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Comparative Efficacy of Organic Acids and Essential Oils against *Varroa destructor* and their Impacts on Colony Performance and Behavioral Traits in Honeybees (*Apis mellifera* L.) in the Mediterranean Climatic Zone

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Abstract

Varroa destructor is one of the most serious threats to the health, productivity, and survival of honey bee (*Apis mellifera* L.) colonies worldwide. This study evaluated the efficacy of selected organic acids and essential oils against V. destructor under field conditions and assessed their effects on colony development and behavior. The study was conducted in the Aegean Region of Türkiye, characterized by a Mediterranean climate, using 30 naturally Varroa-infested colonies. Treatments were applied in spring and autumn according to the recommended application protocol for each product, while control colonies remained untreated. All treatments reduced mite infestation compared with the control. In spring, oxalic acid showed the highest efficacy against adult mites (87.02%), whereas formic acid was most effective against brood mites (87.52%). In autumn, efficacy against adult mite infestation ranged from 48.54% to 94.00%, with neem oil (94.00%), the herbal oil mixture (89.66%), and thyme oil (75.59%) showing the highest performance. No significant differences were detected among treatments in aggressiveness or grooming behavior, although oxalic acid produced the highest mean grooming activity. Honey yield ranged from 3.83 to 13.52 kg colony^{-1}, with oxalic acid (13.52 kg colony^{-1}) and the herbal oil mixture (11.50 kg colony^{-1}) maintaining productivity comparable to the untreated control (11.89 kg colony^{-1}). Overall, oxalic acid and the herbal oil mixture provided the best balance between Varroa control, colony performance, and honey production, supporting their integration into sustainable Varroa management under Mediterranean conditions.

Introduction

Since the parasitic mite Varroa destructor shifted from its original host, the Asian honey bee, to the European honey bee, it has become one of the greatest threats to honey bee health worldwide. Varroa primarily feeds on the fat body tissue of bees, which is essential for their immunity, detoxification, and survival, rather than solely on their hemolymph as previously thought (Traynor et al., 2020; Ramsey et al., 2019). Varroa destructor not only weakens individual bees and whole colonies by feeding on them directly, but it also spreads several deadly viruses, like deformed wing virus and acute bee paralysis virus, which makes colony losses even worse (Morfin et al., 2023).

Chemical control of Varroa destructor primarily relies on synthetic acaricides. Although these compounds effectively suppress mite populations, their repeated use has raised concerns regarding both honey bee health and long-term treatment efficacy. Locke et al. (2011), demonstrated that tau-fluvalinate treatment can influence viral dynamics in honey bee colonies by affecting both mite populations and host susceptibility to viral infection. Jack et al. (2021) further reviewed evidence that commonly used synthetic acaricides, including amitraz and tau-fluvalinate, may exert sublethal effects on queen development, immune function, and honey bee behavior. Repeated application of the same active ingredients has also promoted the development of resistance in Varroa populations, thereby reducing treatment efficacy and increasing the need for more frequent applications (Gregorc et al.,

2018; Shannon et al., 2025; Jack et al., 2024). In addition, residues of synthetic miticides may accumulate in beeswax and honey, posing long-term risks to colony health and hive product quality (Bahreini et al., 2020; Gregorc et al., 2018). Consequently, the development of safe, effective, and residue-free alternatives has become a major objective of integrated Varroa management. Organic acids have consistently demonstrated effective mite control with minimal residue concerns (Hýbl et al., 2021), whereas several essential oils, including thymol-based products, have shown promising acaricidal activity under field conditions (Sabahi et al., 2017; Alsaadi et al., 2024). Plant-derived compounds are therefore increasingly considered sustainable alternatives because they may reduce the selection pressure associated with resistance to conventional synthetic acaricides (Begna et al., 2023). Organic acids, particularly formic acid and oxalic acid, are among the most widely used compounds for controlling Varroa destructor in honey bee colonies. Formic acid is unique because its volatility enables it to penetrate capped brood cells, allowing it to eliminate both phoretic mites and reproducing mites within sealed brood (Qadir et al., 2021). Transcriptomic and proteomic studies have further shown that formic acid disrupts mitochondrial respiration and oxidative phosphorylation, leading to oxidative stress and activation of detoxification pathways in Varroa mites (Genath et al., 2020). In contrast, oxalic acid acts primarily through contact toxicity and is therefore effective mainly against phoretic mites. Because it cannot penetrate brood cappings, its efficacy is greatest when colonies are broodless (Qadir et al., 2021; Sagona et al., 2022). Among botanical alternatives, thymol and neem oil are two of the most extensively studied essential oils for Varroa control. Thymol exhibits strong acaricidal activity by disrupting the nervous system of Varroa mites, resulting in rapid mortality (Sabahi et al., 2020; Bisrat et al., 2022). Neem oil (*Azadirachta indica*), whose principal bioactive compound is azadirachtin, interferes with mite development and feeding behavior (González-Gómez et al., 2016). In addition to their direct acaricidal activity, essential oils may also influence honey bee behavior. Allam and Zakaria (2009) reported that different essential oils affect sensory and behavioral responses in honey bees, whereas Abd El-Wahab et al. (2012) demonstrated that treatment with selected essential oils enhanced grooming behavior, one of the most important natural defense mechanisms against Varroa infestation.

Evaluating both colony development and behavioral parameters alongside Varroa control performance provides a more holistic assessment of treatment safety and colony welfare. This study aimed to evaluate the efficacy of selected organic acids (formic and oxalic) and essential oils (thyme oil, neem oil, and herbal oil mixture) in controlling Varroa destructor infestations in honey bee colonies under field conditions in İzmir whereas Mediterranean climatic zone of Türkiye. In addition to their miticidal effects, the study also assessed the impact of these treatments on colony population growth, brood development, defensive behavior, grooming behavior, and honey production during the production period.

Material and Methods

The study was conducted at the Aegean Agricultural Research Institute (İzmir, Türkiye) during the spring and autumn seasons. A total of 30 naturally Varroa-infested honey bee colonies (*Apis mellifera* L.) with comparable colony strength, brood area, and queen age were used as experimental material. Before the experiment, colonies were standardized according to routine beekeeping management practices to ensure comparable colony conditions, including adequate food reserves. During the spring treatment periods, all colonies received identical supplemental sugar syrup feeding. Syrup feeding was applied only during the treatment period and was discontinued at the onset of the main nectar flow. No supplemental feeding was provided during the autumn treatment period. Colonies that swarmed, requeened, or died during the experimental period were excluded. All colonies were maintained in standard 10-frame Langstroth hives under identical management conditions.

Experimental Design

The experiment consisted of six treatment groups, each containing five colonies, as shown in Table 1.

Table 1. Experimental Design

Group	Treatment	Method of Application	Application Frequency
F	Formic acid	Evaporation via absorbent pads	4 times, every 4 days in both seasons
O	Oxalic acid	Dribbling between combs using syringe	Once per season
N	Neem oil (*Azadirachta indica*)	Spraying directly on comb surfaces	4 times, every 4 days interval in both seasons
K	Thyme oil (*Thymus vulgaris*)	Absorbent sponges placed on top bars	Twice per season, every 10 days interval
B	Herbal oil mixture	Spraying directly on comb surfaces	4 times, every 4 days interval, in both seasons
C	Control	No treatment applied	–

Preparation and Application of Treatments

The experiment was conducted during two experimental periods. The spring trial was initiated in April (early spring) and continued for one month until the beginning of the main nectar flow, whereas the autumn trial was initiated in September and continued for one month. During the treatment periods, all colonies received identical supplemental feeding with sugar syrup according to standard beekeeping practices. Syrup feeding was provided only during the treatment period and was discontinued at the onset of the main nectar flow to avoid interference with honey production. The preparation and application of the tested compounds were performed according to commonly adopted field practices, manufacturer recommendations (where applicable), and protocols optimized for each treatment. Because the tested compounds differ substantially in their physicochemical properties, persistence, and recommended field application methods, the application frequency, duration, and delivery method were not standardized across treatments but followed the recommended protocol for each compound. Therefore, the treatments were designed to reflect practical field use rather than standardized experimental exposure conditions.

Formic Acid

Formic acid (85%) was diluted with water to obtain a 65% solution. Each colony in the formic acid group received 30 mL of this solution via absorbent pads (7 × 7 cm) placed on top of the brood combs. Applications were made four times at four-day intervals in early spring and late autumn.

Oxalic Acid

A 3.2% oxalic acid sugar solution was prepared by dissolving 32 g of oxalic acid and 200 g sugar in 1 L of warm water. The solution was applied by trickling 3–5 mL between the combs using a syringe without a needle, once in early spring and once in late autumn.

Neem Oil

A 50% neem oil–water emulsion was prepared by mixing 500 mL of neem oil with 500 mL of water. The mixture was sprayed directly onto the comb surfaces (5–6 mL per frame). The application was repeated four times at four-day intervals in both seasons.

Thyme Oil

Small sponges (5 × 5 cm) were impregnated with 15 g thyme oil each and placed above the brood frames under the inner cover. The application was repeated twice per season at ten-day intervals.

Herbal Oil Mixture

A herbal oil–water emulsion was prepared from the distilled waters and essential oils of 27 plant species for using their synergic effects by mixing, including *Thymus vulgaris, Juglans regia, Pinus nigra, Urtica dioica, Matricaria chamomilla, Celtis australis, Juniperus communis, Liquidambar orientalis, Calendula officinalis, Lavandula angustifolia, Melissa officinalis, Mentha piperita, Myrtus communis, Laurus nobilis, Hypericum perforatum, Rosmarinus officinalis, Nigella sativa, Cedrus atlantica, Lavandula stoechas, Foeniculum vulgare, Simmondsia chinensis, Corylus avellana, Sesamum indicum, Amygdalus communis, Prunus americana, Allium sativum* and *Salvia officinalis*. The final 42% oil–water mixture was sprayed (5–6 mL per frame) directly on combs, four times at four-day intervals in both seasons.

Control

Control colonies received no treatment but were handled similarly during each application period to maintain identical disturbance conditions.

Determination of Varroa Infestation

Adult Bee Infestation

Approximately 150–200 adult worker bees were collected from each colony into jars containing detergent solution. After vigorous shaking, mites detached from the bees were filtered through a muslin cloth and counted under a stereomicroscope. The percentage of infestation was calculated using the following formula (modified from Kumova, 1985): $V=E/T\times100$; where V is the percentage of infested bees, T is the total number of mites, and E is the total number of examined bees.

Brood Cell Infestation

A brood comb section of 49 cm² (7 × 7 cm) was cut from each colony. Two hundred sealed brood cells were uncapped, and the number of infested cells was recorded. Infestation percentage was calculated as follows: $K=GS/GV\times100$; where GV is the number of mites found, and GS is the total number of examined cells. Total colony infestation was calculated according to the formula of Kumova (1985), integrating both adult and brood infestation levels.

Evaluation of Treatment Efficacy

The efficacy of treatments against Varroa destructor was calculated using the Henderson–Tilton formula (Henderson and Tilton, 1955): $E(\%)=[1-(Tb\times Ca)(Ta\times Cb)]\times100$; where Ta and Tb represent the mite counts in treated colonies before and after treatment, and Ca and Cb are those in the control colonies before and after treatment.

Colony Population Development

Brood area (cm²) was estimated using the Puchta method (Fresnaye and Lensky, 1961), measuring the

long (a) and short (b) axes of each brood patch and calculating the area as: S=3.14×2a×2b. Adult bee population was determined by counting the number of frames covered with bees at each inspection.

Aggressiveness (Defensiveness) Test

Colony aggressiveness was evaluated following Fıratlı and Budak (1992). A black suede-covered ball (4–5 cm diameter) was swung for one minute at the hive entrance, and the number of stings embedded in the ball was counted after collection in plastic bags.

Grooming Behavior

Grooming activity was evaluated by examining Varroa mites collected on petroleum jelly (Vaseline)-coated bottom boards under a microscope. Mites exhibiting body deformation or missing legs or other appendages were considered damaged as a result of successful honey bee grooming behavior and were recorded as evidence of grooming activity, following previously described methods (Fries et al., 1996; Arechavaleta-Velasco and Guzmán-Novoa, 2001).

Honey Yield

Honey production was measured in November during the final harvest. The honeycombs were weighed before and after honey extraction using a precision scale (± 0.05 kg), and the difference represented the total honey yield per colony (kg colony^{-1}), following the method described by Avni et al. (2009) and Taha and Al-Kahtani (2019).

Statistical Analysis

Data were analyzed using SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). The experiment was conducted using a Completely Randomized Design (CRD). Differences among treatment means were evaluated using one-way analysis of variance (ANOVA), and Duncan's Multiple Range Test was used for post hoc comparisons ($P < 0.05$). Colony development variables were analyzed using the Kruskal–Wallis test, and significant differences were further examined using pairwise Mann–Whitney U tests. Spring and autumn datasets were analyzed separately because the objective of the study was to evaluate treatment performance independently within each season rather than to compare seasonal effects. Therefore, season was not treated as a repeated factor in the statistical analysis. Statistical significance was accepted at $P < 0.05$.

Results and Discussion

Spring Season

Varroa Infestation in Bees

Varroa destructor infestation levels in adult bees and sealed brood cells before and after treatment applications is summarized in Table 2. Total infestation rates were calculated as the means of adult and brood infestation percentages. Significant reductions ($P < 0.05$) were observed in all treatment groups compared to the control.

When adult and brood infestation rates were combined, the total Varroa destructor infestation level decreased significantly in all treatment groups ($P < 0.05$). The greatest reduction was observed in the formic acid (1.25%) and thyme oil (1.96%) groups, while the control colonies showed a marked increase (9.94%) in terms of Varroa infestation.

Based on pre- and post-treatment infestation levels, the calculated efficacy of treatments against Varroa destructor ranged between 53.75–87.02% in adult bees and 60.91–87.52% in brood. The highest efficacy in adult bees was achieved with oxalic acid (87.02%), while the

Table 2. Combined Varroa infestation levels (%) in adult bees and brood before and after treatment (spring season)

Group	Adult Before (%)	Brood Before (%)	Total Before (%)	Adult After (%)	Brood After (%)	Total After (%)	p-value
Formic acid	6.00 ± 2.78	2.76 ± 0.77	4.38	1.20 ± 1.19	1.29 ± 0.67	1.25	0.042 *
Oxalic acid	11.06 ± 1.46	6.61 ± 2.04	8.84	4.40 ± 1.46	2.15 ± 1.28	3.28	0.025 *
Neem oil	9.20 ± 0.86	4.74 ± 1.81	6.97	2.40 ± 1.11	1.85 ± 0.94	2.13	0.034 *
Thyme oil	8.66 ± 0.81	4.90 ± 1.61	6.78	2.00 ± 0.81	1.91 ± 1.13	1.96	0.025 *
Herbal oil mixture	10.93 ± 0.76	6.33 ± 2.03	8.63	4.26 ± 0.76	2.85 ± 2.06	3.56	0.039 *
Control	6.40 ± 1.38	3.14 ± 1.10	4.77	14.00 ± 2.53	5.88 ± 1.21	9.94	0.042 *

highest efficacy in brood was found with formic acid (87.52%), Figure 1.

Colony Development

Significant differences were observed among groups regarding brood area development. The herbal oil mixture and oxalic acid treatments significantly enhanced brood area development ($P < 0.05$), while formic acid, thyme oil, and neem oil treatments negatively affected brood growth compared to the control, Table 3.

All colonies exhibited an increase in adult bee population after treatment. The highest mean increase was observed in the control (1.40 combs) and herbal oil mixture (1.10 combs) groups, followed by oxalic acid and thyme oil treatments. The smallest increase occurred in the formic acid group (0.60 combs). Differences among treatments were statistically significant ($P < 0.05$), Table 4.

Autumn Season

During the autumn season, one colony each from the neem oil (*Azadirachta indica*) and formic acid treatment groups was lost before the application phase, and these colonies were therefore excluded from the experiment. Following the autumn treatments, one colony from the neem oil group and one from the control group also collapsed and were removed from the trial.

These colony losses were not directly attributed to the treatments, as similar mortality occasionally occurs during the late season due to environmental and nutritional stress factors.

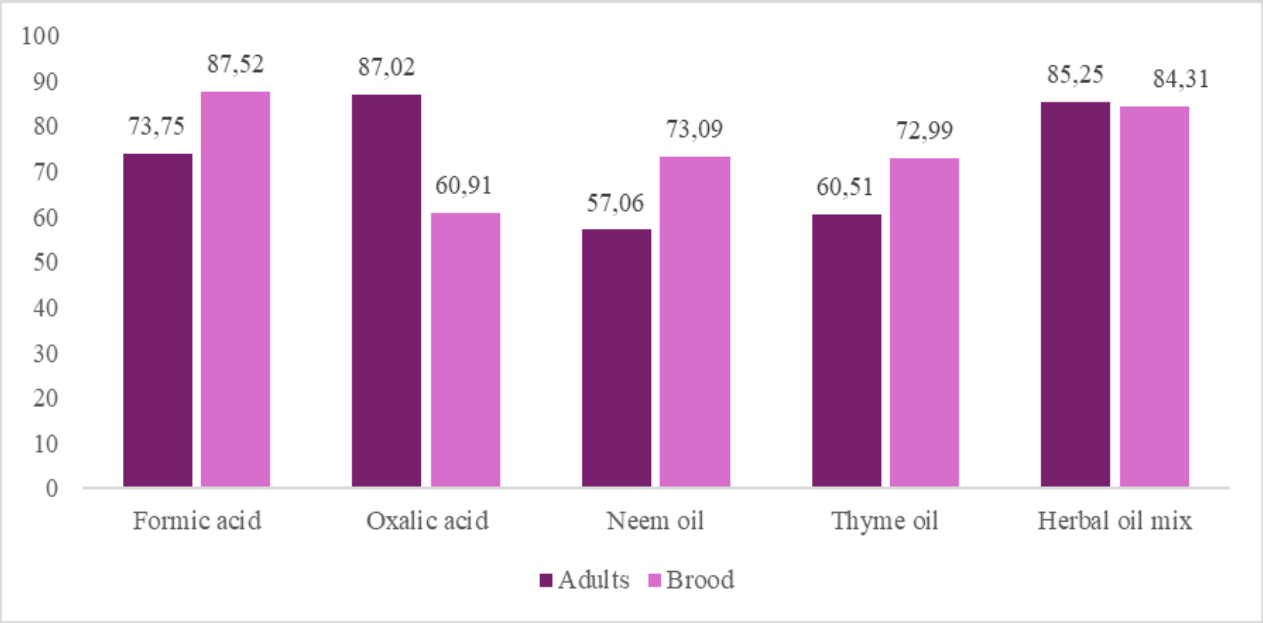

Figure 1. Effect of treatment groups on varroa infestation levels in spring (%)

Table 3. Mean brood area (cm²) of honey bee colonies before and after treatment

Group	Before (cm²)	After (cm²)	Difference (cm²)
Herbal oil mixture	3267.8 ± 120.4^{a}	4910.3 ± 165.2^{a}	+1642.5^{a}
Oxalic acid	2301.8 ± 132.6^{b}	3623.1 ± 141.1^{b}	+1321.3^{a}
Formic acid	3952.9 ± 144.8^{a}	3133.1 ± 126.5^{c}	−819.9^{c}
Thyme oil	3314.0 ± 118.2^{ab}	2473.1 ± 137.8^{c}	−840.9^{c}
Neem oil	3232.0 ± 142.5^{b}	2533.4 ± 131.3^{c}	−695.6^{c}
Control	3582.4 ± 151.7^{a}	4626.7 ± 159.2^{ab}	+1045.3^{b}

Values represent mean ± SE. Different letters within the same column indicate significant differences among treatments

Table 4. Changes in the number of combs covered with adult bees before and after treatment during the spring season

Group	Before	After	Difference
Herbal oil mixture	4.50 ± 0.18^{ab}	5.60 ± 0.22^{a}	+1.10^{a}
Oxalic acid	3.80 ± 0.25^{b}	4.60 ± 0.28^{ab}	+0.80^{ab}
Formic acid	5.00 ± 0.21^{a}	5.60 ± 0.24^{a}	+0.60^{b}
Thyme oil	4.30 ± 0.20^{ab}	5.20 ± 0.26^{ab}	+0.90^{ab}
Neem oil	4.60 ± 0.19^{ab}	5.40 ± 0.23^{ab}	+0.80^{ab}
Control	4.60 ± 0.22^{ab}	6.00 ± 0.27^{a}	+1.40^{a}

Values represent mean ± SE. Different letters within the same column indicate significant differences among treatments

Varroa Infestation in Bees

During the autumn season, Varroa destructor infestation levels decreased significantly in all treated colonies ($P < 0.05$), except for the control group, which exhibited an increase in infestation. The greatest reduction was recorded in the formic acid (−9.66%) and oxalic acid (−8.13%) groups, while the herbal oil mixture and thyme oil treatments achieved moderate reductions. The control colonies showed an increase of approximately 5%, indicating reinfestation in untreated colonies. Table 5 shows the changes in Varroa destructor infestation rates in honey bee colonies during the autumn season. All organic acid and essential oil treatments significantly reduced mite infestation compared to the control ($P < 0.05$). Values are presented as mean ± SE.

The mean percentage of varroa infestation on larvae before application in the test colonies was found to be 6.99%. Due to the decline in colony populations after drug application, samples could not be taken from capped cells. The results of the autumn application were evaluated in terms of the varroa population in adult bees.

Based on the infestation rates of adult bees before and after treatment, treatment efficacy ranged from 48.54% to 94.00%. Statistical analysis revealed that, following treatment, all groups differed significantly from the control group in terms of efficacy values ($P < 0.05$). Neem oil exhibited the highest efficacy (94.00%), followed by the herbal oil mixture (89.66%), thyme oil (75.59%), oxalic acid (58.80%), and formic acid (48.54%). These findings demonstrate that both organic acids and essential oils reduced Varroa destructor infestation under autumn field conditions, with neem oil and the herbal oil mixture showing the greatest acaricidal efficacy.

Colony Development

During the autumn season, colony population parameters declined markedly in all groups due to seasonal conditions and reduced brood activity.

Table 5. Combined Varroa infestation levels (%) in adult bees and brood before and after treatment during the autumn season

Group	Before (%)	After (%)	Difference (%)	p-value
Formic acid	13.83 ± 6.19	4.16 ± 1.66	−9.66 ± 4.63^{b}	0.068
Oxalic acid	12.13 ± 9.76	4.42 ± 4.00	−8.13 ± 6.04^{b}	0.042
Neem oil	8.00 ± 6.77	4.66 ± 2.90	−5.11 ± 5.30^{b}	0.102
Thyme oil	5.06 ± 4.49	2.37 ± 1.86	−3.20 ± 4.20^{b}	0.042
Herbal oil mixture	4.13 ± 4.20	2.35 ± 2.00	−2.13 ± 1.90^{b}	0.041
Control	8.26 ± 3.48	13.33 ± 5.46	+4.99 ± 1.58d	0.068

^{bd} Different superscript letters within the Difference (%) column indicate significant differences among treatment groups ($P < 0.05$). Values are expressed as mean ± SE (n = 5).

However, the magnitude of decrease varied among treatments.

The mean brood area decreased from 1761.18–2124.05 cm² before treatment to 164.32–514.01 cm² after treatment. The lowest reduction was observed in the oxalic acid, thyme oil and herbal oil mixture groups, whereas the highest loss occurred in the formic acid and neem oil groups. Significant within-group reductions in brood area were observed in the herbal oil mixture, thyme oil, and oxalic acid groups ($P < 0.05$), whereas reductions in the neem oil, formic acid, and control groups were not statistically significant, Table 6. Despite the overall decline in brood area, colonies treated with oxalic acid and the herbal oil mixture maintained relatively better population levels compared with the other treatments.

During the autumn season, the number of combs covered with adult bees showed a general decline across colonies, consistent with reduced brood activity typical of this period. However, colonies treated with the herbal oil mixture and oxalic acid maintained stable population levels, whereas those treated with thyme oil exhibited the greatest reduction (−1.6 combs). The formic acid, neem oil, and control groups showed minor decreases, indicating that most treatments did not cause significant stress to the colonies. Statistical evaluation indicated no significant differences among treatments regarding the number of combs covered with adult bees after application ($P > 0.05$). These findings suggest that organic acid and essential oil treatments can be safely used in autumn without major negative impacts on colony strength.

Aggressiveness

The aggressive behavior (stinging response) of the experimental colonies was assessed following each treatment application. When the levels of defensiveness measured immediately after the application of essential oils and organic acids were evaluated, no statistically significant differences were found among treatments or between experimental periods ($P > 0.05$). Although beekeepers often report that thyme oil increases colony aggressiveness, the present study did not confirm this observation statistically. In colonies treated with thyme oil and neem (*Azadirachta indica*) oil, bees were observed to temporarily leave the hive immediately after application. This behavior was attributed to the strong and sharp odor of the essential oils rather than to an increase in true aggressiveness. The measurements indicated that these volatile compounds may have caused short-term irritation, but they did not significantly elevate defensive responses. Overall, similar to the findings in the spring season, no significant differences in colony aggressiveness were detected among treatments or between seasons. These results suggest that both organic acid and essential oil treatments can be safely used without inducing notable defensive or aggressive behavior in honey bee colonies.

Grooming Behavior

The number of *Varroa destructor* mites that had naturally fallen on Vaseline-coated sheets increased after treatment in all colonies, particularly in the thyme

Table 6. Colony population development (brood area, cm²) of honey bee colonies during the autumn season

Group	Before (cm²)	After (cm²)	Difference (cm²)	*p*-value
Herbal oil mixture	1761.18 ± 1037.66	297.51 ± 305.27	−1463.66 ± 992.10	0.043
Thyme oil	2124.05 ± 648.77	514.01 ± 248.93	−1610.03 ± 725.68	0.043
Neem oil	1644.96 ± 848.19	164.32 ± 171.53	−1730.66 ± 697.38	0.109
Oxalic acid	1708.94 ± 403.52	321.85 ± 136.62	−1387.09 ± 470.85	0.043
Formic acid	2022.16 ± 1008.50	217.64 ± 184.50	−1804.51 ± 873.59	0.068
Control	1601.55 ± 538.21	185.45 ± 160.86	−1628.87 ± 242.88	0.068
Mean	1810.47	283.46	—	—

Values are presented as mean ± SE. Mean difference represents the paired difference between pre- and post-treatment brood area. p-values indicate within-group comparisons.

oil, formic acid, and control groups. However, the number of mites showing body deformities (Figure 2)—a measure of grooming activity—did not differ significantly among treatments ($P > 0.05$).

The oxalic acid treatment resulted in the highest level of mite deformation (2.8 mites per colony), suggesting that oxalic acid may enhance grooming behavior by stimulating cleaning reflexes in worker bees, Table 7. These results indicate that although grooming activity varies among treatments, essential oils and organic acids generally do not cause adverse behavioral effects on colonies.

Honey Yield

Honey yield varied considerably among treatment groups, Table 8. The highest mean honey yield was obtained from colonies treated with oxalic acid (13.52 kg), followed by the control (11.89 kg) and herbal oil mixture (11.50 kg) groups. In contrast, the lowest honey yield was recorded in colonies treated with neem oil (3.83 kg), while thyme oil (7.74 kg) and formic acid (7.24 kg) treatments produced intermediate values. The oxalic acid treatment did not reduce colony productivity, indicating its compatibility with honey production. Conversely, colonies treated with neem oil, thyme oil, and formic acid produced lower honey yields compared to the control, possibly due to the repellent odors or mild stress effects associated with these treatments. Despite these reductions, none of the tested natural treatments caused a complete suppression of honey (yield, demonstrating that both organic acids and essential oils can be used safely in Varroa destructor management without substantially impairing honey production.

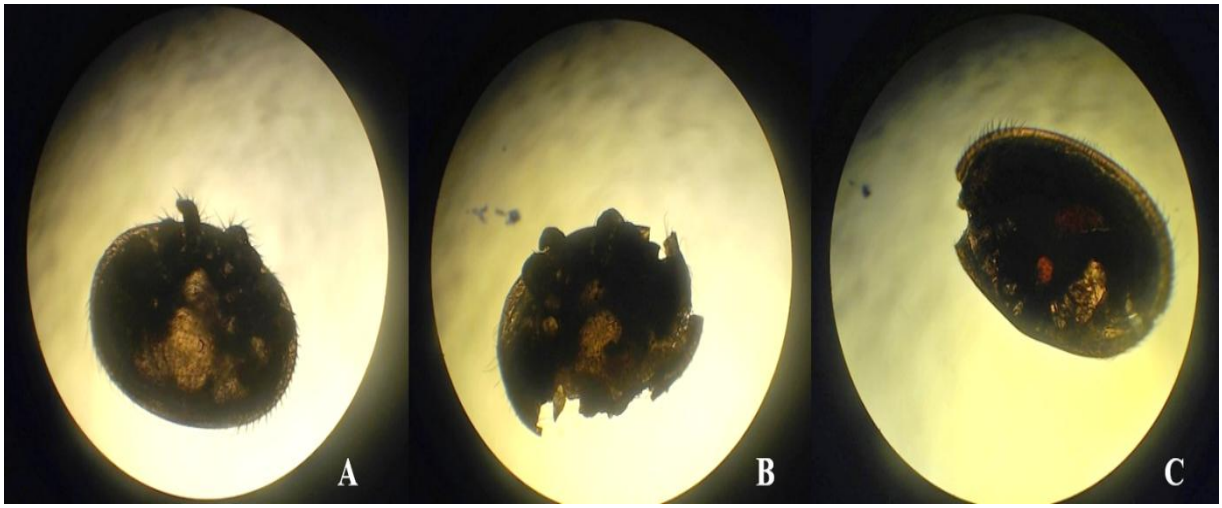

Figure 2. Microscopic observation of body deformities in *Varroa destructor* (original).

Table 7. Number of fallen and deformed *Varroa destructor* mites recorded before and after treatment (autumn season)

Treatment Group	Mites Fallen (Before)	Mites Fallen (After)	Deformed Mites (Mean ± SE)
Herbal oil mixture	10.0	19.6	1.20 ± 0.10
Thyme oil	28.8	43.6	1.40 ± 0.12
Neem oil	20.75	24.0	1.33 ± 0.15
Oxalic acid	9.0	18.2	2.80 ± 0.25
Formic acid	16.75	29.5	1.50 ± 0.11
Control	19.0	30.0	1.00 ± 0.08

Table 8. Mean honey yield (kg) of colonies according to treatment groups

Treatment Group	Honey Yield (kg ± SE)
Herbal oil mixture	11.50 ± 0.42
Thyme oil	7.74 ± 0.35
Neem oil	3.83 ± 0.28
Oxalic acid	13.52 ± 0.47
Formic acid	7.24 ± 0.31
Control	11.89 ± 0.40

Discussion

The present study demonstrated that both organic acids (formic and oxalic acids) and essential oils (thyme, neem, and a herbal oil mixture) were effective in reducing Varroa destructor infestation under field conditions in İzmir, Türkiye whereas in Mediterranean climatic zone. However, their effects on colony development and behavioral parameters differed across treatments and seasons.

Efficacy of Organic Acids and Essential Oils

The efficacy values obtained in this study (48.5–94%) are consistent with previous reports demonstrating that organic acids and essential oils can provide substantial Varroa control without causing major colony losses (Sabahi et al., 2020; Hýbl et al., 2021). Oxalic acid showed the highest mean efficacy, aligning with findings by Sagona et al. (2022), who reported that trickled oxalic acid treatments were highly effective against phoretic mites, particularly in broodless conditions. Formic acid also produced significant reductions, confirming previous reports that it targets mites in the reproductive stage by enabling its volatility to enter closed brood cells (Genath et al., 2020). Among the essential oils tested, thyme oil and the herbal mixture significantly reduced mite infestations, supporting results from Bisrat et al. (2022) and Alsaadi et al. (2024), who observed that thymol and mixed plant oils disrupt mite nervous and respiratory systems while maintaining high bee tolerance. Neem oil achieved high efficacy in the autumn season, consistent with González-Gómez et al. (2016), who found that azadirachtin interferes with mite development and feeding. However, prolonged or high-dose exposure to neem oil may temporarily alter foraging behavior due to its strong odor (Begna et al., 2023). Particularly in the Mediterranean climate zone, the sudden warming of the weather in spring can cause rapid evaporation of formic acid and thyme oil, leading to a risk of concentration within the colony, disturbing the bees, and causing queen bee loss. This risk should not be ignored.

Effects on Colony Development and Productivity

Colony strength and brood area are reliable indicators of the safety of Varroa control agents. In this study, oxalic acid and the herbal oil mixture promoted brood development and maintained population stability, particularly in spring, while formic acid and thyme oil caused mild declines in brood area. These results suggest that while organic acids are generally well-tolerated, formic acid may impose transient stress, especially under warm conditions (Locke et al., 2011; Jack et al., 2024). The moderate reduction observed with thyme oil is consistent with reports that strong essential oil volatiles can temporarily suppress brood-rearing or induce queen avoidance of treated combs (Sabahi et al., 2017; Qadir et al., 2021). Honey yield results further confirmed that oxalic acid and the herbal mixture were compatible with productive colony performance, while neem and thyme oils were associated with reduced honey production. Similar patterns were reported by Bahreini et al. (2020) and Jack et al. (2021), emphasizing that despite their efficacy, some essential oils may temporarily suppress foraging activity or brood care due to their intense odors or irritant effects. Nevertheless, the overall yields in treated groups remained within acceptable ranges, demonstrating that the tested natural compounds can be integrated safely into seasonal management cycles.

Behavioral Responses: Aggressiveness and Grooming

Behavioral evaluations revealed no significant increase in aggressiveness after treatment, suggesting that the tested substances did not disrupt colony temperament. This finding contrasts with anecdotal beekeeping reports of heightened defensiveness following thymol application but aligns with controlled observations by Allam and Zakaria (2009), who found that thymol exposure induced temporary agitation without sustained aggression. The grooming data indicated increased mite drop after treatment in all groups, with oxalic acid-treated colonies exhibiting the highest mean number of deformed mites. However, these differences were not statistically significant among treatments. This supports previous observations by Abd El-Wahab et al. (2012) and Morfin et al. (2023), indicating that natural acaricides may enhance self-cleaning behavior by stimulating cuticular irritation or sensory responses. Grooming behavior is a critical adaptive mechanism that complements chemical control, contributing to colony-level resistance to Varroa infestations.

Seasonal Influence

Seasonal variation significantly influenced both infestation levels and colony dynamics. Spring treatments yielded stronger brood development and higher efficacy values compared to autumn, reflecting the physiological vigor of colonies during active periods. The reduced autumnal brood rearing limited the penetration of some treatments, particularly oxalic acid,

which acts mainly on phoretic mites (Traynor et al., 2020). Nevertheless, all treatments effectively suppressed mite populations, suggesting that integrated, seasonally adjusted organic control strategies can provide sustained protection while minimizing chemical residues in hive products.

A limitation of the present study is that the tested compounds were applied according to their recommended field protocols, which differed in application frequency, duration, and delivery method. Consequently, direct comparisons of miticidal efficacy and colony-level responses among treatments may have been influenced not only by the intrinsic properties of the active substances but also by differences in exposure conditions. Therefore, the findings should be interpreted as a comparison of practical field treatment protocols rather than a strictly standardized comparison of the active compounds themselves. Nevertheless, the results support the integration of organic acids and essential oils into Varroa destructor management programs as environmentally friendly, residue-free alternatives to synthetic acaricides under practical beekeeping conditions. The differential responses observed between seasons and treatment types highlight the importance of context-specific application timing. Combining oxalic acid and plant-based products with non-chemical control measures, such as drone brood removal or screened bottom boards, may enhance long-term efficacy while maintaining colony welfare (Shannon et al., 2025). Future studies should employ standardized exposure conditions to better isolate the effects of individual active ingredients, as well as conduct multi-year trials incorporating molecular and physiological assessments of honey bee health under integrated treatment regimes.

Conclusion

This study demonstrated that organic acids and essential oils can serve as effective and environmentally sustainable alternatives to synthetic acaricides for the control of Varroa destructor in honey bee colonies. Oxalic acid and the herbal oil mixture were the most effective of the treatments, keeping colony strength and productivity up during both seasons. Formic acid and thyme oil showed moderate levels of control, but they caused stress on some colonies for a short time. Neem oil worked better in the fall than in the spring, which suggests that its activity may depend on the season. None of the treatments caused significant changes in aggressiveness or grooming behavior, confirming their safety for honey bee welfare. The results of the honey yield tests showed that oxalic acid and herbal oil mixtures can be used in beekeeping without decreasing productivity. Honey yield results further supported that oxalic acid and herbal oil mixtures can be integrated into beekeeping practices without compromising productivity in the Aegean Region, which is characterized by a Mediterranean climate.

Given the current increase in public awareness and consumption trends regarding health protection and food consumption in relation to organic methods of combating bee pests, it can be anticipated that this approach could enable pesticide-free production of bee products. It could also establish a production model that ensures bee welfare, leaving no residues in the colony and offering higher yield potential. This strategy is an element of both the UN Sustainable Agriculture Goals and the agricultural planning objectives of the 12th Development Plan of Türkiye.

Ethical Statement

The study was conducted in accordance with ethical standards, and all participants provided informed consent prior to data collection.

Conflict of Interest

The authors declare that there are no conflicts of interest related to the execution or reporting of this study.

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