varroa*. According to the results, it was reported that Supatraz killed the mites faster than Apivar and 90% reduction in the number of *Varroa* mites was observed in 50.9 days for Apivar and 28.4 days for Supatraz [33]. In a study conducted in Türkiye, 98.5% efficacy of amitraz, 96.5% efficacy of flumethrin, and 93.2% efficacy of coumaphos were determined. In this study, it was also reported that the desired effect could not be achieved by fumigation of amitraz. This may be attributed to the acquisition of resistance of *Varroa* agents against amitraz and overlooked points in the use of amitraz [34]. The reasons why different researchers obtained different results in their studies with amitraz are; there may be different companies of the amitraz-containing product used, application methods, application time, and environmental conditions during application. In addition, by placing a special apparatus at the tip of the bee smoker we used in the amitraz fumigation method in field studies, the resulting smoke was completely penetrated into the hive. This increased the success rate we achieved with amitraz in field studies.

During the field studies, minimum-maximum temperature values are 10°C-22°C and average humidity is 67%. These values have created the ideal breeding environment for *Varroa* agents. There are clear discrepancies in the efficiency of the Varroosis treatment between the results of our field trial and the articles cited above. There may be many reasons for this situation. The most important of these reasons is that the contents of essential oils vary depending on the season and region. Furthermore, the efficacy of particular components is determined by the evaporation pressure in hives, which varies depending on the time of year and the surrounding humidity and temperature during treatment application.

To sum up, essential oils can be used safely against the phoretic cycle of *Varroa* agents in temperature ranges (10-22°C) in October, which is the beginning of the winter period. In this way, the use of chemical active substances can be prevented by ensuring that honey bee colonies enter the winter season with less *Varroa* load. In this way, honey bee colonies emerge healthier and stronger in the spring.

As a result, according to the findings obtained from our field study, it has been revealed that essential oils can be used instead of chemicals against *Varroa*. More laboratory and field studies are needed in the fight against Varroosis with essential oils. With these studies, essential oils will become more standard and reliable.

Declarations

Availability of Data and Materials: The authors declare that data supporting the study findings are also available from the corresponding author (M. Özüiçli) on reasonable request.

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Competing Interest: The author reports no declarations of interest.

Author Contributions: MÖ planned the study and designed the experiments, YB planned and performed the data analyses. The final version of the manuscript was read and approved by all authors.

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